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SYMPOSIUM REVIEW

Lipid microdomains and the regulation of ion channel function

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Many types of ion channel localize to cholesterol and sphingolipid-enriched regions of the plasma membrane known as lipid microdomains or 'rafts'. The precise physiological role of these unique lipid microenvironments remains elusive due largely to difficulties associated with studying these potentially extremely small and dynamic domains. Nevertheless, increasing evidence suggests that membrane rafts regulate channel function in a number of different ways. Raft-enriched lipids such as cholesterol and sphingolipids exert effects on channel activity either through direct protein–lipid interactions or by influencing the physical properties of the bilayer. Rafts also appear to selectively recruit interacting signalling molecules to generate subcellular compartments that may be important for efficient and selective signal transduction. Direct interaction with raft-associated scaffold proteins such as caveolin can also influence channel function by altering gating kinetics or by affecting trafficking and surface expression. Selective association of ion channels with specific lipid microenvironments within the membrane is thus likely to be an important and fundamental regulatory aspect of channel physiology. This brief review highlights some of the existing evidence for raft modulation of channel function.

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Abbreviations CLIC, chloride intracellular channel; CSD, caveolin scaffolding domain; ENaC, epithelial Na^+ channel; HCN, hyperpolarization-activated cyclic nucleotide-gated; hERG1, human *ether-à-go-go* related gene; K_{ATP}, ATP-sensitive K^+ channel; K_{ir}, inwardly rectifying K^+ channel; K_v, voltage-gated K^+ channel; MAGUK, membrane-associated guanylate kinase; Na_v, voltage-gated Na^+ channel; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; PSD-95, postsynaptic density protein-95; SAP97, synapse-associated protein-97; siRNA, small interfering RNA; SR, sarcoplasmic reticulum; TRP, transient receptor potential.

Introduction

The plasma membrane can consist of in excess of 2000 different species of lipid (Barenholz, 2000). These separate into distinct populations within the bilayer forming a patchwork of different lipid environments at the cell surface. Cholesterol, sphingolipids and phospholipids with saturated acyl tails coalesce to form tightly packed aggregates known as lipid 'rafts' (Simons & Ikonen, 1997; Simons & Toomre, 2000). These represent a relatively rigid, ordered phase of the membrane in contrast to the more fluid bulk of the bilayer where the kinked hydrocarbon chains of the largely unsaturated phospholipids prevent close packing. Multiple types of

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raft are likely to exist based on differences in lipid and protein composition (Pike, 2004), but despite many years of study rafts in native membranes remain relatively controversial in terms of size, stability and physiological importance (Edidin, 2003; Munro, 2003). The only morphologically identifiable raft-like domain is a caveola (Fig. 1). Here, association with the protein caveolin causes the cholesterol- and sphingolipid-enriched regions of the membrane to bulge into the cell forming small (50–100 nm) flask-shaped pits that are clearly visible in scanning or transmission electron microscopy (Razani *et al.* 2002; Cohen *et al.* 2004; Parton & Simons, 2007).

Despite the controversy, membrane rafts have attracted considerable attention in the ion channel field over recent years. This is chiefly because of their apparent ability to selectively aggregate interacting signalling molecules and the implication that they may be involved in the spatial organization of cell signalling pathways (Simons & Toomre, 2000; Patel *et al.* 2008). In addition, a considerable number of channel proteins that associate with caveolar

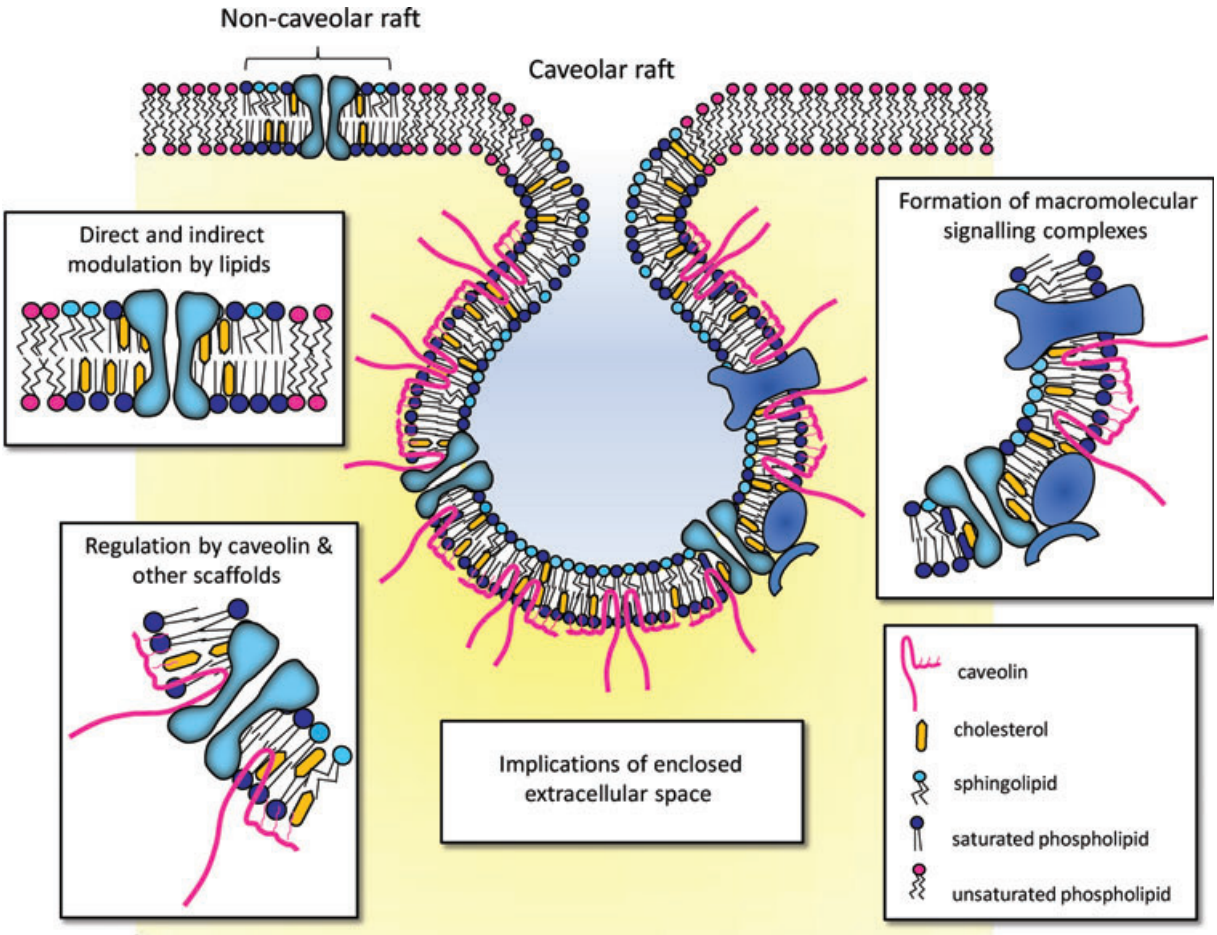

Figure 1. Lipid microdomains potentially influence ion channel activity by a variety of different mechanisms
Cartoon illustrating the segregation of different types of lipids in raft and non-raft regions of the plasma membrane. Cholesterol, sphingolipids and saturated phospholipids aggregate in tightly packed microdomains known as rafts. The lateral association of these lipids in the membrane is driven by tight hydrophilic and hydrophobic van der Waal interactions between sphingolipids and cholesterol (Simons & Ikonen, 1997). Cholesterol tends to increase the packing density in these regions by filling in the spaces between bulky sphingolipids. Sphingomyelin, the most prevalent sphingolipid, localizes predominantly to the outer leaflet of the membrane. In caveolar-type rafts, association with the cholesterol-binding protein caveolin causes the formation of flask-shaped invaginations of the surface membrane. Lipid microdomains may regulate channel function in a number of different ways. This regulation can occur by direct and indirect modulation by lipids; recruitment of interacting molecules to facilitate localized signalling; and/or modulation by caveolae-associated scaffold proteins, such as caveolin. This final form of modulation may be through direct alterations in channel kinetics or through changes in channel trafficking and surface expression. Additionally, the restricted opening at the mouth of the caveola would allow the build up of ions within the caveolar 'pit' to concentrations far in excess of that found in the bulk extracellular fluid. This would additionally influence the activity of proteins localized to these compartments.

rafts interact directly with the cholesterol-binding protein, caveolin. This interaction appears to regulate channel function either directly by altering channel kinetics or indirectly by affecting trafficking and surface expression (Alioua *et al.* 2008; Jiao *et al.* 2008; Garg *et al.* 2009*b*; Lee *et al.* 2009). Raft-enriched lipids such as cholesterol and sphingolipids can also exert effects on channel activity either through direct protein-lipid interactions (Epshtein *et al.* 2009; Fantini & Barrantes, 2009) or by influencing the physical characteristics of the bilayer (Andersen & Koeppe, 2007). The ability of ion channels to associate with specific lipid domains is thus likely to be an important regulatory aspect of channel physiology.

Ion channels and membrane rafts: methods of study

Channel proteins from virtually every class have been reported to associate with lipid microdomains. These include certain voltage-gated (K_v) and inwardly rectifying (K_{ir}) K^+ channels; voltage-gated (Na_v) and epithelial (ENaC) Na^+ channels; L-type Ca^{2+} channels; hyperpolarization-activated cyclic nucleotide-gated (HCN) channels; transient receptor potential (TRP) channels; various connexins; chloride channels; and P2X receptors (Table 1). Evidence for the association of ion channels with membrane rafts comes from three broad approaches: biochemical techniques that exploit the unusual properties of these domains to isolate them and their associated proteins from the bulk of the bilayer; microscopic approaches designed to directly visualize rafts; and functional approaches that destroy these domains and assess the effects on channel activity.

Biochemical isolation of rafts. This is by far the most widely exploited method of identifying proteins that associate with lipid rafts. The tight packing of the lipid acyl chains in these domains results in resistance to solubilization by cold non-ionic detergents (Brown & Rose, 1992), and the high lipid content of these complexes enables them to float to a low density during sucrose gradient centrifugation. Appropriate fractions can then be isolated from the density gradient and proteins associated with these fractions characterized by Western blot analysis. The most widely used detergent-based method consists of solubilization of membranes with 1% Triton X-100 at 4°C, although other methods have been described using lower detergent concentrations or other non-ionic detergents such as Chaps, Lubrol and Brij-98 (Pike, 2004).

Different detergents, and indeed different concentrations of the same detergent, produce rafts of very different protein and lipid composition (Pike, 2004; Babiychuk & Draeger, 2006). This may reflect the inherent heterogeneity of membrane rafts and the ability of some detergents to produce more 'pure' raft fractions by efficiently removing contaminating non-raft proteins and lipids. Equally, however, it may mean that some detergents selectively extract subsets of proteins and lipids from rafts, leaving behind a domain that bears little resemblance to its *in vivo* form. Problems such as these led to the development of non-detergent based methods for the isolation of rafts (Smart *et al.* 1995; Song *et al.* 1996). These essentially use sonication to disrupt the membrane followed by sucrose density centrifugation to separate the buoyant low-density raft component. While this method suffers from none of the potential selective extraction that plagues detergent-based methods, there is evidence that contaminating non-raft proteins may stay associated at the periphery of rafts and float with them to the low density layers (Foster *et al.* 2003; Pike, 2004). In general, it seems that biochemical isolation should be seen as a starting point for assessing the association of specific proteins with rafts and that, where possible, additional techniques should be employed to confirm the interaction.

Direct visualization: microscopic methods. Much of the controversy surrounding the existence of non-caveolar rafts in native cell membranes stems from the lack of suitable, non-invasive imaging techniques that allow direct visualization of these potentially extremely small (estimates range between 5–200 nm) and dynamic domains. Evidence for this form of microdomain is based largely on analysis of non-random clustering of fluorescently labelled raft-associated proteins in the membrane and the segregation of these clusters away from non-raft markers (Varma & Mayor, 1998; Kenworthy *et al.* 1999; Sharma *et al.* 2004). Additional evidence for the existence of distinct lipid microenvironments comes from monitoring changes in the diffusion behaviour of proteins in the bilayer (Pralle *et al.* 2000; Meder *et al.* 2006) or from the use of fluorescent probes such as 6-acyl-dimethylaminonapthalene (Laurdan) that are sensitive to changes in membrane fluidity (Gaus *et al.* 2003).

Morphologically, caveolae, which represent a subset of membrane raft, are much easier to study as they resemble small, stable pits on the membrane surface that are easily recognized in electron micrographs. These pits label heavily for caveolae marker proteins such as caveolin, and the direct visualization of proteins within these distinctive membrane compartments through immunogold electron microscopy provides perhaps the most convincing evidence for channel association with lipid microdomains. A subpopulation of $Ca_v1.2$ L-type Ca^{2+} channels localize with a muscle-specific isoform of caveolin in sarcolemmal caveolae in ventricular cardiomyocytes for example (Balijepalli *et al.* 2006). A particularly useful method here is the immunogold

Table 1. Examples ion channel association with lipid microdomains

Channel	Tissue	Biochemical isolation	Electron microscopy	Association caveolin	Functional effects/Comments	References
$K_v1.3$	Jurkat T-lymphocytes	yes	—	—	Constituitively present in rafts. Hydrolysis of sphingomyelin causes rafts to merge into large ceramide-enriched domains. Association of $K_v1.3$ with ceramide-enriched domains inhibits channel activity	(Bock *et al.* 2003)
$K_v1.4$	Brain HEK293T cells	yes	—	—	Requires PSD-95 for raft targeting	(Wong & Schlichter, 2004)
$K_v1.5$	Heart	yes	yes	?	Cholesterol depletion modulates gating	
	Cell lines	yes	—	yes	Interaction with SAP-97 and caveolin-3 involved in raft targeting. Coexpression with caveolin or addition of exogenous cholesterol causes a depolarizing shift in steady-state activation and inactivation	(Reviewed by Balijepalli & Kamp, 2008)
$K_v2.1$	L cell fibroblasts	yes	—	no	Cholesterol depletion causes hyperpolarizing shift in the inactivation curve	(Martens *et al.* 2000)
	Pancreatic β cells	yes	—	—	Cholesterol depletion reduces current amplitude and causes hyperpolarizing shift in inactivation curve	(Xia *et al.* 2004)
	Oocytes				Regulation of gating by sphingomyelin	(Ramu *et al.* 2006)
$K_v4.2$	Brain	yes	—	—		(Wong & Schlichter, 2004)
$K_v7.1$ (K_vLQT1)	Heart HEK293 cells	yes	—	—		(Balijepalli *et al.* 2007)
$K_v11.1$ (hERG1)	Heart HEK293 cells	yes	—	no	Cholesterol depletion causes positive shift in voltage dependence of activation and accelerates deactivation kinetics	(Balijepalli *et al.* 2007)
BK, hSLO	Aorta	yes	—	yes	Caveolin-regulated surface expression	(Alioua *et al.* 2008)
	Myometrium	yes	—	yes	Knockdown of caveolin with siRNA suppresses total BK current	(Brainard *et al.* 2009)
	Aortic endothelial	yes	—	yes	BK channel inactive under control conditions but activated by cholesterol depletion or knockdown of caveolin	(Wang *et al.* 2005)
$K_{ir}2.1$	Aortic endothelial	yes	—	no	Cholesterol depletion increases current density. Single channel properties unaffected – cholesterol may modulate number of active channels	(Romanenko *et al.* 2002)

Table 1. Continued

Channel	Tissue	Biochemical isolation	Electron microscopy	Association caveolin	Functional effects/Comments	References
$K_{ir}3.1/3.2$	Neurones CHO cells	yes	—	—	Rafts may be involved in surface delivery	(Delling *et al.* 2002)
$K_{ir}4.1$	Astrocytes HEK293 cell line	yes	—	—	Associate with non-caveolar lipid rafts. Cholesterol depletion results in loss of channel activity	(Hibino & Kurachi, 2007)
$K_{ir}6.1$	Aorta	yes	yes	yes	Cholesterol depletion abolishes cAMP/PKA regulation of channel	(Sampson *et al.* 2004, 2007)
	Vascular smooth muscle HEK293 cells				PKC-mediated caveolin-dependent internalization	(Jiao *et al.* 2008)
$K_{ir}6.2$	Heart	yes	—	yes	Currents suppressed by association with caveolin-3, but not caveolin-1	(Garg *et al.* 2009*a*,*b*)
$Na_v1.5$	Heart	yes	—	yes	$G_{\alpha s}$-mediated recruitment of $Na_v1.5$-containing caveolae to surface membrane – increase in current density	(Reviewed by Balijepalli & Kamp, 2008)
ENaC	Cell lines	yes	—	yes	Caveolin-dependent ubiquitination and subsequent internalization	(Hill *et al.* 2007; Lee *et al.* 2009)
$Ca_v1.2$	Pancreatic β cells	yes	—	—		(Xia *et al.* 2004)
	Smooth muscle	yes	—	—		
	Heart	yes	yes	yes	Destruction of caveolae causes loss in β_2AR regulation (neonatal mice myocytes) or enhanced β_2AR regulation (adult rat myocytes)	(Balijepalli *et al.* 2006) (Calaghan & White, 2006)
HCN4	Sinus node	yes	—	yes	Cholesterol depletion causes positive shift in activation; reduces deactivation; loss of β_2AR regulation	(Barbuti *et al.* 2004, 2007)
					Disruption of caveolae by expression of dominant negative caveolin mutants shifts voltage dependence of activation to more negative potentials and increases time constant of activation	(Ye *et al.* 2008)
Connexin-43	Cell lines	yes	—	yes	Sucrose density gradients also suggest that Cx32, Cx36, and Cx46 are targeted to lipid rafts, while Cx26 and Cx50 are specifically excluded	(Schubert *et al.* 2002)
CFTR	Epithelial cells	yes	—	—	Raft association required for CFTR-dependent bacterial internalization and activation of innate immune response	(Kowalski & Pier, 2004)
CLIC4	HEK293 cell line	yes	—	—		(Suginta *et al.* 2001)

Table 1. Continued

Channel	Tissue	Biochemical isolation	Electron microscopy	Association caveolin	Functional effects/Comments	References
TRPC	Submandibular gland cells Smooth muscle Platelets	yes	—	yes	Cholesterol depletion inhibits TRPC1-store operated Ca^{2+} signals	(Kwiatek *et al.* 2006; Pani *et al.* 2009; Sundivakkam *et al.* 2009)
$P2X_1$	Platelets Vascular smooth muscle	yes	—	—	Cholesterol depletion inhibits $P2X_1$-mediated currents and artery contraction	(Reviewed by Garcia-Marcos *et al.* 2009)
$P2X_3$	Neurones	yes	—	—		
$P2X_4$	Epithelial cells	yes	—	—		
$P2X_7$	Submandibular gland cells	yes	—	yes	Cholesterol depletion inhibits $P2X_7$-mediated lipid signalling	

labelling of sheets of plasma membrane ripped from the surface of cells directly onto electron microscopy grids (Prior *et al.* 2003). In this plane, caveolae appear as circular, donut-shaped structures that label for caveolin. This method not only allows visualization of the plasma membrane in two dimensions over relatively large areas, but also permits quantitative analysis of clustering through the distribution of gold particles. This has been used to assess the association of the pore-forming subunit of the vascular ATP-sensitive K^+ (K_{ATP}) channel, Kir6.1, with aortic caveolae (Sampson *et al.* 2007).

Functional approaches. These approaches usually involve the destruction of cholesterol-enriched microdomains either through the depletion of cellular cholesterol by agents such as methyl-β-cyclodextrin, or, in the case of caveolae, by use of small interfering RNA (siRNA) to knock down caveolin isoforms. This has been a useful technique to assess potential roles of rafts and/or cholesterol and caveolins in channel modulation, although methyl-β-cyclodextrin has been reported to have potentially important general effects on membrane and cell function (see, for example, Rodal *et al.* 1999; Kwik *et al.* 2003).

The hydrolysis of sphingomyelin, another important component of membrane rafts, has also been used to alter functional raft properties. Hydrolysis of sphingomyelin by the action of sphingomyelinase generates ceramide, which self-associates to form small ceramide-enriched microdomains that then merge into large ceramide-enriched platforms (Bollinger *et al.* 2005). This change in lipid (and probably protein) organization within the membrane upon addition of exogenous sphingomyelinase can be used to assess the functional role of sphingolipids/ceramides (Bock *et al.* 2003). Sphingomyelinase is also naturally activated by a number of pro-apoptotic stimuli suggesting this membrane reorganization may represent a general mechanism in signal transduction.

Regulation of channel function by lipid microdomains

Modulation by lipids. Ion channels are both directly and indirectly sensitive to the lipid composition of the membrane in which they are embedded. Caveolar and non-caveolar rafts form tightly packed aggregates of cholesterol and sphingolipids, chiefly sphingomyelin and glycosphingolipids, and this lipid composition influences physical characteristics of the membrane such as thickness, curvature and the ability to bend and compress. Every time a channel protein undergoes a conformational change it causes a local disturbance in the surrounding bilayer. The overall energetic cost of a channel transition will thus include not only the intrinsic channel activation energy, but also energy associated with membrane deformation (reviewed by Andersen & Koeppe, 2007). The energetics and kinetics of channel gating, for example, will therefore be modulated by the local lipid environment and this may account for some of the changes in kinetic behaviour seen upon altering cholesterol levels in the membrane (see Table 1).

In some cases, there is evidence for direct modulation of channel activity through interaction with surrounding lipids. The optical isomer of cholesterol, epicholesterol, which essentially mimics the effects of cholesterol on general membrane properties, is unable to substitute for cholesterol in its modulatory effects on inwardly rectifying K^+ channels (Romanenko *et al.* 2002). This suggests that cholesterol exerts direct effects on K^+ channel activity and indeed recent studies have identified specific residues in the cytosolic C-terminal domain of Kir2.1 that confer cholesterol sensitivity (Epshtein *et al.* 2009). Evidence also suggests that specific interaction between channel proteins and sphingolipids may regulate voltage

sensing and/or channel gating. Treatment of cells with sphingomyelinase, an enzyme that cleaves the positively charged choline group from sphingomyelin, results in a pronounced negative shift in $K_v2.1$ activation and has led to the suggestion that key channel residues may interact with lipid head groups to modify channel activity (Ramu *et al.* 2006).

As discussed above, removal of the choline headgroup from sphingomyelin by the action of sphingomyelinase generates ceramide, which tends to aggregate into large ceramide-enriched membrane domains (Bollinger *et al.* 2005). Association of $K_v1.3$ with ceramide-enriched domains in lymphocytes inhibits whole-cell current amplitude, although whether this effect is due to changes in the lipid environment *per se* is unclear (Bock *et al.* 2003).

A discussion of lipid modulation of channel function would not be complete without mention of the best known lipid modulator, phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) (Suh & Hille, 2008). The compartmentalization of $PI(4,5)P_2$ in membrane rafts is controversial, although recent data using a freeze fracture electron microscopic method suggest it is highly concentrated in the rim of caveolae in cultured fibroblasts and mouse smooth muscle (Fujita *et al.* 2009).

Modulation by association: macromolecular signalling complexes. The compartmentation of ion channels with proteins that regulate channel function will clearly have an impact upon channel behaviour. A wealth of signalling proteins aside from ion channels have been shown to accumulate in rafts, including a number of G-protein coupled receptors, various classes of G protein, adenylyl cyclase, protein kinase C, nitric oxide synthase, tyrosine kinases, H-ras and mitogen-activated protein kinase (reviewed by Patel *et al.* 2008). Protein recruitment to lipid microdomains may result from specific targeting signals, such as palmitoylation and myristoylation (Zacharias *et al.* 2002), or from specific transmembrane domain residues (Munro, 1995). Additionally, targeting may be through association with caveolae-associated scaffolding proteins, like caveolin or membrane-associated guanylate kinase (MAGUK) proteins such as postsynaptic density protein-95 (PSD-95) and synapse-associated protein-97 (SAP97), which have been suggested to be important in the localization of $K_V1.4$ and $K_V1.5$, respectively, to membrane rafts (Folco *et al.* 2004; Wong & Schlichter, 2004).

In the heart, activation of L-type Ca^{2+} channels by the stimulation of β-adrenergic receptors and activation of the cAMP/PKA signalling pathway is a well described response to sympathetic activation. Immunoprecipitation studies in mouse ventricular myocytes have identified a macromolecular signalling complex comprising caveolin-3, $Ca_v1.2$ L-type channels and a number of signalling molecules in the β_2-adrenergic pathway (Balijepalli *et al.* 2006). Destruction of caveolar compartments by methyl-β-cyclodextrin-induced cholesterol depletion or siRNA for caveolin-3 perturbs the response of $Ca_v2.1$ to β_2-adrenergic stimulation (Balijepalli *et al.* 2006; Calaghan & White, 2006). A similar form of cAMP signalling compartmentation is seen in vascular smooth muscle where the PKA-dependent regulation of vascular K_{ATP} channels depends upon the integrity of smooth muscle caveolae containing K_{ATP} channel subunits and adenylyl cyclase (Sampson *et al.* 2004).

There is also good evidence in endothelial and smooth muscle cells that caveolae act as integration sites for Ca^{2+} signalling due to their ability to aggregate proteins involved in Ca^{2+} regulation and excitation–contraction coupling (Bergdahl & Sward, 2004). In smooth muscle cells, caveolae are in particularly close association with the peripheral sarcoplasmic reticulum (SR) and appear to be the major site of calcium entry following store depletion (Shaw *et al.* 2006). Depletion of cholesterol has been shown to inhibit TRPC1-related store operated Ca^{2+} entry in smooth muscle and submandibular gland cells. Recent studies suggest a critical role for caveolin-1 in not only retaining TRPC1 channels at distinct junction sites between the plasma membrane and the peripheral SR where it can mediate store-operated calcium entry, but also in regulating TRPC1 activity (Kwiatek *et al.* 2006; Pani *et al.* 2009; Sundivakkam *et al.* 2009).

Aside from roles in caveolae formation and stability, caveolins have been shown to interact with many caveolae-localized signalling molecules (Razani *et al.* 2002; Patel *et al.* 2008). Each caveolin molecule has an unusual hairpin-like topology with a central hydrophobic core embedded in the membrane and the hydrophilic N and C termini free in the cytosol (Fig. 1). The N terminus contains an oligomerization domain that allows interaction with other caveolin molecules and an overlapping region known as the caveolin scaffolding domain (CSD) that is responsible for association with other signalling molecules. The CSD is known to bind to specific sequences on target proteins (ΦXΦXXXXΦ, ΦXXXXΦXXΦ and ΦXΦXXXXΦXXΦ where Φ is an aromatic amino acid (tryptophan, phenylalanine or tyrosine) and X is any amino acid; Couet *et al.* 1997). Interaction with caveolins has been shown to directly modulate the activity of a number of ion channels. Interestingly, the activity of the cardiac K_{ATP} channel (Kir6.2/SUR2A) is suppressed by interaction with caveolin-3, but not caveolin-1, suggesting some specificity in regulation (Garg *et al.* 2009*a*,*b*). Caveolin-3 has also recently been shown to regulate the current density and activation/inactivation kinetics of the HCN4 channel (Ye *et al.* 2008).

The suppressive effects of interaction with caveolin can in some cases be explained by caveolin-regulated

changes in surface expression. Jiao *et al.* demonstrated that activation of protein kinase C facilitates caveolin-1-dependent internalization of vascular K_{ATP} channels (Jiao *et al.* 2008). Association with caveolin-1 has also been shown to negatively regulate ENaC activity by promoting ubiquitination of the channel and subsequent internalization (Lee *et al.* 2009). In cell lines, caveolin-1 has been suggested to play a dual role in regulating surface targeting of the large conductance Ca^{2+}-activated (BK) channel α subunit, Slo1, by maintaining channels in intracellular compartments and/or anchoring channels in the membrane (Alioua *et al.* 2008). In the heart, β-adrenergic stimulation of $Na_v1.5$ activity, which is responsible for the initial upstroke of the cardiac action potential, occurs by both PKA-dependent phosphorylation of channel subunits and by direct PKA-independent $G_{\alpha s}$ modulation of current density. Recent studies indicate that the increase in $Na_v1.5$ current density results from direct interaction between $G_{s\alpha}$ and caveolin-3, which promotes recruitment of $Na_v1.5$-containing caveolae to the surface membrane (reviewed by Balijepalli & Kamp, 2008).

Considerations of an enclosed extracellular compartment. Finally it is worth noting, particularly in the context of ion channel function that the flask-shaped pit formed by a caveola often has a small, restricted opening. This would potentially allow for the build up of ions within the caveolar 'pit' (caveolae have a diameter of 50–100 nm) to concentrations far in excess of what would be experienced in the bulk extracellular fluid. Interestingly, a very recent study by Alday *et al.* (2010) suggests that a subpopulation of cardiac K_v channels may avoid this problem by localizing predominantly to the rim of caveolae. Implications linked to the geometry of caveolae have not been fully investigated but may add an additional layer of complexity when considering the activity of channels localized to these specialized membrane compartments.

Concluding remarks

The idea that the lipid bilayer acts only as an inert solvent for membrane proteins is now superseded by the idea that membrane lipids play an integral part in the regulation of channel function. Regulation of channel activity by $PI(4,5)P_2$ is well characterized, but evidence suggests that raft-associated lipids such as cholesterol and sphingolipids and the lipid domains themselves also directly and/or indirectly regulate channel activity. Much of the information we have so far represents only static snapshots of channel interaction with microdomains, where *in vivo* it is likely that these associations are dynamic and regulated. It may take the development of additional non-invasive imaging technologies before the full dynamic complexity of these regions and their regulatory interactions are fully appreciated.

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