Sphingolipids as Targets for Microbial Infections

Travis J. McQuiston[1], Charles Haller[1] and Maurizio Del Poeta*[1,2]

Departments of [1]Biochemistry & Molecular Biology and [2]Microbiology & Immunology, Medical University of South Carolina, Charleston, South Carolina 29425, USA

Abstract: Sphingolipids had long been regarded as merely structural components of eukaryotic cellular membranes. Research has discovered sphingolipids to have crucial roles in cellular processes as bioactive molecules. Lately, there has also been an increased interest in sphingolipids and sphingolipid-metabolizing enzymes as mediators of microbial pathogenicity and as potential targets for the development of new therapeutics. This minireview will provide a comprehensive analysis of sphingolipid pathways in mammalian and microbial cells, highlighting their uniqueness and discussing their potential as therapeutic targets for microbial infections.

INTRODUCTION

The basic structural component of sphingolipids is a long chain sphingoid base backbone. The linkage of a fatty acid to the 2- amino group of this backbone through an amide bond yields ceramide. Complex sphingolipids are formed with the addition of a polar head group to ceramide via an ester bond at the C-1 position. Due to their chemical structures, sphingolipids are amphipathic molecules with varying degrees of both hydrophobic and hydrophilic characteristics.

Sphingolipid synthesis occurs in all eukaryotic cells and is usually divided into mammalian- and fungal/plant-specific branches. The biosynthetic pathway of protozoa is not conserved, as some protozoa have the biosynthetic pathway similar to mammals while others produce sphingolipids typically observed in fungi/plants. Most bacteria and all viruses are unable to synthesize sphingolipids. However, several pathogenic bacteria that do not synthesize sphingolipids have developed mechanisms to metabolize host sphingolipid. Viruses lack sphingolipid biosynthesis, but host sphingolipids are intricately involved in viral entry, assembly, and budding.

Sphingolipids are common components of membranes and have recently been recognized to function as signaling molecules in a wide variety of signaling pathways (reviewed in [1]. In mammalian cells, bioactive lipids such as ceramide, ceramide-1-phosphate, and sphingosine-1-phosphate exert key roles in the regulation of cell proliferation, stress responses, cell cycle arrest, differentiation, apoptosis, inflammation, and immune responses [2-9]. In microbial cells, sphingolipids have been implicated in the regulation of apoptosis, signal transduction, and pathogenesis [4,7,10]. The role of sphingolipids in membranes has been redefined with the discovery of organized membrane domains called lipid rafts comprised of sphingolipids and cholesterol [11]. These domains are implicated in the initiation of signal transduction, functioning as membrane platforms that aggregate and organize specific populations of receptor molecules into signaling complexes [12-14]. In addition, lipid rafts act as the site of entry for many pathogenic organisms [15-22].

With sphingolipids serving essential roles in cell membrane structure and in cellular processes involving lipid raft organization and function, it should be no surprise that many intracellular microorganisms utilize sphingolipids during infection. The role of sphingolipids during the host-pathogen interaction is now being examined in microbial pathogenesis and is thoroughly discussed in the reviews by Heung *et al.* [23] and Hanada [24]. Serving as essential molecules in the viability and pathogenesis, sphingolipids may be ideal molecular targets for drug development against microorganisms. This mini-review will provide a comprehensive analysis of sphingolipid pathways in mammalian and microbial cells, highlighting their uniqueness and discussing their potential as therapeutic targets for microbial infections.

THE SPHINGOLIPID PATHWAY IN MAMMALS

The synthesis of sphingolipids is well conserved among eukaryotic cells for the first several steps of the pathway. In mammalian cells (see Fig. **1**), the sphingolipid biosynthetic pathway begins with the condensation of serine and palmitoyl Co-A to form ketodihydrosphingosine (KetohydroSPH) by the activity of serine palmitoyltransferase (SPT). SPT has at least two non-identical protein subunits in mammals coded by the genes serine palmitoyltransferase long chain base subunit 1 (*SPTLC1*) and serine palmitoyltransferase long chain base subunit 2 (*SPTLC2*) [25]. These genes are homologous to long chain base 1 (*LCB1*) and long chain base (*LCB2*) that were originally cloned from the yeast *Saccharomyces cerevisiae* [26]. KetodihydroSPH is reduced to dihydrosphingosine (DihydroSPH) by 3- keto-dihydrosphingosine reductase (3KSR). 3KSR is the gene product of the follicular lymphoma variant translocation (*FVT*) gene, which has been isolated in both human and mice [27]. Dihydroceramide synthase (DH-Cer synthase) can acylate DihydroSPH to produce dihydroceramide (DihydroCer). Six mammalian DihydroCerS enzymes are now recognized to convert

*Address correspondence to this author at the Department of Biochemistry & Molecular Biology, Medical University of South Carolina, 173 Ashley Avenue, BSB 503, Charleston, SC 29425, USA; Tel: (843) 792-8381; Fax: (843) 792-8565; E-mail: delpoeta@musc.edu

1389-5575/06 $50.00+.00

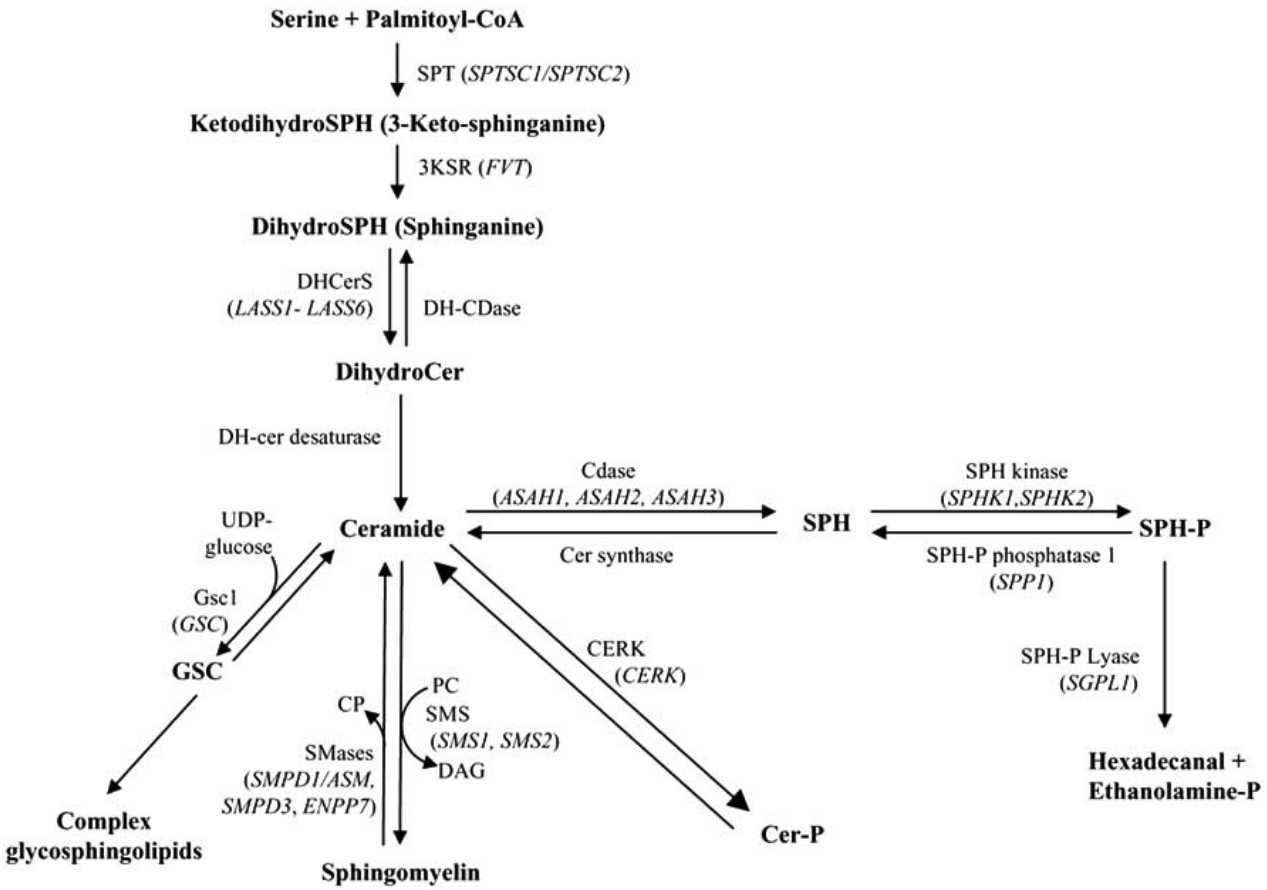

Fig. (1). The sphingolipid pathway of mammals. A scheme for the biosynthetic pathway as observed in mammalian cells. The gene(s) for each of the enzymes is parentheses. Question marks indicate genes and enzymes that have not been isolated, identified or characterized. SPT, Serine palmitoyltransferase; *SPTLC1-2,* serine palmitoyltransferase, long chain base subunit 1 and 2 genes; KetodihydroSPH, ketodihydrosphingosine; 3KSR, 3- keto-dihydrosphingosine reductase; FVT, follicular lymphoma variant translocation gene; DihydroSPH, dihydrosphingosine; DH-Cer synthase, dihydroceramide synthase; LASS1-5, longevity assurance gene homologs; DH-CDase, dihydroceramidase; DihydroCer, dihydroceramide; DH-Cer desaturase, dihydroceramide desaturase; CDase, ceramidase; *ASAH1-3*, N-acylsphingosine amidohydrolase 1- 3 genes; Cer synthase, Ceramide synthase; SPH, sphingosine; SPH kinase, sphingosine kinase; *SPHK1-2*, sphingosine kinase 1 and 2 genes; SPH-P phosphatase, sphingosine-1-phosphate phosphatase 1; *SPP1*, sphingosine-1-phosphate phosphatase 1 gene; SPH-P, sphingosine-1-phosphate; SPH-P lyase, sphingosine phosphate lyase; *SGPL1*, sphingosine phosphate lyase 1 gene; Gcs1, glucosylceramide synthase 1; *GCS*, glucosylceramide synthase 1 gene; GSC, glucosylceramide; PC, phosphatidylcholine; SMS, sphingomyelin synthase; *SMS1-2*, sphingomyelin synthase 1-2 genes; DAG, diacylglycerol; SMase, sphingomyelinase. *SMPD1, 3,* sphingomyelin phosphodiesterase 1, 3 genes; *ASM*, acid sphingomyelinase gene; *ENPP7*, ectonucleotide pyrophosphatase/phosphodiesterase 7 gene; CP, choline phosphate; CERK, ceramide kinase; *CERK*, ceramide kinase gene. Adapted from references [3,23].

DihydroSPH to DihydroCer (reviewed in [28]. As homologs of the *S. cerevisiae* longevity-assurance gene 1 (*LAG1*) and longevity-assurance gene cognate 1 (*LAC1)*, which are necessary long-chain ceramides formation [29,30], mammalian genes coding for DH-Cer synthase are classified as longevity-assurance gene homologs (*LASS*). Four of the six DH-Cer synthase genes, *LASS1 [31]*, *LASS4 [32], LASS5* [32-34] and *LASS6 [35]* have been characterized.

The production of ceramide has governed a lot of attention since it was shown to induce apoptosis [6]. In addition to this important function, ceramide serves as the building block for the biosynthesis of additional sphingolipids and, therefore, its production is vital to sphingolipid biosynthesis. First, ceramidase can remove the fatty acid from ceramide to produce sphingosine (SPH), which, in turn, can serve as the substrate for sphingosine kinase (SPH kinase) to make sphingosine-1-phosphate (SPH-P) (Fig. **1**). Two mammalian isoforms of SPH kinase, encoded by *SPHK1* and *SPHK2,* have been identified [36,37]. SPH-P is dephosphorylated by sphingosine-1-phosphate phosphatase 1 (SPP-P phosphatase 1) that is encoded by *SPP1* [38,39] to yield SPH. Alternatively, SPH-P can be degraded by the action of sphingosine-1-phosphate lyase 1 (SPH-P lyase 1), which is encoded by *SGPL1*, to form hexadecanal and ethanolamine phosphate [40,41].

Second, sphingomyelin synthase (SMS) transfers a choline phosphate (CP) head group from phosphatidyl-choline (PC) to ceramide, producing sphingomyelin and diacylglycerol (DAG). Two SMS genes, *SMS1* and *SMS2*, have been identified in mammalian cells, with SMS1 associating with the Golgi apparatus and SMS2 localized at the cell membrane [42]. Since ceramide is a pro-apoptotic

and DAG is an anti-apoptotic molecule, and their levels is regulated by SMS [43,44], the modulation of SMS activity may exert a synergistic affect on either cell death (SMS down-regulation) or cell proliferation (SMS up-regulation), with important implications for SMS in controlling tissue regeneration [45] and cell transformation [46].

Sphingomyelin can be re-converted to ceramide by sphingomyelinases (SMases) (Fig. **1**). To date, there have been four different SMases found in mammalian cells. The sphingomyelin phosphodiesterase 1 (*SMPD1)*/ acid sphingomyelinase (*ASM)* gene codes for an acid SMase, also referred to as lysosomal SMase (L-SMase), and a zinc-dependent acid SMase, often named secretory SMase (S-SMase) [47-51]. Using the genomic sequence of bacterial sphingomyelinase, two putative mammalian genes, *SMPD2* and *SMPD3*, were identified and suggested to function as neutral sphingomyelinases [52,53]. However, further investigations into the properties of these prospective neutral sphingomyelinases revealed that only *SMPD3* encodes for a true neutral sphingomyelinase, nSmase 2 [54,55]. An alkaline SMase found predominately in the intestinal tract has been found in several mammals and is encoded by *ENPP7* in humans [56-59].

In addition to serving as the substrate for SMS, ceramide can be used by glucosylceramide synthase 1(Gsc1) to form glucosylceramide (GSC) [60,61], which, in turn, is used to produce complex glycosphingolipids. Finally, in mammalian cells, ceramide can also be phosphorylated by ceramide kinase (CERK) to form ceramide-1-phosphate (Cer-P) [62]. Cer-P regulates inflammatory responses by directly

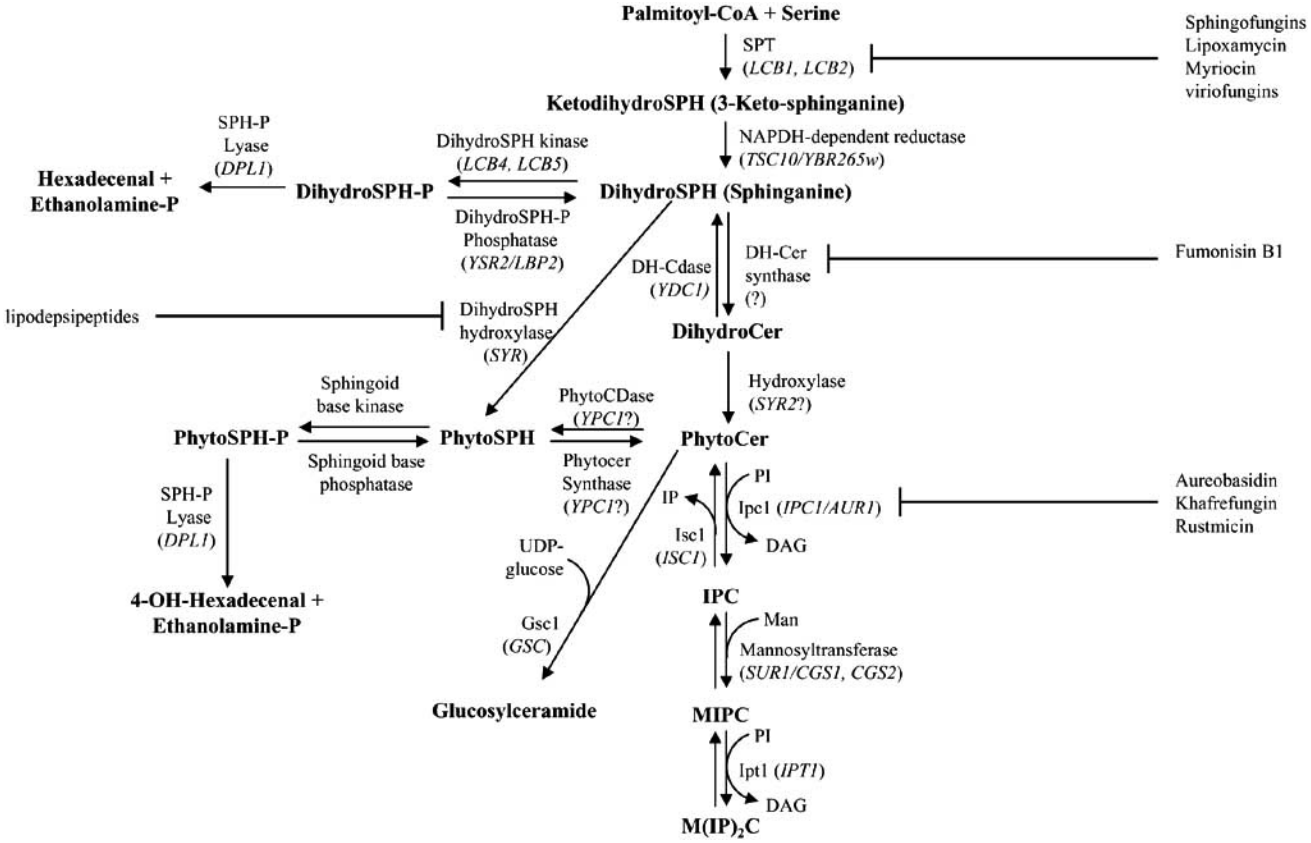

Fig. (2). The sphingolipid pathway of fungi. A scheme for the biosynthetic pathway as observed in fungi/plant cells. The gene(s) for each of the enzymes is parentheses. Question marks indicate genes and enzymes that have not been isolated, identified or characterized. SPT, serine palmitoyltransferase; *LCB1-5*, long chain base 1- 5 genes; DH-CDase, dihydroceramidase; KetodihydroSPH, ketodihydrosphingosine; *TSC10*, temperature-sensitive csg2 delta suppressor 10 gene; DihydroSPH, dihydrosphingosine; DihydroSPH kinase, dihydrosphingosine kinase; DihydroSPH-P phosphatase, dihydrosphingosine phosphate phosphatase; DihydroSPH-P, dihydrosphingosine phosphate; DihydroSPH-P lyase, dihydrosphingosine phosphate lyase; *DPL1,* dihydrosphingosine phosphate lyase 1 gene; DihydroSPH hydroxylase, dihydrosphingosine hydroxylase; *SUR2* (also called *SYR2*) suppressor of rvs161 and rvs167 mutations; DH-Cer synthase, dihydroceramide synthase; DH-CDase, dihydroceramidase; *YDC1,* yeast dihydroceramide synthase 1 gene; DihydroCer, Dihydroceramide; DihydroCer hydroxylase, dihydroceramide hydroxylase; PhytoCer, phytoceramide; PhytoCDase, phytoceramidase; *YPC1*, yeast phytoceramidase; PhytoSPH-P, phytosphingosine phosphate; PI, phosphatidylinositol; Ipc1, inositol phosphorylceramide synthase 1; *IPC1* (also called *AUR1*), inositol phosphorylceramide gene;; DAG, diacylglycerol; Isc1, inositol phosphosphingolipids-phospholipase C 1; IP, inositol-phosphate; IPC, inositol phosphorylceramide; Man, mannose; *SUR1* (also called *CSG1*), suppressor of rvs161 and rvs167 mutations 1 gene; *CSG2*, calcium sensitive growth 2 gene; MIPC, mannose-inositol-P-ceramide; Ipt1, inositolphosphotransferase 1; Adapted from reference [3,23].

activating cytosolic phospholipase A2 (cPLA2) [8,63], the major phospholipase responsible for producing arachidonic acid utilized during eicosanoid biosythesis [64-67].

The exploitation of mammalian sphingolipids and the associated metabolizing enzymes by microorganisms, especially those lacking the ability to synthesize sphingolipids, requires researchers to examine the host sphingolipid pathway for possible therapeutic targets to prevent successful infection. For instance, several pathogens including Sindbis virus [68] and HIV-1 [69-71] enter host cells via lipid rafts that are comprised of SM and induce host SMase activity. This activation of SMase elevates host ceramide levels and initiates a pro- apoptotic signaling cascade. Thus, an understanding of the metabolic changes in the host sphingolipid pathway occurring during infection could lead to the development of new therapeutic strategies against such viruses and perhaps also against other microbes sharing similar mechanisms of interaction.

THE SPHINGOLIPID PATHWAY IN FUNGI

The first steps of fungal sphingolipid synthesis are identical to the mammalian sphingolipid previously discussed (compare Fig. **1** with Fig. **2**). Divergence between mammalian and fungal sphingolipid pathways occurs following the synthesis of DihydroSPH. At this junction in fungal cells, DihydroSPH is either hydroxylated by dihydrosphingosine hydroxylase (DihydroSPH hydroxylase) to form phytosphingosine (PhytoSPH) [72] or used by dihydroceramide synthase (DihydroCer synthase) to produce dihydroceramide (DihydroCer). Currently, the utilization of PhytoSPH or DihydroCer by fungi to create phytoceramide (PhytoCer) is undetermined, although an alkaline ceramidase encoded by the *YPC1* gene has been discovered to catalyze both the breakdown and synthesis of PhytoCer [73-75].

The key difference between the fungal and mammalian sphingolipid pathway is the transfer of inositol phosphate (IP) from phosphotidylinositol (PI) to PhytoCer to produce inositol- phosphorylceramide (IPC) [76-79]. IPC synthase, commonly referred to as Ipc1, is encoded by *IPC1,* also called aureobasidin resistance gene 1 *(AUR1*), was first isolated in *S. cerevisae* [80]. Concomitantly to the formation of IPC, Ipc1 also produces a molecule of DAG. Thus, the Ipc1 reaction represents the fungal counterpart of mammalian sphingomyelin synthase. Similar to its mammalian counterpart, Ipc1 modulates the level of DAG in fungal organisms, such as *S. cerevisiae* [81] and in *C. neoformans* [82]. Phytoceramide can be transformed into glucosylceramide by glucosylceramide synthase encoded by *GCS1* gene. Recently it has been proposed a key role for *GCS1* in the regulation of pathogenicity of *Cryptococcus neoformans* [83].

IPC can undergo mannosylation to form MIPC. It is likely that two non-essential genes, suppressor of rvs161 and rvs167 mutations (*SUR1*)/calcium-sensitive gene 1 (*CSG1*) [84,85] and calcium-sensitive gene 2 (*CSG2*) [86,87], encode for the enzyme responsible for the mannosylation of IPC. Deletion of either of these genes prevents MIPC synthesis and leads to accumulation of IPC [88,89]. In the final step of the fungal sphingolipid biosynthesis, the addition of

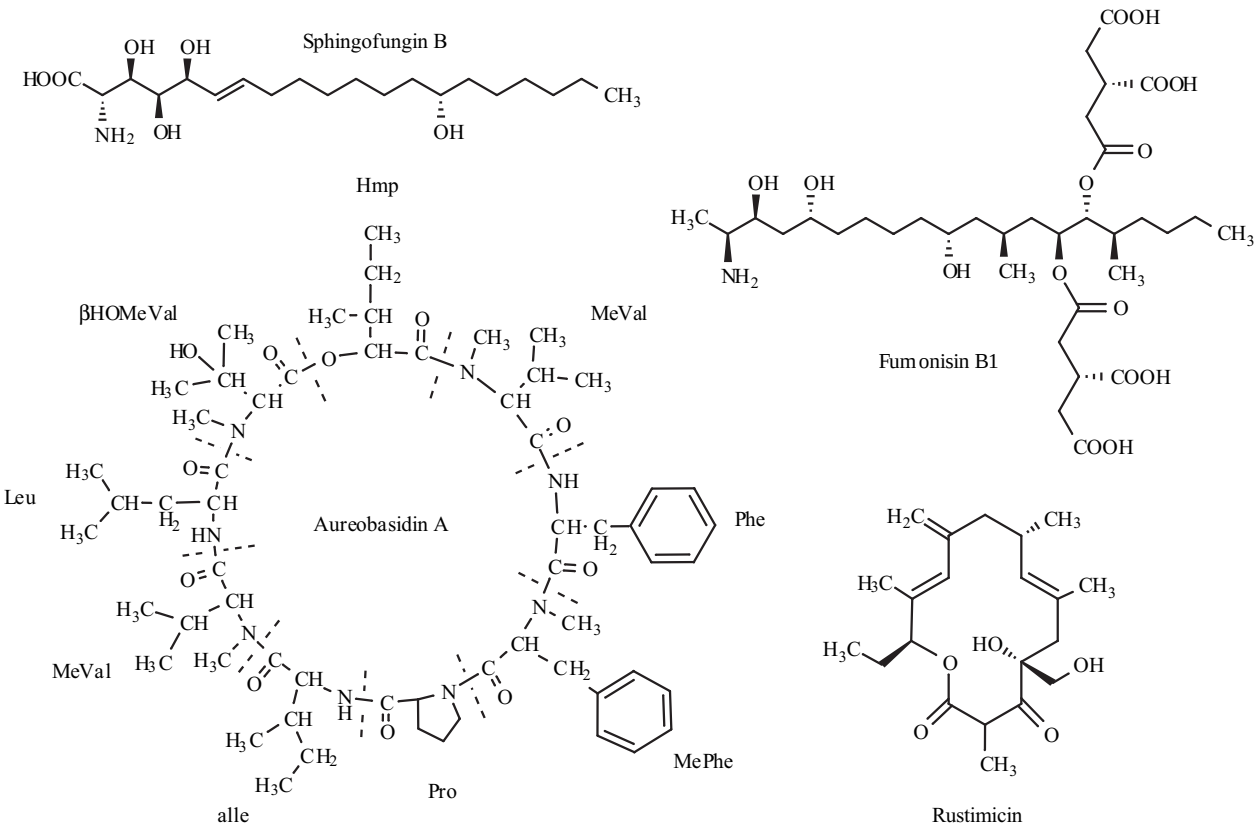

Fig. (3). Antifungal compounds targeting the sphingolipid pathway. Shown are the chemical structures of a few compounds inhibiting specific enzymes of the sphingolipid biosynthesis.

inositol phosphate to MIPC yields M(IP)2C. The production of M(IP)2C is catalyzed by inositol phosphotransferase 1 (Ipt1) [90]. Although M(IP)2C comprises 75% of the sphingolipids in *S. cerevisae*, *IPT1* is not required for viability, perhaps because the deletion of *IPT1* leads to an increase in MIPC, which may compensate for the loss of M(IP)2C [90,91]. Complex sphingolipids, such as IPC, MIPC and M(IP)2C can be converted to phytoceramide by inositol phosphosphingolipid phospholipase C (Isc1) [92], an enzyme which is not essential for viability but it may be important for the yeast cell responses under stress conditions [93,94].

Several compounds have been found to inhibit the first steps of the sphingolipid biosynthetic pathway shared by both mammals and fungi. Due to the commonality of the first steps, targeting these steps is not ideal as the host sphingolipid pathway could also be blocked. These compounds include sphingofungins [95-97], lipoxamycin [98-100], myriocin [101-103] and viriofungins [104] that target the rate- determining activity of SPT as therapeutic drugs (Fig. **2** and **3**). *Pseudomonas syringae* produces two groups of cyclic lipodepsipeptides (LDPs), which include syringomycins, that inhibit the production of phytosphingosine. However, these peptides also target host cell membranes and have been found to lyse red blood cells [105]. Obviously, this drawback makes it impossible to use LDPs clinically. Fumonisin B1 is a mycotoxins produced by the corn pathogen *Fusarium verticillioides* (formerly *Fusarium moniliforme*). Fumonisin B1 inhibits ceramide synthase and is toxic and carcinogenic to rodents and farm animals [106-108]. Fumonisin B1 has also been suggested to cause human esophageal cancer in southern Africa, Brazil and China [109-111]. The lack of selectivity of fumonisin B1 against microbial cells and the magnitude of the side effects prevents its usage as a therapeutic compound.

As the product of the first fungal-specific step of the sphingolipid biosynthesis, regulation of IPC has recently governed a lot of scientific attention. Several compounds, such as aureobasidin, khafrefungin and rustmicin, have been discovered to inhibit Ipc1 activity [80,112-115] with no affect on mammalian SMS activity in vitro (Del Poeta, unpublished results). Aureobasidin and rustmicin (Fig. **3**) show potential as therapeutic compounds in animal models [114,116-118]. In addition, combining the administration of Ipc1 inhibitors with compounds targeting other fungal pathways may increase the antifungal activity of these inhibitors in the treatment of fungal infections, hence reducing their collateral effects. For example, the efficacy of rustmicin in *S. cerevisiae* increases with disruption of pleiotropic drug resistance gene (PDR5) [114]. Also, aureobasidin with the mammalian multidrug resistance modulator verapamil decreased the MIC by approximately 15-fold in *A. fumigatus* [119]. The synergistic combination of aureobasidin with verapamil is intriguing because Aspergillus species are intrinsically resistant to Ipc1 inhibitors due to increased efflux [120] and offers hope to treating Aspergillus infections with Ipc1 inhibitors in combination with existing drugs.

IPC1 has a significant role in the pathogenesis of *C. neoformans.* Ipc1 activity has been demonstrated to regulate the ability of the fungus to grow in acidic pH conditions [4], melanin formation [82,121] and production of antiphagocytic protein 1 (App1*)* [122]. *C. neoformans* replicates at a higher frequency in host macrophages when contained in larger, more spacious phagolysosome in comparison to smaller, less spacious phagolysosomes [4,123]. Interestingly, the increase in phagolysosome size was associated with upregulation of Ipc1 activity and increased intracellular growth [4]. Thus, Ipc1 activity may contribute to the survival and proliferation of *C. neoformans* within macrophages. This hypothesis is supported by recent microarray studies showing that the level of *IPC1* mRNA is increased 4.1-fold in intracellular compared to extracellular *C. neoformans* [124]. Since the discovery of Ipc1 inhibitors, pharmaceutical companies have yet to develop new and less toxic compounds targeting Ipc1 and other fungal-specific sphingolipids-metabolizing enzymes essential for fungal virulence and viability.

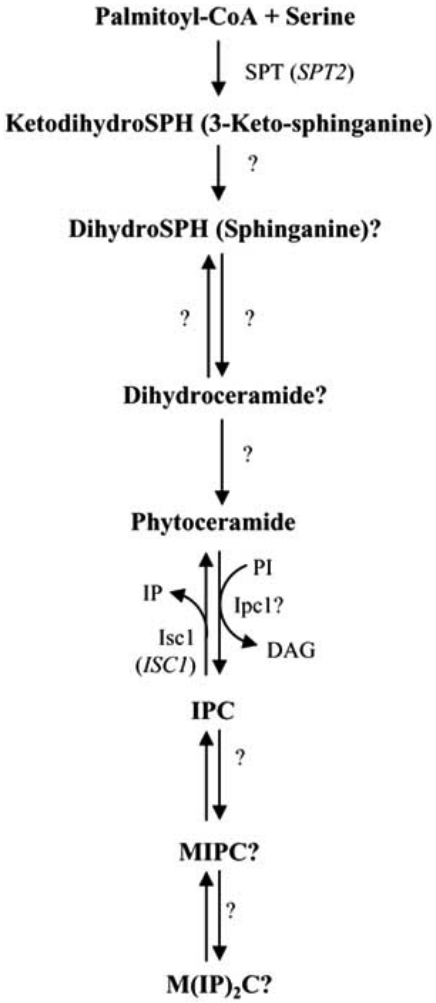

Fig. (4). The sphingolipid pathway of *Leishmania* species. A scheme for the biosynthetic pathway as observed in Leishmania species. The gene(s) for each of the enzymes is parentheses. Question marks indicate genes and enzymes that have not been isolated and identified. See Fig. **2** for abbreviations.

THE SPHINGOLIPID PATHWAYS OF PROTOZOA

A vast number and highly diverse group of unicellular organisms are classified as protozoa. Related only by

taxonomy, the sphingolipid pathway of a protozoan is dependant on the organism and is not conserved among all members of the subkingdom. For example, *Plasmodium* species, the etiologic agents of malaria, produce mammalian-like sphingolipids while kinetoplastid protozoa, most notably *Leishmania* and *Trypanosoma* species, have fungal-like sphingolipids. With the sequencing of the *Plasmodium falciparum* clone 3D7 genome [125] and the *Leishmania major* (Friedlin strain) genome [126] completed, the fields of genomics and proteomics can be used to help elucidate the sphingolipid-metabolizing enzymes of these protozoa.

The sphingolipid metabolism of *Plasmodium* is poorly understood and research has only begun to define the pathway. Biosynthetic activities associated with the mammalian sphingolipid pathway are present during the intraerythrocytic stage of *Plasmodium falciparum*. In this stage, the activities of sphingomyelin synthase (SMS) [127,128], the neutral sphingomyelinase (nSMase) encoded by *PfNSM* [129,130] and glucosylceramide synthase (GCS) [131] have been detected. The stage-specific export of SMS has been linked to the proper development of the tubulo-vesicular membrane (TVM) and host protein and nutrient uptake. [127,132-134]. In connection to these SMS findings, it has been proposed that *Plasmodium* nSMase degrades host SM into ceramide, which would then serve as substrate for *Plasmodium* SMS and GCS in the TVM [129]. Since mature erythrocytes do not have nor require sphingolipid synthesis [135], the inhibition of enzymes essential to the intraerythrocytic stage of *Plasmodium* species could specifically kill the protozoan without affecting the host cell.

Kinetoplastid protozoa, such as *Leishmania* and *Trypanosoma* species, have been found to possess sphingolipids similar to those in fungi. The *SPT2* gene was identified through homology analysis with fungal and mammalian cells [136]. The construction of an *SPT2*-deficient mutant revealed that sphingolipids were not required for procyclic promastigote viability or growth [136]. However, the loss of *de novo* sphingolipid synthesis prevented the proper differentiation into the infectious metacylic form and resulted in the death of the protozoa [136]. The failure to differentiate corresponded to disruption of the membrane trafficking of glycoprotein metalloprotease gp63 and irregular relocalization of lipophosphglycan (LPG) into lipid rafts [137], both of which sustains parasite survival and alteration of the host immune response [138,139]. With intracellular amastigote lacking any SPT activity, it can be concluded that the requirement of *de novo* sphingolipid synthesis is stage-dependent and is essential to the transition from the promastigote to the infectious amastigote.

L. donovani promastigotes have been found to synthesize the fungal sphingolipid IPC, comprising approximately 5-10% of the total cell lipids [140]. In addition, lipid rafts of *Leishmania* are enriched with IPC [141,142]. However, an *IPC1* gene has yet to be identified in *Leishmania* species. Zhang *et al.* discovered that both wild- type amastigotes and *SPT2*-null mutant amastigotes, which are deficient in de novo sphingolipid synthesis, to possess IPC molecules that were created from host sphingolipids [143]. This group has hypothesized that the ability of *Leishmania* to utilize host sphingolipids to produce IPC might be important for the viability of the intracellular parasite. The existence of a host sphingolipid salvage- and- remodeling mechanism is an intriguing discovery that can be exploited to combat *Leishmania* infection. Similar to the fundamental theory behind targeting fungal-specific sphingolipid pathway in antifungal therapies, therapeutic methods can target the enzyme responsible for the observed IPC synthesis in *Leishmania* without detrimental effects to the host cell.

THE SPHINGOLIPID PATHWAY IN BACTERIA AND VIRUSES

Most bacteria and viruses are unable to synthesize sphingolipids. However, some pathogenic bacteria have developed mechanisms to scavenge host sphingolipids during infection. One of these pathogens is *Chlamydia trachomatis*. Despite lacking the necessary sphingolipid-metabolizing enzymes [144], the cell wall of *C. trachomatis* contains *host* sphingomyelin that it obtains by intercepting exocytic vescicles en route from the *trans*-Golgi complex to the plasma membrane [145,146]. Using a Chinese hamster ovary (CHO) cells incapable of synthesizing sphingolipids due to a temperature-sensitive mutation in the *SPT* gene, it was demonstrated that host sphingolipids are required for the intracellular growth of *C. trachomatis* [147]. Thus, inhibiting the incorporation of host sphingolipids into chlamydial inclusions and the subsequent utilization of these molecules by the bacterium may prevent bacterial colonization and/or intracellular proliferation. Further research is required to determine the bacterial mechanism(s) responsible for the interruption of the exocytic pathway and how this incorporation exerts a protective mechanism.

The dysregulation of the host sphingolipid enzymes has been found to be involved in the pathogenesis of several microbes. For example, *Mycobacterium tuberculosis* inhibits the activity of sphingosine kinase, which produces SPH-P. SPH-P levels regulate cytosolic calcium concentrations [148], and an increase in cytosolic calcium is required to trigger antimicrobial activity in human macrophages against the bacterium [149-153]. Without the production of S1P, cytosolic calcium levels do not increase during *M. tuberculosis* infections and phagosome-lysosome fusion is inhibited. By averting the phagolysosome, *M. tuberculosis* is able to survive and proliferate within the macrophages. Thus, the bacterial component or derivative responsible for the inhibition of host SPH kinase would make an ideal therapeutic target against *M. tuberculosis*. Another possibility to combat *M. tuberculosis* would be to increase activation of the second messenger kinase Erk1/2, which in turn activates SPH kinase by phosphorylating Ser255 [154]. Therefore, this mechanism of activation could be used as a therapeutic target to increase the production of SPH-P to initiate the formation of the phagolysosome.

Some obligate intracellular bacteria and enveloped viruses utilize host lipid rafts to gain entry into host cells for replication. The infection of these pathogens that utilize lipid rafts may be prevented through universal means by altering the sphingolipid composition of host lipid rafts that would prevent the entry of the pathogen into host cells. For instance, the disruption of lipid rafts by cholesterol

depletion using beta-cyclodextrins has been shown to inhibit HIV-1 infection [19,21], which requires the binding of viral glycoprotein gp120 of HIV-1 to the lipid raft-associated CD4 to gain entry into T cells [73,155-157].

Along with cholesterol, sphingolipid are components of lipid rafts and they could be targeted to prevent infections. Increasing the ceramide content of lipid rafts indirectly by upregulating *de novo* ceramide biosynthesis through pharmacological manipulation and directly by the addition of exogenous ceramide and purified sphingomyelinase has been found to prevent HIV-1 infection [158]. This finding suggests that altering the sphingolipid and cholesterol concentration in lipid rafts of host cells may preclude entry of obligate intracellular pathogens and, thus, these compounds may hold great promise as new therapeutic strategies.

CONCLUDING REMARKS

Our understanding of the significance of sphingolipids in cell biology has vastly expanded over the past 20 years. Sphingolipids are now acknowledged as vital structural and signaling components of the cell membranes. As such, it is not surprising that the disruption of sphingolipid biosynthesis has been implicated in the human pathology of cancer, diabetes, neurodegenerative disorders, inflammation and infectious diseases. Many infectious microorganisms utilize and manipulate sphingolipid pathways for invasion, survival, and colonization/proliferation in a host cell. With emergence of drug-resistant organisms to current drugs, the efficacy of drug treatments will come to an abrupt halt. As basic science continues to unveil the importance of sphingolipids (especially as antifungal agents), the pharmaceutical industry should utilize this knowledge to produce new and more specific therapeutics.

Intriguingly, scientists have begun to convince the agricultural industry of the importance of sphingolipids. The recognition of microbial sphingolipids by plant surface proteins has been found to elicit a defense response in the plant and confer resistance to the pathogen [159-165]. In this field, where genetic engineering is more developed, perhaps it could be possible to create plants that are 'super resistant' to microbes by overexpressing genes coding for these resistance proteins. Some plants produce defensins in response to plant pathogens. Defensins exert antimicrobial activities [166,167] by binding to complex sphingolipids such as IPCs [168] and glucosylceramides [166] without apparent toxic effects on mammalian cells. Thus, in addition to the importance of plant defensins to the agricultural industry, studies are warranted to explore the potential use of plant defensins in human subjects against microbial pathogens producing IPCs.

As our comprehension of the complexity of sphingolipids increase, we need to broaden their potential application in novel therapeutic strategies. Sphingolipids, and sphingolipid-metabolizing enzymes, are very promising molecular targets for drug development against infectious microorganisms because they are actively involved in many patho-biological processes of the microbial-host cross-talk.

ACKNOWLEDGEMENTS

We would like to thank all members of the Del Poeta's and Luberto's laboratories for helpful discussion. Special thanks to Dr. Zdzislaw Szulc for helping with chemical structures.

Maurizio Del Poeta is supported by the Burroughs Wellcome Fund, the National Institute of Health (NIH) Grant AI56168 and the Centers of Biomedical Research Excellence (COBRE) Program of the National Center for Research Resources Grant RR17677 (Project 2). Maurizio Del Poeta is a Burroughs Wellcome New Investigator in Pathogenesis of Infectious Diseases.

REFERENCES

[1] Futerman, A. H.; Hannun, Y. A. *EMBO Rep.*, **2004**, *5*, 777.
[2] Hannun, Y. A.; Luberto, C. *Trends Cell. Biol.*, **2000**, *10*, 73.
[3] Hannun, Y. A.; Luberto, C.; Argraves, K. M. *Biochemistry*, **2001**, *40*, 4893.
[4] Luberto, C.; Toffaletti, D. L.; Wills, E. A.; Tucker, S. C.; Casadevall, A.; Perfect, J. R.; Hannun, Y. A.; Del Poeta, M. *Genes Dev.*, **2001**, *15*, 201.
[5] Luberto, C.; Kraveka, J. M.; Hannun, Y. A. *Neurochem. Res.*, **2002**, *27*, 609.
[6] Obeid, L. M.; Linardic, C. M.; Karolak, L. A.; Hannun, Y. A. *Science*, **1993**, *259*, 1769.
[7] Obeid, L. M.; Hannun, Y. A. *J. Cell. Biochem.*, **1995**, *58*, 191.
[8] Pettus, B. J.; Bielawska, A.; Subramanian, P.; Wijesinghe, D. S.; Maceyka, M.; Leslie, C. C.; Evans, J. H.; Freiberg, J.; Roddy, P.; Hannun, Y. A.; Chalfant, C. E. *J. Biol. Chem.*, **2004**, *279*, 11320.
[9] Spiegel, S.; Cuvillier, O.; Edsall, L. C.; Kohama, T.; Menzeleev, R.; Olah, Z.; Olivera, A.; Pirianov, G.; Thomas, D. M.; Tu, Z.; Van Brocklyn, J. R.; Wang, F. *Ann. N. Y. Acad. Sci.*, **1998**, *845*, 11.
[10] Cheng, J.; Park, T. S.; Fischl, A. S.; Ye, X. S. *Mol. Cell. Biol.*, **2001**, *21*, 6198.
[11] Simons, K.; Ikonen, E. *Nature*, **1997**, *387*, 569.
[12] Grassme, H.; Bock, J.; Kun, J.; Gulbins, E. *J. Biol. Chem.*, **2002**, *277*, 30289.
[13] Grassme, H.; Jekle, A.; Riehle, A.; Schwarz, H.; Berger, J.; Sandhoff, K.; Kolesnick, R.; Gulbins, E. *J. Biol. Chem.*, **2001**, *276*, 20589.
[14] Grassme, H.; Jendrossek, V.; Bock, J.; Riehle, A.; Gulbins, E. *J. Immunol.*, **2002**, *168*, 298.
[15] Bavari, S.; Bosio, C. M.; Wiegand, E.; Ruthel, G.; Will, A. B.; Geisbert, T. W.; Hevey, M.; Schmaljohn, C.; Schmaljohn, A.; Aman, M. J. *J. Exp. Med.*, **2002**, *195*, 593.
[16] Choi, K. S.; Aizaki, H.; Lai, M. M. *J. Virol.*, **2005**, *79*, 9862.
[17] Cuadras, M. A.; Greenberg, H. B. *Virology*, **2003**, *313*, 308.
[18] Joki-Korpela, P.; Marjomaki, V.; Krogerus, C.; Heino, J.; Hyypia, T. *J. Virol.*, **2001**, *75*, 1958.
[19] Liao, Z.; Cimakasky, L. M.; Hampton, R.; Nguyen, D. H.; Hildreth, J. E. *AIDS Res. Hum. Retroviruses*, **2001**, *17*, 1009.
[20] Lim, K. I.; Yin, J. *Biotechnol. Bioeng.*, **2005**, *90*, 694.
[21] Manes, S.; del Real, G.; Lacalle, R. A.; Lucas, P.; Gomez-Mouton, C.; Sanchez-Palomino, S.; Delgado, R.; Alcami, J.; Mira, E.; Martinez, A. C. *EMBO Rep.*, **2000**, *1*, 190.
[22] Triantafilou, K.; Triantafilou, M. *Virology*, **2003**, *317*, 128.
[23] Heung, L. J.; Luberto, C.; Del Poeta, M. *Infect. Immun.*, **2006**, *74*, 28.
[24] Hanada, K. *Jpn. J. Infect. Dis.*, **2005**, *58*, 131.
[25] Hanada, K.; Hara, T.; Nishijima, M. *J. Biol. Chem.*, **2000**, *275*, 8409.
[26] Buede, R.; Rinker-Schaffer, C.; Pinto, W. J.; Lester, R. L.; Dickson, R. C. *J. Bacteriol.*, **1991**, *173*, 4325.
[27] Kihara, A.; Igarashi, Y. *J. Biol. Chem.*, **2004**, *279*, 49243.
[28] Futerman, A. H.; Riezman, H. *Trends Cell. Biol.*, **2005**, *15*, 312.
[29] Schorling, S.; Vallee, B.; Barz, W. P.; Riezman, H.; Oesterhelt, D. *Mol. Biol. Cell*, **2001**, *12*, 3417.
[30] Guillas, I.; Kirchman, P. A.; Chuard, R.; Pfefferli, M.; Jiang, J. C.; Jazwinski, S. M.; Conzelmann, A. *EMBO J.*, **2001**, *20*, 2655.

[31] Venkataraman, K.; Riebeling, C.; Bodennec, J.; Riezman, H.; Allegood, J. C.; Sullards, M. C.; Merrill, A. H., Jr.; Futerman, A. H. *J. Biol. Chem.*, **2002**, *277*, 35642.

[32] Riebeling, C.; Allegood, J. C.; Wang, E.; Merrill, A. H., Jr.; Futerman, A. H. *J. Biol. Chem.*, **2003**, *278*, 43452.

[33] Lahiri, S.; Futerman, A. H. *J. Biol. Chem.*, **2005**, *280*, 33735.

[34] Xu, Z.; Zhou, J.; McCoy, D. M.; Mallampalli, R. K. *J. Lipid Res.*, **2005**, *46*, 1229.

[35] Mizutani, Y.; Kihara, A.; Igarashi, Y. *Biochem. J.*, **2005**, *390*, 263.

[36] Kohama, T.; Olivera, A.; Edsall, L.; Nagiec, M. M.; Dickson, R.; Spiegel, S. *J. Biol. Chem.*, **1998**, *273*, 23722.

[37] Liu, H.; Sugiura, M.; Nava, V. E.; Edsall, L. C.; Kono, K.; Poulton, S.; Milstien, S.; Kohama, T.; Spiegel, S. *J. Biol. Chem.*, **2000**, *275*, 19513.

[38] Le Stunff, H.; Peterson, C.; Thornton, R.; Milstien, S.; Mandala, S. M.; Spiegel, S. *J. Biol. Chem.*, **2002**, *277*, 8920.

[39] Mandala, S. M.; Thornton, R.; Galve-Roperh, I.; Poulton, S.; Peterson, C.; Olivera, A.; Bergstrom, J.; Kurtz, M. B.; Spiegel, S. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 7859.

[40] Van Veldhoven, P. P.; Gijsbers, S.; Mannaerts, G. P.; Vermeesch, J. R.; Brys, V. *Biochim. Biophys. Acta*, **2000**, *1487*, 128.

[41] Zhou, J.; Saba, J. D. *Biochem. Biophys. Res. Commun.*, **1998**, *242*, 502.

[42] Huitema, K.; van den Dikkenberg, J.; Brouwers, J. F.; Holthuis, J. C. *EMBO J.*, **2004**, *23*, 33.

[43] van Helvoort, A.; van't Hof, W.; Ritsema, T.; Sandra, A.; van Meer, G. *J. Biol. Chem.*, **1994**, *269*, 1763.

[44] Marggraf, W. D.; Kanfer, J. N. *Biochim. Biophys. Acta*, **1984**, *793*, 346.

[45] Miro-Obradors, M. J.; Osada, J.; Aylagas, H.; Sanchez-Vegazo, I.; Palacios-Alaiz, E. *Carcinogenesis*, **1993**, *14*, 941.

[46] Luberto, C.; Hannun, Y. A. *J. Biol. Chem.*, **1998**, *273*, 14550.

[47] Spence, M. W.; Byers, D. M.; Palmer, F. B.; Cook, H. W. *J. Biol. Chem.*, **1989**, *264*, 5358.

[48] Quintern, L. E.; Schuchman, E. H.; Levran, O.; Suchi, M.; Ferlinz, K.; Reinke, H.; Sandhoff, K.; Desnick, R. J. *EMBO J.*, **1989**, *8*, 2469.

[49] Schuchman, E. H.; Suchi, M.; Takahashi, T.; Sandhoff, K.; Desnick, R. J. *J. Biol. Chem.*, **1991**, *266*, 8531.

[50] Schissel, S. L.; Schuchman, E. H.; Williams, K. J.; Tabas, I. *J. Biol. Chem.*, **1996**, *271*, 18431.

[51] Schissel, S. L.; Keesler, G. A.; Schuchman, E. H.; Williams, K. J.; Tabas, I. *J. Biol. Chem.*, **1998**, *273*, 18250.

[52] Hofmann, K.; Tomiuk, S.; Wolff, G.; Stoffel, W. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 5895.

[53] Tomiuk, S.; Hofmann, K.; Nix, M.; Zumbansen, M.; Stoffel, W. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 3638.

[54] Sawai, H.; Domae, N.; Nagan, N.; Hannun, Y. A. *J. Biol. Chem.*, **1999**, *274*, 38131.

[55] Marchesini, N.; Luberto, C.; Hannun, Y. A. *J. Biol. Chem.*, **2003**, *278*, 13775.

[56] Wu, J.; Cheng, Y.; Palmberg, C.; Bergman, T.; Nilsson, A.; Duan, R. D. *Biochim. Biophys. Acta*, **2005**, *1687*, 94.

[57] Duan, R. D.; Hertervig, E.; Nyberg, L.; Hauge, T.; Sternby, B.; Lillienau, J.; Farooqi, A.; Nilsson, A. *Dig. Dis. Sci.*, **1996**, *41*, 1801.

[58] Duan, R. D.; Cheng, Y.; Hansen, G.; Hertervig, E.; Liu, J. J.; Syk, I.; Sjostrom, H.; Nilsson, A. *J. Lipid Res.*, **2003**, *44*, 1241.

[59] Cheng, Y.; Nilsson, A.; Tomquist, E.; Duan, R. D. *J. Lipid Res.*, **2002**, *43*, 316.

[60] Ichikawa, S.; Sakiyama, H.; Suzuki, G.; Hidari, K. I.; Hirabayashi, Y. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 4638.

[61] Leipelt, M.; Warnecke, D.; Zahringer, U.; Ott, C.; Muller, F.; Hube, B.; Heinz, E. *J. Biol. Chem.*, **2001**, *276*, 33621.

[62] Sugiura, M.; Kono, K.; Liu, H.; Shimizugawa, T.; Minekura, H.; Spiegel, S.; Kohama, T. *J. Biol. Chem.*, **2002**, *277*, 23294.

[63] Pettus, B. J.; Bielawska, A.; Spiegel, S.; Roddy, P.; Hannun, Y. A.; Chalfant, C. E. *J. Biol. Chem.*, **2003**, *278*, 38206.

[64] Clark, J. D.; Lin, L. L.; Kriz, R. W.; Ramesha, C. S.; Sultzman, L. A.; Lin, A. Y.; Milona, N.; Knopf, J. L. *Cell*, **1991**, *65*, 1043.

[65] Clark, J. D.; Schievella, A. R.; Nalefski, E. A.; Lin, L. L. *J. Lipid Mediat. Cell. Signal*, 1995, *12*, 83.

[66] Kramer, R. M.; Sharp, J. D. *FEBS Lett.*, **1997**, *410*, 49.

[67] Leslie, C. C. *J. Biol. Chem.*, **1997**, *272*, 16709.

[68] Demeule, M.; Jodoin, J.; Gingras, D.; Beliveau, R. *FEBS Lett.*, **2000**, *466*, 219.

[69] Claus, R.; Russwurm, S.; Meisner, M.; Kinscherf, R.; Deigner, H. P. *Curr. Drug Targets*, **2000**, *1*, 185.

[70] Jana, A.; Pahan, K. *J. Neurosci.*, **2004**, *24*, 9531.

[71] Rivas, C. I.; Golde, D. W.; Vera, J. C.; Kolesnick, R. N. *Blood*, **1994**, *83*, 2191.

[72] Cliften, P.; Wang, Y.; Mochizuki, D.; Miyakawa, T.; Wangspa, R.; Hughes, J.; Takemoto, J. Y. *Microbiology*, **1996**, *142* (Pt. 3), 477.

[73] Maddon, P. J.; Dalgleish, A. G.; McDougal, J. S.; Clapham, P. R.; Weiss, R. A.; Axel, R. *Cell*, **1986**, *47*, 333.

[74] Mao, C.; Xu, R.; Bielawska, A.; Obeid, L. M. *J. Biol. Chem.*, **2000**, *275*, 6876.

[75] Mao, C.; Xu, R.; Szulc, Z. M.; Bielawska, A.; Galadari, S. H.; Obeid, L. M. *J. Biol. Chem.*, **2001**, *276*, 26577.

[76] Smith, S. W.; Lester, R. L. *J. Biol. Chem.*, **1974**, *249*, 3395.

[77] Angus, W. W.; Lester, R. L. *Arch. Biochem. Biophys.*, **1972**, *151*, 483.

[78] Hackett, J. A.; Brennan, P. J. *FEBS Lett.*, **1977**, *74*, 259.

[79] Becker, G. W.; Lester, R. L. *J. Bacteriol.*, **1980**, *142*, 747.

[80] Heidler, S. A.; Radding, J. A. *Antimicrob. Agents Chemother.*, **1995**, *39*, 2765.

[81] Cerbon, J.; Falcon, A.; Hernandez-Luna, C.; Segura-Cobos, D. *Biochem. J.*, **2005**, *388*, 169.

[82] Heung, L. J.; Luberto, C.; Plowden, A.; Hannun, Y. A.; Del Poeta, M. *J. Biol. Chem.*, **2004**, *279*, 21144.

[83] Rittershaus, P. C.; Kechichian, T. B.; Allegood, J.; Merrill, A. H. Jr.; Hennig, M.; Luberto, C.; Del Poeta, M. *J. Clin. Invest.*, **2006**, in press.

[84] Desfarges, L.; Durrens, P.; Juguelin, H.; Cassagne, C.; Bonneu, M.; Aigle, M. *Yeast*, **1993**, *9*, 267.

[85] Beeler, T. J.; Fu, D.; Rivera, J.; Monaghan, E.; Gable, K.; Dunn, T. M. *Mol. Gen. Genet.*, **1997**, *255*, 570.

[86] Zhao, C.; Beeler, T.; Dunn, T. *J. Biol. Chem.*, **1994**, *269*, 21480.

[87] Beeler, T.; Gable, K.; Zhao, C.; Dunn, T. *J. Biol. Chem.*, **1994**, *269*, 7279.

[88] Uemura, S.; Kihara, A.; Inokuchi, J.; Igarashi, Y. *J. Biol. Chem.*, **2003**, *278*, 45049.

[89] Haak, D.; Gable, K.; Beeler, T.; Dunn, T. *J. Biol. Chem.*, **1997**, *272*, 29704.

[90] Dickson, R. C.; Nagiec, E. E.; Wells, G. B.; Nagiec, M. M.; Lester, R. L. *J. Biol. Chem.*, **1997**, *272*, 29620.

[91] Leber, A.; Fischer, P.; Schneiter, R.; Kohlwein, S. D.; Daum, G. *FEBS Lett.*, **1997**, *411*, 211.

[92] Sawai, H.; Okamoto, Y.; Luberto, C.; Mao, C.; Bielawska, A.; Domae, N.; Hannun, Y. A. *J. Biol. Chem.*, **2000**, *275*, 39793.

[93] Vaena de Avalos, S.; Okamoto, Y.; Hannun, Y. A. *J. Biol. Chem.*, **2004**, *279*, 11537.

[94] Vaena de Avalos, S.; Su, X.; Zhang, M.; Okamoto, Y.; Dowhan, W.; Hannun, Y. A. *J. Biol. Chem.*, **2005**, *280*, 7170.

[95] Zweerink, M. M.; Edison, A. M.; Wells, G. B.; Pinto, W.; Lester, R. L. *J. Biol. Chem.*, **1992**, *267*, 25032.

[96] Horn, W. S.; Smith, J. L.; Bills, G. F.; Raghoobar, S. L.; Helms, G. L.; Kurtz, M. B.; Marrinan, J. A.; Frommer, B. R.; Thornton, R. A.; Mandala, S. M. *J. Antibiot. (Tokyo)*, **1992**, *45*, 1692.

[97] VanMiddlesworth, F.; Giacobbe, R. A.; Lopez, M.; Garrity, G.; Bland, J. A.; Bartizal, K.; Fromtling, R. A.; Polishook, J.; Zweerink, M.; Edison, A. M.; Rozdilsky, W.; Wilson, K. E.; Monaghan, R. L. *J. Antibiot. (Tokyo)*, **1992**, *45*, 861.

[98] Mandala, S. M.; Frommer, B. R.; Thornton, R. A.; Kurtz, M. B.; Young, N. M.; Cabello, M. A.; Genilloud, O.; Liesch, J. M.; Smith, J. L.; Horn, W. S. *J. Antibiot. (Tokyo)*, **1994**, *47*, 376.

[99] Whaley, H. A. *J. Am. Chem. Soc.*, **1971**, *93*, 3767.

[100] Whaley, H. A.; Sebek, O. K.; Lewis, C. *Antimicrob. Agents Chemother.*, **1970**, *10*, 455.

[101] Chen, J. K.; Lane, W. S.; Schreiber, S. L. *Chem. Biol.*, **1999**, *6*, 221.

[102] Kluepfel, D.; Bagli, J.; Baker, H.; Charest, M. P.; Kudelski, A. *J. Antibiot. (Tokyo)*, **1972**, *25*, 109.

[103] St-Jacques, M. *J. Org. Chem.*, **1973**, *38*, 1253.

[104] Mandala, S. M.; Thornton, R. A.; Frommer, B. R.; Dreikorn, S.; Kurtz, M. B. *J. Antibiot. (Tokyo)*, **1997**, *50*, 339.

[105] Dalla Serra, M.; Fagiuoli, G.; Nordera, P.; Bernhart, I.; Della Volpe, C.; Di Giorgio, D.; Ballio, A.; Menestrina, G. *Mol. Plant Microbe Interact.*, **1999**, *12*, 391.

[106] Riley, R. T.; Enongene, E.; Voss, K. A.; Norred, W. P.; Meredith, F. I.; Sharma, R. P.; Spitsbergen, J.; Williams, D. E.; Carlson, D. B.; Merrill, A. H., Jr. *Environ. Health Perspect.*, **2001**, *109* (Suppl. 2), 301.

[107] Voss, K. A.; Riley, R. T.; Norred, W. P.; Bacon, C. W.; Meredith, F. I.; Howard, P. C.; Plattner, R. D.; Collins, T. F.; Hansen, D. K.; Porter, J. K. *Environ. Health Perspect.*, **2001***, 109* (Suppl. 2), 259.

[108] Dragan, Y. P.; Bidlack, W. R.; Cohen, S. M.; Goldsworthy, T. L.; Hard, G. C.; Howard, P. C.; Riley, R. T.; Voss, K. A. *Toxicol. Sci.*, **2001***, 61*, 6.

[109] Wang, H.; Wei, H.; Ma, J.; Luo, X. *J. Environ. Pathol. Toxicol. Oncol.*, **2000***, 19*, 139.

[110] Chelule, P. K.; Gqaleni, N.; Dutton, M. F.; Chuturgoon, A. A. *Environ. Health Perspect.*, **2001***, 109*, 253.

[111] van der Westhuizen, L.; Shephard, G. S.; Scussel, V. M.; Costa, L. L.; Vismer, H. F.; Rheeder, J. P.; Marasas, W. F. *J. Agric. Food Chem.*, **2003***, 51*, 5574.

[112] Hashida-Okado, T.; Ogawa, A.; Endo, M.; Yasumoto, R.; Takesako, K.; Kato, I. *Mol. Gen. Genet.*, **1996***, 251*, 236.

[113] Mandala, S. M.; Thornton, R. A.; Rosenbach, M.; Milligan, J.; Garcia-Calvo, M.; Bull, H. G.; Kurtz, M. B. *J. Biol. Chem.*, **1997***, 272*, 32709.

[114] Mandala, S. M.; Thornton, R. A.; Milligan, J.; Rosenbach, M.; Garcia-Calvo, M.; Bull, H. G.; Harris, G.; Abruzzo, G. K.; Flattery, A. M.; Gill, C. J.; Bartizal, K.; Dreikorn, S.; Kurtz, M. B. *J. Biol. Chem.*, **1998***, 273*, 14942.

[115] Harris, G. H.; Shafiee, A.; Cabello, M. A.; Curotto, J. E.; Genilloud, O.; Goklen, K. E.; Kurtz, M. B.; Rosenbach, M.; Salmon, P. M.; Thornton, R. A.; Zink, D. L.; Mandala, S. M. *J. Antibiot. (Tokyo)*, **1998***, 51*, 837.

[116] Endo, M.; Takesako, K.; Kato, I.; Yamaguchi, H. *Antimicrob. Agents Chemother.*, **1997***, 41*, 672.

[117] Takesako, K.; Kuroda, H.; Inoue, T.; Haruna, F.; Yoshikawa, Y.; Kato, I.; Uchida, K.; Hiratani, T.; Yamaguchi, H. *J. Antibiot. (Tokyo)*, **1993***, 46*, 1414.

[118] Yoshikawa, Y.; Ikai, K.; Umeda, Y.; Ogawa, A.; Takesako, K.; Kato, I.; Naganawa, H. *J. Antibiot. (Tokyo)*, **1993***, 46*, 1347.

[119] Gottesman, M. M.; Pastan, I. *Annu. Rev. Biochem.*, **1993***, 62*, 385.

[120] Zhong, W.; Jeffries, M. W.; Georgopapadakou, N. H. *Antimicrob. Agents Chemother.*, **2000***, 44*, 651.

[121] Heung, L. J.; Kaiser, A. E.; Luberto, C.; Del Poeta, M. *J Biol Chem.*, **2005***, 280*, 28547.

[122] Mare, L.; Iatta, R.; Montagna, M. T.; Luberto, C.; Del Poeta, M. *J. Biol. Chem.*, **2005***, 280*, 36055.

[123] Lee, S. C.; Kress, Y.; Zhao, M. L.; Dickson, D. W.; Casadevall, A. *Lab. Invest.*, **1995***, 73*, 871.

[124] Fan, W.; Kraus, P. R.; Boily, M. J.; Heitman, J. *Eukaryot. Cell*, **2005***, 4*, 1420.

[125] Gardner, M. J.; Shallom, S. J.; Carlton, J. M.; Salzberg, S. L.; Nene, V.; Shoaibi, A.; Ciecko, A.; Lynn, J.; Rizzo, M.; Weaver, B.; Jarrahi, B.; Brenner, M.; Parvizi, B.; Tallon, L.; Moazzez, A.; Granger, D.; Fujii, C.; Hansen, C.; Pederson, J.; Feldblyum, T.; Peterson, J.; Suh, B.; Angiuoli, S.; Pertea, M.; Allen, J.; Selengut, J.; White, O.; Cummings, L. M.; Smith, H. O.; Adams, M. D.; Venter, J. C.; Carucci, D. J.; Hoffman, S. L.; Fraser, C. M. *Nature*, **2002***, 419*, 531.

[126] Ivens, A. C.; Peacock, C. S.; Worthey, E. A.; Murphy, L.; Aggarwal, G.; Berriman, M.; Sisk, E.; Rajandream, M. A.; Adlem, E.; Aert, R.; Anupama, A.; Apostolou, Z.; Attipoe, P.; Bason, N.; Bauser, C.; Beck, A.; Beverley, S. M.; Bianchettin, G.; Borzym, K.; Bothe, G.; Bruschi, C. V.; Collins, M.; Cadag, E.; Ciarloni, L.; Clayton, C.; Coulson, R. M.; Cronin, A.; Cruz, A. K.; Davies, R. M.; De Gaudenzi, J.; Dobson, D. E.; Duesterhoeft, A.; Fazelina, G.; Fosker, N.; Frasch, A. C.; Fraser, A.; Fuchs, M.; Gabel, C.; Goble, A.; Goffeau, A.; Harris, D.; Hertz-Fowler, C.; Hilbert, H.; Horn, D.; Huang, Y.; Klages, S.; Knights, A.; Kube, M.; Larke, N.; Litvin, L.; Lord, A.; Louie, T.; Marra, M.; Masuy, D.; Matthews, K.; Michaeli, S.; Mottram, J. C.; Muller-Auer, S.; Munden, H.; Nelson, S.; Norbertczak, H.; Oliver, K.; O'Neil, S.; Pentony, M.; Pohl, T. M.; Price, C.; Purnelle, B.; Quail, M. A.; Rabbinowitsch, E.; Reinhardt, R.; Rieger, M.; Rinta, J.; Robben, J.; Robertson, L.; Ruiz, J. C.; Rutter, S.; Saunders, D.; Schafer, M.; Schein, J.; Schwartz, D. C.; Seeger, K.; Seyler, A.; Sharp, S.; Shin, H.; Sivam, D.; Squares, R.; Squares, S.; Tosato, V.; Vogt, C.; Volckaert, G.; Wambutt, R.; Warren, T.; Wedler, H.; Woodward, J.; Zhou, S.; Zimmermann, W.; Smith, D. F.; Blackwell, J. M.; Stuart, K. D.; Barrell, B.; Myler, P. J. *Science*, **2005***, 309*, 436.

[127] Elmendorf, H. G.; Haldar, K. *J. Cell. Biol.*, **1994***, 124*, 449.

[128] Ansorge, I.; Jeckel, D.; Wieland, F.; Lingelbach, K. *Biochem. J.*, **1995***, 308* (Pt. 1), 335.

[129] Hanada, K.; Mitamura, T.; Fukasawa, M.; Magistrado, P. A.; Horii, T.; Nishijima, M. *Biochem. J.*, **2000***, 346* (Pt. 3), 671.

[130] Hanada, K.; Palacpac, N. M.; Magistrado, P. A.; Kurokawa, K.; Rai, G.; Sakata, D.; Hara, T.; Horii, T.; Nishijima, M.; Mitamura, T. *J. Exp. Med.*, **2002***, 195*, 23.

[131] Couto, A. S.; Caffaro, C.; Uhrig, M. L.; Kimura, E.; Peres, V. J.; Merino, E. F.; Katzin, A. M.; Nishioka, M.; Nonami, H.; Erra-Balsells, R. *Eur. J. Biochem.*, **2004***, 271*, 2204.

[132] Elmendorf, H. G.; Haldar, K. *EMBO J.*, **1993***, 12*, 4763.

[133] Lauer, S. A.; Ghori, N.; Haldar, K. *Proc. Natl. Acad. Sci. USA*, **1995***, 92*, 9181.

[134] Lauer, S.; VanWye, J.; Harrison, T.; McManus, H.; Samuel, B. U.; Hiller, N. L.; Mohandas, N.; Haldar, K. *EMBO J.*, **2000***, 19*, 3556.

[135] Chasis, J. A.; Schrier, S. L. *Blood*, **1989***, 74*, 2562.

[136] Zhang, K.; Showalter, M.; Revollo, J.; Hsu, F. F.; Turk, J.; Beverley, S. M. *EMBO J.*, **2003***, 22*, 6016.

[137] Denny, P. W.; Goulding, D.; Ferguson, M. A.; Smith, D. F. *Mol. Microbiol.*, **2004***, 52*, 313.

[138] McConville, M. J.; Schnur, L. F.; Jaffe, C.; Schneider, P. *Biochem. J.*, **1995***, 310* (Pt. 3), 807.

[139] Proudfoot, L.; Nikolaev, A. V.; Feng, G. J.; Wei, W. Q.; Ferguson, M. A.; Brimacombe, J. S.; Liew, F. Y. *Proc. Natl. Acad. Sci. USA*, **1996***, 93*, 10984.

[140] Kaneshiro, E. S.; Jayasimhulu, K.; Lester, R. L. *J. Lipid Res.*, **1986***, 27*, 1294.

[141] Ralton, J. E.; Mullin, K. A.; McConville, M. J. *Biochem. J.*, **2002***, 363*, 365.

[142] Denny, P. W.; Field, M. C.; Smith, D. F. *FEBS Lett.*, **2001***, 491*, 148.

[143] Zhang, K.; Hsu, F. F.; Scott, D. A.; Docampo, R.; Turk, J.; Beverley, S. M. *Mol. Microbiol.*, **2005***, 55*, 1566.

[144] Stephens, R. S.; Kalman, S.; Lammel, C.; Fan, J.; Marathe, R.; Aravind, L.; Mitchell, W.; Olinger, L.; Tatusov, R. L.; Zhao, Q.; Koonin, E. V.; Davis, R. W. *Science*, **1998***, 282*, 754.

[145] Hackstadt, T.; Scidmore, M. A.; Rockey, D. D. *Proc. Natl. Acad. Sci. USA*, **1995***, 92*, 4877.

[146] Hackstadt, T.; Rockey, D. D.; Heinzen, R. A.; Scidmore, M. A. *EMBO J.*, **1996***, 15*, 964.

[147] van Ooij, C.; Kalman, L.; van, I.; Nishijima, M.; Hanada, K.; Mostov, K.; Engel, J. N. *Cell. Microbiol.*, **2000***, 2*, 627.

[148] Spiegel, S.; Milstien, S. Sphingosine 1-phosphate, a key cell signaling molecule. *J. Biol. Chem.*, **2002**, *277*, 25851.

[149] Kim-Park, W. K.; Moore, M. A.; Hakki, Z. W.; Kowolik, M. J. *Ann. N. Y. Acad. Sci.*, **1997***, 832*, 394.

[150] Denlinger, L. C.; Fisette, P. L.; Garis, K. A.; Kwon, G.; Vazquez-Torres, A.; Simon, A. D.; Nguyen, B.; Proctor, R. A.; Bertics, P. J.; Corbett, J. A. *J. Biol. Chem.*, **1996***, 271*, 337.

[151] Watanabe, N.; Suzuki, J.; Kobayashi, Y. *J. Biochem. (Tokyo)*, **1996***, 120*, 1190.

[152] Tapper, H. *J. Leukoc. Biol.*, **1996***, 59*, 613.

[153] Chen, B. C.; Chou, C. F.; Lin, W. W. *J. Biol. Chem.*, **1998***, 273*, 29754.

[154] Pitson, S. M.; Moretti, P. A.; Zebol, J. R.; Lynn, H. E.; Xia, P.; Vadas, M. A.; Wattenberg, B. W. *EMBO J.*, **2003***, 22*, 5491.

[155] Cinek, T.; Hilgert, I.; Horejsi, V. *Immunogenetics*, **1995***, 41*, 110.

[156] McDougal, J. S.; Maddon, P. J.; Dalgleish, A. G.; Clapham, P. R.; Littman, D. R.; Godfrey, M.; Maddon, D. E.; Chess, L.; Weiss, R. A.; Axel, R. *Cold Spring Harb. Symp. Quant. Biol.*, **1986***, 51 Pt 2*, 703.

[157] Parolini, I.; Sargiacomo, M.; Lisanti, M. P.; Peschle, C. *Blood*, **1996***, 87*, 3783.

[158] Finnegan, C. M.; Rawat, S. S.; Puri, A.; Wang, J. M.; Ruscetti, F. W.; Blumenthal, R. *Proc. Natl. Acad. Sci. USA*, **2004***, 101*, 15452.

[159] Gomes, E.; Sagot, E.; Gaillard, C.; Laquitaine, L.; Poinssot, B.; Sanejouand, Y. H.; Delrot, S.; Coutos-Thevenot, P. *Mol. Plant Microbe Interact.*, **2003***, 16*, 456.

[160] Molina, A.; Segura, A.; Garcia-Olmedo, F. *FEBS Lett.*, **1993***, 316*, 119.

[161] Molina, A.; Garcia-Olmedo, F. *Plant J.*, **1993***, 4*, 983.

[162] Garcia-Olmedo, F.; Molina, A.; Segura, A.; Moreno, M. *Trends Microbiol.*, **1995***, 3*, 72.

[163] Molina, A.; Mena, M.; Carbonero, P.; Garcia-Olmedo, F. *Plant Mol. Biol.*, 1997*, 33*, 803.

[164] Molina, A.; Garcia-Olmedo, F. *Plant J.*, **1997***, 12*, 669.

[165] Segura, A.; Moreno, M.; Molina, A.; Garcia-Olmedo, F. *FEBS Lett.*, **1998***, 435*, 159.

[166] Thevissen, K.; Warnecke, D. C.; Francois, I. E.; Leipelt, M.; Heinz, E.; Ott, C.; Zahringer, U.; Thomma, B. P.; Ferket, K. K.; Cammue, B. P. *J. Biol. Chem.*, **2004***, 279*, 3900.

[167] Thomma, B. P.; Cammue, B. P.; Thevissen, K. *Planta*, **2002***, 216*, 193.

[168] Thevissen, K.; Cammue, B. P.; Lemaire, K.; Winderickx, J.; Dickson, R. C.; Lester, R. L.; Ferket, K. K.; Van Even, F.; Parret, A. H.; Broekaert, W. F. *Proc. Natl. Acad. Sci. U. S. A.*, **2000***, 97*, 9531.

Received: September 16, 2005 Revised: December 10, 2005 Accepted: December 11, 2005