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LETTER TO THE EDITOR

In Plant and Animal Cells, Detergent-Resistant Membranes Do Not Define Functional Membrane Rafts

Membrane rafts or lipid rafts were first postulated to explain the difference in plasma membrane organization of polarized epithelial cells and differential targeting of lipids and proteins to their apical and baso-lateral sides (Simons and van Meer, 1988; Brown and Rose, 1992). Rafts, areas enriched in certain lipids (cholesterol and sphingolipids), were thought to be identical with detergent-resistant membranes (DRMs) or detergent-insoluble membranes (DIMs) (Brown and Rose, 1992). Detergent resistance subsequently became the main criterion for the identification of rafts. In more recent years, most researchers in the mammalian field have come to the conclusion that detergent resistance is not a valid criterion for defining functional membrane rafts. We find the plant science literature to be lagging behind in its treatment of DRM methodology. Although membrane rafts, or lateral membrane compartments, exist in some form as functional entities in plant and fungal cells, it is important to recognize that they are not equivalent to DRMs and should not be defined as such.

The catchy name raft was given by Simons and Ikonen (1997) because these postulated structures were imagined to float as small liquid ordered areas within the larger part of the liquid disordered plasma membrane like rafts on water. Since individual mammalian rafts could not be visualized by light microscopy, the criterion for their existence originally was almost exclusively based on detergent insolubility (Brown and Rose, 1992). Thus, whatever could not be solubilized from membranes (for example with 1% Triton X-100 at 4°C) was assumed to be localized within membrane rafts, and the corresponding fractions were called DRMs or DIMs. Doubts were raised, however, that DRMs may not correspond to any specific membrane structure but simply reflect the fact that different membrane proteins and lipids, even if homogenously distributed, are differentially solubilized by detergents. Heerklotz (2002) convincingly demonstrated that clustering within membranes is the consequence of and not the prerequisite for differential detergent extraction and concluded that "…detergent resistant membranes should not be assumed to resemble biological rafts in size, structure, composition or even existence." Munro (2003) criticized profoundly the detergent method and showed, in probably the most cited review in the field, that DRM preparations in the electron microscope look like large continuous membrane units with holes and do not at all resemble anything that would be expected to represent small sized membrane microdomains/rafts.

Due to these doubts, vividly discussed, for example, during the Euro Conference on Microdomains, Lipid Rafts, and Caveolae in 2003 in Porto (Portugal), with ~150 participants, the following conclusion was drawn and published: "At this meeting, a general consensus that emerged about the nature of a raft in a cell membrane is summarized as follows. Considering the complexity of the system and the poorly understood nature of DRM formation, it is unlikely that DRMs that are derived from cells reflect some preexisting structure or organization of the membrane" (Zurzolo et al., 2003). Subsequently, the leading groups in the field started to accept this consensus. For example, Deborah Brown, the pioneer of the DRM concept, as well as the methodology (Brown and Rose, 1992), agreed that DRMs had been overinterpreted: "Recent findings have shown that DRMs are not the same as preexisting rafts, prompting a major revision of the raft model" (Brown, 2006). Also, Kai Simons, who has published seminal work on the concept of membrane rafts in mammalian cells and in yeast, stated in a *Journal of Cell Biology* interview (Sedwick, 2008), "The methodology everyone initially used to study lipid rafts was detergent resistance: if you put Triton on a membrane, any material that was insoluble at four degrees was considered a part of a lipid raft. Of course this was too simple-minded. People would try to manipulate things to get the protein they worked on to be insoluble. This was easy to do; you just had to use a little less detergent. So this led to an adverse reaction and the whole idea of lipid rafts became controversial." In a recent review by Simons and Gerl (2010), the authors discuss the shortcomings of the detergent extraction method and state that "whereas physiologically induced changes in DRM composition can reflect lateral biases in the membrane, detergent solubilization is an inherently artificial method."

The first publications on the possible existence of rafts in plant cells, based exclusively on detergent extraction, appeared in 2004 and 2005 (Mongrand et al., 2004; Borner et al., 2005) (i.e., after the above mentioned meeting in Portugal). Since then, it has been stated repeatedly that plants possess rafts and these statements typically have been based on DRM methodology (although some exceptions are noted below). Severe criticisms of indiscriminate DRM methodology still come almost exclusively from people working with mammalian cells. Critical voices from the plant camp remain rare and timid. Among them, the most pronounced one pointing out that there are pitfalls in the detergent extraction method has been that of Mongrand et al. (2010). But even there after a certain warning, the authors discuss DIMs as membrane platforms and rely on their usefulness.

The question may be asked whether the behavior of plant membranes toward detergents differs from that of animal membranes. Keeping in mind their specific lipid composition, this seems highly unlikely. Biochemical studies using quantitative proteomics and sterol-disrupting agents have shown unambiguously that DRMs in plant membranes are preparative fractions; they contain proteins that could be components

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of sterol-dependent microdomains but also enclose a large fraction of copurifying proteins and contaminants. Thus, resistance to Triton X-100 cannot by itself be a criterion for microdomain localization (Kierszniowska et al., 2009).

Especially striking is the situation in yeast cells. In *Saccharomyces cerevisiae*, three well-defined nonoverlapping lateral membrane compartments have been characterized: MCC (membrane compartment of Can1, the Arg transport protein), MCP (membrane compartment of Pma1, plasma membrane H^+-ATPase), and MCT (membrane compartment of the TORC2 complex) (Malinska et al., 2003; Berchtold and Walther, 2009). Certain proteins of the first two domains, as well as proteins that are homogenously distributed in the plasma membrane, such as Hxt1 and Gap1 (Lauwers et al., 2007), behave as DRM constituents. Thus, if a DRM fractionation is performed with yeast, proteins of three different membrane localities, which before the addition of Triton were clearly separated, will merge together. Even the most elaborate -omics analysis of this fraction will not give rise to real scientific progress.

In recent years, it has been documented by fluorescence microscopy that plasma membranes of plant and fungal cells are laterally compartmented (some examples, see Sutter et al., 2006; Grossmann et al., 2007; Berchtold and Walther, 2009; Lherminier et al., 2009; Raffaele et al., 2009; Boutté et al., 2010; Haney and Long, 2010; Opekarová et al., 2010). Especially the work of S. Mongrand's group (Raffaele et al., 2009) clearly demonstrated that the membrane protein remorin is organized in patches with a size of ~70 nm and that the protein becomes homogenously distributed when the amount of sterols in the membranes is reduced by methyl-β-cyclodextrin. This has been demonstrated by electron microscopic localizations of immunogold-labeled protein. It would be interesting to see how the critical detergent concentration required for solubilizing remorin changes when it redistributes in the membrane. Such an effect has been shown in yeast, for example, when MCC proteins redistribute in response to changes in membrane potential (Grossmann et al., 2007). Similarly in plants, the solubility of some membrane proteins changes in *Arabidopsis thaliana* (Keinath et al., 2010) and tobacco (*Nicotiana tabacum*; Stanislas et al., 2009) in response to treatments mimicking pathogen infections. Using detergents in this way can be useful since the change in detergent concentration required for protein extraction indicates that the membrane environment of the protein and possibly its localization with respect to membrane microdomains has been changed. The plant proteins residing in such lateral membrane compartments are often related to specific functions, such as nodulation of legumes by nitrogen fixing bacteria (Haney and Long, 2010), intercellular virus movement (Raffaele et al., 2009), or endocytotic turnover of membrane proteins (Grossmann et al., 2008). In *S. cerevisiae*, one of these compartments was identified with plasma membrane invaginations, such as typically seen after freeze fracturing in bacterial, fungal, and algal cells (Strádalová et al., 2009).

After the rapid development of imaging techniques in the last two decades, direct visualizations of plasma membrane compartments rather than attempts for their biochemical characterization/isolation seem to represent the most potent approach (Simons and Gerl, 2010). For characterizing plasma membranes of mammalian cells, single particle movement (Kusumi et al., 2005) has been a valuable alternative but for obvious reasons is not applicable for cell wall–protected cells. Due to various technical difficulties, the situation is analogous with stimulated emission depletion (Eggeling et al., 2009) and other types of superresolution microscopy, as well as with total internal reflection fluorescence microscopy (Gutierrez et al., 2010), although first attempts to employ total internal reflection fluorescence microscopy in mapping the distributions of proteins in the yeast plasma membrane have been performed (F. Spira, N. Mueller, and R. Wedlich-Söldner reported last October at the Workshop "Patchy Prague 2010"). The main established procedures to identify lateral plasma membrane compartments in plant and fungal cells to date thus remain confocal fluorescence microscopy and transmission electron microscopy coupled with immunogold procedures (Raffaele et al., 2009).

Fungal and plant membrane microdomains as visualized to date clearly differ from mammalian rafts in size and local stability. The mammalian rafts have been shrinking over time from up to several hundred nanometers in original reports, to minute membrane areas of <20 nm that are highly dynamic in position and composition. Now they are considered to be generally not much larger than protein complexes surrounded by specific lipid shells (Anderson and Jacobson, 2002; Edidin, 2003; Simons and Gerl, 2010). By contrast, plant and fungal membrane microdomains appear to be up to several hundred nanometers large and generally quite stable in location. In this context, we are concerned to some extent that the newly described lateral membrane compartments in plant and fungal cells are called membrane rafts or lipid rafts. Rafts, after all, are supposed to be moving. Membrane microdomains might be a more adequate name. But much more importantly, membrane compartmentation in plant cells, as well as in any cell, must no longer be based on resistance to detergent extraction.

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