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REVIEW ARTICLE

The effect of membrane domains on the G protein–phospholipase Cβ signaling pathway

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Abstract

The plasma membrane serves as a barrier to limit the exit and entry of components into and out of the cell, offering protection from the external environment. Communication between the cell and the external environment is mediated by multiple signaling pathways. While the plasma membrane was historically viewed as a lipid bilayer with freely diffusing proteins, the last decade has shown that the lipids and proteins in the plasma membrane are organized in a non-random manner, and that this organization can direct and modify various signaling pathways in the cell. In this review, we qualitatively discuss the ways that membrane domains can affect cell signaling. We then focus on how membrane domains can affect a specific signaling pathway – the G protein–phospholipase Cβ pathway and show how membrane domains can play an active role in directing or redirecting G protein signals.

Keywords: *lipid signaling; lipid rafts; caveolae; G protein coupled receptors; calcium signaling*

What are membrane domains?

The definition of membrane domains is fuzzy at best. It can range from organized layers of annular lipids around a monomeric or multimeric protein, to aggregates of ordered phase lipids or large, organized protein domains. In this review, our working definition is that membrane domains are complexes of integral and peripheral proteins with varied and specific compositions, and that the different domains serve specific cellular functions. The composition of membrane domains is presumed to vary widely depending on the individual cell, its proximity to adhesion sites and cytoskeletal elements, the point of cell cycle, and other dynamic processes such as endocytosis and delivery of vesicular cargo from endosomes. Modes of formation and control of domain composition is an interesting question and out of the scope of this review.

For this review, we will only consider membrane domains that contain proteins and not those comprised entirely of lipids and other non-protein components. Membrane domains lacking proteins (lipid domains) have been well characterized in model systems, but their presence and dynamics in cell membranes is still controversial (for a review see Edinin, 2003). Lipid domains are defined as aggregates of ordered phase lipids that are phase-separated from the surrounding fluid phase lipids (Figure 1 and Pierini and Maxfield, 2001). Lipids that have a tendency to be in ordered phases at physiological temperatures are those with saturated chains, such as sphingolipids which are prevalent in

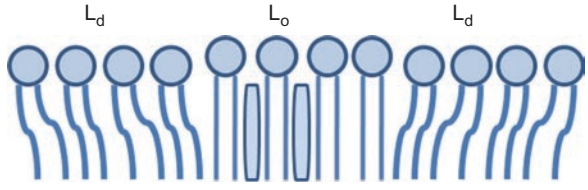

Figure 1. Depiction of a single leaflet of a lipid bilayer with lipids in the liquid-disordered (L_d) phase and a liquid-ordered (L_o) phase. The liquid-ordered phase is seen as lipids with aligned chains that are promoted by saturated or trans-unsaturated hydrocarbon chains. Stabilizing the aligned chains are steroid molecules (rectangular objects) that can intercalate between the hydrocarbon chains.

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rafts. Steroids such as cholesterol, which have rigid, hydrophobic rings, tend to stiffen the neighboring lipid chains and participate in domain formation (Figure 1).

While lipid domains are well-established in model systems, they are difficult to detect in living cells. Additionally, the amount of one of the key components used to form rafts in model membranes (i.e. sphingolipids) may not be high enough in the inner leaflet of the plasma membrane for domains to form (Wang and Silvius, 2001; Munro, 2003; Silvius, 2003). However, it is entirely possible that the lipid/steroid composition required for the formation of rafts in model system is not applicable in cells, and that composition of non-protein membrane domains differs. Most likely, transient lipid and non-protein aggregates form, dissolve and reform depending on the dynamics of the local environment. These transient domains may then organize larger protein complexes.

It is unclear whether aggregates of lipids can immerse specific membrane proteins, or whether integral proteins are responsible for organizing their surrounding the lipids/steroids. It is possible that some lipids that make stable van der Waals contacts with the hydrophobic portion of a specific set of integral proteins (Figure 2) and/or make energetic ionic or hydrogen bonds with the surface amino acid sides (e.g. Soubias *et al.*, 2006), allowing an annular lipid layer to form around the protein that may extend into the surrounding membrane. Such extended interactions are unlikely. On the other hand, certain proteins are more likely to partition into the more ordered lipid domains and others prefer more fluid environment.

Lipid domains will certainly affect signals from the contained lipids and their associated pathways by sequestering the signaling lipid. These signals would either be enhanced or attenuated depending on the ability of the processing protein to partition into the domain and access the lipid. Lipid domains may also be instrumental in organizing peripheral membrane proteins that are post-synthetically modified with hydrocarbon chains (for overview see Magee, 1990). The nature of the lipid modification will determine whether a protein is contained or excluded from the domain. Since lipid domains are aggregates of ordered phase lipids, proteins that have been modified with long, saturated hydrocarbon chains, such as palmitoyl, should tend to partition into the domain. This preferential partitioning would reduce the diffusion coefficient of the attached protein, affect local concentration of the protein within domain, and affect accessibility of potential binding partners. Additionally, binding partners modified with saturated acyl chains will experience a local crowding effect and increased encounter rate. These rates would depend on the rates of partitioning in and out of the domains, the diffusion coefficient and concentration. Alternately, proteins that are modified with unsaturated chains, such as prenyl groups, are expected to be excluded from lipid ordered domains. Thus, the association of a protein with a saturated modification would be expected to have a reduced association with a protein with an unsaturated chain due to differences in localization. Interestingly, the α and $\beta\gamma$ subunits of heterotrimeric G proteins have different types of modifications; Gα subunits can be reversibly modified with two saturated hydrocarbon chains while G$\beta\gamma$ subunits are irreversibly modified with unsaturated prenyl groups (e.g. Wedegaertner *et al.*, 1995; Moffett *et al.*, 2000), suggesting that domains would interfere with association of the subunits.

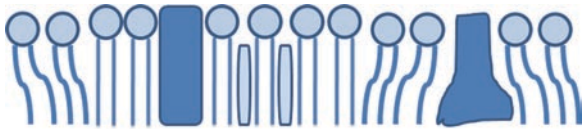

Figure 2. Depiction of a single leaflet of a bilayer containing proteins (black objects) that partition into either the L_d or L_o phases, showing how fluid-phase hydrocarbon chains can accommodate proteins with more irregular hydrophobic surfaces and ones that are shorter in length.

Unlike lipid domains, protein domains are less controversial because some are large enough to be detected by physical methods. The size and composition of protein domains range from small protein oligomers to large protein complexes. Additionally, the dynamics of association and dissociation of proteins in and out of the domains is also expected to vary considerably. These different environments can be assessed by diffusion measurements. Because the diffusion coefficient depends on the hydrodynamic radius of the molecule, larger protein complexes should diffuse significantly slower than free monomeric proteins. Experimental results show that some proteins diffuse rapidly with diffusion coefficient similar to lipids, while others diffuse very slowly, and their mobility is out of the detection range of most available methods, such as fluorescence recovery after photobleaching and fluorescence correlation spectroscopy (see Lakowicz, 1999). However, many membrane proteins show complex diffusion behavior suggesting that these proteins experience multiple environments that promote variable mobility: ranging from free diffusion to immobile aggregates (Kenworthy *et al.*, 2004; Lenne *et al.*, 2006; Suzuki *et al.*, 2007; Day and Kenworthy, 2009b). These observations suggest that a population of these membrane proteins might be localized in quasi-stable aggregates with other proteins and possibility also with lipid domains. It is possible that proteins sample different environments and are constantly exchanging between different domains. For peripheral proteins that partition into lipid ordered domains, it is possible that the "corralled" protein can diffuse freely within the domains, showing a free diffusion using small timescale methods but limited diffusion when

longer timescales are monitored (Figure 3). Presently, because the timescale and distribution of many different membrane protein environments exist, it is impossible to formulate general predictions about the mobility of most membrane proteins. With the recent advances in methods to deconvolve the distribution of the diffusive population of membrane proteins, this might be possible in the near future.

The most prominent and stable membrane domains are caveolae. Caveolae are protein domains found in many mammalian cell lines and appear as flask-shaped invaginations by electron microscopy (Figure 4, for reviews see Anderson, 1998; Schlegel *et al.*, 1998; Anderson and Jacobson, 2002; Liu *et al.*, 2002). Functionally, caveolae are thought to be involved in clarthin-independent internalization and recycling of specific membrane proteins, and in organization of signaling proteins (discussed below). Caveolae are formed from caveolin-1, although in muscle cells, caveolin-3 replaces caveolin-1 as the structural element of these domains. Caveolae are rich in cholesterol (Pike *et al.*, 2002) and may participate in cholesterol homeostasis. Additionally, it has been proposed that certain lipids and/or cholesterol may be required for cholesterol entry (Anderson and Jacobson, 2002). Caveolin-1 is anchored to the membrane surface by three palmitoyl groups, and also contains a scaffold domain that can potentially interact with specific proteins.

Signaling proteins can localize to caveolae through binding to the scaffolding domain of caveolin-1 and this localization can be further stabilized if the protein is modified with saturated hydrocarbon chains, since caveolae are expected to entrap ordered lipids. Proteins that are modified with unsaturated hydrocarbon chairs are not expected to localize in caveolae domains (McCabe and Berthiaume, 2001). It is possible that functionally related proteins have different types of modifications and their probable localization to caveolae may differ. As discussed below, the components in the G protein signaling system are a good example of this scenario.

Some mammalian cells are rich in caveolae domains whereas caveolae are absent in other cell types. This suggests that caveolae are expected to play a role in modifying, but not directing cellular events. We discuss below how their presence impacts the G protein–phospholipase Cβ (PLCβ) signaling system.

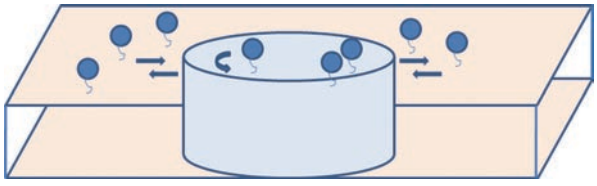

Figure 3. Diffusion of proteins that are anchored to the membrane by a hydrocarbon chain modification outside and within a membrane domain. Corralling or fencing of the proteins may not affect the rate of diffusion compared to the rate outside the domain when measured using methods that do not detect long range mobility. When the mobility of a longer distance is measured, then the rate of diffusion will depend on the rates of partitioning in and out of the domain.

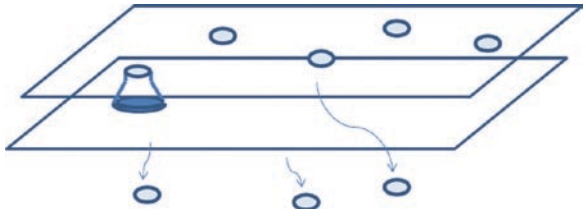

Figure 4. Caveolae domains are seen as flask-shaped invaginations on the plasma membrane surface, and are responsible for non-clathrin mediated endocytosis.

The PLCβ–G protein signaling system

The G protein–PLCβ signaling pathway is initiated when an extracellular agonist binds to its specific G protein coupled receptor (GPCR). GPCRs are seven transmembrane receptors and comprise the largest family of receptors in mammalian cells (Pierce *et al.*, 2002). Agonist binding to the receptor induces a conformational change in the receptor that allows it to catalyze the exchange of GDP for GTP on the α subunit of a heterotrimeric G-protein (Figure 5). Heterotrimeric G-proteins consist

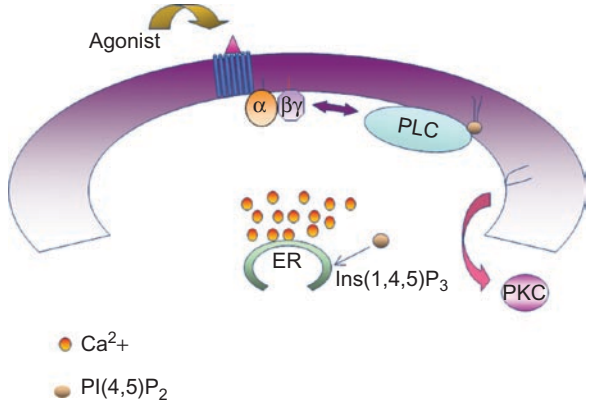

Figure 5. The PLCβ–G protein signaling pathway showing how the binding of an extracellular to a transmembrane GPCR will activate a G protein heterotrimer whose Gα or Gβγ subunits can bind to and activate PLCβ. Activated PLCβ will hydrolyze $PI(4,5)P_2$, releasing $Ins(1,4,5)P_3$ into the cytosol, where it diffuses to the endoplasmic reticulum (ER) causing the release of calcium ions into the cytosol. The lipid portion from $PI(4,5)P_2$ hydrolysis, diacylglyercol, activates protein kinase C (PKC).

of an α subunit that contains the guanine nucleotide binding site and a non-dissociable βγ dimer. Gα subunits have been divided into four subfamilies: $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_q$, and $G\alpha_{12}$; each family is coupled to specific GPCRs and effectors (for reviews see Aasheim *et al.*, 1997; Hildebrandt, 1997; Birnbaumer, 2007). This coupling defines the pathway a signal will follow, although some cross-over between pathways appears to occur. Activated or GTP-bound Gα subunits have a greatly reduced affinity for Gβγ subunits (see Glaser *et al.*, 1996; Runnels and Scarlata, 1999), which allows Gα and Gβγ to interact with a select group of intracellular effector proteins and change their catalytic activity. Gα subunits have an intrinsic GTPase activity and hydrolysis of GTP to GDP promotes its reassociation to Gβγ subunits and cessation of the signal. Additionally, there are proteins called "GAPs" or GTPase-activating proteins that can inactivate Gα subunits by binding to Gα(GTP) and enhancing its GTPase activity. GAPs allow rapid shut-off of the signal (see Berman and Gilman, 1998).

PLCβ enzymes are the main effectors of the $G\alpha_q$ family of G proteins. The $G\alpha_q$ family transduces signals from agents such as angiotensin II, catecholamines, endothelin 1 and prostaglandin $F_{2.}$ Activation of $G\alpha_q$ results in activation of PLCβ. PLC enzymes catalyze the hydrolysis of the signaling lipid phosphatidyinositol 4,5 bisphosphate ($PI(4,5)P_2$) to generate the second messengers, diacylglycerol and 1,4,5 inositol trisphosphate ($Ins(1,4,5)\ P_3$), which activate protein kinase C (PKC) and cause the release of Ca^{2+} from intracellular stores, respectively (see (Rebecchi and Pentylana, 2000; Suh *et al.*, 2008) and Figure 5). There are four isoforms of PLCβ (PLCβ1-4) that are all strongly activated by $G\alpha_q$. Additionally, PLCβ2, and to a lesser extent PLCβ3, can be activated by Gβγ dimers. Thus, agonists coupled to other Gα family members can activate PLCβ through released Gβγ subunits.

It is important to note that there is redundancy in some G protein signaling pathways. Many receptors are coupled to multiple Gα families and the selection of one pathway over another is sometimes unclear (see Kroeze *et al.*, 2003). Also, GPCRs can form homo- and heterodimers and potentially higher order structures (Angers *et al.*, 2002). These dimers have the potential of coupling to different and/or multiple Gα families. Adding to this complexity is the promiscuity of Gβγ subunits. While activation of effectors by a specific Gα subunit is highly selective, effector activation by Gβγ subunits is highly promiscuous; almost all types of Gβγ can activate a Gβγ effector (see Myung *et al.*, 2006). Since Gβγ subunits have the potential to be released by any type of Gα, any signal through a GPCR can activate any Gβγ effector. Taking into account the many ways in which a G protein signal can be delocalized, it is unclear how unique signals can be selected. While much is known about the nature of the potential pathways a particular signal may follow, the actual pathway of a signal will depend on the local concentrations of G proteins, the affinity for the particular partner, and the rates of activation and deactivation.

PLCβ signaling in cells – evidence for preformed complexes

For PLC to be activated and metabolize $PI(4,5)P_2$, several events must occur. An extracellular agonist must bind to its specific GPRC and generate a conformational change in the receptor that alters its interaction with Gα. This change in interaction allows for GDP/GTP exchange on Gα which changes its association with Gβγ, allowing exposure of protein regions that result in activation of PLCβ.

The interactions involved in the GPCR–$G\alpha_q$Gβγ–PLCβ pathway could occur for proteins and lipids that are freely diffusing, or for components that are completely localized in a specific domain. Indirect evidence that members of the G protein–PLCβ signaling pathway are localized in domains first appeared in *in vitro* studies. Ross and coworkers used kinetic methods to show that the $G\alpha_q$ activation cycle is so fast it must remain bound to receptor (Berstein *et al.*, 1992). Using a series of purified proteins, we have found that in addition to the high affinity $G\alpha_q$(GTP) binding site on PLCβ, PLCβ also contains binding sites for $G\alpha_q$(GDP), Gβγ and RGS4 that have affinities which are only 20–100-fold lower than that of the primary site (Dowal *et al.*, 2001). These secondary sites would be expected to promote self-scaffolding of the proteins into signaling complexes. Additionally, these secondary sites appear in other proteins. $G\alpha_i$(GDP) has a high affinity binding site for Gβγ and also a lower affinity site that can bind a second Gβγ (Wang *et al.*, 2009b). In the activated state, $G\alpha_i$ is still capable of binding Gβγ both without and with bound PLCβ (Wang *et al.*, 2009a). There is also evidence that GPCRs complex with G protein heterotrimers in the deactivated state, for example, the bradykinin receptor is associated with $G\alpha_q$Gβγ in resting cells (Philip *et al.*, 2007). Taken together, *in vitro* studies support the idea that receptor–G protein complexes can also be associated with PLCβ.

There is also evidence that components of the PLCβ–$G\alpha_q$ signaling pathway are organized into preformed signaling complexes in cells consisting of receptor–G protein and PLCβ. We have found that PLCβ is complexed with $G\alpha_q$ on the plasma membrane of PC12 and HEK293 cells in the basal state and stimulation does not appear to affect the degree of association (Dowal *et al.*, 2006). Additionally, the amount of $G\alpha_q$–PLCβ complex on the plasma membrane surface remains constant without translocation of cytosolic PLCβ to the membrane surface. A lack of translocation is surprising since the cellular concentration of $G\alpha_q$ exceeds PLCβ and the affinity between

$G\alpha_q$ and PLCβ increase 40-fold upon activation (Runnels and Scarlata, 1999). In contrast, using fast optical kinetic measurements, Hille's group found an increase in FRET between PLCβ and $G\alpha_q$ with stimulation, suggesting an increase in association or a conformational change between the associated proteins (Jensen *et al.*, 2009). These fast events might be missed using steady state FRET measurements. Another possible explanation is that under certain environmental conditions G proteins and PLCβ dissociate with stimulation.

Along with measurements of $G\alpha_q$–PLCβ association in cells, studies of G protein dissociation have been carried out, but have given varied results. Devreotes and coworkers used FRET methods to show that $G\alpha_i G\beta\gamma$ in COS cells dissociate upon stimulation (Janetopoulos *et al.*, 2001). In contrast, Lohse and colleagues also used FRET-based methods to show that $G\alpha_q$ and Gβγ remain bound throughout the activation cycle (Bunemann *et al.*, 2003; Frank *et al.*, 2005). Both Berlot and Lambert found that the ability of G protein heterotrimers to dissociate depends on the G protein family and that $G\alpha_q$ and $G\alpha_i$ were much less likely to dissociate than $G\alpha_s$ (Hughes *et al.*, 2001; Hynes *et al.*, 2004a; 2004b; Digby *et al.*, 2006; Hein *et al.*, 2006). The extent of association between G proteins and receptors is also unclear. $G\alpha_s$ appears to internalize with activation of the β-adrenergic receptor (Hynes *et al.*, 2004b) although this is not the general case for G proteins. Stimulation of the bradykinin type II receptor causes detachment of the receptor from its bound G proteins, leaving $G\alpha_q$ and Gβγ on the plasma membrane (Philip *et al.*, 2007). As detailed below, we have found that the propensity of G protein subunits to dissociate depends on whether the complex is incorporated into a higher order membrane signaling domains. Thus, although PLCβ may exist in preformed signaling complexes consisting of a GPCR dimer–$G\alpha_q G\beta\gamma$–PLCβ in the basal state, the extent of complex dissociation upon stimulation may depend on the nature of the proteins and the local environment.

Does incorporation in membrane domains affect PLCβ-G protein signaling?

Based on the discussions above, it is quite likely that membrane domains either directly or indirectly affect PLCβ signaling. The presence of lipid domains and caveolae appear to alter the diffusion of several types of GPCRs (for review see Day and Kenworthy, 2009a). However, the effect of domains on diffusion has been assessed by cholesterol depletion which dissolves both lipid and caveolae domains, although cholesterol effects appear to vary depending on the method used for depletion.

Caveolae have been found to have a profound effect on $G\alpha_q$–PLCβ signaling properties, as seen in studies using Fisher rat thyroid (FRT) cells. These cells lack caveolin-1 and do not exhibit caveolae domains. FRT cells can be stably transfected with caveolin-1 and give structures on the plasma membrane that have the same morphology as caveolae domains; this cell line has been used extensively for caveoale studies (Chung *et al.*, 1995; Lipardi *et al.*, 1998; Mora *et al.*, 1999; Kim *et al.*, 2002). Using this model system, the association between $G\alpha_q$ and Gβγ has been studied using FRET. In wild-type FRT cells, $G\alpha_q$ and Gβγ remain associated, while in FRTcav+ cells $G\alpha_q$ and Gβγ separate with carbachol stimulation (Sengupta *et al.*, 2008). Thus, caveolae appear to directly affect the interaction between these G protein subunits.

Murthy and Makhlouf (2000) measured the relative strength of association of different Gα families and caveolae from cell extracts. They found that only the $G\alpha_q$ family could specifically bind to caveolin-1. Binding was only observed when $G\alpha_q$ was in the activated state. These observations led the authors to propose that activation of $G\alpha_q$ causes it to move into caveolae domains while other G protein types remain outside the domains. These observations can be compared with studies of Oh and Schnitzer (2001), who observed $G\alpha_q$ but not $G\alpha_i$ or $G\alpha_s$ in caveolae domains, and our lab, which observed a high degree of colocalization between caveolin-1 and $G\alpha_q$ in both unstimated and stimulated FRTcav+ (Sengupta *et al.*, 2008).

Since caveolin-1 has a high affinity for $G\alpha_q$ in the basal state that increases greatly with stimulation, then what happens to their associated Gβγ subunits upon activation? FRET measurements suggest that $G\alpha_q$ and Gβγ separate in the presence of caveolae (Sengupta *et al.*, 2008). It is therefore possible that Gβγ subunits are released from caveolae upon stimulation (Figure 6). Support for this idea comes from the large increase in mobility of Gβγ subunits upon stimulation of FRTcav+ cells. Thus, the strong

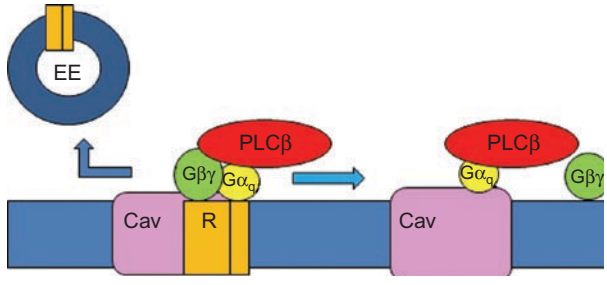

Figure 6. Changes in caveolae–$G\alpha_q$ interactions with stimulation. In the resting state, the receptor (R)–$G\alpha_q G\beta\gamma$ heterotrimer–PLCβ complex resides in caveolae (Cav) domains. Upon stimulation, the receptors move into early endosomes (EE) and strong interactions between $G\alpha_q$ and Gβγ are diminished. This loss in affinity between the G protein subunits allows activated $G\alpha_q$ to strongly interact with caveolin-1, stabilizing both its localization and its activated state. Since Gβγ does not interact with caveolin proteins, the loss of $G\alpha_q$ and receptor interactions destabilizes its caveolae localization resulting in its release from caveolae.

interactions between $G\alpha_q$ and $G\beta\gamma$ in the deactivated state are transferred to $G\alpha_q$ and caveolin-1 in the activated state. It is notable that Oh and Schnitzer (2001), whose studies suggested that $G\alpha_q$ localizes in caveolae domains but $G\beta\gamma$ does not, postulated that the $G\alpha_q$ contained in caveolae may not be associated to $G\beta\gamma$. Most likely, the reason for this discrepancy is that during cell disruption $G\beta\gamma$ becomes released from caveolae domains.

The effects of caveolae on PLCβ function

The separation of $G\alpha_q$ from $G\beta\gamma$ due to caveolin-1 has functional effects on downstream signals. In general, deactivation of $G\alpha_q$ subunits is dependent on its GTPase activity, which can be promoted by GAPs, such as RGS4 (regulators of G protein signaling) that return $G\alpha_q$ to its GDP-bound state (Berman and Gilman, 1998; Berman *et al.*, 1996). We postulate that caveolin-1 inhibits the intrinsic GTPase activity of $G\alpha_q$, although this has not yet been verified experimentally. Thus, caveolae may serve the opposite function as RGS proteins by stabilizing the activated state of $G\alpha_q$. It is also possible that caveolin-1 occludes the $G\alpha_q$ binding site for $G\beta\gamma$, RGS and other deactivating proteins.

Stabilization of activated $G\alpha_q$ would prolong activation of its effectors, such as PLCβ. Using intracellular Ca^{2+} release as an indicator of PLCβ activity, we have observed a prolonged Ca^{2+} response in FRTcav+ cells with $G\alpha_q$ stimulation as compared to the rapid response seen in FRTwt cells (Figure 7 and Sengupta *et al.*, 2008). This result correlates well with enhanced $G\alpha_q$ activation by caveolin-1.

Caveolae could also impact $G\beta\gamma$ signaling. Release of $G\beta\gamma$ from caveolae domains would attenuate activation of $G\beta\gamma$ effectors localized in the domains and activation of effectors that are not localized in the domains. $G\beta\gamma$ signals would also be highly delocalized when compared to $G\beta\gamma$ signals on membranes that do not contain caveolae where the heterotrimer may remain associated. We have found that the sustained Ca^{2+} response seen in the presence of caveolae is more pronounced when monitored using PLCβ2, which is activated by both $G\alpha_q$ and $G\beta\gamma$, as opposed to PLCβ1, which is only activated by $G\alpha_q$.

As mentioned above, it is sometimes difficult to determine selectivity of G protein signals. There are several types of GPCRs that have the ability to activate multiple families of G proteins (e.g. the cannabinoid receptor type 1 (AT1) can activate $G\alpha_q$ as well as $G\alpha_{i/o}$). As mentioned, $G\beta\gamma$ subunits can potentially be released by the activation of any Gα family member. How is specificity achieved? One possibility is that receptors that are localized in caveolae have preferential access to $G\alpha_q$ as opposed to other Gα families that do not localize in caveolae. In this way, cells that contain caveolae have better signal selection. Excess $G\beta\gamma$ released from caveolae would diminish signaling from Gα subunits located outside caveolae domains as well as prompt activation of $G\beta\gamma$ effectors. Alternately, in cells that do not contain caveolae, $G\alpha_q$ may remain complexed to $G\beta\gamma$ subunits with stimulation thereby only allowing activation of $G\beta\gamma$ effectors that are already bound to the heterotrimer. Thus, caveolae can enhance selectivity of Gα subunits but reduce selectivity of $G\beta\gamma$ effectors (Figure 8).

PLCβ has been found to partition equally into raft and non-raft domains. While preferential partitioning to caveolae has not yet been determined, PLCβ is likely to be bound to $G\beta\gamma$ subunits which reside in caveolae domains. Membrane localization of a second effector of $G\alpha_q$ (Ballou *et al.*, 2003; 2006; Lu *et al.*, 2005), phosphadtylinositol 3-kinase (PI3K), has not yet been determined. PI3K is predominantly cytosolic but translocates to the plasma membrane upon stimulation and its distribution in the plasma membrane is punctuated, suggesting preferential association with specific structures. PI3K is

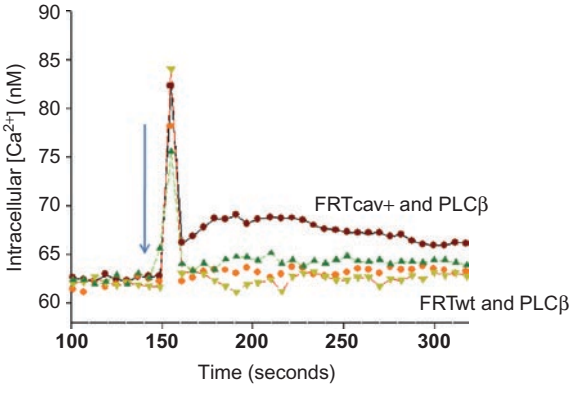

Figure 7. Release of Ca^{2+} in FRTwt and FRTcav+ cells with stimulation by acetylcholine. The timescale of release and recovery are similar in both cell lines, unless PLCβ is overexpressed, in which case a sustained, elevated Ca^{2+} is observed. Since PLCβ is expressed at lower amounts than $G\alpha_q$, additional PLCβ is required to generate enough Ca^{2+} to detect increased amounts of activated $G\alpha_q$ due to its stabilization by caveolin-1 (figure adapted from Sengupta *et al.*, 2008).

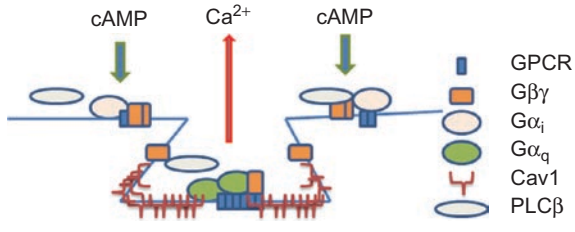

Figure 8. The manner in which caveolae could allow for specific signaling for receptors that are coupled to both $G\alpha_q$, which activates PLCβ, generating increased intracellular Ca^{2+}, and $G\alpha_i$, which inhibits adenylyl cyclase (not shown), decreasing the cellular level of cAMP. These differences are also impacted by the release of $G\beta\gamma$ from caveolae domains following $G\alpha_q$ activation, thereby increasing the response of $G\beta\gamma$-coupled effectors.

a family of enzymes that plays a key role in the trafficking of intracellular proteins (for review see Katso *et al.*, 2001). PI3K uses the same substrate as PLCβ2, PI(4,5) P_2. Activation of $G\alpha_q$ enhances PLCβ but attenuates the activity of PI3K, presumably to allow for more PLCβ substrate. In cells, we find a degree of interaction between $G\alpha_q$ and PLCβ, and between $G\alpha_q$ and PI3K (Golebiewska and Scarlata, 2008). However, we could not detect any interaction between PLCβ and PI3K either in resting or stimulated cells, suggesting that separate pools of G protein and the two effectors, (i.e. $G\alpha_q$–PLCβ and $G\alpha_q$–PI3K) but larger complexes containing G proteins and the two effectors do not exist. These studies support the notion that the plasma membrane contains distinct and different membrane domains with specific functions.

What are the repercussions of confinement in membrane domains?

Since members of the PLCβ signaling system are localized in domains under many environmental conditions, can we delineate the role of domain localization and signaling? There are two extreme conditions. First, all of the members in the pathway can be freely diffusing in the membrane and on its surface. In this case, the rate of each activation step will depend on the diffusion constants of the species, their on-rates and the number of encounters. The signal would have the opportunity to affect multiple pathways and will be delocalized in the cell.

Suppose instead that the proteins are contained in a preformed signaling complex. Then the rate of the signal would be as fast as the conformational changes that accompany activation. In this case the signal would be completely confined to the protein composition and stoichiometry of the domain. Also, each signaling domain would have an independent function whose signals would affect other components in the cell by the small, diffusible second messengers generated (e.g. cAMP, Ins(1,4,5) P_3). Potentially, it should be possible to argue whether or not members of the G protein–PLCβ signaling pathway are localized in domains using a kinetic argument by monitoring the rate of Ins(1,4,5)P_3 production. This prediction would require knowing the endogenous concentrations of the proteins and their cellular distributions which is not yet possible. It would also require knowing their diffusion coefficients and the on- and off-rates for the protein–protein interactions, as well as for ligand binding, GDP/GTP exchange, and the rate of PI(4,5)P_2 catalysis. Additionally, the number, affinities, and types of competing proteins in the vicinity must be known. However, if the proteins are in a preformed complex, then analysis is greatly simplified. The concentrations of the proteins and competing proteins are immaterial since only the composition of the domain is important. The rate of partitioning out of the domain can be assumed to be much longer than the rate of partitioning into the domains and the proteins can be considered to be kinetically trapped in the domains. This trapping fits in well with very slow diffusion rates of GPCRs, $G\alpha_q$ and Gβγ in resting cells (e.g. Philip *et al.*, 2007; Sengupta *et al.*, 2008). The rate of PI(4,5)P_2 generation will only depend on the rate of ligand binding to the GPCR, the rate of GDP/GTP exchange on Gα, the rate of PLCβ activation and the rate of catalysis. In this way, the localization of proteins in preformed domains may provide a basis for analysis of signaling in more complex cellular environments.

Concluding statements and future directions

In some ways, membrane domains can be thought of as micro-organelles in cells whose function, while variable, can center on stabilizing and organizing proteins involved in lipid signaling, as well as processes such as endocytosis and trafficking. The composition of membrane domains may vary widely depending on the particular function of the domain. The potential functions of caveolae domains are expected to have a more narrow distribution of functions since only a subset of membrane proteins have caveolin binding motifs. Significant challenges will be in determining the distribution of these domains, the factors that control their individual protein composition, and the movement of proteins in and out of them.

Until recently, most of the data pertaining to the composition of membrane domains have been based on methods that involve cell fractionation. However, the results of these studies can vary with the temperature at which the procedure was carried out, the type of detergent used and other extraction conditions, since these will influence the degree of lipid domain solubilization. To complicate analysis, caveolae domains and other lipid domains are usually found in the same extraction fractions, complicating determination of protein localization. Moreover, since caveolae are involved in endocytosis, they can be found in internal pools, and cell fractionation cannot distinguish between the plasma membrane signaling and internal signaling platforms. However, advances in fluorescence imaging and protein labeling will allow studies in living and intact cells which will certainly lead to a better characterization of membrane domains.

We focused this review on $G\alpha_q$-coupled receptors that are found in caveolae, but it is possible that many are localized outside of these domains. The factors that regulate GPCR localization are unclear. Since GPCRs form homo- and hetero-dimers, it is possible that one of the subunits would predominate in localizing, and these preferences will most likely be uncovered in the near

future. It is probable that $G\alpha_q$ associates with receptors that are not in caveolae domains based on the much weaker affinity between unactivated $G\alpha_q$ and caveolin-1. The ability of $G\alpha_q$ to bind to target that has dimerized with a non-$G\alpha_q$ GPCR is additionally unclear.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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