Published in final edited form as:
Mol Membr Biol. 2004 ; 21(3): 193–205. doi:10.1080/09687680410001700517.

Plasma membrane microdomains: organisation, function and trafficking

Alex J. Laude and **Ian A. Prior**
The Physiological Laboratory, University of Liverpool, Crown Street, Liverpool, L69 3BX, UK

Summary

The plasma membrane consists of a mosaic of functional microdomains facilitating a variety of physiological processes associated with the cell surface. In most cells, the majority of the cell surface is morphologically featureless leading to difficulties in characterising its organisation and microdomain composition. The reliance on indirect and perturbing techniques has led to vigourous debate concerning the nature and even existence of some microdomains. Recently, increasing technical sophistication has been applied to study cell surface compartmentalisation providing evidence for small, short-lived clusters that may be much less than 50nm in diameter. Lipid rafts and caveolae are cholesterol-dependent highly ordered microdomains that have received most attention in recent years, yet their precise roles in regulating functions such as cell signalling remains to be determined. Endocytosis of lipid rafts/caveolae follows a clathrin-independent route to both early endosomes and non-classical caveosomes. The observation that a variety of cellular pathogens localise to, and internalise with these microdomains provides an additional incentive to characterise the organisation, dynamics and functions of these domains.

Keywords

lipid rafts; caveolae; cholesterol; compartmentalized signalling; endocytosis

Introduction

There is a general consensus that the plasma membrane is compartmentalised into functional microdomains that facilitate processes such as cell signalling, cell adhesion and membrane trafficking. As discussed later, microdomains may consist of anything from a short-lived cluster of several protein and lipid molecules to large, stable, organised domains many tens or hundreds of nanometers in diameter. The key problem in this area of research is imaging resolution; there is no substitute for actually being able to see compartments to convince us of their existence and to help to provide models for their organisation and function. Gross cell surface compartmentalisation such as apical and basolateral domains of epithelial cells have been well characterised, partly due to the clearly defined boundaries between the compartments that are visible at the light microscope level. Similarly, microdomains such as caveolae and clathrin-coated vesicles, 50-150nm invaginations visible at the electron microscope level, are well-characterised organelles. The key question is: how is the remaining morphologically featureless plasma membrane organised? This area can represent up to 90-95% of the cell surface in fibroblasts for example, and therefore may well represent the site where many of the cell surface functions are occurring. This review will outline some of the current models of plasma membrane organisation and the techniques being used to probe the cell surface at high resolution. We have focussed in particular on the cholesterol

Corresponding Author: Ian A. Prior Phone: +44 151 794 5332 Fax: +44 151 794 4434 iprior@liverpool.ac.uk.

Europe PMC Funders Author Manuscripts

rich microdomains: lipid rafts and caveolae, highlighting research into their organisation, function and trafficking. A series of excellent recent reviews have been published in this area including (Simons and Toomre, 2000, London, 2002, Edidin, 2003, Nichols, 2003, Parton and Richards, 2003, Silvius, 2003, van Deurs *et al.*, 2003, Vereb *et al.*, 2003).

Plasma membrane microdomains

The Singer-Nicholson fluid mosaic model accurately predicted the general organisation of proteins and lipids within membranes (Singer and Nicholson, 1972); however, one feature of the model is undergoing reassessment. The model predicted free rotational and lateral diffusion of proteins and lipids within the plane of the membrane resulting in their random distribution. As discussed below, a number of lines of evidence suggest that the movement of most proteins within the plasma membrane is partially restricted over the nanometre scale; and that this lateral heterogeneity in membrane organisation is a result of protein-protein, protein-lipid and lipid-lipid interactions.

Lipid rafts

A mechanism proposed to induce lateral heterogeneity is described by the lipid raft hypothesis (Simons and Ikonen, 1997). Lipid rafts are envisaged to be enriched in cholesterol and saturated lipids such as sphingolipids that are packed together to form a highly ordered structure distinct from the surrounding sea of disordered, predominantly unsaturated, lipid species. The raft hypothesis encompasses both leaflets of the plasma membrane despite the fact sphingolipids are only found on the exoplasmic leaflet. Glycosphingolipids typically possess a long chain fatty acid that is proposed to be able to interdigitate with an inner leaflet raft structure enriched in saturated lipids, cholesterol and peripheral proteins anchored with saturated acyl chains. The highly ordered nature of this domain thus provides a mechanism for sorting proteins and lipids depending on their ability to intercalate into this close-packed structure. Exofacial proteins tethered by glycophosphatidylinositol (GPI)-anchors, cytofacial proteins anchored by saturated palmitoyl or myristoyl groups and cholesterol-binding proteins are all predicted to preferentially segregate into lipid rafts (Figure 1).

The hypothesis represented a synthesis of a number of important observations made over the previous decade. Firstly, sphingolipids are enriched within the apical plasma membrane of polarised epithelial cells (Simons and van Meer, 1988), and therefore a lipid-based sorting mechanism was proposed to exist at the level of the Golgi complex (Simons and Wandinger-Ness, 1990). Secondly, the highly ordered structure of lipid rafts renders them partially insoluble in cold, non-ionic Triton X-100 detergent compared to the more disordered surrounding plasma membrane (Brown and Rose, 1992). Thirdly, work with model membranes containing mixtures of saturated lipids and cholesterol showed that cholesterol promoted a ‘liquid-ordered’ state of tightly packed, but laterally mobile lipids (Ipsen *et al.*, 1987). These membranes exhibited detergent resistance analogous to that seen with intact cells (Schroeder *et al.*, 1994, Ahmed *et al.*, 1997), suggesting that lipids alone mediate the insolubility of cellular domains. Finally, cholesterol-depleting agents inhibited physiological processes indicating a role for cell surface cholesterol in regulating signalling (Rothberg *et al.*, 1990).

Cholesterol-sensitive detergent-insolubility became the defining feature of lipid rafts and together with the propensity of these microdomains to float in sucrose gradients, provided a simple mechanism to isolate and characterise raft proteins and lipids. Raft residents defined by these criteria include GPI-anchored proteins (Schroeder *et al.*, 1994), many acylated signalling proteins including Src family kinases (Song *et al.*, 1997), G proteins (Song *et al.*, 1996, Melkonian *et al.*, 1999, Moffet *et al.*, 2000) and nitric oxide synthase (Shaul *et al.*,

1996), growth factor receptors (Liu *et al.*, 1996, Waugh *et al.*, 1999), integrins (Baron *et al.*, 2003), and cholesterol-binding proteins including caveolin (Sargiacomo *et al.*, 1993) and Sonic Hedgehog (Rietveld *et al.*, 1999). Recent systematic proteomic analysis of cholesterol-sensitive detergent-insoluble fractions, revealed a 10-fold enrichment of signalling proteins in rafts versus total membranes (Foster *et al.*, 2003). Lipidomic analysis revealed that in addition to enrichment for cholesterol, sphingolipids and saturated phospholipids, they are specifically enriched in phosphatidylserine, phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) and arachidonic acid (Pike and Casey, 1996, Fridriksson *et al.*, 1999, Pike *et al.*, 2002). The presence of many signalling proteins and lipid cofactors within lipid rafts suggest a role as a signalling platform where their close proximity acts to increase the efficiency, specificity and regulation of signalling cascades (Brown and London, 1998, Simons and Toomre, 2000).

Illusive rafts?

Although there is a significant amount of literature citing raft localisation and raft involvement in many physiological processes, controversy still surrounds the exact nature of these domains (Edidin, 2003, Munro, 2003). This is due to the reliance to date on indirect, perturbing techniques to study them and the apparent lack of consensus amongst other, microscopy-based techniques in resolving their size and distribution. Of particular concern is how closely detergent-insoluble fractions relate to putative lipid raft domains *in vivo*. Several lines of evidence indicate the care that must be taken when interpreting detergent-insolubility data. Firstly, not all detergent-insoluble proteins exhibit cholesterol-sensitive insolubility (Foster *et al.*, 2003); non-detergent based confirmatory experiments should be routine. Secondly, detergent extraction is done on ice, this promotes more ordered lipid phase behaviour than seen at physiological temperatures and probably results in increased association of lipids with liquid-ordered detergent-insoluble domains (Shogomori and Brown, 2003). Thirdly, detergent:lipid ratios have not been standardised and are not typically well controlled, potentially resulting in differential solubilisation efficiencies between experiments (Edidin, 2003, Shogomori and Brown, 2003). This may explain some discrepancies in apparent raft localisation (McCabe and Berthiaume, 2001, Wang *et al.*, 2001). Related to this, a number of detergents are now being employed, some of which only poorly solubilise putative non-raft domains, producing significant heterogeneity between raft preparations (Schuck *et al.*, 2003). In addition, partial mixing of raft proteins from separate raft domains has been detected following Triton X-100 solubilisation (Madore *et al.*, 1999); this may be of relevance to immuno-precipitation studies where detergent solubilisation may promote more extensive protein-protein interactions. Finally, work with lipid raft model membranes has shown that Triton X-100 can induce domain formation, implying that detergent-insoluble domains bear little resemblance to their original size, distribution and conformation (Heerklotz, 2002). Evidence of this can also be seen with microscopic examination of isolated fractions, which reveals a broad size range of 0.1-1 μm diameter vesicles that are likely to represent a coalescence of smaller raft domains (Shogomori and Brown, 2003).

The other pillar of raft identity is cholesterol dependence; cholesterol depletion is often used to confirm raft localisation and function in a physiological process. Typically, acute depletion is performed, however the effects are not restricted to disrupting raft function, it has also been shown to inhibit clathrin-mediated endocytosis (Rodal *et al.*, 1999, Subtil *et al.*, 1999), prevent exocytic SNARE complex formation (Lang *et al.*, 2001), and delocalise plasma membrane $PI(4,5)P_2$ (Pike and Miller, 1998). The potential of this non-specificity for causing misinterpretation of functional data was particularly highlighted in a recent study that showed that sequestration of cell surface $PI(4,5)P_2$, and the consequent reorganisation

of the actin cytoskeleton, mimic many of the effects seen with acute cholesterol depletion (Kwik *et al.*, 2003).

As a result of these concerns, it became increasingly evident that new, more direct techniques needed to be developed to monitor the organisation and dynamics of lipid rafts and other cell surface microdomains within the context of an intact cell. As discussed later, many of these new approaches together with research using model membrane systems, have strengthened the case for the existence of lipid raft domains and support the usefulness of detergents for preliminary experiments, by providing independent confirmation of protein and lipid segregation as envisioned by the raft hypothesis.

Caveolae

Caveolae are specialized uncoated cell surface invaginations, typically 50-70 nm in diameter, that were first identified 50 years ago using electron microscopy (Palade, 1953, Yamada, 1955). Their relative abundance is cell type dependent, in lymphocytes and most neurones they are absent, whilst in muscle, endothelia and adipocytes they are very abundant representing up to 35% of the cell surface (Parton and Richards, 2003). In most cells, caveolae are simple vesicular invaginations; however, they can also form extensive branching networks within some cell types. The best characterised of these are found in differentiating muscle where they may be involved in the biogenesis of the T-tubular network (Ishikawa, 1968). Apart from their morphology, the other defining features of caveolae are the presence of the protein caveolin (Kurzchalia *et al.*, 1992, Rothberg *et al.*, 1992), and enrichment of many of the same molecular components present in lipid rafts, resulting in similar detergent-insolubility and cholesterol dependence (Tran *et al.*, 1987, Rothberg *et al.*, 1990). Due to their biochemical similarity to lipid rafts, caveolae can be viewed as a specialised sub-type of lipid rafts.

Caveolin is a 21kDa, triply palmitoylated, cholesterol binding protein with unusual membrane topology in that it forms an intra-membrane hairpin loop leaving both termini extending into the cytosol (Figure 1). There are three isoforms; caveolin-1 and caveolin-2 are found in most caveolae-containing cell types, whilst caveolin-3 is restricted to muscle cells (Parton, 1996). After synthesis in the endoplasmic reticulum (ER), caveolin oligomerises as it traffics through the Golgi, becoming increasingly detergent-insoluble before transport to the cell surface (Monier *et al.*, 1995). Hetero-oligomerisation of caveolins drives the formation of caveolae (Fra *et al.*, 1995). Caveolin-1 is essential for this (Galbiati, 1998), whereas, caveolin-2 facilitates the process (Lahtinen *et al.*, 2003, Sowa *et al.*, 2003), but is not essential since caveolae are still found in caveolin-2 null mice (Razani *et al.*, 2002b).

Many proteins reportedly interact with caveolin via the scaffolding domain, a 20 amino acid region immediately N-terminal to the intramembrane loop, suggesting a general regulatory or chaperone role (Okamoto *et al.*, 1998). Many other functions have also been attributed to caveolae and caveolin including endocytosis, endothelial transcytosis, regulation of cell signalling processes and cholesterol transport and homeostasis (van Deurs *et al.*, 2003). Their role in signal transduction and endocytosis will be discussed in more detail in later sections.

The biochemical similarities between caveolae and lipid rafts mean that they co-purify together, and share many of the same molecular markers used for detection with fluorescence microscopy. This has led to some confusion over the extent of the contribution of caveolin and caveolae versus lipid rafts in regulating many of these processes, particularly since their abundance is so variable between cell types. Recently, knockout mice have been generated for all three caveolin isoforms. Perhaps the most significant observation

is that none of these genes are essential for the development of viable and fertile adults, (Hagiwara *et al.*, 2000, Drab *et al.*, 2001, Galbiati *et al.*, 2001, Razani *et al.*, 2001, Razani *et al.*, 2002b). Knockout mice did however show a series of severe abnormalities; for example, caveolin-1 null mice exhibited a 50% reduction in life span (Park *et al.*, 2003), cardiac and pulmonary defects partly resulting from impaired nitric oxide and calcium signalling (Drab *et al.*, 2001), reduced albumin uptake (Schubert *et al.*, 2001), increased serum triglyceride levels (Razani *et al.*, 2002a), and inefficient GPI-anchored protein transport (Sotgia *et al.*, 2002). The latter defects are consistent with proposed roles of caveolae and caveolin in regulating fatty acid and cholesterol trafficking.

The role of caveolin in cholesterol transport and homeostasis is perhaps fundamental to mechanisms involved in plasma membrane organisation. Caveolin binds to and its expression is regulated by cholesterol (Murata *et al.*, 1995, Bist *et al.*, 1997, Trigatti *et al.*, 1999, Fra *et al.*, 2000). Overexpression of caveolin-1 increases free cholesterol transport to the plasma membrane (Smart *et al.*, 1996, Fielding *et al.*, 1999). Complexes of caveolin and cholesterol have also been found in the cytosol (Uittenbogaard *et al.*, 1998), further intimating a role in intracellular cholesterol transport. This suggests that caveolin could play an important role in regulating plasma membrane organisation and in particular the distribution of both caveolae and lipid rafts. Support for this idea came in recent studies using a dominant-negative N-terminally truncated caveolin-3 mutant, cav^{DGV}. Cav^{DGV} localises to and stimulates the formation of lipid droplets: organelles enriched in triglycerides and cholesterol esters; and reduced the efficiency of cholesterol transport to the cell surface (Pol *et al.*, 2001). This mutant impairs lipid raft and caveolar organisation and abundance and caused a specific inhibition of signalling from Ras proteins resident in lipid rafts but not Ras proteins localised to non-raft microdomains (Roy *et al.*, 1999). Therefore, caveolin may play an important indirect role in many cellular processes by helping to maintain cholesterol-dependent microdomains that, as discussed earlier, are concentrated with a wide variety of regulatory proteins and lipids.

Membrane skeletons, picket fences and other microdomains

Recent work has demonstrated the importance of proteins in organising the cell surface through modulating the diffusion of proteins and lipids within the plane of the membrane (Ritchie *et al.*, 2003). Single-particle tracking of fluorescently- or 40 nm gold-labelled proteins or lipids allows direct observation of their diffusion characteristics within living cells and the generation of 2-D spatial maps describing cell surface organisation. The key findings are that firstly, the membrane cytoskeleton, consisting of actin and actin-binding proteins, acts to partially corral membrane proteins within large macrodomains (Kusumi *et al.*, 1993, Edidin *et al.*, 1994, Sako and Kusumi, 1994; Figure 1). Actin depolymerisation tends to increase compartment size whereas actin stabilisation increases residency time within these compartments (Fujiwara *et al.*, 2002). Secondly, although this organisation is generated on the inner plasma membrane leaflet, its effects are also seen on the external leaflet. L-α-dioleoylphosphatidylethanolamine (DOPE), an unsaturated phospholipid probe, displayed ‘hop-diffusion’ within the external leaflet of NRK cells similar to that seen for a transmembrane protein and likewise relied on the integrity of the actin cytoskeleton (Fujiwara *et al.*, 2002). It was proposed that transmembrane proteins anchored to the cytoskeletal framework could generate this type of compartmentalised diffusion by providing obstacles that would impair escape from the macrodomain. In effect, proteins and lipids randomly bounce around within the macrodomain until they find a gap to exit into the neighbouring macrodomain. Modelling indicated that only 20-30% of the macrodomain boundary would need to be occupied by anchored transmembrane proteins to recapitulate the observed results (Fujiwara *et al.*, 2002). With this additional level of cytoskeleton-based membrane organisation, as proteins and lipids condense to form larger complexes, there

would be a dramatic reduction in their ability to transit between macrodomains. This mechanism for restricting rapid long-range diffusion of protein/lipid complexes and microdomains involved in for example, signalling, cell adhesion or cytokinesis would help to ensure retention of spatial information within these processes.

Other microdomains have also been detected using similarly direct analytical techniques. Two studies, using either electron microscopy or fluorescence recovery after photobleaching (FRAP) analysis, detected different Ras isoforms clustered in distinct non-cholesterol-dependent signalling microdomains on the inner plasma membrane surface (Niv *et al.*, 2002, Prior *et al.*, 2003). Galectin-1, a cytosolic protein-binding lectin, was required to stabilise activated H-ras microdomains. In contrast, the polybasic domain of K-ras was postulated to help to organise its own microdomains via electrostatic interactions with acidic phospholipids, in a similar way to that proposed following biophysical studies using the myristoylated and polybasic domain-containing MARCKS protein (Murray *et al.*, 1997, Murray *et al.*, 1999). Similar cholesterol-independent, non-raft domains were also visualised using fluorescence energy transfer (FRET) (Zacharias *et al.*, 2002).

There are likely to be a range of interactions that modulate cell surface organisation. Whilst the domain structure of lipids envisaged by the raft hypothesis may help to generate segregation at one level, it is clear that protein-protein and protein-lipid interactions, such as those just described, provide additional levels of organisation both inside and outside of raft domains. The dynamics of these interactions and overall plasma membrane organisation represent the new frontier for research in this field. In order to study them, new techniques have had to be applied to study the cell surface at much higher resolution than simple detergent-insolubility, sucrose gradients and immunofluorescence experiments.

High-resolution studies of the cell surface

A variety of new techniques have been used to study the cell surface and reconstitute the interactions occurring within it. The aim of these techniques has been to resolve the size, distribution and dynamics of microdomains that are typically predicted to be below the resolution of the light microscope (<250 nm). Probably the most noticeable feature of the many studies performed to date is that although most of them detect microdomains, there is an apparent lack of agreement over their size and distribution (sizes range from <5nm up to 750nm diameter, Table 1). Possible reasons for this include: differences in imaging resolution, sample complexity, activation state, assay temperature, cell type and association dynamics of different microdomain markers.

Model membrane studies

Model membranes have shown the propensity for mixtures of sphingolipids and phospholipids to segregate into domains consisting of gel states with little lipid mobility and fluid domains with significant lipid mobility (London, 2002). Cholesterol acts to reduce the ordering of the gel state to make them ‘liquid-ordered’ i.e. lipids are tightly packed but exhibit rapid lateral diffusion (Ipsen *et al.*, 1987).

Recent fluorescence-quenching experiments, have tried to reconstitute extracellular leaflet composition to investigate the requirements for lipid raft formation. Membranes containing cholesterol, unsaturated phospholipids and saturated phospholipids or sphingolipids generated cholesterol-dependent microdomains enriched for cholesterol and the saturated lipid species; whereas unsaturated lipids exhibited low affinity for these domains (Silvius *et al.*, 1996, Ahmed *et al.*, 1997, Wang *et al.*, 2000, Xu and London, 2000). Fluorescence microscopy of similar model bilayers or giant unilamellar vesicles, revealed that these domains were massive (>10μm diameter), relatively stable, cholesterol-dependent,

detergent-insoluble, exhibited trans-bilayer coupling and accumulated GPI-anchored Thy-1 (Dietrich *et al.*, 2001a, Dietrich *et al.*, 2001b, Samsonov *et al.*, 2001). These domains are much bigger than anything seen *in vivo* and probably reflect the lack of complexity in the system relative to the cell surface where for example, proteins may provide additional levels of regulation. Perhaps for similar reasons, model membranes composed of inner leaflet lipids, phosphatidylserine and phosphatidylethanolamine with physiological levels of cholesterol, failed to generate microdomains (Wang and Silvius, 2001). The mechanisms regulating inner leaflet raft formation remain to be elucidated.

Cellular studies

Biochemical and advanced microscopy techniques have been applied directly to cells to try to determine the size and distribution of lipid rafts and other microdomains in native membranes. Cross-linking extracellular leaflet membrane proteins in BHK or CHO cells, revealed cholesterol-dependent clusters typically containing at least 15 molecules of GPI-anchored folate receptor or recombinant GPI-anchored growth hormone, suggesting very small (<5 nm) raft domains (Friedrichson and Kurzchalia, 1998). Small (20-30 nm) and stable, cholesterol-dependent domains were also detected with photonic force microscopy that measures the viscous drag exerted by the membrane on a laser trapped protein (Pralle *et al.*, 2000).

Rapid, close-range associations can be detected by FRET, this works by monitoring fluorophore density-dependent changes in the efficiency of donor/acceptor fluorophore interactions that occur within a range of <5-10 nm. Results using FRET have been inconsistent, possibly due to differences in labelling efficiencies of domain markers. Conventional hetero-FRET using antibody-bound or cholera toxin-bound fluorophores, failed to detect FRET between lipid and protein markers of lipid rafts (Kenworthy and Edidin, 1998, Kenworthy *et al.*, 2000). In contrast, homo-FRET of GFP-tagged folate receptor detected clustering in 70 nm, cholesterol-sensitive domains (Varma and Mayor, 1998). Subsequent sophisticated modelling of this and similar recent data dramatically reduced the predicted size of these domains to <5nm and consisting of at most 4 molecules (Sharma *et al.*, 2004). The different homo- and hetero-FRET observations may reflect differences in labelling efficiencies; there is a two-fold greater probability of getting simultaneous labelling of a single domain in homo-FRET versus hetero-FRET. In addition, using the intrinsic fluorescence of GFP-tagged proteins avoids the problems of relatively low labelling seen with antibody labelling, giving greater power to detect very small or unstable domains.

Recent two-photon analysis using the fluorescent probe Laurdan, investigated the extent of membrane fluidity in cellular domains visible at the light microscope level (Gaus *et al.*, 2003). Laurdan has been extensively used in model membrane studies because it distributes non-specifically throughout membranes and the wavelength of its fluorescent emission is influenced by the extent of membrane fluidity in its immediate microenvironment. Gaus and colleagues used this to show that stable (>30 seconds) cholesterol- and temperature-sensitive raft domains typically occupied 10-15% of the macrophage cell surface at 37°C and were particularly enriched within filopodia and sites of cell-cell contact. The study may have partially underestimated the extent of lipid raft coverage since it was limited to the spatial and temporal resolution of the two-photon microscope however it provides clear evidence for the existence of membrane fluidity differences within the plasma membrane and the necessary role of cholesterol in promoting more ordered lipid microdomain formation consistent with the raft hypothesis.

Electron microscopy of fixed plasma membrane fragments coupled with statistical analysis of the gold labelling patterns, detected a variety of inner leaflet microdomains (Prior *et al.*,

2003). Microdomain markers and full-length Ras proteins were imaged using 2 nm and 5 nm gold directly conjugated to antibodies. Both lipid raft and non-raft targetted GFP-tagged peptides were detected in 34-44nm clusters. Clustering of the raft-localised protein was cholesterol-dependent and modelling indicated that lipid rafts were typically 44nm in diameter and occupied 35% of the BHK cell surface.

Single-particle tracking of phospholipids, GPI-anchored proteins and raft lipids has detected a variety of compartment sizes ranging from 30-750 nm in diameter, dependent on cell type and imaging rate (Ritchie *et al.*, 2003). The hop-diffusion visualised in these studies was shown to be actin dependent and generated the picket-fence model of plasma membrane organisation described earlier. Recent studies looked at the dynamics of microdomain association and found a hierarchy of stabilised states ranging from small, unstable rapidly diffusing microdomains to stable, condensed signalling domains (Subczynski and Kusumi, 2003). The hop-diffusion of a raft-localised GPI-anchored protein slowed upon ligand binding and occasionally became immobile consistent with a progression from a small/unstable microdomain to a stabilised signalling microdomain upon ligand binding (Subczynski and Kusumi, 2003). Similar transient confinement zones had been characterised previously in a variety of cell types and were found to be cholesterol and actin-dependent domains, stable for several seconds (Simson *et al.*, 1995, Sheets *et al.*, 1997, Simson *et al.*, 1998, Dietrich *et al.*, 2002) and are believed to be sites of productive signalling (Subczynski and Kusumi, 2003).

Whilst there is some variation in estimates of microdomain size and stability, most data points towards small (<50nm) relatively unstable microdomains with a secondary macrodomain organisation imposed by the cytoskeletal framework. Condensation of more stable microdomains would be stimulated by protein interactions such as ligand binding and this may temporarily restrict the lateral diffusion of these complexes.

Microdomains and cell signalling

Microdomains are believed to regulate signalling by providing concentrated microenvironments enriched for lipid and protein components of signalling pathways. Activation-induced aggregation and stabilisation of these microdomains together with increased association of receptors and downstream effectors would then promote signal integration (Simons and Toomre, 2000).

The difficulty in separating lipid rafts from caveolae has caused problems in determining their respective roles in physiological processes. Immunoprecipitation experiments indicated that many signalling proteins could interact with caveolin via its scaffolding domain, in many cases inhibiting their activity and suggesting a direct regulatory role for caveolin (Lisanti *et al.*, 1994). Perhaps the best evidence for this kind of direct regulation is seen with endothelial nitric oxide synthase (eNOS) signalling, discussed below. However, localisation studies found less than expected enrichment of signalling proteins in caveolae and suggested that in most cell types non-caveolar lipid rafts may represent the predominant location of these proteins (Huang *et al.*, 1997, Nomura *et al.*, 1997, Ringerike *et al.*, 2002). The microdomain requirements for some signalling pathways are described below; whilst lipid rafts and caveolae are implicated in all of these, more research is needed to define what role they play in facilitating signalling.

Lymphocyte signalling

Lymphocytes possess no caveolae and represent the best example of a system with a specific requirement for lipid rafts in facilitating signalling. Immunoglobulin E (IgE) receptor and T cell antigen receptor (TCR) signalling in T-cells both involve the formation of multisubunit

immune recognition receptor complexes that associate with raft-localised downstream non-receptor tyrosine kinases. Binding of multivalent IgE induces cross-linking of IgE receptors resulting in increased detergent insolubility and redistribution of GPI-anchored proteins and raft lipids so that they all co-localise in patches visible by immunofluorescence (Thomas *et al.*, 1994, Stauffer and Meyer, 1997, Sheets *et al.*, 1999), downstream signalling is inhibited by cholesterol depletion (Field *et al.*, 1995). Similar events occur in T cell signalling following activation of TCR. Crosslinking of lipid rafts or TCR increases TCR detergent resistance, recruits downstream adaptors and effectors to these domains and promotes TCR signalling (Montixi, 1998, Xavier *et al.*, 1998, Janes *et al.*, 1999). Palmitoylation and raft targetting of downstream adaptors and effectors are essential for TCR signalling (Kabouridis *et al.*, 1997, Zhang *et al.*, 1998). However, the role of lipid rafts in modulating TCR receptor signalling has been called into question (Germain, 2001), primarily due to the indirect techniques used. Recent microscopic and cell fractionation experiments found that raft markers were not enriched with TCR signalling complexes (Harder and Kuhn, 2000, Bunnel *et al.*, 2002). This suggests that lipid rafts may be required for initiation rather than maintenance of receptor clusters, or that raft-localised signal complexes segregate away from the general raft population forming a specialised signalling raft subcompartment.

EGF receptor (EGFR) and Ras signalling

Another example of reversible association with signalling microdomains can be seen in the EGFR-Ras-Raf signalling pathway. Caveolae, lipid rafts and non-cholesterol dependent microdomains have all been implicated in regulating this pathway. EGFR is found in caveolae and lipid rafts (Ringerike *et al.*, 2002); an extracellular juxtamembrane sequence elements is required for this association (Yamabhai and Anderson, 2002). EGFR signalling is enhanced by cholesterol depletion, possibly due to the increase in cell surface associated EGFR (Furuchi and Anderson, 1998, Ringerike *et al.*, 2002). Downstream Ras signalling is also hyperactivated by cholesterol depletion (Furuchi and Anderson, 1998), although an analysis of Ras isoform specific signalling revealed that cholesterol depletion specifically inhibited H-ras but not K-ras signalling (Roy *et al.*, 1999). Further analysis using biochemical and microscopic approaches showed that K-ras predominantly signals from non-raft microdomains (Prior *et al.*, 2001, Niv *et al.*, 2002, Prior *et al.*, 2003). In contrast, H-ras is present in cholesterol-dependent and non-raft microdomains; activation shifts the equilibrium in favour of residence in non-raft signalling domains distinct from K-ras domains (Figure 3; Prior *et al.*, 2001, Niv *et al.*, 2002, Prior *et al.*, 2003). Therefore, activated EGFR is found in lipid rafts/caveolae and aspects of Ras signalling rely on these microdomains, but association is reversible and subsequent signalling predominantly occurs in non-cholesterol dependent microdomains.

eNOS signalling

Caveolae are particularly abundant in endothelial cells and caveolin has been proposed to be an important negative regulator of eNOS activity (Figure 3). eNOS generates nitric oxide (NO), a short lived second messenger that regulates vascular tone, vascular permeability and angiogenesis. Several lines of evidence support the role of caveolae in facilitating eNOS signalling. Plasma membrane eNOS is predominantly targetted to caveolae by N-terminal myristoylation and palmitoylation (Shaul *et al.*, 1996), although some evidence suggests that cell confluence can influence the extent of eNOS-caveolin co-localisation (Fleming and Busse, 2003). Calcium-calmodulin is an important positive regulator of eNOS activity and caveolae are sites of concentrated calcium influx (Isshiki and Anderson, 1999). The caveolin scaffolding domain (CSD) of caveolin-1 interacts with eNOS, antagonising calmodulin binding and inhibiting its activity (Garcia-Cardena *et al.*, 1997), reducing vascular permeability and vasodilation *in vivo* (Bucci *et al.*, 2000). Caveolin-1 knockout mice exhibited enhanced basal and stimulated eNOS activity further confirming a role for

caveolin in regulating eNOS activity (Drab *et al.*, 2001, Razani *et al.*, 2001). However, the general reliance of many studies on detergent-insolubility and gradient purification and the putative role of caveolin in cholesterol transport and therefore indirectly regulating cell surface organisation means that more work needs to be done to unequivocally assign a role for caveolin in direct regulation of eNOS.

Lipid raft and caveolar endocytosis

Internalisation of lipids rafts and caveolae mediates entry of glycosphingolipids, GPI-anchored proteins, ligands such as folic acid, albumin and growth hormone and various pathogens including cholera toxin, and viruses such as SV40 and HIV (Parton and Richards, 2003). Lipid raft/caveolar endocytosis is clathrin-independent and relies on src-family kinases, actin reorganisation and dynamin-2 (Parton *et al.*, 1994, Oh *et al.*, 1998, Pelkmans *et al.*, 2002). An alternative pinocytic pathway trafficking GPI-anchored proteins has also been identified that is constitutive and dynamin-independent (Sabharanjak *et al.*, 2002, Nabi and Le, 2003).

Caveolae and lipid rafts are biochemically similar suggesting that they should internalize via a common pathway (Nabi and Le, 2003). FRAP studies using caveolin-1-GFP indicated that only a minor pool of cell surface caveolae are actively endocytosing (Thomsen *et al.*, 2002). In addition, lowering or increasing caveolin-1 expression respectively promotes or retards endocytosis of caveolar/raft-localised autocrine motility factor (Le *et al.*, 2002). These data are consistent with the proposal that caveolin-1 is a negative regulator of lipid raft endocytosis by stabilising raft domains on the cell surface (Le *et al.*, 2002). The relative contributions of caveolae versus lipid rafts in mediating non-clathrin dependent endocytosis remains to be accurately determined.

The intracellular itinerary and function of these non-clathrin dependent pathways is currently the subject of intense investigation. Cholera toxin and SV40 virus have been extensively used to map the intracellular itinerary of caveolar/raft endocytosis. Video microscopy showed that after SV40 binding to MHC class I molecules on the cell surface, there is extensive actin reorganisation before recruitment of dynamin and budding of caveolin-labelled SV40 vesicles (Pelkmans *et al.*, 2001, Pelkmans *et al.*, 2002). The virus initially traffics to a ‘caveosome’, a neutral pH compartment that contains no fluid phase markers indicative of constitutive uptake, or EEA1 and transferrin - markers for the early endosome and the clathrin-mediated endocytic pathway. The virus subsequently sorts away from caveolin-GFP into tubular extensions that detach and traffic along microtubules to the ER where it translocates into the cytosol (Pelkmans *et al.*, 2001).

Cholera toxin has been used as a marker for caveolar endocytosis because the B subunit of cholera toxin binds to the sphingolipid GM1, a lipid raft/caveolae resident lipid (Holmgren, 1973) and early electron microscopy studies showed labelling in caveolae (Montesano *et al.*, 1982). However, it was also found that the toxin can also be internalised by the clathrin-dependent pathway (Nichols *et al.*, 2001, Torgersen *et al.*, 2001), and cholera toxin was found in EEA1-positive early endosomes (Montesano *et al.*, 1982, Tran *et al.*, 1987, Parton and Richards, 2003). It is now believed that cholera toxin internalises via both clathrin-mediated and caveolae-mediated routes and that they generally traffic to separate destinations because only caveolar-mediated endocytosis generated toxic cholera toxin (Orlandi and Fishman, 1998). This was supported by a recent study that directly compared SV40 and cholera toxin internalisation. Following inhibition of clathrin-mediated uptake, cholera toxin localised to caveolin-1 positive caveosomes (Nichols, 2002).

Recent work has provided strong evidence for integration of the caveolar/raft endocytic pathway with the classical endocytic pathway. Fluorescent glycosphingolipids are

internalised by a clathrin-independent, dynamin-2-dependent pathway; at early time points these lipids co-localize with caveolin whereas, later they co-localise with transferrin-containing endosomes (Sharma *et al.*, 2003). In some cells there is a partial overlap of EEA1 and caveolin-1 staining indicating that caveolin may also occupy sub-compartments within the classical endocytic system (Parton and Richards, 2003). Together these data reveal that whilst clathrin-mediated endocytosis is specifically targeted to early endosomes, caveolar/raft endocytosis is targeted to both the caveosomes and classical early endosomes (Figure 4). The mechanisms determining sorting and which organelle is preferentially targetted remain to be determined. Finally, a non-clathrin, non-dynamin dependent endocytic pathway mediating raft-localised GPI-anchored protein uptake has also been characterised (Sabharanjak *et al.*, 2002). This pathway probably represents a pinocytic uptake pathway and targets to perinuclear recycling endosomes (Figure 4).

Conclusions and future directions

Investigations into cell surface compartmentalisation are still at an early stage; microscopy and model membrane techniques have detected a variety of microdomains and provided insights into their organisation. The intrinsic properties of some lipids provide the capacity to segregate within the plane of the membrane, proteins provide additional regulation of this phenomenon and also may be able to directly generate their own microdomains. Cholesterol-rich microdomains, have been implicated in regulating a wide variety of cellular processes however it is still not clear exactly how lipid organisation and caveolin regulate many of these phenomena. It seems likely that combinations of protein and lipid interactions are required for the formation, stabilisation and regulation of these domains and the actin cytoskeleton appears to play an important role in many of these steps. The small size and apparent transient nature of microdomains have provided severe technical challenges, and new protocols are still needed for selectively manipulating the organisation and function of specific types of microdomain. However, new techniques are already allowing lipid rafts, caveolae and other microdomains to be discriminated from each other in intact cells providing new avenues for research into their individual functions. It will be especially interesting to determine functions and mechanisms regulating the recently identified, non-cholesterol dependent microdomains. Finally, the non-clathrin-dependent internalisation of lipid rafts and caveolae is the preferred route of some pathogens, characterising this pathway further remains an important challenge. Whilst the focus has been on cell surface microdomains, recent research has also begun to understand the importance of similar scales of compartmentalisation within intracellular organelles for regulating cell processes. Discovering the precise role that membrane compartmentalisation plays in regulating physiological processes represents the key challenge for all future studies.

Acknowledgments

We gratefully acknowledge the Wellcome Trust and the Royal Society for funding work in our laboratory, and thank Dr. Sylvie Urbé for helpful comments and discussion.

References

Ahmed SN, Brown DA, London E. On the origin of sphingolipid-cholesterol rich detergent-insoluble domains in cell membranes: physiological concentrations of cholesterol and sphingolipid induce formation of a detergent-insoluble, liquid-ordered lipid phase in model membranes. Biochemistry. 1997; 36:10944–10953. [PubMed: 9283086]

Baron W, Decker L, Colognato H, ffrench-Constant C. Regulation of integrin growth factor interactions in oligodendrocytes by lipid raft microdomains. Current Biology. 2003; 13:151–155. [PubMed: 12546790]

Bist A, Fielding PE, Fielding CJ. Two sterol regulatory element-like sequences mediate up-regulation of caveolin gene transcription in response to low density lipoprotein free cholesterol. Proceedings of the National Academy of Sciences USA. 1997; 94:10693–10698.

Brown DA, London E. Functions of lipid rafts in biological membranes. Annual Review of Cell and Developmental Biology. 1998; 14:111–136.

Brown DA, Rose JK. Sorting of GPI-anchored proteins to glycolipids-enriched membrane subdomains during transport to the apical cell surface. Cell. 1992; 68:533–544. [PubMed: 1531449]

Bucci M, Gratton JP, Rudic RD, Acevedo L, Roviezzo F, Cirino G, Sessa WC. *In vivo* delivery of the caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation. Nature Medicine. 2000; 6:1362–1367.

Bunnel SC, Hong DI, Kardon JR, Yamazaki T, McGlade CJ, Barr VA, Samelson LE. T cell receptor ligation induces the formation of dynamically regulated signaling assemblies. Journal of Cell Biology. 2002; 158:1263–1275. [PubMed: 12356870]

Dietrich C, Bagatolli LA, Volovyk ZN, Thompson NL, Levi M, Jacobson K, Gratton E. Lipid rafts reconstituted in model membranes. Biophysical Journal. 2001a; 80:1417–1428. [PubMed: 11222302]

Dietrich C, Volovyk ZN, Levi M, Thompson NL, Jacobson K. Partitioning of Thy-1, GM1, and cross-linked phospholipid analogs into lipid rafts reconstituted in supported bilayer model membranes. Proceedings of the National Academy of Sciences USA. 2001b; 98:10642–10647.

Dietrich C, Yang B, Fujiwara T, Kusumi A, Jacobson K. Relationship of lipid rafts to transient confinement zones detected by single particle tracking. Biophysical Journal. 2002; 82:274–284. [PubMed: 11751315]

Drab M, Verkade P, Elger M, Kasper M, Lohn M, Lauterbach B, Menne J, Lindschau C, Mende F, Luft FC, Schedl A, Haller H, Kurzchalia TV. Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. Science. 2001; 293:2449–2552. [PubMed: 11498544]

Edidin M. The state of lipid rafts: from model membranes to cells. Annual Review of Biophysics and Biomolecular Structure. 2003; 32:257–283.

Edidin M, Zuniga MC, Sheetz MP. Truncation mutants define and locate cytoplasmic barriers to lateral mobility of membrane glycoproteins. Proceedings of the National Academy of Sciences USA. 1994; 91:3378–3382.

Field KA, Holowka D, Baird B. Fc epsilon RI-medieated recruitment of p53/56lyn to detergent resistant membrane domains accompanies cellualar signalling. Proceedings of the National Academy of Sciences USA. 1995; 92:9201–9205.

Fielding CJ, Bist A, Fielding PE. Intracellular cholesterol transport in synchronized human skin fibroblasts. Biochemistry. 1999; 38:2506–2513. [PubMed: 10029545]

Fleming I, Busse R. Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. American Journal of Physiology - Regulatory Integrative and Comparative Physiology. 2003; 284:R1–R12.

Foster LJ, De Hoog CL, Mann M. Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. Proceedings of the National Academy of Sciences USA. 2003; 100:5813–5818.

Fra AM, Pasqualetto E, Mancini M, Sitia R. Genomic organization and transcriptional analysis of the human genes coding for caveolin-1 and caveolin-2. Gene. 2000; 243:75–83. [PubMed: 10675615]

Fra AM, Williamson E, Simons K, Parton RG. *De novo* formation of caveolae in lymphocytes by expression of VIP21-caveolin. Proceedings of the National Academy of Sciences USA. 1995; 92:8655–8659.

Fridriksson EK, Shipkova PA, Sheets ED, Holowka D, Baird B, McLafferty FW. Quantitative analysis of phospholipids in functionally important membrane domains from RBL-2H3 mast cells using tandem high-resolution mass spectrometry. Biochemistry. 1999; 38:8056–8063. [PubMed: 10387050]

Friedrichson T, Kurzchalia TV. Microdomains of GPI-anchored proteins in living cells revealed by crosslinking. Nature. 1998; 394:802–805. [PubMed: 9723622]

Fujiwara T, Ritchie K, Murakoshi H, Jacobson K, Kusumi A. Phospholipids undergo hop diffusion in compartmentalized cell membrane. Journal of Cell Biology. 2002; 157:1071–1081. [PubMed: 12058021]

Furuchi T, Anderson RGW. Cholesterol depletion of caveolae causes hyperactivation of extracellular signal-related kinase. Journal of Biological Chemistry. 1998; 274:30636–30643.

Galbiati F. Targeted downregulation of caveolin-1 is sufficient to drive cell transformation and hyperactivate the p42/44MAP kinase cascade. EMBO Journal. 1998; 17:6633–6648. [PubMed: 9822607]

Galbiati F, Engelman JA, Volonte D, Zhang XL, Minetti C, Li M, Hou H, Kneitz B, Edelmann W, Lisanti MP. Caveolin-3 null mice show a loss of caveolae, changes in the microdomain distribution of the dystrophin-glycoprotein complex, and t-tubule abnormalities. Journal of Biological Chemistry. 2001; 276:21425–21433. [PubMed: 11259414]

Garcia-Cardena G, Martasek P, Masters BS, Skidd PM, Couet J, Li S, Lisanti MP, Sessa WC. Dissecting the interaction between nitric oxide synthase (NOS) and caveolin. Functional significance of the non caveolin binding domain *in vivo*. Journal of Biological Chemistry. 1997; 272:25437–25440. [PubMed: 9325253]

Gaus K, Gratton E, Kable EPW, Jones AS, Gelissen I, Kritharides L, Jessup W. Visualizing lipid structure and raft domains in living cells with two-photon microscopy. Proceedings of the National Academy of Sciences USA. 2003; 100:15554–15559.

Germain RN. The T cell receptor for antigen: signaling and ligand discrimination. Journal of Biological Chemistry. 2001; 276:35223–35226. [PubMed: 11435448]

Hagiwara Y, Sasaoka T, Araishi K, Imamura M, Yorifuji H, Nonaka I, Ozawa E, Kikuchi T. Caveolin-3 deficiency causes muscle degeneration in mice. Human Molecular Genetics. 2000; 9:3047–3054. [PubMed: 11115849]

Harder T, Kuhn M. Selective accumulation of raft-associated membrane protein LAT in T cell receptor signaling assemblies. Journal of Cell Biology. 2000; 151:199–208. [PubMed: 11038169]

Heerklotz H. Triton promotes domains formation in lipid raft mixtures. Biophysical Journal. 2002; 83:2693–2701. [PubMed: 12414701]

Holmgren J. Comparison of the tissue receptors for *Vibrio cholerae* and *Escherichia coli* enterotoxins by means of gangliosides and natural cholera toxoid. Infection and Immunity. 1973; 8:851–859. [PubMed: 4206342]

Huang C, Hepler JR, Chen LT, Gilman AG, Anderson RG, Mumby SM. Organization of G proteins and adenylyl cyclase at the plasma membrane. Molecular Biology of the Cell. 1997; 8:2365–2378. [PubMed: 9398661]

Ipsen JH, Karlstrõm G, Mouritsen OG, Wennerstrõm H, Zuckermann MJ. Phase equilibrium in the phosphatidylcholine-cholesterol system. Biochimica et Biophysica Acta. 1987; 905:162–172. [PubMed: 3676307]

Ishikawa H. Formation of elaborate networks of T-system tubules in cultured skeletal muscle with special reference to the T-system formation. Journal of Cell Biology. 1968; 38:51–66. [PubMed: 5691978]

Isshiki M, Anderson RG. Calcium signal transduction from caveolae. Cell Calcium. 1999; 26:201–208. [PubMed: 10643558]

Janes PW, Ley SC, Magee AI. Aggregation of lipid rafts accompanies signaling via the T cell antigen receptor. Journal of Cell Biology. 1999; 147:447–461. [PubMed: 10525547]

Kabouridis PS, Magee AI, Ley SC. S-acylation of LCK protein tyrosine kinase is essential for its signalling function in T lymphocytes. EMBO Journal. 1997; 16

Kenworthy AK, Edidin M. Distribution of a glycophosphatidylinositol-anchored protein at the apical surface of MDCK cell examined at a reolution of <100Å using imaging fluorescence resonance energy transfer. Journal of Cell Biology. 1998; 142:69–84. [PubMed: 9660864]

Kenworthy AK, Petranova N, Edidin M. High-resolution FRET microscopy of cholera toxin B-subunit and GPI-anchored proteins in cell plasma membranes. Molecular Biology of the Cell. 2000; 11:1645–1655. [PubMed: 10793141]

Kurzchalia TV, Dupree P, Parton RG, Kellner R, Virta H, Lehnert M, Simons K. VIP21, a 21-kD membrane protein is an integral component of *trans*-Golgi-network-derived transport vesicles. Journal of Cell Biology. 1992; 118:1003–1014. [PubMed: 1512286]

Kusumi A, Sako Y, Yamamoto M. Confined lateral diffusion of membrane receptors as studied by single particle tracking (nanovid microscopy). Effects of calcium-induced differentiation in cultured epithelial cells. Biophysical Journal. 1993; 65:2021–2040. [PubMed: 8298032]

Kwik J, Boyle S, Fooksman D, Margolis L, Sheetz MP, Edidin M. Membrane cholesterol, lateral mobility, and the phosphatidylinositol 4,5-bisphosphate-dependent organization of cell actin. Proceedings of the National Academy of Sciences USA. 2003; 100:13964–13969.

Lahtinen U, Honsho M, Parton RG, Simons K, Verkade P. Involvement of caveolin-2 in caveolar biogenesis in MDCK cells. FEBS Letters. 2003; 538:85–88. [PubMed: 12633858]

Lang T, Bruns D, Wenzel D, Riedel D, Holroyd P, Thiele C, Jahn R. SNAREs are concentrated in cholesterol-dependent clusters that define docking and fusion sites for exocytosis. EMBO Journal. 2001; 20:2202–2213. [PubMed: 11331586]

Le PU, Guay G, Altschuler Y, Nabi IR. Caveolin-1 is a negative regulator of caveolae-mediated endocytosis to the endoplasmic reticulum. Journal of Biological Chemistry. 2002; 277:3371–3379. [PubMed: 11724808]

Lisanti MP, Scherer PE, Tang ZL, Sargiacomo M. Caveolae, caveolin and caveolin-rich membrane domains: A signalling hypothesis. Trends in Cell Biology. 1994; 4:231–235. [PubMed: 14731661]

Liu P, Ying Y, Ko YG, Anderson RG. Localization of platelet-derived growth factor-stimulated phosphorylation cascade to caveolae. Journal of Biological Chemistry. 1996; 271:10299–10303. [PubMed: 8626598]

London E. Insights into lipid raft structure and formation from experiments in model membranes. Current Opinion in Structural Biology. 2002; 12:480–486. [PubMed: 12163071]

Madore N, Smith KL, Graham CH, Jen A, Brady K, Hall S, Morris R. Functionally different GPI proteins are organized in different domains on the neuronal surface. EMBO Journal. 1999; 18:6917–6926. [PubMed: 10601014]

McCabe JB, Berthiaume LG. N-terminal protein acylation confers localization to cholesterol, sphingolipid-enriched membranes but not to lipid rafts/caveolae. Molecular Biology of the Cell. 2001; 12:3601–3617. [PubMed: 11694592]

Melkonian KA, Ostermeyer AG, Chen JZ, Roth MG, Brown DA. Role of lipid modifications in targeting proteins to detergent-resistant membrane rafts. Many proteins are acylated, while few are prenylated. Journal of Biological Chemistry. 1999; 274:3910–3917. [PubMed: 9920947]

Moffet S, Brown DA, Linder ME. Lipid-dependent targeting of G proteins into rafts. Journal of Biological Chemistry. 2000; 275:2191–2198. [PubMed: 10636925]

Monier S, Parton RG, Vogel F, Behlke J, Henske A, Kurzchalia TV. VIP21-caveolin, a membrane protein constituent of the caveolar coat, oligomerizes *in vivo* and *in vitro*. Molecular Biology of the Cell. 1995; 6:911–927. [PubMed: 7579702]

Montesano R, Roth J, Robert A, Orci L. Non-coated membrane invaginations are involved in binding and internalization of cholera and tetanus toxins. Nature. 1982; 296:651–653. [PubMed: 7070509]

Montixi C. Engagement of T-cell receptor triggers its recruitment to low-density detergent-insoluble membrane domains. EMBO Journal. 1998; 17:5334–5348. [PubMed: 9736612]

Munro S. Lipid rafts: elusive or illusive? Cell. 2003; 115:377–388. [PubMed: 14622593]

Murata M, Peranen J, Schreiner R, Wieland F, Kurzchalia TV, Simons K. VIP21/caveolin is a cholesterol binding protein. Proceedings of the National Academy of Sciences USA. 1995; 92:10339–10343.

Murray D, Arbuzova A, Hangyas-Mihalyne G, Gambhir A, Ben-Tal N, Honig B, McLaughlin S. Electrostatic properties of membranes containing acidic lipids and adsorbed basic peptides: theory and experiment. Biophysical Journal. 1999; 77:3176–3188. [PubMed: 10585939]

Murray D, Ben-Tal N, Honig B, McLaughlin S. Electrostatic interaction of myristoylated proteins with membranes:simple physics, complicated biology. Structure. 1997; 5:985–989. [PubMed: 9309215]

Nabi IR, Le PU. Caveolae/raft-dependent endocytosis. Journal of Cell Biology. 2003; 161:673–677. [PubMed: 12771123]

Nichols BJ. A distinct class of endosome mediates clathrin-independent endocytosis to the Golgi complex. Nature Cell Biology. 2002; 4:374–378.

Nichols BJ. Caveosomes and endocytosis of lipid rafts. Journal of Cell Science. 2003; 116:4707–4714. [PubMed: 14600257]

Nichols BJ, Kenworthy AK, Polischuk RS, Lodge R, Roberts TH, Hirshberg K, Phair RD, Lippincott-Schwartz J. Rapid cycling of lipid raft markers between the cell surface and golgi complex. Journal of Cell Biology. 2001; 153:529–542. [PubMed: 11331304]

Niv H, Gutman O, Kloog Y, Henis Y. Activated K-ras and H-ras display different interactions with saturable nonraft sites at the surface of live cells. Journal of Cell Biology. 2002; 157:865–872. [PubMed: 12021258]

Nomura R, Inuo C, Takahashi Y, Asano T, Fujimoto T. Two-dimensional distribution of Gi2α in the plasma membrane: a critical evaluation by immunocytochemistry. FEBS Letters. 1997; 415:139–144. [PubMed: 9350984]

Oh P, McInitosh DP, Schnitzer JE. Dynamin at the neck of caveolae mediates their budding to form transport vesicles by GTP-driven fission from the plasma membrane of endothelium. Journal of Cell Biology. 1998; 141:101–114. [PubMed: 9531551]

Okamoto T, Schlegel A, Scherer PE, Lisanti MP. Caveolins, a family of scaffolding proteins for organizing “preassembled signaling complexes” at the plasma membrane. Journal of Biological Chemistry. 1998; 273:5419–5422. [PubMed: 9488658]

Orlandi PA, Fishman PH. Filipin-dependent inhibition of cholera toxin: evidence for toxin internalization and activation through caveolae-like domains. Journal of Cell Biology. 1998; 141:905–915. [PubMed: 9585410]

Palade GE. Fine structure of blood capillaries. Journal of Applied Physics. 1953; 24:1424.

Park DS, Cohen AW, Frank PG, Razani B, Lee H, Williams TM, Chandra M, Shirani J, De Souza AP, Tang B, Jelicks LA, Factor SM, Weiss LM, Tanowitz HB, Lisanti MP. Caveolin null (-/-) mice show dramatic reductions in life span. Biochemistry. 2003; 42:15124–15131. [PubMed: 14690422]

Parton RG. Caveolae and caveolins. Current Opinion in Cell Biology. 1996; 8:542–548. [PubMed: 8791446]

Parton RG, Joggerst B, Simons K. Regulated internalization of caveolae. Journal of Cell Biology. 1994; 127:1199–1215. [PubMed: 7962085]

Parton RG, Richards AA. Lipid rafts and caveolae as portals for endocytosis: new insights and common mechanisms. Traffic. 2003; 4:724–738. [PubMed: 14617356]

Pelkmans L, Kartenbeck J, Helenius A. Caveolar endocytosis of simian virus 40 reveals a new two-step vesicular-transport pathway to the ER. Nature Cell Biology. 2001; 3:473–483.

Pelkmans L, Puntener D, Helenius A. Local actin polymerization and dynamin recruitment in SV40-induced internalization of caveolae. Science. 2002; 296:535–539. [PubMed: 11964480]

Pike LJ, Casey L. Localization and turnover of phosphatidylinositol 4,5-bisphosphate in caveolin-enriched membrane domains. Journal of Biological Chemistry. 1996; 271:26453–26456. [PubMed: 8900109]

Pike LJ, Han X, Chung KN, Gross RW. Lipid rafts are enriched in arachidonic acid and plasmenylethanolamine and their composition is independent of caveolin-1 expression: a quantitative electrospray ionization/mass spectrometric analysis. Biochemistry. 2002; 41:2075–2088. [PubMed: 11827555]

Pike LJ, Miller JM. Cholesterol depletion delocalizes phosphatidylinositol bisphosphate and inhibits hormone-stimulated phosphatidylinositol turnover. Journal of Biological Chemistry. 1998; 273:22298–22304. [PubMed: 9712847]

Pol A, Luetterforst R, Lindsay MR, Heino S, Ikonen E, Parton RG. A caveolin dominant-negative mutant associates with lipid bodies on the caveolin-cycling pathway and induces intracellular cholesterol imbalance. Journal of Cell Biology. 2001; 152:1057–1070. [PubMed: 11238460]

Pralle A, Keller P, Florin EL, Simons K, Horber JK. Sphingolipid-cholesterol rafts diffuse as small entities in the plasma membrane of mammalian cells. Journal of Cell Biology. 2000; 148:997–1008. [PubMed: 10704449]

Prior IA, Harding A, Yan J, Sluimer J, Parton RG, Hancock JF. GTP-dependent segregation of H-ras from lipid rafts is required for biological activity. Nature Cell Biology. 2001; 3:368–375.

Prior IA, Muncke C, Parton RG, Hancock JF. Direct visualization of Ras proteins in spatially distinct cell surface microdomains. Journal of Cell Biology. 2003; 160:165–170. [PubMed: 12527752]

Razani B, Combs TP, Wang XB, Frank PG, Park DS, Russel RG, Li M, Tang B, Jelicks LA, Scherer PE, Lisanti MP. Caveolin-1 deficient mice are lean, resistant to diet-induced obesity, and show hypertriglyceridemia with adipocyte abnormalities. Journal of Biological Chemistry. 2002a; 277:8635–8647. [PubMed: 11739396]

Razani B, Engelman JA, Wang XB, Schubert W, Zhang XL, Marks CB, Macaluso F, Russel RG, Li M, Pestell RG, Di Vizio D, Hou H, Kneitz B, Lagaud G, Christ GJ, Edelmann W, Lisanti MP. Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. Journal of Biological Chemistry. 2001; 276:38121–38138. [PubMed: 11457855]

Razani B, Wang XB, Engelman JA, Battista M, Lagaud G, Zhang XL, Kneitz B, Hou H, Christ GJ, Edelmann W, Lisanti MP. Caveolin-2-deficient mice show evidence of severe pulmonary dysfunction without disruption of caveolae. Molecular Cell Biology. 2002b; 22:2329–2344.

Rietveld A, Neutz S, Simons K, Eaton S. Association of sterol- and glycophosphatidylinositol-linked proteins with Drosophila raft microdomains. Journal of Biological Chemistry. 1999; 274:12049–12054. [PubMed: 10207028]

Ringerike T, Blystad FD, Levy FO, Madshus IH, Stang E. Cholesterol is important in control of EGF-receptor kinase activity but EGF receptors are not concentrated in caveolae. Journal of Cell Science. 2002; 115:1331–1340. [PubMed: 11884532]

Ritchie K, Iino R, Fujiwara T, Murase K, Kusumi A. The fence and picket structure of the plasma membrane of live cells as revealed by single molecule techniques. Molecular Membrane Biology. 2003; 20:13–18. [PubMed: 12745919]

Rodal SK, Skretting G, Garred Ø, Vilhardt F, van Deurs B, Sandvig K. Extraction of cholesterol with methyl-β-cyclodextrin perturbs formation of clathrin-coated endocytic vesicles. Molecular Biology of the Cell. 1999; 10:961–974. [PubMed: 10198050]

Rothberg KG, Heuser JE, Donzell WC, Ying YS, Glenney JR, Anderson RGW. Caveolin, a protein component of caveolae membrane coats. Cell. 1992; 68:673–682. [PubMed: 1739974]

Rothberg KG, Ying YS, Kamen BA, Anderson RG. Cholesterol controls the clustering of the glycophospholipid-anchored membrane receptor for 5-methyltetrahydrofolate. Journal of Cell Biology. 1990; 111:2931–2938. [PubMed: 2148564]

Roy S, Luetterforst R, Harding A, Apolloni A, Etheridge M, Stang E, Rolls B, Hancock JF, Parton RG. Dominant-negative caveolin inhibits H-ras function by disrupting cholesterol-rich plasma membrane domains. Nature Cell Biology. 1999; 1:98–105.

Sabharanjak S, Sharma P, Parton RG, Mayor S. GPI-anchored proteins are delivered to recycling endosomes via a distinct cdc42-regulated clathrin-idependent pinocytic pathway. Developmental Cell. 2002; 2:411–423. [PubMed: 11970892]

Sako Y, Kusumi A. Compartmentalized structure of the plasma-membrane for receptor movements as revealed by a nanometer-level motion analysis. Journal of Cell Biology. 1994; 125:1251–1264. [PubMed: 8207056]

Samsonov AV, Mihalyov I, Cohen FS. Characterization of cholesterol-sphingomyelin domains and their dynamics in bilayer membranes. Biophysical Journal. 2001; 81:1486–1500. [PubMed: 11509362]

Sargiacomo M, Sudol M, Tang Z, Lisanti MP. Signal transducing molecules and glycosyl-phosphatidylinositol-linked proteins form a caveolin-rich insoluble complex in MDCK cells. Journal of Cell Biology. 1993; 122:789–807. [PubMed: 8349730]

Schroeder R, London E, Brown DA. Interactions between saturated acyl chains confer detergent resistence on lipids and GPI-anchored proteins: GPI-anchored proteins in liposomes and cells show similar behaviour. Proceedings of the National Academy of Sciences USA. 1994; 91:12130–12134.

Schubert W, Frank PG, Razani B, Park DS, Chow CW, Lisanti MP. Caveolae-deficient endothelial cells show defects in the uptake and transport of albumin in vivo. Journal of Biological Chemistry. 2001; 276:48619–48622. [PubMed: 11689550]

Schuck S, Honsho M, Ekroos K, Shevchenko A, Simons K. Resistance of cell membranes to different detergents. Proceedings of the National Academy of Sciences USA. 2003; 100:5795–5800.

Schutz GJ, Kada G, Pastushenko VP, Schindler H. Properties of lipid microdomains in a muscle cell membrane visualized by single molecule microscopy. EMBO Journal. 2000; 19:892–901. [PubMed: 10698931]

Sharma DK, Choudhary A, Singh RD, Wheatley CL, Marks DL, Pagano RE. Glycosphingolipids internalized via caveolar-related endocytosis rapidly merge with the clathrin pathway in early endosomes and form microdomains for recycling. Journal of Biological Chemistry. 2003; 278:7564–7572. [PubMed: 12482757]

Sharma P, Varma R, Sarasij RC, Ira, Gousset K, Krishnamoorthy G, Rao M, Mayor S. Nanoscale organization of multiple GPI-anchored proteins in living cell membranes. Cell. 2004; 116:577–589. [PubMed: 14980224]

Shaul PW, Smart EJ, Robinson LJ, German Z, Yuhanna IS, Ying Y, Anderson RG, Michel T. Acylation targets endothelial nitric-oxide synthase to plasmalemmal caveolae. Journal of Biological Chemistry. 1996; 271:6518–6522. [PubMed: 8626455]

Sheets ED, Holowka D, Baird B. Critical role for cholesterol in Lyn-mediated tyrosine phosphorylation of FcεRI and their association with detergent-resistant membranes. Journal of Cell Biology. 1999; 145:877–887. [PubMed: 10330413]

Sheets ED, Lee GM, Simson K, Jacobson K. Transient confinement of a glycosylphosphatidylinositol-anchored protein in the plasma membrane. Biochemistry. 1997; 36:12449–12458. [PubMed: 9376349]

Shogomori H, Brown DA. Use of detergent to study membrane rafts: the good, the bad, and the ugly. Biological Chemistry. 2003; 384:1259–1263. [PubMed: 14515986]

Silvius JR. Role of cholesterol in lipid raft formation: lessons from lipid model systems. Biochimica et Biophysica Acta. 2003; 1610:174–183. [PubMed: 12648772]

Silvius JR, del Giudice D, Lafleur M. Cholesterol at different bilayer concentrations can promote or antagonize lateral segregation of phospholipids of differing acyl chain length. Biochemistry. 1996; 35:15198–15208. [PubMed: 8952467]

Simons K, Ikonen E. Functional rafts in cell membranes. Nature. 1997; 387:569–572. [PubMed: 9177342]

Simons K, Toomre D. Lipid rafts and signal transduction. Nature Reviews: Molecular Cell Biology. 2000; 1:31–39.

Simons K, van Meer G. Lipid sorting in epithelial cells. Biochemistry. 1988; 27:6197–6202. [PubMed: 3064805]

Simons K, Wandinger-Ness A. Polarized sorting in epithelia. Cell. 1990; 62:207–210. [PubMed: 2196994]

Simson R, Sheets ED, Jacobson K. Detection of temporary lateral confinement of membrane proteins using single-particle tracking analysis. Biophysical Journal. 1995; 69:989–993. [PubMed: 8519998]

Simson R, Yang B, Moore SE, Doherty P, Walsh FS, Jacobson KA. Structural mosaicism on the submicron scale in the plasma membrane. Biophysical Journal. 1998; 74:297–308. [PubMed: 9449330]

Singer SJ, Nicholson GL. The fluid mosaic model of the structure of cell membranes. Science. 1972; 175:720–731. [PubMed: 4333397]

Smart EJ, Ying Y, Donzell WC, Anderson RGW. A role for caveolin in transport of cholesterol from endoplasmic reticulum to plasma membrane. Journal of Biological Chemistry. 1996; 271:29427–29435. [PubMed: 8910609]

Song KS, Sargiacomo M, Galbiati F, Parenti M, Lisanti MP. Targeting of a G alpha subunit (Gi1 alpha) and c-Src tyrosine kinase to caveolae membranes: clarifying the role of N-myristoylation. Cell and Molecular Biology Noisy le grand. 1997; 43:293–303.

Song SK, Li S, Okamoto T, Quilliam LA, Sargiacomo M, Lisanti MP. Co-purification and direct interaction of Ras with caveolin, an integral membrane protein of caveolae microdomains. Detergent-free purification of caveolae microdomains. Journal of Biological Chemistry. 1996; 271:9690–9697. [PubMed: 8621645]

Sotgia F, Razani B, Bonuccelli G, Schubert W, Battista M, Lee H, Capozza F, Schubert AL, Minetti C, Buckley JT, Lisanti MP. Intracellular retention of glycophosphatidyl inositol-linked proteins in caveolin-deficient cells. Molecular Cell Biology. 2002; 22:3905–3926.

Sowa G, Pypaert M, Fulton D, Sessa WC. The phosphorylation of caveolin-2 on serines 23 and 36 modulates caveolin-1-dependent caveolae formation. Proceedings of the National Academy of Sciences USA. 2003; 100:6511–6516.

Stauffer TP, Meyer T. Compartmentalized IgE receptor-mediated signal transduction in living cells. Journal of Cell Biology. 1997; 139:1447–1454. [PubMed: 9396750]

Subczynski WK, Kusumi A. Dynamics of raft molecules in the cell and artificial membranes: approaches by pulse EPR spin labeling and single molecule optical microscopy. Biochimica et Biophysica Acta. 2003; 1610:231–243. [PubMed: 12648777]

Subtil A, Gaidarov I, Kobylarz K, Lampson MA, Keen JH, McGraw TE. Acute cholesterol depletion inhibits clathrin-coated pit budding. Proceedings of the National Academy of Sciences USA. 1999; 96:6775–6780.

Thomas JL, Holowka D, Baird B, Webb WW. Large-scale co-aggregation of fluorescent lipid probes with cell surface proteins. Journal of Cell Biology. 1994; 125:795–802. [PubMed: 8188747]

Thomsen P, Roepstorff K, Stahlhut M, van Deurs B. Caveolae are highly immobile plasma membrane microdomains which are not involved in constitutive endocytic trafficking. Molecular Biology of the Cell. 2002; 13:238–250. [PubMed: 11809836]

Torgersen ML, Skretting G, van Deurs B, Sandvig K. Internalization of cholera toxin by different endocytic mechanisms. Journal of Cell Science. 2001; 114:3737–3747. [PubMed: 11707525]

Tran DJ, Carpentier JL, Sawano F, Gorden P, Orci L. Ligands internalised through coated or non-coated invaginations follow a common intracellular pathway. Proceedings of the National Academy of Sciences USA. 1987; 84:7957–7961.

Trigatti BL, Anderson RGW, Gerber GE. Identification of caveolin-1 as a fatty acid binding protein. Biochemical and Biophysical Research Communications. 1999; 255:34–39. [PubMed: 10082651]

Uittenbogaard A, Ying YS, Smart EJ. Characterization of a cytosolic heat-shock protein caveolin chaperone complex. Involvement in cholesterol trafficking. Journal of Biological Chemistry. 1998; 275:25595–25599. [PubMed: 10833523]

van Deurs B, Roepstorff K, Hommelgaard AM, Sandvig K. Caveolae: anchored, multifunctional platforms in the lipid ocean. Trends in Cell Biology. 2003; 13:92–100. [PubMed: 12559760]

Varma R, Mayor S. GPI-anchored proteins are organized in submicron domains at the cell surface. Nature. 1998; 394:798–801. [PubMed: 9723621]

Vereb G, Szöllösi J, Matkó J, Nagy P, Farkas T, Vigh L, Matyus L, Waldmann TA, Damjanovich S. Dynamic yet structured: the cell membrane three decades after the Singer-Nicholson model. Proceedings of the National Academy of Sciences USA. 2003; 100:8053–8058.

Wang TY, Leventis R, Silvius JR. Fluorescence-based evaluation of the partitioning of lipids and lipidated peptides into liquid-ordered microdomains: a model for molecular partitioning into “lipid rafts”. Biophysical Journal. 2000; 79:919–933. [PubMed: 10920023]

Wang TY, Leventis R, Silvius JR. Partitioning of lipidated peptide sequences into liquid-ordered lipid domains in model and biological membranes. Biochemistry. 2001; 40:13031–13040. [PubMed: 11669641]

Wang TY, Silvius JR. Cholesterol does not induce segregation of liquid-ordered domains in bilayers modeling the inner leaflet of the plasma membrane. Biophysical Journal. 2001; 81:2762–2773. [PubMed: 11606289]

Waugh MG, Lawson D, Hsuann JJ. Epidermal growth factor receptor activation is localized within low-buoyant density, non-caveolar membrane domains. Biochemical Journal. 1999; 337:591–597. [PubMed: 9895306]

Xavier R, Brennan T, Li Q, McCormack C, Seed B. Membrane compartmentation is required for efficient T cell activation. Immunity. 1998; 8:723–732. [PubMed: 9655486]

Xu X, London E. The effect of sterol structure on membrane lipid domains reveals how cholesterol can induce lipid domain formation. Biochemistry. 2000; 39:843–849. [PubMed: 10653627]

Yamabhai M, Anderson RGW. Second cysteine-rich region of epidermal growth factor receptor contains targeting information for caveolae/rafts. Journal of Biological Chemistry. 2002; 277:24843–24846. [PubMed: 12023273]

Yamada E. The fine structure of the gall bladder epithelium of the mouse. Journal of Biophysical and Biochemical Cytolology. 1955; 1:445–458.

Zacharias DA, Violin JD, Newton AC, Tsien RY. Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells. Science. 2002; 296:913–916. [PubMed: 11988576]

Zhang W, Trible RP, Samelson LE. LAT palmitoylation: its essential role in membrane microdomain targeting and tyrosine phosphorylation during T cell activation. Immunity. 1998; 9:239–246. [PubMed: 9729044]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

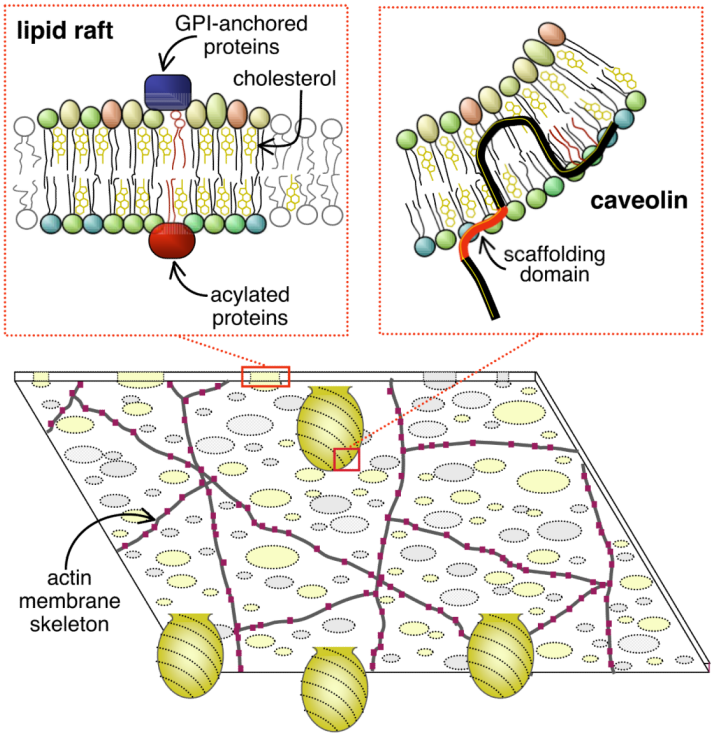

Figure 1.
Plasma membrane compartmentalisation.
Plasma membrane microdomains exist within a secondary macro-organisation imposed by the actin cytoskeleton and anchored transmembrane proteins. A variety of types of microdomain have been identified, however their size, organisation and distribution remain to be accurately determined. Caveolae and lipid rafts are cholesterol-dependent microdomains whose organisation is proposed to facilitate sorting of proteins and lipids that can intercalate into the highly ordered structure. Caveolin drives the formation of caveolae and is proposed to interact with a wide variety of proteins via the caveolin scaffolding domain. This Figure is reproduced in colour in Molecular Membrane Biology on-line.

Europe PMC Funders Author Manuscripts

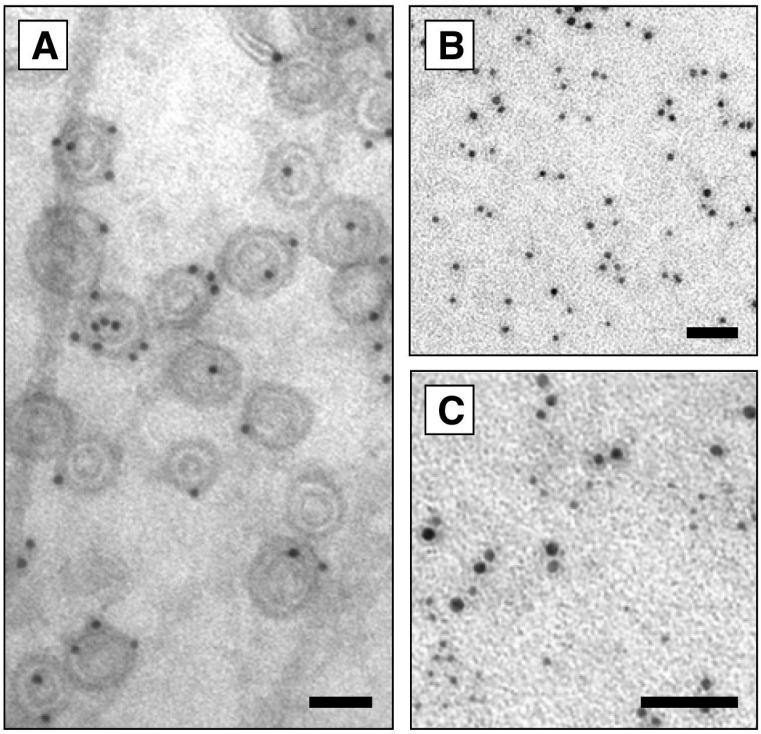

Figure 2.
Electron microscopic imaging of microdomains
Electron microscopy of isolated plasma membrane sheets provides nanoscale resolution for characterising microdomains. Caveolae (A) possess distinctive morphology but in most cell types the majority of the cell surface is morphologically featureless (B, C). Single (B) or double gold labelling (C) of microdomain markers and proteins of interest coupled with spatial statistical analysis of clustering patterns allows the size and abundance of microdomains and the degree of protein co-localisation to be determined (Prior *et al.*, 2003). Bars = 50nm.

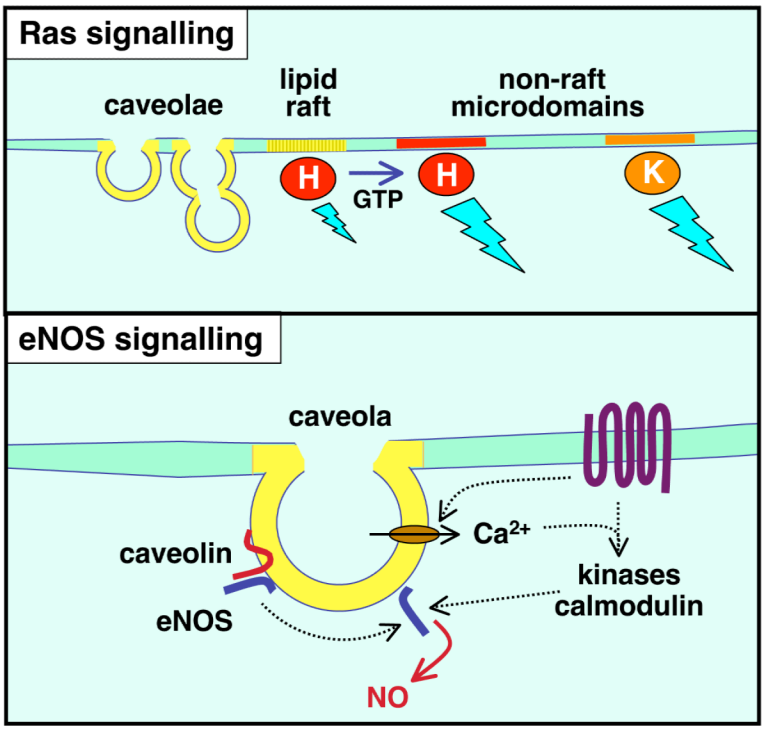

Figure 3.
Compartmentalised cell signalling.
Ras isoform signalling is regulated by precise microlocalisation. Disruption of fibroblast lipid rafts/caveolae inhibits H-ras but not K-ras signalling. H-ras activation promotes migration from caveolae and lipid rafts to non-raft signalling microdomains that are distinct from K-ras signalling domains. eNOS signalling is regulated by caveolin; cell stimulation promotes calcium entry and eNOS/caveolin dissociation allowing eNOS activation and nitric oxide (NO) production. Many regulators and targets of eNOS/NO have been localised to caveolae/lipid rafts. This Figure is reproduced in colour in Molecular Membrane Biology on-line.

Europe PMC Funders Author Manuscripts

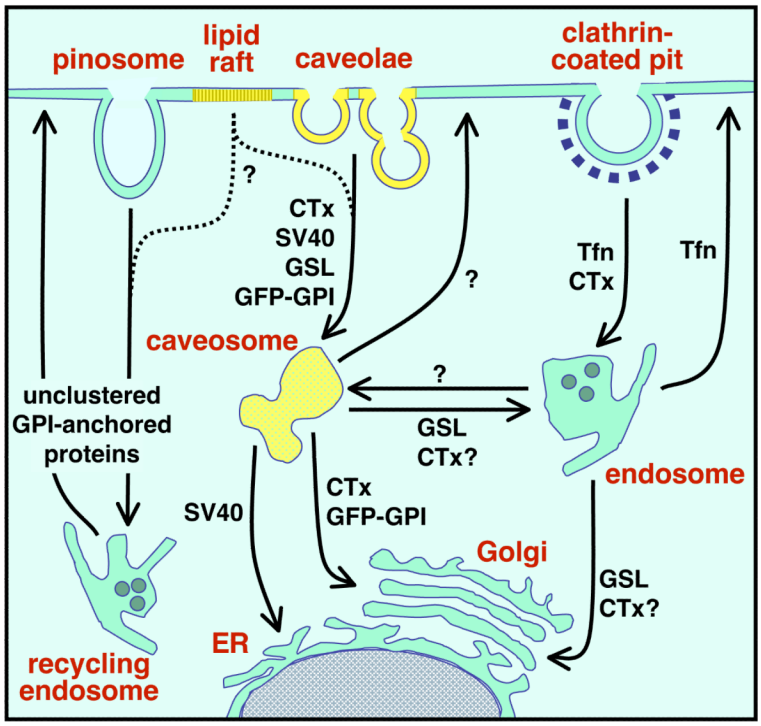

Figure 4.

Endocytosis of caveolae and lipid rafts.

After internalisation lipid raft and caveolae-derived vesicles fuse with caveosomes where their cargo is sorted for delivery to the endoplasmic reticulum (ER), Golgi and the classical endocytic system. The connections between caveosomes and the classical endocytic sytem are poorly defined; studies of glycosphingolipid (GSL) trafficking represent the best example of a putative link. Unlike the other pathways, pinocytosis is dynamin-independent and represents an alternative route for GPI-anchored protein endocytosis; this pathway ultimately merges with the classical endocytic system in perinuclear recycling endosomes. Abbreviations: Cholera Toxin, CTx; Transferrin, Tfn. This Figure is reproduced in colour in Molecular Membrane Biology on-line.

Europe PMC Funders Author Manuscripts

Table 1

Techniques used to study cell surface microdomains

Technique	Marker	Microdomain	Domain size	Reference
Hetero-FRET	GM1, Folate Receptor (FR), CD59, 5′NT	raft/caveolae	0	Kenworthy *et al.* (1998, 2000)
Cross-linking	FR, GPI-Growth Hormone	raft/caveolae	<5nm	Friedrichson and Kurzchalia (1998)
Homo-FRET	FR	raft/caveolae	<5nm, 70nm	Sharma *et al.* (2004), Varma and Mayor (1998)
Electron microscopy	GFP-Ras, GFP-Ras-targetting domains	non-raft raft	34nm 44nm	Prior *et al.* (2003)
Laser trap	Placental alkaline phosphatase, haemagglutinin	raft/caveolae	50nm	Pralle *et al.* (2000)
Single particle tracking	CD44, DOPE Thy-1, GM1	actin macrodomains raft/caveolae	41-750nm 260-370nm	Ritchie *et al.* (2003) Sheets *et al.* (1997)
Single dye tracking	DMPE	raft/caveolae	700nm	Schutz *et al.* (2000)