Are rafts involved in T-cell receptor signalling?

Introduction to the Talking Point on the involvement of lipid rafts in T-cell activation

Lipid rafts are envisioned as cell-membrane domains with distinctive physical properties and lipid compositions. Their existence and proposed functions in cellular processes are a matter of intense debate. Raft involvement at T-cell receptor (TCR) activation sites in the plasma membrane is an intense focus of the research community. In the accompanying Talking Points in this issue of *EMBO reports*, Anne Kenworthy and Hai-Tao He & Didier Marguet present differing views on this topic, debating both the existence of lipid rafts and their contribution to T-cell triggering (He & Marguet, 2008; Kenworthy, 2008).

The membranes of eukaryotic cells contain a complex mixture of glycerophospholipids, sphingolipids and cholesterol, and the chemistry of these lipid species has profound effects on the physical properties of the membrane. The phosphoglycerate backbones of different glycerophospholipids harbour the different functional head groups bound to the phosphate. The two remaining hydroxyl groups are esterified by acyl chains of varying lengths and degrees of saturation. Sphingolipids contain the aliphatic amino-alcohol sphingosine, which is coupled to an acyl chain through an amide bond. One of the sphingosine hydroxyls contains either a sugar or a phosphorylcholine moiety to generate glycolipids or sphingomyelin, respectively. Sphingolipid hydrocarbon chains are usually saturated. The hydrophobic steroid-core ring of cholesterol consists of three six-carbon rings and one five-carbon ring, and, as such, has a rigid planar structure. The lipid composition of cell membranes is indeed complex, with hundreds of lipid species that are asymmetrically distributed in the two leaflets of the lipid bilayer. The fundamental issue of whether specific lipid species have a laterally inhomogeneous distribution—for example, by forming specific assemblies—stands at the centre of the debate about raft existence and function (Munro, 2003; Simons & Ikonen, 1997).

Using artificial model membranes to mimic rafts in cell-membrane domains has been a successful approach (Brown & London, 1997). Indeed, in such membranes, cholesterol and saturated phosphatidylcholine/sphingolipids form domains of higher packing density, in the so-called liquid-ordered (L_o) phase. In appropriate lipid mixtures, L_o phases can coexist with a liquid-disordered (L_d) phase that is formed by unsaturated phosphatidylcholine species. In these model membranes, L_o domains cover large areas that can easily be monitored. However in native cell membranes, L_o domains have proven difficult to measure accurately. The presence and properties of L_o domains have frequently been investigated by assessing the clustering behaviour of putative raft components. These clusters of raft

components were shown to exist, but were found to be small (in the range of a few nanometres; Sharma *et al*, 2004). The recent introduction of fluorescent lipid dyes that report on the ordering of cell membranes has allowed ordered domains to be directly distinguished from less-ordered domains of cellular plasma membranes. Using this approach, it has been shown that TCR signalling sites in the T-cell plasma membrane specifically adopt an ordered state—a key physical property of raft membrane domains (Gaus *et al*, 2005).

This and other lines of evidence (as discussed by He and Marguet) have been used to argue for a functional role of lipid rafts in TCR triggering. It has been proposed that TCR signalling components are compartmentalized by association with ordered raft domains. Indeed, TCR signals are transduced by microclusters on engagement with complexes of cognate peptide-major histocompatibility complex (Campi *et al*, 2005). Many TCR-associated signalling components—such as the activator protein tyrosine kinases LCK and FYN and the signalling adaptor linker for activation of T cells (LAT)—are anchored in the plasma membrane. Signalling proteins downstream from the TCR form clusters on TCR triggering. These signalling clusters are transiently associated with TCR microclusters, but individual components show differential behaviour in terms of their temporal association with, and dissociation from, the TCR microclusters (Bunnell *et al*, 2002). What is the contribution of lipid rafts to the formation of these microdomains? Do they—as Hai-Tao He & Didier Marguet propose—act as a ‘selective glue’ that contributes specifically to positively or negatively regulating the interactions between membrane-associated signalling partners?

Taking an opposing view, Kenworthy points out the gaps in the chain of arguments that support the involvement in TCR signalling of rafts in the T-cell plasma membrane. Her main criticism is that the different models used to describe lipid rafts determine, to a large extent, the approaches used to address their functions. She proposes a focus on putative raft-resident molecules, and argues that their properties and membrane environment need to be characterized. This approach will certainly aid in refining the models that describe lateral cell-membrane organization. Similarly, a detailed elucidation of the molecular lipid composition of plasma membrane domains involved in TCR signalling will hold the key to the unbiased assessment of the role of rafts in this process. With the advent of new technologies for obtaining a comprehensive overview of membrane ‘lipidomes’ (Ejsing *et al*, 2006), it will soon be possible to make conclusions about these fundamental issues.

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For more discussion on this topic, see also:
 He H-T, Marguet D (2008) T-cell antigen receptor triggering and lipid rafts: a matter of space and time scales. This issue p525.
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 Kenworthy AK (2008) Have we become overly reliant on lipid rafts? This issue p531.
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