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REVIEW

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# Lipid rafts in plants

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**Abstract** About two decades ago a provocative hypothesis evolved suggesting that the plasma membrane (PM) of mammalian and probably other eukaryotic cells constitutes a mosaic of patches comprising particular molecular compositions. These scattered lipid bilayer microdomains are supposedly enriched in sterols as well as sphingolipids and depleted in unsaturated phospholipids. In addition, the PM microdomains are proposed to host glycosyl-phosphatidylinositol-anchored polypeptides and a subset of integral and peripheral cell surface proteins while excluding others. Though the actual in vivo existence of such "lipid rafts" remains controversial, a range of fundamental biological functions has been put forward for these PM microenvironments. A variety of recent studies provide preliminary evidence that lipid rafts may also occur in plant cells.

**Keywords** Lipid rafts · Plasma membrane · Lipid microdomains · Detergent resistant membranes · Sterols · Sphingolipids

**Abbreviations** DRM: Detergent-resistant membrane · FRET: Fluorescence resonance energy transfer (a technique to determine protein-protein interactions via radiation-less energy transfer between fluorophore-tagged polypeptides) · FRAP: Fluorescence recovery after photobleaching (a technique to study lateral protein movement) · GPI: Glycosylphosphatidylinositol · PM: Plasma membrane · SNARE: Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor

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## Lipid rafts : a hot and timely cell biological topic

"It's lovely to live on a raft". It seems that not only the protagonist of Mark Twain's enchanting classic "The Adventures of Huckleberry Finn" (Twain 1885) enjoys the ease and freedom of rafting, but, according to the opinion of a continuously growing group of scientists, also a considerable amount of eukaryotic plasma membrane (PM)-resident proteins. This rising attention in the lipid raft concept within the scientific community is convincingly documented by the steadily increasing number of publications that contain the respective term in the title, abstract or key-words (Fig. 1). Though the hypothesis already evolved in the late 1980s and early 1990s, the term "lipid raft" was coined in a 1997 publication (Simons and Ikonen 1997), and since then rapidly raised in popularity (Fig. 1). The increasing awareness of lipid rafts is probably fuelled by the broad range of essential cellular tasks that are attributed to these PM microdomains. Proposed biological roles include signal transduction (Simons and Toomre 2000), regulation of exocytosis (Salaün et al. 2004), endocytosis (Parton and Richards 2003) and apoptosis (Garcia et al. 2003), actin cytoskeleton organization (Falk et al. 2004; Wickström et al. 2003) as well as subversion of lipid raft function for pathogen entry (Rosenberger et al. 2000; Lafont et al. 2004). Despite the fact that the concept is also subject to extensive criticism (see below) the lipid raft enthusiasm has recently reached the plant sciences, too (Martin et al. 2005).

In this review, we summarize current experimental evidence for the possible existence and probable biological functions of lipid rafts with a particular emphasis on plants. We describe major techniques currently used to analyze proposed lipid microdomains in animal and plant cells and discuss the concerns associated with the respective procedures. Finally, we provide an outlook on future experimental routes that may contribute to solve the pivotal question whether or whether not lipid raft-like PM microdomains do exist in planta.

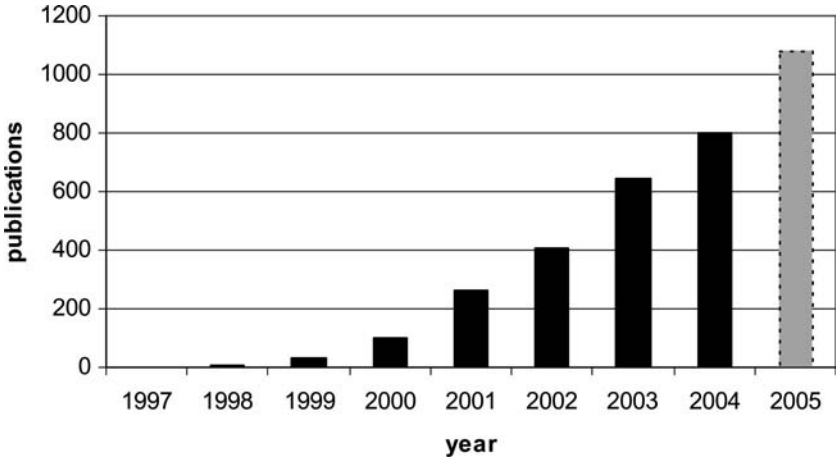


**Fig. 1** Manifestation of the term "lipid raft(s)" in scientific literature. The ISI "Web of Science" database was examined year by year for occurrence of the term "lipid raft(s)" in the "topic" (equals title, key words, and abstract) search field. The number of hits is plotted against the respective year. The value for 2005 is extrapolated based on the data from January to May 2005. Please note that within the same timeframe incidence of the control term "plasma membrane" increased only slightly from ~3,500 to ~3,900 publications/year

## What are lipid rafts (supposed to be)?

In eukaryotes, lipids fulfill numerous roles: they form indispensable hydrophobic barriers (membranes) for cellular compartments, function as energy store, signaling molecules, and defence compounds and are employed for post-translational protein modification. In plants, lipids additionally represent essential components of cutins and waxes that protect the plant against the environment. Biological membranes are composed of many lipid types including phospholipids (e.g. phosphatidylcholine), sphingolipids (e.g. glycosphingolipids and sphingomyelin) and sterols (e.g. cholesterol). The lipid raft hypothesis suggests that the various types of lipids are not uniformly distributed in eukaryotic PMs but spatially organized in lateral patches of distinct molecular makeup. Initially inspired by the finding that the interior and exterior leaflet of mammalian PM bilayers vary in lipid composition (especially in polarized epithelial cells; Simons and van Meer 1988), Simons and Ikonen (1997) proposed that dynamic entities enriched in sterols and sphingolipids, the so-called lipid rafts, are present in the outer (exoplasmic) leaflet of the PM. It was also suggested that the molecular composition of lipid rafts further differs from the remainder of the PM by hosting a specific subset of integral and membrane-associated proteins including glycosylphosphatidylinositol (GPI)-anchored polypeptides, while excluding others (Fig. 2). The lipid raft hypothesis is experimentally supported by studies with artificial membranes in which the major mammalian sterol, cholesterol, but also yeast and plant sterols were shown to promote the formation of microdomains that are reminiscent of the presumptive lipid rafts (Xu et al. 2001; reviewed in Silvius 2003). In addition, a range of biochemical, immunological and biophysical methods provides further evidence for the existence of PM microdomains. Firstly, sphingolipid/cholesterol-rich liposomes were found to be insoluble in mild nonionic detergents such as Triton X-100 at 4°C. Such detergent-insoluble low density membrane fractions are thought to reflect the in vivo composition of lipid microdomains (Brown and Rose 1992; Schroeder et al. 1994; Simons and Ikonen 1997; Brown and London 1998). Proteins presumably interacting with or residing in lipid rafts are therefore often enriched and identified based on their ability to float in vitro on gradients with detergent-resistant membranes (DRMs) that were derived from cell lysates treated with Triton X-100. Further experimental approaches to prove the in vivo existence of rafts focused in the past on biochemical und immunological cross-linking of probably raft-associated proteins (e.g. Chamberlain et al. 2001; Kasahara et al. 2002), indirect visualization of presumptive lipid rafts by fluorescence resonance energy transfer (FRET) (e.g. Nichols 2003), as well as direct visualization via protein immunolocalization (e.g. Oliferenko et al. 1999), atomic force microscopy (reviewed in Henderson et al. 2004), and single particle tracking (e.g. Pralle et al. 2000). While the results of the majority of these studies were, in principle, consistent with the existence of lipid rafts, the employed techniques and interpretation of the respective data are nonetheless subject to extensive criticism (see below).

It seems that the main forces enabling the formation of rafts would be lipid–lipid interactions. Sphingolipids are able to associate with each other through interactions between their carbohydrate heads and their long, predominantly saturated, lipid hydrocarbon chains, while cholesterol molecules are supposed to serve as spacers to fill voids between sphingolipids (Simons and Ikonen 1997). Cholesterol and sphingolipids containing saturated hydrocarbon chains assemble to form tightly packed sub-domains corresponding to the so-called "liquid-ordered" phase biophysically characterized in model and biological membranes (Ahmed et al. 1997; Brown and London 1998; London and Brown 2000; Xu et al. 2001). These lipid rafts are supposed to float freely

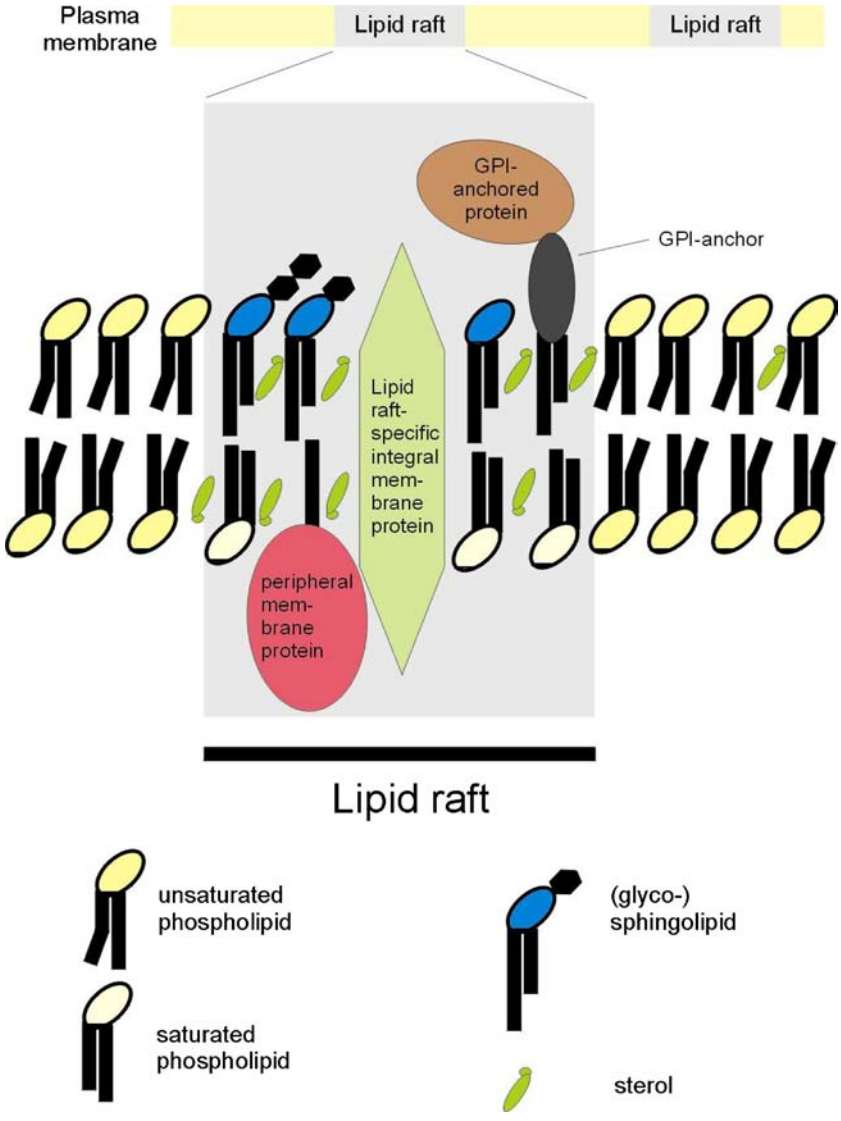


**Fig. 2** Schematic representation of presumed lipid raft organization. According to the lipid raft hypothesis, rafts (gray boxes) are PM patches characterized by a particular molecular composition. They are supposed to be enriched in saturated phospholipids, sterols, and sphingolipids and assumed to harbor a subset of membrane-associated polypeptides, including GPI-anchored proteins, peripheral and integral PM proteins

in the surrounding membrane which is more fluid and analogous to the so-called "liquid disordered" phase. At the molecular level, the higher fluidity is thought to be the consequence of the high surface area occupied by unsaturated phospholipids as compared to the dense packing of the sphingolipid-cholesterol assemblies. Recruitment of proteins to lipid rafts is likely to affect their function in several ways. Firstly, the concentration of different proteins in rafts could facilitate homo- and heteromeric polypeptide interactions. Secondly, the ordered lipid environment might directly modulate the activity of presumptive protein complexes, e.g. by modification and/or stabilization of their conformation (Opekarova and Tanner 2003).

Though a consensus has not yet been reached in this respect, it emerges that individual rafts in resting mammalian cells are small entities (~5–50 nm) containing possibly thousands of lipid molecules but probably only a moderate number of proteins (Varma and Mayor 1998; Simons and Toomre 2000; Füllekrug and Simons 2004; Glebov and Nichols 2004; Sharma et al. 2004). Accordingly, direct visualization of individual lipid rafts using conventional light- or epifluorescence microscopy (resolution >300 nm; Varma and Mayor 1998) has not been successful in many cases (Simons and Toomre 2000; Glebov and Nichols 2004). However, apparent stimulus-dependent aggregation of rafts to microscopically visible units has been described in various cell types and upon a range of cues (Tanimura et al. 2003; Gekara and Weiss 2004; Triantafilou et al. 2004). It is conceivable that the small size of individual rafts in resting cells is important to keep raft-associated proteins in an inactive state. Upon stimulation, rafts may cluster to form a larger platform where functionally related proteins can interact (Simons and Toomre 2000; Garcia et al. 2003). Glycoprotein-binding proteins (lectins) were recently suggested to contribute to the constitution of these stimulus-dependent microdomain assemblies, for example via crosslinking of PM-resident glycoproteins (Füllekrug and Simons 2004).

## Lipid rafting: proposed biological roles of lipid microdomains

Despite the wealth of information on presumptive lipid raft composition and structure, the precise functional roles of these lipid microdomains are still subject to considerable debate (Shin and Abraham 2001). Many cellular tasks have been ascribed to sterol-rich lipid microdomains, including such diverse processes as signal transduction, polarized secretion, membrane transport, transcytosis across epithelial monolayers, cytoskeletal organization, apoptosis, generation of cell polarity and the entry of infectious organisms in living cells (Bagnat and Simons 2002; Rosenberger et al. 2000; Simons and Ikonen 1997).

Sterol-rich lipid rafts have been implicated in transmembrane signal transduction because of the recruitment and concentration of various receptors and signaling components within DRMs (reviewed in Simons and Toomre 2000). Receptors for various growth factors and hormones, including epidermal growth factor, platelet-derived growth factor and insulin have been localized to presumptive lipid microdomains (Okamoto et al. 1998). In addition, many signaling molecules such as receptor tyrosine kinases, protein kinase C isoforms, mitogen-activated protein (MAP) kinases, adenylyl cyclase, lipid signaling intermediates and heterotrimeric G protein α subunits have been shown to be enriched in DRMs (Okamoto et al. 1998). It is therefore thought that lipid rafts may represent "signaling platforms" in which various components of signal transduction cascades are locally condensed (Hoessli et al. 2000).

A subset of soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, involved in membrane fusion events at the PM, have been reported to be recruited and enriched in DRMs prompting a potential role of lipid rafts in exocytosis (Chamberlain et al. 2001; Xia et al. 2004; reviewed in Salaün et al. 2004). SNARE domain-containing proteins syntaxin 1-A, SNAP-25 and VAMP-2 were found to be enriched in DRMs of rat adrenal medulla PC12 tumor cells as well as rodent pancreatic β-cells (Chamberlain et al. 2001; Xia et al. 2004). In the case of the latter cell type, pharmacological depletion of membrane cholesterol resulted in redistribution of SNARE proteins from the presumptive lipid rafts and an increase in glucose-mediated insulin secretion and single cell exocytic events (Xia et al. 2004). In contrast, Chamberlain et al. (2001) reported that the SNARE-domain proteins, when excluded from the lipid rafts in neuroendocrine PC12 cells, led to a decrease in regulated dopamine exocytosis from these cells. Taken together, these results may indicate that, depending on the cell type, localization of SNARE proteins in PM microdomains may either have a stimulatory or inhibitory effect on secretion.

Another function attributed to lipid rafts is their potential involvement in cytoskeletal organization by association with actin-rich regions of the cell (Falk et al. 2004; Oliferenko et al. 1999; Simpson-Holley et al. 2002; Wickström et al. 2003). Human endostatin for example, a naturally occurring inhibitor of angiogenesis capable of inhibiting tumor growth and metastasis in various animal models, was shown to connect with lipid rafts and induce reorganization of actin cytoskeleton via downregulation of RhoA, a member of the Rho GTPase family (Wickström et al. 2003). Removal of endostain from raft fractions using cholesterol chelators resulted in the inhibition endostatin-induced actin reorganization (Wickström et al. 2003).

Recently, programmed cell death (apoptosis) also has been implicated with lipid rafts (reviewed in Garcia 2003). Many cell death receptors and their cognate ligands were reported to be enriched in DRMs (Ayllon et al. 2002; Gajate and Mollinedo 2005; Scheel-Toellner et al. 2004). It has been suggested that lateral association of rafts concentrates the receptors and their cognate ligands in the lipid microdomains and may thus trigger a potent apoptotic response (Gajate and Mollinedo et al. 2005). This role of PM microdomains is further supported by the fact that raft disruption leads to a significant delay in spontaneous apoptosis of human neutrophils (Scheel-Toellner et al. 2004).

PM microdomains have also been suggested to act as portals for pathogen entry and import of certain macromolecules into host cells and their subsequent translocation to various subcellular sites (Rosenberger et al. 2000). Although these microdomains are thought to comprise only a small percentage of the cell surface area, the local enrichment of a subset of PM-resident proteins may render them suitable targets for microbes to associate and communicate with their target cells. While lipid microdomains presumably provide a functional platform for signaling events that also regulate defensive responses at the cell periphery, various pathogens including bacteria, viruses and eukaryotic parasites appear to have evolved strategies to evade these host immune responses by hijacking the very rafts for survival and/or completion of their life cycle (Rosenberger et al. 2000; Shin and Abraham 2001).

Disruption of raft integrity by pharmacological means has been proven to interfere with host cell entry of various bacterial pathogens (Lafont 2004). For example, endocytosis of *Campylobacter jejuni*, a causative organism of diarrhea, into host intestinal cells was inhibited when sterol-rich caveolae (invaginated cave-like structures in the PM of animal cells thought to represent a sub-type of lipid rafts) were disrupted with cholesterol chelators (Wooldridge et al. 1996). Similarly, the uptake of *Mycobacterium tuberclulosis*, the causal agent of tuberculosis, depends on the presence of cholesterol-rich microdomains in the PM of host macrophages. Internalization of the mycobacteria was inhibited when the macrophages were depleted of cholesterol (Gatfield and Pieters 2000). Entry of FIMH (encoding an adhesin)-expressing *Escherichia coli* into mast cells after binding to the raft-associated protein CDC48 allows the survival of this opportunistic

pathogen that causes extra-intestinal infections in immuno-compromised patients. In contrast, disruption of rafts by β-methyl-cyclodextrin resulted in the inhibition of *E. coli* uptake (Shin et al. 2000).

Early stages of viral entry into human cells frequently involve binding of viral particles to cell surface receptors followed by subsequent admission into the host cell. These host receptors, among others include epithelial growth factor receptor (EGFR) and integrins (Wang et al. 2003). Recently, human cytomegalovirus (HCMV), an opportunistic pathogen causing birth defects in newborn babies and diseases in immuno-compromised individuals was shown to induce the translocation of an integrin (αvβ3) into lipid rafts. There, the αvβ3 integrin interacts with EGFR to form multimeric complexes and triggers downstream signaling cascades that enable viral entry specifically within lipid rafts and not at random sites (Wang et al. 2005). Similarly, human immunodeficiency virus (HIV-1) uses lipid rafts for nearly all stages of its life cycle, including initial entry into host mucosal cells and subversion of host cell signaling for replication and immune evasion (Alfsen et al. 2001; Peterlin et al. 2003). Treatment of HIV particles with raft-disrupting drugs renders the virus incompetent for cell entry (Guyader et al. 2002). Finally, parasitic protozoae like *Toxoplasma gondii* and *Plasmodium falciparum*, have also been shown to exploit rafts for their intracellular survival and/or to modulate host responses (Shin and Abraham 2001).

## To raft or not to raft? That is the question!

Though the lipid raft concept represents a very appealing hypothesis and despite the fact that during the last decade, a substantial amount of data has been accumulated that would at least be compatible with the existence of lipid rafts, there has also been substantial criticism that basically challenges the very existence of lipid rafts. The censure centers around the point that many of the experimental methods used to study lipid microdomains are indirect and thus prone to potential misinterpretation of the respective data. This particularly applies to the popular procedure of isolating DRMs. The detergent commonly used for this practice is Triton X-100, a substance recently blamed even to promote the formation of the lipid microdomains it was (and usually still is) generally claimed to extract (Heerklotz 2002). The non-physiological temperature (4°C) used to extract DRMs is a further concern associated with this method since lipid phase behavior is highly temperature-dependent and reduction in temperature alone could potentially induce alterations in overall lipid organization (Munro 2003). In conclusion, there remain serious doubts whether DRMs isolated by treatment with cold Trition X-100 reflect in any way the in vivo situation. Similar worries apply to the method of pharmacological cholesterol depletion, e.g. by application of cholesterol synthesis inhibitors or direct extraction via methyl-β-cyclodextrin. Besides the anticipated effects on lipid rafts, perturbation of the PM cholesterol content might impinge on other sterol functions thereby potentially obscuring the interpretation of data obtained in this type of experiments (Munro 2003). It is further criticized that more direct methods of lipid raft visualization like electron microscopy, atomic force microscopy, single particle tracking and FRET have not yet reached a consensus with respect to size or even existence of these microdomains (Laude and Prior 2004; Pierce 2004; reviewed in Munro 2003).

Although the extensive criticism does not generally preclude the existence of lipid rafts per se it challenges scientists to consider also alternative explanations for the observed phenomena. For example, based on findings in model membranes (reviewed in Silvius 2003) the classical lipid raft concept argues for co-presence of proteins in particular lipid fractions being the consequence of a distinct lipid composition of such microdomains. However, in vivo it might be exactly the other way around. The observed lipid composition is possibly just the direct or indirect consequence of the formation of specific protein complexes (Epand 2004; Hammond et al. 2005). Only future can tell whether PM microdomains bearing a specific subset of proteins indeed exist in vivo and whether lipids or proteins or both constitute the actual "driving force" for the establishment of such subcellular microenvironments.

## Veggy oil: DRMs as first indication for the potential existence of lipid rafts in plants

As stated above, several methods can, in principle, provide evidence for the existence of lipid rafts. Like in the animal field, the examination of low-density DRMs has been proven to be the most popular technique to study putative lipid microdomains in plants. Peskan et al. (2000) reported for the first time the isolation of Triton X-100-insoluble PM vesicles from a higher plant species (*Nicotiana tabacum*; tobacco). The detergent-resistant leaf membrane fraction was enriched for a distinct subset of proteins, excluding the majority of PM-resident proteins. Among the Triton X-100 insoluble polypeptides, six putative GPI-anchored proteins were identified by their release in the aqueous phase upon phosphatidylinositol-specific phospholipase C digest. In addition it was immunologically shown that ~15% of the heterotrimeric G-protein β subunit is present in the tobacco DRM fraction (Peskan et al. 2000).

Two-dimensional thin layer chromatography revealed the lipid composition of PMs derived from either 5-day-old etiolated bean hooks or 9-week-old Arabidopsis leaves, revealing a significantly enhanced sterol content in the thale cress samples (Bérczi and Horvath 2003). Previously, it was found that ascorbate-reducible b-type cytochrome (cytochrome $b_{561}$) could be easily

solubilized by Triton X-100 from the bean PMs, whereas seemingly the same protein appeared resistant to solubilization in Arabidopsis PMs (Bérczi et al. 2001). The authors speculated that the differential Triton X-100 solubility could be the consequence of the formation of sterol-containing lipid rafts in the Arabidopsis membrane (Bérczi and Horvath 2003).

These pioneering findings were recently extended by a set of related studies who all employed protein mass spectrometry to get a glance at the polypeptide composition of the Triton X-100 insoluble PM fraction of various plant species (Mongrand et al. 2004; Shahollari et al. 2004; Borner et al. 2005; Table 1). Using thale cress (*Arabidopsis thaliana*) and mustard (*Sinapis alba*) cotyledons as biological source material, Shahollari et al. (2004) found a particular enrichment in potential signaling components, including a range of leucine rich repeat receptor kinases and other kinases, several small GTP-binding proteins and, reminiscent of the findings by Peskan et al. (2000), the β subunit of heterotrimeric G-proteins. These findings would be compatible with a pivotal role for plant lipid microdomains as signal relays, one of the recurrently proposed functions for lipid rafts in animal cells (Hoessli et al. 2000; Simons and Toomre 2000; see above).

Using a comparable approach, the molecular lipid and polypeptide composition of DRMs isolated from tobacco cell cultures (BY2 cells) and *N. tabacum* leaf material was studied (Mongrand et al. 2004). Triton X-100-insoluble membrane fractions were highly enriched in glycosylceramide (a sphingolipid) as well as several sterols (stigmasterol, sitosterol, 24-methylcholesterol and cholesterol), whereas PM-typical phospho- and glycoglycerolipids were largely excluded from the DRMs. Moreover, the proportion of saturated fatty acids was significantly higher in the glycerolipids of the DRMs as compared to the PM. Based on the results of SDS-PAGE and subsequent liquid chromatography followed by tandem mass spectrometry (LC-MS/MS), PM-resident $H^+$ ATPase isoforms, an aquaporin and the oligogalacturonic acid binding protein called remorin were identified as present in DRMs of both tobacco leaves and cultured BY2 cells. Interestingly, the authors also demonstrated immunologically that an NADPH oxidase isoform, NtrbohD, whose expression is specifically triggered upon treatment with pathogen elicitors, is recruited to the tobacco DRMs upon elicitation. The small G-protein NtRac5, assumed to be a negative regulator of NADPH oxidase, accumulated likewise in this lipid fraction.

In a similar study, Borner et al. (2005) examined DRMs derived from *A. thaliana* callus membranes and determined lipid as well as protein composition of the Triton X-100-insoluble fraction. Using gas chromatography coupled to mass spectrometry, they found that the DRMs exhibit four to five fold higher sterol-to-protein and sphingolipid-to-protein ratios, respectively, than the average Arabidopsis membrane. In a comparative proteomics approach by using two-dimensional difference gel electrophoresis, the authors identified a range of polypeptides that were specifically enriched in DRMs. Mass spectrometry based on LC-MS/MS revealed, amongst others, presence of eight GPI-anchored proteins, several $H^+$ ATPase isoforms as well as a plant homolog of flottilin, a protein proposed to be also associated with lipid microdomains in mammalian cells (Table 1; Salzer and Prohaska 2001).

## Animal and plant DRMs : alike but distinct

Taken together, the analysis of Triton X-100-insoluble membrane fractions of plant cells revealed similar to the results obtained in animal cells evidence for the existence of lipid microdomains that include a subset of PM proteins and exclude others. Some plant DRM-resident polypeptides were identified in independent studies (e.g. PM-ATPase, ERD4, SKU5, aquaporins, and 14–3-3 proteins), strengthening the notion that these proteins are possibly associated with a particular lipid fraction (Table 1). Notably, similar sets of proteins appear to be present in DRMs of animal and plant cells including GPI-anchored polypeptides, PM-ATPase, and signaling molecules (Table 1). Though there is not yet a full consensus in this respect, plant DRMs, like their animal counterparts, appear to be enriched in sterols and sphingolipids or might at least possess elevated sterol/protein and sphingolipid/protein ratios, respectively. In addition to these seeming communities, plant DRMs exhibit also some apparent differences. For example, tobacco DRMs show higher buoyant densities than respective fractions isolated from yeast and animal cells, possibly due to a lower lipid/protein ratio of the plant DRMs (Mongrand et al. 2004). Furthermore, plant sterols and sphingolipids exhibit a much greater structural diversity than their animal and yeast counterparts (Hartmann 1998; Sperling and Heinz 2003). In contrast to yeast and animal cells where one sterol dominates (ergosterol or cholesterol, respectively) at least five sterols (stigmasterol, 24-methyl-cholesterol, sitosterol, campesterol and cholesterol) have been found in plant DRMs (Mongrand et al. 2004; Borner et al. 2005).

## Beyond detergent insolubility: further evidence for the existence of plant PM microdomains

Besides detergent insolubility, which is suspected to be subject to various artefacts (Heerklotz 2002; Munro 2003; see above), further experimental routes provide additional evidence for the existence of lipid microdomains in plant cells. These include FRET studies, stimulus-specific coalescence of fluorescently tagged PM proteins as well as filipin staining directly visualizing local sterol accumulation. Vermeer et al. (2004) used multimode FRET between differently lipidated green fluorescent protein (GFP) variants to probe PM orga-

**Table 1** Compilation of proteins in plant DRMs identified by protein mass spectrometry

| Protein annotation | Predicted molecular mass (kDa) | Species | Accession number(s) | Subcellular localization or mode of membrane asscociation [a] | Source material | References |
|---|---|---|---|---|---|---|
| AtPGP4/AtMDR4 PGP/MDR protein | 139.4 | *At* | At2g47000 | Unknown | *At* callus | Borner et al. (2005) |
| AtPGP11/AtMDR8 PGP/MDR protein | 138.1 | *At* | At1g02520 | Unknown | *At* callus | Borner et al. (2005) |
| Plasma membrane ATPase 4 | 105.2 | *Np* | Q03194 | PM | *Nt* BY2 cells | Mongrand et al. (2004) |
| Plasma membrane ATPase 1 | 105.1 | *Le* | P22180 | PM | *Nt* leaf | Mongrand et al. (2004) |
| Plasma membrane ATPase | ~105 | *At* | At2g18960, At4g30190, At3g47950, At2g07560, At3g42640, At1g80660, At5g62670 | PM | *At* callus | Borner et al. (2005) |
| Plasma membrane ATPase | ~104 | *At* | At1g17260, At1g80660, At2g18960, At2g24520, At2g07560, At4g30190, At3g47950, At5g62670 | INT | *At* cotyledons | Shahollari et al. (2004) |
| Phospholipase D Δ | 98.9 | *At* | At4g35790 | PER | *At* cotyledons | Shahollari et al. (2004) |
| Putative receptor kinase (ACR4) | 98.2 | *At* | At3g59420 | INT | *At* cotyledons | Shahollari et al. (2004) |
| S locus receptor kinase (ARK3) | 96.4 | *At* | At4g21380 | INT | *At* cotyledons | Shahollari et al. (2004) |
| V0 ATPase subunit | ~93.5 | *At* | At2g21410, At4g39080 | EM/PM | *At* callus | Borner et al. (2005) |
| EF hand protein | 90.2 | *At* | At1g05150, At2g32450 | PER | *At* cotyledons | Shahollari et al. (2004) |
| TOC75 chloroplast outer envelope protein | 89.6 | *At* | At3g46740 | CHL | *At* callus | Borner et al. (2005) |
| Kinase | 84.6 | *At* | At1g16760 | INT | *At* cotyledons | Shahollari et al. (2004) |
| GPDL1 glycerophosphodiesterase-like 1 protein | 84.5 | *At* | At5g55480 | PM (GPI) | *At* callus | Borner et al. (2005) |
| ATP synthase α-subunit | 86.2 | *At* | At2g07698 | MIT | *At* callus | Borner et al. (2005) |
| ERD4 early responsive to hydration stress protein | 82.3 | *At* | At1g30360 | Unknown | *At* callus | Borner et al. (2005) |
| ERD4 early responsive to hydration stress protein | 82.3 | *At* | At1g30360 | INT | *At* cotyledons | Shahollari et al. (2004) |
| Heat shock cognate protein 80 | 80.1 | *Le* | P36181 | CYT | *Nt* BY2 cells | Mongrand et al. (2004) |
| Leucin-rich repeat receptor kinase | 77.6 | *At* | At3g14350 | INT | *At* cotyledons | Shahollari et al. (2004) |
| HIPL1 hedgehog-interacting protein-like 1 | 75.2 | *At* | At1g74790 | PM (GPI) | *At* callus | Borner et al. (2005) |
| Glycosyl hydrolase family 3 protein | 72.9 | *At* | At5g04885 | PM (GPI) | *At* callus | Borner et al. (2005) |
| Leucin-rich repeat receptor kinase | 71.7 | *At* | At2g26730 | INT | *At* cotyledons | Shahollari et al. (2004) |
| Heat shock cognate protein 2 | 70.7 | *Le* | P27322 | CYT | *Nt* leaf | Mongrand et al. (2004) |
| Heat shock cognate protein 2 | 70.7 | *Le* | P27322 | CYT | *Nt* BY2 cells | Mongrand et al. (2004) |
| Leucin-rich repeat receptor kinase | 70.4 | *At* | At3g17840 | INT | *At* cotyledons | Shahollari et al. (2004) |
| Leucin-rich repeat receptor kinase | 69.4 | *At* | At3g08680 | INT | *At* cotyledons | Shahollari et al. (2004) |
| V1 ATPase subunit (AtVHA-A) | 69.1 | *At* | At1g78900 | EM/PM | *At* callus | Borner et al. (2005) |
| V ATP synthase subunit A | 68.7 | *Bn* | Q39291 | PM | *Nt* BY2 cells | Mongrand et al. (2004) |
| V ATP synthase subunit A | 68.5 | *Bv* | Q39442 | VAC | *Nt* leaf | Mongrand et al. (2004) |
| Dynamin-like protein | 68.2 | *At* | P42697 | CYT/PM | *Nt* BY2 cells | Mongrand et al. (2004) |
| Leucin-rich repeat receptor kinase | 67.7 | *At* | At3g02880 | INT | *At* cotyledons | Shahollari et al. (2004) |

**Table 1** (Contd.)

| Protein annotation | Predicted molecular mass (kDa) | Species | Accession number(s) | Subcellular localization or mode of membrane asscociation [a] | Source material | References |
|---|---|---|---|---|---|---|
| Leucin-rich repeat receptor kinase | 67.4 | *At* | At5g16590 | INT | *At* cotyledons | Shahollari et al. (2004) |
| SKS-1 | 66.1 | *At* | At4g25240 | PM (GPI) | *At* callus | Borner et al. (2005) |
| Jacalin lectin family protein | 66.1 | *At* | At3g16460 | ER/VAC | *At* callus | Borner et al. (2005) |
| SKU5 | 65.8 | *At* | At4g12420 | PM (GPI) | *At* callus | Borner et al. (2005) |
| SKU5 | 65.6 | *At* | At4g12420 | INT | *At* cotyledons | Shahollari et al. (2004) |
| DIMINUTO protein | 65.4 | *At* | Q39085 | ER | *Nt* leaf | Mongrand et al. (2004) |
| FAD-binding domain protein | 60.5 | *At* | At4g20830 | Unknown | *At* callus | Borner et al. (2005) |
| Calnexin-like protein | 60.5 | *At* | At5g07340 | PER | *At* cotyledons | Shahollari et al. (2004) |
| β-glucosidase | 60.0 | *At* | At1g66270 | ER/VAC | *At* callus | Borner et al. (2005) |
| Calcium-dependent kinase (CPK21) | 59.9 | *At* | At4g04720 | PER | *At* cotyledons | Shahollari et al. (2004) |
| ATP synthase β-subunit 1 | 59.8 | *At* | At5g08670 | MIT | *At* callus | Borner et al. (2005) |
| Kinase | 56.8 | *At* | At4g35230 | PER | *At* cotyledons | Shahollari et al. (2004) |
| V1-ATPase subunit (AtVHA-B2) | 54.4 | *At* | At4g38510 | EM/PM | *At* callus | Borner et al. (2005) |
| V1-ATPase subunit (AtVHA-B1) | 54.1 | *At* | At1g76030 | EM/PM | *At* callus | Borner et al. (2005) |
| CBL-interacting protein kinase 9 | 50.5 | *At* | At1g01140 | PER | *At* cotyledons | Shahollari et al. (2004) |
| CBL-interacting protein kinase 8 | 50.4 | *At* | At4g24400 | PER | *At* cotyledons | Shahollari et al. (2004) |
| AtFlot1 (related to flottilin) | 52.3 | *At* | At5g25250 | Unknown | *At* callus | Borner et al. (2005) |
| Tubulin β-1 chain | 49.9 | *Gm* | P12459 | CYT | *Nt* BY2 cells | Mongrand et al. (2004) |
| Elongation factor 1-α | 49.3 | *Dc* | P29521 | CYT/PM | *Nt* BY2 cells | Mongrand et al. (2004) |
| Elongation factor 1-α | 49.3 | *Dc* | P29521 | CYT/PM | *Nt* leaf | Mongrand et al. (2004) |
| Ankyrin kinase IV | 49.3 | *At* | At1g14000 | PER | *At* cotyledons | Shahollari et al. (2004) |
| RuBisCo large subunit | 48.9 | *Ab* | O78262 | CHL/CYT | *Nt* leaf | Mongrand et al. (2004) |
| Kinase | 47.6 | *At* | At1g63500 | PER | *At* cotyledons | Shahollari et al. (2004) |
| Leucin-rich repeat receptor kinase | 47.1 | *At* | At1g13230 | INT | *At* cotyledons | Shahollari et al. (2004) |
| Kinase (similar to ATMRK1) | 46.0 | *At* | At3g01490 | PER | *At* cotyledons | Shahollari et al. (2004) |
| Stomatin-like | 45.0 | *At* | At4g27585 | Unknown | *At* callus | Borner et al. (2005) |
| Unknown protein | 44.3 | *At* | At1g29980 | PM (GPI) | *At* callus | Borner et al. (2005) |
| V1-ATPase subunit (AtVHA-C) | 42.6 | *At* | At1g12840 | EM/PM | *At* callus | Borner et al. (2005) |
| V1-ATPase subunit (AtVHA-B3) | 36.3 | *At* | At1g20260 | EM/PM | *At* callus | Borner et al. (2005) |
| Carbonic anhydrase | 34.5 | *Nt* | P27141 | CHL/CYT/PM | *Nt* leaf | Mongrand et al. (2004) |
| Receptor-like protein | 32.8 | *At* | At5g41290 | PM (GPI) | *At* callus | Borner et al. (2005) |
| Jacalin lectin family protein | 32.1 | *At* | At3g16420 | ER/VAC | *At* callus | Borner et al. (2005) |
| Putative hypersensitive-induced response proteins | ~31.6 | *At* | At3g01290, At1g69840, At5g62740 | Unknown | *At* callus | Borner et al. (2005) |
| Putative hypersensitive-induced response proteins | ~31 | *At* | At5g62740, At3g01290, At1g69840 | PER | *At* cotyledons | Shahollari et al. (2004) |
| Aquaporin (PIP1B) | 30.8 | *At* | At2g45960 | PM | *At* callus | Borner et al. (2005) |
| Propable aquaporin (PIP) | 30.7 | *Le* | Q08451 | PM | *Nt* BY2 cells | Mongrand et al. (2004) |
| Propable aquaporin (PIP) | 30.7 | *Le* | Q08451 | PM | *Nt* leaf | Mongrand et al. (2004) |
| Aquaporin (PIP1A) | 30.7 | *At* | At3g61430 | PM | *At* callus | Borner et al. (2005) |
| Aquaporin (PIP3) | 30.0 | *At* | At4g35100 | PM | *At* callus | Borner et al. (2005) |
| Aquaporin (PIP3) | 30.0 | *At* | At4g35100 | INT | *At* cotyledons | Shahollari et al. (2004) |

**Table 1** (Contd.)

| | | | | | | |
|---|---|---|---|---|---|---|
| Aquaporins (PIPs) | ~30 | *At* | At1g01620, At2g45960, At2g16850, At2g39010, At3g53420, At4g23400 | INT | *At* cotyledons | Shahollari et al. (2004) |
| Syntaxin 71 | 29.9 | *At* | Q9SF29 | PM | *Nt* BY2 cells | Mongrand et al. (2004) |
| Putative porin | 29.6 | *At* | At5g67500 | MIT/CHL | *At* callus | Borner et al. (2005) |
| cAMP-dependent kinase | 29.5 | *At* | At2g20040 | INT | *At* cotyledons | Shahollari et al. (2004) |
| 14-3-3-like protein 16R | 28.9 | *St* | P93784 | CYT/PM | *Nt* BY2 cells | Mongrand et al. (2004) |
| Putative quinone reductase | 28.7 | *At* | At4g36750 | Unknown | *At* callus | Borner et al. (2005) |
| 14-3-3-like protein A | 28.6 | *Nt* | P93342 | CYT/PM | *Nt* leaf | Mongrand et al. (2004) |
| 14-3-3 protein (GRF6) | 28.0 | *At* | At5g10450 | PER | *At* cotyledons | Shahollari et al. (2004) |
| RPS3A/RPS3C ribosomal protein S3 | ~27.7 | *At* | At2g31610, At5g35530 | CYT | *At* callus | Borner et al. (2005) |
| GTP-binding protein (Rab2) | 23.1 | *At* | At4g17170 | PER | *At* cotyledons | Shahollari et al. (2004) |
| Ras-related GTP-binding proteins | ~23 | *At* | At3g09910, At4g18430, At1g56330, At1g02130 | PER | *At* cotyledons | Shahollari et al. (2004) |
| GTP-binding protein (Rab 1c) | 22.3 | *At* | At4g17530 | PER | *At* cotyledons | Shahollari et al. (2004) |
| GTP-binding protein (SAR1B) | 22.0 | *At* | At1g56330 | PER | *At* cotyledons | Shahollari et al. (2004) |
| Putative quinone reductase (FQR1) | 21.8 | *At* | At5g54500 | Unknown | *At* callus | Borner et al. (2005) |
| Remorin | 21.7 | *St* | P93788 | PM | *Nt* leaf | Mongrand et al. (2004) |
| Remorin | 21.7 | *St* | P93788 | PM | *Nt* BY2 cells | Mongrand et al. (2004) |
| Remorins | ~20 | *At* | At2g45820, At3g48940 | PER | *At* cotyledons | Shahollari et al. (2004) |
| Lipid transfer protein-like | 20.2 | *At* | At1g27950 | PM (GPI) | *At* callus | Borner et al. (2005) |
| GTP-binding protein (SAR1A) | 13.8 | *At* | At1g02620 | PER | *At* cotyledons | Shahollari et al. (2004) |

*At*,*Arabidopsis thaliana*; *As*,*Abies sachalinensis*; *Bn*,*Brassica napus*; *Bv*,*Beta vulgaris*; *Dc*,*Daucus carota*; *Gm*,*Glycine max*; *Le*,*Lycopersicon esculentum*; *Nt*,*Nicotiana tabacum*; *Np*,*Nicotiana plumbaginifolia*; *St*,*Solanum tuberosum*[a] According to information provided in the respective reference. Abbreviations are as follows: *CHL* chloroplast, *CYT* cytosol, *EM* endomembranes, *ER* endoplasmic reticulum, *INT* integral membrane protein, *GPI* GPI-anchored protein, *MIT* mitochondria, *PER* peripheral membrane protein, *PM* plasma membrane, *VAC* vacuole

nization in cowpea protoplasts. Translational fusions of cyan fluorescent protein (CFP) to the hypervariable region of a small G-protein of maize (supposed to mediate fusion protein palmitoylation) and of yellow fluorescent protein (YFP) to the N-myristoylation motif of the calcium-dependent protein kinase (CDPK) of tomato were employed for these set of experiments. Various state-of-the-art FRET techniques like acceptor photobleaching (APB), fluorescence spectral imaging microscopy (FSPIM) and fluorescence lifetime imaging microscopy (FLIM) revealed significant FRET efficiencies for the above mentioned donor–acceptor fluorophore pair, suggesting that the two fluorescent fusion proteins reside in very close spatial proximity. Additionally, fluorescence recovery after photobleaching (FRAP) experiments on partially acceptor-bleached protoplasts revealed slow re-quenching of donor fluorescence by diffusion of unbleached acceptor molecules and restored FRET, suggesting that the lipidated CFP-YFP fusion protein complex is relatively stable. Low lateral protein mobility as, for example, visualized by delayed FRAP of fluorescently labeled proteins assumed to be associated with lipid rafts has been previously reported (Oliferenko et al. 1999; Shvartsman et al. 2003; Tanimura et al. 2003; Triantafilou et al. 2004). Notably, the observed low lateral mobility appeared in some instances constitutive (Oliferenko et al. 1999; Shvartsman et al. 2003) while in other cases reduced protein agility was stimulus-dependent and correlated with local protein aggregation in microscopically visible patches (Tanimura et al. 2003; Gekara and Weiss 2004; Triantafilou et al. 2004).

Two independent studies reported recently the pathogen-triggered focal accumulation of components of a presumptive ancient plant defence pathway in the PM of the leaf epidermis (Assaad et al. 2004; Bhat et al. 2005). These proteins include the Arabidopsis SNARE domain-bearing syntaxin *At*PEN1, originally identified in a genetic screen for so-called ''non-host'' resistance mutants (Collins et al. 2003), as well as the corresponding barley homolog, *Hv*ROR2 and the heptahelical *Hv*MLO protein. *Hv*ROR2 and *Hv*MLO are supposed to represent positive and negative regulatory elements of a basal defence pathway against powdery mildew attack that operates at the cell periphery (reviewed in Panstruga 2005). Fluorescently tagged versions of all three proteins (*At*PEN1 as well as *Hv*ROR2 and *Hv*MLO) are each virtually evenly distributed in the PM of unchallenged (healthy) leaf epidermal cells but focally accumulate at attempted fungal pathogen entry sites, thereby defining a stable PM microdomain of circular appearance and approximately 3–10 μm diameter (Fig. 3). Reminiscent of lipid rafts, this lateral membrane heterogeneity comprises a subset of PM-resident proteins (including the above mentioned cytochrome $b_{561}$ that appears to reside in DRMs; Bérczi et al. 2001) and excludes others (Bhat et al. 2005). FRAP analysis and genetic interference with the actin cytoskeleton revealed that focal accumulation is triggered once and does not require actin cytoskeleton function (Bhat et al. 2005). Though the authors of the two studies did not provide any data (apart from filipin-based staining of local sterol accumulation) that presents a direct link between PM protein accumulation and formation of lipid macrodomains, such a connection appears conceivable. Firstly, plasmolysis experiments demonstrated that the focal protein accumulation indeed occurs in the PM and not in the apoplastic space as integral part of a cell wall apposition (Bhat et al. 2005). Besides, dynamic stimulus-triggered accumulation of proteins in lipid rafts or even aggregation of presumptive lipid rafts per se has been reported before (Tanimura et al. 2003; Gekara and Weiss 2004; Triantafilou et al. 2004; see above). In addition to microscopically visible protein accumulation, the low level of lateral mobility of the plant polypeptides at the focal accumulation sites is likewise reminiscent of findings in human cells. Finally, various components of the exocytic machinery including SNARE domain proteins like syntaxins have been suggested to reside in lipid rafts in animal cells (Chamberlain et al. 2001; Xia et al. 2004; reviewed in Salaün et al. 2004). Further experimentation using transgenic lines expressing epitiope-tagged protein variants will be necessary to resolve whether components of basal defence like syntaxins and MLO proteins reside in a lipid domain of distinct molecular composition. Possibly, particular biotic and/or abiotic cues are necessary to recruit these proteins to lipid microdomains. Precedence for such a dynamic stimulus-triggered alteration in subcellular protein localization, here from a soluble to potentially membrane-associated polypeptide, is provided by Arabidopsis nitrilase which becomes immunologically apparent in a Triton X-100 insoluble pellet fraction upon herbicide- induced cell death (Cutler and Somerville 2005). However, preliminary evidence that DRM-associated proteins may also constitutively reside in discrete regions of the PM is provided by immunolocalization of a remorin isoform in tomato root tips (Bariola et al. 2004). Remorins constitute a family of plant-specific coiled-coil forming oligomeric and filamentous proteins which notably appear to represent abundant constituents of Arabidopsis and tobacco DRMs (Table 1; Mongrand et al. 2004; Shahollari et al. 2004; see above).

The polyene antibiotic filipin has been previously used to stain bulk and locally enriched cholesterol in animal and fungal cells (Castanho et al. 1992; del Pozo et al. 2004; Martin and Konopka 2004; Takeda et al. 2004). Direct visualization of local sterol accumulation in plant cells via filipin staining has to our knowledge only been reported in two cases to date. Grebe et al. (2003) used filipin to examine early endocytic sterol trafficking in Arabidopsis roots. The authors found that the polyene antibiotic specifically labels 3-β-hydroxysterols such as the prevalent plant sterols campesterol, sitosterol and stigmasterol. Bhat et al. (2005) employed filipin staining in the context of plant–microbe interactions. Pronounced filipin fluorescence occurred at the tips of fungal invasion

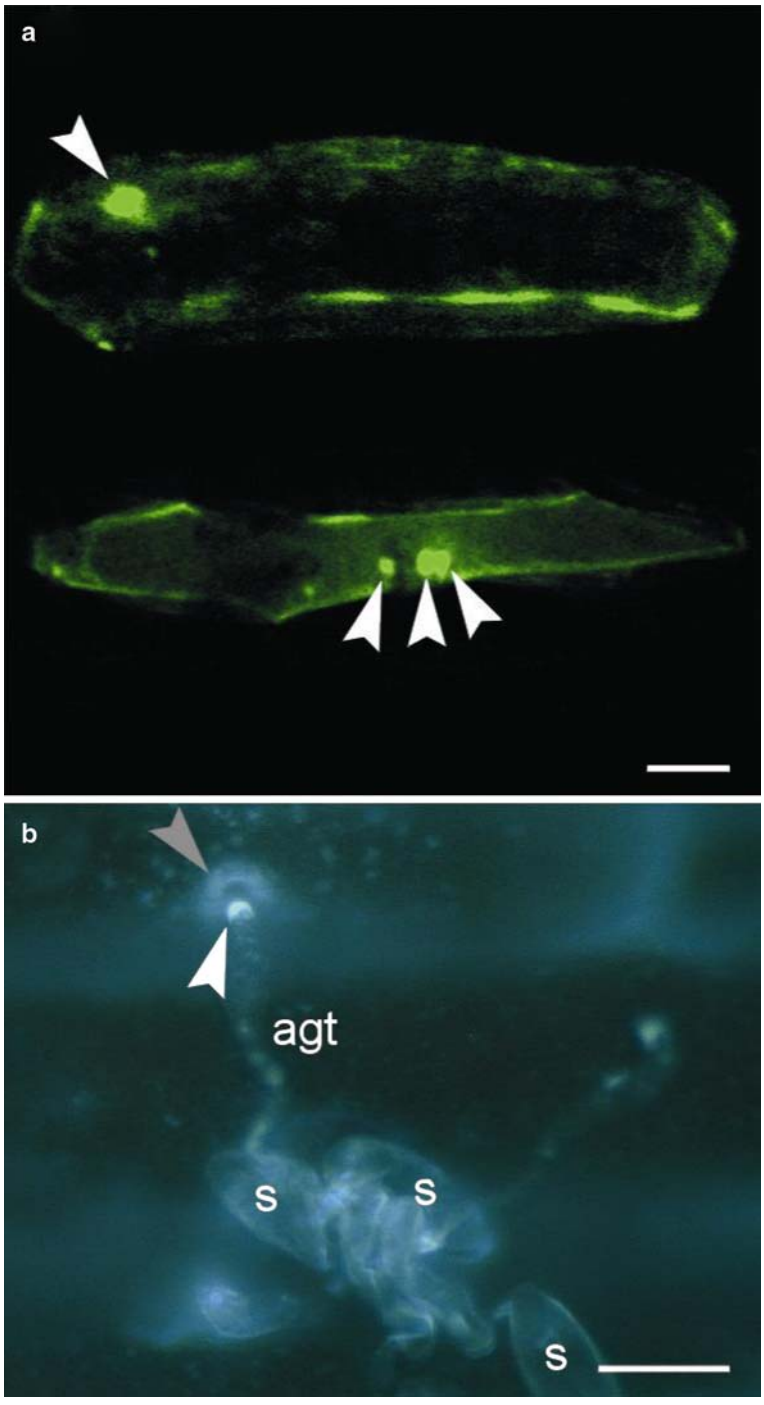


**Fig. 3** Evidence for the existence of PM microdomains in plants. **a** Focal protein accumulation at attempted fungal entry sites. Reminiscent of the proposed stimulus-dependent aggregation of lipid rafts in human/animal cells, fluorescently (GFP) tagged heptahelical barley MLO (*upper cell*) and ROR2 syntaxin (*lower cell*) accumulate at attempted pathogen entry sites (indicated by *white arrowheads*) of single barley epidermal cells transiently expressing the respective genes. Please note that fungal infection structures are not visible on this composed confocal micrograph. *Scale bar* 20 μm. **b** Filipin staining of fungal sporelings and around prospective host cell entry sites. Powdery mildew sporelings (*s*) were germinated on barley leaves and specimens subsequently stained with filipin. Prominent staining at the tip of the appressorial germ tube (*agt*) is indicated by a *white arrowhead*. Please also note the more diffuse circular halo-like staining on the plant side surrounding the contact site with the fungal germ tube (indicated by *gray arrowhead*). The micrograph was taken at 16 h post spore inoculation. *Scale bar* 20 μm

structures and, on the plant side, at the respective attempted pathogen entry sites (Bhat et al. 2005; Fig. 3). It remains to be shown whether this apparent local sterol accumulation on the plant side indicates the aggregation of a plant lipid raft-like microdomain or whether the fluorescence reflects the early release of sterol-enriched fungal PM-derived material at the prospective host cell invasion site, potentially serving a role for manipulation of the host PM.

GPI-anchored proteins represent a structurally and functionally diverse group of post-translationally-modified membrane proteins that exclusively localize to the outer leaflet of the PM in a variety of eukaryotic cells. A range of studies suggest that most (if not all) GPI-anchored proteins may be associated with presumptive lipid rafts (Brown and Rose 1992; Schroeder et al. 1994; Varma and Mayor 1998). Elortza et al. (2003) used a combination of biochemistry, mass spectrometry and computational sequence analysis to experimentally identify 44 GPI-anchored proteins in an *A. thaliana* membrane fraction. Three of these 44 polypeptides, Hedgehog-interacting protein-like 1, SKU5, and a

glycerophosphodiesterase, were independently recognized as residents of plant DRMs, supporting the idea that GPI-anchored proteins also reside in lipid microdomains in planta (Table 1). Notably and in accordance with a potential localization in scattered PM microdomains, at least two GPI-anchored plant proteins, the above-mentioned SKU5 and COBRA, have been shown to exhibit either a non-uniform or polarized subcellular localization, respectively (Schindelman et al. 2001; Sedbrook et al. 2002).

## Outlook: the future's bright. The future's (possibly) greasy

Current experimental lines have not led to a consensus about the existence or non-existence of lipid microdomains in vivo in any eukaryotic organism. It is also not clear how proteins are potentially recruited to these microenvironments, whether they possibly share the same type of rafts and whether their assumed association with raft domains is stable or transient (Shvatsnman et al. 2003). Thus, innovative experimental approaches are required to enlighten the conundrum from novel angles. Immediate visualization of lipid molecules could potentially serve as a groundbreaking method for the direct observation of lipid rafts in intact tissues with minimal external interference. Tagging of the relatively small lipid moieties with bulky fluorescent labels (like BODIPY, NBD etc.) has proven unsuitable as it profoundly affects the structure and behavior of natural lipids (Chattopadhyay 1990; Pagano et al. 1991; van Meer and Liskamp 2005). Recently, creative chemistry resulted in the development of a general tag for labeling lipids with minimal interference of their natural conformation (Kuerschner et al. 2005). These brightly fluorescent pentaene-fatty acids represent mimics of regular fatty acids that coupled with advanced cell biology (i.e two-photon excitation microscopy to minimize UV-mediated damage), allow excellent in vivo observation of lipid organization, traffic and metabolism. After incorporation into living cells, pentaene-tagged lipids showed convincing similarity to the structure of membrane lipids such as sphingomyelin, as opposed to lipids labeled with the erstwhile used NBD or BODIPY tags (van Meer and Liskamp 2005). Visualization and characterization of membrane lipids by fluorescent pentaene tagging thus probably might become pivotal to identify and understand the different biological roles attributed to lipid rafts. Co-localization of various raft-associated proteins, identified by biochemical methods, and pentaene-labeled membrane lipids may shed new light on the protein composition of the presumptive lipid rafts. Potential raft coalescence and dynamic protein sorting following different types of biotic and/or abiotic cues could be visualized in cells bearing the pentaene-tagged membrane lipids in conjunction with expression of fluorescently-tagged proteins of interest.

Plant mutants deficient in sterol or sphingolipid biosynthesis also represent potentially powerful tools for studying the consequences of altered sterol and/or sphingolipid levels on the chemical composition, physical properties and biological functions of membranes (Hartmann 1998; Clouse 2002; Dunn et al. 2004; Zheng et al. 2005). In *Saccharomyces cerevisiae*, this kind of mutants has been previously employed to study lipid microdomain function during mating (Bagnat and Simons 2002). A range of Arabidopsis mutants defective at various steps of in planta sterol biosynthesis have been recently isolated which are each characterized by specific alterations of their sterol profile (Clouse 2002). A comprehensive collection of mutants defective in the biosynthesis of sphingolipids are currently being characterized in detail in the context of an ongoing Arabidopsis 2010 project (http://bio.usuhs.mil/2010.html) While it appears as an obvious experimental route to engage these mutants for the analysis of presumptive plant lipid microdomains, this approach has not been followed up widely yet. The only example we are aware of concerns the polarized subcellular localization of auxin efflux carriers, PIN1 and PIN3. While both polypeptides are normally positioned in *hyd1* and *hyd2/fk*, two mutants with altered sterol composition (Souter et al. 2002), the two proteins mislocalize in *sterol methyltransferase 1* (*smt1*$^{orc}$) plants—a mutant deficient in an enzyme that catalyzes an early step of sterol biosynthesis (Willemsen et al. 2003). Notably, preliminary data indicate that the above mentioned syntaxin *At*PEN1 (Collins et al. 2003) is normally recruited to fungal attack sites in *smt1*$^{orc}$ mutants (Willemsen et al. 2003; R. Bhat, R. Panstruga, unpublished results). These results indicate that particular phenotypes may only become apparent in a subset of mutants with altered sterol composition. Further thorough analysis of plants with altered sterol and/or sphingolipid makeup is required to reveal whether the structure of presumptive plant lipid microdomains is affected in any of these mutants. It remains a caveat of such genetic studies that the altered sterol composition, like pharmacological perturbation of membranes by compounds like methyl-β-cyclodextrin, likely affects general parameters of the lipid bilayer like fluidity etc., thereby complicating the interpretation of the data obtained in this type of experiments.

Additionally, unbiased genetic studies might help to uncover the molecular principle of unequal protein distribution in the plant PM, like for example, the focal stimulus-dependent microdomain formation seen at sites of attempted pathogen entry (Assaad et al. 2004; Bhat et al. 2005). The analysis of an EMS-mutagenized population of transgenic Arabidopsis lines expressing fluorophore-tagged *At*PEN1 syntaxin for individuals that show aberrant focal protein accumulation upon fungal spore inoculation is currently in progress (D. Meyer, P. Schulze-Lefert, personal communication). Likewise, a chemical genetics approach (Blackwell and Zhao 2003) is followed up to identify low molecular weight compounds that may interfere with this pathogen-triggered process (D. Meyer, P. Schulze-Lefert; personal communication).

## Conclusions

Based on a variety of experimental approaches there is accumulating evidence indicating the possible existence of PM microdomains with a particular lipid and protein makeup in plant cells. These presumptive plant lipid microdomains appear to differ from their animal counterparts in the lipid composition. The presence of similar sets of proteins in animal and plant DRMs (e.g. signaling molecules like receptor-like kinases and G-proteins, GPI-anchored proteins, proton pump and flottilin) indicates the likely conservation of lipid microdomain composition in higher eukaryotes. Likewise, biological roles of presumptive plant PM micro-environments appear similar to those proposed for animal lipid rafts and may comprise establishment of cellular polarity, signaling, exocytosis, and subversion for pathogen entry.

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