Supplementary Box 1 | **Rafts in the tree of life**

Although the general fluid bilayer organization of biological membranes is nearly completely conserved throughout the kingdoms of life, the specific lipid and protein compositions of cellular membranes vary dramatically between various life forms. Recent findings suggest that membrane domains are prevalent throughout the evolutionary tree, suggesting that lateral heterogeneity is a broad organizing principle for biological membranes.

Rafts in prokaryotes – Most prokaryotes do not produce cholesterol or sphingomyelin, instead relying on sterol analogs[1] or Lipid A[2] that may function as physical surrogates of key raft constituents. Hopanoids, for instance, fluidize the bacterial membrane and drive formation of ordered membrane domains[2]. Borrelia, a genus of bacteria obtaining cholesterol from the host, shows distinct domains of high lipid order[3] that are required for bactericidal activity by host factors[4]. Comparing the DRM proteome of Borrelia[5] with that of mammalian cells has provided insight in how membrane domains composition has evolved from prokaryotes to eukaryotes. Membrane heterogeneity is specifically maintained in distinct parts of the bacterial cells. For instance, bacterial flagella are rich in saturated and depleted with unsaturated lipids, which supports the formation of ordered membrane environments in the flagella and this environment is vital for its function[6].

Rafts in fungi and plants – Fungal membranes possess broadly similar lipid classes to those found in metazoans. For instance, the dominant sterol is ergosterol[7], which like cholesterol has the ability to interact with sphingolipids and form ordered membrane domains[8]. Such domains have been implicated in several processes, including growth and death of the yeast cell[9]. In plants, on the other hand, a complex mixture of sterols (usually called phytosterols) is present and interactions of these sterols with lipids (mostly ceramides) and proteins can lead to membrane structuring[10].

Rafts in animals – As highlighted before, plasma membranes of mammalian cells are marvellously capable of undergoing microscopic phase separation in the absence of cortical actin cytoskeleton[11]. Recent labelling and observation technologies have also enabled us to probe lateral heterogeneity in the plasma membrane of live mammalian cells[12-14]. However, it is not the only structure accommodating domains. Very surprisingly, most direct evidence of liquid-liquid phase separation in live cells occurs in vacuoles[15]. Mitochondria or mitochondria-associated membranes[16] and exosomes[17] have recently been shown to be enriched in DRMs, as well.

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