SPHINGOLIPID VARIETY, BIOSYNTHESIS AND REGULATION

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Chapter

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ABSTRACT

Sphingolipids constitute a ubiquitous class of lipids present in all eukaryotes as well as in several prokaryotes and even some viruses. Their structure is peculiar among other classes of lipids with a backbone made of a sphingoid base bearing an N-linked fatty acid. This structure, known as ceramide, is further modified by means of polar head substitutions. Head substitutions, fatty acids of different lengths and sphingoid bases variants generate a great assortment of sphingolipid isoforms (sphingolipidome), which is highly diversified between organisms, kingdoms and cell types. Sphingolipids are essential for organism development and cell growth since complex sphingolipids are major components of biological membranes and involved in membrane domain organization and receptor signaling. Sphingoid bases are instead involved in cell signaling, cell growth and stress responses. Several key enzymes of the biosynthetic pathway are highly conserved and finely regulated to generate key "structural orthologs" that seem to be shared among organisms. In this chapter, we describe step by step the sphingolipid biosynthetic pathway, and via a continuous parallel between plant, yeast and animal, we will review the sphingolipid functions in different organisms and cells.

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1. INTRODUCTION

The assortment of sphingolipids (sphingolipidome) is highly diversified between organisms, kingdoms and cell types; however, several key enzymes of the biosynthetic pathway are highly conserved and several "structural orthologs" seem to be shared among organisms (Figure 1). The sphingolipid family gathers ceramides and complex sphingolipids derived from ceramide by addition of a variety of head substitutions on the first carbon (C1). For example, in the model plant Arabidopsis, the substitution of glucose or glucosylinositol-phosphate leads to the formation of glucosyl-ceramide (GluCer) and glucosyl-inositol-phosphoryl-ceramide (GIPC), respectively. GluCer and GIPC represent around 40 and 50% respectively of the sphingolipidome of Arabidopsis seedlings (Markham, Li et al., 2006). GluCer is absent in *Saccharomyces cervisiae*, which only produces Inositolphosphoryl-ceramide (IPC) and Mannosylated Inositolphosphoryl ceramide (MIPC) (Lester and Dickson 2001). However, homology search using BLAST revealed that several other fungi species contain GluCer synthase enzymes. In animal cells, GluCer is also synthetized, but rapidly converted in more complex glycosphingolipids.

Sphingomyelin (SM) is another abundant sphingolipid found only in animal cell membranes. SM is characterized by the presence of phosphocholine as head group, instead of a carbohydrate molecule like in other sphingolipids. SM is greatly enriched in the multilayered myelin sheath surrounding the axons of neurons, where it participates in the propagation of the electrical impulses. It has been proposed that the intertwining hydrocarbon chains of SM strengthen myelin sheath packing.

Besides different head substitution, variability is also observed in FA chain length and LCB and/or FA saturation and hydroxylation, leading to a multiplicity of sphingolipid isoforms. In particular, hydroxylation of FA and LCB moieties is a hallmark of sphingolipids. Sphingolipids are the lipid with the highest level of hydroxylation in plants. Sphingolipidome, with more than 160 different molecules, appears to be more diversified in plants compared to animals and yeast (Markham and Jaworski 2007).

2. SPHINGOLIPID BIOSYNTHESIS

2.1. The Biosynthesis of the Fatty Acyl Chain

Sphingolipids, as many other lipids, contain a fatty acyl chain. Fatty acids (FA) of 16 or 18 carbon atoms (C16, C18) are synthetized by the fatty acid synthase complex and released for further use in the form of Acyl-CoAs. By definition, fatty acids of C16 or C18 are named LCFA for long chain fatty acid. The fatty acid synthase complex is localized in plant chloroplasts or animal mitochondria. LCFA-CoA can be further elongated to VLCFA-CoA (very long chain fatty acids) in the ER by the elongase complex. The elongase complex is composed of 4 enzymes that act in a sequential mode to add 2 carbons to the LCFA-CoA. Multiple cycles lead to VLCFA-CoA from 20C up to 26C (Oh, Toke et al., 1997; Gaigg, Neergaard et al., 2001) (Han, Gable et al., 2002). In Arabidopsis these enzymes are: 3-keto acyl-CoA synthase (KCS) (Joubes, Raffaele et al., 2008), 3-keto acyl-CoA reductase (KCR) (Beaudoin, Wu et al., 2009), 3-hydroxy acyl-CoA dehydratase (HCD/PASTICCINO2) (Bach,

Michaelson et al., 2008), and the enoyl-CoA reductase (ECR/CER10) (Zheng, Rowland et al., 2005). VLCFA-CoA can then be incorporated in membrane lipids (sphingolipids, phospholipids); in storage lipids (triacylglycerides) or extracellular protective lipid (waxes and suberin).

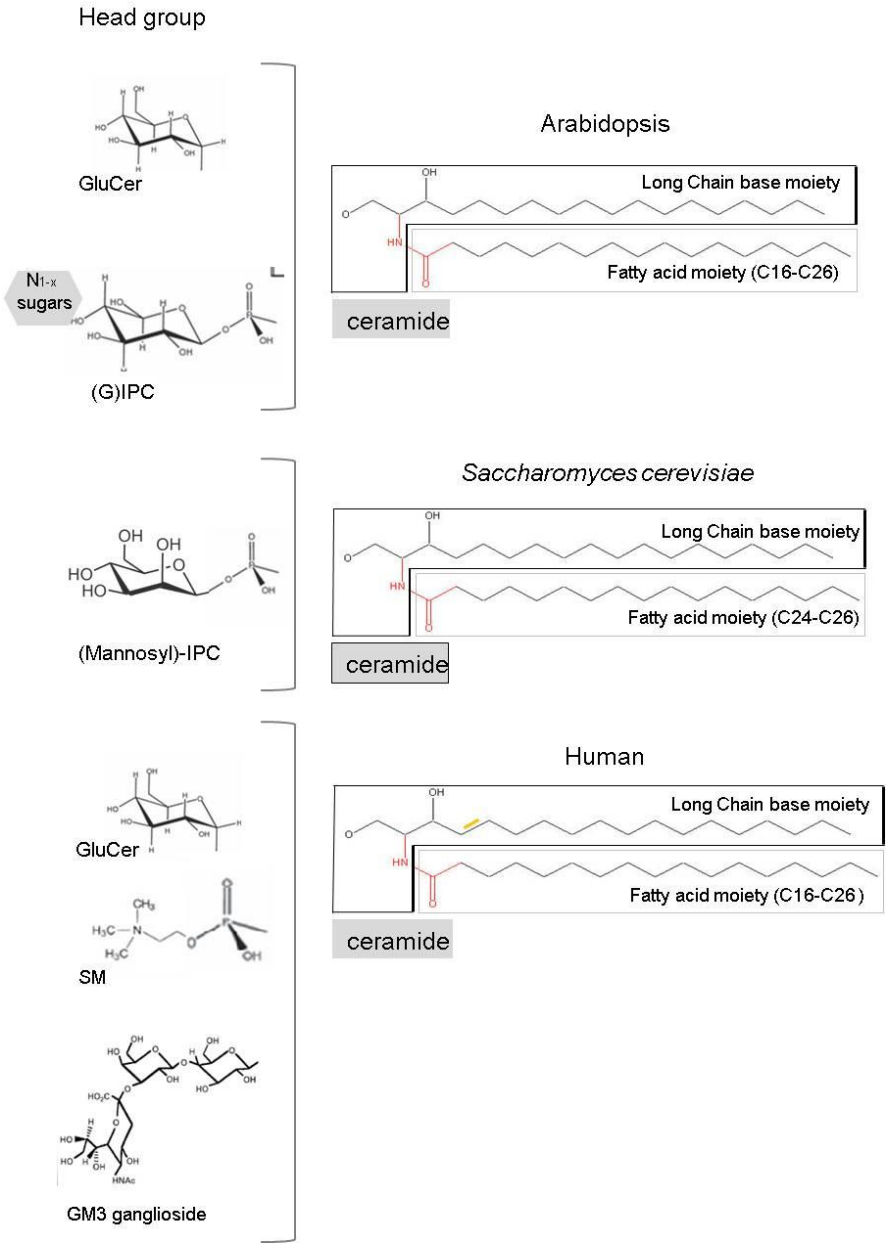

Figure 1. Sphingolipid structure and diversity. A common ceramide backbone is shared among the different organisms. Ceramide has two moieties: a Long Chain Base (LCB) and an N-linked fatty acid. Polar head groups (left) and fatty acyl chain length (right) are indicated for Arabidopsis, Saccharomyces cerevisiae and humans. Ceramide structure is from Pata et al., 2010.

The length of the FA chain is a critical feature of sphingolipids. In both animals and plants, it can be long (LCFA: C16-18), or very long (VLCFA: C20-26), while in yeast, sphingolipids only contain VLCFA (C24-26). Sphingolipid biosynthesis is therefore tightly associated with FA elongation in yeast, while in plants and animals, two branched pathways exist, one linked to FA elongation, and the other not. In both animals and plants, LCFA-C18 and VLCFA-C24 are both significantly abundant and they may fulfill different functions. Phospholipids may also contain VLCFA but the great majority of them contain two symmetric C16/C18 chains. Finally, most of the FAs associated with sphingolipids are hydroxylated and saturated or mono-unsaturated.

In summary sphingolipids are characterized by very long, saturated or monounsaturated and hydroxylated acyl chains.

2.2. Sphingoid Bases Variants: Hydroxylation Is Essential in Plants while Saturation Predominates in Animals

The first step of the biosynthesis of the sphingoid backbone is the condensation of a serine with palmitoyl-CoA, via a serine palmitoyl transferase (SPT) (Table 1, Figure 2). The product is a long chain base called 3-ketosphinganin, which is immediately reduced by the ketosphinganin reductase (KSR) to sphinganine (Table 1). Genes encoding SPT are present in a large number of species from bacteria to humans and highly conserved among eukaryotes (Sperling and Heinz 2003; Lynch and Dunn 2004). The importance of sphingolipids in cell biology is highlighted by the deleterious consequence of the complete knockout of *SPT*. Mutations in SPT subunits have been involved in hereditary neuropathy in humans (Dawkins, Hulme et al., 2001). The complete knockout of *SPT* resulted in lethality in mice, yeast and plants (Hojjati, Li et al., 2005; Chen, Han et al., 2006) revealing that LCBs are essential molecules for cell viability. SPT functions as a heterodimer, and a 20% reduction of its activity by the inactivation of one subunit is sufficient for impairing cell expansion in Arabidopsis (Chen, Han et al., 2006). A complete list of all enzymes involved in the biosynthesis and modifications of sphingolipids in Arabidopsis and their subcellular localization is listed in Table 1. The LCB moiety always contains 18 carbon atoms chain that can be hydroxylated and desaturated. Such chemical modifications add structural variability to sphingolipids. A total extract of LCBs from Arabidopsis reveals a majority of di- and tri-hydroxylated LCBs (d18 or t18). Di-hydroxylation is associated with the presence of hydroxyl groups on C1 and C3, corresponding to the serine and palmitoyl-CoA precursors, respectively, while the third hydroxylation occurs later in the pathway on C4 by a C4-hydroxylase (Figure 2). In Arabidopsis, two redundant genes, *SBH1* and *SBH2* (for sphingoid base hydroxylase 1 and 2, Table 1), encode a C4-hydroxylase. Double mutants as well as RNAi suppression lines revealed the physiological and metabolic relevance of this hydroxyl group (Chen, Markham et al., 2008). The complete loss of t18 (tri-hydroxyl LCB) resulted in both cell division and cell expansion defects, stressing the importance of LCB hydroxylation in sphingolipid cellular function. Moreover, the total content of sphingolipids in *sbh* mutants was globally modified with higher levels of d18 (di-hydroxyl LCB), as predicted. Unexpectedly, these mutants also showed higher levels of sphingolipids with shorter fatty acyl chains. These observations suggest that the 3-hydroxyl configuration (t18) is preferentially combined with VLCFA, while d18 is mainly associated with LCFA. Chen at al.

proposed that the presence of tri-hydroxyl groups would be an important structural determinant for channelling VLCFA downstream in complex sphingolipids (Chen, Markham et al., 2008). Indeed, in a total extract of *Arabidopsis* seedlings, most of the GluCer containing d18 is associated with C16 FA to form d18/C16-GluCer. It is not true for GIPC which overall contains mostly t18 LCB even in C16-GIPC. The d18 LCB are thus channeled in C16 GluCer while t18 are mostly associated with VLCFA- GluCer and GIPC. The fact that in *sbh* mutants d18/C16 GluCer and GIPC are increased 10-fold, support the role of C4-hydroxylation in channeling t18 species into VLCFA-complex sphingolipids.

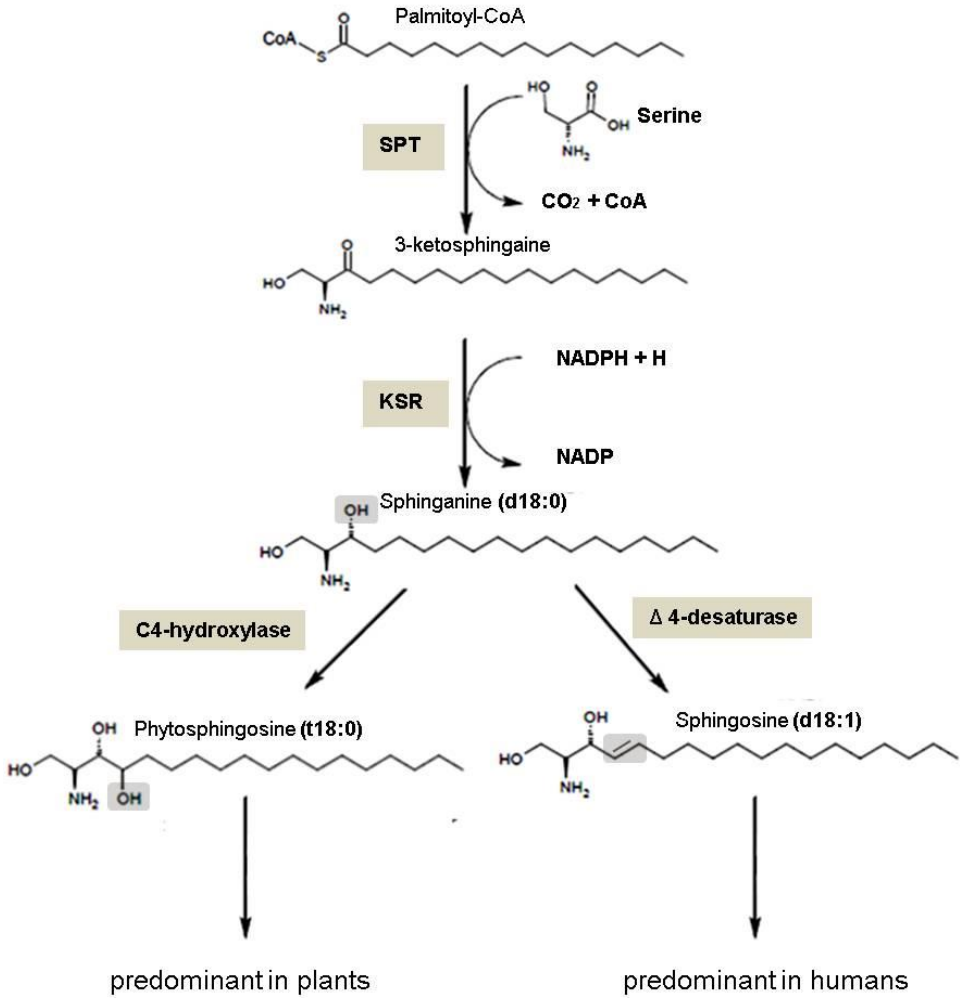

Figure 2. Sphingoid base biosynthesis. SPT for serine palmitoyl transferase. KCR for keto sphinganine reductase. d18:0, t18:0, d18:1 nomenclature indicates the number of -OH (di or tri) and the number of double bound. Predominant LCBs species in animals and plants are indicated.

LCBs can also be unsaturated with 1 or 2 double bonds on sphinganine to produce d18:1, d18:2 or on phytosphingosine to produce t18:1, or t18:2. Desaturation can occur at C4 in *trans* configuration or on C8 in both *cis* and *trans* configurations. The enzymes responsible for these desaturations are the Δ4- or Δ8-desaturases. Combination of these different LCB

modifications (hydroxylation and desaturation) with their respective stereoisomers results in a subset of 9 different molecules (Figure 3). The Δ4-desaturase (DES4) was recently reported to be expressed in specific tissues, mainly pollen and at low level in flowers (Michaelson, Zauner et al., 2009). The complete knockout of the DES4 does not lead to any developmental defect. However the *des4* sphingolipidome of flower tissue revealed low levels of C16-GluCer, while VLCFA-GluCer and VLCFA-GIPC levels were unaffected, indicating that Δ4-desaturated LCBs are preferentially combined with C16 rather than with longer fatty acyl chains. Interestingly, mutation of the *Drosophila* LCB desaturase homolog DES1 caused defective spermatogenesis in flies confirming the importance of unsaturated LCB in development (Endo, Akiyama et al., 1996). It has to be underlined that the C4-hydroxylation and Δ4-desaturation occur on the same site, suggesting that the respective enzymes may act in a competitive manner for their substrate. Interestingly, while Δ4-desaturation does not seem to be essential in Arabidopsis, it is prevalent in animal sphingolipids, leading to the formation of sphingosine (SPH), an important signal molecule (see below) (Figure 2). On the other hand, Δ8-desaturation, either in *cis* or *trans* configuration, is very common in plant sphingolipids (Sperling, Franke et al., 2005). The proportion of LCB and their *cis/trans* isomers is highly variable between plant species. For example, 20% of the total GluCer in Arabidopsis leaves is t18:1$\Delta 8^{E}$, while the t18:1$\Delta 8^{Z}$ is only 3.4% of GluCer. In maize leaves, the levels of t:18:1 GluCer is low compared to Arabidopsis, furthermore the relative distribution of the *E/cis* and *Z/trans* are inverted with respectively 0.2% and 1% of total GluCer, reviewed by (Pata, Hannun et al., 2010). To date, the exact order in which C-4 hydroxylation, Δ4 and Δ8 desaturations occur is not yet fully defined, and the substrate specificities of each step remains poorly known. A possible sequence of the enzymatic activities along the pathway, as proposed by Pata et al., 2009 is shown in Figure 3.

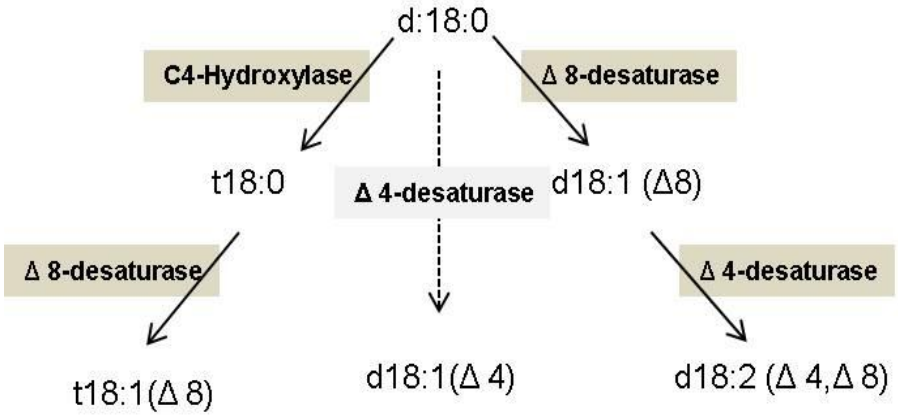

Figure 3. LCBs modifications. Possible sequential order of LCB modifications. The pathway does not take into account the different possible isomers.

2.3. Ceramide Synthesis and Inhibition, an Essential Component of Life Span

After LCB synthesis, the next step in sphingolipid biosynthetis involves the condensation of a LCB with a fatty acyl chain to form ceramide (Cer). The enzyme responsible for this

reaction is the ceramide synthase (CerS). The CerS gene was originally isolated during a screen designed to identify genes that were differentially expressed cells that had undergone low or high number of divisions and that could thus be associated with longevity (Egilmez, Chen et al., 1989). Like most cells, yeast cells are subjected to aging, which is defined as the total number of budding events (cell divisions). A gene, whose expression was high in early generations, decreased linearly during aging until being undetectable at final age (around 15 divisions), was characterized and named LAG1, for longevity assurance gene (D'Mello N., Childress et al., 1994). *lag1* mutant displayed no defect in size, shape, budding pattern or rate of division; however, it exhibited increased life span corresponding to an average of 25 divisions (D'Mello N., Childress et al., 1994). Considering the negative correlation between LAG1 expression level and the increased life span, the author proposed that Lag1p could be directly or indirectly responsible for the average of 20-cell cycle limit in yeast life span. Another gene displaying a significant homology with LAG1 was found in yeast and named LAC1. The double knockout *lag1/lac1* was either unviable or had severe growth defects depending on the strain background (Barz and Walter 1999). Both proteins were localized in the ER and the viable double mutant combination displayed inability to transport GPI-anchored proteins from the ER to the Golgi, a defect associated with sphingolipid levels (Barz and Walter 1999). Indeed, LAG1 and LAC1 disruption resulted in reduced sphingolipid biosynthesis, because both genes were found to code for Acyl-CoA dependent ceramide synthases (Guillas, Kirchman et al., 2001; Schorling, Vallee et al., 2001).

Mammals, like humans or mice, have six different LAG1 homologs named LASS1-6 (for longevity assurance) that code for six different ceramide synthases (CerS1-6). The different enzymes display different substrate specificity, related in particular to the fatty acyl chain (Pewzner-Jung, Ben-Dor et al., 2006; Mizutani, Mitsutake et al., 2009) (Table 2). CerS3 is the only ceramide synthase utilizing a broad range of FA substrates, mainly C18 and C24, but also medium chains (C14, C16). Surprisingly, mammalian CerS, with the exception of CerS1, present an extra domain. This domain is a bona fide homeodomain except that it defines a very divergent subclass. (Venkataraman and Futerman 2002; Riebeling, Allegood et al., 2003; Holland, Booth et al., 2007). Although bona fide domain structure prediction studies suggest that they form three alpha helices in the correct conformation to bind DNA, no evidence for a DNA binding or transcription factor activity has been demonstrated yet (Pewzner-Jung, Ben-Dor et al., 2006). However, ceramide synthases are ER transmembrane proteins and predicted to be inside the ER lumen. It is therefore unlikely that they could be in contact with DNA. Yeast and plants LAGs protein do lack this homeodomain.

The high number of CerSs in humans likely reflects tissue specific functions. CerS have been mainly characterized in cell cultures and a knockout model is only available for CerS2 (Table 2) (Pewzner-Jung, Park et al., 2010). The cerS2$^{-/-}$ mice could survive almost 2 years (like the wild type) but exhibited important liver dysfunction. Specifically, hepatocytes cerS2$^{-/-}$ presented a shortened life span, with defective cellular regeneration that caused the development of liver carcinoma (Pewzner-Jung, Park et al., 2010). CerS3 (Table 2) was found to be highly expressed in the skin (Riebeling, Allegood et al., 2003), which was correlated to the involvement of VLCFA-ceramides in the impermeable barrier function of the skin (Coderch, Lopez et al., 2003). LCFA-ceramide, namely C16-ceramide (Table 2), is the most abundant sphingolipid species in non-neuronal tissues, and its accumulation has been associated, in several cases, with the induction of apoptosis and cell stress (Eto, Bennouna et al., 2003; Osawa, Uchinami et al., 2005; Senkal, Ponnusamy et al., 2011). Tumor cells such

as head and neck squamous carcinoma, melanoma and Lewis lung carcinoma, also contained high levels of C16-Cer (Koybasi, Senkal et al., 2004) and the accumulation of such short species were associated with drug resistance and cell death (Ponnusamy, Meyers-Needham et al., 2010). However, length of fatty acyl chain of sphingolipids could have opposite effects on carcinoma depending wether it occurred in the lung and colon carcinoma (Hartmann, Lucks et al., 2012).

Our knowledge of the role of ceramide synthase activity was not only inferred from genetic studies, but also from the use of specific toxin-derived inhibitors. The fungus *Fusarium moniliforme* is a common contaminant of maize (*Zea Mays*) intended for human and animal consumption (Marasas, Kriek et al., 1984). The presence of *F. moniliforme* in maize has been correlated with high incidence of human oesophageal cancer in southern Africa and outbreaks of leukoencephalomalacia in horses (Pienaar, Kellerman et al., 1981; Rabie, Marasas et al., 1982; Gelderblom, Jaskiewicz et al., 1988). The toxic and carcinogenic contaminants were isolated as Fumonisins (made by fusarium moniliforme) by (Gelderblom, Jaskiewicz et al., 1988). Fumonisin B1 (FB1) was found to be the most abundant and active fumonisin, inducing liver toxicity and cancer after 1 month of treatment in rats (Gelderblom, Jaskiewicz et al., 1988). The hepatotoxicity was correlated with both apoptosis and necrosis. Additionally, FB1 caused the appearance of foci of cell proliferation and carcinogenesis (Gelderblom, Kriek et al., 1991; Gelderblom, Lebepe-Mazur et al., 2001). *In vitro* studies in rat hepatocytes revealed that FB1 targets sphingolipid biosynthesis by increasing LCB levels and reducing the ceramides and complex sphingolipids levels (Yoo, Norred et al., 1992; Merrill, van Echten et al., 1993). It was also shown that FB1 could directly inhibit the enzymatic activity of ceramide synthase *in vitro*, and the potency of the inhibition was correlated with the accumulation of free LCBs and acyl-chain substrates (Merrill, Wang et al., 1993). Elevated level of sphingosine in urine was therefore used as a diagnostic indicator of FB1 consumption and the toxicity associated with altered sphingolipid metabolism.

In Arabidopsis, 3 LAG1 homologs are present in the genome and were named LOH1-3, for LAG One Homolog). Ceramides represent only 1-2% of total sphingolipids extract, as they are rapidly converted into more complex forms. Two major classes of ceramides exist in the Arabidopsis sphingolipidome: ceramide and hydroxyl-ceramides (hCer), the latter containing α-hydroxylated FAs (Lynch and Dunn 2004; Markham and Jaworski 2007). α-hydroxylation is however mostly found in complex sphingolipids downstream of ceramide (see below), suggesting that either only α-hydroxy-Cer pool is used for downstream biosynthesis of complex sphingolipids or that α-hydroxylation occurs on a fraction of ceramide pool and independently on the whole pool of complex sphingolipids. The biological role of α-hydroxylation is unknown, but it is tempting to speculate that it plays a structural role in mediating interactions between lipids or between lipids and proteins within the membrane layer. Arabidopsis mutants depleted in very long acyl chain sphingolipids were non-viable contrary mutants lacking long acyl chain sphingolipids demonstrating the importance of the length of acyl chain (Markham, Molino et al., 2011). VLCFA-sphingolipids were in particular involved in sorting specific material from the TGN towards the PM *in vivo* (Markham, Molino et al., 2011). VLCFA-sphingolipid depletion modified the dynamic of specific endomembrane compartments in particular for vesicle fusion during exocytosis and cytokinesis (Molino, Van der Giessen et al., 2014).

2.4. Complex Sphingolipids in Plants

Downstream from ceramide synthase, several enzymes transfer a head group to the C1 of the ceramide backbone (Figure 1). Yeast, mammals and plants share a common head substitution but show also some specificity. In yeast, the IPC-synthase transfers a phosphorylinositol group from a phosphatidylinositol (PI) to ceramide to form Inositolphosphoryl-ceramide (IPC). In mammals, sphingomyelin synthases (SMS) transfer a phosphocholine from phosphatidylcholine to ceramide to produce sphingomuelin (SM), the most abundant mammalian sphingolipid. Apart from SM, mammals synthesize a neutral complex sphingolipid, glucosylceramide (GluCer) by transferring glucose to ceramide via glucosyceramide synthase (GCS). In plants, two major complex sphingolipids are found: a glucosylated ceramide (GluCer) and a glucosylated inositolphosphoryl-ceramide (GIPC). In Arabidopsis, GluCer-synthase (GCS) is encoded by a unique essential gene, and was recently shown to be important for Golgi morphology and protein secretion (Melser, Batailler et al., 2010).

Furthermore, there are three functional IPC-synthases (Mina, Okada et al., 2010). The first IPC-synthase identified was the gene product of ERH1 (enhancing RPW8-mediated HR-like cell death) (Wang, Yang et al., 2008). ERH1 was identified in a screening for mutations that enhanced hypersensitive response during pathogen infection (see below). The mutant *erh1* did not display an obvious reduction in GIPC, probably because of redundancy with the two other IPC synthases, however it accumulated ceramides which are considered as the main cause of the increased cell death responses in the mutant.

GIPC and GluCer can be distinguished by their head groups as well as by their fatty acid and LCB contents. A complete sphingolipid profile of Arabidosis provided a detailed picture of the diversity of sphingolipids among the four classes of molecules; Cer, α-hydroxy-Cer (containing α-hydroxylated fatty acid), GluCer and GIPC (Markham, Li et al., 2006). Four major observations can be made from these profiles. Firstly, t18 is the predominant form in both GluCer and GIPC, representing 90% of the total LCBs species. Secondly, t18 preferentially associates with VLCFA-Sphingolipids and d18 with LCFA-Sphingolipids. Thirdly, Cer can be α-hydroxylated or not (Cer versus hydroxy-Cer), while complex GluCer and GIPC only contain α-hydroxy FA. Finally, we have two major forms of GluCer: d18-LCFA(C16), t: 18-VLCFA(C24), and only one major form of GIPC: t:18 VLCFA(C24).

Sphingolipid distribution could also differ between different tissues suggesting specialized metabolic pathways. In plants, sphingolipids showed qualitative and quantitative variations according to the different tissues (Islam, Jacquemot et al., 2012; Tellier, Maia-Grondard et al., 2014). GIPCs and GluCer showed for instance quantitative variations between seedling and seed extracts however seeds but not oil showed specific accumulation of a new amine derived GIPC (Tellier, Maia-Grondard et al., 2014). The presence of specific sphingolipid species in specific tissues raises questions about their roles that need to be addressed.

3. LOCALISATION OF SPHINGOLIPID BIOSYNTHETIC ENZYMES AND SPHINGOLIPID TRANSPORT

Yeast IPCS, the human sphingomyelin synthase 1 (SMS1) and human GCS are all found in the Golgi (Levine, Wiggins et al., 2000; Huitema, van den Dikkenberg et al., 2004; Tafesse, Huitema et al., 2007), while the human SMS2 is at the plasma membrane (PM) (Yamaji and Hanada 2015). Interestingly in Arabidopsis, LOHs and GCS localize in the ER (Marion, Bach et al., 2008), IPCS-ERH1 has been instead localized at the trans Golgi network (TGN) (Wang, Yang et al., 2008). These different localizations indicate that the compartmentalization of the sphingolipid pathway and the channeling of ceramide into GluCer or IPC would depend on ceramide transport. Direct (non- vesicular) translocation of ceramide to the Golgi was proposed to occur thanks to ceramide transfer protein CERT (Cer transfer) (Hanada, Kumagai et al., 2003). CERT contains a ceramide binding domain (START) and a plekstrin (PH) domain, needed for binding to PtdIP (phosphatidylinositol-phospate) at the Golgi. It's integrity is essential for the synthesis of SM. Localization of a fluorescent Cer to the Golgi was also impaired in Hela cells expressing mutated CERT. CERT could also extract Cer from ER membranes *in vitro* demonstrating its role as ceramide carrier.

In Arabidopsis, a ceramide transfer protein has not been identified yet. However, a putative sphingosine-transfer protein has been isolated in a screening for mutants with accelerated cell death symptoms and named *acd11* (for accelerated cell death) (Brodersen, Petersen et al., 2002). ACD11 is part of the GLTP protein family (glycolipid transfer protein) and at least two other homologs are present in the Arabidopsis genome, but the precise biochemical activity of these proteins awaits further work (Table 1).

4. SPHINGOLIPIDS ARE MAJOR MEMBRANE LIPIDS

Lipids are a vast class of molecules, which cover different cellular functions such as energy storage, protective barriers, membranes biogenesis and dynamics, and intracellular signaling.

The majority of lipids that build up a biological membrane are amphiphilic, with a hydrophilic head and a lipophilic tail. The polar head interacts with water while the hydrophobic tails tend to self-associate to avoid water, leading to the spontaneous formation of a bilayer which maximizes these hydrophilic/hydrophobic interactions. The propensities of lipids to self-assemble in simple or more complex structures could be one of the major mean of determining the different shapes found within the cells. Lipids can be organized as a flat surface for plasma membrane, spherical and the tubular structures in the vesicular system, and more intricate geometry in ER (endoplasmic reticulum) and Golgi (Melser et al., 2010). Sphingolipids play major roles in biological membrane and participate in membrane organization. Specific and diverse PM structures such as plasmodesmata of plant tissues or immunological synapsis of animal cells from the immunity system have been shown to need sphingolipids for both structural and functional meaning (Gombos, Kiss et al., 2006; Grison, Brocard et al., 2015). Sphingolipids play essential roles in PM receptor clustering and

signaling and malfunctioning of these processes have been associated with pathology. (Lauwers and Andre 2006; Lingwood and Simons 2010; Molino and Galli 2014).

The best way to look at the intracellular organization is to consider the possible routes followed by a plasma membrane (PM)-targeted protein after its synthesis in the ER. In a simplified view, after synthesis in the ER membranes a PM-protein usually reaches the Golgi apparatus. The Golgi is composed of a *cis* part which is in continuity with ER membranes, a medial, and a *trans* part which constitutively delivers proteins to all post-Golgi compartments, including the plasma membrane, the different endosome populations, and the prevacuolar and vacuolar compartments. Such a step by step transport defines the eukaryotic secretory pathway (Figure 4).

Table 1. Enzymes and genes from *Arabidopsis thaliana* involved in sphingolipid biosynthesis

ENZYME	Name	Gene	Localization	References
serine palmitoyl transferase (heterodimeric)	LCB1	At4g36480	ER	Chen et al. 2006
	LCB2a	At5g23670	ER	Tamura et al. 2001
	LCB2b	At3g48780		Teng et al. 2008
ceramide synthase	LOH1	At3g25540	ER	Markham, Molino et al. 2010
	LOH2	At3g19260	ER	Markham, Molino et al. 2010
	LOH3	At1g13580	ER	Markham, Molino et al. 2010
inositol phosphoryl ceamide synthase	IPCS	At2g37940	Golgi	Wang et al. 2008
		At2g29525 At3g54020	? mitocondria	
glucosyl inositol phophoryl ceramide synthase?	GIPCS?	unknown	unknown	Mina et al. 2010
glucosyl ceramide synthase	GCS	At2g19880	ER	Melser et al. 2009
ceramidase	CDase	At4g22330	ER/Golgi	Pata et al. 2008
glucosyl ceramidase	GlcCDase	unknown	unknown	---
glycolipid transfer protein	GLTP1	At2g33470	ER/Golgi	West et al. 2008
glycolipid binding-transporter	?	at4g39670	unknown	--
sphingosine transfer protein	ACD11	At2g34690	Cytosol	Brodersen et al. 2002

Table 1. (Continued)

ENZYMES	Name	Gene	Location	References
ketosphinganine reductase	KSR	At3g06060	ER	Chao et al. 2011
		At5g19200		Chao et al. 2011
LCB C4-hydroxylase	SBH1	At1g69640	ER/Golgi	Sperling et al. 2001
	SBH2	At1g14290		Chen et al. 2008
LCB Δ8-desaturase	SLD1, DES8	At3g61580	ER/Golgi	Sperling et al. 1998
		At2g46210		García-Maroto et al. 2007
				Marion et al. 2008
LCB Δ4-desaturase	DES4	At4g04930	unknown	Ternes et al. 2002
				Bedia et al. 2005
ceramide kinase	ACD5	At5g51290	unknown	Liang et al. 2003
sphingosine-1P phosphatase	SSPase	At3g58490	unknown	Worrall et al. 2008
				Nakamura et al. 2007
sphingosine-1P lyase	DPL1	At1g27980	ER	Tsegaye et al. 2007
	SPHK1	At4g21540	Tonoplast	Worrall et al. 2008
sphingosine kinase	SPHK2	At2g46090	Mitochondria	Imai and Nishiura 2005
LCB kinase	LCBK1	At5g23450	Plasma membrane	Benschop 2007
	LCBK2	At5g51290	unknown	

Subcellular purification of different intracellular membranes has revealed different lipid composition (Moreau, Bessoule et al., 1998; Warnecke and Heinz 2003). In particular, phospholipids are the most abundant species in membranes, whereas sphingolipids and sterols can be very low or absent (such as in the ER membranes), or representing up to 25% each of total lipid levels (as in the plasma membrane). Apart from the plasma membrane, sphingolipids are enriched also in the late compartments and in the plant tonoplast, where

they represent 10-20% of the total membrane lipids (Figure 4) (Moreau, Bessoule et al., 1998; Warnecke and Heinz 2003). Golgi and late secretory compartments contain discrete amounts of both sterols and sphingolipids, with a composition showing more similarity to the PM than to the ER membranes. Membrane lipids appear thus compartmentalized in the cell with a specific gradient of sphingolipid and sterol along the secretory pathway with the highest accumulation at the PM. Interestingly, while sphingolipids are enriched in the late compartments of the secretory pathway, most of the biosynthesis is localized to the ER (Table 1). There are however few exceptions with the glycosylation of Ceramide to form GlcCer that occurs in the cytosolic leaflet of early Golgi cisternae. Once formed, GluCer is the transported across the Golgi either via a vesicular pathway or with the lipid transfer protein known as FAPP2 (D'Angelo, Uemura et al., 2013). Interestingly, these two differently transported GlcCer pools follow two different glycosylation mechanisms, and in different Golgi subdomains. In particular, GlcCer transported by direct protein transfer is used to synthesize glycosphingolipids known as globosides in the TGN while vesicular transported GlcCer is used in the Golgi cisternae for ganglioside production, such as that of GM3 (D'Angelo, Uemura et al., 2013).

Table 2. Ceramide synthases

	Enzyme	Gene	FA substrate
Human	CerS1	Lass1	C18
	CerS2	Lass2	C22-C24
	CerS3	Lass3	C18,C24, all medium chain
	CerS4	Lass4	C20, few C22-C24
	CerS5	Lass5	C16
	CerS6	Lass6	C14-C16
S. Cerevisiae	Lag1	LAG1	Mostly C26
	Lac1	LAC1	Mostly C26
A. Thaliana	LOH1	loh1	C22-C26
	LOH2	loh2	Mostly C16
	LOH3	loh3	C22-C26

5. SPHINGOLIPID HOMEOSTASIS IN CELL GROWTH

Pools of different LCBs, ceramide and complex sphingolipids are highly dynamic. In mammals, activation of sphingomyelinase and ceramidase resulted in the accumulation of ceramide and LCBs respectively, both involved in growth arrest and cell death (Mao and Obeid 2008). Several studies indicateed that high levels of LCBs (especially sphingosine, SPH) and ceramides were associated with growth arrest, while phosphorylated sphingosine (S1P) induced cell proliferation (Ping and Barrett 1998). Thus, the relative level of ceramide and SPH versus S1P seems to associate with opposite cellular effects leading to the idea that sphingolipids may act as a rheostat system regulating the cellular switch between survival or death, reviewed by (Hannun and Obeid 2008).

Catabolism of complex sphingolipids is better understood in humans, and several autosomal recessive mutations have been reported and classified as sphingolipidosesbecause they lead to sphingolipid accumulation. Degradation of ceramide is achieved by ceramidase, sphingomylein by sphingomyelinase, glucosylceramide by glucocerebrosidase, and galactosylceramide by galactosylceramidases. Mutations of glucosylceramidase induce the Gaucher disease, which is the most common lysosomal storage disease. In patients with Gaucher disease, GluCer accumulates in spleen, liver, kidneys, lungs and brain, eventually causing organ degeneration (Grabowski, Gatt et al., 1990). Genetic defects of sphingo-myelinase causes Niemann-Pick type A and B diseases, characterized by the accumulation of sphingomyelin and hepatic and neuronal degeneration (Schuchman 2010). Ceramidase mutations cause the Faber disease, characterized by progressive body deformation and, 20 pain because of ceramide accumulation in joints and the development of granulomas (Ozkara; Molino and Galli 2014).

In plants the degradative enzymes for GluCer and GIPC (analog of sphingomyelinase) have not yet been found. Three putative ceramidases (CDase) are present in the Arabidopsis genome (Table 1), but they have not been characterized yet. The only plant ceramidase so far characterized is the rice OsCDase1. It localizes to the ER and Golgi and can hydrolyze a fluorescent ceramide analog *in vitro* (Pata, Wu et al., 2008). However, no ceramidase activity against endogenous Cer was detected. On the contrary, OsCDase1 expression in yeast CDases knockout induced an increase of C-26-ceramide. This observation suggested that OsCDase1, displays a reverse ceramidase activity, resulting in an Acyl-CoA independent ceramide synthesis, similar observation where made for yeast YPC1p and YDC1p (Jiang, Kirchman et al., 2004).

The balance between ceramide synthesis and degradation appears to be essential for the homeostasis of the three signaling molecules Cer, SPH and S1P. Several stresses induce degradation of sphingomyelin and accumulation of ceramide. Known factor that have been associated with ceramede accumumation are: proapoptotic cytokine (TNF-α, IL-1β, FAS ligand, interferon γ), chemotherapeutic agents that induces DNA damage-mediated apoptosis, UV, ionizing irradiation and serum deprivation (Mao and Obeid 2008). Sphingosine and ceramide have been reported to induce both cell growth arrest and apoptosis (Pettus, Bielawski et al., 2003), (Futerman and Hannun 2004). In particular, in several carcinogenic cell lines, SPH was found to be an efficient inducer of cell death. SPH inhibits protein kinase C (PKC), the tyrosine kinase SRC (sarcoma), several Ca^{++}-Calmodulin dependent kinases (Jefferson and Schulman 1988; Igarashi, Hakomori et al., 1989) and mitogen activated

kinases ERK1/2 (Jarvis, Fornari et al., 1997). Apart from targeting cytosolic proteins, SPH also alters membrane systems. High levels of SPH increase permeability of lysosomes (Kagedal, Zhao et al., 2001) and mitochondria (Cuvillier and Levade 2001) which respectively release cathepsins and Cytochrome C to the cytosol that in turn induces apoptosis. Treatment of Hela cells with C-6 ceramide induces endogenous increase of SPH followed by Golgi fragmentation and apoptosis (Hu, Xu et al., 2005). Overexpression of ceramidases also caused SPH increases and a similar effect on Golgi fragmentation leading to apoptosis (Markham, Li et al., 2006).

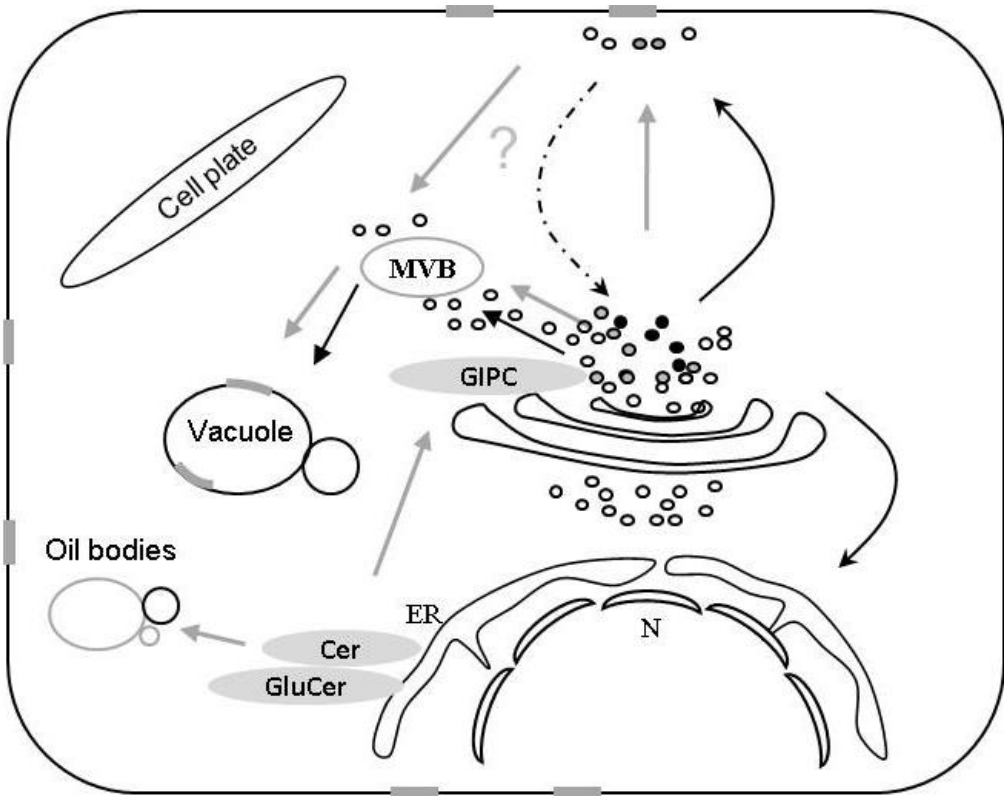

Figure 4. Sphingolipids distribution in plant secretory pathway. Model underlining the sphingolipid distribution along the plant secretory pathway (in gray). Plant Cer, GluCer, and GIPC localization is shown, according to the localization of biosynthetic enzymes. Arrows consider the possible vesicular transport of sphingolipid (grey arrows and question marks).

6. Sphingolipids Are Associated with Pathogen Infection in Plants and Animals

Sphingolipids play roles in signal transduction pathways regulating stress responses in eukaryotes. In plants LCB modifications including phosphorylation as well as unsaturation were shown to take part in the tolerance to environmental extreme and responses to chilling (Chen, Markham et al., 2012; Dutilleul, Benhassaine-Kesri et al., 2012). In the other side,

modulation of the sphingolipid metabolism was associated with responses to heat stress in yeast (Sun, Miao et al., 2012).

Several Arabidopsis mutations modifying sphingolipid homeostasis enhanced sensitivity to pathogen infections. Upon infection, plant responses include generation of H_2O_2 at fungal penetration sites and triggering of the hypersensitive response (HR). HR is a form of programmed cell death at the infection site (Levine, Tenhaken et al., 1994). The gene *RPW8* confers resistance to powdery mildew, a disease caused by several fungi targeting hundreds of dicotyledonous plants. In Arabidopsis, the Columbia (Col 0) accession does not have a *RPW8* gene while WS accession contains two *RPW8* copies. EMS mutagenesis on a Col 0 background overexpressing RPW8 enabled to find mutations that suppress the resistance conferred by RPW8 (Wang, Yang et al., 2008). Such a screen identified the plant IPC-synthase ERH1, as already mentioned, *erh1* mutation prompted the accumulation of high level of ceramides explaining the spontaneous induction of cell death. Similarly, the ceramide kinase ACD5 (for accelerated cell death) (Brodersen, Petersen et al., 2002), as well as a sphingosine transfer protein ACD11 (Liang, Yao et al., 2003), were identified in a screen for mutations that cause HR responses in the absence of pathogens. Altogether these observations indicated that any mutation resulting in increased levels of ceramide, possibly at the ER, induces cell growth arrest and PCD in plant as in mammals. Ceramide phosphorylation, or its transport out of the ER, seems to be protective factors against HR, since they would prevent Cer accumulation (Liang, Yao et al., 2003).

As already mentioned, fungal infection was shown to directly modify Cer levels by inhibition of ceramide synthase activity. Mycotoxins such as AAL and FB1 inhibit CerS and induce cell death. Several pieces of evidence suggested that in plants, the bioactive molecules leading to cell death are accumulated free LCBs. AAL induced death symptoms can be suppressed by concomitant pharmacological treatment with Myriocin, an inhibitor of the SPT (Spassieva, Markham et al., 2002), which abolished free LCB accumulation. Furthermore an *lcb1* mutant, impaired in SPT activity, was not able to accumulate free LCBs and was resistant to FB1 treatment. Application of exogenous LCBs (d18:0, t:18:0, t:18:1) induced ROS (reactive oxygen species) production and pro-death responses (Shi and Wei 2007). The LCB-dependent induction of cell death was found to be dependent upon nuclear Ca^{++} release (Lachaud, Da Silva et al., 2009). Studies in tobacco on the role of sphingolipids in plant immunity against mycotoxins suggest that LCBs modulate the salicylic acid-dependent responses (Rivas-San Vicente, Larios-Zarate et al., 2013). Finally, a MYB transcription factor (AtMYB30), a positive regulator of cell death in plants during HR responses, has been shown to directly increase the transcription of the genes encoding for the 4 core enzyme of the elongase complex. The increase of VLCFA elongation would directly impact on VLCFA-bearing lipids among them the sphingolipids, as demonstrated by (Raffaele, Vailleau et al., 2008). The authors suggested that acyl chain length would be a major determinant in modulating cell death and defense responses. Alternatively VLCFA modifications could alter pathogen recognition at the membrane by modifying structural properties of plasma membrane lipids. It can also not be excluded that the increase of VLCFA simply enhanced accumulation of epicuticular waxes and increase the epidermal physical barriers preventing pathogen entry.

Interestingly enough, human viruses are known to use membranes and their components (including sphingolipids) in different steps of their life cycle including attachment, intracellular transport, and budding, reviewed in(Schneider-Schaulies and Schneider-

Schaulies 2015). Examples for sphingolipid-dependent virus entry are found for human immunodeficiency virus (HIV), which besides its protein receptors also interacts with glycosphingolipids (GSLs) (Sorice, Garofalo et al., 2001) as well as the rhinovirus, which promotes the formation of ceramide- and glycosphingolipids-enriched platforms (Dreschers, Franz et al., 2007). Furthermore viruses can also manipulate sphingolipid metabolism as for example influenza A virus, which activates sphingosine kinase 1 and the transcription factor NF-κB (Seo, Pritzl et al., 2013).

7. SPHINGOLIPID PHOSPHORYLATION: THE SPHINGOSINE-P PARADIGM IN ANIMALS AND PLANTS

Phosphorylated LCBs (LCB-P) and corresponding kinases have been so far identified in all organisms. In yeast reversible phosphorylation is carried out by two LCB-kinases, LCB4p and LCB5p (Nagiec, Skrzypek et al., 1998) while dephosphorylation is carried out by the LCB phosphatases LCB3p and YSR3p (Figure 5). LCB-P can also be degraded by the LCB phosphate-lyase DPL11p, which irreversibly breaks down the LCBs-P in ethanolamine-phosphate and a hexadecanal aldhyde (Figure 5). While LCB3p, YSR3p and DPL1p are localized to perinuclear and cortical ER, LCB4p and LCB5p are found only at the cortical ER and Golgi (Hait, Fujita et al., 2002; Iwaki, Sano et al., 2007). Single mutant strains for any of these LCB-P related genes resulted in wild type phenotype. On the contrary double mutant *dpl1/lcb3* was not viable but could be rescued by *lcb4* mutation in the triple *dpl1/lcb3/lcb4* mutant (Nagiec, Skrzypek et al., 1998). LCB-P accumulation was thus correlated with growth inhibition (Figure 5), which was confirmed by LCB4 or LCB5 overexpression in *dpl1lcb3/lcb4* mutant that increased LCB-P levels and arrested growth (Nagiec, Skrzypek et al., 1998). A number of studies suggested that LCB-P could also have a role in several stress responses in yeast. For example heat stress induced LCB-P increase and *lcb3* mutants, who accumulate LCB-P, survived better upon heat shock, while *lcb3* overexpression resulted in lower LCB-P and higher sensitivity to temperature (Mandala, Thornton et al., 1998). Altogether these observations suggested that LCB-P plays a dual role according to its concentration in the cell. A moderated increase of LCB-P would correlate with a protective role during stress responses, while greater accumulation of LCB-P would correlate with growth arrest.

It has been reported that G1 cell cycle arrest induced by rapamycin involved lcb4p degradation (Iwaki, Sano et al., 2007). Thus, it is possible that LCB-Ps are also involved in the maintenance of G1 arrest. A similar function was proposed for the mammalian S1P. In mammals, overexpression of SPHK1, a homologue of Lcb4p, results in a decreased number of cells in G0/G1 and an increased number of cells in S phase, suggesting that LCB-P could be involved in G1-to-S transition (Jenkins and Hannun 2001).

In Arabidopsis, four LCBs kinases have been identified: Sphk1 and sphk2 (for sphingosine kinase 1 and 2), and LCBK1, LCBK2 (for LCB kinase 1 and 2) (Table 1). They have different subcellular localizations involving tonoplast, mitochondria and PM (Table 1). LCBs-P can be dephosphorylated by the phosphatases SPPase (sphingosine phosphatase) and hydrolyzed by the unique lyase atDPL1 in the ER (Tsegaye, Richardson et al., 2007). Arabidopsis *dpl1* mutant does not accumulate much LCB-P or LCB. This observation

suggests that LCB-P degradation via AtDPL1 does not represent a major pathway to control LCB/LCB-P levels in normal growth conditions. However, LCB and LCB-P accumulation are associated with stress conditions and cell death. The Arabidopsis *dpl1* mutant showed a hypersensitive response to FB1, which correlates with an accumulation of both LCB and LCB-P. Thus, AtDPL1 activity seems to become important in situations of stress leading to high LCB accumulation. It has to be noted that phosphorylated LCBs cannot be directly incorporated into ceramide, but can be reversibly dephosphorylated into LCB and then incorporated into sphingolipids, providing a different pathway to control LCB-P accumulation.

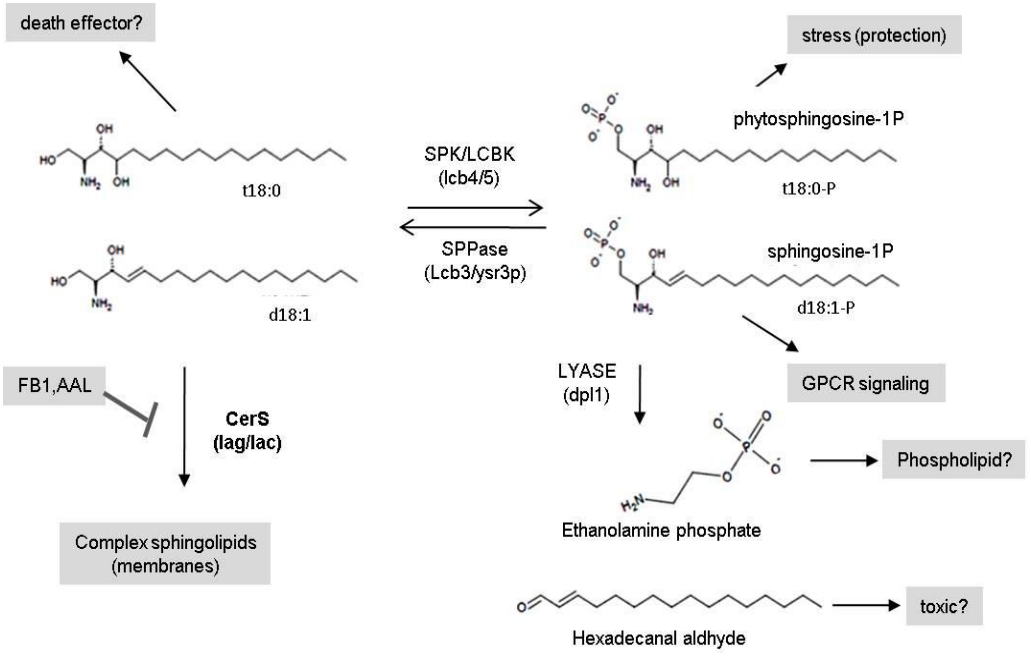

Figure 5. LCB phosphorylation pathway. LCBs can be phosphorylated and dephosphorylated by sphingosine- or LCB-kinases and sphingosine-phosphatase (SPPase) respectively. Only phosphorylated LCBs are substrate of degradation by the lyase DPL1. The interconnection of the phosphorylation pathway with the ceramide synthase is shown. In boxes, molecule functions in different organisms have been indicated. Interrogation points represent speculations.

Sphingosine phosphate (S1P) has been associated with cell proliferation in animals. Originally, S1P was shown to induce mitosis in fibroblasts (Zhang, Desai et al., 1991). Later, the pro-mitotic activity was associated with increases in Ca^{++} release *via* a non-identified mechanism which is phosphatidyl-inostol-1,4,5-P-independent (Mattie, Brooker et al., 1994). Numerous studies then revealed that S1P could directly interact at the plasma membrane with several G protein coupled receptors (GPCR), leading to their activations. At least five different S1P-GPCR mechanism mediating proliferative responses have been described in animals (Mao and Obeid 2008; Rosen, Gonzalez-Cabrera et al., 2009) that involve angiogenesis, vascular tissue development, immune responses and neuronal development. In human cell lines, S1P was also found to bind to and modulate the activity of a histone deacetylase in the nucleus (Hait, Allegood et al., 2009) and a E3 Ubiquitin ligase complex of

tumor necrosis factor receptor associated factor 2 (TRAF2) in the cytosol (Alvarez, Harikumar et al., 2010). S1P signaling system was also associated with several physiological process as well as human pathology especially inflammation process and inflammatory disease, immunity cells physiology (Garris, Blaho et al., 2014; Gonzalez-Cabrera, Brown et al., 2014) as well as cancer (Zhang, Wang et al., 2014).

In plants, the pharmacological treatment with S1P induces stomata closure (Ng, Carr et al., 2001). The S1P effect was abolished in G protein mutant, indicating that S1P activity is dependent on G protein-coupled receptor activity in plants (Coursol, Fan et al., 2003). The role of S1P was confirmed by the fact that the *sphk1* mutant or SPHK1 overexpression showed respectively lower and higher sensitivity to stomatal opening after abscissic acid stimulation (Worrall, Liang et al., 2008). The activity of the SPHK1 is correlated with stomatal sensitivity to ABA. ABA inhibition of seed germination was also much less pronounced in *sphk1* mutants. However the analysis of Arabidopsis Δ4-desaturase mutant lacking both sphingosine and S1P revealed no developmental defect (including stomata closure) (Michaelson, Zauner et al., 2009). The latest observation strongly suggests that another mechanism may be responsible for stomata closure (Lynch, Chen et al., 2009). Since SPHK1 seems to use a variety of LCBs as substrate, different free LCBs than S1P could be responsible for ABA-related responses.

8. REGULATION OF SPHINGOLIPID METABOLISM

The homeostasis of the different sphingolipids needs to be tightly controlled to ensure cell viability. While in plants the regulation of key enzymes of the pathway remains largely unknown, in animals and yeast several important regulatory mechanisms have been uncovered.

An important breakthrough in the understanding of homeostasis and regulation of sphingolipid metabolism was recently achieved thanks to a global yeast genetic interaction study aiming to understand the role of ORM proteins, which are involved in several human inflammatory diseases. There are 2 *ORM* genes (*ORM1* and 2) in Arabidopsis, yeast and three in humans (Han, Lone et al., 2009; Breslow 2013; Kimberlin, Majumder et al., 2013). They are ER-localized proteins involved in protein folding and unfolding responses (Han, Lone et al., 2010). ORM proteins were found to be negative regulators of the serine palmitoyl transferase (SPT), the first step of sphingolipid biosynthesis (Breslow, Collins et al., 2010). Characterization of ORM-SPT complex allowed the isolation of SAC1, a phosphoinositide phosphatase involved in protein secretion in response to nutrients in both animals and yeast (Rohde and Cardenas 2003; Blagoveshchenskaya and Mayinger 2009). Interestingly, while both SAC1 and ORMs have a negative effect on SPT, they bind SPT independently, suggesting that they probably act as two independent regulatory mechanisms. Conversely, yeast *orm1/2* presented an increased SPT activity and an accumulation of LCB but not ceramides. This observation suggests that LCB and ceramide pools are not simply interconnected and in balance, but that CerS activity is also tightly regulated (Breslow, Collins et al., 2010). In Arabidopsis, small subunits of SPT (ssSPT) also limit sphingolipid biosynthesis and therefore modulate FB1 sensitivity and are essential for male gametophyte viability (Kimberlin, Majumder et al., 2013).

The activity of ORM and its inhibitory effect on SPT depends upon ORM hyperphosphorylation. Interestingly, ORM hyperphosphorylation is also sensitive to ceramide synthesis. In yeast ceramide synthase mutant *lac1*, ORMs phosphorylation was increased and SPT activity decreased (Breslow, Collins et al., 2010). This important observation suggests that ceramide levels could regulate ORM activity, defining a negative feedback loop that prevents ceramide accumulation.

In yeast, ceramide synthase activity requires another cofactor, LIP1 (for lag1/lac1 interacting protein), essential for CerS activity. LIP1 copurified with Lag1p and Lac1p (Vallee and Riezman 2005). *In vivo* labeling experiments showed that *lip1* was impaired in ceramide synthesis, also with a sphingolipid profile similar to *lac1/lag1*. The exact mechanism by which LIP1 works is not clear but it has been suggested that LIP1 could be important for specifying ceramide synthase substrate specificities. In fact, the purification of ceramide synthase complex revealed several protein associations: two Lip1p proteins could bind Lag1p–Lac1p heterodimers or Lag1p/Lac1p homodimers (Vallee and Riezman 2005). Thus, the different complexes could have different enzymatic activities, i.e., different specificities for acyl chain length or LCB.

Plant and animal genomes do not seem to encode any LIP1p related genes (data not shown). However, a different mechanism involving enzymatic channeling could regulate sphingolipid biosynthesis. It was recently demonstrated that the VLCFA-ceramide synthase CerS2, but not the LCFA-CerS4, interacted specifically with elongase enzymes allowing efficient incorporation of very long acyl chain-CoA into sphingolipids (Ohno, Suto et al., 2010). In yeast, the target of rapamycin complex 2 (TORC2) was recently found to control *de novo* ceramide synthesis (Aronova, Wedaman et al., 2008). Arnova et al. isolated a conditional allele with a mutation in AVO3 (*avo3*), a subunit of the TORC2 complex that grows normally at 25°C, but slows down at 30°C. The authors found that a few hours after transfer to 30° the synthesis of ceramides was strongly reduced. Furthermore the overexpression of a target of TOR, the kinase YPK2, reverted *avo3* phenotypes and ceramide depletion, suggesting that YPK2 kinase activity is acting in the pathway downstream of TOR and upstream of ceramide synthase. Another interesting result regarded calcineurin, a conserved Ca++/Calmodulin-regulated phosphatase that inhibits several targets of TOR. Deletion of regulatory subunit of the calcineurin could also restore proper ceramide synthesis in *avo3* (Aronova, Wedaman et al., 2008).

In conclusion, TOR is a kinase involved in stress sensing in all eukaryotes and a master regulator acting upstream Orms proteins in the signaling cascade regulating sphingolipids metabolism (Liu, Huang et al., 2012). Such a master regulator also controls actin polarization and endocytosis via unclarified mechanisms (Rispal, Eltschinger et al., 2015). Sphingolipids were also shown to be required for endocytosis and actin organization (Zanolari, Friant et al., 2000).

Interestingly enough, processes that maintain lipid and protein clustering and thus the formation of membrane domains have been proposed to be involved in cortical actin (Goswami, Gowrishankar et al., 2008). Thus, it is plausible that the control of membrane lipid biosynthesis is a basic process regulating membrane composition, tension and organization necessary for different plasma membrane activities, as well as intracellular trafficking pathways.

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