thematic review

*Thematic Review Series: Lipids and Lipid Metabolism in the Eye*

# Regulating survival and development in the retina: key roles for simple sphingolipids

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**Abstract** **Many sphingolipids have key functions in the regulation of crucial cellular processes. Ceramide (Cer) and sphingosine (Sph) induce growth arrest and cell death in multiple situations of cellular stress. On the contrary, sphingosine-1-phosphate (S1P), the product of Sph phosphorylation, promotes proliferation, differentiation, and survival in different cell systems. This review summarizes the roles of these simple sphingolipids in different tissues and then analyzes their possible functions in the retina. Alterations in proliferation, neovascularization, differentiation, and cell death are critical in major retina diseases and collective evidence points to a role for sphingolipids in these processes. Cer induces inflammation and apoptosis in endothelial and retinal pigmented epithelium cells, leading to several retinopathies. S1P can prevent this death but also promotes cell proliferation that might lead to neovascularization and fibrosis. Recent data support Cer and Sph as crucial mediators in the induction of photoreceptor apoptosis in diverse models of oxidative damage and neurodegeneration, and suggest that regulating their metabolism can prevent this death. New evidence proposes a central role for S1P controlling photoreceptor survival and differentiation. Finally, this review discusses the ability of trophic factors to regulate sphingolipid metabolism and transactivate S1P signaling pathways to control survival and development in retina photoreceptors.**—Rotstein, N. P., G. E. Miranda, C. E. Abrahan, and O. L. German. **Regulating survival and development in the retina: key roles for simple sphingolipids.** ***J. Lipid Res.*** **2010.** 51: **1247–1262.**

**Supplementary key words** apoptosis • cell signaling • ceramides • eye • sphingosine phosphate • photoreceptor

When asked about the roles of cellular lipids, the first thought usually coming to our minds relates to their functions as structural membrane components and energetic fuels. However, the findings of the previous half century have extended these roles, shedding light on their indisputable relevance as signaling molecules controlling key aspects of cellular life and development. The identification of diacylglycerol and inositol 1,4,5 triphosphate (1) as second messengers seemed at the time a peculiarity of these lipids. The subsequent findings of the roles of arachidonic acid metabolites (2), platelet activating factor (3), and other minor inositol lipids (4) in cell signaling began to establish that being intracellular messengers was not an oddity but a habitual task for lipids in cells. The family of lipid signaling molecules largely expanded in the last two decades, when several sphingolipids were successively shown to regulate a multiplicity of cell functions. Sphingosine (Sph) was the first sphingolipid to be established as a bioactive lipid, involved in the regulation of protein kinase (PK)C activity (5). Ceramide (Cer) and sphingosine-1-phosphate (S1P) were next shown to signal a myriad of cell functions and the number of sphingolipid molecules with bioactive properties has gone on enlarging in recent years (6, 7).

Though a vast literature exists on the functions of sphingolipids in several tissues, less is known concerning its roles in the eye, and in the retina in particular. Accumulation of sphingolipids originated in defects in the lysosomal

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*Manuscript received 23 October 2009 and in revised form 25 January 2010.*

*Published, JLR Papers in Press, January 25, 2010*
*DOI 10.1194/jlr.R003442*





Abbreviations: AMD, age-related macular degeneration; BDNF, brain derived neurotrophic factor; Cer, ceramide; CerK, Cer kinase; CerKL, CerK like; CERT, Cer transport protein; C1P, ceramide-1-phosphate; CNS, central nervous system; DHA, docosahexaenoic acid; ER, endoplasmic reticulum; GDNF, glial derived neurotrophic factor; GCS, glucosylceramide synthase; GlcCer, glucosylceramide; JNK, c-*jun* N-terminal kinase; MAPK, mitogen-activated protein kinase; NGF, nerve growth factor; NF-κB, nuclear factor κB; OS, outer segment; PI3K, phosphatidylinositol 3-kinase; PK, protein kinase; RPE, retinal pigmented epithelium; SAPK, stress-activated protein kinase; S1P, sphingosine-1-phosphate; S1P1,2,3,4,5, S1P receptors 1, 2, 3, 4, 5; SMase, sphingomyelinase; Sph, sphingosine; SphK, sphingosine kinase; TGF, tumor growth factor; TAK-1, TGF-β activated kinase 1; TNF, tumor necrosis factor.

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enzymes responsible for their degradation, occurs in several inherited diseases, and is often associated with retinal impairment and vision loss due to retina neuronal cell death (8–11); however, the mechanisms leading to this death are uncertain. In this review, we will focus on the so-called "simple sphingolipids" Cer, Sph, and S1P, as opposed to sphingolipids with more complex headgroups (12). After briefly summarizing their metabolism, functions, and established signaling pathways in different tissues, our purpose is to put together and discuss the recently uncovered information that highlights their possible functions in the retina.

## THE SPHINGOLIPIDS: A VAST FAMILY

Sphingolipids were first discovered in 1884 by J. L. W. Thudichum in the nervous tissue and then established as essential components of all eukaryotic cell membranes. As other membrane components, sphingolipids are amphipatic molecules, their hydrophobic region made up by a sphingoid long chain base, to which a long-chain fatty acid is attached. Though over 60 different sphingoid bases have been reported (13), Sph, a straight long-chain amino-alcohol with 18–20 carbon atoms (indicated in red in **Fig. 1**) and a trans double bond between C-4 and C-5, is the most frequently found in mammalian sphingolipids.

Attachment of a fatty acid to Sph through an amide linkage (Fig. 1) originates a highly hydrophobic molecule, Cer (14, 15). Its fatty acids are of variable length (C2 to C28) and usually saturated, though certain specialized cells as spermatozoa, have very long-chain (C24 to C34) polyunsaturated fatty acids (16, 17). Cer is the backbone of many complex sphingolipids, formed by attachment of different headgroups at C-1. Addition of phosphorylcholine forms sphingomyelin (SM) (Fig. 1) whereas attachment of a sugar, such as glucose or galactose, is the first step in the formation of many complex glycosphingolipids.

Cer is generated by two main enzymatic mechanisms, its synthesis de novo, which takes place in the endoplasmic reticulum (ER) and the so called "SM cycle" (**Fig. 2**). The latter occurs in different cell compartments through the hydrolysis of SM by diverse sphingomyelinases (SMases), the acidic and neutral isoforms being activated in response to many extracellular stimuli in different cell compartments such as lysosomes, endosomes, and the plasma membrane (7). Both pathways may be differentially or simultaneously induced to achieve a similar biological purpose and which one prevails depends on the stimuli and the cell type.

Catabolism of Cer occurs through several pathways. Ceramidases deacylate Cer to produce Sph (Fig. 2). Sph serves as a precursor to resynthesize Cer, a reaction catalyzed by Cer synthase, or is further phosphorylated to S1P by Sph kinase (SphK). S1P can be transformed back to Sph and then to Cer by S1P phosphatase and Cer synthase or it can generate ethanolamine phosphate and hexadecenal, the main exit route of sphingolipid metabolism (Fig. 2). Cer can also be phosphorylated by Cer kinase to generate ceramide-1-phosphate (C1P). In the Golgi, Cer can be converted to SM through the addition of phosphorylcholine catalyzed by SM synthase. Glycosyltransferases attach sugar moieties to Cer to generate galactosylceramides, in the ER or glycosylceramides, in the Golgi (18). These reactions are key steps in the generation of more complex glycosphingolipids.

## CER, A DEADLY SECOND MESSENGER

Cer has a central role in sphingolipid metabolism and plays many relevant physiological functions (6, 7, 19–22). This demands keeping a tight control of its cellular level; hence, the routes for Cer generation and removal respond to a variety of cell stimuli that control the activities of at least 26 different enzymes in diverse intracellular locations (6, 23, 24).

Many Cer actions can be ascribed to its role as a membrane component. Due to its chemical structure that allows for tight packing, Cer regulates membrane properties,

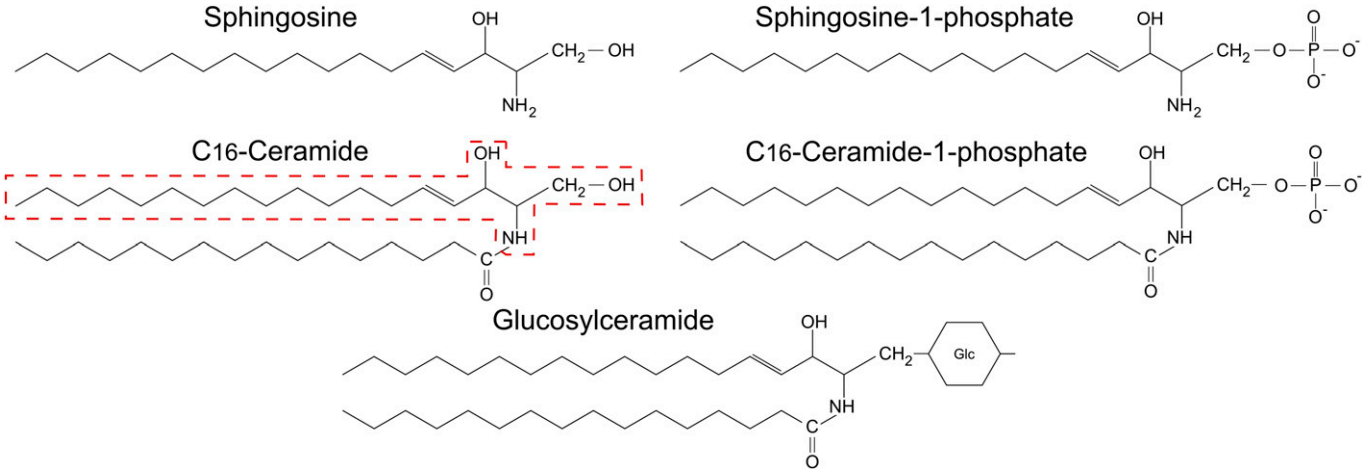


**Fig. 1.** Chemical structures of sphingolipids. The structures of different sphingolipids types are shown. The sphingoid base present in all these molecules is indicated in red. Glc, glucose.

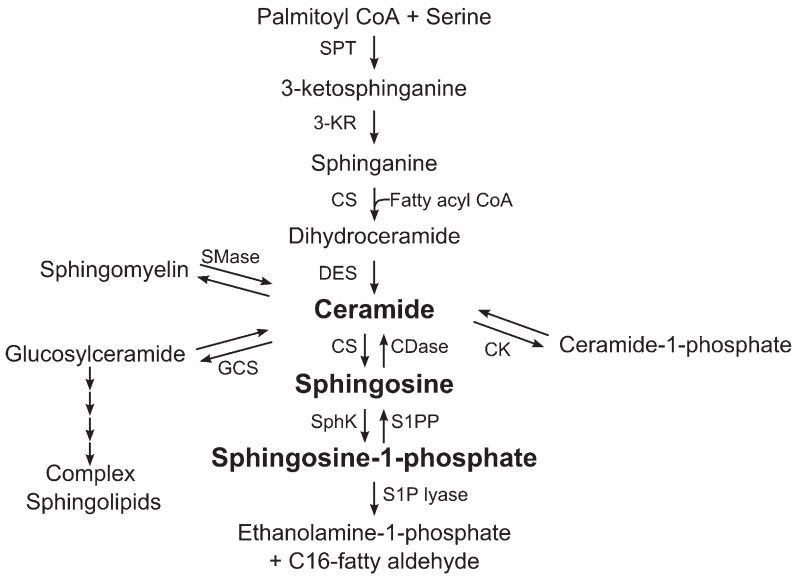


**Fig. 2.** The complex metabolic pathways of sphingolipid metabolism. The metabolic pathways and enzymes leading to the synthesis of the major sphingolipids classes are shown. Cdase, ceramidase; CK, ceramide kinase; CS, ceramide synthase; DES, dihydroceramide desaturase; GCS, glucosylceramide synthase; KR, ketosphinganine reductase; S1PP, sphingosine-1-phosphate phosphatase; S1P lyase, sphingosine-1-phosphate lyase; SMase, sphingomyelinase; SphK, sphingosine kinase; SPT, serine palmitoyl transferase.

increasing acyl chain order and altering membrane permeability (25). Its exogenous addition or generation through SMase activity leads to lateral phase separation and the enlargement and clustering of preexistent nanodomains, promoting raft fusion (12, 26–28) and giving rise to Cer-enriched platforms that facilitate the clustering of receptor molecules and their ligands. Thus, Cer-induced clustering of the CD95/Fas death receptor (29, 30) precedes the induction of apoptosis, evidencing the close relationship between Cer's structural role and modulation of cell death.

In addition to this structural role, Cer is a well-established second messenger. It is rapidly produced upon a myriad of cell stimuli to participate in the regulation of cell cycle arrest, differentiation, senescence, and apoptosis by activating a diverse set of protein kinases and phosphatases, which in turn regulate a variety of signaling cascades (6, 27, 31). One of Cer's best recognized functions is being a mediator of cell death. Multiple endogenous and exogenous stimulants including tumor necrosis factor (TNF)-α, FAS ligands, anticancer drugs, oxidative stress, ionizing and ultraviolet (UV) radiation, and serum or trophic factor deprivation (6, 7, 19–21) increase Cer intracellular levels, and this increase precedes the onset of apoptosis in many cell types (32, 33).

The choice among the diverse intracellular pathways and biological outcomes activated by Cer depends on its levels, the molecular species formed, the cell type and age, the subcellular localization, the expression of specific receptors, and the selective targets coupled to specific signaling pathways (22). Cer differentially modifies the activity of a large number of intracellular effectors (31, 34) (**Fig. 3**). To trigger apoptosis, it might bind directly to cathepsin D, phospholipase A2, c-Raf, PKC α and ζ, and some cytoskeletal and stress proteins (6, 7, 20, 27, 35) and has been suggested to modulate the activity of different kinases such as c-*jun*-N terminal kinase (JNK), MAPK, PKC, Akt, phosphatidylinositol 3-kinase (PI3K), TGF-β activated kinase 1 (TAK-1), and kinase supressor of Ras (36). Cer also promotes the nuclear translocation of nuclear factor κB (NF-κB) (37), binds and activates Ser-Thr phosphatases (23, 34), and activates several proteases involved in apoptotic pathways, such as caspase-3 (38), caspase-8 (39), and cathepsin D (40).

Cer plays relevant roles in the nervous system, which depend on the developmental stage and Cer concentration; low Cer levels protect neurons from apoptosis (41, 42), whereas high Cer concentrations activate it (43, 44). Mounting evidence suggests Cer increase contributes to the etiology of several pathologies. Cer accumulates in the brain during aging and might promote inflammation and endocytic abnormalities (45, 46). Elevated Cer levels have been found in the brain in patients affected by Alzhei-

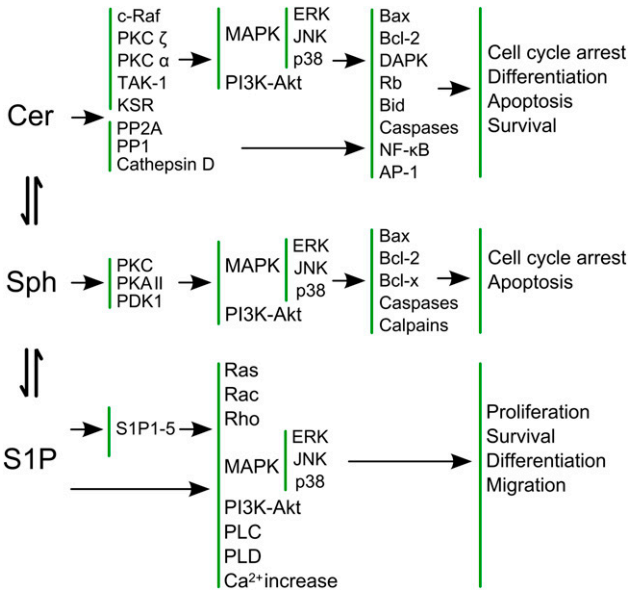


**Fig. 3.** Multiple targets signaling pathways are regulated by simple sphingolipids to achieve their biological outcomes. Ceramide (Cer), sphingosine (Sph), and sphingosine-1-phosphate (S1P) regulate directly or indirectly a large diversity of intracellular targets and signaling pathways that lead to different biological outcomes.

mer's disease and recent data involves Cer in amyloid β peptide generation (22). Hence, understanding the signaling pathways activated by Cer to induce neuronal death might provide clues for treating neurodegenerative diseases.

## SPH, A FURTHER MEDIATOR OF CELL DEATH

Different apoptotic agents increase cellular Sph levels in the early steps of the apoptotic pathway (47–49). This increase arises mainly in deacylation of Cer in mammalian cells, in which Sph is not formed de novo, in a reaction catalyzed by ceramidases (50). These enzymes comprise a heterogeneous family made up of alkaline, acidic, and neutral isoforms (51, 52) and their activity increases upon apoptotic injuries that augment Sph levels (53, 54). Exogenous Sph also induces apoptosis and inhibits proliferation in many cell types (38, 55, 56), supporting its role as a signaling molecule.

The intracellular accumulation of Sph activates several targets and signal transduction pathways (Fig. 3). Sph inhibits PKC (35, 57) and activates PKA type II and mammalian 3-phosphoinositide-dependent protein kinase-1, which regulates the activity of protein kinases like AMPc- and GMPc-dependent protein kinases, several PKCs, and PKC-related kinase isoforms (58–60). Interestingly, Sph can bind to nuclear receptors such as the steroidogenic factor-1 (61) and has also been reported to activate synaptic vesicle exocytosis by promoting SNAP Receptors (SNARE) assembly (62).

Sph activates multiple signaling pathways to induce cell death, which sometimes differ from those activated by Cer. Sph strongly inhibits the extracellular signal-regulated kinase (ERK1/2) (63) and the Akt kinase pathway (64), and activates the p38-MAPK, which has been linked to induction of apoptosis (65). The mitochondrial pathway is also involved in Sph induced-apoptosis (48, 49). In addition, several lysosomal proteases participate in Sph-induced programmed cell death (66). In conclusion, Sph, as Cer, is an apoptotic mediator that recruits several pathways and organelles to lead to cell death.

## S1P, AN EXTRACELLULAR AND INTRACELLULAR MESSENGER

S1P, the product of Sph phosphorylation, is a bioactive lysophospholipid that regulates multiple processes indispensable for cellular homeostasis, including growth and survival, proliferation, differentiation, migration, and immune function (67–72). This diversity of functions enlightens S1P's crucial role in diseases such as cancer, atherosclerosis, angiogenesis, inflammation, and autoimmunity, among others (71, 72).

S1P synthesis is catalized by Sph kinases (SphKs), which have two mammalian isoforms, SphK1 and SphK2, that differ in their sequence, catalytic and kinetics properties, subcellular localization, developmental and tissue expression, and, puzzlingly, in their biological functions. SphK1 is mainly cytosolic and growth factors promote its translocation to the plasma membrane (73, 74) where its substrate, Sph, is found (70). SphK1 is responsible for S1P-mitogenic and anti-apoptotic effects; its exogenous expression induces cell proliferation and pro-survival signals whereas its downregulation activates proapoptotic events (75). In contrast, SphK2 is a putative BH3-only protein that inhibits cell growth and enhances apoptosis (76–78). Stress promotes its translocation from the cytosol to the ER and/or nucleus to stop proliferation and induce $Ca^{2+}$ mobilization, leading to apoptosis (76–79). Targeting SphK1 to the ER turns its action from anti- to pro-apoptotic (78), implying the site of synthesis is critical for determining its biological functions.

S1P is unique as a signaling molecule because it mediates its biological effects by acting both as an intracellular second messenger (80) and as an extracellular ligand that binds to and activates G protein-coupled membrane receptors. Most of S1P's ability to control cell growth and survival seems to depend on S1P intracellular signaling. Survival factors and cytokines signal through receptors such as tyrosine kinase receptors to increase SphK1 activity, either by enhancing its transcription or through its rapid activation and translocation to the plasma membrane. SphK1 activation leads to S1P accumulation, which turns on signaling pathways regulating calcium mobilization, DNA synthesis, cell growth, tumorigenesis, and proliferation and suppression of apoptosis (81–85).

Newly synthesized S1P can also be secreted to the extracellular milieu acting in an autocrine/paracrine fashion to activate S1P receptors (86, 87). This S1P "inside-out" signaling regulates cytoskeleton rearrangements and cell movement (71). S1P high plasma levels (0.1–1 μM) and its relatively elevated critical micellar concentration (about 12–14 μM at physiological pH) allow S1P to act as an effective first messenger because the bloodstream concentration of its monomers are well above the $K_D$ for S1P receptors (71, 88, 89). S1P receptors are a family of five high-affinity G protein-coupled membrane proteins, referred to as S1P1–5, members of the previously called Edg receptor family, encoded by the endothelial/differentiation gene (Edg) (81, 90). They are ubiquitously found throughout the body and all of them are expressed in the central nervous system (CNS) (91). Their activation regulates cell migration, angiogenesis, and heart embryonic development (24). The processes they stimulate depend on the different, and at times overlapping, subsets of heterotrimeric G-proteins they turn on. This downstream G protein-mediated signaling controls activation of the small GTPases of the Rho family, particularly Rho and Rac, MAPK, adenilate cyclase, phospholipase C and D, PI3K, and JNK, and processes such as $Ca^{2+}$ mobilization (92–94) (Fig. 3).

S1P suppresses apoptosis induced by multiple insults in many cell types (71, 83, 95–99). SphK1 is frequently overexpressed in cancer cells, preventing their apoptosis (71) and suggesting a central role for S1P in cancer cell survival (100).

The mechanisms of intracellular action of S1P are still elusive (Fig. 3). S1P enhances $Ca^{2+}$ mobilization (101–105) and stimulates the ERK/MAPK pathway to promote cell growth and survival (95). It may also activate the PI3K/Akt pathway (106) to affect the balance between pro- and anti-apoptotic Bcl-2 proteins (95, 107) and activator protein 1 and NF-κB, which promote survival (108).

S1P has multiple roles in the CNS. S1P1 receptor is highly expressed in specific brain regions during neurogenesis (109) and S1P1-null and Sphk1/Sphk2 double-null embryos have major defects in neurogenesis, suggesting their relevance in this process (110–112). Acting through S1P receptors, S1P stimulates neural stem/progenitor cell proliferation and promotes their differentiation into neurons and astrocytes (113). Deletion of SphK1 or S1P3 in a mouse model of Sandhoff disease results in a milder disease course, with decreased glial proliferation (114), endorsing a significant role for S1P in glial proliferation in physiological and pathological processes. The relevance of S1P in neuronal and glial survival is beginning to be uncovered. Cerebellum and astroglial cells release S1P, which might act in an autocrine/paracrine manner in the CNS to promote survival and growth at various stages of neuronal development (115).

## LIFE AND DEATH DECISIONS: THE SPHINGOLIPID RHEOSTAT

A striking feature underscoring the complexity of sphingolipid signaling is the antagonism between the cellular responses regulated by Sph and its immediate precursor Cer, and those controlled by S1P. Different cell stressors activate Cer and Sph biosynthetic enzymes to increase their levels, which then signal pro-apoptotic and growth inhibitory effects. On the contrary, growth factors upregulate SphK levels to increase intracellular content of S1P, which then promotes proliferation and cell survival. The high conservation of these sphingolipid second messengers, from yeast to man, highlights their biological relevance. Because Cer, Sph, and S1P are readily interconverted depending on cell conditions, maintaining a dynamic equilibrium and a fine-tuning in their levels is crucial for cell functioning. This has led to the "sphingolipid rheostat" concept, which proposes that favoring the synthesis of either sphingolipid and the consequent activation/inactivation of opposing signaling pathways has crucial and opposing effects on cell fate (81).

Accumulating data endorse this model and underscore the pathological relevance of imbalances in sphingolipid levels in cancer, cardiovascular pathologies disorders, and allergies, among others (116, 117). Brains of Alzheimer's disease patients show increased acid SMase activity, higher Cer and Sph levels, and decreased S1P content. Abeta oligomers increase acid SMase activity and Cer levels in neuronal cells, triggering apoptosis, whereas pretreatment with recombinant acid ceramidase prevents it (118). These findings underline the significance the sphingolipid-rheostat concept might have in the design of new therapeutical approaches for treating relevant human pathologies.

## GLUCOSYLCERAMIDE, AN ESCAPE ROUTE IN THE APOPTOTIC PATHWAY

Alternative routes complement the sphingolipid rheostat, contributing to lower Cer levels, among them channeling Cer to the synthesis of complex sphingolipids such as glucosylceramide (GlcCer), the simplest glycosphingolipid. Its synthesis by Cer glucosylation is catalyzed by glucosylceramide synthetase (GCS), whose expression and activity is modulated by multiple compounds (119).

GlcCers participate in cellular processes such as cell proliferation, oncogenic transformation, differentiation, and tumor metastasis (119, 120). Modulation of GCS can significantly affect the apoptotic mechanism (119). Many chemotherapeutical drugs increase Cer to trigger tumoral cell death; favoring a decrease in ceramide levels provides an escape route to avoid apoptosis. This strategy is implicated in development of drug resistance (121–124); several chemotherapy-resistant cancer cell types increase GCS activity and/or expression to enhance Cer metabolization to GlcCer (125). GCS regulation of Cer levels is also relevant for cell survival during development (126, 127) and for neural differentiation, contributing to regulate the rate of axonal (128–130) and dendritic growth (131, 132). However, too much GlcCer can impair neuronal development. Its defective degradation in Gaucher's disease results in neurological symptoms because its buildup in the brain might induce neurodegeneration by alterating calcium homeostasis (133, 134) In conclusion, GlcCer is another sphingolipid with a role in the regulation of cell survival and functioning.

## SPHINGOLIPIDS IN THE EYE

The knowledge concerning sphingolipids in different tissues and systems has explosively expanded in recent years. The increased awareness of the diversity of sphingolipid functions has driven current research on their roles in the eye, and new evidence already points to a key role of sphingolipids in controlling crucial cellular processes in retina neurons both during normal development and in pathological situations.

Little is known regarding sphingolipid composition and enzymatic activities in the retina. Cer and Sph are present in rat retina, in which 79% of Cer molecules contained 16:0 and 18:0, whereas the remaining 21% had very long-chain fatty acids (20:0 to 24:2) (135). In bovine retina rod outer segments (OSs) Cer is also found, enriched in polyunsaturated fatty acids (136). Lipid phosphate phosphatases that dephosphorylate S1P have been identified in rod OSs and their activities are modulated by Cer and Sph (137, 138). More information on the composition and metabolism of sphingolipids in the retina is required as it

would provide an essential framework to unravel their roles in this tissue.

## CERAMIDE IN THE RETINA

The earliest indirect evidence suggesting a role for Cer in retinal pathologies was the increased Cer levels in brains of patients with the juvenile form of Batten disease, in which neuronal apoptosis in the retina and brain leads to blindness and cognitive decline (139) and in retinas of patients with Farber disease, which is also associated with blindness (140, 141). Acharya et al. (142) provided the first direct proofs of the involvement of simple sphingolipids in the death of retinal neurons. Using a *Drosophila* model of retinal degeneration, their initial work revealed that Cer had a crucial role in controlling fly photoreceptor survival; keeping Cer levels low through transgenic expression of a neutral ceramidase or by preventing de novo biosynthesis of Cer suppresses retinal degeneration in *Drosophila* phototransduction mutants. Increased ceramidase expression facilitates the turnover of light-activated rhodopsin helping endocytosis of the faulty rhabdomeres, the set of membranes housing the visual signaling machinery, in *Drosophila* mutant photoreceptors; the decrease in Cer levels might contribute to this process, perhaps by modifying membrane structure (143). Noteworthy, overexpression of neutral ceramidase, even in tissues distant from photoreceptors, prevents their death (144). A recent work pointed to an additional role of Cer in controlling intracellular signalling in *Drosophila* photoreceptors. In mutants lacking Cer kinase, the resulting Cer accumulation alters phosphatidylinositol biphosphate ($PIP_2$) membrane distribution, leading to loss of phospholipase C activity and consequent failure in phototransduction, accompanied by severe degeneration of photoreceptors; overexpression of ceramidase in these mutants leads to Cer decrease and rescues PLC activity (145). Mouse embryos lacking Cer transport protein (CERT), which transfers Cer from the ER to the Golgi, accumulate Cer in mitochondria in cells from the optic cup and this accumulation alters mitochondria structure (146). These results underscore the significance of controlling Cer levels to regulate membrane processes and thus prevent the death of retina photoreceptors.

The finding that mutations in a novel Cer kinase (CerK) homologous gene, the CerK-like protein (CerKL) cause an autosomal recessive form of retinitis pigmentosa (RP) (147, 148) provided the first direct link between sphingolipid metabolism and human retinal degeneration. Early macular degeneration and cone and rod involvement are common outcomes of the two CERKL mutations identified. Conversion of Cer by CerK to ceramide-1-phosphate (C1P), which has anti-apoptotic properties, is emerging as an important factor in the regulation of apoptosis (149–151). CerKL was initially expected to act as a second, specific retinal CerK; however, it does not phosphorylate Cer (152) and its deletion in mice affects neither Cer nor C1P levels in the retina, suggesting it might use a novel lipid substrate, which has proved elusive (153). In spite of this, overexpression of CerKL isoforms protects cells from apoptosis induced by oxidative stress (152). CerKL, like other RP-related proteins, has a nuclear localization signal that might be responsible for its retention in the nucleolus, a finding that suggests relevant though still unclear roles for CerKL in retinal function (154).

Collective evidence draws attention to the importance of Cer in the etiology of several retinal pathologies such as age-related macular degeneration (AMD). Impairment of retinal pigment epithelium (RPE) cells and their consequent death are primary events in the development of AMD, and Cer has been shown to participate in activating RPE cell death. These cells possess the enzymes for Cer synthesis (155), which is stimulated by oxidative stress (156), and enhanced endogenous synthesis or Cer exogenous addition induce RPE cell apoptosis, particularly in nonpolarized RPE cells, as those found in late stages of AMD (155–158). Oxidative stress and oxidized LDL, which are involved in the pathogenesis of AMD, increase Cer levels in RPE cells, augmenting their levels of reactive oxygen species and leading to mitochondrial permeabilization and caspase-3 activation that precede their apoptosis (159). Additional evidence supporting Cer as an essential signal in the activation of apoptosis in RPE cells is the increase in its intracellular levels after laser injury, which induces apoptosis of these cells (160). Cer might also lead to visual disfunctions by modifying membrane processes. Autosomal dominant Stargardt-like macular degeneration is associated to mutations in elongation of very long-chain fatty acid-4, and heterozygous mice carrying one of these mutations show progressive photoreceptor degeneration and altered visual function. Because this mutation is associated to a decrease in Cer content in skin leading to loss of its barrier function, it might be proposed that a similar Cer decrease might affect retinal function (161, 162). In Best macular dystrophy, a juvenile onset form of macular degeneration, Cer accumulation alters a chloride channel activity, altering fluid transport and enhancing inflammation in the retina (163).

Alterations in sphingolipid metabolism are also present in diabetic retinopathy; decreased Cer levels and a concomitant increase in GlcCer content are observed in retinas of animals subjected to experimentally-induced diabetes (135). Neutral and acid SMases are rapidly activated in human retinal endothelial cells, the vascular tissue affected in retinopathy, by pro-inflammatory cytokines, which are increased in diabetic eyes; noteworthy, reduction of inflammation and retinal pathological angiogenesis by docosahexaenoic acid (DHA), the major retina polyunsaturated fatty acid, has been proposed to involve downregulation of SMases expression and activity in these cells (164). Cer might participate in pericyte apoptotic death, an early event in diabetic retinopathy (165). Incubation of cultured bovine retinal pericytes with palmitate leads to Cer accumulation, NF-κB activation, and increased apoptosis, which is prevented by inhibiting Cer synthesis (166). This suggests that the high available levels of fatty acids, a common feature of poorly controlled diabetes, might increase Cer levels and promote pericyte death. As a whole, these data point to the involvement of Cer in the triggering of inflam-

mation and apoptosis in cell types whose alteration is a central event in major retina diseases.

Photoreceptor death by apoptosis is a hallmark of most retinal degeneration disorders (167–170). Oxidative stress has been proposed to have a decisive role in activating photoreceptor death; reactive oxygen species are a common feature and act as key signaling molecules in driving apoptosis in both in vivo and in vitro models of retinal disease (171–174). Cer is a well-established signal of oxidative stress-induced apoptosis (175) and recent evidence implies it as an essential second messenger in the activation of this death in photoreceptors. Work from our laboratory has recently demonstrated that Cer is an endogenous mediator of apoptosis in cultured retinal neurons subjected to oxidative damage (176). Addition of $C_2$-Cer triggers photoreceptor apoptosis in culture, whereas inhibition of de novo synthesis of Cer protects photoreceptors from apoptosis induced by oxidative stress (paraquat)-induced apoptosis (**Fig. 4**) and by the lack of trophic factors during their early development in vitro (176). Studies done by Sanvicens and Cotter (177) reveal Cer acts as a key mediator in oxidative stress (nitric oxide)-induced apoptosis in a cone-like cell line, retina derived 661W cells; in this cell type, SM hydrolysis by acid SMase is responsible for Cer generation. Additional support to Cer's role as a death mediator in the retina was provided by Mandal et al. (178), who found that both $H_2O_2$ treatment of 661W cells and photooxidative damage of albino rat retinas induced by intense light exposure lead to a rapid SMase activation followed by an increase in Cer content; in addition, intense light stress upregulates the expression of Cer biosynthetic genes. As a whole, these evidences point toward a key role for Cer as a common mediator of photoreceptor apoptosis in diverse situations of cellular stress.

An intimate connection has been demonstrated in several model systems between Cer and the mitochondrial pathway of apoptosis; mitochondrial production of Cer induces Bax oligomerization (179) and Cer increases outer membrane permeability to several proteins, including cytochrome c (180), leading to cell death. The mitochondrial pathway seems also to participate in Cer-induced apoptosis in photoreceptors. $C_2$-Cer induces mitochondrial membrane depolarization, which precedes apoptosis in cultured rat photoreceptors; DHA, a photoreceptor trophic factor (181, 182), prevents this depolarization by upregulating Bcl-2 expression, thus rescuing photoreceptors from Cer- and paraquat-induced apoptosis (176, 183) (Fig. 4). In 661W cells, Cer overproduction induces deregulation of mitochondrial $Ca^{2+}$ homeostasis, which precedes the cytosolic increase of $Ca^{2+}$ levels and the subsequent activation of the calpain apoptotic pathway. Preventing Cer increase by inhibiting acid SMase abolishes these changes (177). As a whole, these data imply Cer acts upstream of mitochondrial membrane permeabilization in photoreceptors.

Sphingolipids' role as membrane structural components is also relevant for the regulation of photoreceptor functionality; cyclic nucleotide-gated channels, essential for signal transduction in photoreceptors, are associated with membrane domains enriched in these lipids, and inhibition of sphingolipid synthesis impairs their activation and promotes their delocalization (184).

Cer might induce death in retina neuronal types other than photoreceptors. Increased Cer levels have been recently shown not only in photoreceptors but also in other retinal layers in an in vivo model of retinal detachment and this increase correlates with photoreceptor apoptosis (185). $C_2$-Cer induces the apoptosis of cultured amacrine cells (176); however, inhibition of endogenous Cer synthesis hardly prevents their oxidative-stress-induced apoptosis, suggesting that Cer might not be the principal mediator in the triggering of amacrine cell apoptosis. Noteworthy, $C_2$-Cer has the opposite effect on newborn retinal ganglion cells, promoting their survival and potentiating the survival effects of insulin and basic fibroblast growth factor (186).

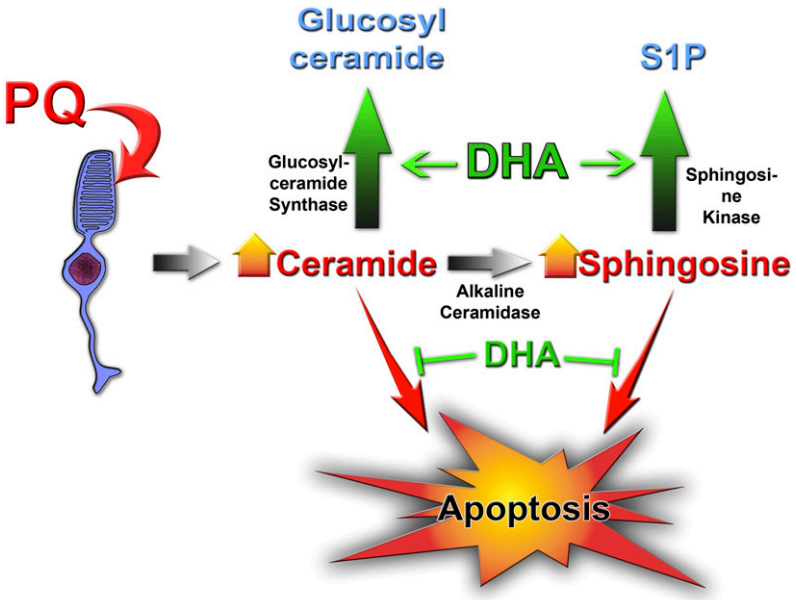


**Fig. 4.** Sphingolipid mediators leading to oxidative stress-induced apoptosis of photoreceptors and to docosahexaenoic acid protection. Paraquat (PQ)-induced oxidative stress promotes the de novo synthesis of ceramide (Cer), which is at least partially metabolized to sphingosine (Sph) by alkaline ceramidase and both Cer and Sph then induce the apoptotic death of photoreceptors. Docosahexaenoic acid (DHA) protects photoreceptors from oxidative stress-induced apoptosis by stimulating the metabolic pathways that decrease Cer levels, upregulating and/or activating glucosylceramide synthase and sphingosine kinase 1 to enhance the synthesis of glucosylceramide and S1P, respectively.

The above evidence points to a central role for Cer in controlling photoreceptor death and supports the proposal that lowering Cer levels might prevent photoreceptor death. Work from our laboratory has shown that photoreceptor trophic factors such as DHA selectively activate enzymes that have Cer as a substrate in order to prevent photoreceptor apoptosis (Fig. 4). DHA upregulates transcription of enzymes such as GCS and SphK1 (187, 188) to promote GlcCer or S1P synthesis, respectively, thus lowering Cer levels (176, 188). New and exciting evidence from Strettoi's group suggests manipulation of Cer metabolism might indeed render promising therapeutical benefits in retinal degenerations. Cer levels double in rd10 mouse retinas between P14 to P30, the period of maximum photoreceptor death; in contrast, Cer levels in wild-type mice retinas from the same age periods remain much lower. Administering inhibitors in the eye to inhibit Cer synthesis markedly reduces the amount of pycnotic photoreceptors found at day 10, prolongs survival of photoreceptors up to P24, and preserves the electroretinogram response (189, 190). These data strongly support the proposal that controlling Cer metabolism might provide new therapeutical approaches for the treatment of retinal degenerations.

## SPHINGOSINE IN THE RETINA

Whereas a Sph-enriched diet enhances photoreceptor degeneration in *Drosophila* phototransduction mutants (142), Sph functions in the mammalian retina were virtually unknown. Recent data from our laboratory demonstrates that Sph acts as a mediator of photoreceptor apoptosis, together with Cer, in retina neurons in culture (Fig. 4) (191). Oxidative stress rapidly increases [$^3$H]Sph levels in cultured photoreceptors, whereas inhibiting Sph synthesis with an alkaline ceramidase inhibitor prevents the apoptosis of photoreceptors induced by Cer or by oxidative stress (191). The easy interconversion between Cer and Sph has made it complex to distinguish their individual roles and effects. Though Sph may be converted back to Cer to induce apoptosis, inhibiting this conversion does not prevent apoptosis in several cell lines (49, 192, 193); conversely, blocking Sph synthesis attenuates apoptosis due to different death stimuli (194, 195). Exogenous addition of Sph leads to photoreceptor apoptosis and inhibiting Sph metabolization to Cer does not prevent this death (191), implying Sph is by itself a mediator of apoptosis. Exogenous Sph induces mitochondrial depolarization, cytochrome C release, and production of reactive oxygen species in photoreceptors, whereas inhibiting Sph synthesis preserves mitochondrial membrane integrity (191). These results are consistent with the involvement of the mitochondrial pathway in Sph-induced apoptosis in several cell types; mitochondria are among the major cellular sources of reactive oxygen species, and both Sph and Cer have been shown to increase their generation (196). Sph downregulates Bcl-2 levels (197), induces cytochrome c release from mitochondria, and activates caspases (48). Bcl-xl overexpression prevents the onset of apoptosis without blocking Sph generation, suggesting Sph is produced upstream of mitochondria permeabilization and this permeabilization is required for Sph-induced death (49).

As reported for Cer, DHA prevents Sph-induced apoptosis (Fig. 4). Noteworthy, this DHA protection is blocked when SphK1 activity is inhibited (191), implying that a decrease in Sph levels through its phosphorylation to S1P is required for DHA protective effect. Hence, we propose that increased oxidative stress in different pathological conditions might promote the synthesis of Cer in photoreceptors, which is then at least partially metabolized to Sph. Both sphingolipids might then act as second messengers, inducing mitochondrial dysfunction, increased generation of reactive oxygen species, and consequent inflammation (198), activating apoptosis in photoreceptors.

## SPHINGOSINE-1-PHOSPHATE IN THE RETINA

Pathological scar tissue production (fibrosis) and choroidal and retinal neovascularization contribute to numerous ocular disorders and are directly linked to visual loss in AMD and other macular disorders, and recent evidence indicates S1P has a role in these processes. S1P has been recently proposed as a novel fibrotic mediator in the eye; human RPE cells, which are key clinical targets in the treatment of subretinal fibrosis, produce and respond to S1P, which promotes proliferation, myofibroblast transformation, collagen production, and pro-fibrotic protein expression (199). These data suggest that S1P might participate in the ocular fibrogenic cascade and contribute to scar formation in ocular diseases. On the other hand, due to its multiple roles, S1P can also have beneficial functions, protecting RPE cells from Cer-induced apoptosis (155). Hence, regulating sphingolipid metabolism might provide a useful tool for preventing the death of RPE cells, which is a key change in ocular diseases such as AMD. Retina and choroidal neovascularization are directly related to visual loss in AMD; S1P stimulates this neovascularization whereas its selective binding with a monoclonal antibody significantly reduces the damage (200, 201). Survival of retinal endothelial cells depends on SphK activity (202) and S1P, acting through its S1P2 receptor, participates in pathological retinal angiogenesis caused by ischemic retinopathy (203). Lipopolysaccharide-induced inflammation increases S1P levels in rat retina, promoting astrogliosis (204), suggesting S1P might also participate in retina inflammation. Collectively, these data imply that control of S1P levels might be successfully used for treating ocular diseases involving neovascularization.

S1P has a crucial role in the regulation of proliferation both during development and in adult tissues (110, 205–207). Work done in our laboratory has shown that S1P plays multiple and prominent roles in the development of photoreceptors. S1P acts as a mitogen in rat retina neuronal cultures, increasing the proliferation of photoreceptor progenitors at early culture times and then stimulating their differentiation (188), establishing S1P as a molecular

cue that promotes the proliferation and eventual differentiation as photoreceptors of these progenitors. This finding is particularly relevant since the recent discovery of stem cells in the retina, which might provide a therapeutic option to replace lost neurons. Information on the clues required for controlling its proliferation and further differentiation into photoreceptors is a prerequisite to take advantage of these cells for this regenerative purpose.

S1P is involved in neuritogenesis, promoting either neurite extension or retraction, depending on the type of activated S1P receptor (69, 73, 208). In Xenopus retina, it participates in controlling axonal outgrowth, eliciting repulsive responses in growth cones (69). S1P is also a critical promoter of photoreceptor differentiation (188). In vivo, differentiation of these cells involves several steps. Once photoreceptor progenitors exit the cell cycle, they first develop a distal connecting cilium and then start expressing opsin and accumulating membranes at the cilium tip, to finally form the rhodopsin-containing disks and the OS characteristic of mature photoreceptors. Differentiation appears to be arrested in retina neurons in vitro; photoreceptors show a poor opsin expression, diffusely distributed over the entire plasmalemma and fail to develop apical processes (**Fig. 5A**, C). S1P increases the synthesis of proteins characteristic of OS, such as opsin and peripherin, and advances the development of OS, inducing the accumulation of membranes at the end of photoreceptor cilia and promoting the colocalization in them of opsin and peripherin (Fig. 5B, D) as occurs during differentiation in vivo (188). S1P enhances rhodopsin transport and uptake and esterification of DHA, the major fatty acid component, in rod OS membranes and participates in lipid and rhodopsin trafficking to OS in the frog retina (209). Blocking this trafficking with brefeldin A completely inhibits S1P effects on the morphogenesis of rat photoreceptor OS (188). Opsin and peripherin are essential for the formation of OS and their accumulation might be a prerequisite for building these structures; rhodopsin null mice and homozygous peripherin/*rds*-knockout mice that do not synthesize peripherin/*rds* fail to form OS and undergo a slow degeneration (210–212). Hence, S1P controls key steps in the formation of photoreceptor OS; it increases the levels of proteins and lipids that are indispensable building blocks of these specialized structures and promotes morphogenesis, targeting these proteins and lipids to the tip of the cilia for the assembly of OS.

New data points to a key role of S1P in promoting photoreceptor survival. Pretreatment with S1P protects photoreceptors from retinal detachment-induced apoptosis (185). Recent work from our laboratory shows that S1P prevents photoreceptor death during development in culture [(188); Figs. 5F, H], which otherwise occurs when cultured in a chemically defined media, lacking their specific trophic factors (181, 213) (Figs. 5E, G). Interestingly, intense light exposure in albino rat retinas upregulates the expression of S1P metabolic genes such as SphK1, and S1P receptors such as S1P3 and S1P5 in albino rat retina (178), in what might be an attempt to activate neuroprotective pathways upon photooxidative damage. Our preliminary data shows that exogenous S1P addition protects photoreceptors from oxidative stress-induced apoptosis (214), supporting a role for S1P as a mediator of photoreceptor survival.

As described above, S1P acts both as an extracellular ligand and an intracellular second messenger, in a receptor dependent and independent manner, respectively. Increasing evidence shows that there is a mutual cross-talk between S1P and growth factor-activated signaling cas-

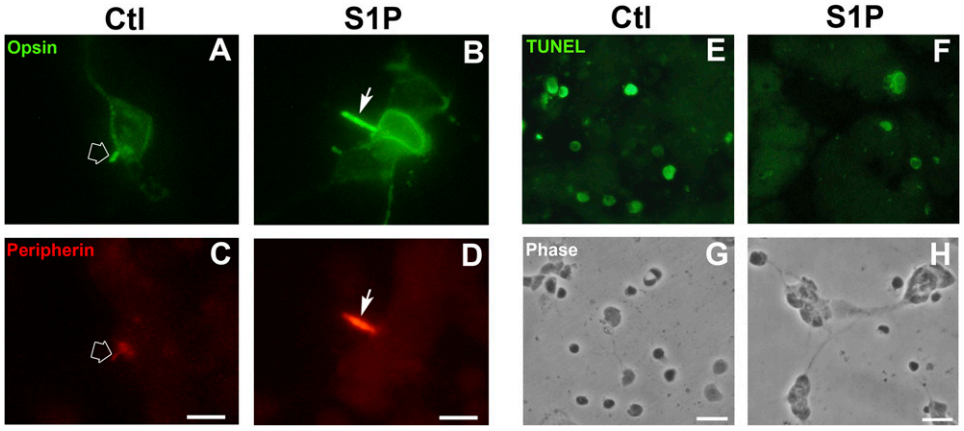


**Fig. 5.** S1P promotes the differentiation and survival of photoreceptors. The effects of S1P were analyzed in day 6, rat retina neuronal cultures treated without (Ctl) and with S1P. As observed in fluorescent micrographs (A–D) showing opsin (A, B) and peripherin (C, D) expression, S1P promotes the formation of rudimentary outer segments ("apical processes"), intensely stained with opsin and peripherin (thin arrows in B, D); in contrast, only labeled cilia are observed in controls (wide open arrows in A, C). E, F: Fluorescent micrographs showing Tunel labeling evidence that photoreceptors cultured in media lacking their trophic factors degenerate during development in culture (E); S1P markedly prevents their apoptosis, decreasing the amount of TUNEL-labeled cells (F). G, H: Phase micrographs. Scale bars, 5 μm in A–D; 10 μm, in E–H . (A–D, Taken from "Sphingosine-1-phosphate is a key regulator of proliferation and differentiation in retina photoreceptors", Invest. Ophthalmol. Vis. Sci. 50, 4416-4428 (2009), copyright holder: Association for Research in Vision and Ophthalmology).

cades (86). Among the identified trophic factors for retina photoreceptors, glial derived neurotrophic factor (GDNF) promotes progenitor proliferation, whereas DHA stimulates survival and differentiation (181, 213, 215, 216). The close intersection between their biological functions and those of S1P led us to investigate whether they stimulated S1P synthesis to make use of it as a second messenger to elicit their biological effects. Further experiments evidence that inhibiting S1P synthesis blocks GDNF mitogenic effect and the enhancing effect of DHA on survival and differentiation, whereas S1P addition restores DHA effects. Moreover, both DHA and GDNF upregulate SphK1 levels in photoreceptors and also enhance its translocation to the plasma membrane (188). This implies that enhanced transcription and higher activity of SphK1 combine to increase S1P levels (**Figs**. 5 and **6**).

Activation of S1P2 and S1P3 has been established to mediate cell proliferation and survival (91, 217, 218). Preliminary data from our laboratory show that treatment of photoreceptors with a S1P3 antagonist blocks the antiapoptotic effect of S1P, but not that of DHA, upon oxidative stress (214). These results suggest that S1P might have dual roles in photoreceptors (Fig. 6). It can act operate as a second messenger whose synthesis is activated by trophic factors and can then be released to act in a autocrine/paracrine manner as an extracellular signal, through the activation of G-protein coupled membrane receptors, to activate intracellular pathways leading to proliferation, survival, and differentiation of photoreceptors. Hence, the available data support the proposal that S1P acts as a central mediator in establishing the final number of photoreceptors in the retina; it initially regulates the proliferation of photoreceptor neuroblasts and once they leave the cell cycle, it promotes photoreceptor survival and advances their differentiation. Photoreceptor trophic factors such as GDNF and DHA elicit their biological effects by stimulating the synthesis of S1P, which then acts as a key second messenger in the development of photoreceptors.

The mechanisms involved in S1P actions in retinal neurons have still to be uncovered. Recent studies in chick amacrine cells point to an S1P-mediated increase in calcium levels in dendrites and cytosol by S1P receptor-independent and -dependent mechanisms, respectively (219, 220). Amacrine cells express S1P1 and S1P3 and their activation by S1P induces an inward cation current, though cytosolic $Ca^{2+}$ increase not only arises in external $Ca^{2+}$ influx but also in its release from intracellular stores. S1P binding to its receptors also stimulates SphK activity, and newly produced S1P leads to a sustained $Ca^{2+}$ influx. This complex S1P-mediated mechanism might participate in neurotransmitter release and signaling in the inner retina (220). Identifying the intracellular pathways activated by S1P still requires intensive research.

## GLUCOSYLCERAMIDE IN THE EYE

Lysosomal storage diseases, which arise from dysregulated sphingolipid metabolism, like Farber's disease (acid ceramidase), Tay-Sachs/Sandhoff (hexosaminidase A or B), Krabbe's (galactosylceramidase), Niemann Pick (sphingomyelinase) and Gaucher's (glucosyl-ceramidase) disease are associated with retinal impairment and visual dysfunction. GlcCer accumulation in patients with Gaucher's disease ultimately results in vision loss due to retinal neuronal cell death (11). As mentioned before, elevated GlcCer seems to be a major mediator of diabetic retinopathy, signaling stress responses and/or insulin resistance and contributing to the pathogenesis of diabetic retinopathy; inhibition of GlcCer synthesis increases insulin sensitivity in retinal neurons and reduces neuronal death in the diabetic rat retina (135). However, GlcCer seems to have opposite functions in retina photoreceptors. An increase in GlcCer synthesis is involved in DHA protection from Cer- and oxidative stress-induced apoptosis in photoreceptors (Fig. 3). DHA induces the differential transcription of several genes in fetal human retina, including the gene coding for GCS (187). Inhibiting Cer glucosylation blocks DHA protection of photoreceptors against oxidative stress, suggesting that an increased synthesis and/or activity of GCS are involved in DHA antiapoptotic effect by providing a route for decreasing Cer levels (176). Further understanding of the roles that (glyco) sphingolipid enzymes and their metabolites have in the retina may offer new targets for the treatment of retinal diseases.

## FINAL CONCLUSIONS

Exciting new findings have added up in recent years that underscore the relevance of controlling the different

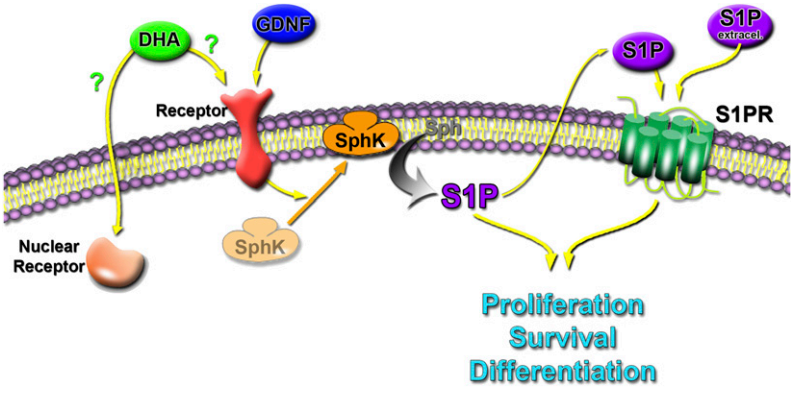


**Fig. 6.** S1P acts as an extracellular ligand and an intracellular second messenger to promote proliferation, survival, and differentiation in photoreceptors. Photoreceptor trophic factors, such as GDNF and DHA, upregulate SphK1 and might induce its translocation and consequent activation to the plasma membrane, where it catalyzes Sph phosphorylation to augment S1P levels. S1P might then act as an intracellular second messenger or be released to the extracellular milieu to act as a first messenger, in an autocrine/paracrine manner, activating S1P receptors (S1PR, at least S1P3) in photoreceptor plasma membrane. In these dual roles, S1P leads to the activation and inhibition of different intracellular targets to promote the proliferation, survival, and differentiation of photoreceptors.

pathways in sphingolipids metabolism as a novel and promising therapeutical approach for the treatment of retina neurodegenerative diseases. Alterations in proliferation, neovascularization, differentiation, and cell death are critical events in major diseases of the retina, such as AMD, diabetic retinopathy, Stargardt's disease, and retinitis pigmentosa, and sphingolipids have been show to control these processes in most of the cell types in the eye whose dysfunction leads to retina degeneration. Death of RPE cells and choroidal and retinal neovascularization are fundamental in the etiology of macula degeneration, neovascularization also contributing to ischemic retinopathies, and sphingolipids seem to be actively involved in these pathological events. Cer participates in the induction of RPE cell apoptosis whereas S1P acts as a double-edged sword, preventing this death but also promoting endothelial proliferation that might lead to fibrosis and neovascularization. These findings strongly support that dysregulation of sphingolipid metabolism might contribute to the development of retina degeneration. They also suggest the therapeutical potential of manipulating sphingolipid metabolic reactions to prevent this degeneration. A careful control of Cer and S1P levels and interconversion would be required to achieve this purpose, in order to promote protection but avoid potentially noxious proliferation.

Sphingolipids are also central players in orchestrating the fate of photoreceptors whose preservation is crucial to prevent visual loss. The scenery and the actors participating in this complex play are presently getting clearer. In several situations of cell stress, such as oxidative damage, lack of trophic factors, and during retina neurodegenerative diseases, Cer synthesis is activated, either by a de novo biosynthetic pathway or by SM hydrolysis, this apparently depending on the cell type and the kind of stress faced by the cells. Cer can be subsequently metabolized to Sph, a reaction in which alkaline ceramidase has a relevant role, at least in rat retina photoreceptors. Both Cer and Sph then act as key mediators in the activation of apoptotic death. Keeping low Cer and Sph intracellular levels is essential for preserving photoreceptor survival. This can be achieved by inhibiting Cer synthesis, a strategy already shown to rescue photoreceptors in both in vivo and in vitro models. Photoreceptor trophic factors seem to target specific enzymes to modulate sphingolipid metabolism in order to avoid cell death. DHA upregulates the transcription and/or activity of enzymes that lower Cer and Sph levels, such as GCS, which catalyzes Cer glucosylation to GlcCer, and SphK1, which promotes the phosphorylation of Sph generated from Cer hydrolysis to synthesize S1P. Either reaction decreases the intracellular content of Cer and Sph, thus rescuing photoreceptors from their apoptotic death. Because S1P has roles in the control of cell survival and growth opposite to those of Cer and Sph, favoring S1P synthesis provides the combined benefits of lowering Cer and Sph levels and increasing those of a pro-survival factor, which would thus enhance protection. Moreover, new data proposes S1P as an essential second messenger, whose synthesis is enhanced by photoreceptor trophic factors, such as GDNF and DHA, in order to control key processes in the development of photoreceptors.

Further research is required to uncover the roles and pathways activated by the different members of the sphingolipid family to control death, survival, proliferation, and development in the retina. Information on the changes in tissue levels of these simple sphingolipids in pathological situations is still lacking. Knockout transgenic animal models for sphingolipid genes and receptors are presently available (21) and their use, and that of animals with tissue-specific deletion of enzymes and/or receptors in those cases in which the proteins involved are essential during embryonic or early postnatal development, would be of immense help to provide new insights on the relevance of these molecules in physiological and pathological processes affecting retinal neurons. The available evidence emphasizes the key role of sphingolipid molecules and their relative balance in regulating the final fate of photoreceptors. It also endorses the proposal that modulating the activity of the enzymes involved in sphingolipid metabolism may provide novel and promising therapeutical tools for controlling photoreceptor cell fate, preventing death, and restoring the photoreceptors lost in retinal neurodegenerative diseases. Identifying molecules capable of regulating sphingolipid metabolism, either by inhibiting or activating key enzymes such as Cer synthases, ceramidases, and SphK, or by regulating their transcription, might provide new useful tools for treating retina neurodegenerations and tilt the intracellular scale toward photoreceptor survival.

The authors thank Dr. Luis Politi for helpful discussion and suggestions.

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