Lipid Rafts in Plants

Current thinking on lipid membrane organization has evolved from the seminal fluid mosaic model proposed by Singer and Nicolson (1972). Subsequent studies on plasma membranes suggest that biological membranes are not a simple homogeneous layer of proteins and lipids, but rather are organized into discrete regions that can be characterized by distinct lipid and protein content. These membrane microdomains have been named ''lipid rafts.'' The lipid raft hypothesis implies that lipids and proteins are not uniformly distributed in the membrane as proposed in the fluid mosaic membrane model, but instead are organized into discrete ''islands.'' The focus of this *High Impact* article is a paper on the isolation and characterization of putative lipid rafts published in our January 2005 issue, titled ''Analysis of Detergent-Resistant Membranes in Arabidopsis. Evidence for Plasma Membrane Lipid Rafts,'' by Borner et al. As of December 2006, this article had 34 citations according to Thompson ISI (Thompson ISI Web of Science, http://www.isinet.com).

BACKGROUND

In a study conducted on the formation of the two layers of polarized cell membranes in mammalian epithelial cells, Simons and van Meer (1988) noted that the apical and basal plasma membranes varied in lipid composition, suggesting an organization of the membranes into distinct regions rather than homogeneous layers. The term ''lipid raft'' was coined to describe the sterol- and sphingolipid-enriched microdomains present in the apical plasma membrane. These membrane microdomains have since been proposed to provide dynamic scaffolding for a variety of cellular processes, including signaling and stress responses (both biotic and abiotic) and cellular trafficking. The working definition of lipid rafts is biochemical, with lipid rafts forming a liquid-ordered phase within the membrane that allows them to be readily isolated due to their nonionic detergent insolubility (e.g. Triton X-100).

Lipid rafts have since been isolated in yeast and plants. Peskan et al. (2000) were the first to isolate detergent insoluble membrane fraction from plants (tobacco [*Nicotiana tabacum*] leaves), within which they identified proteins typically found in animal raft regions, including heterotrimeric G-proteins, as well as proteins with glycosylphosphatidylinositol (GPI) anchors. The lipid composition of detergent-resistant membranes (DRMs) in tobacco was analyzed in a study by Mongrand et al. (2004), and, as with animal and yeast lipid rafts (where cholesterol is not only a marker of lipid rafts in animal cells but a requirement), it was found that plants rafts also include sterols and sphingolipids and largely exclude phospholipids (Mongrand et al., 2004). As discussed in more detail below, this observation was confirmed and further refined in a study by Borner et al. (2005).

Uncertainty about the existence of lipid rafts persists predominantly due to isolation procedures. A common protocol for DRM isolation involves the detergent Triton X-100 at 4°C. However, low temperatures can alter lipid behavior in membranes, and detergents have been implicated in promoting the formation of lipid microdomains (for review, see Bhat and Panstruga, 2005). Both low temperature and detergents can also affect proteins. On the other hand, DRMs isolated from animal, yeast, and plant systems contain similar classes of lipids as well as proteins, suggesting they are not artifacts arising from isolation procedures. A recent study comparing the two most common isolation procedures—detergent solubilization and sonication—yielded membrane fractions with similar lipid composition but differed somewhat in the protein composition (García-Marcos et al., 2006). An additional complicating factor is that proteins present in lipid rafts might be underrepresented in the total membrane protein composition, hindering the identification of low-abundance proteins. Moreover, the plasma membrane protein composition can vary due to tissue type, developmental stage, and environmental conditions.

WHAT WAS SHOWN

Borner et al. (2005) developed an elegant method to support the hypothesis that DRMs were indeed derived from distinct regions within the membrane and not from an isolation artifact. One consensus from earlier lipid raft studies is the presence of GPI-anchored proteins. GPI-anchored proteins have been identified previously in Arabidopsis (*Arabidopsis thaliana*; Sherrier et al., 1999) and the GPI-proteome predicted (Borner et al., 2002). Many of these predicted 250 GPI-anchored proteins were later confirmed using a proteomics approach (Borner et al., 2003). In their article, Borner et al. (2005) utilized this information to generate Arabidopsis plants containing an immunodetectable GPI-anchored reporter protein that could be followed throughout the purification process. The GPI-anchored reporter protein was enriched in the DRMs relative to the starting material, indicating that the procedure of membrane solubilization was successful and leads to a specific subset of membrane proteins. This also demonstrated that the addition of the GPI anchor targeted the reporter protein to the plasma membrane.

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Borner et al. (2005) proceeded to isolate DRMs from a mixture of organellar membranes from Arabidopsis callus tissues, and analyzed the proteins within these domains by quantitative two-dimensional electrophoresis and liquid chromatography-tandem mass spectrometry. The quantitative proteomics approach was taken to allow contaminants in the DRMs to be distinguished from truly enriched proteins. Most of the 40 plus specifically enriched proteins were known plasma membrane proteins, strongly suggesting that the DRMs are derived from the plasma membrane and not another cellular membrane. Earlier studies used purified plasma membranes for the DRM isolation; thus, it was not possible to conclude that the plasma membrane specifically contributed to the DRMs. The DRM-enriched proteins found by Borner et al. (2005) included several ATPases, plasma membrane aquaporins, and GPI-anchored proteins, analogs of which have been shown to be present in yeast and animal DRMs. The plant-specific arabinogalactan glycoproteins were also shown to be enriched in DRMs. Both this study in Arabidopsis callus tissues and earlier ones in tobacco leaf and suspension cultures (Mongrand et al., 2004) indicate that plant DRMs are able to recruit a specific subset of plasma membrane proteins while excluding others. This protein discrimination process in the formation of lipid rafts is an area of current interest. A possible role of lipid modification in protein targeting was suggested by Borner et al. (2005), who found that several of the DRM-enriched proteins contain putative acylation motifs.

An analysis of the lipid composition of Arabidopsis callus DRMs revealed a 4- to 5-fold increase in sterol and sphingolipids relative to the total plasma membrane. This increase is similar to that observed in tobacco by Mongrand et al. (2004) as well as animal DRMs, lending further support to elevated sterol and sphingolipid content being one of the defining characteristics of lipid rafts.

THE IMPACT

In a follow-up study to Mongrand et al. (2004), Morel et al. (2006) chose to use tobacco Bright Yellow-2 (BY-2) suspension cells to further examine the lipid and protein composition of plant DRMs. The rational for using BY-2 cells is that they are undifferentiated, which the authors felt would give more meaningful results by eliminating the possible complications of tissue type and developmental stage. The lipid content and ultrastructure of the BY-2 plasma membrane were characterized previously (Mongrand et al., 2004). Morel et al. (2006) analyzed the BY-2 DRM proteome by three protein detection methods, leading to the identification of 145 proteins. Examination of the functional and physiochemical characteristics of the proteins provided a more detailed picture of the classes of proteins recruited to the DRMs, giving a glimpse of the possible function of these microdomains. An increase in proteins involved in signaling and stress responses (both biotic and abiotic), cellular trafficking, and cell wall metabolism was observed, supporting the proposal that lipid rafts are signaling platforms involved in the above-mentioned processes. Morel et al. (2006) also looked for posttranslational modifications of the proteins found in the microdomains and found four with GPI-anchors, while 22 others seemed to undergo predicted palmitoylation and/or myristoylation, giving support to the proposal that protein modification could be in involved in partitioning of proteins within the different regions of the plasma membrane.

The major lipid composition of lipid rafts in plants is sterols and sphingolipids. It would then follow that mutations in the synthesis of these two classes of lipid may lead to disruptions in the formation of lipid rafts. One component of sphingolipids is very-long-chain fatty acid (VLCFA) moieties, which, in part, determine the physical characteristics of the lipid. Enzymes of the yeast VLCFA biosynthetic pathway have been localized to the endoplasmic reticulum, where lipid rafts have been predicted to form, and have been demonstrated to be necessary for the proper formation of lipid rafts (Gaigg et al., 2006). An alteration to plant sphingolipids due to a mutation in the VLCFA synthesis pathway was the focus of a study by Zheng et al. (2005). Arabidopsis plants with a mutation in enoyl-CoA reductase (ECR) displayed not only morphological abnormalities, but also changes in the VLCFA content of seed triacylglycerols and sphingolipids as well as changes in membrane trafficking during cell expansion in leaf pavement cells. An ECR-GFP fusion protein was localized throughout the endoplasmic reticulum, consistent with the position of the yeast enzymes. The authors hypothesize that the observed defect in membrane trafficking in expanding pavement cells could be due to an alteration in endocytosis. A very similar result was seen by Grebe et al. (2003) in their study on sterol trafficking when plants were treated with the vesicle trafficking inhibitor brefeldin A. Zheng et al. (2005) further postulate that the VLCFA composition of sphingolipids could play a critical role in the formation of lipid rafts and that an explanation of the ERC mutant phenotype may stem from a lack of coordination of raft-related activities in membrane trafficking.

As mentioned above, a possible function for lipid rafts is involvement in trafficking. One subset of proteins that has been found in DRM microdomains of both plants (Mongrand et al., 2004; Morel et al., 2006) and animals is intercellular trafficking proteins, including those involved in SNAREs involved in membrane fusions. Plant SNAREs have been shown to be involved in many activities beyond the conventional actions of vesicular trafficking, including pathogen resistance, stomatal movements, and gravisensing (for review, see Pratelli et al., 2004). A recent study by Sutter et al. (2006) also suggests that SNAREs could be involved in the positioning of ion channels (KAT1 K^+

channel) within the plasma membrane. In that study, transiently expressed AtKAT1 in tobacco was found in moderately detergent-resistant membrane fractions, possibly suggesting its location within lipid rafts.

CONCLUSION

Future studies that delve deeper into the properties of the lipid raft-associated proteins should enable the discovery of what triggers the formation of these regions. More studies of sphingolipid and sterol mutants will also aid in this and determine the importance of these lipids in the formation of DRMs.

A recent study in mouse (*Mus musculus*) membranes used transmission electron microscopy to visualize markers specific to lipid rafts and to nonraft regions (Lillemeier et al., 2006). The majority of plasma membrane-associated proteins were found separated into ''protein islands'' surrounded by predominantly protein-free, low-cholesterol membrane regions. All six isoforms of actin were associated with these protein islands, suggesting a role for actin in the formation and persistence of the domains. It will be of interest to determine if these domains are also present in plants.

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