University of Gothenburg
Faculty of Science

2009

Interplay Between Phospholipids and Digalactosyldiacylglycerol in Phosphate Limited Oats

Henrik Tjellström

UNIVERSITY OF GOTHENBURG

Akademisk avhandling för filosofie doktorsexamen in växtmolekylärbiologi, som enligt beslut i lärarförslagsnämnden i biologi kommer att offentligt försvaras fredagen den 27:e mars 2009, klockan 10.15 i föreläsningssalen, Institutionen för Växt-och Miljövetenskaper, Carl Skottbergsgata 22B, 413 19 Göteborg

Examinator: Professor Adrian K. Clarke

Fakultetsopponent: Dr Sébastien Mongrand, CNRS, Université Victor Segalen Bordeaux 2, Bordeaux, France

Göteborg, Mars 2009 ISBN: 978-91-85529-27-8

Interplay Between Phospholipids and Digalactosyldiacylglycerol in Phosphate Limited Oats

Henrik Tjellström

University of Gothenburg
Dept of Plant and Environmental Sciences
Box 461, SE-405 30 Gothenburg, Sweden

ABSTRACT

Phosphate is an essential nutrient. In most soils it is limiting, which has resulted in that phosphate is supplied as fertilizer to increase crop yield. Through evolution, plants have adapted several mechanisms to increase phosphate uptake from the soil and to household with acquired phosphate. A recent discovered house-holding mechanism is that plants utilize the phosphate bound in the headgroups of phospholipids: under phosphate-limiting conditions, phospholipids can be replaced by the non-phosphate containing lipid digalactosyldiacylglycerol (DGDG), previously assumed to reside in plastid membranes. The extra-plastidial phospholipid-to-DGDG replacement occurs in plasma membrane, tonoplast and mitochondria and has led to discoveries of new enzymes and metabolic pathways in plants.

This thesis reports that phosphate limitation-induced biochemical and lipid compositional changes in oat root plasma membranes occur prior to any morphological changes in the oat. The phospholipase kinetics suggests that the plasma membrane is continuously supplied with phospholipids and that the products of plasma membrane lipase activities, phosphatidic acid and diacylglycerol, both are removed from the membrane. Furthermore, the phospholipid-to-DGDG replacement is reversible and when phosphate is resupplied the proportion of phospholipids increases and DGDG decreases in the oat root plasma membrane.

Membrane lipids are more than a two dimensional liquid where membrane proteins reside. The specific lipid composition and distribution enables the membrane to function as a barrier to solutes and the interactions between lipids and proteins are important for the correct function. The lateral and transversal lipid distribution in oat root plasma membranes shows that DGDG does not replace phospholipids molecule for molecule; whereas phospholipids occur in both leaflets of the plasma membrane, DGDG is almost exclusively localized in the cytosolic leaflet. Model membrane studies suggests that one of the reasons that DGDG is absent in the apoplastic leaflet is its incompatibility to properly interact with the high sterol content of this leaflet.

The oat seed contains enough phosphate to complete an entire generation without any exogenously supplied phosphate. The overall seed yield is much lower in phosphate-limited oat compared to fully fertilized oat, but the seed quality (starch, β-glucan, lipid, soluble protein) is very similar, including that the phospholipids-to-DGDG replacement is absent from the mature oat seeds, here membrane lipid composition is conserved. Oat thus produce a few seeds of acceptable quality rather than more seeds of poor quality.

Keywords: acyl chain order; *Avena sativa*; DGDG; digalactosyldiacylglycerol; oat; liquid order; phosphate; phospholipase; plasma membrane; stress

Göteborg, Mars 2009 ISBN: 978-91-85529-27-8

Interplay Between Phospholipids and Digalactosyldiacylglycerol in Phosphate Limited Oats

This thesis is based on the data presented and discussed in the following papers, throughout the thesis referred to by roman numerals

Paper I Andersson MX, Larsson KE, **Tjellström H**, Liljenberg C, Sandelius AS *(*2005); Phosphate-limited oat: The plasma membrane and the tonoplast as major targets for phospholipid-to-glycolipid replacement and stimulation of phospholipases in the plasma membrane. *J. Biol. Chem.* 280: 522-525 *

Paper II **Tjellström H**, Andersson MX, Larsson KE, Sandelius AS (2008), Membrane phospholipids as a phosphate reserve: the dynamic nature of phospholipid-to-digalactosyl diacylglycerol exchange in higher plants. *Plant Cell & Environ.* 31:1388-1398 **

Paper III **Tjellström H**, Hellgren LI, Wieslander Å, Sandelius AS (2009) Lateral and transversal lipid distribution in oat root plasma membranes during phosphate limitation. *Manuscript*

Paper IV **Tjellström H**, Sandelius AS (2009) The effects of phosphate limitation on yield and seed quality in oat (*Avena sativa* L. var Belinda). *Manuscript*

Papers not included in this thesis:

Larsson KE, Kjellberg JM, **Tjellström H**, Sandelius AS (2007); LysoPC acyltransferase/ PC transacylase activities in plant plasma membrane and plasma membrane-associated endoplasmic reticulum. *BMC Plant Biology* 7:64

Porsbring T, Blanck H, **Tjellström H**, Backhaus T (2009); Toxicity of the pharmaceutical clotrimazole to marine microalgal communities*. Aquatic Toxicology* 91:203-211

CONTENTS

ABBREVIATIONS

16:0	Hexadecanoic acid (palmitic acid)
18:0	Octadecanoic acid (stearic acid)
18:1	cis-9-octadecenoic acid (oleic acid)
18:2	All-cis-9,12-octadecadienoic acid (linoleic acid)
18:3	All- cis-9,12,15-octadecatrienoic acid (linolenic acid)
ASG	Acylated sterolglucosides
ACP	Acyl carrier protein
CDP	Cysteine diphosphate
Cer	Ceramide
CoA	Coenzyme A
DAG	Diacylglycerol
DGDG	Digalactosyldiacylglycerol
DRM	Detergent resistant membrane
ER	Endoplasmic reticulum
EST	Expressed sequence tag
FA	Fatty acid
GGGT	Galactolipid:glactolipidglucosyl transferase
GlcCer	Glucosylceramind
GPI	Glycosylphosphatidylinositol
GPAT	glycerol-3-phosphate acyl transferase
GIPC	glycosyl inositol phosphorylceramides
IE	Inner envelope
Lo	Liquid order
Ld	Liquid disorder
LPAT	Lyso phosphatidic acid acyl transferase
LPCAT	Lyso phosphatidylcholine acyltransferse
LPEAT	Lyso phosphatidylethanolamine acyltransferse
MAM	Mitochondria associated membrane
MGDG	Monogalactosyldiacylglycerol
NPC4	Non specific phospholipase C 4
OE	Outer envelope
PA	Phosphatidic acid
PAM	Plasma membrane associated membrane
PAP	Phosphatidic acid phosphatase
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PLA	Phospholipase A
PLAM	Plastid associated membrane
PLB	Phospholipase B
PLC	Phospholipase C
PLD	Phospholipase D
PS	Phosphatidylserine
SG	Sterolglucosides
TLC	Thin layer chromatography
UDP	Uridine diphosphate

1. INTRODUCTION

A membrane delimits the cell from the surrounding environment and inside the eukaryotic cell, other membranes delimits different compartment from the cytosol. The compartments have different cellular functions and require different concentration of ions, proteins, lipids and other molecules. The membranes are primarily composed of lipids and proteins and their composition varies between membranes. The lipid composition of membranes differs with plant development and between tissues and it adapts to changes in the environment.

Phosphate is limiting in most soils. Contrary to animals, plants cannot move to more favorable conditions and have therefore developed strategies to cope with the immediate environment. In phosphate-limited soils, the root elongates and the root exudes organic acids to facilitate uptake.
Recently, a house-holding mechanism involving phospholipids as a phosphate reserve was identified in plants. The mechanism replaces phospholipids with the galactolipid digalactosyldiacylglycerol (DGDG) and during phosphate-limited conditions, the replacement occurs in plasma membranes, mitochondria and tonoplasts.

The aim of this thesis is to elucidate some of the mechanisms behind and consequences for the plant plasma membrane when phospholipids are replaced with DGDG. A key step in the use the phospholipids as a phosphate reserve, is the removal of the phosphate moiety form the phospholipid. This investigation was initiated in **Paper I** and extended to include different plant ages and different degrees of phosphate limitation in **Paper II**. The transient nature of the phospholipid-to-DGDG exchange was investigated in **Paper II**. The specific localization of DGDG in the plasma membrane was examined in **Paper III**, with analyzes of lateral and transversal distribution of the lipids. Furthermore, the consequence of elevated levels of DGDG in the plasma membrane was also investigated in **Paper III**, using model membranes. Previously it had been noted that oat grown without any exogenously supplied phosphate were able to produce grains. In **Paper IV**, the quantity and quality of grains produced in oat cultivated with different levels of phosphate was examined.

2. BACKGROUND

2.1 The biological membranes

All living cells are surrounded by biological membrane and all eukaryotic cells have membranes that delimit their organelles from the cytosol. The main role of membranes is to create a barrier between the cytosol and intra-cellular compartments and the outside of the cell. The biological membrane model that has received the most recognition was put forward by Singer and Nicholson in 1972 and is usually referred to "the fluid mosaic model". Singer and Nicholson proposed that in aqueous environments polar lipids spontaneous arrange themselves in bilayers (Singer and Nicholson, 1972).

The two major groups of components in biological membranes are proteins and lipids. Proteins provide structural support to the cell and the membranes. In the membrane, proteins catalyze enzymatic reactions, facilitate transport of molecules across membranes and receive and propagate signals.

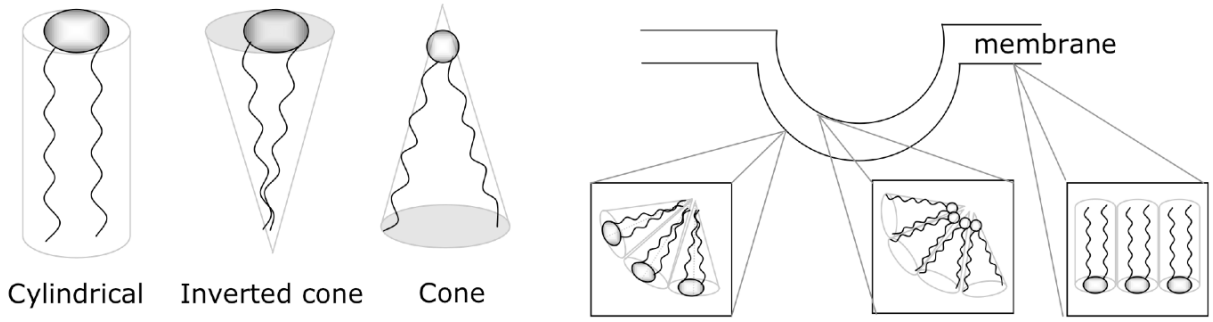


Figure 1. Shapes and curvature of lipids. Dependent on the size of the headgroup (spheres) and the degree of unsaturation of the acyl chains lipids adopt different conformations in membranes. Enrichment of inverted or cone shaped lipids can induce curvature of the membrane.

Lipids are soluble in organic solvents such as heptane, hexane, chloroform or methanol. Membrane lipids are usually comprised of two distinctive parts: one polar (hydrophilic) and one non-polar (hydrophobic). There are several different classes of membrane lipids, which differ in their basic molecular structure; glycerolipids, sphingolipids and sterols. The lipids in these groups can have quite different properties, such as chemical structure, shape, charge, polarity and rigidity. Depending on the arrangements of lipids with different properties and proteins, membranes can adopt different curvature and impose different conditions on its surroundings (Figure 1).

The role of organelles is to organize, compartmentalize and regulate enzymatic activities. Organelles have different lipid and protein compositions, ions and co-factors. A biological membrane is not a tight seal; it is a selective barrier, which means that water, nutrients, signaling molecules and other small molecules can pass a membrane from the outside to inside of the cell or between different compartments in the cell.

2.2 The plant cell

Plant cells differ from other eukaryotic cells in several ways. The most profound is the presence of plastids, cell walls and a large central vacuole. Plastids originated as prokaryotic photosynthetic bacteria, which, according to the endosymbiotic theory were engulfed by a eukaryotic cell (Mereschkowski, 1905; Kim and Archibald, 2008). The plastid contains several functions central to plant cell metabolism and development. The plastids in green tissues are called chloroplast and contain the photosynthetic machineries

Plants have a rigid cell wall, primarily made up of the linear polymer cellulose (Somerville, 2006). The central plant vacuole can occupy as much as

90% of the cell volume. One of the most important functions of vacuoles is to regulate the osmotic pressure against the cell wall. This pressure, turgor, is the foundation for the rigidity of well-watered plants.

2.3 The plasma membrane

The plasma membrane is the cell's outer membrane, it controls when and how molecules and signals are transported and propagated into and out of the cell. All solutes taken up by the cell must be actively or passively transported though the plasma membrane.

The integrity of the plasma membrane is determined by its protein and lipid composition. When plasma membranes from different plant specis are compared the polypeptide patterns seem to be more conserved than the lipid compositions. (Larsson et al., 1990). The most abundant lipids in the plasma membrane are glycerophospholipids, sterols, sterol derivatives and sphingolipids. Digalactosyl-diacylglycerol (DGDG) is a minor constituent under normal growth conditions, but during phosphate-limited conditions it comprises up to 25 mol% of the root plasma membrane (Andersson et al., 2003; **Papers I and III**).

In order to study the plasma membrane it is necessary to be able to isolate highly purified plasma membrane. This is accomplished by aqueous polymer two-phase partitioning (Kjellbom and Larsson, 1984; Sandelius and Morré, 1989)

2.4 Phosphate limited plant cultivation

Phosphate is required in numerous molecules and processes in the cell and is a limiting factor in most of the arable land. Plants have evolved several strategies to obtain phosphate. These include longer roots and root hairs (Andersson et al., 2003; Li et al., 2006). The increase in root hairs greatly contribute to an increase in surface area during phosphate limiting conditions compared to fully fertilized growing conditions (Jungk, 2001). Other strategies involve increased mycorrhizal symbiosis and exudation of organic acid into the soil to liberate bound phosphate (Raghothama, 1999; Raghothama and Karthikeyan, 2005).

Phosphate is used as a fertilizer in agriculture to increase crop yields. Most of this phosphate is mined as rock phosphate. The rock phosphate is, like fossil oil, a finite resource and it has been proposed that there will be a shortage of rock-phosphate in 50-100 years. (Runge-Metzger, 1995; Vance et al., 2003). Once distributed in the field, surplus phosphate binds to aluminum or iron complexes, which renders the phosphate inaccessible to plants, or is leached into the ground water and oceans and cannot be processed and recovered to be reused. It is therefore vital that we learn how phosphate is utilized in the plants, so that we can household and use the available phosphate in the best way possible.

All of the papers in this thesis deal with oat that has been subjected to different levels of phosphate limitation. Oat seeds were imbibed in de-ionized water over night and planted in vermiculite. Vermiculite is an inert material suitable for nutrition studies. All nutrients available to the plants are supplied as nutrient solution mixed from inorganic salts, as previously described (Norberg and Liljenberg, 1991). Oats were grown in a growth chamber with 16/8 h and

18°/15°C day/night regime with humidity set at 70%. To induce phosphate limiting conditions potassium phosphate was replaced by potassium chloride. Several different concentrations of inorganic phosphate were used during the different experiment, ranging from 0 mM to 1.5 mM. The different concentrations are referred to as a percentage, with 100% representing 1.5 mM inorganic phosphate.

2.5 Why oats?

Oat in a self-pollinating monocotyledon, which grows in temperate climate and is tolerant to cold and wet climate. All of these traits make it an ideal crop for Swedish climate and is frequently used in Swedish agriculture as a rotational crop. Oat is used as feedstock for animals and as oatmeal for humans and is rich in the soluble fiber β-glucan (3-4% w/w), which has the beneficial health properties of lowering cholesterol (Brown et al., 1999). It is also used as a thickening agent in low fat foods (Bräutigam et al. 2005) The dicotyledon *Arabidopsis thaliana* has become the weapon of choice for plant researchers to elucidate plant functions. It has a relative short generation time with high seed production and a sequenced genome. The major drawback of working with oat is that its hexaploid genome is not sequenced. Expressed sequence tag (EST) libraries are available to some extent (Bräutigam el al., 2005), but classic plant molecular biology in oat is still quite hampered. Advantage of oats compared to Arabidopsis includes larger root and shoot biomass to facilitate large-scale isolation of membranes. The oat seed contains enough phosphate to sustain an entire generation (**Paper IV**).

Even though much knowledge and information can be extracted from studies using Arabidopsis there are differences between species. To understand oat during phosphate limitation, it is necessary to work with oat. Initially, we used the oat variety Vital (**Papers I and II**) but decided to switch to Belinda (**Papers II-IV**) due to that Vital was removed from the agricultural market and we wanted to work with a variety relevant for Swedish agriculture.

3. MEMBRANE LIPIDS

As mentioned in the previous sections there are several different classes of membrane lipids. In this section, their synthesis and bilayer properties will be discussed.

3.1 Glycerolipids

Glycerolipids share a similar backbone consisting of a glycerol molecule. They have two acyl chains, usually 16 or 18 carbons long, bound to two of the three hydroxyl-groups on the glycerol backbone through an ester bond. These two positions are referred to *sn*-1 and *sn*-2. At the third hydroxyl-group (*sn*-3) there can be a myriad of different polar molecules such as sugars, phosphocholine, phosphoethanolamine and phosphate to name a few.

Dependent on the degree of desaturation of the acyl chains at position *sn*-1, *sn*-2 and the polar moiety attached to the *sn*-3 position, the glycerolipids have quite different properties such as, size, shape and charge (Figure 1).

The fatty acid synthetase (FAS) in the plastid stroma, produces 16:0-ACP (acyl carrier protein) and 18:0-ACP (Ohlrogge and Browse, 1995), which is desaturated to 18:1-ACP by FAB2 (Lightner et al., 1994). The 16:0 and 18:1 acyl chains are transferred from ACP to Coenzyme A (CoA) and exported to the ER as acyl-CoA to react with glycerol-3-phosphate to produce phosphatidic acid (PA). In the past years a second pathway for exporting fatty acids from the chloroplast has come to light, namely that newly synthesized acyl chains are initially incorporated into the phospholipid phosphatidylcholine (PC; Bates et al., 2007). The authors propose an acyl editing mechanism involving lyso PC acyl transferases for incorporation of acyl chains into PC.

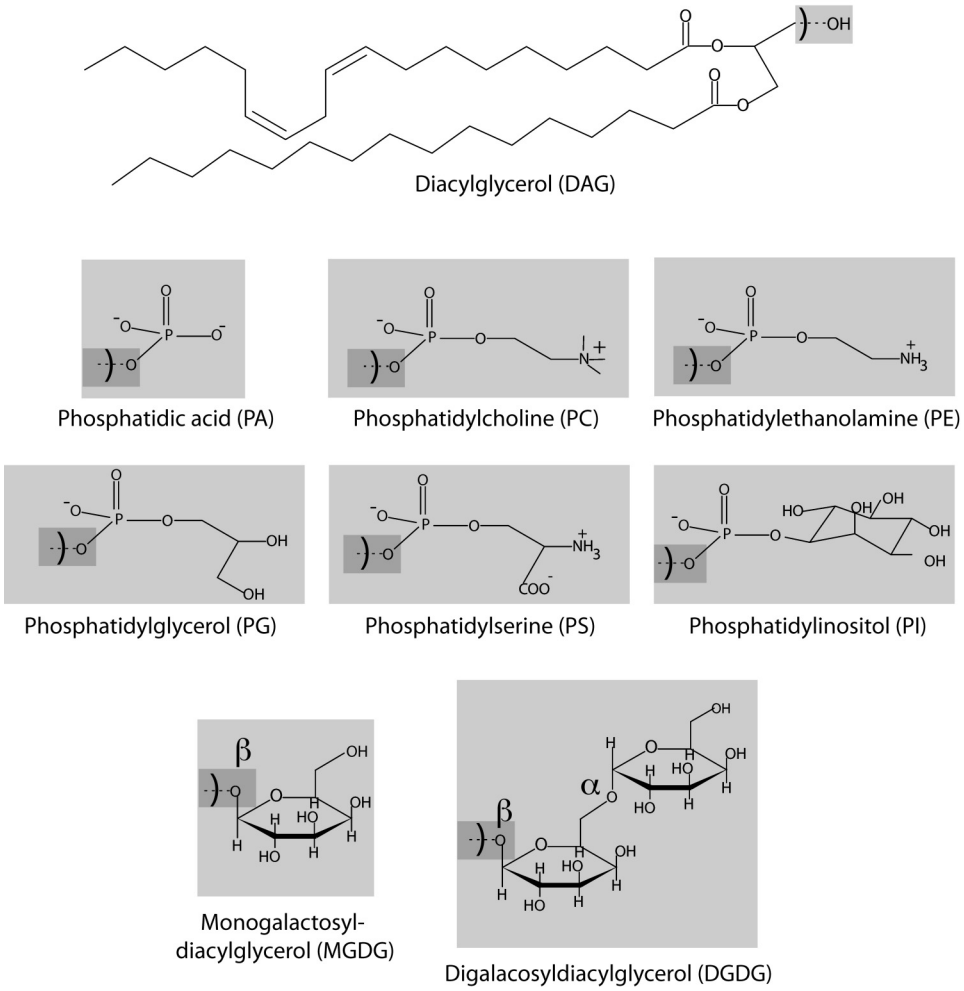


Figure 2. Structures of the common glycerolipids in plants. All gycerolipids are based on a diacylglycerol backbone. The shaded regions indicate the different headgroups common in plant glycerolipids

3.2 Phospholipids

Glycerophospholipids are the most prevalent membrane lipid in animal cells and in extra plastidial membranes in plant cells. In this thesis glycerophospholipids will be referred to as phospholipids. The polar headgroup is made up of a phosphate to which one of several other molecules can be bound, such as choline, ethanolamine, serine and glycerol, to produce an array of phospholipids (Figure 2). Beside the function as major membrane constituents, phospholipids serve as signaling molecules, protein co-factors and as substrates in various reactions. More and more cellular responses are attribute to various phospholipids signaling, such as programmed cell death (Park et al., 2004) and root elongations (Li et al., 2006). Especially PA seems to be involved in many different responses and for reviews on the subject on PA as a singling molecule see (Munnik, 2001; Munnik and Testerink, 2008)

Most of the plant cell phospholipids are syntheses in the ER with the initial step being the formation of lyso-PA from acyl-CoA and glycerol-3-phosphate via glycerol-3-phosphate acyl transferase (GPAT; 1 in Figure 3). A second acyl chain is added by lyso PA acyl transferase (LPAT; 2 in Figure 3) to produce PA. From PA, numerous pathways are possible; phosphatidylglycerol (PG) and phosphatidylinositol (PI) are produced by cytidine-diphosphatediacylglycerol (CDP-DAG; produced from PA) and glycerol or inositol, respectively (3 in Figure 3). Phosphatidylserine (PS) is synthesized in similar reactions with serine and UDP-DAG (4 in Figure 3). Phosphatidylethanolamine (PE) is produced through decarboxylation of PS, (5 in Figure 3). Diacylglycerol (DAG) is produced from PA by a phosphatidic acid phosphatase (PAP) (6 in Figure 3). The Kennedy pathway produces PC and PE by the transfer of phosphocholine or phosphoethanolamine from CDP-phosphocholine or CDP-phosphoethanolamine to DAG (7 and 8 in Figure 3), for details see Ohlrogge and Browse, (1995). In yeast, PE can be converted to PC by a series of three methylations, but if this is actually true for plants is not certain (Kanipes and Henry, 1997; 9 in Figure 3).

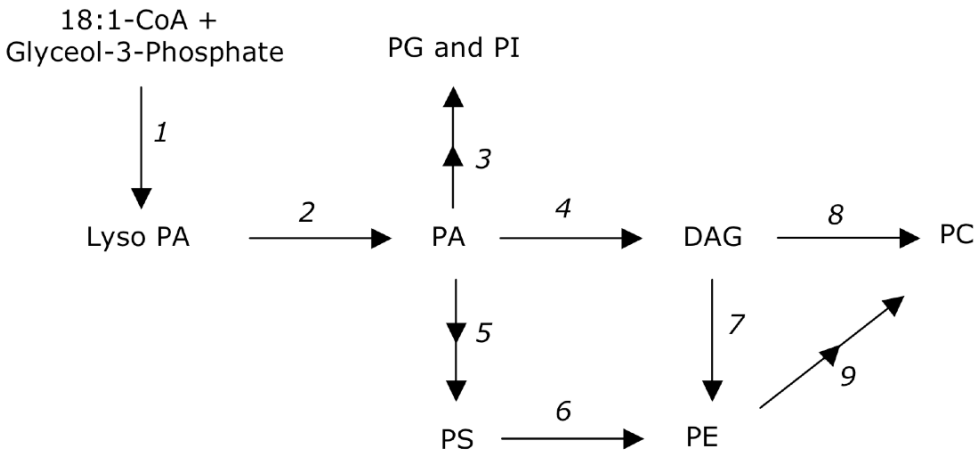


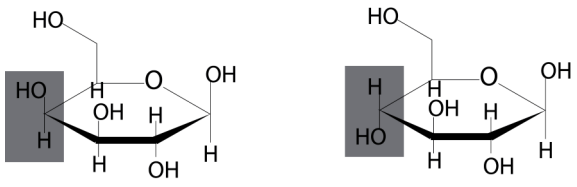
Figure 3. Pathway of phospholipid synthesis in ER. See text for the identification of the indicated reaction

Desaturation of phospholipid acyl groups in ER occur primarily by FAD2 where a double bond is introduced into 18:1 at position Δ12 to form 18:2 (Okuley et al., 1994) and FAD3 introduces a third double bond at position Δ15 to form 18:3 (Browse et al., 1993). Both FAD2 and 3 only desaturate acyl chains attached to lipids in a membrane and have a preference to PC compared to other phospholipids (Ohlrogge and Browse, 1995).

3.3 Galactolipids

The galactolipids MGDG and DGDG (Figure 2) are the most abundant lipids on earth and comprises circa 70% of thylakoid lipids. These lipids are almost exclusively found in photosynthesizing organisms, which indicate that they are important in the photosynthetic machinery. Photosynthetic organisms have greater requirements of membrane lipids than other eukaryotes due to the large surface area of the thylakoid membrane. Galactolipids make up the majority of lipids in the chloroplast but since glucose and not galactose is the most abundant sugar in the plant cell, it has been puzzling why MDGD and DGDG are galactolipids and not glucolipids. Structural information regarding Photosystem I (Jordan et al., 2001) and light harvesting complex II (Nussberger et al., 1993) revealed close interaction between the galactolipid headgroup and proteins (Kelly and Dörmann, 2004). In transgenic Arabidopsis producing *glucose*-galactose-DAG instead of *galactose*-galactose-DAG (DGDG) displayed hampered photosynthetic activity (Hölzl et al., 2006). Galactose and glucose have different orientation of the C4 hydroxyl group (Figure 4) and there are indications that the specific orientation of the C4 hydroxyl group in galactose is involved in stabilizing the LHCII (Hölzl et al., 2006). With an increasing number of structures of membrane bound proteins solved by x-ray crystallography, more lipids are recognized as an intricate and vital part of membrane proteins (Nussberger et al., 1993; Jordan et al., 2001).

In plants there are two different pathways by which galactolipids are produced: the eukaryotic and the prokaryotic pathway. In the former the glycerolipid backbone is assembled in the ER and then transported to the plastid for further synthesis to MGDG and DGDG. It is not entirely clear which lipid(s) is/are transported from ER to plastids, PA (Awai et al., 2006; Lu et al., 2007; Benning 2008; **Paper II**), DAG (Williams et al., 2000); **Paper II**), lysoPC (Mongrand et al., 1997; 2000; Moreau et al., 1998) and PC (Roughan et al. 1980; Hellgren and Sandelius, 2001a; Andersson et al., 2004).



Galactose Glucose

Figure 4. Galactose and glucose. Shaded region "highlights" the difference in hydroxyl group orientation between galactose and glucose.

In the other pathway, the prokaryotic, the galactolipid backbone is assembled inside the plastid followed by the formation of MGDG and DGDG. By analyzing the fatty acid composition of galactolipids it is possible to identify if the galactolipid backbone is assembled in the ER or the plastid (Heinz and Roughan, 1983; Mongrand et. al, 1998). Galactolipids completely assembled in the plastid have C16 fatty acids at the *sn*-2 position; usually 16:3 while ER derived galactolipids have C18 as the most abundant fatty acid in the *sn*-2 position, usually 18:3. Plants with only the eukaryotic pathway are referred to as 18:3-plants and plants with both the eukaryotic and prokaryotic pathways are referred to as 16:3-plants. In 16:3 plants, a PAP also generates DAG but the assembly of PA occurs entirely inside the plastid. The resulting DAG is the backbone for galactolipids synthesis. The main galactolipid MGDG is synthesized by the addition of galactose from UDP-galactose onto a DAG backbone (Neufeld and Hall, 1964; Ongun and Mudd, 1968) and DGDG is synthesized by the addition of an additional glucose from UDP-galactose onto MGDG (Dörmann et al., 1999; Kelly et al., 2003). Most plants (including oats) are 18:3-plants but Arabidopsis is a 16:3-plant (Mongrand et al., 1998).

3.4 Sphingolipids

Sphingolipids have three basic components; a polar headgroup, a long chain amino alcohol and a fatty acid (Figure 5). Sphingolipids with a polar headgroup consisting different sugar compounds such as mannose, galactose or glucose are referred to as cerebrosides. Ceramides with a polar headgroup of phosphorylated inositol and complex sugar moieties are referred to as phytoglycolipids or glycosyl inositol phosphorylceramides (GIPC; Lester and Dickson, 1993; Sperling and Heinz, 2003; Markham et al., 2006).

The first step in sphingolipid synthesis is the condensation of an acyl-CoA and a serine, which occur in the cytosolic leaflet of the ER. A ceramide is formed by N-acylation of the nitrogen on the serine (Lynch and Fairfield, 1993; Lynch, 2000). In animal cells, glucosylceramind synthetase is localized in the cytosolic leaflet of the Golgi apparatus (Futerman and Pagano, 1991; Jeckel et al., 1992; Marks et al., 1999), but in plants a fluorescent protein tagged enzyme appeared to be localized in the ER (Hillig et al., 2003). Further additions of sugars to the GlcCer headgroup occur at the luminal leaflet of the Golgi apparatus (Pomorski et al., 2001). There are several other modifications to sphingolipids other than alteration of the glucosylated-head-groups such as desaturation of the acyl chains, normally at positions $\Delta 4$ and $\Delta 8$, and hydroxylation. For details regarding sphingolipid metabolism see the reviews by Sperling and Heinz (2003) and Lynch and Dunn (2004).

Standard lipid isolation protocols are based on chloroform/methanol mixtures (Bligh and Dyer, 1959; Kates, 1986), which extract neutral lipids, sterols, galactolipids and most phospholipids. Some sphingolipids are extracted, but not GIPC. This may lead to a systematic underestimation of the sphingolipids content in membranes. Protocols were developed and evaluated to analyze the GIPC content in plant tissues (Markham et al., 2006). When one of the GIPC optimized protocols for plant tissue was used on oat root plasma membranes, no

significant amount of GIPCs were detected (**Paper III**). The functional and structural aspects of sphingolipids in a membrane environment will be discussed in a later section ("…along the membrane")

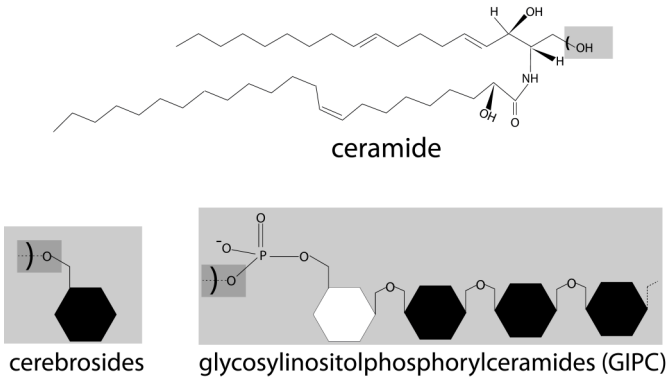


Figure 5. Structures of sphingolipids. All sphingolipids are based on a ceramide backbone to which polar headgroups are attached. White hexagon represents inositol and black hexagons other sugars (glucose, galactose, etectera)

3.5 Sterols

Sterols and sterol derivatives are major components of the plant plasma membrane (Yoshida and Uemura, 1986; Norberg and Liljenberg 1990; Hartmann and Benveniste, 1987; Benveniste, 2004; **Papers I and III**) and can function as substrate in the cellular development process. (Heinz et al., 1975; Heinz, 1996; Warnecke et al., 1997; Peng et al., 2002). All sterols share a four ring-structure; to which 8-10 carbons are attached at carbon 17 (C17) and a hydroxyl group at C3 (Figure 6). The hydroxyl group is the polar headgroup of sterols and interacts with the polar headgroups of glycerolipids and sphingolipids at membrane surfaces. Free sterols are synthesized through the isoprenoid pathway in the ER, for review see Benveniste, (2004). The main functions of free sterols are to fine-tune the biophysical properties of membranes such as fluidity and permeability (Hartmann, 1998; Ikonen, 2008). The three major plant sterols in oat root plasma membranes are campesterol, β-sitosterol and stigmasterol (**Papers I and III**; Figure 6). The reason why plants have several types of free sterols is not known, but some different properties have been observed between the different sterols. Campesterol and β-sitosterol reduce the fluidity and permeability of membranes in a similar fashion to cholesterol, where as stigmasterol has the opposite effect and reduce the order of the acyl chains (Hellgren and Sandelius, 2001b; **Paper III**).

In plants have several sterol molecular species while in animals there is only cholesterol (Benveniste, 2004). Free sterols are synthesized in the ER but are rapidly transported to Golgi apparatus and finally to the plasma membrane via the secretory pathway. In barley roots and other tissues the proportion of free sterols increases from ER-to-Golgi-to-plasma membrane (Heinz et al., 1975). It has been postulated that regions in the ER contain a high proportion of sterols to facilitate protein insertion (Bretscher and Munro, 1993)

Sterol derivatives have additional moieties attached to the sterol backbone via an ether bond to the hydroxyl group at C3 (Figure 7). Sterol glycosylation

which, produces sterol glucosides (SG) is localized in the plasma membrane (Marie et al., 1978) but some activity has been found to occur in the tonoplast and Golgi apparatus (Green, 1983). In oats, steryl glucosides are synthesized by membrane bound enzymes, which transfer glucose from UDP-glucose to a sterol backbone (Warnecke and Heinz, 1994). The most common sugar in SG is glucose, but others also occur (Heinz, 1996). Steryl glucosides can be further acylated to form acylated steryl glucosides (ASG; Heinz et al., 1975). Information regarding the synthesis of ASG is a bit scarce and there is no consensus regarding which substrate donates the acyl chain to SG to form ASG. In *Vicia faba* and carrot, steryl glucoside acyl transferases were soluble and the acyl chain was donated by galactolipids with a preference of DGDG over MGDG (Eichenberger and Siegrist, 1975; Heinz et al., 1975). However, in *Solanum melongena* the activity was membrane associated and the acyl chain originated from phospholipids and not galactolipids (Potocka and Zimowsk, 2008). Acylation of sterols produce steryl esters, but these are not structural membrane lipids and are out of scope the for this thesis.

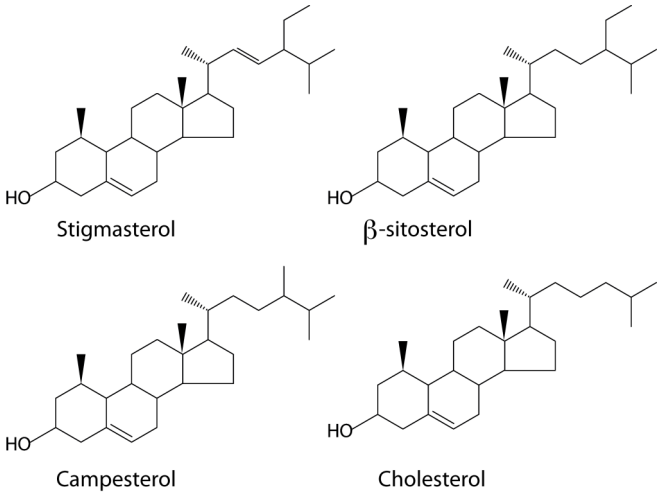


Figure 6. Structures of common plant sterols and cholesterol

The functional information of sterol derivatives is also somewhat limited. SG can act as primer molecule for cellulose synthase (Peng et al., 2002). ASG replace phospholipids in detergent resistant membranes (DRMs, see section "…along the membrane") in oat roots during phosphate limiting conditions (**Paper III**).

A potential problem in sterol analysis on isolated membranes is the technique used to isolate the membrane fraction. During protoplast isolation from leaf cells, most if not all free sterols disappears (Kesselmeier et al., 1987). A possibility is that they are converted to SG to be used as a primer for cellulose synthesis to counter act the cell wall degrading enzymes employed in protoplast isolation. When plant tissue is homogenized with an ultra-turax or in a blender (which could be considered as a mechanical damage) similar responses might be an issue. However, since mechanical isolations usually are done at 4° C, the

activities of the enzymes involved (UDP-glucose: sterol glucosyltransferase and cellulose synthase) are hampered. A possibility is that they are converted to SG to be used as a primer for cellulose.A possibility is that they are converted to SG to be used as a primer for cellulose synthesis to counter act the cell wall degrading enzymes employed in protoplast isolation. When plant tissue is homogenized with an ultra-turax or in a blender (which could be considered as a mechanical damage) similar responses might be an issue. However, since mechanical isolations usually are done at 4° C, the activities of the enzymes involved (UDP-glucose: sterol glucosyltransferase and cellulose synthase) are hampered. It is thus possible that the free sterol:sterol derivatives ratio in isolated membranes are a reflection of the membrane isolation technique and not necessarily an accurate description of the membrane composition. Comparisons of sterol and sterol derivatives from protoplasts, homogenized and untreated plant tissue should give some insights on how to limit isolation induced alterations in lipid composition.

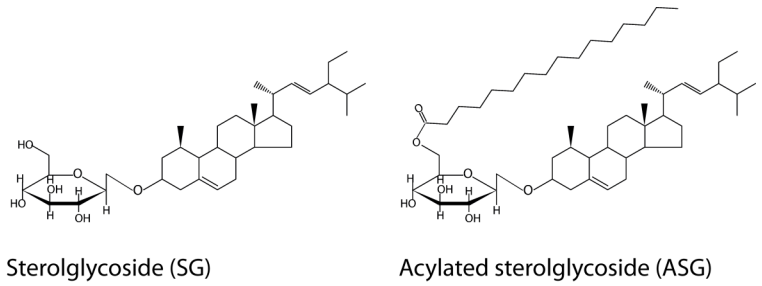


Figure 7. Structures of sterol derivatives.

4. THE PHOSPHATE CONNECTION

During phosphate-limited growth conditions, a significant proportion of the phosphate in phospholipids are used as a phosphate reserve (Essigmann et al., 1998; Härtel et al., 2000; Andersson et al., 2003; **Papers I-III**). The liberated phosphate is used in molecules and processes that cannot be replaced by phosphate-free alternaties, such as DNA, ATP or phosphorylation of proteins, to name a few. During this process the galactolipid DGDG replaces phospholipids in many different membranes. In this section I will address our and others results on phosphate limitation in the context of membrane lipids.

4.1 Phosphate limitation induces DGDG synthesis

For a long time galactolipids were believed to be restricted to plastids. By default, researchers ascribed DGDG as plastidial contamination when detected in isolated extra-plastidial membrane fractions. In the past decade it has become clear that DGDG is a native constituent of extra-plastidial membranes especially during phosphate limiting conditions (Härtel et al., 2000; Andersson et al., 2003; Jouhet et al., 2003; 2004; Gaude et al., 2004; Nakamura et al., 2005; **Papers I-III).**

DGDG is formed by glycosylation of MGDG (Dörmann et al., 1999). In Arabidopsis there are three genes responsible for MGDG synthetase, MDG1, MGD2 and MGD3 (Dörmann et al., 1999; Awai et al., 2001; Kelly and Dörmann, 2004). MGD1 is localized in the inner plastid envelope (type A) and MDG2 and MGD3 (type B) are in the outer envelope (Awai at al., 2001). In the 16:3 plant Arabidopsis, type A MDG-synthetase produces the majority of MGDG in photosynthetic tissues. Type B have a preference for eukaryotic lipids, which have the glycerol backbone assembled in the ER, and has higher expression in non-green tissue (Awai et al., 2001). Several mutants in galactolipid synthesis have been identified in Arabidopsis. In the *mdg1* mutant there was as reduction of 75% of MDGD in the leaves, indicating that MDG1 is responsible for the majority of the MGDG in Arabidopsis (Awai et al., 2001).

DGDG is synthesized by DGD1 and DGD2 (Kelly et al., 2003). In the *dgd1* mutant the amount of DGDG was reduced by 90%, indicating that DGD1 is the enzyme that synthesizes the bulk of the thylakoid DGDG during normal growing conditions (Dörmann et al., 1995). The *dgd1* mutant has a severe phenotype with stunted growth, pale leaves, reduced photosynthetic capacity and a "wrapped" thylakoid structure (Dörmann et al., 1995). The *pho1* mutant is deficient in xylem loading of phosphate and thus, no phosphate can be transported to the leaves (Poirier et al., 1991). When the two mutants dgd1 and pho1 were crossed, the DGDG levels were restored up to 60% of wild type levels (Härtel et al., 2000). This resulted in the identification of DGD2, a second gene involved in DGDG synthesis, which is highly active during phosphate-limited conditions (Kelly et al., 2003). The *dgd2* mutant showed no visible phenotype under normal growth conditions (Kelly et al., 2003). In the double mutant *dgd1dgd2,* there were minute proportions of DGDG present. Both *dgd1* and *dgd2* were null mutations (Kelly et al., 2003) and thus there must be a third enzyme with the capacity of synthesizing DGDG. This enzyme is galactolipid:glactolipid-glucosyltransferase (GGGT). This enzyme,catalyzes the formation of DGDG and DAG from two MGDG molecules (van Besouw and Wintermans, 1978). However, it is not believed to be involved in the bulk synthesis of DGDG, as DGDG synthesized by GGGT have the two galactose moieties in a different conformation of compared to "normal" DGDG (Figure 4).

All of the enzymes involved in DGDG syntheses during phosphate limitation are localized in the outer envelope: MGD2, MGD3, DGD1 and DGD2 (Kelly et al., 2003; Kelly and Dörmann, 2004). In oat, all lipids precursors for MGDG synthase are derived from the ER since there is no active prokaryotic pathway for galactolipid synthesis. The current model on how DGDG is synthesized during phosphate limited conditions is depicted in figure 8 (see also **Paper II**).

Arabidopsis, *Phaseolus vulgaris* (bean) and oat show increased levels of DGDG in roots and shoots under phosphate limiting conditions compared to fully fertilized plants (Härtel et al., 2000; Andersson et al., 2003; Li et al., 2006b; Russo et al., 2007; **Paper II**). The increase in DGDG is also evident in cell cultures of Arabidopsis and *Acer pseudoplatanus* (sycamore maple) from phosphate limiting compared to fully fertilized conditions (Jouhet et al., 2003; Jouhet et al., 2004). To further elucidate if the increase of DGDG during

phosphate limitation is a universal feature, we cultivated several additional different plant species representing both 16:3 and 18:3 plants and analyzed shoots and roots for DGDG content (**Paper II**). All shoots and roots showed an increase in DGDG content when the plants were grown on phosphate-limited media compared to fully fertilized conditions (except for Phleum vulgare). The changes in shoots were less pronounced, due to the abundance of chloroplast DGDG in shoot tissue (**Paper II**). These results significantly expand the list of plant species that increases the DGDG content during phosphate limited conditions compared to fully fertilized conditions

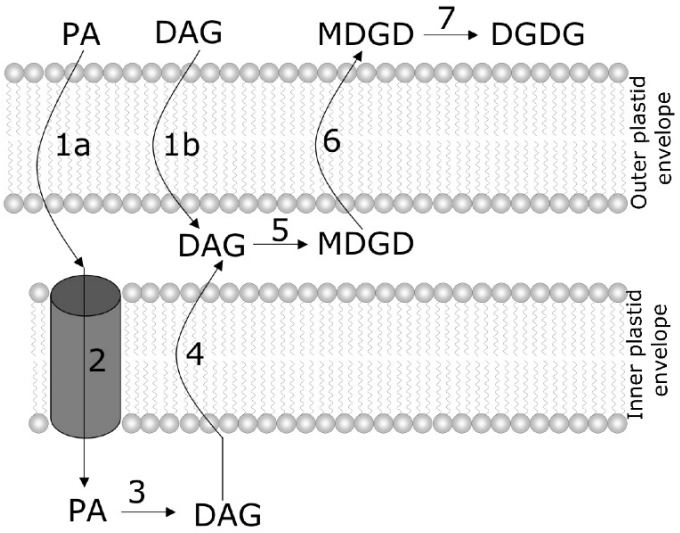


Figure 8. DGDG synthesis during phosphate limited cultivation of oats. PA and DAG removed from the plasma membrane are transported to the plastid envelope, where they are transported through the outer envelope (**1a & 1b**). PA is further transported into the stroma possibly via the TGD-complex (**2**) followed by dephosphorylation by a stroma localized PAP (**3**). DAG is then transported to the intermembrane space (**4**) where a galactose is added from UDP-galactose by type B MDGD-synthetase (**5**). After transfer of MDGD to the cytosolic leaflet of the outer envelope (**6**); a second galactose is added from UDP-galactose by dgd2 (**7**).

In Arabidopsis leaves, phosphate limitation caused an increased ratio of 16C/18C fatty acids (Härtel et al., 2000). In oat tissue there is a decrease in 18:3 and an increase in 16:0 and 18:2 fatty acids (**Paper I**) and similar changes in DGDG fatty acid composition were observed in the roots of the plants investigated in **paper II**. All investigations thus show that DGDG from phosphate limited cultivation has a fatty acid compositions more similar to phospholipids compared to thylakoid DGDG.

There are several reports on the presence of a glucose moiety in the diglycosyldiacylglycerols (Jamieson and Reid, 1976; Gaude et al., 2004; **Paper II**). It ranges from 2-24 mol% but without any real consensus as to the abundance of glucose in the diglycosylacylglycerol in plant species, tissue or growing conditions.

When lipid analyses are done on tissues, it is not possible to determine if the increase in DGDG is in the plastid or in extra plastidial membranes, but it is safe to say that the increase of DGDG in phosphate limited plants appears to be a wide-spread feature in plants.

4.2 Extra-plastidial DGDG

To further analyze the localization of DGDG in plant cells, several different techniques can be used to fractionate the plant cell into organelles and individual membrane fractions. Most fractionation protocols are based on either density (differential centrifugation) or surface properties. Aqueous two-phase partition has been extensively used to isolate highly purified plasma membranes (Kjellbom and Larsson, 1984; Palmgren et al., 1990; Andersson et al., 2003; Mongrand et al., 2004; **Papers I-III**) but it can also be used to separate several different cellular membranes using a 10-step counter current procedure (Larsson et al., 2007; **Paper I).**

In highly purified plasma membranes from oat roots, the proportion of DGDG in the glycerolipid fraction increased from 8 to 70 mol% when the oat variety Vital was grown in phosphate free media compared to fully fertilized conditions (Andersson et al., 2003). A similar response was also evident in the shoots. When the DGDG increased, all phospholipid classes in the plasma membrane decreased. in oat grown under phosphate limiting condition compared to fully fertilized cultivation conditions (Andersson et al., 2003).

In oat root plasma membrane isolated from oat grown under phosphate-limited conditions, the fatty acid composition of DGDG was very different from chloroplast DGDG and more similar to that of plasma membrane phospholipids (Andersson et al., 2003; **Paper I**.

DGDG has also been found in the mitochondrion of Arabidopsis cell cultures (Jouhet et al., 2004) and in periobacteroid membrane of nitrogen fixing nodule of soybean and lotus (Gaude et al., 2004). To asses if any additional extra plastidial membranes could replace phospholipids with DGDG, we fractioned microsomes from oat roots grown with or without phosphate with a 10-step counter current two-phase partitioning (**Paper I**). A significant proportion of DGDG was found also in the tonoplast wheras ER and Golgi membranes were clearly less affected by phosphate limitation in this respect.

Two decades ago, a few researchers found DGDG in extraplastidial membranes and did not regard it as plastid contamination due to the lack of correlation between the proportions between DGDG and MDGD which would indicated plastid contamination (Liljenberg and Kates, 1985; Rochester et al., 1987). In the recent years, DGDG is frequently detected in plasma membranes isolated from fully fertilized plants and is regarded as a natural lipid constituent of pant plasma membrane (Mongrand et al., 2004; Laloi et al., 2007; Lefebvre et al., 2007; **Papers I-III**).

A more in-depth analysis of the lipid compositions in the plasma membrane during phosphate limiting conditions is presented in **papers I and III**. Both papers show that the major changes are an increase in DGDG and a reduction of phospholipid in phosphate limited oats. Furthermore, other sugar-containing lipids increased (glucosylcerobrocides, SG and ASG). The differences in lipid compositions between **paper I and III** can probably be attributed to that different oat varieties and lipid extraction protocols were used in the two studies. Inherent problems with chloroform:methanol lipid extraction protocols are that

they do not extract all sphingolipids resulting in an underestimation of the sphingolipid content (Sperling et al., 2005). Protocols designed to extract highly polar sphingolipids from leaf tissue (Markham et al., 2006) was used to extract lipids in **paper III**.

4.3 Degradation of phospholipids

Phospholipases are a group of enzymes that hydrolyze specific bonds in phospholipids. There are four different classes; A, B, C and D and their modes of actions are shown in figure 9. Phopholipase A (PLA) hydrolyses the acyl chains in phospholipids rendering a lyso-PL and a free fatty acid. Within the PLA class there are two different types of enzyme; PLA_1, which hydrolyses the acyl chain at the *sn*-1 position and PLA_2, which hydrolyses the acyl chain in the *sn*-2 position. Furthermore, PLB has similar mode of action as PLA but has no specificity for either *sn*-1 or *sn*-2 position. Phospholipase C (PLC) removes the headgroup of PC (Nakamura et al., 2005) and phopshoinositol lipids (Pical et al., 1992). Phospholipase D (PLD; Wang, 2000) hydrolyses the bond on the other side of the phosphate producing PA and part of the headgroup (choline, ethanolamine, inositol, glycerol etc.). PLD has a second mode of action, base exchange, which exchange the headgroup of the phospholipid to a primary alcohol (Ella et al., 1994). The base exchange is also involved in headgroup conversion between PS and PE, which normally occurs in ER but also can take place in plasma membrane (Vincent et al., 1999). Phosphatidic acid phosphatase (PAP) removes the phosphate from PA resulting in free phosphate and DAG.

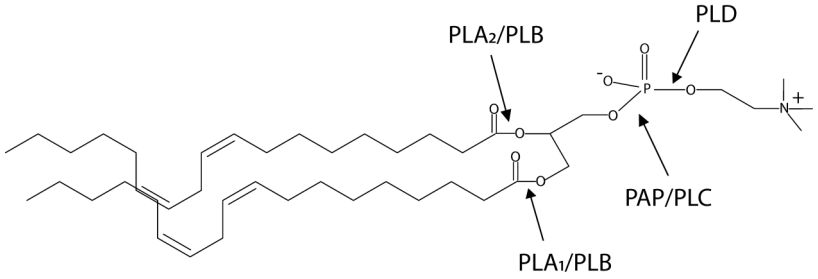


Figure 9. Mode of action for different phospholipases

Since there is a 75% reduction of the oat root plasma membrane-localized phospholipids during phosphate limited conditions ,we decided to analyze the occurrence of phospholipases in the oat root plasma membrane (**Papers I-II**). When radiolabeled PC, PE or PA were supplied to plasma membrane isolations, there was an eight to ten fold increase in PLD and PAP activities in plasma membrane isolated from phosphate limited compare to fully fertilized oat (**Papers I-II**). In Arabidopsis, phosphate limitation induced putative non-specific-PLC4 (NPC4) in the plasma membrane (Nakamura et al., 2005). The antibody, raised towards the 120 amino acids of the C-terminal of NPC4, reacted with phosphate limited oat plasma membrane, indicating that an NPC4 ortholog is also present in oat (**Paper I**). Contrary to the findings in phosphate limited

Arabidopsis; no PLC activity could be detected in fractions isolated from oat during phosphate limited (**Papers I and II**).

To identify the enzymes involved in phosphate limited oat plasma membrane, all plasma membrane proteins from phosphate-limited oat were solubilized with deoxycholate and separated with gel electrophoresis. The gel was cut into 24 pieces and proteins were extracted. Each of the fractions was assayed for PLC, PLD and PAP activity. The highest activity for PLD and PAP were located in the same fraction. In this fraction, no PLC activity could be detected but western blot analysis and mass spectrometry revealed the presence of NPC4 (Figure 8 in **Paper I**).

A complication in detecting any NPC4 activity in phosphate limited oat root plasma membrane is the differences in enzyme activity between PLD and PLC. The PLD activity in phosphate limited oat plasma membrane is 50 times higher than apparent endogenous PLC activity in the plasma membrane of phosphate limited Arabidopsis (Nakamura et al., 2005; **Papers I and II**). If NPC4 in oat has comparable enzyme kinetics to NPC4 in Arabidopsis, then any PLC activity would be masked by the PLD activity in phosphate limited oat root plasma membrane. To further investigate the presence of any PLC activity in phosphate limited oat plasma membrane, a competition assay was used. The purpose of this assay is to saturate the reaction with exogenously supplied unlabeled PA, thus diluting the radiolabeled PA produced by PLD from radiolabeled PC. With a large pool of non-radiolabeled PA and a small pool of radiolabeled PC, the production of radiolabeled DAG via PLD and PAP would be slowed down due to the competition between radiolabeled and non-radiolabeled substrates. When non-radiolabeled PA was added to radiolabeled PC, there was only a slight decrease in radiolabeled DAG formation indicating that PLC is not involved in phospholipid degradation in phosphate limited oat plasma membrane (Tjellström and Sandelius, unpublished).

In Arabidopsis, 12 different PLDs have been identified (Wang, 2005) and at least two, PLDζ1 and PLDζ2, are involved in metabolizing PC to PA in response to phosphate limitation (Wang, 2001; Li et al., 2006; Li et al., 2006b). PLDζ1 is localized in the plasma membrane while PLDζ2 is localized in the tonoplast and more specifically to an area loosely associated with mitochondria (Yamayro). Thus PLDζ1 is a likely candidate for the PLD activity in oat root plasma membrane. However, the molecular mass of PLDζ1 (123kDa) is quite different from the region on the native gel electrophoresis, where PLD activity was detected in the 50-55 kDa region (Figure 8 in **Paper I**). This indicates that there are other phospholipases than PLDζ1 involved in degrading phospholipids in oat root plasma membrane from phosphate-limited oat. Mass spectrometry analysis on fractions 10-13 (Fig 8 in **Paper I**) revealed several putative additional phosphoesterases and phospholipases from phosphate limited oat root plasma membrane. In order for the cell to utilize the phosphate in phospholipids, it needs to be liberated. PLD does not fulfill this function as it results in the formation of the phospholipid PA but there is no accumulation of PA in the plasma membrane of phosphate-limited oat (**Paper I**). In the measurement of PAP in **paper II,** the activity is not high enough to metabolize the PA produced

by PLD. In short, PLD produces more PA than PAP can handle. This is true in assays were PC is supplied exogenously (**Papers I-II**) but there is always the possibility that we misjudge the enzyme activities. Perhaps the activities of PLD and PAP will match each other *in vivo*, when the substrate resides in the plasma membrane. If the lipase assays reflect the true activities, there will be an accumulation of PA in the plasma membrane, if PA is not removed and transported from the plasma membrane to another membrane with additional PAP activity.

The similarities in fatty acid composition between plasma membrane localized phospholipids and extra-plastidial DGDG (Andersson et al.,2003; **Paper I**) infers that DGDG is synthesized on a DAG backbone that is derived from plasma membrane phospholipids; the plant recycles the DAG backbone (**Paper II**). Which lipid and how the transport proceeds between plasma membrane and the plastid envelopes, is obscure. In **paper II** we propose that PA or DAG is transported from the plasma membrane via ER (possibly via membrane contact sites). PA could be transported into the plastid stroma via the TGD complex (Benning, 2008). PA is degraded to DAG by a chloroplast inner envelope localized PAP (Lu et al., 2007; Benning, 2008). In **Paper II** we suggest that this DAG is used as the backbone for the phosphate limited induced synthesis of DGDG via MGDG synthetase type B (MDG2/3) and DGDG synthetase (DGD1/2).

There is a phospholipase activity in the plasma membrane of phosphate-limited oat even when though the absolute amount of phospholipids is strongly reduced (**Papers I-II**). Phospholipids are constantly supplied to the plasma membrane as vesicles transporting components to the membrane and vesicle cargo to the cellular exterior. This could explain the constant degradation of phospholipids in the plasma membrane in phosphate limited oat (**Paper II**).

4.4 Phosphate threshold

When plants are grown in climate chambers they are quite pampered; light, temperature, humidity and nutrients are controlled. They are never exposed to drought, wind, rain, pathogens or a cloudy day unless the experimental design requires it or unintended problems occur. To simulate phosphate-limiting conditions we omitted phosphate from the nutrient media. However, it is not very probable that plants would ever encounter such extreme growing conditions. Therefore we decided to investigate at what level of phosphate availability the phosphate limitation occurred. We have analyzed growth (roots and shoots), seed quality (starch, β-glucan lipids, soluble protein, phosphate, C/N ratio), seed quantity, phospholipase activities in the plasma membrane and DGDG/PC ratio from oat cultivated with different levels of phosphate (**Paper II and IV**). In Arabidopsis the phosphate threshold for DGDG accumulation was between 0.1 and 1 mM exogenously supplied phosphate after the Arabidopsis plants were primed by pre-cultivativtion on phosphate rich media for ten days prior to transfer to phosphate limited media (Härtel et al., 2000). Oat has a relatively large seed, compared to Arabidopsis and contain approximately 1.5 μmol phosphate (var. Belinda) (**Paper IV**). Oat is able to sustain normal shoot growth without visible symptoms for up to two weeks without supplied

phosphate. There are however clear symptoms of phosphate limitation when roots are analyzed for length, DGDG/PC ratio and plasma membrane localized phospholipase activities, which are all increased after two weeks of phosphate free cultivation (**Paper II**). However, oat is able to sustain an entire generation and produce seeds without any exogenously supplied phosphate (**Paper IV**).

In the experiments with different degree of phosphate limitation the % represent phosphate content in the nutrient solution (100% =1.5 mM phosphate). Plants grown without phosphate (0%) have shorter, thinner and more rigid leaves. In plants grown in 3% phosphate for 4 weeks, the shoots became as tall as in oat grown with 100% phosphate, but the roots showed increase length, which is an indication of phosphate limitation (Andersson et al., 2003; Li et al., 2006; **Paper II**). At 5% phosphate in the nutrient solution, the roots did not elongate more that the control plants (100%; (**Paper II**). During the first four weeks of cultivation,10% phosphate (0.15 mM) is enough to sustain similar growth as with the oat supplied with 100% phosphate (**Paper II**). During longer cultivation times, differences regarding shoot length and biomass became more pronounced and there were clear differences between the different phosphate limitation treatments (**Paper IV**).

The phospholipid-to-DGDG exchange was monitored in oat roots by measuring the DGDG/PC ratio at seven time points with ten different concentration of phosphate (**Paper II**). The phosphate threshold for oat (var. Belinda) was at 30% phosphate. With 10 % phosphate there was a clear increase in DGDG/PC ratio, after 15 days of growth. The times and nutrient solution correlated better to a PLD activity rather than a plasma membrane localized PAP activity, indicating the need for additional PAP activity to supply DAG for DGDG synthesis (**Paper II**).

Plants were also maintained in the growth chamber to complete one generation. This resulted in that that oat grown in all different levels of phosphate (0, 3, 5, 10, 30 and 100% phosphate) set seeds. After seven weeks the 0% phosphate treatment begun to set seeds and within one week the plants cultivated in 3, 5 and 10% followed. Plants grown in 30 and 100% phosphate lagged another two weeks behind. After 5 months, seeds were collected and analyzed (**Paper IV**).

Measurements of total seed yield (i.e. mass and number of seeds per plant) indicated that more than 30% phosphate does not improve the yield (**Paper IV**). Between 0 and 10%, the plant tissue (not including seeds) was almost devoid of phosphate, most of the phosphate hade been allocated to the seed (**Paper IV**). Starch content showed a gradual increase from 0-to-100% phosphate, but total % of carbon remained similar. Other parameters investigated, lipids, β-glucan, protein and C/N ratio showed small differences between the different growth conditions. It should be noted that the polar lipid composition seems to be unaffected between the seeds from the different degree of phosphate limitation. Apparently, in seeds, the phosphate allocated into phospholipids remains constant (per dry weight) regardless of the amount of phosphate in the seed. The differences in phosphate contents between the different seeds are correlated to the amount of phosphate in the water-soluble faction (**Paper IV**). It is clear

that during phosphate limiting growth condition, membranes in vegetative tissues, replace phospholipids with DGDG to utilize the phosphate elsewhere, but during seed formation, phosphate is re-allocated to phospholipids and there appears to be no phospholipid-to-DGDG replacement regardless of the degree of phosphate limitation (**Paper IV**).

4.5 The transient nature of phospholipids

To investigate if the phosphate limitation induced phospholipid-to-DGDG replacement was reversible, we re-supplied phosphate to the growth media of phosphate limited plants (**Paper II**). Lipid class analysis showed that after 7 days, the levels of all lipid classes had begun to adapt to the new growing conditions (**Paper II**). The most dramatic effect was on DGDG and phospholipids. Phospholipid content had tripled and DGDG content had halved in oat root plasma membrane after one week of phosphate resupply (**Paper II)**. Since the root mass had increased after resupplied phosphate, there would have been an increase in "new" plasma membranes that would be largely devoid of DGDG. It is therefore uncertain whether the reduction of the proportion of DGDG in is due to removal of DGDG from the "old" plasma membrane or a dilution due to the phospholipid rich "new" plasma membrane.

In experiment where radiolabel phosphate was supplied to 4 weeks old oat plants cultivated with or without phosphate there was a rapid incorporation of phosphate into phosphate limited plants compared to fully fertilized plants (**Paper II**). For phosphate limited oat the majority of the radiolabel in the lipid fraction was initially recovered in PC. At later time points PE+PG (which co-migrated on the TLC plate) became the predominantly labeled lipid classes. Uptake of radiolabel phosphate in fully fertilized plants showed that only minute proportions of the radiolabel was recovered in PC, instead almost all of the lipid incorporated radiolabel was recovered in PE+PG fraction. This corresponds well with previous reports of the turnover of PE+PG, which increased while turnover of PC decreased with age in pea leaves (Hellgren and Sandelius, 2001b). Phosphatidic acid (PA) remained at a constant low level in both treatments, probably because PA is an intermediate and plays a central role in the phospholipid synthesis pathway and are involved in many signaling events which require tight control (Munnik and Testerink, 2008).

4.6 Acyl transferase activities

Lyso phospholipids acyltransferases is a group of enzymes, which are involved in creating and maintaining phospholipid diversity (Hishikawa et al., 2008). The mode of action is an exchange of an acyl chain from a phospholipid with an acyl chain from acyl-CoA. Phosphate limited oat plasma membranes have three-fold higher lysoPC acyltransferase (LPCAT) and lysoPE acyltransferase (LPEAT) activity compared to plasma membrane from fully fertilized oat (Tjellström unpublished). This indicates that modification of exiting phospholipids in the membrane in part of the response to phosphate limitation.

It has been shown that the synthesis of ASG involves an SG acyl transferase activity (Heinz et al., 1975; Heinz, 1996). The relative proportion of

ASG is higher in plasma membrane from phosphate-limited compared to fully fertilized oats **(Paper III)**. In the former, the SG acyl transferases activity showed a three-fold increase in activity compared to the latter, using acyl-CoA as substrate (Tjellström, unpublished).

5. MOVEMENTS OF LIPIDS…

In the cell there are requirements for lipids to be transported between, across and along the membranes. The lipid compositions of plant membranes are constantly changing and any lipid compositions reported here and elsewhere are merely an average of membranes from different cell types at a specific time and growing conditions.

5.1 …between membranes

In the basic textbook model, vesicles transport both membrane constituents (lipids and membrane proteins) and cargo (proteins, ions, sugars, etcetera), between the compartments of the eukaryotic cell. Many of the membrane constituents are made in the ER and transported as vesicles to plasma membrane and tonoplast via the Golgi apparatus. Also, there is evidence emerging of a vesicle transport route, similar to the secretory pathway, inside chloroplasts (Morré et al., 1991; Räntfors et al., 2000; Andersson and Sandelius, 2004; Andersson et al., 2001). The vesicular transports to the plasma membrane influence the localization of lipid in the plasma membrane. Lipids localized in the cytosolic leaflet of the Golgi apparatus will initially end up in the cytosolic leaflets in the plasma membranes and lipids in the leaflet facing the Golgi lumen facing will end up in the apoplastic leaflet, as depicted in figure 10.

Lipid can also be transported at membrane contact sites, which are connections between membranes, where the ER membrane usually acts as one of the membranes. Mitochondria-associated membranes (MAMs) are the connections between ER and the mitochondria in animal cells (Vance, 1990) and yeast (Gaigg et al., 1995). They are involved in transporting PS to the mitochondria. After carboxylation to PE, this lipid is transported to the ER via MAM (Achleitner et al., 1999). MAM are also involved in facilitating Ca^{2+} transportation between ER and mitochondrion in HeLa and rat liver cells via a voltage dependent anion channel mediated by the chaperone glucose response protein 75 and an inositol 1,4,5-triphosphate receptor (Szabadkai et al., 2006).
The plant ER contains many discrete functional domains (Staehelin, 1997) but most of the domains are not very well characterized, thus many functions are rather speculative. Experiments using optical tweezers demonstrate that there is a physical connection between plastids and ER. The plastid associated membranes (PLAM) are probably attached via a protein-protein interaction (Figure 11). The interaction could only be disrupted after treatment with trypsine and are believed to be involved in transport of lipids to and from the ER and plastids (Andersson et al., 2007).

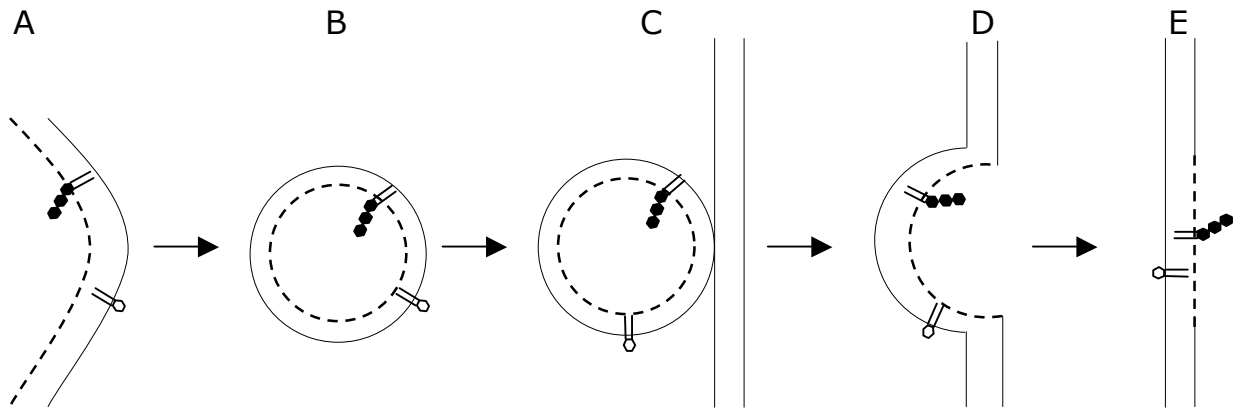


Figure 10. Generation of lipid asymmetry via the secretory pathway. Multiple sugar moieties are added to ceramides in the lumen leaflet of the Golgi apparatus (**A**). In vesicle transported from late Golgi to plasma membrane the Golgi lumen leaflet is the inside leaflet of the vesicle (**B** & **C**). Fusion with the plasma membrane results in that the Golgi lumen leaflet ends up as the apoplastic leaflet of the plasma membrane (**D** & **E**). Due to the large and polar headgroup (black hexagons) it is unlikely that the lipid can move between the leaflets. The dashed line represent membrane that originates from the lumenal leaflet of the Golgi apparatus and solid lines represent membrane that originates from the cytosolic leaflet of the Golgi apparatus.

The transport requirements, varies dependent on different circumstances. In early development there are increased requirements for import and exports of solutes and lipids between the ER and the chloroplast. There is an increase in young compared to old pea chloroplasts in lyso-phospholipid acyl transferase activity (Kjellberg et al., 2000) and furthermore, the number of contact sites between ER and chloroplasts was also higher in young compared to old Arabidopsis chloroplasts (H Tjellström, R Wellander, M Göksör and AS Sandelius unpublished). These experiments support that PLAMs are involved in chloroplast/ER transport.

The connection between ER and plasma membrane (plasma membrane associated membranes; [PAM]), was first discovered in yeast (Pichler et al., 2001) but is also present in plants, which was visualized by confocal microscopy (Figure 11; A-C from Larsson et al., 2007). Biochemical studies on PAM showed enrichment in LPCAT activity compared to the bulk plasma membrane, indicating the possible involvement of PAM in membrane remodeling (Larsson et al., 2007; Hishikawa et al., 2008).

Lipid transfer proteins are proteins that bind lipids in a hydrophobic pocket and are able to transport lipid monomers across an aqueous environment between different membranes. The lipid binding protein could work as a shuttle or as a bridge between the two membranes (Figure 12). It has been proposed that the actual connections between membranes in membrane contact sites involved lipid binding proteins.

A PLAM fraction was isolated from rapidly expanding pea leaves with a protocol initially developed for MAM isolation (Graigg et al., 1995; Andersson et al., 2007). The PLAM sub-fraction had a different polypeptide pattern and lipid

composition compared to ER and chloroplast envelope membranes (Andersson et al., 2007). In an attempt to identify any bridging-complex proteins or lipid metabolizing enzymes involved in the PLAM-chloroplast interactions, membrane factions from ER, PLAM, outer envelope (OE) and inner envelope (IE) of the chloroplast were analyzed. A few proteins, including several unidentified ones, were most abundant in PLAM, but no plausible candidates for bridging-complex have yet been identified (Bräutegam, Breuers, Tjellström, Froehlich, Ohlrogge, Sandelius and Weber, manuscript).

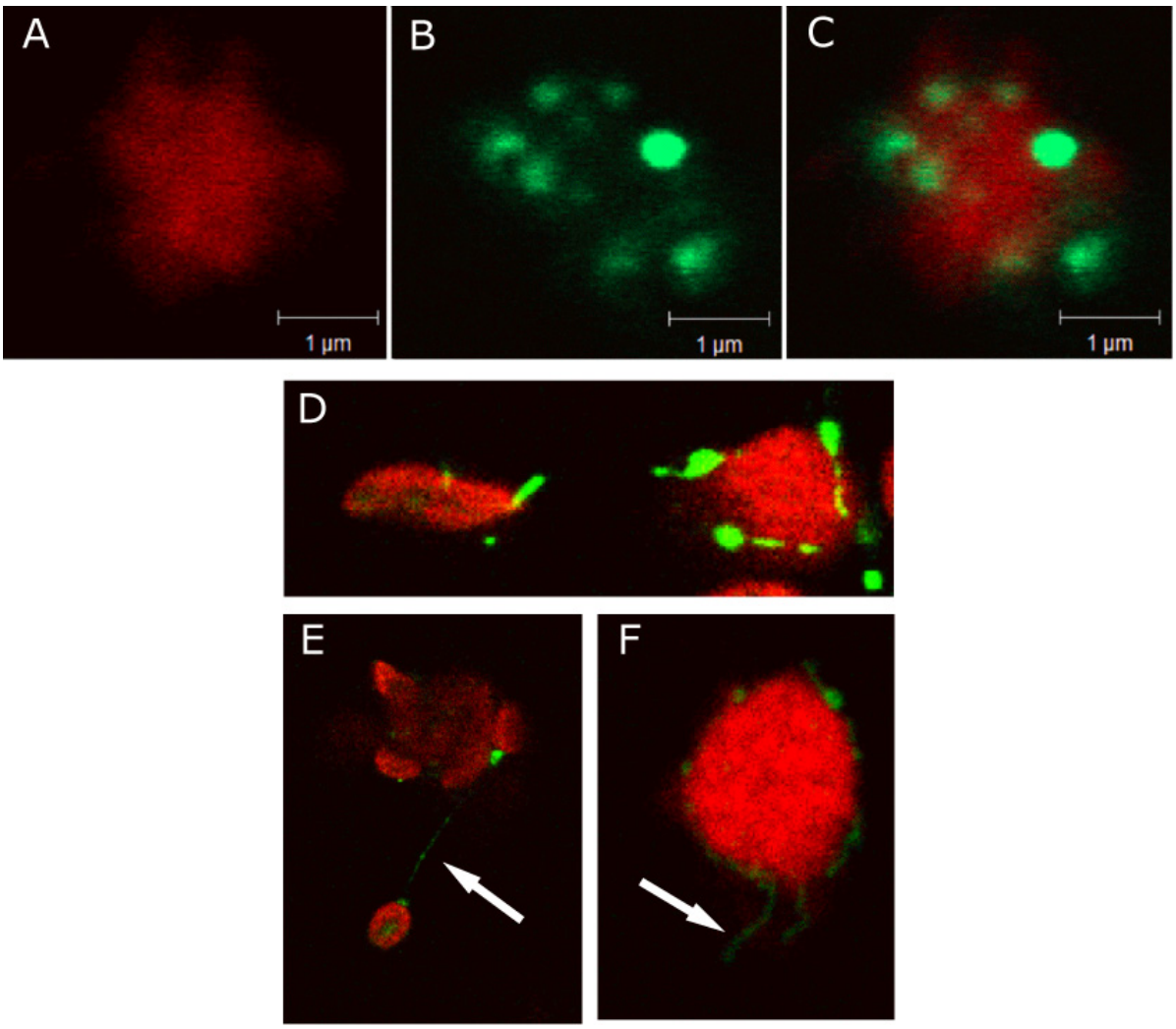


Figure 11. Visualization of membrane contact sites. Plasma membrane associated membranes (PAM) in transgenic Arabidopsis containing green fluorescent protein (GFP) in the ER lumen. Isolated plasma membrane vesicles are visualized by the red fluorescent dye FM 4-64 (**A**), ER is visualized in green by GFP in the ER lumen (**B**) and the overlay demonstrates co-localization (**C**). Plastid associated membranes (PLAM) in transgenic Arabidopsis (**D & E**) are visualized by GFP. In *Pisum sativum* (garden pea) (**F**), where the ER membrane is visualized by the green fluorescent dye $DiOC_6$ (**F**). In **C-F** chloroplasts are visualized through chlorophyll auto fluorescence. Panels A-C are from Larsson et al., (2007),BMC Plant Biology **7:** 64

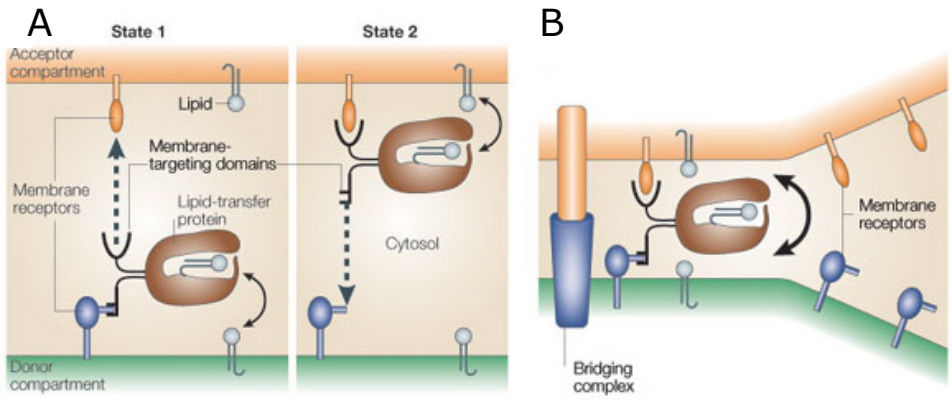


Figure 12. Possible mechanisms for lipid trafficking at membrane contact sites. A lipid transfer protein binds to a donor compartment via a membrane receptor and extracts a lipid (**A**, *state 1*). The lipid transfer protein transfers the lipid through the cytosol, binds to a receptor at the acceptor compartment and inserts the lipid into the acceptor compartment's cytosolic leaflet (**A**, *state 2*). In (**B**) a bridging complex binds to two membranes, generating a physical interaction between the donor and acceptor membrane. Lipids are transported via a lipid transport protein, bound to both compartments. From Holthuis and Levine (2005) Nature Reviews Molecular Cell Biology **6:** 209-220; with minor modifications.

5.2 …across membranes

Biological membranes are composed of two lipid leaflets with embedded and associated proteins. The two leaflets have different lipid compositions and this is important for the membrane to function as a "water-tight" barrier (Hill and Zeidel, 2000). Hill and co-workers found that the exo-plastic (apoplastic) leaflet had 18-fold reduction in water permeability compared to the cytosolic and is the major water barrier in the plasma membrane of epithelial cells (Hill and Zeidel, 2000; Krylov et al., 2001).

Lipid asymmetry is generated in the membrane as lipids are synthesized. At the site of lipid synthesis, lipids are inserted in the membrane resulting in a local increase of the specific lipid in the synthesizing leaflet. It is necessary for membranes to be able to facilitate distribution of the newly synthesized lipids within the membrane, otherwise the membrane will grow asymmetrically. According to the fluid mosaic mode proposed by Singer and Nicholson (1972), membranes are a two dimensional liquid in which the lateral movement of lipids is somewhat unrestricted. However, the transport of lipid over the membrane from one leaflet to another (flip-flop) is much slower. Spontaneous flip-flop is thermodynamically unfavorable in lipid vesicles, due to the fact that the polar headgroups have to move through the hydrophobic center of the lipid bilayer. There are however difference between different lipid species, headgroup size and polarity. As a general rule of thumb, lipids with larger and more polar headgroups, such as PC and DGDG, takes longer time for the flip-flop movement than for a lipid with a small and less polar headgroups such as ceramides (Contreras et al., 2003). In liposomes, the flip-flop movement for phospholipid analogues was drastically increased when protein helices were included. The flip-flop rates were both dependent on the type and concentration of the helices (Kol

et al., 2001, 2003). A corresponding mechanism could be present in biological membranes but it is generally assumed that the lipid asymmetry is generated by enzymes that facilitate a higher lipid transport rate than the spontaneous flip-flop movement. The enzymes that are involved in maintaining the transversal asymmetry of phospholipids are called flipases and flopases. Flipases move lipids from the cytosolic leaflet to the non-cytosolic leaflet and flopases move lipids from the non-cytosolic leaflet to the cytosolic leaflet. Several enzymes that are involved in maintaining the PL asymmetry have been identified from human, yeast cell (Devaux et al., 2006). To date, only one has been identified in plants, ALA1. It is believed to be involved in aminophospholipid (PE and PS) translocation in Arabidopsis during cold stress (Gomes et al., 2000). In erythrocytes, PS is almost exclusively located in the cytosolic leaflet. As a result of ageing or cell death PS accumulate in the exoplasmic leaflet and this results in removal of the erythrocytes by macrophages (Zachowski, 1993). Scramblases are the third class of lipid translocators that transports lipids across membranes and as the name infers, they scramble/shuttle the lipids unspecifically between the cytosolic and the apoplastic leaflet (Zwaal et al., 2005).

The modes of action for the flipase, flopase or the scramblases are not known, there are two models that appeal to me see figure 13 (Vishwakarma et al., 2005). In the first model a membrane protein is modified so that the polar lipid headgroup can interact with the transmembrane region, which enables movements of lipids from cytosolic to apoplastic leaflet through lateral movement in the membrane (Figure 13A). The other model proposes that a protein forms a pore in the membrane where the polar headgroup can move through the pore while the acyl chains are inside the membrane (Figure 13B). The selectivity of the transporters is probably based on headgroup size, shape and polarity. The mechanism presented in figure 13A could be involved in reducing tension between the two leaflets for examples during lipid synthesis, which cause asymmetric lipid distribution. It is probably more rapid compared to the pore model but with reduced selectivity.

Under phosphate limited growing conditions, oat root plasma membrane retains the phospholipid asymmetry (**Paper III**) but the proteins involved here are not yet identified. Ongoing proteomics of plasma membrane isolated from oat grown with and without phosphate using iTRAQ profiling will hopefully reveal candidate proteins.

A key step in assessing lipid asymmetry in the plasma membrane is to isolate highly purified plasma membranes exposing either the cytosolic or the apoplastic (exofacial) leaflet. For plant plasma membranes this is accomplished with aqueous polymer two-phase partitioning (Palmgren et al., 1990).

It is possible to deduce the lipid asymmetry in membranes by analyzing the rate by which a fluorescent lipid analogue is translocated between the two leaflets. The environments inside and outside the membrane vesicle are different; in one the fluorescent probe fluoresces and in the other the fluorescence is quenched. By comparing the differences in transportation rate; both from inside to outside and vice versa it is possible to deuces the asymmetry of the lipid (Zachowski, 1993; Lenoir et al., 2007).

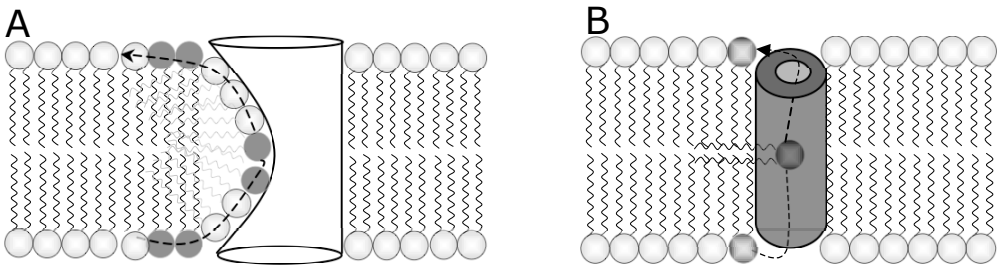


Figure 13. Models for transversal movements of lipids. A protein interacts with the polar headgroups so that the two leaflets connects, which facilitates transversal movement via lateral diffusion (**A**). In (**B**) a protein pore shields the polar headgroup from the hydrophobic interior during the lipid movement between the leaflets (**B**).

An alternative method is to modify the lipids with chemicals or enzymes (Zachowski, 1993). The labeling agent is added to vesicles exposing the cytosolic leaflet and vesicles exposing the apoplastic (exofacial) leaflet. The amount of labeling agent is quantified in both vesicle populations, which corresponds to the relative distribution of the analyzed lipid between the leaflets (Zachowski, 1993). To determine the lipid distribution over the plasma membrane we used lipid specific antibodies, chemical labeling and a phospholipases assay using PLA_2 (**Paper III**). When two different surfaces are labeled the membrane environments will inherently be different. The two vesicle populations expose different surfaces and present different environments to the agent and thus there are two different environments for the agent to work in. Therefore there are uncertainties in interpreting asymmetry.

In animals, glycolipids are primarily localized in the exofacial leaflet of the plasma membrane. The reason for this originates from the site of synthesis of complex sphingolipids in animal cells two or more sugar-moieties in the headgroup (Jeckel et al., 1992; Schulte and Stoffel, 1993; Burger et al., 1996). They are added to the lipid backbone in the lumenal leaflet of the Golgi apparatus and due to the large and polar headgroup, they cannot spontaneously transverse the Golgi membrane (van Meer and Holthuis, 2000). The sphingolipid transport from Golgi to the plasma membrane occurs via vesicular transport or via transfer proteins (Holthuis and Levine, 2005). The Golgi lumen leaflet will end up as the apoplastic leaflet and the cytosolic Golgi leaflet becomes the cytosolic leaflet of the plasma membrane (Figure 10).

The transmembrane distribution of DGDG and GlcCer were analysed by lipid specific antibodies (**Paper III**). DGDG was almost exclusively localized in the cytosolic leaflet. With the assumption that a lipid with multiple sugars primarily localizes in the apoplastic leaflet (c.f. above), the almost exclusive cytosolic localization of DGDG was unexpected (**Paper III**). When the site of synthesis of DGDG and possible transports routes are taken into account the localization of DGDG makes more sense. DGDG is synthesized in the cytosolic leaflet of the outer plastid envelope (Froehlich et al., 2001; Kelly et al., 2003), possibly

followed by transport to the outer leaflet of the ER via contact sites. Regardless of whether the transports from the ER to the plasma membrane are via PAM (Larsson et al., 2007) or through a vesicular mechanism (Bonifacino and Glick, 2004), DGDG will end up in the cytosolic leaflet of the plasma membrane, at least 90% in root plasma membrane in phosphate limited oat.

For GlcCer, the distribution in the plasma membrane shifted towards the apoplastic leaflet but in phosphate limited oat the majority of GlcCer was now localized in the cytosolic leaflet (**Paper III**), which agrees with the literature on animals that GlcCer, which can transverse membranes and are present in both membrane leaflets (van Meer and Holthuis, 2000).

Most of the information on phospholipid asymmetry over the plasma membrane is derived from animal cells (Lenoir et al., 2007) and even though there are some general "truths" such as that aminophospholipids are primarily localized in the exofacial leaflet, there are not really any consensus between different species, tissues and cell types (Devaux, 2006).

Phospholipid asymmetry was analyzed by assessing the degradation rate in an assay when the substrates (i.e. endogenous phospholipids) were limiting when exogenous phospholipase was added (**Paper III)**. The leaflet with the highest concentration of phospholipids will also have the faster reduction of phospholipid. After evaluating PLA_2 and PLD as possible phospholipases it was decided that PLA_2 was more suitable. The main reason was that we had obseved an increased PLD activity in the plasma membrane of phosphate limited oat (**Papers I and II**) which could have interfered with the measurements. In our study, no endogenous PLA_2 activity was detected in either plasma membrane isolated from oat grown with or without phosphate.

The only previously published study on phospholipid asymmetry in plant plasma membranes showed no differences between the cytosolic and apoplastic leaflets of mung bean hypocotyl plasma membrane (Takeda and Kasamo, 2001). In oat, there is an asymmetrical distribution of phospholipids in root plasma membrane, where 65 % are localized in the cytosolic leaflet. When oat is grown without phosphate in the nutrient solution, only 25 % of the phospholipids remain compared to fully fertilized oats, but the phospholipid distribution between the plasma membrane leaflets remained the same as in the fully fertilized oat (**Paper III**). As phospholipid degradation is accompanied by DGDG accumulation, it would be logical to expect that the DGDG replaces the phospholipids, but this was not the case. The phospholipids are reduced from both sides of the plasma membrane but the replacing DGDG only accumulate in the cytosolic leaflet (**Paper III**).

The asymmetry of sterols and sterol derivatives has not been studied extensively in either animal or plant cells. The polar headgroup in free sterols (-OH) is small, which allows the lipid to move through the hydrophobic area of the lipid bilayer. Due to sterol-sphingolipid interactions (Ikonen, 2008) free sterols are believed to have an asymmetry shifted towards the exofacial leaflet (Devaux et al., 2006). However a recent reports indicate that the majority of cholesterol (60-70 %) is located in the cytosolic leaflet of in the plasma membrane of Chinese hamster ovary cells (Mondal et al., 2009). Sterol derivatives in plant

plasma membranes such as SG and ASG, in plant plasma membranes have a glucose moiety at the 3'-hydroxyl group. This results in a larger and more polar headgroup than free sterols, which hinders movement between the leaflets.

To assess sterol distribution the chemical agent Filipin III was used. Filipin III labeling could not discriminate between free sterols and sterol derivatives (ASG and SG; Kleinschmidt et al., 1972; **Paper III**), so we could only obtain the information on the total distribution of the sterols backbone (total sterols) for the oat plant plasma membranes (**Paper III**). At high concentration (>10mg/mL) and long incubation times (>15 min) with Filipin III, lipid vesicles become leaky (Kleinschmidt et al., 1972). It has been shown that under these conditions, Filipin III can react to sterols located on the inside of the plasma membrane vesicles. We used a short incubation time (5 min) and a low concentration of Filipin (2 μg/ml), which according to experiments on liposomes, does not permeate the membrane (Kleinschmidt et al., 1972; **Paper III**). The distribution on total sterols was largely unaffected by phosphate limited growth. Since we are unable to discriminate between the different sterol classes (free sterols, SG and ASG), it is possible that individual sterol classes display other asymmetries than the bulk sterols. There is a slight increase of ASG in the plasma membrane of phosphate-limited oats and since total sterols are more abundant in the apoplastic leaflet there is probably an increase in ASG in the apoplastic leaflet in plasma membrane in phosphate-limited oat compared to fully fertilized oat. **Paper III** clearly shows that during phosphate limited conditions, phospholipids are replaced by different lipids in the two plasma membrane leaflets: DGDG in the cytosolic leaflet and ASG in the apoplastic leaflet. ASG has a smaller headgroup and DGDG has a larger headgroup than most phospholipids they are replacing. With increased proportion of DGDG in the cytosolic leaflet and increased ASG in the apoplastic leaflet then the plasma membrane will get an increased ability to bend inwards and perhaps facilitate endocytosis (Figure 1).

5.3…along membranes

The lateral lipid movements are, contrary to the transversal lipid movement, relatively unrestricted. Membranes are not a homogeneous "liquid" were proteins reside. Lipids are also organized along the membrane. In a lipid mixture, certain lipids tend to aggregate into patches, both large and small.

When cells were labeled with florescent probes, sensitive to the order of the lipid environment, the fluorescent probe associated to membranes domains with higher liquid ordered phase (Lo) compared to the rest of the membrane, which are more liquid disorder (Ld; Kim et al., 2007). In a Lo phase the lipid bilayer is more rigid and ordered compared to Ld. In model membranes sterols and sphingolipids have a tendency to cluster together and induce Lo. Patches in Lo-phase in the plasma membrane, are usually referred to as rafts (Simons and Ikonen, 1997). In the plasma membrane, rafts are ascribed to be involved in numerous functions, such as exocytosis, endocytosis, cytoskeleton organization, signaling (Simons and Toomre, 2001; Parton and Richards, 2003; Salaun et al., 2004).

The size, lipid and protein composition of the different phases (Lo and Ld) in the membrane varies depending on tissue and species and there is no real consensus on how many or how large they are or even if they exist in other membranes besides the plasma membrane. Labeling with the fluorescent dye Laurdan indicate that Lo domains can comprise of as much as 38 % of the plasma membrane of human cell line A431 (Kim et al., 2007).

When plasma membranes are treated with high concentration of non-ionic detergent (usually 1% Triton X-100) at 4°C, certain membrane portions are resistant to the detergent and remain unsolubilised. This membrane fraction is usually referred to as detergent resistant membrane (DRMs) (Simons and Ikonen, 1997). Ever since the first DRMs were isolated and characterized there has been a debate whether DRMs actually represents membrane rafts or if they are artificial membrane fractions created as a result of the treatment with detergent at low temperature (Munro, 2003; Lingwood and Simons, 2007). The main evidence that DRMs are a representative *in-vitro* fraction of rafts is that lipids that induce Lo domains are found in both rafts and DRMs (Munro, 2003). In DRMs there is an enrichment of sterols and sphingolipids, although one report indicates that glycosphingolipids are not essential for the formation of DRMs (Ostermeyer et al., 1999). These lipids induce Lo phase in model membranes when analyzed with Lo phase sensitive probes (Duggan et al., 2008).

Several reports on plant DRM proteomics have revealed that several signaling proteins are raft-associated (Shahollari et al., 2004; Borner et al., 2005, Morel et al., 2006; Lefebvre et al., 2007). The mechanisms or physical constrains that guide raft-resident proteins to rafts are not completely understood. The thickness of the Lo phase domains in liposomes was calculated to 48 Å compared to 39 Å for the Ld by using lipid mixtures known to induce Lo and Ld respectively in combination with transmembrane helices with different length (McIntosh et al., 2003; Allende et al., 2004). It has been proposed that the thickness of the membrane would guide helices of specific length of either Lo or Ld membranes. However, experimental data on both plasma membrane (Ld) and DRMs (Lo) on tobacco plasma membrane and DRMs show that the transmembrane helixes have similar length (20-21 amino acids) and similar hydrophobicity according to the GRAVY index (Morel et al., 2006).

Interactions between saturated acyl chains of glycerolipids, sphingolipids and sterols are the basis for the stability of rafts and DRMs. Hydrogen-bonding between the headgroups and hydrophobic interactions are the major chemical interactions that hold rafts together (Ikonen, 2008). The relative small polar headgroup of sterols (hydroxyl-group) is insufficient to shield the sterol ring structure from the aqueous environment. Close interactions with neighboring lipids with large headgroups provide additional shielding. Large headgroups like PC can provide shielding for two sterols while smaller headgroups like PE only provide sufficient shielding for a single sterol (Ikonen, 2008).

The stability of rafts and DRMs is ascribed to sterols and sphingolipids. Methyl-β-cyclodextrin is an oligosaccharide that binds sterols, and it can be used to extract sterols from the membrane. Experiments using cyclodextrin have illuminated the importance of sterols in raft/DRM stability (Roche et al., 2008).

Some reports indicate that cyclodextrin also can bind phospholipids (Quinn and Wolf, 2009), but when cyclodextrin was applied to plasma membranes from BY2 cell cultures, only free sterols were extracted to any significant extent (Roche et al., 2008) As a result of the sterol depletion, the detergent resistance of the DRMs were abolished (Roche et al., 2008). There were only minor proportions of sterol derivates extracted by cyclodextrin. The functions of ASG and SG are not well understood, but they can reduce the phase transition and modulate the fluidity in lipid bilayers (Mudd and Kleinschmidt, 1970).

One of the objectives in **paper III** was to investigate what happens to the phospholipids in DRMs when the DRMs are isolated from plasma membrane from phosphate limited oat. Is there a phospholipid-to-DGDG exchange in DRMs? We examined DRMs isolated from phosphate limited oat root plasma membrane and found that the phospholipid to DGDG replacement that is evident in the bulk plasma membrane does not occur in DRMs. Instead of DGDG, phospholipids were replaced by ASG (**Paper III**).

In DRMs isolated from phosphate limited oat root plasma membrane only 5% of the lipids were glycerolipids compared to 15 % of the DRM lipids in fully fertilized oat. This did not influence the yield of the DRM isolation, both plasma membranes had an average yield of 10% (w/w, protein) indicating that the integrity of the DRMs are not influenced by the proportions of glycerolipids (**Paper III**). It is clear that DGDG is less associated to DRMs than phospholipids. Weather this is due to the headgroup or the higher degree of unsaturation in the acyl chains in DGDG compared to phospholipids is in part addressed in the next section on lipid-lipid interaction.

It is likely that DRMs represent a fraction of the plasma membrane with many similarities to rafts, regarding lipid and protein composition, but not enough information is available to conclude that DRMs actually are isolated membrane rafts. At best, DRMs represent a mixture of all rafts in all cell types used for the fractionation. Thus, the DRMs presented in **paper III** are a mixture of all rafts in all root cell plasma membranes.

6. LIPID-LIPID INTERACTION

Lipids interact with other lipids and proteins to generate a biological membrane Lipid-lipid interactions can be measured between headgroups or between acyl chains, but the headgroup influence how the acyl chains interact with each other and vice versa. The extent of the Lo (rafts) or Ld can be detected with fluorescent probes sensitive to the lipid order such as Laurdan (Parasassi et al., 1991) or bis-pyren probes (Sunamoto et al., 1980; Xiang, 1993). Laurdan senses the dipole moment of water molecules in its vicinity, which is correlated to the free volume between the headgroups of the lipids (Parasassi et al., 1991; Parasassi et al., 1998). *Bis*-pyren probes sense the order between the acyl chains in the lipid bilayer. In DGDG rich liposomes, there is a reduced order both between the headgroups (Szilágyi et al., 2008) and between acyl chains (Hincha et al., 1998; **Paper III**) compared to a liposomes with low DGDG content. The

studies of model membranes with DGDG showed that DGDG increases membrane permeability of solutes compared to PC, but not as much as MGDG (Hincha et al., 1998). Beside the difference in headgroup moieties between phospholipids and galactolipids, there are differences in the degree of unsaturation of the acyl chains. In chloroplast MGDG and DGDG the majority of acyl chains are 18:3 and 16:3 (Mongrand et al., 1998) and a high degree of unsaturation have a destabilizing effect on membranes (Popova and Hincha, 2005). Plasma membrane phospholipids contain on the other hand primarily 16:0 and 18:2 acyl chains (Rochenster et al., 1987; Norberg and Liljenberg, 1991; Andersson et al., 2003; **Paper I)**

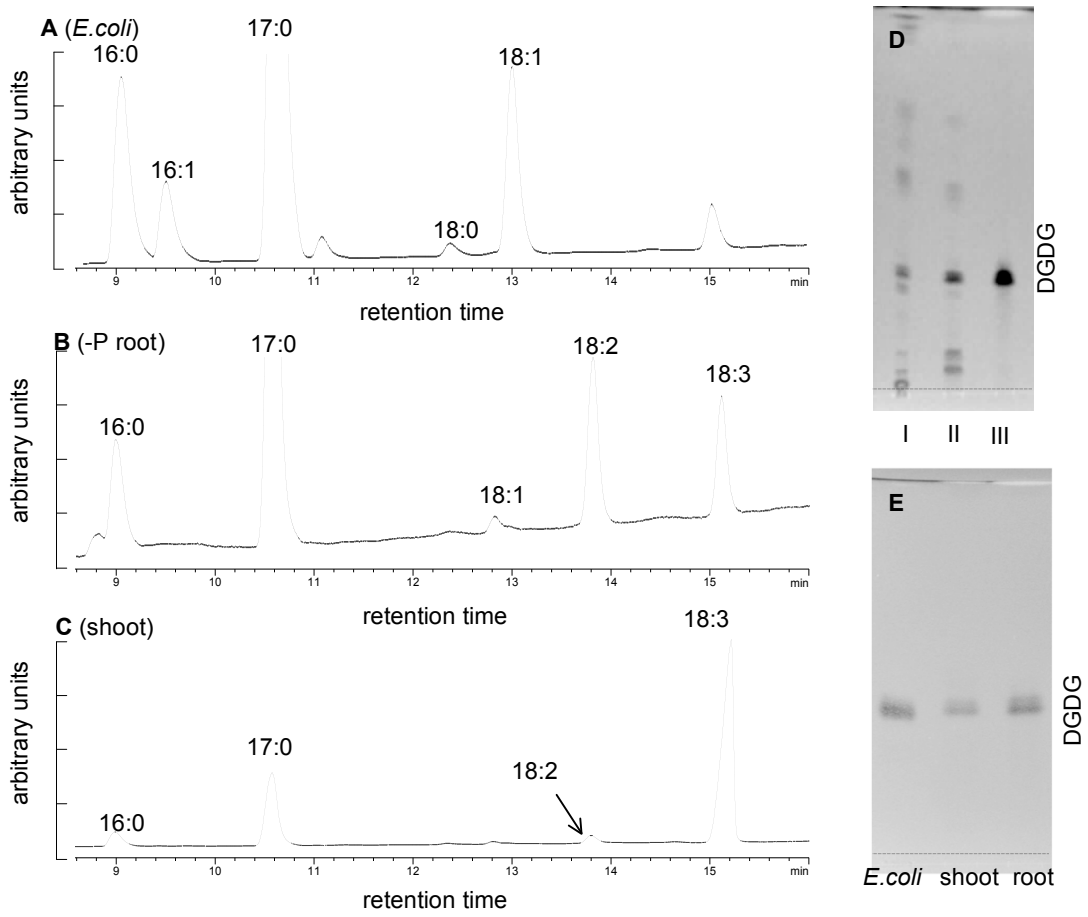


Figure 14. Different types of DGDG. The acyl chain composition of DGDG isolated from transgenic *E.coli* (**A**) roots from phosphate limited oat (**B**) and shoots for fully fertilized oat (**C**). Panl **(D)**, shows the stepwise purification of root DGDG on TLC; I: crude lipid extract, II: after solid phase extraction-purification and III: after preparative TLC. **Panel E,** HP-TLC of the three different DGDGs. DGDG was identified by co-chromatography of authentic lipid standards. Tabulated values of acyl chain compositions are presented in **Paper III.**

Bis-pyrene probes contain pyrene moiety (four fused benzene rings) attached to the end of each acyl chain of a lipid molecule. Pyrene probes respond to the free volume between the acyl chains in the lipid bilayer (Sunamoto et al., 1980; Xiang, 1993). More free volume between the acyl chains correlates to a

less ordered membrane, which reflects a membrane that is also more permeable to small molecules (Xiang, 1993). The probes can assss the acyl chain order at different depths in a liposome monolayer.

In **paper III**, PC with pyrene probes that sense below C9 or C16 were used. To provide DGDG batches with different acyl chain compositions for the studies on DGDG behavior in liposomes, DGDG was isolated from transgenic *Escherichia coli* (Wikström et al., 2009), shoots of fully fertilized oat and roots from phosphate-limited oat (Figure 14). All three batches of DGDG lowered the order between the acyl chains in PC liposomes and the effects were most pronounced with the shorter *bis*-pyren probe sensing at a shallow depths in the liposome leaflet. However, importantly, DGDG isolated with the acyl chain composition most similar to plasma membrane phospholipids (from phosphate limited oat roots) had the least impact (**Paper III**).

Since both DGDG and free sterols reduce acyl chain order, it was concluded in **paper III** that a membrane with high DGDG and free sterol contents in the same leaflet would create a membrane with too low acyl chain order. This would result in membrane that would be more permeable to solutes, which would be detrimental to the cell (Xiang, 1993). In the plant plasma membrane this is avoided as DGDG and sterols are enriched in opposite leaflets in the root plasma membranes (**Paper III** c.f. above).

DGDG has a larger headgroup than phospholipids and this influenced and reduced the acyl chain order in the cytosolic leaflet of phosphate limited oat compared to fully fertilized oat (**Paper III**). To compensate the reduced acyl chain order in the cytosolic leaflet it is possible that the increase of ASG in the apoplastic leaflet increases the order in the apoplastic leaflet.

7. FINAL REMARKS

Some of the questions in this section have been addressed in previous sections but I wish to highlight what I see as the most interesting questions and ideas that have been raised from the work presented.

What is the function of DGDG in the plasma membrane? We know that during phosphate limited growth it substitutes phospholipids (Andersson et al., 2003; **Papers I-III**). DGDG has also been detected in fully fertilized plasma membrane in many studies from different plants and tissues, (Liljenberg and Kates, 1985; Norberg and Liljenberg, 1991; Andersson et al., 2003; Mongrand et al., 2004; Lefebvre et al., 2007; **Papers I-III**).There is some evidence that points to that it could function as an acyl chain donor to SG to produce ASG (Heinz et al., 1975; Potocka and Zimowsk, 2008). ASG is one of the lipids that have been neglected in the plant lipid field. It is synthesized by acylation of SG, but the localization of the enzyme(s) and the identity of the substrate(s), which donates the acyl chain to SG, seem to vary from species to species. As far as ASG function and how it behaves in a membrane environment, the information is almost non-existent. The small headgroup of ASG hints that it is likely be a cone-shaped lipid. It would be interesting to see which lipids ASG associates within a

liposome preparation, does it partition into liquid order or liquid disorderd domains?

The identities of the lipids transported during the phospholipid-to-DGDG are still not identified. Although acyl chain composition of plasma membrane localized DGDG from phosphate-limited oat have similarities to phospholipid acyl chain composition, it is not proven that the glycerol backbone from phospholipids are used for DGDG synthesis. Also the transport routes and the identity of the transported lipids (PA, DAG) are not yet identified. By following the phospholipid glycerol backbone it would be possible to elucidate the fate of the glycerol backbone during phosphate limited growth. It is also not known what happens to plasma membrane localized DGDG when phosphate is resupplied to phosphate-limited oat. In addition to a dilution effect (cf. above), there is also the possibility that DGDG is degraded by galactolipases and removed from the plasma membrane.

In animals, DAG binds to protein kinase C which activates a cellular response, but protein kinase C have not been found in plants, so the function of DAG as a signaling molecule or as a membrane constituent is largely unknown. Under normal growing conditions, any DAG in the plasma membrane is rapidly phosphorylated by DAG-kinase, rendering PA (Munnik and Testerink, 2008) With a increase in PAP activity in phosphate limited oat plasma membrane this would result in a futile cycle where DAG is phosphorylated and PA is de-phosphorylated and it seems highly unlikely that this would be the case.

One of the more annoying questions that I have entertained for a long time now is: what is the phosphate liberated from the phospholipids used for? My usual answer is that it is used for molecules, which have no suitable non-phosphate containing alternative, such as ATP and DNA. I believe that this hold true to some extent but it would be worth wile to investigate what the initial phosphate, liberated from phospholipids, is used for.

8. ACKNOWLEDGMENTS

Over the past years, during my time at Botan there are many people that have helped and encouraged me whom I wish to extend my gratitude to.

None of this would have been possible without the excellent guidance and support given to my by my supervisor **Anna Stina Sandelius**. Thank you for your endless enthusiasm, regarding science in general and membranes and lipids in particular. In our Tuesday meetings, you have engaged me in scientific discussions, helping me to develop my scientific thinking, without spoon-feeding me. Thank you for all your hard work in editing and proofreading posters, manuscripts and thesis.

Mats Andersson for taking me under your wing when I first arrived in the lab, where you have been my lab-supervisor, providing useful hints and advice and for fruitful collaborations and for proof reading my thisis.

Åke Wieslander for introducing me to the biophysical aspects of membrane biology and for helping me to design the pyrene experiments. For your insightful comments and suggestions regarding recent and past literature and also for proofreading my thesis. Thank you **Mats Ellerstöm and Lennart Adler** for your help and involvement in the annual reference group meeting.

Thank you **Lars Hellgren** for sphingolipid analysis and **Karin Larsson** and **Tobias Porsbring** for fruitful collaborations and all the other people that have come to work in the lab with their various lipid projects: **Olga, Johanna S, Melanie, Mats B and Robert.**

John Ohlrogge and the people in his lab who took care of me during my visit to East Lansing and MSU; **Phil, Mike, Doug, Tim, Isabel, Katrin, Weli, Shannon, Dezi, Fred and Youghua.**

Working at Botan is more than science. Time spent on lunches, coffee breaks, parties, pubs or just chatting in the hallways always feels like time well spent. Thank you **Lars Sjögren** for walks in the snow along the "prison yard" at Tegenzee. Along with your passion for metal and concerts you are a great friend to me. Thank you, **Johan Fredin** for the times when we were roommates and for wonderful sport discussions. Thank you, **Anders**, **Patrik**, **Fredrik**, **Henrik A** and **Adrian** for interesting discussions at coffee breaks and lunches. Thank you **Jossan, Sara** and **Johanna B** for organizing various social events. Thank you **Sven**, **David**, **Inga**, and **Niclas** for helping me with this, that and the other.

I also wish to thank my friends and family whom make me flourish in their proximity

Johan Flykt for being my eternal wingman, to share pain, sweat, lactic acid, numb thighs, cramps and other sensations that makes riding road bikes the best thing ever. For many great moments at Statoil Hellingsjö, where they have the good candy, and other fantastic gas stations that we have visited on our bike tours to Varberg, Borås, Ullared Hällingsjö, the 11% climb at Edsås or round Vättern. For our endless debates regarding various topics; insurance companies, TV-license or why trams don't take visa.
Jonas Stenberg for pushing me to do things that I normally would not do. We accumulated many fun stories to tell in Thailand, cab ride to Fisherman's Village and back to Bangkok (although you slept most of it), camping at the beach on the outskirts of Phi-Phi and the escalator at NaNa plaza. Thank you for the time that we lived together, it was a lot of fun and very kind of you to take me in and to share your friends and life with me. You are the most generous and giving person I will ever know! It pains me to say that in the end you are right, --"det löser sig".

Friends that have stuck by my side since primary school whom I don't hang out with enough, but when we meet we have a connection that make me feel truly blessed:
Thank you; **Mikael Wigart** for always encouraging me to come to England and visit. whether we meet in Hull drinking pangalactic gurgleblasters at Spiders or in Bristol drinking rum from Guatemala or at the olympic stadium in Athens I always have the best of times when I am with you; **Victor Stålblad** for the years at Kobbarnasväg, having a drink of lemonade at your place (which flavor?) or a coffee at Mauritz to discuss our latest and somewhat elitist insights into shoes; **Marin Hennung** for wonderful memories playing "brödra glofen", pinball at Beckmans and snooker at Paul's billiards; **Theo Gabay** for our love of movies and for our ability to end up at the same place in the world (London or mid-west America) at the same time without knowing it; **Constantin Primo** for the long nights with Resident evil, slashing zombies like there's no tomorrow.

Thank you **mom** and **dad** for raising me in an environment full of love, encouragement and support. My older sister **Katarina Egg-Olofsson** and her family, **Måns**, **Agnes**, **Sofia** and **Martin** and my older brother **Thomas Tjellström** and his family **Camilla**, **Julia** and **Gustav** who always look out for me and engaging me for various building and digging projects over the years, which I really enjoy.

Financial support was provided from the Swedish Council for Forestry and Agricultural Research (FORMAS), The Royal Society of Arts and Sciences in Göteborg (KVVS) and Carl Trygger's Science Foundation. PA Larssons fond, Adlerbertska forskningsfonden and Hvitfeldtska stiftelsen

9. REFERENCES

Achleitner G, Gaigg B, Krasser A, Kainersdorfer E, Kohlwein SD, Perktold A, Zellnig G, Daum G (1999) Association between the endoplasmic reticulum and mitochondria of yeast facilitates interorganelle transport of phospholipids through membrane contact. Eur. J. Biochem. **264:** 545-553

Allende D, Vidal A, McIntosh TJ (2004) Jumping to rafts: gatekeeper role of bilayer elasticity. Trends Biochem. Sci. **29:** 325-330

Andersson MX, Göksor M, Sandelius (2007) Optical Manipulation Reveals Strong Attracting Forces at Membrane Contact Sites between Endoplasmic Reticulum and Chloroplasts. J. Biol. Chem. **282:** 1170-1174

Andersson MX, Kjellberg JM, Sandelius AS (2001) Chloroplast biogenesis. Regulation of lipid transport to the thylakoid in chloroplasts isolated from expanding and fully expanded leaves of pea. Plant Phys. **127:** 184-193

Andersson MX, Kjellberg JM, Sandelius AS (2004) The involvement of cytosolic lipases in converting phosphatidyl choline to substrate for galactolipid synthesis in the chloroplast envelope. Biochim. Biophys. Mol. Cell Biol. Lipids **1684:** 46-53

Andersson MX, Sandelius AS (2004) A chloroplast-localized vesicular transport system: a bio-informatics approach. BMC Genomics **5**

Andersson MX, Stridh MH, Larsson KE, Lijenberg C, Sandelius AS (2003) Phosphate-deficient oat replaces a major portion of the plasma membrane phospholipids with the galactolipid digalactosyldiacylglycerol. FEBS Letters **537:** 128-132

Awai K, Maréchal E, Block MA, Brun D, Masuda T, Shimada H, Takamiya K, Ohta H, Joyard J (2001) Two types of MGDG synthase genes, found widely in both 16:3 and 18:3 plants, differentially mediate galactolipid synthesis in photosynthetic and nonphotosynthetic tissues in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. U.S.A **98:** 10960-10965

Awai K, Xu CC, Tamot B, Benning C (2006) A phosphatidic acid-binding protein of the chloroplast inner envelope membrane involved in lipid trafficking. Proc. Natl. Acad. Sci. U.S.A **103:** 10817-10822

Bates PD, Ohlrogge JB, Pollard M (2007) Incorporation of Newly Synthesized Fatty Acids into Cytosolic Glycerolipids in Pea Leaves Occurs via Acyl Editing. J. Biol. Chem. **282:** 31206-31216

Benning C (2008) A role for lipid trafficking in chloroplast biogenesis. Progr. Lip. Res. **47:** 381-389

Benveniste P (2004) Biosynthesis and accumulation of sterols Annu. Rev. Plant Biol. **55:** 429-457

Bligh E, Dyer W (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 911-917

Bonifacino JS, Glick BS (2004) The mechanisms of vesicle budding and fusion. Cell **116:** 153-166

Borner GHH, Sherrier DJ, Weimar T, Michaelson LV, Hawkins ND, MacAskill A, Napier JA, Beale MH, Lilley KS, Dupree P (2005) Analysis

of detergent-resistant membranes in Arabidopsis. Evidence for plasma membrane lipid rafts. Plant Phys. **137:** 104-116

Bretscher M, Munro S (1993) Cholesterol and the Golgi apparatus. Science **261:** 1280

Brown L, Rosner B, Willett WW, Sacks FM (1999) Cholesterol-lowering effects of dietary fiber: a meta-analysis. Am J Clin Nutr **69:** 30-42

Browse J, McConn M, James D, Jr., Miquel M (1993) Mutants of Arabidopsis deficient in the synthesis of alpha-linolenate. Biochemical and genetic characterization of the endoplasmic reticulum linoleoyl desaturase. J. Biol. Chem. **268:** 16345-16351

Bräutigam M, Lindlof A, Zakhrabekova S, Gharti-Chhetri G, Olsson B, Olsson O (2005) Generation and analysis of 9792 EST sequences from cold acclimated oat, Avena sativa. BMC Plant Biol. **5:** 18

Burger KN, van der Bijl P, van Meer G (1996) Topology of sphingolipid galactosyltransferases in ER and Golgi: transbilayer movement of monohexosyl sphingolipids is required for higher glycosphingolipid biosynthesis. J. Cell Biol. **133:** 15-28

Contreras FX, Villar A-V, Alonso A, Kolesnick RN, Goni FM (2003) Sphingomyelinase Activity Causes Transbilayer Lipid Translocation in Model and Cell Membranes. J. Biol. Chem. **278:** 37169-37174

Devaux PF, Lopez-Montero I, Bryde S (2006) Proteins involved in lipid translocation in eukaryotic cells. Chem. Phys. Lipids **141:** 119-132

Duggan J, Jamal G, Tilley M, Davis B, McKenzie G, Vere K, Somekh M, O'Shea P, Harris H (2008) Functional imaging of microdomains in cell membranes. Euro. Biophys. J. **37:** 1279-1289

Dörmann P, Balbo I, Benning C (1999) Arabidopsis galactolipid biosynthesis and lipid trafficking mediated by DGD1. Science **284:** 2181-2184

Dörmann P, Hoffmann S, Balbo I, Benning C (1995) Isolation and characterization of an Arabidopsis mutant deficient in the thylakoid lipid digalactosyl diacylglycerol. Plant Cell **7:** 1801-1810

Eichenberger W, Siegrist HP (1975) Steryl glycoside acyltransferase from carrots. FEBS Letters **52:** 153-156

Ella KM, Meier GP, Bradshaw CD, Huffman KM, Spivey EC, Meier KE (1994) A Fluorescent assay for agonist- activated phospholipase D in mammalian cell extracts. Anal. Biochem. **218:** 136-142

Essigmann B, Guler S, Narang RA, Linke D, Benning C (1998) Phosphate availability affects the thylakoid lipid composition and the expression of SQD1, a gene required for sulfolipid biosynthesis in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U.S.A. **95:** 1950-1955

Froehlich JE, Benning C, Dormann P (2001) The Digalactosyldiacylglycerol (DGDG) Synthase DGD1 Is Inserted into the Outer Envelope Membrane of Chloroplasts in a Manner Independent of the General Import Pathway and Does Not Depend on Direct Interaction with Monogalactosyldiacylglycerol Synthase for DGDG Biosynthesis. J. Biol. Chem. **276:** 31806-31812

Futerman A, Pagano R (1991) Determination of the intracellular sites and topology of glucosylceramide synthesis in rat liver. Biochem. J. **280:** 295-302

Gaigg B, Simbeni R, Hrastnik C, Paltauf F, Daum G (1995) Characterization of a microsomal subfraction associated with mitochindria of the yeast, Saccharomyces cerevisiae. Involvement in synthesis and import of phospholipids into mitochondria. Biochim. Biophys. Acta **1234:** 214-220

Gaude N, Tippmann H, Flemetakis E, Katinakis P, Udvardi M, Dörmann P (2004) The Galactolipid Digalactosyldiacylglycerol Accumulates in the Peribacteroid Membrane of Nitrogen-fixing Nodules of Soybean and Lotus. J. Biol. Chem. **279:** 34624-34630

Gomes E, Jakobsen MK, Axelsen KB, Geisler M, Palmgren MG (2000) Chilling Tolerance in Arabidopsis Involves ALA1, a Member of a New Family of Putative Aminophospholipid Translocases. Plant Cell **12:** 2441-2454

Green J (1983) The Golgi apparatus. In: Isolation of membranes and organelles from plant cells. Academic Press, London

Hartmann M-A (1998) Plant sterols and the membrane environment. Trends Plant Sci. **3:** 170-175

Hartmann M-A, Benveniste P (1987) Plant membrane sterols: isolation, identification and biosynthesis. Methods enzymol. **148:** 632-650

Heinz E (1996) Plant Glycolipids: structure, isolation and analysis *in* - Advances in lipid methodology - three. The Oily Press

Heinz E, Dieler HP, Rullköter J (1975) Enzymatic acylation of sterly glycosides. Zeitschrift für Pflanzenphysiologie **75:** 78-87

Heinz E, Roughan P (1983) Similarities and differences in lipid metabolism of chloroplasts isolated from 18:3 and 16:3 plants. Plant Physi. **72:** 273-279

Hellgren LI, Sandelius AS (2001a) Age dependent variation in membrane lipid synthesis in leaves of garden pea (*Pisum sativum L.*). J. Exp. Bot.**52:** 2275-2282

Hellgren LI, Sandelius AS (2001b) The impact of different phytosterols on the molecular dynamics in the hydrophobic/hydrophilic interface phospahtidylcholine liposomes. Physiol Plant **113:** 23-32

Hill WG, Zeidel ML (2000) Reconstituting the Barrier Properties of a Water-tight Epithelial Membrane by Design of Leaflet-specific Liposomes. J. Biol. Chem. **275:** 30176-30185

Hillig I, Leipelt M, Ott C, Zähringer U, Warnecke D, Heinz E (2003) Formation of glucosylceramide and sterol glucoside by a UDP-glucose-dependent glucosylceramide synthase from cotton expressed in Pichia pastoris. FEBS Letters **553:** 365-369

Hincha DK, Oliver AE, Crowe JH (1998) The effects of chloroplast lipids on the stability of liposomes during freezing and drying. Biochim. Biophys. Acta - Biomembranes **1368:** 150-160

Hishikawa D, Shindou H, Kobayashi S, Nakanishi H, Taguchi R, Shimizu T (2008) Discovery of a lysophospholipid acyltransferase family essential for membrane asymmetry and diversity. Proc. Natl. Acad. Sci. U.S.A. **105:** 2830-2835

Holthuis JCM, Levine TP (2005) Lipid traffic: Floppy drives and a superhighway. Nat. Rev. Mol. Cell Biol. **6:** 209-220

Härtel H, Dörmann P, Benning C (2000) DGD1-independent biosynthesis of extraplastidic galactolipids after phosphate deprivation in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America **97:** 10649-10654

Hölzl G, Witt S, Kelly AA, Zahringer U, Warnecke D, Dormannn P, Heinz E (2006) Functional differences between galactolipids and glucolipids revealed in photosynthesis of higher plants. Proc. Natl. Acad. Sci. U.S.A. **103:** 7512-7517

Ikonen E (2008) Cellular cholesterol trafficking and compartmentalization. Nat Rev Mol Cell Biol **9:** 125-138

Jamieson GR, Reid EH (1976) The sugar components of the galactosyl diglycerides from green plants. Phytochemistry **15:** 135-136

Jeckel D, Karrenbauer A, Burger KN, van Meer G, Wieland F (1992) Glucosylceramide is synthesized at the cytosolic surface of various Golgi subfractions. J. Cell Biol. **117:** 259-267

Jordan P, Fromme P, Witt HT, Klukas O, Saenger W, Krausz N (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution. Nature **411:** 909-917

Jouhet J, Eric M, Richard B, Jacques J, Maryse AB (2003) Transient increase of phosphatidylcholine in plant cells in response to phosphate deprivation. FEBS Letters **544:** 63-68

Jouhet J, Marechal E, Baldan B, Bligny R, Joyard J, Block MA (2004) Phosphate deprivation induces transfer of DGDG galactolipid from chloroplast to mitochondria. J. Cell Biol. **167:** 863-874

Jungk A (2001) Root hairs and the acquisition of plant nutrients from soil. J. Plant Nutr. Soil Scien. **164:** 121-129

Kanipes M, Henry S (1997) The phospholipid methyltransferases in yeast. Biochim Biophys Acta. **1348(1-2):** 134-141

Kates M (1986) Techniques in Lipidology: Isolation, Analysis and Identification of Lipids. Elsevier Science Publishers, Berlin. **2nd ed**

Kelly AA, Dörmann P (2004) Green light for galactolipid trafficking. Curr. Opin. Plant Biol. **7:** 262-269

Kelly AA, Froehlich JE, Dörmann P (2003) Disruption of the Two Digalactosyldiacylglycerol Synthase Genes DGD1 and DGD2 in Arabidopsis Reveals the Existence of an Additional Enzyme of Galactolipid Synthesis. Plant Cell **15:** 2694-2706

Kesselmeier J, Eichenberger W, Urban B (1987) Sterols and sterylglycosides of oats Avena sativa. Distribution in the leaf tissue and medium-induced glycosylation of sterols during protoplast isolation. Physiol Plant **70:** 610-616

Kim E, Archibald J (2008) Diversity and Evolution of Plastids and Their Genomes *in* The Chlorplast eds: Sandelius AS and Aronsson H. Springer

Kim HM, Choo H-J, Jung S-Y, Ko Y-G, Park W-H, Jeon S-J, Kim CH, Joo T, Cho BR (2007) A Two-Photon Fluorescent Probe for Lipid Raft Imaging: C-Laurdan. ChemBioChem **8:** 553-559

Kjellberg JM, Trimborn M, Andersson M, Sandelius AS (2000) Acyl-CoA dependent acylation of phospholipids in the chloroplast envelope. Biochimica Biophysica Acta **1485:** 100-110

Kjellbom P, Larsson C (1984) Preparation and polypeptide composition of chlorophyll-free plasma membranes from leaves of light-grown spinach and barley. Plant. Physiol. **62:** 501-509

Kleinschmidt MG, Chough KS, Mudd JB (1972) Effect of Filipin on Liposomes Prepared with Different Types of Steroids. Plant Physiol. **49:** 852-856

Kol MA, de Kroon AIPM, Rijkers DTS, Killian JA, de Kruijff B (2001) Membrane-spanning peptides induce phospholipid flop: A model for phospholipid translocation across the inner membrane of E-coli. Biochemistry **40:** 10500-10506

Kol MA, van Laak ANC, Rijkers DTS, Killian JA, de Kroon AIPM, de Kruijff B (2003) Phospholipid Flop Induced by Transmembrane Peptides in Model Membranes Is Modulated by Lipid Composition. Biochemistry **42:** 231-237

Krylov AV, Pohl P, Zeidel ML, Hill WG (2001) Water Permeability of Asymmetric Planar Lipid Bilayers: Leaflets of Different Composition Offer Independent and Additive Resistances to Permeation. J. Gen. Physiol. **118:** 333-340

Laloi M, Perret A-M, Chatre L, Melser S, Cantrel C, Vaultier M-N, Zachowski A, Bathany K, Schmitter J-M, Vallet M, Lessire R, Hartmann M-A, Moreau P (2007) Insights into the Role of Specific Lipids in the Formation and Delivery of Lipid Microdomains to the Plasma Membrane of Plant Cells. Plant Physiol. **143:** 461-472

Larsson C, Möller I, Widell S (1990) Introduction to the Plant Plasma Membrane – Its Molecular Composition and Organization *in* The plant plasma membrane Springer-Verlag

Larsson K, Kjellberg JM, Tjellström H, Sandelius A (2007) LysoPC acyltransferase/PC transacylase activities in plant plasma membrane and plasma membrane-associated endoplasmic reticulum. BMC Plant Biol. **7:** 64

Lefebvre B, Furt F, Hartmann M-A, Michaelson LV, Carde J-P, Sargueil-Boiron F, Rossignol M, Napier JA, Cullimore J, Bessoule J-J, Mongrand S (2007) Characterization of Lipid Rafts from Medicago truncatula Root Plasma Membranes: A Proteomic Study Reveals the Presence of a Raft-Associated Redox System. Plant Physiol. **144:** 402-418

Lenoir G, Williamson P, Holthuis JCM (2007) On the origin of lipid asymmetry: the flip side of ion transport. Curr Opin Chem Biol **11:** 654-661

Lester R, Dickson R (1993) Sphingolipids with inositolphosphate-containing head groups. Adv Lipid Res. **26:** 253-274

Li M, Qin C, Welti R, Wang X (2006) Double Knockouts of Phospholipases D ζ1 and D ζ2 in Arabidopsis Affect Root Elongation during Phosphate-Limited Growth But Do Not Affect Root Hair Patterning. Plant Physiol. **140:** 761-770

Li M, Welti R, Wang X (2006b) Quantitative Profiling of Arabidopsis Polar Glycerolipids in Response to Phosphorus Starvation. Roles of Phospholipases D ζ1 and D ζ2 in Phosphatidylcholine Hydrolysis and Digalactosyldiacylglycerol Accumulation in Phosphorus-Starved Plants. Plant Physiol. **142:** 750-761

Lightner J, Wu J, Browse J (1994) A Mutant of Arabidopsis with Increased Levels of Stearic Acid. Plant Physiol. **106:** 1443-1451

Liljenberg C, Kates M (1985) Changes in lipid composition of oat root membranes as a function of water-deficit stress. Can. J. Biochem. Cell Biol. **63:** 77-84

Lingwood D, Simons K (2007) Detergent resistance as a tool in membrane research. Nat Protoc **2:** 2159-2165

Lu BB, Xu CC, Awai K, Benning C (2007) TGD3, an ATPaw protein of arabidopsis, functions in ER-to-plastid lipid trafficking. Faseb Journal **21:** A236-A236

Luckey M (2008) Membrane structural biology: with biochemical and biophysical foundation. Cambridge University Press

Lynch DV (2000) Enzymes of sphingolipid metabolism in plants. Methods Enzymol. **311:** 130-149

Lynch DV, Dunn TM (2004) An introduction to plant sphingolipids and a review of recent advances in understanding their metabolism and function. New Phytol. **161:** 677-702

Lynch DV, Fairfield SR (1993) Sphingolipid Long-Chain Base Synthesis in Plants (Characterization of Serine Palmitoyltransferase Activity in Squash Fruit Microsomes). Plant Physiol. **103:** 1421-1429

Marie A, Hartmann B, Benveniste P (1978) Sterol biosynthetic capability of purified membrane fractions from maize coleoptiles. Phytochemistry **17:** 1037-1042

Markham JE, Li J, Cahoon EB, Jaworski JG (2006) Separation and identification of major plant sphingolipid classes from leaves. J. Biol. Chem. **281:** 22684-22694

Marks DL, Wu K, Paul P, Kamisaka Y, Watanabe R, Pagano RE (1999) Oligomerization and Topology of the Golgi Membrane Protein Glucosylceramide Synthase. J. Biol. Chem. **274:** 451-456

McIntosh TJ, Vidal A, Simon SA (2003) Sorting of Lipids and Transmembrane Peptides Between Detergent-Soluble Bilayers and Detergent-Resistant Rafts Biophysical Journal **85:** 1656-1666

Mereschkowski C (1905) Über Natur und Ursprung der Chromatophoren im Pflanzenreiche. Biol Centralbl **25:** 593–604

Mondal M, Mesmin B, Mukherjee S, Maxfield FR (2009) Sterols Are Mainly in the Cytoplasmic Leaflet of the Plasma Membrane and the Endocytic Recycling Compartment in CHO Cells. Mol. Biol. Cell **20:** 581-588

Mongrand S, Bessoule JJ, Cassagne C (1997) A re-examination in vivo of the phosphatidylcholine- galactolipid metabolic relationship during plant lipid biosynthesis. Biochem. J. **327:** 853-858

Mongrand S, Bessoule JJ, Cabantous F, Cassagne C (1998) The C-16 : 3/C-18 : 3 fatty acid balance in photosynthetic tissues from 468 plant species. Phytochemistry **49:** 1049-1064

Mongrand S, Cassagne C, Bessoule JJ (2000) Import of lyso-phosphatidylcholine into chloroplasts likely at the origin of eukaryotic plastidial lipids. Plant Physiology **122:** 845-852

Mongrand S, Morel J, Laroche J, Claverol S, Carde J-P, Hartmann M-A, Bonneu M, Simon-Plas F, Lessire R, Bessoule J-J (2004) Lipid Rafts in Higher Plant Cells: Purification and characterization of Triton X-100 insoluble microdomains from tobacco plasma membrane. J. Biol. Chem. **279:** 36277-36286

Moreau P, Bessoule JJ, Mongrand S, Testet E, Vincent P, Cassagne C (1998) Lipid trafficking in plant cells. Prog. Lipid Res. **37:** 371-391

Morel J, Claverol S, Mongrand S, Furt F, Fromentin J, Bessoule JJ, Blein JP, Simon-Plas F (2006) Proteomics of plant detergent-resistant membranes. Mol Cell Proteomics **5:** 1396-1411

Morré DJ, Morré JT, Morré SR, Sundqvist C, Sandelius AS (1991) Chloroplast Biogenesis - Cell-Free Transfer of Envelope Monogalactosyl-glycerides to Thylakoids. Biochim Biophys Acta **1070:** 437-445

Mudd JB, Kleinschmidt MG (1970) Effect of filipin on the permeability of red beet and potato tuber discs. Plant Physiol. **45:** 517-518

Munnik T (2001) Phosphatidic acid: an emerging plant lipid second messenger. Trends Plant Sci. **6:** 227-233

Munnik T, Testerink C (2008) Plant phospholipid signaling - 'in a nutshell'. J. Lipid Res.**:** R800098-JLR800200

Munro S (2003) Lipid rafts: Elusive or illusive? Cell **115:** 377-388

Nakamura Y, Awai K, Masuda T, Yoshioka Y, Takamiya K-i, Ohta H (2005) A novel Phosphatidylcholine-hydrolyzing Phospholipase C induced by phosphate starvation in Arabidopsis. J. Biol. Chem. **280:** 7469-7476

Neufeld E, Hall C (1964) Formation of galactolipids by chloroplasts. Biochem Biophys Res Commun. 1964;14:503-8. **14**

Norberg P, Liljenberg C (1991) Lipids of plasma membranes prepared from oat Root cells : Effects of induced water-deficit tolerance. Plant Physiol. **96:** 1136-1141

Nussberger S, Dörr K, Wang DN, W. K (1993) Lipid-protein Interactions in Crystals of Plant Light-harvesting Complex. J. Mol. Biol. **234:** 347-356

Ohlrogge J, Browse J (1995) Lipid biosynthesis. Plant Cell **7:** 957-970

Okuley J, Lightner J, Feldmann K, Yadav N, Lark E, Browse J (1994) Arabidopsis FAD2 Gene Encodes the Enzyme That Is Essential for Polyunsaturated Lipid Synthesis. Plant Cell **6:** 147-158

Ongun A, Mudd JB (1968) Biosynthesis of Galactolipids in Plants. J. Biol. Chem. **243:** 1558-1566

Ostermeyer AG, Beckrich BT, Ivarson KA, Grove KE, Brown DA (1999) Glycosphingolipids Are Not Essential for Formation of Detergent-resistant Membrane Rafts in Melanoma Cells. Methyl-β-cyclodextrins does not affect

cell surface transport of GPI-anchored proteins. J. Biol. Chem. **274:** 34459-34466

Palmgren MG, Askerlund P, Fredrikson K, Widell S, Sommarin M, Larsson C (1990) Sealed inside-out and right-side-out plasma membrane vesicles-optimal conditions for formation and separation. Plant Physiol. **92:** 871-880

Parasassi T, De Stasio G, Ravagnan G, Rusch RM, Gratton E (1991) Quantitation of lipid phases in phospholipid vesicles by the generalized polarization of Laurdan fluorescence. Biophys. J. **60:** 179-189

Parasassi T, Krasnowska EK, Bagatolli L, Gratton E (1998) Laurdan and Prodan as Polarity-Sensitive Fluorescent Membrane Probes. J Fluoresc **8:** 365-373

Park J, Gu Y, Lee Y, Yang Z, Lee Y (2004) Phosphatidic Acid Induces Leaf Cell Death in Arabidopsis by Activating the Rho-Related Small G Protein GTPase-Mediated Pathway of Reactive Oxygen Species Generation. Plant Physiol. **134:** 129-136

Parton RG, Richards AA (2003) Lipid Rafts and Caveolae as Portals for Endocytosis: New Insights and Common Mechanisms. Traffic **4:** 724-738

Peng L, Kawagoe Y, Hogan P, Delmer D (2002) Sitosterol-beta -glucoside as Primer for Cellulose Synthesis in Plants. Science **295:** 147-150

Pical C, Sandelius AS, Melin PM, Sommarin M (1992) Polyphosphoinositide Phospholipase-C in Plasma-Membranes of Wheat (Triticum-Aestivum L) - Orientation of Active-Site and Activation By Ca2+ and Mg2+. Plant Physiol. **100:** 1296-1303

Pichler H, Gaigg B, Hrastnik C, Achleitner G, Kohlwein SD, Zellnig G, Perktold A, Daum G (2001) A subfraction of the yeast endoplasmic reticulum associates with the plasma membrane and has a high capacity toi synthesize lipids. Eur. J. Biochem. **268:** 2351-2361

Poirier Y, Thoma S, Somerville C, Schifelbein J (1991) A mutant of Arabidopsis deficient in xylem loading of phosphate. Plant Physiol. **97:** 1087-1093

Pomorski T, Hrafnsdottir S, Devaux PF, van Meer G (2001) Lipid distribution and transport across cellular membranes. Semin. Cell Dev. Biol. **12:** 139-148

Popova AV, Hincha DK (2005) Effects of the sugar headgroup of a glycoglycerolipid on the phase behavior of phospholipid model membranes in the dry state. Glycobiology **15:** 1150-1155

Potocka A, Zimowsk J (2008) Metabolism of conjugated sterols in eggplant. Part 2. Phospholipid:steryl glucoside acyltransferase. Acta Biochim Pol **55** 135-140

Quinn PJ, Wolf C (2009) The liquid-ordered phase in membranes. Biochim. Biophys. Acta - Biomembranes **1788:** 33-46

Raghothama K, Karthikeyan A (2005) Phosphate Acquisition. Plant and Soil **274:** 37-49

Raghothama KG (1999) Phosphate Acquisition. nu. Rev. Plant Physiol. Plant Mol. **50:** 665-693

Roche Y, Gerbeau-Pissot P, Buhot B, Thomas D, Bonneau L, Gresti J, Mongrand S, Perrier-Cornet J-M, Simon-Plas F (2008) Depletion of phytosterols from the plant plasma membrane provides evidence for disruption of lipid rafts. FASEB Journal**:** fj.08-111070

Rochester CP, Kjellbom P, Andersson B, 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

Roughan PG, Holland R, Slack CR (1980) Role of chloroplasts and microsomal fractions in polar-lipid synthesis from (carbon-14-labeled) acetate by cell-free preparations from spinach (Spinacia oleracea) leaves. Biochem. J. **188:** 17-24

Runge-Metzger A (1995) Closing the cycle: Obstacles to efficient P management for improved global food security. In Phosphorus in the Global Environment: Transfers, cycles and Management. John Wiley, New York

Russo MA, Quartacci MF, Izzo R, Belligno A, Navari-Izzo F (2007) Long- and short-term phosphate deprivation in bean roots: Plasma membrane lipid alterations and transient stimulation of phospholipases. Phytochemistry **68:** 1564-1571

Räntfors M, Evertsson I, Kjellberg JM, Sandelius AS (2000) Intraplastidial lipid trafficking: Regulation of galactolipid release from isolated chloroplast envelope. Physiol. Plant **110:** 262-270

Salaun C, James DJ, Chamberlain LH (2004) Lipid Rafts and the Regulation of Exocytosis. Traffic **5:** 255-264

Sandelius A, Morré D (1989) Plasma membrane isolation. *in* "The plant plasma membrane". Springer-Verlag; eds. C.Larsson and IM Möller

Sandelius AS, Hellgren LI (1990) Metabolism and *in vivo* transport of phosphatidylinositol in hypocotyls of dark grown soybean (*Glycine max*). *In* PJ Quinn, JL Harwood, eds, Plant Lipid Biochemistry, Structure and utilisation. Portland Press Ltd., London, pp 257-259

Schulte S, Stoffel W (1993) Ceramide UDPgalactosyltransferase from myelinating rat brain: purification, cloning, and expression. Proc. Natl. Acad. Sci. U.S.A. **90:** 10265-10269

Shahollari B, Peskan-Berghöfer T, Oelmuller R (2004) Receptor kinases with leucine-rich repeats are enriched in Triton X-100 insoluble plasma membrane microdomains from plants. Physiologia Plantarum **122:** 397-403

Simons K, Ikonen E (1997) Functional rafts in cell membranes. Nature **387:** 569-572

Simons K, Toomre D (2001) Lipid rafts and signal transduction (vol 1, pg 31, 2000). Nat. Rev. Mol. Cell Biol. **2:** 216-216

Singer SJ, Nicholson GL (1972) The Fluid Mosaic Model of the Structure of Cell Membranes. Science **175:** 720-731

Somerville C (2006) Cellulose Synthesis in Higher Plants. Annual Review of Cell and Developmental Biology **22:** 53-78

Sperling P, Franke S, Lüthje S, Heinz E (2005) Are glucocerebrosides the predominant sphingolipids in plant plasma membranes? Plant Physiol. Biochem. **43:** 1031-1038

Sperling P, Heinz E (2003) Plant sphingolipids: structural diversity, biosynthesis, first genes and functions. Biochim. Biophys. Acta-Mol. Cell Biol. Lipids **1632:** 1-15

Staehelin LA (1997) The plant ER: A dynamic organelle composed of a large number of discrete functional domains. Plant J. **11:** 1151-1165

Sunamoto J, Iwamoto K, Kondo H, Shinkai S (1980) Liposomal Membranes: VI. Polysaccharide-Induced Aggregation of Multilamellar Liposomes of Egg Lecithin. J. Biochem. **88:** 1219-1226

Szabadkai G, Bianchi K, Varnai P, De Stefani D, Wieckowski MR, Cavagna D, Nagy AI, Balla T, Rizzulo R (2006) Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels. J. Cell Biol. **175:** 901-911

Szilágyi A, Selstam E, Åkerlund H-E (2008) Laurdan fluorescence spectroscopy in the thylakoid bilayer: The effect of violaxanthin to zeaxanthin conversion on the galactolipid dominated lipid environment. Biochim. Biophys. Acta - Biomembranes **1778:** 348-355

Takeda Y, Kasamo K (2001) Transmembrane topography of plasma membrane constituents in mung bean (Vigna radiata L.) hypocotyl cells - I. Transmembrane distribution of phospholipids. Biochim. Biophys. Acta - Biomembranes **1513:** 38-48

van Besouw A, Wintermans JFGM (1978) Galactolipid formation in chloroplast envelopes: I. Evidence for two mechanisms in galactosylation. Biochim. Biophys. Acta - Lipids and Lipid Metabolism **529:** 44-53

van Meer G, Holthuis JCM (2000) Sphingolipid transport in eukaryotic cells. Biochim. Biophys. Acta - Molecular and Cell Biology of Lipids **1486:** 145-170

van Meer G, Voelker DR, Feigenson GW (2008) Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol **9:** 112-124

Vance CP, Uhde-Stone C, Allan DL (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phyto. **157:** 423-447

Vance JE (1990) Phospholipid-synthesis in a membrane-fraction associated with mitochondria. J. Biol. Chem. **265:** 7248-7256

Wang X (2000) Multiple forms of phospholipase D in plants: the gene family, catalytic and regulatory properties, and cellular functions. Prog. Lipid Res. **39:** 109-149

Wang X (2001) Plant phospholipases. Annu. Rev. Plant Physiol. Plant Mol. **52:** 211-231

Wang X (2005) Regulatory Functions of Phospholipase D and Phosphatidic Acid in Plant Growth, Development, and Stress Responses. Plant Physiol. **139:** 566-573

Warnecke DC, Baltrusch M, Buck F, Wolter FP, Heinz E (1997) UDP-glucose:sterol glucosyltransferase: cloning and functional expression in Escherichia coli. Plant Mol. Biol. **35:** 597-603

Warnecke DC, Heinz E (1994) Purification of a Membrane-Bound UDP-Glucose:Sterol β-D-Glucosyltransferase Based on Its Solubility in Diethyl Ether. Plant Physiol. **105:** 1067-1073

Williams JP, Imperial V, Khan MU, Hodson JN (2000) The role of phosphatidylcholine in fatty acid exchange and desaturation in Brassica napus L. leaves. Biochem. J. **349:** 127-133

Wikström M, Kelly AA, Georgiev A, Eriksson HM, Klement MR, Bogdanov M, Dowhan W, Wieslander A (2009) Lipid-engineered Escherichia coli Membranes Reveal Critical Lipid Headgroup Size for Protein Function. J. Biol. Chem. **284:** 954-965

Vincent P, Maneta-Peyret L, Sturbois-Balcerzak B, Duvert M, Cassagne C, Moreau P (1999) One of the origins of plasma membrane phosphatidylserine in plant cells is a local synthesis by a serine exchange activity. FEBS Letters **464:** 80-84

Vishwakarma RA, Vehring S, Mehta A, Sinha A, Pomorski T, Herrmann A, Menon AK (2005) New fluorescent probes reveal that flippase-mediated flip-flop of phosphatidylinositol across the endoplasmic reticulum membrane does not depend on the stereochemistry of the lipid. Org. Biomol. Chem. **3:** 1275-1283

Xiang TX (1993) A computer simulation of free-volume distributions and related structural properties in a model lipid bilayer. Biophys. J.l **65:** 1108-1120

Yoshida S, 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

Zachowski A (1993) Phospholipids in animal eukaryotic membranes: transverse asymmetry and movement. Biochem. J **294:** 1-14

Zwaal RFA, Comfurius P, Bevers EM (2005) Surface exposure of phosphatidylserine in pathological cells. Cell. Mol. Life Sci. **62:** 971-988

10. POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

Under de senaste åren har jag fått frågan: **MEN VAD GÖR DU EGENTLIGEN?** ett antal gånger. Svaret har varierat förvånansvärt mycket med tanke på att jag gjort i stort sett samma sak hela tiden -här är ytterligare en variant:

Runt om nästan allt finns det ett skyddande lager, vi har huden, karboncykeln har lacken och cellen har ett plasmamembran. Även om det finns en massa skäl att gå in på hur en karboncykel är uppbyggd och konstruerad för att bli lätt och vridstyv på samma gång så får det vänta till en annan gång och vi får fokusera på plasmamembranet. Det är uppbyggt av membranlipider och proteiner som skapar en barriär mot omvärlden.

Plasmamembranet är till för att cellen skall kunna behålla vissa ämnen och se till att vissa ämnen inte kommer in. Detta membran är ca en miljondel av tjockleken av en tio-krona. Sammansättningen av membranlipider gör att denna tunna biologiska film klarar av att hålla vissa molekyler utanför och andra innanför och sammansättingen av membranlipider och membranproteiner regleras hela tiden för att vara optimal för rådande omständigheter.

Membranlipider består av två delar som har olika egenskaper: huvudet som är vattenlösligt och svansen som är inte är vattenöslig. Då membranlipider blandas i vatten organiserar sig membranlipiderna så att svansarna pekar mot varandra och huvudgruppen pekar ifrån varandra. Det bildas ett membran, där huvudgrupperna skyddar svansarna från vattnet. När detta membran sluts bildas en sfär, vilket är grunden för bildandet av en cell. I en cell så finns det mer än 1000 olika sorters lipider som delas in i lite större lipidklasser. Dessa klasser baseras i stort sett på vad det är för huvudgrupp och de två huvudgrupper som är mest intressanta för min forskning är fosfolipider som innehåller fosfat och galaktolipider som innehåller galaktos (ett slags socker).

Fosfat är ett ämne som är nödvändigt för alla levande organismer, tillgången är dock begränsande i de flesta jordar. Eftersom växter inte kan flytta på sig till områden med mer fosfat så har de anpassat sig till de rådande förhållandena. Växter har blivit experter på att leta reda på mer fosfat och med att hushålla med den mängd fosfat som finns att tillgå. Genom att låta rötterna växa längre, så kan växten ”leta rätt” på mer fosfat eller så kan rötterna utsöndra vissa syror för att frigöra fosfat som är bunden i marken. En av mekanismerna för fosfathushållning som upptäcktes för tio år sedan är att växten kan använda sig av fosfaten som finns i fosfolipiderna. Då fosfattillgången är riktigt dålig så kommer växten att bryta ned fosfolipidrna och använda fosfaten till andra molekyler och en slags galaktolipid, DGDG, kommer att ersätta fosfolipiderna i membranet. Då havre får växa utan fosfat så ökar mängden DGDG i plasmamembranet med ca 800 % till en total halt av ca 25 mol%, på samma gång ser man en minskning av andelen fosfolipider om man jämför med havre som växt under fosfatrika förhållanden.

Jag har bland annat studerat vilka enzymer som är inblandade i nedbrytningen och frigörandet av fosfaten från fosfolipider i plasmamembranet och korrelerat denna aktivitet till ålder, fosfathalt och mängd DGDG i havre.

Även om havre klarar sig utan tillsatt fosfat i näringslösningen så ser man att den mår mycket bättre om den har tillgång på fosfat. Den växer bättre, producerar mera frön och ser allmänt mer friskare ut än havre som vuxit utan fosfat.

När jag tillförde fosfat till fosfatsvulten havre, kunde jag se att växten tog upp fosfaten och att fosfolipider bildades på nytt. Efter en vecka så började den före detta fosfatsvultna havren att se lite friskare ut. Mängden fosfolipider hade ökat och andelen av DGDG i plasmamembranet hade minskat. Genom att tillföra fosfat så hade jag vänt på förloppet Det visade sig att DGDG är acceptabel som ersättare för fosfolipider, men egentligen bara om nöden kräver det.

Konsekvenserna av hög halt DGDG i plasmamembranet undersöktes genom att mäta hur DGDG interagerade med andra lipider som finns i plasmamembranet. Det visade sig att på ett membran med mycket DGDG har sämre egenskaper än ett membran med lite DGDG. Men detta undviks i havren genom att DGDG har en specifik lokalisering i plasmamembranet som minimerar dess påverkan på plasmamembranets integritet.

Varför ska man bry sig om fosfat? I dagens jordbruk så använder man sig av NPK gödsel, som tillför kväve, fosfat och kalium, för att öka tillväxt och skörd. Det är dock bara en liten del av den tillförda fosfaten som växten tar upp, det mesta försvinner i avrinningen av ytvattnet eller binds upp i marken till järn- eller aluminiumrika jordpartiklar, viket gör den otillgänglig för växten. När fosfat sprids på åkern kommer den del som växterna inte tar upp således inte att kunna återvinnas utan går förlorad. Studier, som denna avhandling är till för att öka vår kunskap om vilka mekanismer som växter använder sig av vid upptag och hushållning av fosfat, då fosfaten som används som gödsel är en ändlig resurs, precis som den fossila oljan. Fosfaten i NPK gödsel bryts i dagbrott i norra Afrika och enligt vissa beräkningar så kommer fosfatmalmen att ta slut om 50-100 år - vad skall vi då använda? Något att tänka på nästa gång som vi tar fram säcken med blåkorn…