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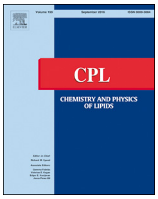

Review

Role of membrane shape in regulating the phosphatidylinositol cycle at contact sites

José Carlos Bozelli Jr., Richard M. Epand*

Department of Biochemistry and Biomedical Sciences, McMaster University, Health Sciences Centre, Hamilton, Ontario, L8S 4K1, Canada

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ABSTRACT

The extensive number of metabolic processes regulated by phosphatidylinositol (PI) phospholipids highlights their physiological importance. The major metabolic pathway for their biosynthesis in cells is the PI-cycle. Contrary to most metabolic cycles, reactions of the PI-cycle occur in two different locations; those are, the plasma membrane (PM) and endoplasmic reticulum (ER). Lipid movement between the two organelles is, therefore, a requirement of the cyclical process. Moreover, in mammals the PI-cycle yield PI molecular species enriched in specific acyl chains, namely 1-stearoyl-2-arachidonoyl acyl chains. Hence, to ensure cycle efficiency and specificity it should take place in specialized regions of PM and ER rather than being randomly distributed among those membranes. Along these lines, ER-PM contact sites have emerged as the location where a number of proteins related to the PI-cycle have been reported to localize. Of importance to this review is the presence of the epsilon isoform of diacylglycerol kinase (DGKε) at ER-PM contact sites. In the PI-cycle DGKε is in part responsible for the acyl chain enrichment of the PI molecular species. However, it has recently been shown that the enzyme can only engage in the PI-cycle upon membrane morphological changes. In this review we will discuss the PI-cycle at ER-PM contact sites and how the generation of membrane negative Gaussian curvature nearby those regions could regulate the cycle. We will focus our discussion on the hypothesis that actin polymerization provides the mechanical force needed to change membrane shape nearby ER-PM contact sites, which will transiently trigger DGKε and, therefore, link enzymatic catalysis and lipid transfer in the PI-cycle.

1. Introduction

The phosphatidylinositol cycle (PI-cycle) plays major roles in signal transduction with many of the lipid intermediates of the cycle being modulators of metabolic activity. A surprising characteristic of this metabolic cycle is that it occurs in two membranes, *i.e.* the endoplasmic reticulum (ER) and the plasma membrane (PM). As a consequence, in order for this series of enzyme-catalyzed reactions to result in a regenerating, cyclical process, some lipid intermediates of the cycle have to be transferred from the ER to the PM, while other intermediates are transferred from the PM to the ER. In addition, the PI and its phosphorylated forms produced in the PI-cycle are enriched with specific acyl chains, namely, 1-stearoyl-2-arachidonoyl (SA) acyl chains. This acyl chain enrichment is in part accounted for by the substrate specificity of the enzymes of the cycle. Hence, it seems reasonable that in order to ensure cycle efficiency and specificity, the machinery needed for catalysis and for the shuttling of intermediates between the membranes have to be confined in specialized regions rather than being uniformly distributed on those membranes.

PI is synthesized in the ER, but is used as a substrate in the PM; therefore it should be transferred from ER to PM. Protein-dependent and –independent (vesicular trafficking) mechanisms have being proposed to account for PI transfer from ER to PM. Interesting, the identified proteins reported to transfer PI localize in specialized junctions of ER and PM, that is, at ER-PM contact sites. On the other side of the cycle there is the movement of diacylglycerol (DAG) and/or phosphatidic

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Abbreviations: DAG, diacylglycerol; DGK, diacylglycerol kinase; DGKε, the epsilon isoform of diacylglycerol kinase; ER, endoplasmic reticulum; E-syts, Extended synaptotagmin; IP3, inositol triphosphate; MCS, membrane contact sites; Nir2, longer version of PITP; PA, phosphatidic acid; PALM, photoactivated location microscopy; PI, phosphatidylinositol; PI-cycle, metabolic cycle to synthesize PI; PIP_2, phosphatidylinositol-4,5-bisphosphate; PITP, PI transfer protein; PLC, phospholipase C; PM, plasma membrane; SA, 1-stearoyl-2-arachidonoyl acyl chains; SAG, 1-stearoyl-2-arachidonoyl-glycerol; SIM, structural illumination microscopy; SOCE, store-operated Ca^{2+} entry; TIRFM, total internal reflection fluorescence microscopy

* Corresponding author at: Department of Biochemistry and Biomedical Sciences, McMaster University, 1280 Main Street West, Hamilton, Ontario, L8S 4K1, Canada.

E-mail address: epand@mcmaster.ca (R.M. Epand).

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acid (PA) from the PM to the ER. The mechanisms for this transfer are less well understood. In mammals, the conversion of DAG to PA within the PI-cycle is catalyzed by the epsilon isoform of diacylglycerol kinase (DGKε). This enzyme plays an important role in the PI-cycle as it is one of the cycle enzymes that contribute to the enrichment of PI and its phosphorylated forms with SA acyl chains. DGKε is a membrane-bound enzyme reported to be found at both membranes where the PI-cycle takes place; that is, PM and ER. However, it is currently unknown where DGKε catalyzes its reaction within the PI-cycle. It is noteworthy, that the enzyme is not uniformly distributed in those membranes, but it is present at ER-PM contact sites (Hozumi et al., 2017). Furthermore, it has recently been shown, using *in vitro* assays in the absence of detergent, that DGKε activity and acyl chain specificity are allosterically regulated by membrane-shape changes, which suggested that the membrane should undergo morphological changes to engage DGKε in the PI-cycle (Bozelli et al., 2018). Here, we will discuss the PI-cycle at ER-PM contacts sites with emphasis on how membrane shape changes in the proximity of these membrane junctions could be produced as well as acting as a regulatory mechanism of the cycle.

1.1. Membrane shape

Membrane shape (morphology) is related to membrane geometry and it could be, generally, described by two principal curvatures (k_1 and k_2) (Fig. 1A) (Zimmerberg and Kozlov, 2005). There are two types of membrane curvature related to shape; those are: (i) mean ($H = (k_1 + k_2)/2$) and Gaussian ($K = k_1 \cdot k_2$). Based on a different combination of these two types of curvatures membranes could acquire four basic shapes (Fig. 1B, C). Of particular importance to this review is the saddle-like shape, which is characterized by a membrane bearing negative Gaussian curvature. The sign negative (or positive) is arbitrary and for a membrane presenting Gaussian curvature, it just means that the two principal curvatures at right angle to each other bear opposite sign. In regard to the regulation of membrane events by its shape, most of the reports in the literature have dealt so far with mean curvature as it is easier to measure than Gaussian curvature. The importance of membrane Gaussian curvature had mainly been used to describe membrane fusion/fission events as well as the formation of bicontinuous cubic phases. However, recently we have shown that membrane negative Gaussian curvature, but not membrane intrinsic curvature, is responsible for allosteric regulate DGKε (Bozelli et al., 2018).

2. Membrane contact sites

In eukaryotic cells, organelles´ contents are enclosed by a membrane, which allows the segregation of specialized biochemical reactions in tailored microenvironments. On one side, the compartmentalization imposed by membranes increases the specificity and efficiency of biochemical reactions. On the other hand, it acts as a barrier for the free movement of the metabolites and signaling events between organelles. To overcome this restriction imposed by membranes and ensure an efficient communication among organelles, specialized membrane

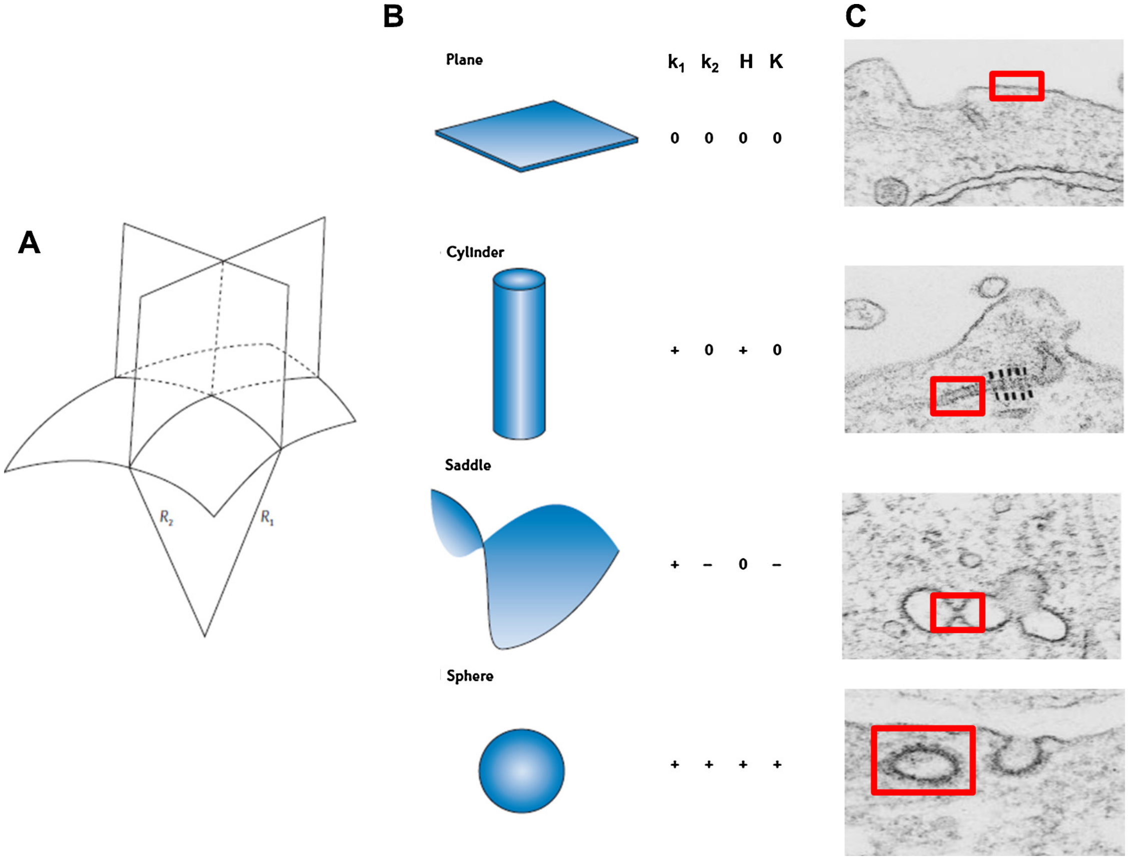

Fig. 1. Membrane shapes. (A) Diagram showing two principal curvatures on a curved surface. For a point under consideration in a curved surface, two principal curvatures can be defined by cross-sectioning the surface with two planes that are perpendicular to the surface and oriented along the two principal directions. The curvature of the surface along the two principle axes can be defined by R_1 and R_2, the radii of circles drawn by extending the arc to be an imaginary full circle. The two principle curvatures k_1 and k_2 are then defined as the reciprocal of the radii of the two circles. (B) The four basic membrane shapes. On left panels are cartoons of different membrane shapes by having different curvatures having a value of 0. The right panels summarize the sign of principal curvatures (k_1 and k_2), mean curvature ($H = (k_1 + k_2)/2$), and Gaussian curvature ($K = k_1 \cdot k_2$) for the respective membrane shapes. (C) Micrographs of biological membranes. Highlighted in red the four basic membrane shapes. (A) and (B) were adapted from Zimmerberg and Kozlov (2006) and used with permission of Springer Nature. (C) was adapted from Iversen et al. 2003, Copyright (2003) National Academy of Sciences.

regions between the organelles are formed; those are collectively called membrane contact sites (MCS) (Helle et al., 2013).

MCS are sites where two membranes come in close proximity (*ca.* 10–40 nm), but are physically distinct as the membranes do not undergo fusion (Eisenberg-Bord et al., 2016). At MCS, the two membranes are physically held together via protein-protein and/or protein-lipid interactions. It is noteworthy that even between the same two organelles MCS could have different tethers and likely functionality (Besprozvannaya et al., 2018). Structurally, MCS are heterogeneous, ranging from two parallel membrane sheets to tubules tightly wrapping against another membrane (organelle) (Eisenberg-Bord et al., 2016). Dynamically, MCS could be formed transiently upon stimulation or present more long-lived dynamics (virtually throughout cell life) (Okeke et al., 2016). Hence, it is reasonable to think about MCS as presenting a variety of functional properties. MCS are widely acknowledged for their role in lipid and Ca^{2+} exchange/regulation and organelle dynamics (fission)/movement (Zhang and Hu, 2016). However, the variety of structural and dynamic properties of MCS indicates that they might play a role in many other cellular functions in health and diseases yet to be discovered.

2.1. ER-PM contact sites

Electron microscopy studies have identified regions where PM and ER were in close proximity (ER-PM contact sites) in skeletal muscles more than 60 years ago (Porter and Palade, 1957). Since then ER-PM contact sites have been reported in a variety of organisms and cell types, indicating that these specialized membrane structures are ubiquitous within a cell. The extent of these contact regions varies. In yeast, the abundance seems to be regulated by ER membrane shape and proteins related with shaping membranes and range from 20 to 40% of the PM area (West et al., 2011). The presence of tethers physically holding together these membranes make their complete separation only possible when all the tether proteins are removed.

ER-PM contact sites are mostly acknowledged for their role in Ca^{2+} signaling/regulation. These junctions have been reported to be responsible for mediating stimulus-contraction in skeletal muscle cells and store-operated Ca^{2+} entry (SOCE) in non-excitable cells (Takeshima et al., 2015). SOCE is a fundamental signaling and ion transport mechanism dependent on direct interactions between ER and PM-resident proteins (Okeke et al., 2016). ER-PM contact sites have also been implicated in the regulation and exchange of lipids. Of particular importance is the finding of proteins involved in PI metabolism at ER-PM contact sites (see below). Indeed, impaired PI signaling was observed upon disruption of ER-PM contact sites (Balla, 2018).

3. The PI-cycle

3.1. A cycle requiring two membranes – ER and PM

It is known that the portion of the PI-cycle that results in the conversion of PA to PI takes place in the ER (Epand et al., 2016). To complete the cycle, PI is converted to DAG in the PM. The one additional required reaction is the conversion of DAG to PA catalyzed by DGKε. It is interesting that DGKε is found on both membranes where the PI-cycle takes place, raising the question of the location where DGKε catalyzes its reaction within the PI-cycle. We will discuss below the location of this step in the PI-cycle, suggesting that this reaction is coupled with the transfer of lipid from the PM to the ER.

3.2. Shuttling lipids between the two membranes of the PI-cycle

As lipids present low water solubility the transport of lipids between membranes should be mediated either by transfer proteins or vesicular trafficking. One of the most acknowledged functions ascribed to MCS is lipid exchange. There are several proteins that facilitate the transfer of lipids between the ER and PM that are located at MCS (Cockcroft and Raghu, 2018). In this section we will discuss the mechanisms of transferring lipid intermediates of the PI-cycle between ER and PM and *vice-versa* in light of ER-PM contact sites.

3.2.1. PI-transfer proteins

One of the mechanisms of transfer of PI from the ER to PM is through transfer proteins, which extracts a lipid from one membrane and delivers to another membrane. Several proteins have been reported to be involved with the shuttling of PI from ER to PM (Lev, 2010). One such protein is the phosphatidylinositol transfer protein (PITP), which is 271 residues long. With regard to the PI-cycle, a larger version of PITP (Nir2), containing 1,244 residues and a membrane binding domain, is of particular importance (see below) (Lev, 2010). Another protein, TMEM24, has been suggested to transfer PI from ER to PM in order to maintain the PIP2 pool in the PM, needed for insulin secretion via exocytosis (Balla, 2018).

Upon receptor stimulation and phospholipase C (PLC) activation, MCS are transiently formed between the ER and PM (Okeke et al., 2016). PLC activation increase the pool of DAG in the PM through PIP_2 hydrolysis, which leads to the recruitment of Nir2, which is anchored to the ER through the interaction with ER-resident proteins, VAP-A(B). There is evidence that the Nir2 protein is located at MCS (Kim et al., 2015; Hsieh et al., 2017). In addition to being recruited to ER-PM contact sites, Nir2 could help to stabilize these sites via its interaction with DAG and/or PA. The protein has been shown to transfer PI from the ER to the PM in exchange for PA moving in the opposite direction (Chang and Liou, 2015; Kim et al., 2013, 2015). This exchange of lipids could be sufficient by itself to transfer lipids in both directions between the PM and ER to complete the PI-cycle. However, it is currently unknown whether Nir2 bears acyl chain specificity for the lipids it transfer between the membranes, which is known to be an important feature of the PI-cycle.

3.2.2. Extended synaptotagmins (E-syts)

Another lipid intermediate of the PI-cycle that has been reported to be transported by transfer proteins was DAG. E-syts are integral membrane proteins that are found in the ER. There are 3 isoforms (E-syts 1–3), all involved in the translocation of DAG from the PM to the ER at elevated levels of Ca^{2+} due to the presence of multiple C-2 domains (domains that can bind Ca^{2+}) (Balla, 2018). E-syts binds PM PIP_2 in the presence of Ca^{2+}, tethering the ER to PM (forming ER-PM contact sites) (Bian et al., 2018; Lev, 2010). The ER-PM distance at E-syts MCS is cytosolic Ca^{2+}-dependent, with a shorter distance at higher Ca^{2+} concentrations (Fernández-Busnadiego et al., 2015). The E-syts mediated ER-PM contact sites present a different architecture from SOCE. Electron micrographs showed that the later present filamentous structures bridging the membranes while the former present an electron dense layer, highlighting the structural heterogeneity of MCS (Fernández-Busnadiego et al., 2015).

3.2.3. PIPerosomes (Vesicular transfer)

Alternative to protein mediated lipid exchange between membranes; there is also evidence for PI transfer via a unique kind of small highly mobile membrane compartment containing PI as well as the enzyme responsible for the final step in the biosynthesis of PI. These structures have been termed PIPerosomes (Kim et al., 2011) and have been suggested to carry PI from the ER, where it is synthesized, to the PM. Rab10, a small GTPase, has been shown to facilitate the movement of these particles (English and Voeltz, 2013). Rab10 can cause ER tubule extension and fusion with adjacent ER regions. During membrane fusion the membrane undergoes several morphological changes, with fusion intermediates presenting negative Gaussian curvature. In the PI-cycle the presence of membranes with this particular type of curvature could also serve to activate DGKε (see below).

3.2.4. Calcium and lipid signaling at contact sites

The relationship between the PI-cycle and Ca^{2+} homeostasis has long been acknowledged. Ca^{2+} activates PLC, an enzyme of the PI-cycle, located in the PM. The action of PLC results in the hydrolysis of PIP_2 to produce inositol triphosphate (IP3) which is an effector for opening Ca^{2+} channels in the ER. The Ca^{2+} released from the ER could, then, further activate PLC to make the system self-reinforcing. It is currently understood that this interrelationship could take place at ER-PM contact sites. One of the most studied ER-PM contact sites is SOCE, which is formed via a physical interaction between STIM1, an ER-resident protein, and Orai1, a PM-resident channel forming protein, and is implicated in Ca^{2+} homeostasis. In addition to interacting with Orai1, STIM1 has a polybasic domain reported to be important for binding PM PIP_2. Orai1 calcium channels are activated by STIM1 when the Ca^{+2} concentration in the ER lumen is reduced.

Ca^{2+} is not only transported at contact sites but can also control the nature and efficiency of lipid transfer between membranes within the cell (Balla, 2018). At high calcium concentration the E-syts proteins can facilitate the translocation of DAG. Cells that lacked all three isoforms of E-syts showed defects in removing DAG from the PM (Saheki et al., 2016). PLC-catalyzed hydrolysis destroys PIP_2 that is required for the binding of E-syts to the membrane. In the end it is observed that the expression of E-syts is not essential. Mice devoid of all three isoforms of E-syts are viable, grow normally and are fertile (Sclip et al., 2016). The apparent lack of a more altered phenotype can be explained by compensatory or alternative mechanism for lipids transfer at the ER-PM contact site (Sclip et al., 2016; Tremblay and Moss, 2016). Hence, it is not surprising that Ca^{2+} and lipid exchange/regulation seems to be one of the best characterized functions of MCS.

3.2.5. DGKε: linking enzymatic catalysis and lipid transfer in the PI-cycle

DGKε has a particularly important role in the PI-cycle. It has been shown *in vitro* and *in vivo* that it bears specificity for arachidonoyl-containing forms of DAG, which suggested that it is responsible in part for the enrichment of PI and its phosphorylated forms with SA acyl chains (Rodriguez de Turco et al., 2001; Milne et al., 2008; Epand et al., 2016). Lipid transfer in the PM to ER direction can involve DAG and/or PA; that is, DGKε substrate and product, respectively. This is in part due to the finding that DGKε is present at both membranes where the PI-cycle takes place (see below) and there have been reported proteins that could mediate the movement of both DAG and PA in this direction (see above). However, recent findings suggest that DGKε could be a potentially important link between membranes for the PI-cycle. It has been shown that in the absence of detergents, DGKε is active and specific only in curved membranes (Bozelli et al., 2018). The type of membrane curvature identified to modulate DGKε activity and specificity is similar to the one found in membrane fusion/fission intermediates; *i.e.*, a membrane bearing negative Gaussian curvature. Although membrane fusion does not take place at MCS, MCS have been reported to determine sites of membrane fission/fusion machinery assembly (Helle et al., 2013). Hence, the answer to the question of which lipid is transferred may be that it is both and the lipid transfer and the DAG kinase step in the PI-cycle are inter-related.

4. Location of DGKε in cells

DGKε has been described to be located in different regions of the cell; although most evidence points to ER and/or PM. In most cell types the natural level of expression of DGKε is low; therefore, most of the studies reporting its localization used cells transfected with DGKε. In addition, in most studies a tag was attached to the protein to probe its location. For instance, in COS-7 cells GFP fused DGKε has been identified in the ER (Kobayashi et al., 2007), which was shown to be targeted through the hydrophobic N-terminal segment of the enzyme (Matsui et al., 2014). In NIH 3T3 cells flag tagged DGKε has been localized in the PM. This has been observed by fluorescence microscopy, showing the appearance of an N-terminal FLAG-tag P33A mutant of DGKε on the exterior of cells as well as by subcellular fractionation and by affinity purification of the PM (Decaffmeyer et al., 2008).

DGKε expression levels in the brain is somewhat higher than in other tissues (Goto and Kondo, 2004; Goto et al., 2006). The higher expression of DGKε in the brain and the availability of a high affinity, high specificity antibody to DGKε has allowed for the localization of DGKε within cerebellar Purkinje cells of rats (Hozumi et al., 2017). It is noteworthy that in these cells DGKε has been localized to subsurface cisterns, which are ER-PM contact sites of neurons and primary dendrites (Tao-Cheng, 2018). Furthermore, in these cells high resolution immunohistochemistry revealed that DGKε was localized adjacent to the IP3-receptor-1, providing additional evidence for a linkage between DGKε and the PI-cycle. Moreover, in dendrites of these cells DGKε was also located in close apposition to the enzyme diacylglycerol lipase-α (Hozumi et al., 2017). When diacylglycerol lipase-α acts on the substrate for DGKε it produces 2-arachidonoyl glycerol, a major cannabinoid in the brain. Hence, in the brain DGKε and diacylglycerol lipase-α may compete for the substrate SAG to enter the PI-cycle or to produce a ligand for the cannabinoid receptor.

Although DGKε is reported to be a membrane bound enzyme, there is a report showing that DGKε is localized on actin stress fibers in vascular smooth muscle cells (Nakano et al., 2009). Such subcellular localization of DGKε has not been widely reported. However, it is possible that there is a smaller localized concentration of DGKε on actin fibers in other cell types, but the phenomenon is more prevalent in muscle cells because of the greater abundance of actin fibers in this cell type.

5. Regulation of DGKε by membrane shape

In mammals the diacylglycerol kinase (DGK) family of enzymes is comprised by at least 10 isoforms as well as species made by alternative splicing (Shulga et al., 2011). Of those, DGKε is the only one reported to be permanently associated with membranes and this isoform also lacks a putative regulatory domain (Epand et al., 2016). Its importance for the PI-cycle is in part ascribed to its substrate acyl chain specificity and its contribution for the enrichment of PI molecular species with SA acyl chains. There are various membrane bound enzymes which the activity has been reported to be regulated by the lipid composition and/or membrane physical properties to which they are bound. However, until recently there was no report of the modulation of the activity of enzymes by the membrane´s shape. By employing a systematic variation of membrane lipid composition and physical properties it was shown that DGKε has low activity and acyl chain specificity in locally flat membranes, even if those mimic the lipid composition of ER and PM (Bozelli et al., 2018). However, upon membrane shape changes the activity and substrate acyl chain specificity was significantly increased. In particular, the requirement of negative Gaussian curvature was identified to modulate the activity and substrate acyl chain specificity of the enzyme. It was also shown that the enzyme stabilizes the formation of membranes containing such structures. Based on these findings it was proposed that the enzyme might not catalyze its reaction within the PI-cycle in stable regions of either ER or PM. Its function would be transiently triggered by membrane sites bearing negative Gaussian curvature; for instance, nearby junction sites between these two organelles where membrane shape changes have been reported and/or at local sites where membrane fusion/fission takes place. Hence, the modulation of DGKε activity and acyl chain specificity by membrane morphological changes could be seen as a mechanistic way of the cells to link this enzyme catalysis with lipid shuttling in order to enforce an efficient PI-cycle and signaling.

6. Actin and membrane shape

The PM undergoes continuous dynamic change in morphology together with changes in the underlying cortical actin cytoskeleton (Saarikangas et al., 2010). Polymerization of actin filaments that are adjacent to the PM causes the membrane to acquire negative Gaussian curvature that facilitates a number of cellular processes including migration, morphogenesis, endocytosis, protrusions and invaginations. Actin polymerization provides the driving force for the bending of membranes (Carlsson, 2018). We hypothesize that the induction of these membrane morphological changes by actin polymerization, known to facilitate other biological processes, are also important to produce the membrane environment for the activation and acquisition of substrate specificity of DGKε.

In addition to actin there are other proteins that can participate in modulating membrane bending. For example, the involvement of clathrin and Bar domain proteins in promoting actin polymerization at the base of clatherin-coated pits has been recently elucidated (Almeida-Souza et al., 2018).

7. PI-cycle and actin polymerization

The PI-cycle is linked to actin polymerization through PM PIP_2 binding to one of a group of proteins, such as profilin (Sohn and Goldschmidt-Clermont,1994), that are released from actin (Lassing and Lindberg, 1988; Senju and Lappalainen, 2019). Neomycin, that has a high affinity for PIP_2 and interferes with the PI-cycle, inhibits the polymerization of actin in platelets (Lassing and Lindberg, 1988) showing the dependence of actin polymerization on PIP_2. The structural features of the actin-binding proteins that allow them to bind to PIP_2 have been studied (Janmey et al., 1999). Profilin is not unique in the role of affecting both actin polymerization and the PI-cycle. Other proteins that serve this function by binding to both PIP_2 and to actin include α-actinin, gelsonin, protein 4.1, cofilin, gCap39 and MARKS (Sohn and Goldschmidt-Clermont, 1994).

8. Actin-modulated shape changes around ER-PM contact sites

Several biological processes, including the functioning of the PI-cycle, are dependent on the special organization of the ER-PM contact sites. Regulation of the morphology of these contact sites will be determined by ER shaping proteins (Jozsef et al., 2014), junctional tethers (Chang et al., 2013; Giordano et al., 2013) and the cortical cytoskeleton (Dingsdale et al., 2013; Wang et al., 2014; Zhang et al., 2016; van Vliet et al., 2017; Morone et al., 2006). A recent study of the morphology of the ER-PM contact sites using a combination of specialized microscopy techniques has yielded morphological information at high resolution (Hsieh et al., 2017). This work employed photoactivated location microscopy (PALM), total internal reflection fluorescence microscopy (TIRFM) and structural illumination microscopy (SIM) to achieve resolution beyond the diffraction limit of light microscopy. The study, using HeLa cells, showed that the ER-PM contact sites are elongated in shape with dimensions around 120 nm x 80 nm (Hsieh et al., 2017). Furthermore it was shown that cortical actin fibers contribute to the spatial distribution and stability of these junctions. Latrunculin B, an actin polymerization inhibitor, caused ER-PM contact sites to double their lateral mobility in the membrane and there was even a 35% reduction in contact site density. The ER-PM contact sites were not super imposed on actin fibers but rather in close proximity to them indicating that actin had an important role in the location and functioning of contact sites. Additional functional assays showed that actin fibers and the action of Nir2 at the ER-PM contact site are required for PIP_2 homeostasis (Hsieh et al., 2017). Latrunculin B treatment resulted in Nir2 not being stably located to contact sites. As a consequence Latrunculin B treatment suppressed the replenishment of PIP_2 at the PM after receptor stimulation. Interestingly, STIM1 functioning at ER-PM contact sites is not affected by latrunculin B. The differing requirements for the actin cytoskeleton between STIM1 and Nir2 may reflect that Nir2 is a soluble protein, while STIM1 is not. This study concluded that it is likely that functions of the ER-PM contact sites "*can be differentially regulated through dynamic actin remodeling during cellular processes*" (Hsieh et al., 2017).

9. Model for the functioning of DGKε in the PI-cycle

DGKε is found in different locations in cells. One of these locations is the ER-PM contact site. Several processes are coordinated at the ER-PM interface. At such location DGKε could function in the PI-cycle both to catalyze one of the steps of that cycle, as well as to simultaneously move the substrate DAG produced by PLC in the PM to PA in the ER. It has been shown that in absence of detergents, DGKε is active and shows substrate acyl chain specificity only in membranes having negative Gaussian curvature (Bozelli et al., 2018). Many cell membranes are largely flat, including the plasma membrane. The ER can form highly curved tubular membranes, but the cytosolic side of these tubes has a positive membrane curvature that does not promote the activity or specificity of DGKε. Thus, there must be mechanisms to bend the membrane in order to engage DGKε in the PI-cycle. One possibility would be the use of mechanical forces provided by actin polymerization. Actin plays an important role in shaping the membranes around the ER-PM contact sites. Actin polymerization is able to bend the membrane to acquire negative Gaussian curvature and is sensitive to the presence of PIP_2 that is hydrolyzed by PLC to generate DAG, the substrate for DGKε. Hence, the coupling of lipid conversion in the PM with actin polymerization provides the driving force to membrane shape changes; which would ultimately regulate DGKε activation and substrate acyl chain specificity in the PI cycle. In addition to being a substrate for DGKε, the DAG itself has strong intrinsic negative curvature tendency and its formation as a result of the activation of PLC may also contribute to membrane bending. There are several pathways to move lipids to and from the ER and PM. The presence of this redundancy may be the result of the importance of lipid movement to cell functioning and/or inter-organelle communication, so that there are alternative pathways that can function if one is aberrant.

Conflict of interests

We have no conflict of interest with the material in this review.

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