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The Plant Plasma Membrane

Springer

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Preface

The spontaneous formation of lipid-bounded compartments is one of the preconditions for the genesis of the earliest living cells. Such membranous structures retain essential components, serve as a barrier to intrusion of external contaminants, and, via differential diffusion of ions, generate transient electrochemical gradients that can energize selective exchange processes. In plant cells, the outer barrier plasmalemma, or plasma membrane, is a highly elaborated structure that functions as the point of exchange with adjoining cells, cell walls, and the external environment. Transactions at the plasma membrane include uptake of water and essential mineral nutrients, gas exchange, movement of metabolites, transport and perception of signaling molecules, and initial responses to external biota. Central to all of these processes is the formation of a chemiosmotic gradient across the plasma membrane that results from ATP-driven proton extrusion. This gradient generates a net negative charge on the inner surface of the membrane and a delta pH of 1.5–2. Selective channels and carriers harness this electromotive force to control the rates and direction of movement of small molecules across the membrane barrier and manipulate the turgor that maintains plant form and drives plant cell expansion. Where required, ATP-dependent transporters mobilize the movement of essential molecules against the gradient.

However, it is erroneous to view the plasma membrane as just a diffusion barrier studded with transport proteins. Like other cellular membranes, the plasma membrane provides an environment in which molecular and macromolecular interactions can occur more efficiently. This is primarily a result of the enhanced efficiency of diffusional interactions taking place in two dimensions, the clustering of proteins in oligomeric complexes via protein–protein or protein–lipid interactions for more efficient retention of biosynthetic intermediates, and the anchoring of protein complexes to enhance regulatory interactions. Coupling of signal perception at the membrane surface with intracellular second messengers also necessarily involves transduction across the plasma membrane. Finally, the generation and ordering of the external cell walls involve processes mediated at the plant cell surface by the plasma membrane.

This volume is divided into three parts. Part I, consisting of five chapters, describes the basic mechanisms that regulate all plasma membrane functions. Chapter "Lipids of the Plant Plasma Membrane" by Furt et al. describes the most fundamental aspect of the plasma membrane – its lipid composition and the ordering of membrane lipids into leaflets and domains. The chapter "Plasma Membrane Protein Trafficking" by Peer describes the mechanisms by which proteins are trafficked to and from the plasma membrane. The chapter "The Plasma Membrane and the Cell Wall" by Sampathkumar et al. describes the role of the plasma membrane in cell wall production as well as the interactions between the plasma membrane surface and the cell walls during development. The chapter "Plasmodesmata and non-cell autonomous signaling in plants" by Lee et al. describes the plasmodesmal structures that provide unique regulated conduits that can partially bridge cell wall barriers to provide direct intercellular interactions. The chapter "Post-translational Modifications of Plasma Membrane Proteins and Their Implications for Plant Growth and Development" by Luschnig and Seifert details the regulatory posttranslational modifications made to many plasma membrane proteins.

Part II describes plasma membrane transport activity. Chapter "Functional Classification of Plant Plasma Membrane Transporters" by Schulz provides an overview of the structure and classification of plasma membrane transporters and uses structural characteristics to classify these proteins into groups. In the chapter "Plasma Membrane ATPases" by Palmgren et al., a similar structural analysis is combined with functional analyses derived from experimental results to describe the ATPases that export protons and calcium at the plasma membrane. Chapter "Physiological Roles for the PIP Family of Plant Aquaporins" by Vera-Estrella and Bohnert uses a similar approach to characterize the aquaporin intrinsic membrane protein channels that transport water and other small molecules in and out of the cell. In chapters "The Role of Plasma Membrane Nitrogen Transporters in Nitrogen Acquisition and Utilization" by Tsay and Hsu, "Plant Plasma Membrane and Phosphate Deprivation" by Nussaume et al., "Biology of Plant Potassium Channels" by Hedrich et al., "Mechanism and Evolution of Calcium Transport Across the Plant Plasma Membrane" by Connorton et al., "Sulfate Transport" by Hawkesford, "Metal Transport" by Atkinson, and "Organic Carbon and Nitrogen Transporters" by Tegeder et al., the regulated transport of nitrogen, phosphorus, potassium, calcium, sulfur, metals, and cellular metabolites across the plasma membrane are described. Chapter "ABC Transporters and Their Function at the Plasma Membrane" by Knöller and Murphy returns to a more structural approach to describe what is currently know about the plasma membrane ATP-binding cassette transporters of the ABCB and ABCG subfamilies. The transporter part of the book is rounded out by a description of hormone transport in chapter "Hormone Transport" by Kerr et al.

Part III of the book describes signaling interactions at the plasma membrane, with chapters describing hormone signaling (chapter "Plant Hormone Perception at the Plasma Membrane" by Pandey), light sensing (chapter "Light Sensing at the Plasma Membrane" by Christie et al.), lipid signaling (chapter "The Hall of Fame:

Lipid Signaling in the Plasma Membrane" by Im et al.), abiotic stress responses (chapter "Plasma Membrane and Abiotic Stress" by Barkla and Pantoja), and biotic interactions (chapter "The Role of the Plant Plasma Membrane in Microbial Sensing and Innate Immunity" by Nürnberger and Küfner).

Although these topics have been the subject of many current and past reviews, they are given a unique treatment in this volume, as we have made an effort to concentrate on events and mechanisms that occur at the plasma membrane rather than discuss mechanisms that occur throughout plant cells. It is hoped that this effort will provide the reader with a strong sense of the unique role that the plasma membrane plays in plant physiology and development. Further, the authors of the individual chapters have made an effort to identify areas where there are substantial gaps in our understanding of mechanisms sited on this critical cellular structure. Finally, we hope to convince the reader that a more complete knowledge of plasma membrane structure and function is essential to current efforts to increase the sustainability of agricultural production of food, fiber, and fuel crops.

Lafayette, USA A. Murphy
1 May 2010

Contents

Section I
Plasma Membrane Structure and Basic Functions

Lipids of the Plant Plasma Membrane

Fabienne Furt, Françoise Simon-Plas, and Sébastien Mongrand

Abstract The plasma membrane (PM) is arguably the most diverse membrane of the plant cell. Furthermore, the protein and lipid composition of the PM varies with cell type, developmental stage, and environment. Physical properties of lipids and associate proteins allow the formation of a barrier that is selectively permeable to macromolecules and solutes. As the plasma membrane delineates the interface between the cell and the environment, it is the primary part of signal recognition and transduction into intracellular responses for nutritional uptake/distribution, environmental responses, and developmental signaling. Many essential PM functions are carried out by proteinaceous components. However, PM lipids play a crucial role in determining cell structures regulating membrane fluidity and transducing signals. The composition and physical state of the lipid bilayer influence lipid–protein and protein–protein associations, membrane-bound enzyme activities, and transport capacity of membranes. Analyses of membrane function require highly selective and efficient purification methods. In this chapter, we first briefly review the methods to isolate PM from plant tissue and describe the lipid content of purified membranes. We further examine the involvement of different lipid species on signaling events that allow the plant cell to cope with environmental fluctuations. Finally, we discuss how regulated segregation of lipids inside the PM is of crucial importance to understand signaling mechanisms.

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1 Biochemical Analysis of Plant Plasma Membrane

1.1 Isolation of Highly Purified Plasma Membrane Fractions from Plant Tissues

Isolating highly purified fractions of organelles or membranes from other cellular compartments is a key requirement for in-depth identification and characterization of membrane proteins and lipids. PM fractions were first purified from microsomal membranes (a mix of various cellular membranes) by their high density on flotation gradient after high-speed ultracentrifugation. This approach has been shown to inefficiently fractionate the PM from other membranes, particularly the tonoplast. Higher efficiency partial separations of PM vesicles by free flow electrophoresis have also been reported (for review Canut et al. 1999).

In the early 1980s, Larsson developed an effective tool for preparative isolation of PM fractions by partitioning microsomal fractions in aqueous polymer two-phase systems using aqueous solutions of polyethylene glycol (PEG) and dextran (Widell et al. 1982). The method is rapid and uses only standard laboratory equipment. The separation is continued on after stirring, the system spontaneously forms two phases, and microsomal membranes separate according to differences in surface properties rather than in size and density. As PM vesicles are more negatively charged than most other cellular membranes, they are recovered into the upper phase. Up to 98% purity can be reached with this method. This technique therefore represents an attractive alternative to conventional fractionation protocols and has been shown to be effective for multiple plant tissues (root, leaves, etc.). The respective proportions of the two polymers, pH, and the ionic strength of the aqueous phase are the crucial parameters to ensure PM purity (Larsson et al. 1987).

The outer (apoplastic) side of the PM bilayer is negatively charged. Consequently, PM vesicles purified by two-phase partition are mostly sealed in a right-side-out topology. Morphological studies of highly purified PM fractions pelleted by centrifugation, fixed by chemical or high-pressure freeze substitution, and embedded in resin showed that these fractions contained mostly membrane vesicles ranging from 50 to 500 nm in diameter, should the majority of which exhibited a diameter between 200 and 300 nm. Higher magnifications showed that the membrane leaflets were highly contrasted and 8 nm thick, which corresponds to in situ observations of PM in intact tissues (Fig. 1).

1.2 Lipid Content of Plant Plasma Membrane

The lipid-to-protein mass ratio in the plant PM is ca. 1. However, considering that the average lipid molecular mass is far below than the average molecular mass of protein, the lipid-to-protein molar ratios in the PM range from 50:1 to 100:1. Analyses of highly purified PM lipid extracts are performed by thin layer

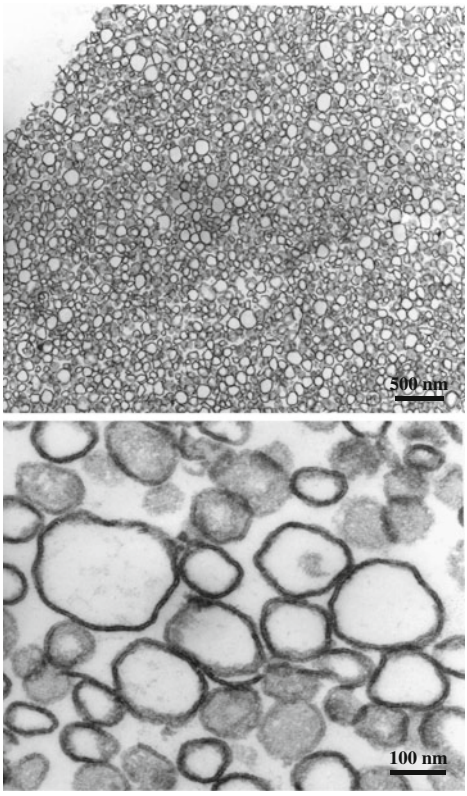

Fig. 1 Electron microscopy observation of a two-phase partition highly purified plasma membrane fraction isolated from tobacco leaves

chromatography (TLC), gas chromatography (GC), high-pressure liquid chromatography (HPLC), and GC/HPLC coupled to mass spectrometer. More recently, mass spectrometry approaches have been adapted to "lipidomic" analysis. For instance, tandem mass spectrometry (MS/MS) or MS^3 strategies that can simultaneously identify multiple lipid species are required because they provide structural information regarding polar head groups, length, unsaturation of fatty acid chains, and presence of glycosyl units in lipid molecules. However, as there are often close to 1,000 lipid species in a single cell (Van Meer 2005), even MS/MS methods are not sufficient to fully resolve the complexity of lipid mixtures. Therefore, although the results of lipid analyses from several plant species have been available for many years, a complete characterization of the plasma membrane is still lacking.

Three main classes of lipids exist in the PM: glycerolipids (mainly phospholipids), sterols, and sphingolipids (Fig. 2). Except for complex sphingolipids, which are synthesized in the *trans*-Golgi network, most lipids are assembled in the endoplasmic reticulum and are transported through the secretory pathway to the PM (Van Meer and Sprong 2004). Briefly, fatty acids are synthesized in plastids and mainly exported to the ER S-acylated to coenzyme A to enter the Kennedy pathway for phospholipids (see for review Bessoule and Moreau 2004) and sphingolipids pathway (see for review Pata et al. 2010).

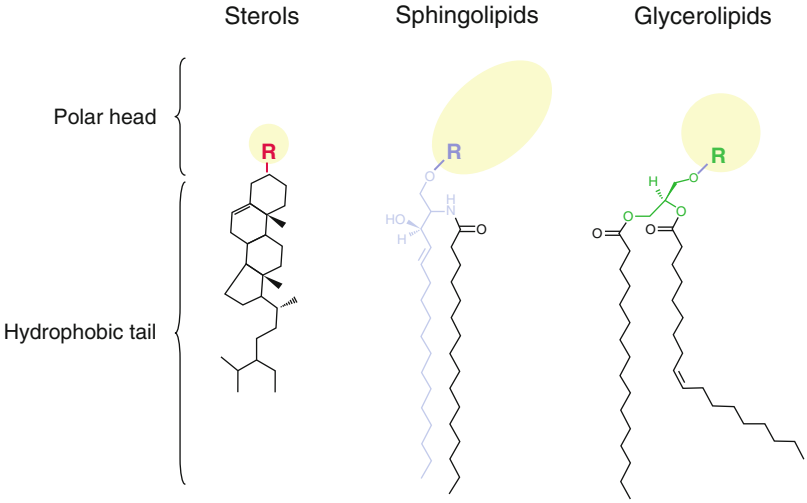

Fig. 2 Chemical features of the three major classes of plant plasma membrane lipids. The various polar heads are represented in *gray*

A great diversity is observed in PM lipid composition across plant species (e.g., Uemura and Steponkus 1994; Uemura et al. 1995) and within the different organs of a given plant species (e.g., Sandstrom and Cleland 1989). However, compared with other cellular membranes, the PM is always strongly enriched in sterols and sphingolipids with a sterol-to-phospholipid ratio ranging from 0.6 to 1.5 (Table 1). PM lipids are generally classified by abundance as well as by structure: the most abundant are often referred to as "structural lipids" and less abundant as "signaling lipids." These two categories are somewhat artificial as several lipids referred to as examples of abundant lipids may exhibit signal-transducing function. This chapter focuses on the biosynthesis of signaling lipids rather than on the synthesis of structural lipids (see for review Bessoule and Moreau 2004; Pata et al. 2010), and clustering of lipid and protein in PM microdomains.

1.2.1 Glycerolipids

Glycerolipids are tripartite molecules made up of a head group nucleated by a glycerol moiety to which two fatty acyl chains are esterified at positions *sn1* and *sn2* as shown in Fig. 2. The third position consists of a hydroxyl group to form diacylglycerol (DAG) and is further modified to form the different classes, namely, glycolipids and phospholipids (Fig. 3). With the notable exception of PM-localized digalactosyl diacylglycerol (DGDG), glycoglycerolipids are mostly present in plastids. DGDG replaces phospholipids in the PM bilayer during plant phosphate deprivation to preserve the integrity of the membrane and remobilize the phosphate pool (e.g., Andersson et al. 2005; Tjellstrom et al. 2008).

Table 1 Lipid content of plant plasma membrane, expressed as percent of total lipids

	Vigna radiata hypocotyl	*Secale cereale* leaves	*Secale cereale* leaves	*Avena sativa* coleoptyle	*Avena sativa* roots	Spring oat leaves	Winter oat leaves	*Hordeum vulgare* roots	*Zea mays* roots	*Arabidopsis thaliana* leaves	*Solanum tuberosum* leaves	*Solanum commersonii* leaves	*Nicotiana tabacum* BY-2 cells	*Nicotiana tabacum* leaves
PL	48.9	31.7	36.6	41.7	50.1	28.2	28.9	45	43.9	46.8	46.4	48.3	40.3	38.5
SL	6.8	16.2	16.4	26.1	10.1	27.2	30.4	8	6.8	7.3	6.5	6.1	11.3	20.6
St	43.6	52.1	46.6	32.2	39.7	41.3	39.1	43	49.3	46	45	41.6	26.7	22.6
Other	0.7	–	0.4	–	–	2.7	1.7	4	–	–	2.1	4	6.6	5.8
St/PL	0.9	1.6	1.3	0.8	0.8	1.5	1.3	0.9	1.1	1	1	0.9	0.7	0.6
References	Yoshida and Uemura (1986)	Lynch and Steponkus (1987)	Uemura and Steponkus (1994)	Sandstrom and Cleland (1989)	Sandstrom and Cleland (1989)	Uemura and Steponkus (1994)	Uemura and Steponkus (1994)	Brown and Dupont (1989)	Bohn et al. (2007)	Uemura et al. (1995)	Palta et al. (1993)	Palta et al. (1993)	Mongrand et al. (2004)	Mongrand et al. (2004)

PL phospholipid, *SL* sphingolipids, *St* sterols

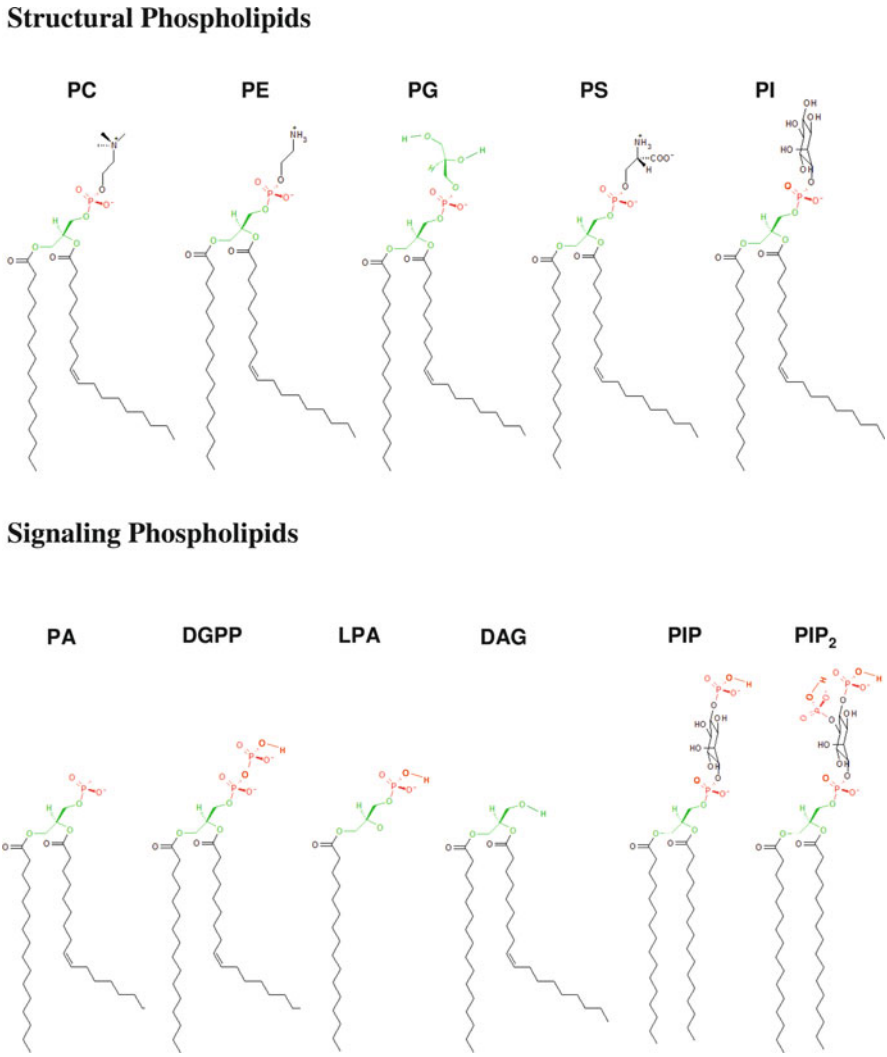

Fig. 3 Chemical structure of the *structural* and *signaling* phospholipids. Abbreviations: *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PS* phosphatidylserine, *PI* phosphatidylinositol, *PA* phosphatidic acid, *DGPP* diacylglycerolpyrrophosphate, *LPA* lysophosphatidic acid, *PIP* phosphatidylinositol monophosphate, *PIP2* phosphatidylinositol bisphosphate

Structural Glycerolipids

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) represent up to 68–80% of structural phospholipids. The remainder consists of phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidic acid (PA) (Fig. 3; Table 2). Phospholipid fatty acid composition is well conserved

Table 2 Structural phospholipid content of plant plasma membrane, expressed as percent of total lipids

	Vigna radiata hypocotyle	*Secale cereale* leaves	*Secale cereale* leaves	Oat roots	Spring oat leaves	*Hordeum vulgare* roots	*Zea mays* roots	*Zea mays* roots	*Arabidopsis thaliana* leaves	*Solanum tuberosum* leaves	*Solanum commersonii* leaves	Cauliflower bud
PC	32.7	46.7	36.3	33.8	35.1	36.9	39	36	35.5	38.4	42.6	39
PE	38	34.4	33.9	31.2	29.2	32.1	35	32.3	38.9	35.7	36.2	35
PG	4.5	5.7	8.2	13.9	6.6	3	14	12.1	9	3.9	3.7	14
PI	5.3	2.2	7.4	4.8	9.4	7.6	9	10.5	10.3	7.5	5.5	9
PS	3.1	4.7	–	–	–	7.1	–	9.1	6.4			
PA	16.4	5.4	10.4	16.3	15.3	6.3	4	–	–	10.2	9.5	4
Other	–	<0.9	3.8	–	4.5	7	–	–	–	4.3	2.6	
References	Yoshida and Uemura (1986)	Lynch and Steponkus (1987)	Uemura and Steponkus (1994)	Norberg and Liljenberg (1991)	Uemura and Steponkus (1994)	Brown and Dupont (1989)	Grandmougin et al. (1989)	Bohn et al. (2007)	Uemura et al. (1995)	Palta et al. (1993)	Palta et al. (1993)	Wright et al. (1982)

PC phosphatidylcholine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PS* phosphatidylserine, *PI* phosphatidylinositol, *PA* phosphatidic acid, *DGPP* diacylglycerolpyrophosphate, *LPA* lysophosphatidic acid

among plant species, with palmitic (16:0) and linoleic (18:2) acids being the primary species (20–60%), and linolenic (18:3) acid representing another 7–26% (Table 3). Mass spectrometry analyses have shown that palmitic acid and linoleic acid are often associated (35–50% of total phospholipids), followed by 18:1/18:3 and 18:2/18:2 associations. Several lines of evidence suggest that temperature and osmotic stress produce changes in the fluidity of plant cell membranes that initiate cellular responses to modify the extent of unsaturation of fatty acids, mostly of phospholipids (for review Mikami and Murata 2003).

Phosphoinositides

Phosphoinositides (PIs) or inositolphospholipids constitute a minor fraction of total cellular lipids in all eukaryotic cells and play an important regulatory role in cell physiology. The repertoire of cellular processes known to be directly or indirectly controlled by this class of lipids continues to expand. Through interactions mediated by their headgroups, which can be reversibly phosphorylated to generate up to seven species, PIs play a fundamental part in controlling membrane interfaces (van Leeuwen et al. 2004). HPLC analysis of plants cells identify six different isoforms: PI(4)P, PI(4,5)P_2, PI(3)P, P(5)P, PI(3,4)P_2, and PI(3,5)P_2 (Irvine et al. 1989; Brearley and Hanke 1992; Meijer et al. 2001) (Fig. 4a). The enzymes that catalyze PI synthesis and hydrolysis, as well as the genes that encode them, have been characterized plants and are briefly described below.

PI(3)P and PI 3-Kinase

Depending on plant species, PI(3)P represents 2–15% of monophosphate phosphoinositides (Munnik et al. 1998, 2000). In animals, PI 3-kinases are classified into three distinct families according to their biochemical properties. Plant enzymes only belong to the third group containing a C2 domain, a helical domain, and a catalytic domain, but no RAS-binding domain. Three genes have been cloned in *Arabidopsis thaliana* (Welters et al. 1994). Moreover, only PI is phosphorylated to give PI(3)P (for review Mueller-Roeber and Pical 2002). PI (3)P can bind to FYVE protein domain, and chimeric GFP translational fusions indicate that PI(3)P localizes to the pre-vacuolar compartment and endosomes, suggesting a role for this lipid in vacuolar trafficking (Vermeer et al. 2006). PI(3)P is also likely to be involved in stress-related signaling as its levels increase after NaCl treatment in *Chlamydomonas* (Meijer et al. 2001), and inhibitors of PI 3-kinase prevent reactive oxygen species production and stomatal closure in guard cells (Park et al. 2003; Jung et al. 2002).

Table 3 Fatty acid composition of plasma membrane phospholipids from various plants and tissue

	Vigna Unguiculata hypocotyles						Oat roots		Maize roots					*Solanum tuberosum* leaves		*Solanum comersonii* leaves	
	PE	PC	PG	PI	PS	PA	PC	PE	PC	PE	PG	PI	PS	PC	PE	PC	PE
16:0	42	26.1	67.1	43.2	18.5	35.3	27.2	32.3	30.8	35	47.8	50.2	23.7	36.5	39.9	28.2	31.7
18:0	4.2	8.7	5.7	7.3	6.9	4.6	0.3	0.4	1	0.5	2.8	5.8	3	4.3	3.6	4.2	3.2
20:0	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7	2.4	1.7	1.5
16:1	–	–	–	–	–	–	–	–	–	–	–	–	–	0.6	0.5	0.7	0.5
18:1	8	11.6	5.6	8.7	5.9	9	1.7	1.3	5.3	2.5	5.4	4.2	2.3	4.7	2.2	12.3	5.2
20:1	9	1.2	–	–	14.4	0.7	–	–	–	–	–	–	–	–	–	–	–
22:1	4	1.1	0.5	–	8.3	0.5	–	–	–	–	–	–	–	–	–	–	–
18:2	23.4	24.4	8.5	16	19.3	25.4	54.1	50.4	61.2	60.2	42.3	38.2	36	34.4	35.9	33.3	40.5
20:2	0.3	0.4	4.2	2.6	0.8	0.7	–	–	–	–	–	–	–	–	–	–	–
22:2	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–
16:3	–	–	–	–	–	–	1.6	1.3	–	–	–	–	–	–	–	–	–
18:3	19.3	23.3	7.3	22.2	26.2	24.2	15.9	12.9	1.8	1.7	1.7	1.1	1.1	18.4	15.1	19	17
20:3	1.5	3.5	2	–	0.4	0.1	–	–	–	–	–	–	–	–	–	–	–
Others	–	–	–	–	–	–	1.8	3	–	–	–	–	27.8	–	–	–	–
References	Yoshida and Uemura (1986)						Norberg and Liljenberg (1991)		Bohn et al. (2007)					Palta et al. (1993)			

PC phosphatidylcholine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *PS* phosphatidylserine, *PI* phosphatidylinositol, *PA* phosphatidic acid, *DGPP* diacylglycerolpyrophosphate, *LPA* lysophosphatidic acid

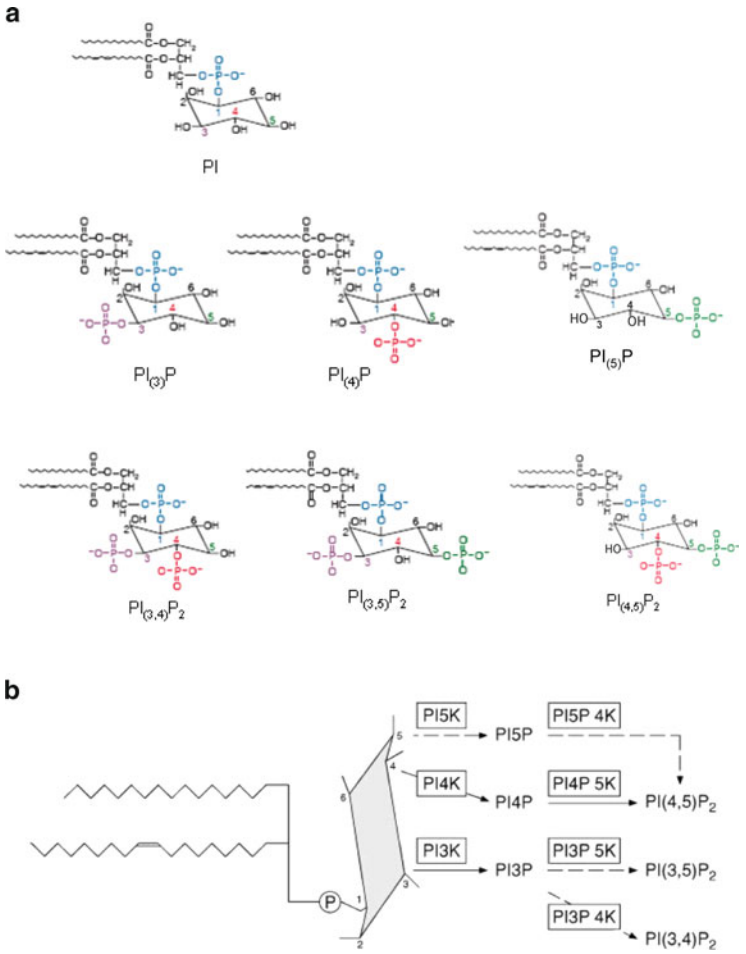

Fig. 4 (**a**) Chemical structure of the different isomers of polyphosphoinositides. (**b**) Different phosphoinositide kinases responsible for the phosphorylation of the inositol ring

PI(4)P and PI 4-Kinase

PI(4)P is the major isomer of monophosphate phosphoinositides, with up to 80% of them in *Chlamydomonas* (Meijer et al. 2001). All PI 4-kinase isoforms display a conserved catalytic domain in the C-terminus of protein. The *Arabidopsis* genome contains eight genes encoding type II PI 4-kinase (MW 55–70 kDa, membrane associated, and inhibited by calcium and adenosine) and four genes encoding putative enzymes of type III [MW 68–230 kDa, Pleckstrin domain (PH) or novel homology (NH), and an α-helix]. PI(4)P levels vary in response to elicitors, cold, sorbitol (Van deer Luit et al. 2000; Ruelland et al. 2002; Pical et al. 1999; Cho et al. 1993) and generally correlate with PI(4,5)P_2 levels, suggesting a tight control of PI pools.

PI(5)P

PI(5)P is a minor isomer (3–18% in *Chlamydomonas*), and no PI 5-kinase has been isolated to date in plants. Few data are available concerning its cellular role, albeit a transient increase is observed after an NaCl treatment of *Chlamydomonas* (Meijer et al. 2001).

PI(4,5)P_2 and PIP Kinase

Three isomers of PIP_2 are detected in plants, with PI(4,5)P_2 being by far the most abundant except in *Commelina communis*, where PI(3,4)P_2 is the major isoform (Parmar and Brearley 1993). PIP_2 is synthesized by a large set of PIP kinases which all share a conserved catalytic domain. Fifteen genes encode putative PIP kinases in the *Arabidopsis* genome. Among them, four are more similar to animal class III PI(3)P 5-kinases with a PI(3)P-binding FYVE domain and a catalytic domain at the C-terminus of proteins. The others can be classified into two subgroups, which are distinct from animal PIP kinases. Subgroup A contains an incomplete dimerization domain and a catalytic kinase domain at the C-terminus. Subgroup B displays a complete dimerization domain and eight repetitive MORN (membrane occupation and recognition nexus) motifs. To date, only *Arabidopsis* PIPK10 and PIP5K1 have been thoroughly characterized (Elge et al. 2001; Perera et al. 2005; Mikami et al. 1998), although kinase activity has been detected in PM fractions (Sandelius and Sommarin 1986). Recent evidence suggests that PI(4,5)P_2 resides in cellular pools associated with various fatty acid compositions. These pools may compartmentalize along organellar borders or in microdomains within one membrane (König et al. 2007).

Phosphatidic Acid/Diacylglycerol Pyrophosphate

Phosphatidic acid (PA) has only recently been identified as an important signaling molecule in plants and animals. In plants, its formation is transient and results from the activation of phospholipases C and D (for review Testering and Munnik 2000, 2005). More recently, a novel phospholipid, diacylglycerol pyrophosphate (DGPP), produced through phosphorylation of PA by the novel enzyme PA kinase (PAK), has been found in plants and yeast, but not in higher animals (Zalejski et al. 2005, 2006). No gene encoding a PA kinase has been yet characterized in any organism. PA and DGPP have emerged as second messengers in plant signaling (see the chapter “Plasma Membrane and Abiotic Stress” by Barkla and Pantoja).

1.2.2 Sphingolipids

Sphingolipids consist of a long-chain base (LCB) amidated by a fatty acid and a polar head attached to the alcohol residue of the LCB. There are over 500 different

molecular species of sphingolipids in plant cells (Pata et al. 2010). Greater LCB diversity is seen in plants than in yeast and animals: up to eight different molecular moieties can be formed from the most basic LCB sphinganine which has an acyl chain of 18 carbon atoms with two alcohol residues at C1 and C3 and an amine residue at C2 (Fig. 5). Sphinganine is further modified by hydroxylation at carbon 4 and/or desaturation at C4 or C8. In the latter case, *cis* (*Z*) and *trans* (*E*) isomers are formed. In plants, four LCBs are used to form complex sphingolipids, namely, (*E*)-sphing-8-enine (d18:18*E*), 4*E*,8*E*-sphinga-4,8-dienine (d18:24*E*,8*E*), (4*E*,8*Z*)-sphinga-4,8-dienine (d18:24*E*,8*Z*), and (*E*)-4-hydroxysphing-8-enine (t18:18*E*) (reviewed in Sperling and Heinz 2003; Pata et al. 2010) (Fig. 5), whereas animals and yeast used (*E*)-sphing-4-enine (d18:14*E*) and 4-hydroxysphinganine (t18:0),

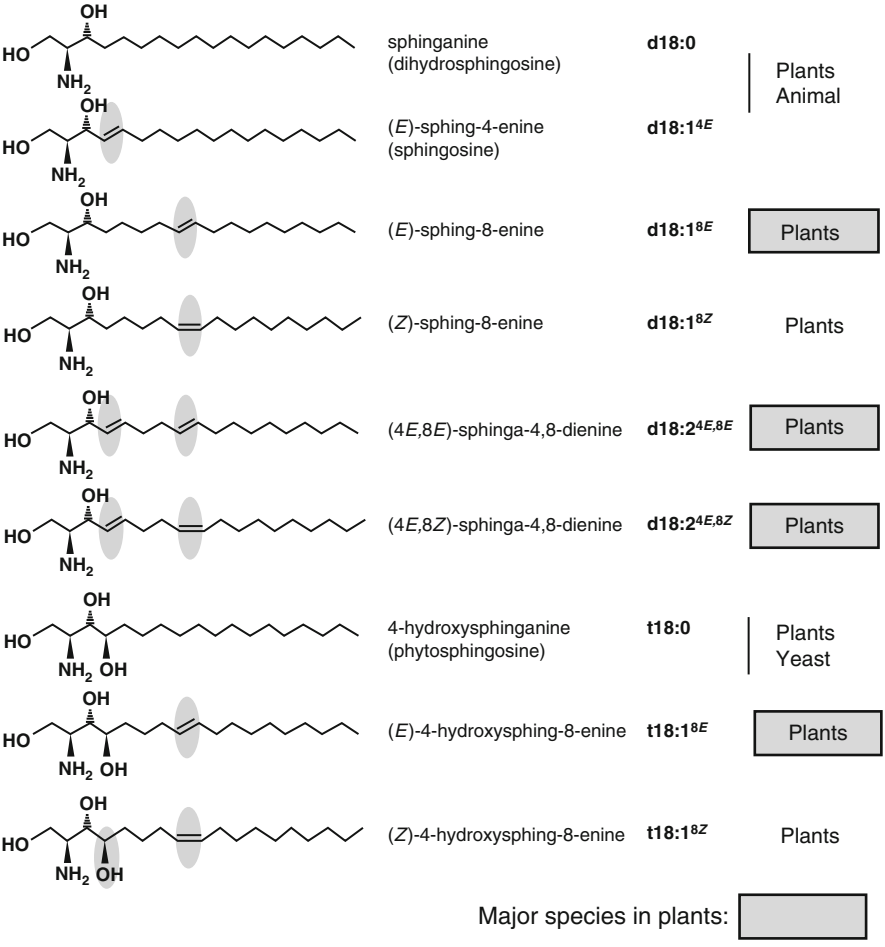

Fig. 5 Structures of long-chain bases (LCB) from animal, yeast, and plants (trivial names are given in *parenthesis*)

respectively. Acyl chains amidated to plant LCBs vary from 16 to 26 carbon atoms. They can be α-hydroxylated and are generally saturated.

Depending on the chemical nature of the polar head at C1 position of the LCB, two types of complex sphingolipids can be obtained: the cerebrosides with one or more glycosyl residues and the inositol phosphorylceramides (IPCs), which are formed by the addition of an inositol monophosphate group. IPC can be further polyglycosylated to form glycosyl inositol phosphorylceramides (GIPCs), previously called "phytoglycolipid" (Carter et al. 1958). One must emphasize that GIPCs are atypical lipids because they are insoluble in chloroform/methanol, a solvent system generally used to extract a broad spectrum of biological lipids. For this reason, GIPCs were long considered as minor plant sphingolipids. However, by using a single-solvent system with reversed-phase high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry detection, the complex mixture of plant sphingolipids has been recently deciphered (Markham and Jaworski 2007; Markham et al. 2006), and it now appears that GIPCs are the most abundant sphingolipids in *Arabidopsis thaliana* leaves. Indeed, hexosehexuronic-inositolphosphoceramides (GIPCs), monohexosylceramides (GluCers), and ceramides accounted for ca. 64%, 34%, and 2% of the total sphingolipids, respectively. Extraction and separation of sphingolipids from soybean and tomato showed that the neutral sphingolipids consisted of ceramide and monohexosylceramides; however, the major polar sphingolipid was found to be *N*-acetyl-hexosaminehexuronic-inositolphosphoceramide (Markham et al. 2006; Markham and Jaworski 2007). GIPC family can reach a great complexity and up to 13 different polar heads have been described (for review Pata et al. 2010). Sphingolipids are mainly associated with the PM (Sperling and Heinz 2003) although there is no report of the exact sphingolipid composition of the plant PM per se. Other minor sphingolipids, such as free long-chain bases and their phosphorylated derivates, have been detected and are likely to be involved in signaling (see the chapter "Plasma Membrane and Abiotic Stress" by Barkla and Pantoja).

1.2.3 Plant Sterols

Sterols are isoprenoids formed by a cylcopentaperhydrophenantren moiety, made up of four rigid rings, that is hydroxylated at position 3 (Fig. 2). Animal and yeast membranes incorporate only one sterol (cholesterol and ergosterol, respectively), but plant membranes contain multiple sterol species. Phytosterols differ by the number and the position of double bonds in the cycle, and by the nature (branched vs. linear, saturated vs. unsaturated) of the lateral chain inserted at the C17 position (reviewed in Hartmann and Benveniste 1987; Mongrand et al. 2004; Lefebvre et al. 2007; Palta et al. 1993). Phytosterols are derived from cycloartenol, a polycyclic sterol intermediate unique to plants, which is further alkylated at the C24 position to produce a triene. A C14 sterol reductase and an 8-7 isomerase act on the triene to yield 24-methylenelophenol, a branch point in the pathway. Parallel pathways lead to the synthesis of membrane sterols, sitosterol, and campesterol. Campesterol also

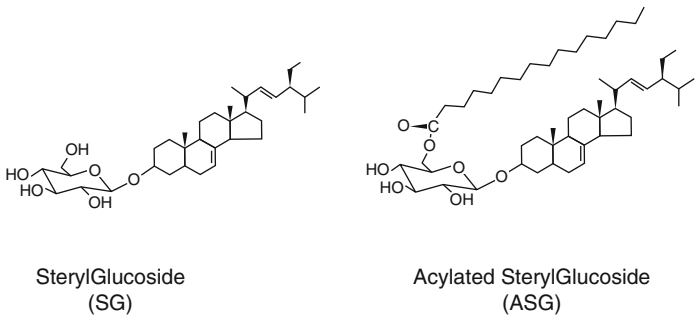

Fig. 6 Structure of conjugated phytosterols found in plant plasma membrane

serves as the progenitor of brassinosteroid hormones that are required for normal plant development.

Phytosterols can be acylated by a sugar (primarily glucose, but also mannose, xylose, and galactose) to form steryl glycosides (SGs), which can be further acylated to form acylated steryl glycosides (ASGs, Fig. 6). In the Solanaceae, the acyl chain is mainly palmitic acid (65%), but linoleic acid can also be detected. Steryl conjugates are also found in some bacteria such as *Helicobacter* (Haque et al. 1995) and *Spiroplasma* (Patel et al. 1978). Free sterols esterified with fatty acids are also detected in plant cells but are rarely constituents of the PM, except in cauliflower (Table 4). Esterified sterols are generally present in cytoplasmic oil bodies.

Free sterols generally represent 70–90% of total sterols in the PM of most plant species and across tissue types (Table 4). For example, in oat roots and coleoptyles, free sterols are predominant, whereas in leaves ASGs represent the major form (Table 4). Free sterols are synthesized in the ER and then transported to the PM by the secretory pathway. Sterol glycosylation to produce SG followed by acylation to form ASG occurs in the PM (Hartmann and Benveniste 1987).

Biochemical analysis coupled with forward and reverse genetic studies of sterol-deficient mutants in *Arabidopsis* revealed that sterols are essential for normal plant development, particularly embryogenesis, cell elongation, and vascular differentiation (reviewed in Clouse 2002). These developmental effects are independent of the conversion of sterol in the brasinosteroid phytohormones (Clouse 2002). The molecular mechanisms underlying these pleiotropic developmental effects are still poorly understood.

Sterols are crucial structural components of membranes because their insertion in the bilayer is known to regulate lipid chain order and modify the thermotropic phase transition between *liquid-disordered* and *solid-ordered* phases by inducing an intermediate *liquid-ordered* phase. This liquid-ordered phase combines a high rotational or translational mobility and a high conformational order in the lipid acyl chain. Sterols therefore increase the bilayer permeability and optimize the mechanical properties of the PM while maintaining its liquid form. The composition of sterol mixtures in the plant PM suggests that sterols regulate the structural and

Table 4 The sterol family in plant plasma membrane, expressed as total sterol content

	Vigna radiata hypocotyle	Seigle leaves	Seigle leaves	Oat coleoptyle	Oat roots	Oat roots	Spring oat leaves	Winter oat leaves	*Zea mays* coleoptyles	*Zea mays* roots	*Solanum tuberosum* leaves	*Solanum commersonii* leaves	Cauliflower bud
Free sterols	91.2	62.8	81.8	60.2	62	73.5	20.3	26.1	89	82.7	9.3	4.1	27
SG	5.3	29	12	22.7	26.7	1	13.6	9.5	3	11.8	19.7	17.9	18.9
ASG	3.5	8.2	6.2	17.1	11.3	26.6	66.1	64.4	8	5.5	71	78	32.5
Esterified sterol	–	–	–	–	–	1.5	–	–	–	–	–	–	21.6
References	Yoshida and Uemura (1986)	Lynch and Steponkus (1987)	Uemura and Steponkus (1994)	Sandstrom and Cleland (1989)	Sandstrom and Cleland (1989)	Norberg and Liljenberg (1991)	Uemura and Steponkus (1994)	Uemura and Steponkus (1994)	Hartmann and Benveniste (1987)	Bohn et al. (2007)	Palta et al. (1993)	Palta et al. (1993)	Wright et al. (1982)

SG steryl glycosides, *ASG* acylated steryl glycosides