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Plant Sphingolipids Today – Are They Still Enigmatic?

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Abstract: Sphingolipids are a diverse group of lipids found in all eukaryotes and some bacteria, consisting of a hydrophobic ceramide and a hydrophilic head group. We have summarised the contemporary understanding of the structure of plant sphingolipids with an emphasis on glucosylceramides and inositolphosphorylceramides. Plant glucosylceramides are important structural components of plasma and vacuole membranes. Inositolphosphorylceramides have been identified as moieties of the glycosylphosphorylinositol (GPI) anchors of plant proteins targeted to the plasma membrane. In the last few years, progress has been made in the cloning of plant genes coding for enzymes involved in sphingolipid metabolism. As found in yeast and mammals, the plant sphingolipid pathway is a potential generator of powerful cell signals. The role of plant sphingolipid metabolites in programmed cell death and calcium influx is discussed.

Key words: Plant sphingolipids, ceramide, sphingolipid signalling, membrane lipids, plant GPI-anchored proteins.

Abbreviations:

ER:	endoplasmic reticulum
FA:	fatty acid
GluCers:	glucosylceramides
GPI:	glycosylphosphorylinositol
InsPCers:	inositolphosphorylceramides
LCB:	long-chain base
PL:	phosphoglycerolipids
SPT:	serine palmitoyltransferase
SAMs:	(dihydro)sphingosine analogue mycotoxins
VLCFA:	very long-chain fatty acids

Introduction

Sphingolipids are widely spread among eukaryotes and some bacteria (Ng and Hetherington, 2001). They were discovered in the pioneering work of Thudichum (1874), more than 100 years ago (reviewed in Hakomori, 1983). He named them after the mysterious Sphinx because their properties were unusual for a lipid. The first studies on plant sphingolipids were focused mainly on their structure and on their role as components of biomembranes (Carter, 1961; Fujino and Ito, 1971; Lynch, 1990). Later on attention was drawn to the enzymes of their biosynthetic pathway (Lynch, 2000) and only recently have reports appeared showing a role for plant sphingolipids in cell signalling (Ng, 2001; Spassieva, 2002).

Plant sphingolipids appear to be significantly variable in their structure (Lynch, 1993 a). Characteristic for plant sphingolipids is the diversity of their ceramide backbone. Plant ceramides show variation depending on the species or the tissue they are isolated from. Thirty different molecular species from rye have been identified using reverse phase HPLC (Cahoon and Lynch, 1991). The core structure of a plant ceramide is the *l*ong-chain *b*ase (LCB) dihydrosphingosine (2-amino-1,3-dihydroxyoctadecane) with amide linked *f*atty *a*cid (FA) (Fig. **1**) (Sullards, 2000). The chain length of the FA component of a plant ceramide can vary from C_{14} to C_{26} and in more than 90% of the cases is α-hydroxylated (Lynch, 1990, 2000). The FA could be saturated or mono-unsaturated, the latter predominantly found in cereals. In maize root plasma membranes glucosylceramides mainly contain saturated, α-hydroxy *v*ery *l*ong-chain *f*atty *a*cids (VLCFA) (Very long-chain fatty acids – C_{20} – C_{26} fatty acids) (Bohn, 2001). The LCB of a plant ceramide, in addition to position 1 and 3, can have one more hydroxy group at position 4 (t18 : 0) (Short description of LCB: d – dihydroxy; t – trihydroxy; the number before the semicolon designates number of C atoms, the number after – degree of unsaturation; suffix – position[s] of the double bound[s]). In plants the unsaturated Δ8 *cis* or *trans* isomers of the LCB with or without Δ4 *trans* unsaturation are often found, while saturated d18 : 0 (dihydrosphingosine) and t18 : 0 (phytosphingosine) are found as minor components (Lynch, 1993 a). This is in contrast to yeast ceramides, where the major LCBs are dihydrosphingosines (d18 : 0, d20 : 0) and phytosphingosines (t18 : 0, t20 : 0), and mammals, where the predominant LCB is sphingosine (d18 : $1^{\Delta 4trans}$) (Merrill, Jr., 2002). All combinations between different LCBs and FAs are possible, which lead to the diversity of the plant ceramides. In grapevine leaves the t18 : 1 LCB is predominantly linked to VLCFA, such as C_{22} to C_{26} (Kawaguchi, 2000). Similar results were presented in the analysis of spinach leaves (Ohnishi, 1983). In the glycosphingolipid fraction, the trihydroxy LCBs are paired mainly with VLCFA $C_{24,}$ while dihydroxy LCBs are exclusively acylated with palmitic acid (C_{16}).

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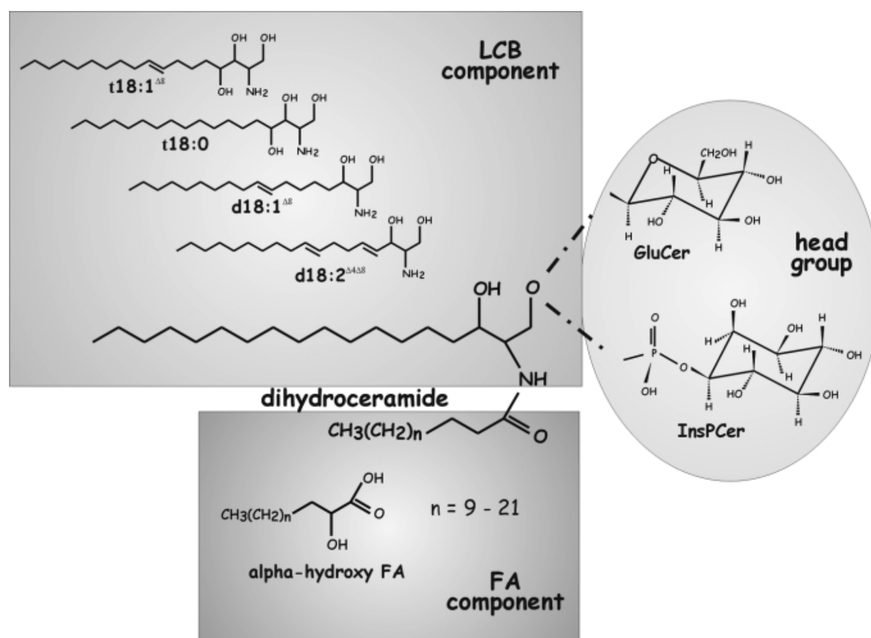

Fig. 1 Structure of a plant sphingolipid. The general structure is based on a hydrophobic ceramide and a hydrophilic head group. In plants ceramide consists of a C_{18} long-chain base (LCB) amide linked to a fatty acid (FA). The LCB component can vary in its degree of unsaturation and hydroxylation. The FA component is predominantly α-hydroxylated and varies in chain length (from C_{14} till C_{26}) and the degree of unsaturation. GluCer – glucosylceramide; InsPCer – inositolphosphorylceramide. d = dihydroxy; t = trihydroxy; the number before the semicolon designates number of C atoms, the number after = degree of unsaturation; suffix = position(s) of the double bound(s).

Depending on the head group attached to the C_1 hydroxyl group of the LCB (Fig. **1**), there are two major groups of plant sphingolipids characterised so far: plant glycosphingolipids (Lynch, 1993 a), including the extensively studied glucosylceramides (GluCers) or cerebrosides, and plant inositolphosphorylceramides (InsPCers) (Lester and Dickson, 1993).

Glycosphingolipids are also called glycosylceramides, using the appropriate trivial name of the mono- or oligosaccharide residue for "glycosyl" e.g. glucosylceramide (Hakomori, 1983). The trivial name cerebroside was originally used to designate ceramide-galactoside from brain. Now the term cerebroside is more generally applied for naturally occurring sphingolipids in which the head group linked to ceramide is a monosaccharide and the LCB and the FA are not specified (Carter, 1965).

Glycosphingolipids

Structure

The best-studied class of sphingolipids in plants is that of the glycosphingolipids (Lynch, 1990; Imai, 1997; Sullards, 2000; Imai, 2000). In grapevine leaves, more than 97% of their sugar component is glucose, the remainder containing mannose and galactose (Kawaguchi, 2000). Mannose was found as a minor sugar component in the diglycosylceramide fraction of spinach leaves and in wheat di-, tri-, and tetraglycosylceramides (Ohnishi, 1983; Fujino, 1985). In the same study it was suggested that all the glycosidic linkages are in the β configuration. In soybean the only sugar component identified so far is glucose (Sullards, 2000).

The most intensively studied and also the most abundant glycosylceramide is monohexoside ceramide. Di-, tri- and tetrahexoside ceramides were reported in cereals (Fujino, 1985). In other studies from the same group, diglycosylceramides were identified in soybean and spinach leaves, but were not further characterised because of the insufficient amounts isolated (Ohnishi and Fujino, 1982; Ohnishi, 1983). These results show the existence of oligoglycosylceramides in plants, but because they are not as abundant as monohexoside ceramides, they are not accurately characterised.

Glycosphingolipids as membrane components

The plasma membrane (7 – 30 mol% from total lipids) and tonoplast (12 – 17 mol% from total lipids) are rich in GluCers compared to the membranes of the chloroplast or mitochondria (Yoshida and Uemura, 1986; Rochester, 1987; Lynch and Steponkus, 1987 a; Sandstrom and Cleland, 1989; Norberg, 1991; Cahoon and Lynch, 1991; Tavernier, 1993). GluCers are believed to stabilise the membranes and reduce their permeability to ions (Boggs, 1987; Lynch, 1993 a). This may explain the high mol% of GluCers in the plasma membrane and tonoplast, because these membranes are known to maintain large ions gradients.

GluCers were found in the intracellular membrane fraction, endoplasmic reticulum (ER) and Golgi, of barley leaf extracts (Rochester, 1987). In spinach, GluCer were found in the chloroplast envelope membranes (Poincelot, 1973). It is difficult to detect GluCer in the chloroplast membranes because they are only 0.2% of the total lipid composition and are found exclusively in the envelope membrane, where they are 2.7% of the total lipid composition. A clean chloroplast envelope preparation was required in order for this lipid class to be detected.

A number of studies pointed out the importance of the lipid composition of the plant plasma and vacuolar membranes for cold tolerance (Lynch and Steponkus, 1987 a; Lynch and Steponkus, 1987 b; Yoshida, 1988; Steponkus and Lynch, 1989; Lynch, 1992; Uemura and Steponkus, 1994; Norberg, 1996; Kawaguchi, 2000). GluCers, together with phosphoglycerolipids (PL) and sterols, are the major components of these membranes. For example, GluCers encompass up to 27 mol% from the total lipids of the plasma membrane of spring oat or

23 mol% of barley and 19.6 weight % of the tonoplast lipids of oat leaves. The structure of the lipids and the proportion of PLs/GluCers/sterols determine the thermotropic phase behaviour of the membrane, which was shown to correlate with its cryostability. Compared to PL, GluCers exhibit high lamella gel-to-liquid crystalline transition temperatures (in the range of 50 – 60 °C). GluCers are considered to be one of the inducers of phase separation in the membranes at low temperature, consistent with the observation of decreased GluCers proportion as a result of cold acclimation. One of the characteristic changes of cold acclimation of winter rye and spring oat is a decreased proportion of GluCer in the plasma membrane (Lynch and Steponkus, 1987 b; Uemura and Steponkus, 1994). The significance of the relative ratio of *trans/cis* isomers at the Δ8 position of the LCB component of the GluCer of grapevine species was pointed out for their cold tolerance (Kawaguchi, 2000). The ratio between *trans* Δ8 and *cis* Δ8 isomers is lower for cold-tolerant varieties. It was shown that chilling-sensitive species contain less *cis* isomers of the trihydroxy LCB components of their GluCer (Imai, 1997). This observation corresponds to the thermotropic characteristic of these isomers. Cerebrosides with *cis* Δ8 unsaturated LCBs, compared to their *trans* isoforms, have lower gel-to-liquid crystalline phase transition temperatures (Ohnishi, 1988).

GluCers influence the liotropic phase behaviour of membranes, which is also important for cold tolerance (Steponkus and Lynch, 1989). The dehydration-induced formation of hexagonal-II phases can compromise the cell compartmentalisation, the most important function of the cell membrane systems, as well as the functionality of membrane proteins. The plasma membrane lipid composition of cold acclimated and non-cold acclimated winter rye differ. This difference results in a predisposition of the liposomes prepared from nonacclimated plants to form lipid domains leading to hexagonal-II phase transition, while acclimated ones do not (Steponkus and Lynch, 1989). From phase behaviour studies of PLs, it is known that the hexagonal-II phase is promoted by the presence in the membrane of nonbilayer-forming lipids, such as phosphatidylethanolamine. Sphingolipids, which are bilayer-forming lipids, were observed to decrease upon cold acclimation. The explanation of this, at first sight an apparent paradox, is that the formation of hexagonal-II phase structures is preceded by demixing of the bilayer and non-bilayer forming lipids in the membrane and, as a result, the non-bilayer forming lipids become enriched in part of the membrane and undergo lamella to hexagonal-II phase transition. Mixtures containing PLs and cerebrosides show decreased gel mixability when the cerebroside proportion is increased, because cerebrosides tend to form a separate gel phase (Steponkus and Lynch, 1989).

Glycosphingolipids as a food source

GluCers are a major glycolipid class of a number of edible plant sources including cereals, legumes, vegetables, and fruits. The distribution of different classes of glycolipids was studied in a variety of edible plant sources (Sugawara and Miyazawa, 1999). In this comprehensive study, including 48 different edible plant sources, it was shown that ceramide monohexoside is the major class, with an average content of 12% among other glycolipids. The amounts of sphingolipids in the edible plants is variable and can be up to 2 µmol/g dry weight, for example in soybean (Ohnishi and Fujino, 1982; Merrill, Jr., 1997).

The work of Sullards et al. (2000) pointed out the importance of the glycosylceramides as an abundant lipid component of different food sources for the human diet and their possible ability to affect diseases such as cancer. Mice with induced colon tumors, but fed with sphingomyelin, showed significant reduction of the disease symptoms (Vesper, 1999; Schmelz, 2001). So far, there is no evidence for the biological activity of plant sphingolipids as part of the animal and human diet and the structure of the plant sphingolipids studied up to now differs from sphingomyelin. On the other hand, preliminary studies showed that wheat and soybean ceramides exhibit comparable toxicity on human adenocarcinoma cell line as ceramide derived from sphingomyelin (Sullards, 2000).

Inositolphosphorylceramides and GPI-anchored Proteins in Plants

Identification and structure of inositolphosphorylceramides

For the first time, Carter and Koob (1969) described InsPCers (Fig. **1**) in plants in their work on plant sphingolipids. The "phytoglycolipid" they described was a ceramide attached through a phosphate diester linkage to a tetrasaccharide unit, consisting of inositol, glucoronic acid, glucosamine and either mannose, arabinose or galactose (Fig. **2a**). The FA component of the ceramide moieties was identified as saturated α-hydroxy VLCFA, C_{22} – C_{26} and the major LCB components as dehydrophytosphingosine (t18 : 1) and phytosphingosine (t18 : 0) (Lester and Dickson, 1993).

InsPCers in plants are not as extensively studied as GluCers. This is mainly because of their higher polarity and less efficient extraction when less polar solvents such as chloroform/methanol are used. Kaul and Lester (1975) applied a relatively mild extraction procedure that yielded sufficient amounts of InsPCers from tobacco leaves for isolation of pure trisaccharide sphingolipids, which fell into two classes depending on the acetylation of the glucosamine moiety, phosphosphingolipid-I and phosphosphingolipid-II. The latter contains nonacetylated glucosamine. The ceramide component of the tobacco leaf InsPCers resembles that described by Carter from soybean. The structure is shown on Fig. **2b**. Characterisation of the saccharide moiety of the plant InsPCers using reverse phase HPLCs resulted in at least 24 different components (Hsieh, 1981). These studies showed that InsPCers are as diverse and probably as wide-spread as GluCers in the plant kingdom. InsPCers, until now, have been mainly characterised in crop plants.

Inositolphosphorylceramides as a part of a glycosylphosphatidylinositol anchor of a protein

GPI-anchored proteins occur widely, most likely universally in eukaryotes (Muniz and Riezman, 2000). They are situated on the surface of all eukaryotic cells. In the last decade GPI-anchored proteins in animals have been studied in detail and they have been linked to different cellular processes, such as signal transduction, cell-cell recognition and nutrient uptake. In yeast, GPI-anchored proteins are known to participate in cell signalling and are associated with the cell wall (de Nobel, 2000). In the last few years attention has been drawn to plant GPI-anchored proteins (Morita, 1996; Oxley and Bacic, 1999; Takos, 2000; Borner, 2002).

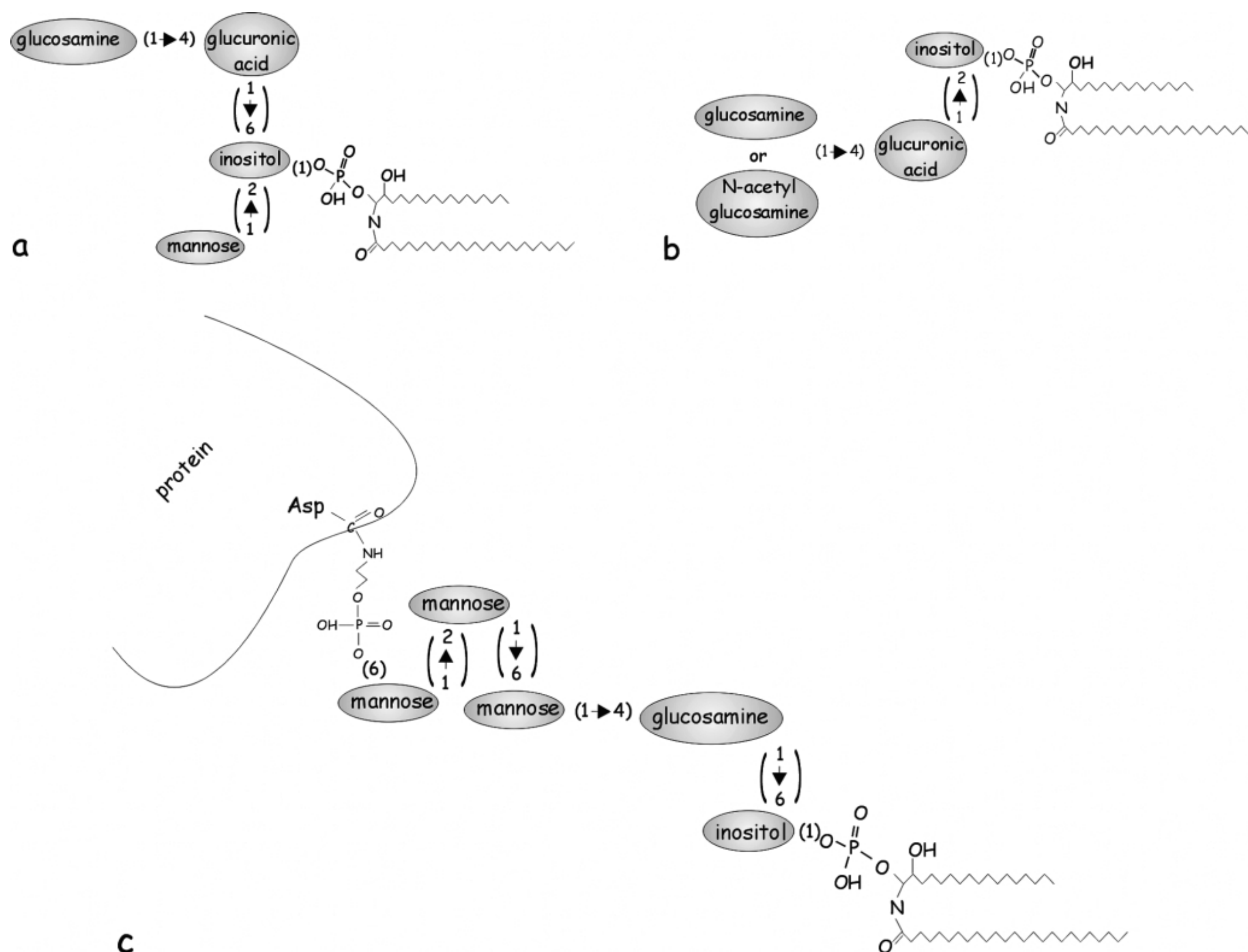

Fig. 2 Structure of the inositolphosphorylceramides: (**a**) identified by Carter and Koob (1969) in soybean and (**b**) identified by Hsieh (1978) in tobacco leaves. (**c**) Glycosylphosphatidylinositol anchor of a protein.

The structure of the GPI-anchored moiety has been characterized in different organisms, including plants. It is based on a conserved oligosaccharide core, the nonreducing end of which is linked via ethanolamine phosphate to the C terminus of a protein (Oxley and Bacic, 1999). An inositol-phospholipid moiety is linked to the reducing end (Fig. **2c**). The lipid can be diacyl-, lysoacyl-, lysoalkyl-glycerol or ceramide. In plants, as in yeast and *Dictyostelium,* the analyzed GPI anchor moieties show ceramide as a lipid component (Morita, 1996; Oxley and Bacic, 1999). More detailed analysis of the plant ceramide component revealed t18 : 0 or t18 : $1^{\Delta 8}$ as the LCB coupled to a VLCFA, tetracosanoic (C_{24}) or docosanoic (C_{22}) acids. As mentioned previously, analysis of InsPCers in plants showed that they are predominantly composed of α-hydroxy VLCFA (Lester and Dickson, 1993). Only a limited number of GPI-anchored moieties have been analysed to date in plants. Further research is required to establish if there is a difference in the hydroxylation of the FA of the InsPCer when it is part of a GPI-anchored moiety.

The first GPI-anchored protein to be identified in plants was alkaline phosphatase from *Spirodela oligorrhiza* (Morita, 1996). GPI anchors have also been found in certain arabinogalactan proteins (Darjania, 2002). Arabinogalactan proteins are a large family of plant proteins implicated in different aspects of growth and development, including cell fate, cell proliferation and expansion. In a recent analysis of the *Arabidopsis* genome database, 210 candidate GPI-anchored proteins have been predicted (Borner, 2002). Together with the above-mentioned arabinogalactan family, 12 other protein families showed the characteristics of potential GPI anchor post-translational modification, among them β-1,3-glucanases, extensins, plasma membrane receptors, lipid transfer proteins, GEPEPs-potential signalling peptides and glycerophosphodiesterases. The putative GPI-anchored proteins identified via database analysis in this genomic study can be broadly categorized as those involved in signalling, adhesion, stress response and cell wall remodelling. These preliminary data require further biochemical studies but indicate that GPI anchor attachment, as a post-translational remodelling, is widespread in *Arabidopsis* and most likely in the whole plant kingdom. This raises the question, is ceramide also widespread as a moiety of GPI anchors,

as in yeast? In yeast, a nonceramide GPI anchor is attached to the protein in the ER and, subsequently in the Golgi, the GPI anchor is remodelled to a ceramide-containing one (Reggiori, 1997; Reggiori and Conzelmann, 1998). Because plant GPI anchors also contain ceramide, it remains to be further investigated if a similar remodelling process takes place in plants. Another similarity between plant and yeast concerning GPI anchor attachment was provided in the study of Takos et al. (2000). The GPI-anchor addition signal of yeast, but not of animals, was recognized in *Nicotiana tabacum*. There are more similarities between yeast and plants in their GPI anchor structure compared to animals. In the future, research parallels with yeast can be helpful to develop new strategies for investigation of plant GPI-anchored proteins.

Lipid Rafts in Plants?

In animal systems, the attachment of a GPI anchor is a post-translational modification that targets proteins to the outer leaflet of the plasma membrane. This protein sorting is associated with detergent-insoluble membrane microdomains, so called lipid rafts (Simons and Ikonen, 1997). In the proposed model, sphingolipids associate laterally with one another, mainly via weak interactions of their carbohydrate head groups. The LCB and FA components of these sphingolipids are predominantly saturated. To complete this packing, the voids between the sphingolipids are filled with cholesterol. These sphingolipid-cholesterol microdomains are located in the outer leaflet of the plasma membrane. In animal systems it is proposed that their formation starts in the Golgi, where sphingolipids are synthesised. Along with GPI-anchored proteins, transmembrane proteins are also associated with lipid rafts (Simons and Ikonen, 1997).

The existence of detergent-insoluble plasma membrane microdomains (rafts) was also demonstrated in tobacco plants (Peskan, 2000). In the same study, it was shown that six GPI-anchored proteins are associated with these domains. Five of them corresponded to previously identified GPI-anchored proteins on the surface of tobacco protoplasts (Takos, 1997).

The lipid composition of the detergent-insoluble plasma membrane domains in plants has not yet been studied. As mentioned above, the thermotropic properties of plant sphingolipids are important for membrane fluidity. Tendency of the plant sphingolipids to form organised domains was studied with the respect to cold tolerance (Yoshida et al., 1988). The same domain promoting properties of plant sphingolipids, however, is likely to be important for raft formation. There are indications that plant GluCers are distributed in the outer leaflet of the plasma membrane (Lynch and Phinney, 1995). Two widespread plant sterols, sitosterol and stigmasterol, have been shown to promote *in vitro* domain formation at even higher levels than cholesterol (Xu et al., 2001). These facts together suggest a similar sphingolipid-sterol domain organisation of the plant plasma membrane and open the door to further intriguing investigations into plant lipid rafts.

Enzymes Involved in Sphingolipid Metabolism in Plants

The first studies on plant sphingolipid metabolism were mainly biochemical. Lynch and co-workers established *in vitro* most of the enzyme activities of the ceramide synthesis pathway (Lynch, 1993 b, 2000). The schematic representation of the proposed pathway is shown in Fig. **3**. Details of the properties of the plant sphingolipid pathway enzymes have been previously reviewed (Lynch, 2000). We will concentrate on the progress made in cloning the genes of the sphingolipid pathway in plants. The cloning of the animal and yeast orthologues and the fact that the *Arabidopsis* genome has been sequenced will, certainly, also lead to progress in the plant field. The first reports of cloned plant genes have already been published.

Serine palmitoyltransferase

The first step of *de novo* sphingolipid biosynthesis is catalyzed by *s*erine *p*almitoyl*t*ransferase (SPT) (Fig. **3**) (Merrill, Jr., 2002). The enzyme catalyzes condensation of L-serine with palmitoyl-CoA. The product of this reaction 3-ketodihydrosphingosine, and the product of the next reductase step, dihydrosphingosine, have an extremely low concentration under normal non-stressful conditions in sphingolipid extracts, e.g. the amount of dihydrosphingosine is 2 – 6 pmol/mg dry weight in different plant sources (Abbas, 1994). The activity of the enzyme is directly affected by the availability of its two substrates. These two facts lead to the conclusion that serine palmitoyltransferase is the rate-limiting enzyme of the pathway and is most likely under complex metabolic regulation, though not yet well understood. In plants, the SPT activity was established *in vitro* in squash fruit microsomes (Lynch and Fairfield, 1993). The SPT activity has been localized to the ER by using differential centrifugation and marker enzymes. The plant enzyme showed selectivity to Palmitoyl-CoA (C_{16}) as a substrate, which corresponds to the C_{18} LCB found in plants.

The genes LCB1 and LCB2 from yeast and mammals have recently been cloned and both were shown to be necessary for SPT enzyme activity (Nagiec, 1994; Hanada, 1997; Weiss and Stoffel, 1997). The interaction between their products was also shown in mammals (Hanada, 2000). The *Arabidopsis* LCB2 homologue has been cloned, expressed in *E coli* and in *lsb2*Δ yeast mutant and its SPT activity shown *in vitro* (Tamura et al., 2000; Tamura et al., 2001). Analyses of mRNA levels in different plant organs revealed overall expression with slightly less LCB2 mRNA detected in seeds. The green fluorescent protein fusion with *Arabidopsis* LCB2 was used to study cellular localization. The results confirmed the ER localization shown previously with enzyme activity assays in squash (Lynch and Fairfield, 1993).

Ceramide synthesis

The other key step of *de novo* ceramide synthesis is the acylation of dihydrosphingosine (d18 : 0) to (dihydro)ceramide (Fig. **3**) (Lynch, 2000; Merrill, Jr., 2002). There are two routes for this synthesis, CoA-dependent and CoA-independent. The latter was shown to be catalyzed by alkaline ceramidases in yeast and mammals and the corresponding genes have been cloned (Mao, 2000 a, b, 2001). Ceramidases are mainly responsible for the breakdown of ceramide (Fig. **3**). Their reverse synthase activity was shown when the CoA-dependent ceramide synthase was inhibited. In the *Arabidopsis* database, one ceramidase homologue has been annotated. The enzyme activity of the proposed plant ceramidase is yet to be established.

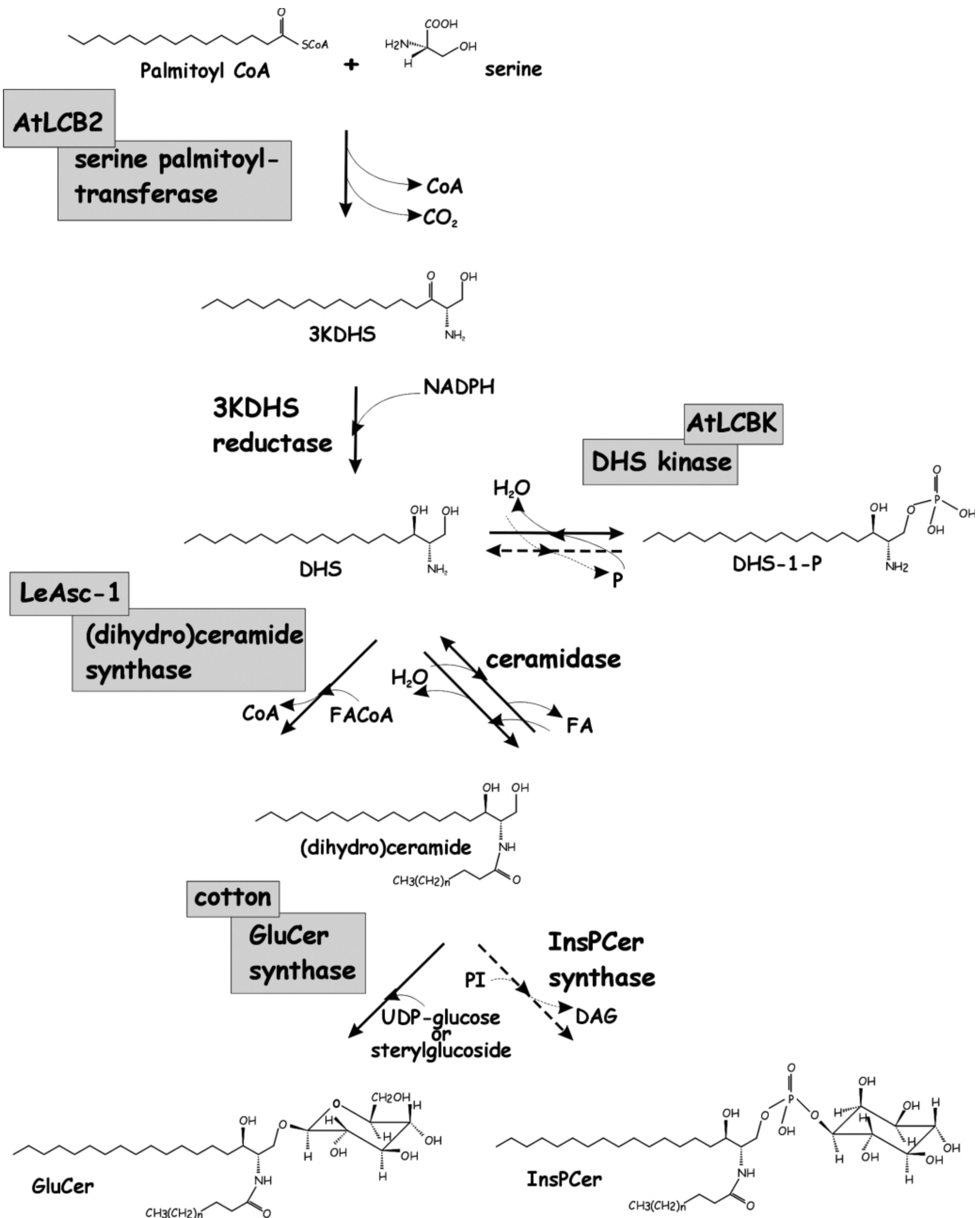

Fig. 3 Plant sphingolipid pathway. The steps from the plant sphingolipid pathway shown in this figure have been demonstrated enzymatically. Enzymes presented in grey boxes are those for which a plant gene has been cloned. FA CoA = fatty acid CoA, 3 KDHS = 3 ketodihydrosphingosine; DHS = dihydrosphingosine; DHS-1-P = dihydrosphingosine 1 phosphate; p = phosphate; PI = phosphatidylinositol; DAG = diacylglycerol; UDP = uridindiphosphate; GluCer = glucosylceramide; InsPCer = inositolphosphorylceramide; n = 9 – 21; AtLCB2 = *Arabidopsis thaliana* LCB2 subunit of serine palmitoyltransferase; AtLCBK = *Arabidopsis thaliana* long-chain base kinase; LeAsc-1 = *Alternaria* stem canker resistance gene from *Lycopersicon esculentum,* a necessary component of (dihydro)ceramide synthase.

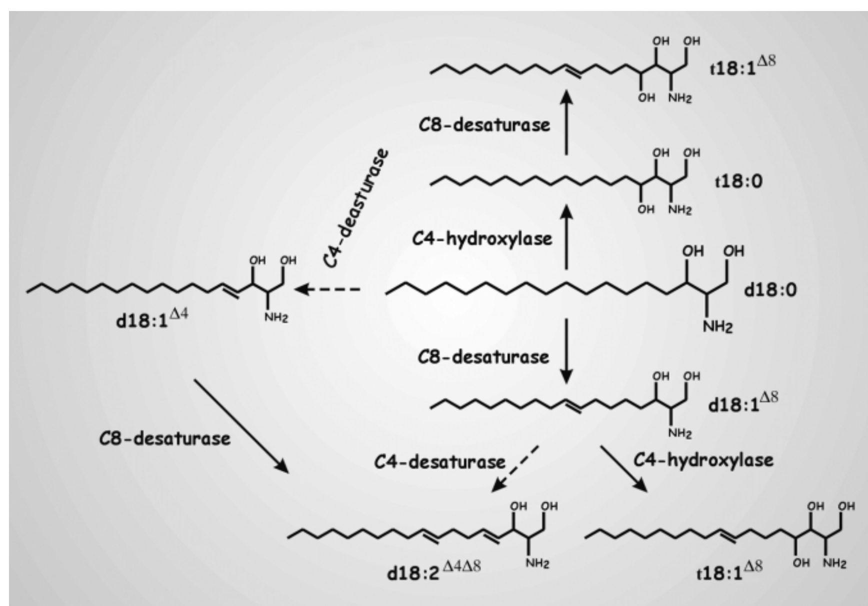

Fig. 4 Possible reactions involved in modification of the LCB component of a plant ceramide. Genes for C_4- and C_8-desaturase and C_4-hydroxylase have been cloned from different plant species. The enzymatic activity of C_8-desaturase and C_4-hydroxylase has been demonstrated via expression of the plant genes in yeast (solid arrows). The enzymatic activity of the plant C_4-desaturase orthologue has not yet been shown (dashed arrows). In plants, the exact order of these reactions is not yet established. Considering existing plant long-chain bases, all possible variations of the reaction order are shown in the figure.

Recently two yeast genes were cloned, *LAG1* and *LAC1*, which were shown to be necessary for CoA-dependent ceramide synthesis (Guillas, 2001; Schorling, 2001). Their function is most likely redundant, because single deletion yeast mutants do not show impaired ceramide synthesis. A tomato homologue of these yeast genes, *Asc-1*, has been cloned recently (Brandwagt, 2000). The tomato *Asc-1* gene can complement growth defects of the *lag1Δ/lac1Δ* yeast double deletion mutant by restoring its *de novo* ceramide synthesis (Spassieva, 2002). Circumstantial evidence from yeast reports recent overexpression studies of the mammalian *UOG1* homologue (Venkataraman, 2002) and our work suggest that, most likely, ceramide synthase is a multi-subunit enzyme and *Asc-1 (LAG1, LAC1, UOG1)* is a necessary component of the (dihydro)ceramide synthase complex. Alternatively, these genes could encode regulatory proteins of (dihydro)ceramide synthase. Cloning of these genes from yeast, plants and mammals is an important step in the direction of elucidating the enzyme responsible for CoA-dependent ceramide synthesis.

(Dihydro)ceramide synthase can utilize different FA CoAs and is most likely a family of isoenzymes (Merrill, Jr., 2002). It has been proposed that incorporation of different FA moieties into ceramides is regulated by (dihydro)ceramide synthase(s) (Venkataraman, 2002). In tomato and *Arabidopsis* there are three *Asc-1* homologous sequences (J. E Markham, University of Groningen, The Netherlands, personal communication). It is possible that they are parts of different enzyme isoforms. In plants, the InsPCers and the GluCers differ in the structure of their ceramide backbone. It remains to be elucidated whether this is a result of the two different pools formed at the ceramide synthase level or is a result of subsequent modifications.

Modification of the LCB component

As described above, plant sphingolipids differ mainly in their ceramide backbone. While the chain length of LCB and FA is most likely determined by SPT and (dihydro)ceramide synthase, the other modifications, such as the degree of unsaturation, additional hydroxylation or methyl branching, require additional corresponding enzymes. In plants, the LCB dihydrosphingosine can be either desaturated at position Δ4 and/or Δ8 or C_4 hydroxylated (Fig. **4**).

LCB C_4 hydroxylase

Recently, two *Arabidopsis* homologues of yeast *SUR2* were identified (Sperling, 2001 b). The yeast Sur2 p is required for hydroxylation at the C_4 position of the sphingoid moiety of a ceramide. The two plant proteins showed C_4 hydroxylase activity in a yeast mutant *(sur2Δ)* lacking C_4 hydroxylation activity, resulting in the formation of C_{18} and C_{20} phytosphingosine. The exact substrate for the plant C_4 hydroxylase (dihydrosphingosine, dihydroceramide or both) was not identified. In yeast, both dihydrosphingosine (d18 : 0) and phytosphingosine (t18 : 0) can serve as substrates for the ceramide synthesis. Deletion of the two genes *LAG1* and *LAC1*, necessary components for ceramide synthase reaction, causes accumulation of d18 : 0 and t18 : 0 (Schorling, 2001). In plants, elevation of both LCBs, d18 : 0 and t18 : 0, was also reported when explants were treated with fumonisin B_1 (Abbas, 1994). This might be an indication that, in plants, as in yeast, C_4-hydroxylation occurs before ceramide synthesis. Alternatively, the observed high levels of phytosphingosine could be a result of ceramide recycling.

The plant Δ8 LCB desaturases

Genes encoding for Δ8 LCB desaturases were identified from *Arabidopsis thaliana* and *Brassica napus* (Sperling, 1998). These genes encode for proteins which are a fusion between cytochrome b_5 and a desaturase. The catalyzed reaction is an electron donor-dependent insertion of a double bond in a lipid chain. The immediate electron donor of many other desaturases is cytochrome b_5. The fusion in the case of the sphingolipid desaturases, as in some other desaturases, may have a functional advantage. The Δ8 LCB desaturases from *Arabidop-*

sis thaliana and *Brassica napus* belong to the cytochrome b_5 superfamily. The studied Δ8 LCB desaturases were assumed to measure Δ6 distance from the oxygen functionalized carbon to the place of the double bond insertion. In plants, only Δ8 unsaturated, and not Δ9 unsaturated, LCBs are found and so it was suggested that C_4-hydroxylation occurs after the introduction of the double bond at the Δ8 position. However, expression of the sunflower Δ8 LCB desaturase orthologue in the yeast *sur2*Δ mutant revealed that, in yeast, the substrates for this enzyme are only phytosphingosines (t18 : 0 and t20 : 0) and not dihydrosphingosines (d18 : 0 and d20 : 0) (Sperling, 2000). It remains to be further established if the Δ8 LCB desaturation precedes or follows the C_4-hydroxylation, or if both pathways coexist in higher plants or if alternatively the plant Δ8 LCB desaturases are not region-specific.

For the first time in the same study it was shown that the enzyme has *cis/trans* Δ8 unselective desaturase activity. It will be interesting to investigate if the enzyme is generally *cis/trans* unselective or if it can change its selectivity towards *cis* isomers upon regulation. As pointed out above, the grapevine leaf plasma membrane cerebrosides change to more $\Delta 8^{cis}$ unsaturation of their LCB as a cold acclimation response (Kawaguchi, 2000).

The Δ4 LCB desaturase

By using a bioinformatics approach a sphingoid Δ4-desaturase family was identified (Ternes, 2002). The gene family includes homologous sequences from animals, plants and fungi. The tomato protein, when expressed in the yeast *sur2*Δ mutant (with only d18 : 0 and d20 : 0 as LCBs), did not show enzymatic activity, while *D. melanogaster, M. musculus* and *H. sapiens* proteins did. One explanation could be that the experimental conditions are not optimal for the plant Δ4 LCB desaturase activity. It is also possible that d18 : 0 and d20 : 0 are not substrates for the tomato Δ4 LCB desaturase.

Hydroxylation at the C_4 position of LCBs in plants and yeast is catalyzed by the Sur2 homologues, the C_4 hydroxylases (Ternes, 2002). Interestingly, the *M. musculus* Δ4-desaturase, together with its desaturase activity, showed C_4 hydroxylase activity as well. These two groups of enzymes belong to the superfamily of membrane-bound desaturases and hydroxylases. All the members of the superfamily show three characteristic histidine boxes. The two above-mentioned groups differ in the spacing between the first and the second histidine box, which likely represents an evolutionary early insertion/deletion event. In addition, the sphingolipid Δ4 and Δ8 desaturases evolved independently. The plant Δ8 desaturases are more similar to the FA Δ5 and Δ6 desaturases and are cytochrome b_5 fusion proteins, while Δ4 desaturases are not and they share only the histidine box sequence motifs (Ternes, 2002).

Dihydrosphingosine kinase

In animals, sphingosine-1-phosphate, a metabolite of ceramide degradation, is an established signalling molecule (Spiegel and Milstien, 2002) and recently has been shown to play a signalling role in plants (Ng and Hetherington, 2001). LCB kinase activity was established in corn shoot microsomes (Crowther and Lynch, 1997). The substrate specificity was characterized *in vitro* and dihydrosphingosine (d18 : 0) and sphingosine ($d18:1^{\Delta 4}$) were shown to be effectively converted to their phosphate derivatives, while phytosphingosine (t18 : 0) was shown not to be a substrate. In the same study, using differential centrifugation, LCB kinase activity was localized to the ER and Golgi. Recently, an *Arabidopsis* LCB kinase has been cloned and expressed in *E. coli* (Nishiura, 2000). The recombinant protein showed LCB kinase activity and utilized *in vitro* ATP and sphingosine as substrates.

Glucosylceramide synthase

GluCers are the most abundant sphingolipids described, so far, in plants. The addition of the glucosyl headgroup is catalyzed by glucosylceramide synthase (Lynch, 2000). The enzymatic activity was found in plasma membrane preparations and two possible glucose donors were described, UDP-glucose and sterylglucoside (Cantatore, 2000). Despite the ubiquity of GluCers in all plants species studied to date, only glucosylceramide synthase from *Gossypium arboreum* (cotton) has been cloned (Leipelt, 2001). The cotton gene belongs to the family of NDP-sugar hexosyltransferases and shows very low sequence similarity to the other glucosylceramide synthases from other organisms. When expressed in a *Pichia pastoris* glucosylceramide-synthase-deficient mutant, the cotton protein showed glucosylceramide synthase activity.

Some aspects of regulation of sphingolipid metabolism in plant

It is important to maintain an appropriate sphingolipid composition of the plasma membrane and tonoplast during membrane biogenesis, therefore, it is possible that *de novo* sphingolipids biosynthesis plays an important role for development of young plant tissue. For example, it has been proposed that changes in the relative growth rate of the tissue are linked to the regulation of SPT activity, the first enzyme of ceramide biosynthesis (Lynch, 1993 a). Another fact supporting this suggestion is from expression studies of Δ8 LCB desaturase from *Borago officinalis* (Sperling, 2001 a). Northern blot analysis revealed that only young leaves showed detectable levels of Δ8 LCB desaturase mRNA. The predominant plant LCB component of the membrane sphingolipids is Δ8 unsaturated. In addition, young tomato leaves are more sensitive to AAL-toxin (an inhibitor of [dihydro]ceramide synthase) (Witsenboer, 1988), which might correspond to the higher sphingolipid requirements of young tomato leaves.

Sphingolipid Signalling in Plants

In yeast and animals sphingolipid metabolism is known as a generator of signals that are involved in different cellular processes (Hannun, 2001; Hannun and Obeid, 2002). Ceramide, sphingosine ($d18:1^{\Delta 4}$) and dihydrosphingosine (d18 : 0) are involved in pro-apoptotic signalling in animal systems, while LCB phosphates are established as regulators of cell proliferation, movement and differentiation. Sphingolipid signals are generated in response to a variety of stress-inducers such as cytokines, chemotherapeutic agents, UV light, ionising radiation or heat. In yeast, dihydrosphingosine (d18 : 0) and phytosphingosine (t18 : 0) are involved in heat stress (Jenkins, 1997).

Free ceramide was identified from different plant sources (alfalfa leaves, rice seeds, wheat and Azuki bean) as a typical sphingolipid (Fujino and Ito, 1971; Ohnishi and Fujino, 1981;

Ohnishi, 1985; Fujino, 1985). In animals, free ceramide is established as an important signalling molecule and, in particular, as an apoptosis trigger (Hannun and Obeid, 2002). Until now, there have been no studies on the signalling role of free ceramide in plants, but this certainly requires future research. Unhydroxylated and α-hydroxylated FA have been identified in cerebrosides, while in ceramides α,β-dihydroxylated FA have been found (Fujino and Ohnishi, 1983). This raises the questions, is this second hydroxyl group important for the function of ceramide in plants, and is there a different enzyme catalyzing the second hydroxylation which cannot recognize cerebrosides as a substrate?

In plants, there is very little known about the role of sphingolipids in cell signalling. Recently, the role of sphingosine-1-phosphate in drought-induced guard cell signalling via calcium mobilisation was shown (Ng, 2001). Sphingosine was virtually absent as a component of the plant sphingolipids (Lynch, 2000) before the study of Ng et al., where they identified its phosphate derivative (Ng, 2001). These authors pointed out that the Δ4 double bond is essential for the signalling role because dihydrosphingosine-1-phosphate was not able to affect stomatal aperture. It will be interesting to check what the effect of other unsaturated LCB-1 phosphates (Δ8 or Δ4/Δ8) will be. The abundance of the Δ8 or Δ4/Δ8 LCB is well established in plant sphingolipids and it is likely that their phosphate derivatives can also have signalling functions.

What Is Known in Plants About Sphingolipids and Cell Death?

There are a number of fungal toxins which can inhibit enzymes of the *de novo* ceramide biosynthesis pathway. Some of them, like Fumonisin B_1 produced by *Fusarium moniliforme* and AAL-toxins produced by *Alternaria alternata* f.sp. *lycopersici*, are structural analogues of dihydrosphingosine (d18:0), so called (dihydro)*s*phingosine *a*nalogue *m*ycotoxins (SAMs) (Brandwagt, 2000). SAMs were shown to inhibit (dihydro)ceramide synthase *in vitro*, in animal and plant microsomal preparations (Merrill, Jr., 1993; Lynch, 1993 b; Gilchrist, 1995). As was previously described, the SAMs can increase the level of dihydrosphingosine (Wang, 1991; Abbas, 1994). The AAL-toxin can alter the profile of *de novo* synthesized sphingolipids depending on the plant genotype (Spassieva, 2002). As described previously, the tomato *Asc-1* gene encodes a necessary component of ceramide synthase. Cells derived from tomato plants with a frame shift in the *Asc-1* gene die in an apoptotic manner when challenged with AAL-toxin (Wang, 1996; Brandwagt, 2000). The profile of *de novo* synthesized sphingolipids of these plants revealed that, after AAL-toxin treatment, the levels of dihydrosphingosine and 3-keto-dihydrosphingosine increased, while the levels of the complex sphingolipids decreased. Simultaneous inhibition of SPT and (dihydro)ceramide synthase with myriocin and AAL-toxin, respectively, substantially reduced the cell death symptoms. These two results suggest that dihydrosphingosine is a possible death signal in the tomato AAL-toxin interaction. However, myriocin was not completely able to rescue the cell death phenotype. An explanation could be that the LCBs derived from the recycling of complex sphingolipids also accumulate and serve as signals. Alternatively, LCBs may not be the only signalling molecules. The results of Tolleson et al. (1999) on induction of apoptosis in human keratinocytes showed that the mechanism could involve accumulation of excess dihydrosphingosine or depletion of ceramide and complex sphingolipids.

The profile of *de novo* synthesized sphingolipids of wild type tomato leaf discs (with intact *Asc-1* gene) treated with AAL-toxin showed alterations, while the leaf discs remained insensitive to the toxin not showing cell death symptoms (Spassieva, 2002). Wild type plants responded to the AAL-toxin treatment with the production of extra, complex sphingolipids. The results from tomato *Asc-1* sphingolipid biosynthesis, with and without AAL-toxin treatment, agree with recent findings of over-expression of *UOG1*, a mammalian homologue of *Asc-1*, in embryonic kidney 293 T cells (Venkataraman, 2002). Over-expression of *UOG1* made the 293 T cells resistant to Fumonisin B_1, similar to the effect of the *Asc-1* on tomato plant resistance to AAL toxin. The 293 T cells also responded to a long-term treatment with Fumonisin B_1 by altering their sphingolipid profile, which channels the ceramide to neutral glycosphingolipids and not to gangliosides. The other observed effect of Fumonisin B_1 on overexpressing *UOG1* 293 T cells was the increased amount of stearic acid (C_{18}) in the ceramides. This agrees with the notion that the (dihydro)ceramide synthesis step determines the FA component of ceramides (Lynch, 1993 a). These latest findings have started to elucidate the functioning of ceramide synthase, but how *Asc-1* "protects" cells from entering the apoptotic pathway while challenged with SAM remains enigmatic.

The downstream signals triggered by Fumonisin B_1 were studied in *Arabidopsis thaliana* plants using knockout mutants disturbed in different cell death signalling pathways (Asai, 2000). The results indicated that SAM-induced cell death pathways involve salicylic acid, jasmonic acid and ethylene signaling, and require light.

Recently, an *Arabidopsis* *a*ccelerated-*c*ell-*d*eath mutant *(acd11)* that encodes for a sphingosine transfer protein was described (Brodersen, 2002). Triggering of the programmed cell death process in the *acd11* mutant requires light and the salicylic acid signalling pathway, but is independent of jasmonic acid and ethylene signalling. *In vitro*, the ACD11 p was able to catalyse intravesicular transfer of sphingosine but not ceramide or glycosphingolipids. Sphingosine in plants is present only in trace amounts (Imai, 1997), so it will be interesting to further investigate the possibility that other LCBs characteristic for plants are substrates for this enzyme.

Concluding Remarks

Plant sphingolipids are involved in different aspects of plant cell metabolism. They are components of the plasma membrane and tonoplast and, as such, play key roles in the characteristics of these biomembranes, e.g. permeability for ions and cryostability. In plants, sphingolipids play a role in protein sorting. They are found in the GPI anchors of proteins and, most likely, they are involved in lipid raft formation. Research on the enzymes of plant sphingolipid metabolism has now received a boost because of the availability of the *Arabidopsis* genome sequence. Plant sphingolipid metabolites have begun to prove themselves as signals involved in programmed cell death and calcium regulation. However, in comparison to yeast and mammalian studies, research on plant ceramide signalling is limited. Are plant sphingolipids still enigmatic? Now, in

plants, they are no longer a mysterious lipid class, but there are many questions yet to be answered.

References

Abbas, H., Tanaka, T., Duke, S., Porter, J., Wray, E., Hodges, L., Sessions, A., Wang, E., Merrill, A. H., and Riley, R. (1994) Fumonisin- and AAL-toxin-induced disruption of sphingolipid metabolism with accumulation of free sphingoid bases. Plant Physiol. 106, 1085 – 1093.

Asai, T., Stone, J. M., Heard, J. E., Kovtun, Y., Yorgey, P., Sheen, J., and Ausubel, F. M. (2000) Fumonisin B1-induced cell death in *Arabidopsis* protoplasts requires jasmonate-, ethylene-, and salicylate-dependent signaling pathways. Plant Cell 12, 1823 – 1836.

Boggs, J. M. (1987) Lipid intermolecular hydrogen bonding: influence on structural organization and membrane function. Biochim. Biophys. Acta 906, 353 – 404.

Bohn, M., Heinz, E., and Luthje, S. (2001) Lipid composition and fluidity of plasma membranes isolated from corn (*Zea mays* L.) roots. Arch. Biochem. Biophys. 387, 35 – 40.

Borner, G. H. H., Sherrier, D. J., Stevens, T. J., Arkin, I. T., and Dupree, P. (2002) Prediction of glycosylphosphatidylinositol-anchored proteins in *Arabidopsis.* A Genomic Analysis. Plant Physiol. 129, 486 – 499.

Brandwagt, B. F., Mesbah, L. A., Takken, F. L., Laurent, P. L., Kneppers, T. J., Hille, J., and Nijkamp, H. J. (2000) A longevity assurance gene homolog of tomato mediates resistance to *Alternaria alternata* f. sp. *lycopersici* toxins and fumonisin B1. Proc. Natl. Acad. Sci. USA 97, 4961 – 4966.

Brodersen, P., Petersen, M., Pike, H. M., Olszak, B., Skov, S., Odum, N., Jorgensen, L. B., Brown, R. E., and Mundy, J. (2002) Knockout of *Arabidopsis* accelerated-cell-death11 encoding a sphingosine transfer protein causes activation of programmed cell death and defense. Genes Dev. 16, 490 – 502.

Cahoon, E. B. and Lynch, D. V. (1991) Analysis of glucocerebrosides of rye (*Secale cereale* L. cv Puma) leaf and plasma membrane. Plant Physiol. 95, 58 – 68.

Cantatore, J. L., Murphy, S. M., and Lynch, D. V. (2000) Compartmentation and topology of glucosylceramide synthesis. Biochem. Soc. Trans. 28, 748 – 750.

Carter, H. E., Hendry, R. A., Nojima, S., Stanacev, N. Z., and Ohno, K. (1961) Biochemistry of the sphingolipids XIII. Determination of the structure of cerebrosides from wheat flour. J. Biol. Chem. 236, 1912 – 1916.

Carter, H. E., Jonson, P., and Weber, E. J. (1965) Glycolipids. Ann. Rev. Biochem. 34, 109 – 142.

Carter, H. E. and Koob, J. L. (1969) Sphingolipids in bean leaves *(Phaseolus vulgaris).* J. Lipid Res. 10, 363 – 369.

Crowther, G. J. and Lynch, D. V. (1997) Characterization of sphinganine kinase activity in corn shoot microsomes. Arch. Biochem. Biophys. 337, 284 – 290.

Darjania, L., Ichise, N., Ichikawa, S., Okamoto, T., Okuyama, H., and Thompson Jr, G. A. (2002) Dynamic turnover of arabinogalactan proteins in cultured *Arabidopsis* cells. Plant Physiol. Biochem. 40, 69 – 79.

de Nobel, H., van Den, E. H., and Klis, F. M. (2000) Cell wall maintenance in fungi. Trends Microbiol. 8, 344 – 345.

Fujino, Y. and Ito, S. (1971) Existance of ceramide in alfalfa leaves. Biochem. Biophys. Acta 231, 242 – 243.

Fujino, Y. and Ohnishi, M. (1983) Sphingolipids in wheat grain. J. Cereal Sci. 1, 159 – 168.

Fujino, Y., Ohnishi, M., and Ito, S. (1985) Further studies on sphingolipids in wheat grain. Lipids 20, 337 – 342.

Gilchrist, D., Wang, H., and Bostock, R. (1995) Sphingosine-related mycotoxins in plant and animal diseases. Can. J. Bot. 73 (Suppl. 1), S459 – S467.

Guillas, I., Kirchman, P. A., Chuard, R., Pfefferli, M., Jiang, J. C., Jazwinski, S. M., and Conzelmann, A. (2001) C26-CoA-dependent ceramide synthesis of *Saccharomyces cerevisiae* is operated by Lag1 p and Lac1 p. EMBO J. 20, 2655 – 2665.

Hakomori, S. (1983) Chemistry of glycosphingolipids. Handbook of lipid research (Hanahan, D. J., ed.), New York: Plemun Press, pp. 1 – 165.

Hanada, K., Hara, T., and Nishijima, M. (2000) Purification of the serine palmitoyltransferase complex responsible for sphingoid base synthesis by using affinity peptide chromatography techniques. J. Biol. Chem. 275, 8409 – 8415.

Hanada, K., Hara, T., Nishijima, M., Kuge, O., Dickson, R. C., and Nagiec, M. M. (1997) A mammalian homolog of the yeast LCB1 encodes a component of serine palmitoyltransferase, the enzyme catalyzing the first step in sphingolipid synthesis. J. Biol. Chem. 272, 32 108 – 32 114.

Hannun, Y. A., Luberto, C., and Argraves, K. M. (2001) Enzymes of sphingolipid metabolism: from modular to integrative signaling. Biochemistry 40, 4893 – 4903.

Hannun, Y. A. and Obeid, L. M. (2002) The Ceramide-centric universe of lipid-mediated cell regulation: Stress encounters of the lipid kind. J. Biol. Chem. 277, 25 847 – 25 850.

Hsieh, T. C., Kaul, K., Laine, R. A., and Lester, R. L. (1978) Structure of a major glycophosphoceramide from tobacco leaves, PSL-I: 2-deoxy-2-acetamido-D-glucopyranosyl(alpha1 leads to 4)-D-glucuronopyranosyl(alpha1 leads to 2)myoinositol-1-O-phosphoceramide. Biochemistry 17, 3575 – 3581.

Hsieh, T. C., Lester, R. L., and Laine, R. A. (1981) Glycophosphoceramides from plants. Purification and characterization of a novel tetrasaccharide derived from tobacco leaf glycolipids. J. Biol. Chem. 256, 7747 – 7755.

Imai, H., Ohnishi, M., Hotsubo, K., Kojima, M., and Ito, S. (1997) Sphingoid base composition of cerebrosides from plant leaves. Biosci. Biotechnol. Biochem. 61, 351 – 353.

Imai, H., Yamamoto, K., Shibahara, A., Miyatani, S., and Nakayama, T. (2000) Determining double-bond positions in monoenoic 2-hydroxy fatty acids of glucosylceramides by gas chromatography-mass spectrometry. Lipids 35, 233 – 236.

Jenkins, G. M., Richards, A., Wahl, T., Mao, C., Obeid, L., and Hannun, Y. (1997) Involvement of yeast sphingolipids in the heat stress response of *Saccharomyces cerevisiae.* J. Biol. Chem. 272, 32 566 – 32 572.

Kaul, K. and Lester, R. L. (1975) Characterization of inositol-containing phosphosphingolipids from tobacco leaves. Plant Physiol. 55, 120 – 129.

Kawaguchi, M., Imai, H., Naoe, M., Yasui, Y., and Ohnishi, M. (2000) Cerebrosides in grapevine leaves: distinct composition of sphingoid bases among the grapevine species having different tolerances to freezing temperature. Biosci. Biotechnol. Biochem. 64, 1271 – 1273.

Leipelt, M., Warnecke, D., Zahringer, U., Ott, C., Muller, F., Hube, B., and Heinz, E. (2001) Glucosylceramide synthases, a gene family responsible for the biosynthesis of glucosphingolipids in animals, plants, and fungi. J. Biol. Chem. 276, 33 621 – 33 629.

Lester, R. L. and Dickson, R. C. (1993) Sphingolipids with inositol-phosphate-containing head groups. Adv. Lipid Res. 26, 253 – 274.

Lynch, D. V. (1993 a) Sphingolipids. In Lipid Metabolism in Plants (Moore, T. S., ed.), Boca Raton: CRC Press, pp. 285 – 308.

Lynch, D. V. (2000) Enzymes of sphingolipid metabolism in plants. In Sphingolipid Metabolism (Merrill, A. H., Jr. and Hannun, Y., eds.), San Diego: Academic Press, pp. 130 – 149.

Lynch, D. V., Caffrey, M., Hogan, J. L., and Steponkus, P. L. (1992) Calorimetric and x-ray diffraction studies of rye glucocerebroside mesomorphism. Biophys. J. 61, 1289 – 1300.

Lynch, D. V., Cahoon, E. B., Fairfield, S. R., and Tannishtha (1990) Glycosphingolipids of plant membranes. In Physical Properties of Membrane Lipids (Quinn, P. J. and Harwood, J. L., eds.), London: Portland Press, pp. 47 – 52.

Lynch, D. V. and Fairfield, S. R. (1993) Sphingolipid long-chain base synthesis in plants. Plant Physiol. 103, 1421 – 1429.

Lynch, D. V. and Phinney, A. J. (1995) The transbilayer distribution of glucosylceramide in plant plasma membrane. Plant Lipid Metabolism (Kader, J. C. and Mazliak, P., eds.), Dordrecht: Kluwer Academic Publishers, pp. 239 – 241.

Lynch, D. V., Spence, R. A., Theiling, K. M., Thomas, K. W., and Lee, M. T. (1993 b) Enzymatic reactions involved in ceramide metabolism. In Biochemistry and Molecular Biology of Membrane and Storage Lipids in Plants (Murata, N. and Somerville, C. R., eds.), Rockville: ASPP, pp. 183 – 190.

Lynch, D. V. and Steponkus, P. L. (1987 a) Plasma membrane lipid alterations associated with cold acclimation of winter rye seedlings (*Secale cereale* L. cv Puma). Plant Physiol. 83, 761 – 767.

Lynch, D. V. and Steponkus, P. L. (1987 b) Thermotropic phase behavior of glucocerebrosides from rye leaves. Cryobiology 24, 555 – 556.

Mao, C., Xu, R., Bielawska, A., and Obeid, L. M. (2000 a) Cloning of an alkaline ceramidase from *Saccharomyces cerevisiae.* An enzyme with reverse (CoA-independent) ceramide synthase activity. J. Biol. Chem. 275, 6876 – 6884.

Mao, C., Xu, R., Bielawska, A., Szulc, Z. M., and Obeid, L. M. (2000 b) Cloning and characterization of a *Saccharomyces cerevisiae* alkaline ceramidase with specificity for dihydroceramide. J. Biol. Chem. 275, 31,369 – 31,378.

Mao, C., Xu, R., Szulc, Z. M., Bielawska, A., Galadari, S. H., and Obeid, L. M. (2001) Cloning and characterization of a novel human alkaline ceramidase. A mammalian enzyme that hydrolyzes phytoceramide. J. Biol. Chem. 276, 26,577 – 26,588.

Merrill, A. H., Jr. (2002) De novo sphingolipid biosynthesis: a necessary, but dangerous pathway. J. Biol. Chem. 277, 25 843 – 25 846.

Merrill, A. H., Jr., Schmelz, E. M., Wang, E., Dillehay, D. L., Rice, L. G., Meredith, F., and Riley, R. T. (1997) Importance of sphingolipids and inhibitors of sphingolipid metabolism as components of animal diets. J. Nutr. 127, 830 – 833.

Merrill, A. H., Jr., van Echten, G., Wang, E., and Sandhoff, K. (1993) Fumonisin B1 inhibits sphingosine (sphinganine) *N*-acyltransferase and de novo sphingolipid biosynthesis in cultured neurons in situ. J. Biol. Chem. 268, 27 299 – 27 306.

Morita, N., Nakazato, H., Okuyama, H., Kim, Y., and Thompson, G. A., Jr. (1996) Evidence for a glycosylinositolphospholipid-anchored alkaline phosphatase in the aquatic plant *Spirodela oligorrhiza.* Biochim. Biophys. Acta 1290, 53 – 62.

Muniz, M. and Riezman, H. (2000) Intracellular transport of GPI-anchored proteins. EMBO J. 19, 10 – 15.

Nagiec, M. M., Baltisberger, J. A., Wells, G. B., Lester, R. L., and Dickson, R. C. (1994) The *LCB2* gene of *Saccharomyces* and the related *LCB1* gene encode subunits of serine palmitoyltransferase, the initial enzyme in sphingolipid synthesis. Proc. Natl. Acad. Sci. USA 91, 7899 – 7902.

Ng, C. K., Carr, K., McAinsh, M. R., Powell, B., and Hetherington, A. M. (2001) Drought-induced guard cell signal transduction involves sphingosine-1-phosphate. Nature 410, 596 – 599.

Ng, C. K. Y. and Hetherington, A. M. (2001) Sphingolipid-mediated signalling in Plants. Ann. Bot. 88, 957 – 965.

Nishiura, H., Tamura, K., Morimoto, Y., and Imai, H. (2000) Characterization of sphingolipid long-chain base kinase in *Arabidopsis thaliana.* Biochem. Soc. Trans. 28, 747 – 748.

Norberg, P., Mansson, J. E., and Liljenberg, C. (1991) Characterization of glucosylceramide from plasma membranes of plant root cells. Biochim. Biophys. Acta 1066, 257 – 260.

Norberg, P., Nilsson, R., Nyiredy, S., and Liljenberg, C. (1996) Glucosylceramides of oat root plasma membranes – physicochemical behaviour in natural and in model systems. Biochim. Biophys. Acta 1299, 80 – 86.

Ohnishi, M. and Fujino, Y. (1981) Chemical composition of ceramide and cerebroside in Azuki bean seeds. Agric. Biol. Chem. 45, 1283 – 1284.

Ohnishi, M. and Fujino, Y. (1982) Sphingolipids in immature and mature soybeans. Lipids 17, 803 – 810.

Ohnishi, M., Imai, H., Kojima, M., Yoshida, S., Murata, N., Fujino, Y., and Ito, S. (1988) Separation of cerebroside species in plants by reversed-phase HPLC and their phase transition temperature. Proc. ISF-JOCS World Congress II, 930 – 935.

Ohnishi, M., Ito, S., and Fujino, Y. (1983) Characterization of sphingolipids in spinach leaves. Biochem. Biophys. Acta 752, 416 – 422.

Ohnishi, M., Ito, S., and Fujino, Y. (1985) Structural characterization of sphingolipids in leafy stems of rice. Agric. Biol. Chem. 49, 3327 – 3329.

Oxley, D. and Bacic, A. (1999) Structure of the glycosylphosphatidylinositol anchor of an arabinogalactan protein from *Pyrus communis* suspension-cultured cells. Proc. Natl. Acad. Sci. USA 96, 14 246 – 14 251.

Peskan, T., Westermann, M., and Oelmuller, R. (2000) Identification of low-density Triton X-100-insoluble plasma membrane microdomains in higher plants. Eur. J. Biochem. 267, 6989 – 6995.

Poincelot, R. P. (1973) Isolation and lipid composition of spinach chloroplast envelop membranes. Arch. Biochem. Biophys. 159, 134 – 142.

Reggiori, F., Canivenc-Gansel, E., and Conzelmann, A. (1997) Lipid remodelling leads to the introduction and exchange of defined ceramides on GPI proteins in the ER and Golgi of *Saccharomyces cerevisiae.* EMBO J. 16, 3506 – 3518.

Reggiori, F. and Conzelmann, A. (1998) Biosynthesis of inositol phosphoceramides and remodelling of glycosylphosphatidylinositol anchors in *Saccharomyces cerevisiae* are mediated by different enzymes. J. Biol. Chem. 273, 30 550 – 30 559.

Rochester, C. P., Kjellbom, P., Andersson, B., and Larsson, C. (1987) Lipid composition of plasma membranes isolated from light-grown barley *(Hordeum vulgare)* leaves: identification of cerebroside as a major component. Arch. Biochem. Biophys. 255, 385 – 391.

Sandstrom, R. P. and Cleland, R. E. (1989) Comparison of the lipid composition of oat root and coleoptile plasma membranes: lack of short-term change in response to auxin. Plant Physiol. 90, 1207 – 1213.

Schmelz, E. M., Roberts, P. C., Kustin, E. M., Lemonnier, L. A., Sullards, M. C., Dillehay, D. L., and Merrill, A. H., Jr. (2001) Modulation of intracellular β-catenin localization and intestinal tumorigenesis *in vivo* and *in vitro* by sphingolipids. Cancer Res. 61, 6723 – 6729.

Schorling, S., Vallee, B., Barz, W. P., Riezman, H., and Oesterhelt, D. (2001) Lag1 p and Lac1 p are essential for the Acyl-CoA-dependent ceramide synthase reaction in *Saccharomyces cerevisae.* Mol. Biol. Cell 12, 3417 – 3427.

Simons, K. and Ikonen, E. (1997) Functional rafts in cell membranes. Nature 387, 569 – 572.

Spassieva, S. D., Markham, J. E., and Hille, J. (2002) The plant disease resistance gene *Asc-1* prevents disruption of sphingolipid metabolism during AAL-toxin induced programmed cell death. Plant J. 32, 561 – 572

Sperling, P., Blume, A., Zahringer, U., and Heinz, E. (2000) Further characterization of Delta(8)-sphingolipid desaturases from higher plants. Biochem. Soc. Trans. 28, 638 – 641.

Sperling, P., Libisch, B., Zahringer, U., Napier, J. A., and Heinz, E. (2001 a) Functional identification of a delta8-sphingolipid desaturase from *Borago officinalis.* Arch. Biochem. Biophys. 388, 293 – 298.

Sperling, P., Ternes, P., Moll, H., Franke, S., Zahringer, U., and Heinz, E. (2001 b) Functional characterization of sphingolipid C4-hydroxylase genes from *Arabidopsis thaliana.* FEBS Lett. 494, 90 – 94.

Sperling, P., Zahringer, U., and Heinz, E. (1998) A sphingolipid desaturase from higher plants. Identification of a new cytochrome b5 fusion protein. J. Biol. Chem. 273, 28 590 – 28 596.

Spiegel, S. and Milstien, S. (2002) Sphingosine 1-phosphate, a key cell signaling molecule. J. Biol. Chem. 277, 25 851 – 25 854.

Steponkus, P. L. and Lynch, D. V. (1989) Freeze/thaw-induced destabilization of the plasma membrane and the effects of cold acclimation. J. Bioenerg. Biomembr. 21, 21 – 41.

Sugawara, T. and Miyazawa, T. (1999) Separation and determination of glycolipids from edible plant sources by high-performance liquid chromatography and evaporative light-scattering detection. Lipids 34, 1231 – 1237.

Sullards, M. C., Lynch, D. V., Merrill, A. H., Jr., and Adams, J. (2000) Structure determination of soybean and wheat glucosylceramides by tandem mass spectrometry. J. Mass Spectrom. 35, 347 – 353.

Takos, A. M., Dry, I. B., and Soole, K. L. (1997) Detection of glycosylphosphatidylinositol-anchored proteins on the surface of Nicotiana tabacum protoplasts. FEBS Lett. 405, 1 – 4.

Takos, A. M., Dry, I. B., and Soole, K. L. (2000) Glycosyl-phosphatidylinositol-anchor addition signals are processed in *Nicotiana tabacum.* Plant J. 21, 43 – 52.

Tamura, K., Mitsuhashi, N., Hara-Nishimura, I., and Imai, H. (2001) Characterization of an *Arabidopsis* cDNA encoding a subunit of serine palmitoyltransferase, the initial enzyme in sphingolipid biosynthesis. Plant Cell Physiol. 42, 1274 – 1281.

Tamura, K., Nishiura, H., Mori, J., and Imai, H. (2000) Cloning and characterization of a cDNA encoding serine palmitoyltransferase in *Arabidopsis thaliana.* Biochem. Soc. Trans. 28, 745 – 747.

Tavernier, E., Le Quoc, D., and Le Quoc, K. (1993) Lipid composition of the vacuolar membrane of *Acer pseudoplatanus* cultured cells. Biochim. Biophys. Acta 1167, 242 – 247.

Ternes, P., Franke, S., Zahringer, U., Sperling, P., and Heinz, E. (2002) Identification and characterization of a sphingolipid delta4-desaturase family. J. Biol. Chem. 227, 25 512 – 25 518.

Thudichum, J. L. W. (1874) Reports of the medical officer of privy council and local government board. N. Ser. III, 113.

Tolleson, W. H., Couch, L. H., Melchior, W. B., Jr., Jenkins, G. R., Muskhelishvili, M., Muskhelishvili, L., McGarrity, L. J., Domon, O., Morris, S. M., and Howard, P. C. (1999) Fumonisin B1 induces apoptosis in cultured human keratinocytes through sphinganine accumulation and ceramide depletion. Int. J. Oncol. 14, 833 – 843.

Uemura, M. and Steponkus, P. L. (1994) A contrast of the plasma membrane lipid composition of oat and rye leaves in relation to freezing tolerance. Plant Physiol. 104, 479 – 496.

Venkataraman, K., Riebeling, C., Bodennec, J., Riezman, H., Allegood, J. C., Sullards, M. C., Merrill, A. H., Jr., and Futerman, A. H. (2002) Upstream of growth and differentiation factor 1 *(uog1),* a mammalian homolog of the yeast longevity assurance gene 1 *(LAG1),* regulates *N*-Stearoyl-sphinganine (C18-(Dihydro)ceramide) synthesis in a Fumonisin B1-independent manner in mammalian cells. J. Biol. Chem. 277, 35 642 – 35 649.

Vesper, H., Schmelz, E. M., Nikolova-Karakashian, M. N., Dillehay, D. L., Lynch, D. V., and Merrill, A. H., Jr. (1999) Sphingolipids in food and the emerging importance of sphingolipids to nutrition. J. Nutr. 129, 1239 – 1250.

Wang, E., Li, J., Bostock, R., and Gilchrist, D. (1996) Apoptosis: a functional paradigm for programmed plant cell death induced by a host-selective phytotoxin and invoked during development. Plant Cell 8, 375 – 391.

Wang, E., Norred, W. P., Bacon, C. W., Riley, R. T., and Merrill, A. H., Jr. (1991) Inhibition of sphingolipid biosynthesis by fumonisins. Implications for diseases associated with *Fusarium moniliforme.* J. Biol. Chem. 266, 14 486 – 14 490.

Weiss, B. and Stoffel, W. (1997) Human and murine serine-palmitoyl-CoA transferase – cloning, expression and characterization of the key enzyme in sphingolipid synthesis. Eur. J. Biochem. 249, 239 – 247.

Witsenboer, H., Schaik, C. E., Bino, R. J., Loffler, H. J. M., Nijkamp, H. J., and Hille, J. (1988) Effects of *Alternaria alternata* f.sp. *lycopersici* toxins at different levels of tomato plant cell development. Plant Science 56, 253 – 260.

Xu, X., Bittman, R., Duportail, G., Heissler, D., Vilcheze, C., and London, E. (2001) Effect of the structure of natural sterols and sphingolipids on the formation of ordered sphingolipid/sterol domains (rafts). Comparison of cholesterol to plant, fungal, and disease-associated sterols and comparison of sphingomyelin, cerebrosides, and ceramide. J. Biol. Chem. 276, 33 540 – 33 546.

Yoshida, S. and Uemura, M. (1986) Lipid composition of plasma membranes and tonoplasts isolated from etiolated seedlings of mung bean (*Vigna radiata* L.). Plant Physiol. 82, 807 – 812.

Yoshida, S., Washio, K., Kenrick, J., and Orr, G. (1988) Thermotropic properties of lipids extracted from plasma membrane and tonoplast isolated from chilling-sensitive mung bean (*Vigna radiata* [L.] Wilczek). Plant Cell Physiol. 29, 1411 – 1416.

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