Structural and signaling role of lipids in plasma membrane repair

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Abstract

The plasma membrane forms the physical barrier between the cytoplasm and extracellular space, allowing for biochemical reactions necessary for life to occur. Plasma membrane damage needs to be rapidly repaired to avoid cell death. This relies upon the coordinated action of the machinery that polarizes the repair response to the site of injury, resulting in resealing of the damaged membrane and subsequent remodeling to return the injured plasma membrane to its pre-injury state. As lipids comprise the bulk of the plasma membrane, the acts of injury, resealing, and remodeling all directly impinge upon the plasma membrane lipids. In addition to their structural role in shaping the physical properties of the plasma membrane, lipids also play an important signaling role in maintaining plasma membrane integrity. While much attention has been paid to the involvement of proteins in the membrane repair pathway, the role of lipids in facilitating plasma membrane repair remains poorly studied. Here we will discuss the current knowledge of how lipids facilitate plasma membrane repair by regulating membrane structure and signaling to coordinate the repair response, and will briefly note how lipid involvement extends beyond plasma membrane repair to the tissue repair response.

Keywords

Lipids; plasma membrane; membrane injury; tissue repair

1. Introduction

The plasma membrane separates the extracellular environment from the cell interior, where biochemical reactions necessary for life occur. The plasma membrane is semi-permeable, allowing the cell to communicate with and utilize resources from its surrounding environment. However, all cells are susceptible to plasma membrane damage, which results in the mixture of the intracellular fluid and extracellular milieu and can result in death if the damage is not rapidly repaired. Plasma membrane repair relies on the coordinated activity of repair machinery, which carries out vesicle fusion to the membrane, membrane shedding,

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and polymerization of F-actin at the site of repair (Horn & Jaiswal, 2018). Repair of injured cells is tied closely to tissue repair and regeneration, as shown by studies demonstrating release of inflammatory mediators, including small molecules, peptides and proteins that signal to cells in the inflammatory and regenerative systems to initiate a tissue level reparative response.

The most abundant component of the cell's plasma membrane is the lipids. Lipids are a class of biomolecules, which are generally insoluble in water, and may refer to fatty acids, sterols, mono-, di-, and triglycerides, as well as phospholipids, among others. While lipids are often ascribed a metabolic role as energy storage molecules, they also play important structural and signaling roles in the cell. Acute and chronic release of lipids and free fatty acids following cell and tissue injury has been widely recognized to be involved in the process of tuning the inflammatory and subsequent tissue repair response. Being the most abundant component of the plasma membrane, lipids are also essential player in the process of plasma membrane repair; however, much of the research committed to identifying the mechanisms of plasma membrane repair has focused on the proteins associated with plasma membrane repair (Cooper & McNeil, 2015).

Lipids contribute to cellular physiology at both an individual and population level. Individual lipids can serve as signaling molecules on their own or through binding proteins, and chemical changes to a single lipid can initiate change in local membrane composition. At the population level, the composition of lipids in a membrane can result in formation of signaling platforms that can change the properties of an entire membrane, enabling the cell to finely tune tension, shape, and rigidity. At each of these levels the structural and signaling aspects of lipids are critical for the cell to mount an efficient response to plasma membrane injury. In this review, we will focus on the role of lipids during plasma membrane repair by discussing their functions as both structural and signaling molecules. We will highlight how lipids respond to injury and facilitate repair both at the level of individual molecules and at the bulk level by collectively altering the plasma membrane form and function.

2.   Plasma membrane lipid composition, organization, and response to injury

The plasma membrane has a unique lipid composition that helps distinguish its structural and functional properties from the other internal membrane-bound compartments. Plasma membrane lipids can be grouped into three classes – glycerophospholipids, sphingolipids, and sterols. Each of these lipids contributes their own qualities that affect the structural and signaling characteristics of the plasma membrane (Nicolson, 2014). In mammalian cells, lipids formed upon the phosphate and glycerol (e.g. diacylglycerol - DAG) backbone are called glycerophospholipids (referred to as phospholipids hereafter) and make up the majority of the plasma membrane. These phospholipids are derived from glycerol-3-phosphate, itself a product of cellular metabolism that is enzymatically modified into phosphatidic acid (PA). From PA, cells generate DAG, or cytidine diphosphate-DAG (CDP-DAG), which serve as inputs into the phospholipid biosynthetic pathways (Figure 1A, B). Changing the head group attached to the DAG backbone creates different phospholipid

species, which include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), and PA (Oropeza, 2017) (Figure 1B). Among these lipids, PC is the most abundant in the plasma membrane and is formed by adding choline to the DAG backbone. It can also be produced by methylation of existing PE, while PE can be produced by the decarboxylation of PS. Further, PE and PC head groups can be cleaved and replaced with serine to produce PS (Oropeza, 2017). The variety of possible phospholipid interconversions, such as these, allows the cell to rapidly change the lipid composition of the plasma membrane. Thus, each of these lipids contributes significantly to the organization, structure, and function of the plasma membrane as a whole.

Plasma membrane lipids containing the alkanoamine sphingosine are called sphingolipids. The primary plasma membrane sphingolipid in mammalian cells is sphingomyelin, which utilizes a ceramide backbone (Merrill Jr, 2008). Sphingomyelin also confers different properties to the plasma membrane than the phospholipids, not least due to its preferential association with sterols (Ramstedt & Slotte, 2006). This preferential association between different lipids results in an important feature of the plasma membrane – its transverse and lateral heterogeneity. Many of the sphingolipids also contain a glycosidically bound carbohydrate moiety causing formation of the glycosphingolipids. Here the sugar (oligosaccharide) moiety faces the extracellular space interacting with other cells and extracellular ligands required for processes such as signaling, cell adhesion or intercellular interactions.

Sterols, or steroid alcohols, are a third type of plasma membrane lipid, with cholesterol being the predominant form found in mammalian cells. Unlike phospholipids and sphingolipids, which are structurally analogous, cholesterol is composed of a steroid backbone that results in a planar and more rigid molecule. While being a mostly non-polar molecule, owing to its steroid backbone and hydrocarbon chain, cholesterol does possess a hydroxyl group opposite the hydrocarbon chain. This structural arrangement is important for the organization of cholesterol in biological membranes as it results in the hydroxyl group associating with the neighboring lipid head groups and water, while the majority of the cholesterol molecule resides within the hydrophobic core of the membrane. In mammalian cells the majority of cellular cholesterol is found in the plasma membrane, where it can make up to 50% of lipid content (Van Meer, Voelker, & Feigenson, 2008). Cholesterol interacts with both phospholipids and sphingolipids, and its interactions with these lipids play a crucial role in determining the overall physical properties of the plasma membrane. For example, when inserted into a region abundant in phospholipids, cholesterol has a rigidifying effect; however, the opposite can be true with sphingolipids. In this case, cholesterol actually increases the fluidity among these lipids, which prevents them from forming a gel-like phase that is incompatible with the overall fluid nature of the plasma membrane (Krause & Regen, 2014). While the structural importance of plasma membrane cholesterol is well appreciated, it can also play a role in signaling. This is due to its protein-binding ability, resulting in protein translocation to the cholesterol-rich regions of plasma membrane (Sheng et al., 2012; Van Meer et al., 2008).

The plasma membrane is not a homogenous mixture of the different lipids described above (Figure 2A). Instead, there exists distinct inter- and intra-leaflet heterogeneity of lipids. This allows local and functional diversity between the two leaflets as well as various parts of the single contiguous plasma membrane (Figure 2B). Phospholipids in particular show inter-leaflet heterogeneity. While PC exists in both the inner and outer leaflet of the plasma membrane, the charged phospholipids – PE, PI, and PS – are almost exclusively maintained within the inner leaflet (Nicolson, 2014; van Meer, 1989). Similar inter-leaflet heterogeneity exists among sphingolipids, with the glycosphingolipids maintained exclusively in the outer leaflet. With an abundance of uncharged (zwitterionic) lipids and smaller amounts of neutral and anionic glycosphingolipids in the outer leaflet, and the negatively charged phosphatidylserine, phosphatidic acid and phosphatidylinositol within the inner leaflet, there exists a charge differential between the two plasma membrane leaflets (Steck & Lange, 2018; Zachowski, 1993). There also exists lateral heterogeneity of lipid composition within each leaflet, which is demonstrated by the formation of lipid microdomains, such as the sphingomyelin and cholesterol-rich domains that exist interspersed among the phospholipids throughout the plasma membrane (Cebecauer et al., 2018; Sezgin, Leventa, Mayor, & Eggeling, 2017) (Figure 2B). These examples of organizational heterogeneity, along with the differences among the lipids that comprise the plasma membrane confer a variety of structural and signaling properties to the plasma membrane and allow the plasma membrane to mount and sustain localized signaling despite being fully interconnected and fluid. In the subsequent sections, we will discuss how this is achieved and utilized by the cell.

The plasma membrane is a dynamic barrier that separates the cell interior from the extracellular space (Figure 2C). Not only is the composition and organization of the plasma membrane in constant flux, the membrane itself also must interact with forces being applied to it from all directions. In some cases, these forces are benign, such as membrane protrusion driven by cortical actin polymerization (Pontes, Monzo, & Gauthier, 2017). However, excessive force applied to the membrane can result in physical damage, such as the shear force applied on the endothelial cells by blood flow, on muscle cells during contraction, on lung alveolar cells during breathing, or by a blunt force trauma to any cell (Cong, Hubmayr, Li, & Zhao, 2017; Demonbreun & McNally, 2016; McNeil & Steinhardt, 2003). While small injuries to the plasma membrane (on the nanometer scale) can be spontaneously resealed due to the line tension imposed by plasma membrane lipids alone (McNeil & Terasaki, 2001), larger membrane injuries (on the micron scale) require a series of coordinated mechanisms to undergo repair. Early observations of cells undergoing plasma membrane repair identified that membrane bound vesicles are involved in the process of membrane resealing (Bi, Alderton, & Steinhardt, 1995). It has since become evident that in addition to resealing, remodeling of the plasma membrane through vesicle fusion (exocytosis), vesicle internalization (endocytosis), and shedding (ectocytosis) also contribute to the repair process (reviewed in (Horn & Jaiswal, 2018)). Each of these pathways that facilitate repair through membrane remodeling also alter the local composition and distribution of plasma membrane lipids in healthy cells (van Meer, 1989) (Figure 2D). Constitutive fusion of biosynthetic vesicles is a major mechanism for delivery of new lipids and proteins, which helps to build and maintain the plasma membrane. This homeostatic process of vesicle fusion that maintains the plasma membrane at rest also enables plasma

membrane repair through regulated fusion of vesicles triggered by calcium influx following plasma membrane injury (Horn & Jaiswal, 2018; McNeil & Steinhardt, 2003).

The role of lipids during plasma membrane repair goes far beyond a passive role in vesicle-mediated delivery of membrane lipids. As structural components of the plasma membrane, lipids are responsible for contributing to membrane tension, rigidity, and overall shape. After an injury, the biophysical properties of the plasma membrane, and the individual lipids themselves, are altered, eliciting changes to membrane rigidity and fluidity. These structural changes to lipids are both necessary for repair and potentially pathogenic if left uncorrected (Sreetama et al., 2018). In addition to their structural role, and often as an extension of it, lipids also provide a signaling function that facilitates plasma membrane repair. Injury to the plasma membrane also changes the biochemical environment within the cell. Influx of calcium from the extracellular space, as well as locally increased oxidation, both trigger lipid signaling that is required for repair. Given their integral role in forming the structural backbone of the membrane, lipids are ideally placed to act as damage sensors, initiating early signaling that sets the stage for subsequent repair machinery. Further, the complexity of signaling afforded by lipids allows the cell to coordinate a highly organized response to injury across space and time (Figure 1B). These observations suggest that lipids are not bystanders during the repair process, but are instead actively involved in organizing the playing field on which repair machinery operates.

3. Structural role for lipids during plasma membrane repair

The physical properties of the plasma membrane are governed in large part by the effect of lipid interactions at the population level. For example, lipid composition, distribution, and inter-lipid interactions actively control the rigidity and tension acting upon the plasma membrane, which in turn regulates cellular functions such as vesicle fusion, cell motility, and membrane resealing (Diz-Muñoz, Fletcher, & Weiner, 2013; Gauthier, Fardin, Roca-Cusachs, & Sheetz, 2011; Togo, Krasieva, & Steinhardt, 2000). The tension forces acting on the plasma membrane are generally applied by three sources: the difference in hydrostatic pressure between the cell interior and extracellular space, the cortical cytoskeletal network, and the extracellular matrix to which the cell is attached (Gauthier, Masters, & Sheetz, 2012; Kozlov & Chernomordik, 2015) (Figure 2B). The plasma membrane itself forms the barrier responsible for the hydrostatic pressure experienced by the cell, while the cortical actin cytoskeleton, focal adhesions, and extracellular matrix regulate the structural tension (Burridge & Guilluy, 2016; Gauthier et al., 2012). As these features change dynamically, tension forces experienced by the plasma membrane are not static and the cell's ability to respond to them appropriately allows for essential functions such as change in morphology, movement, adhering to new substrates, cell division, and membrane fusion. These functions also require modulating the membrane area, such as through the processes of exocytosis and endocytosis or by changing the existing membrane architecture (Kozlov & Chernomordik, 2015; Nassoy & Lamaze, 2012).

While tension acts at the level of an entire membrane, the physical properties of the individual lipids and lipid domains dynamically guide the local changes required for adapting to the change in tension. Lateral movement, rotation, and flipping of lipids between

the leaflets of the plasma membrane are the physical changes that work together to allow the fluid membrane to adapt to the changes in membrane tension (Nicolson, 2014) (Figure 2C). These same processes also work to dynamically control membrane fluidity by regulating the distribution of phospholipids, sphingolipids, and cholesterol in the membrane. Due to the differences in the three-dimensional conformations of membrane lipids, a change in their distribution changes the lipid packing density in a given lipid domain. For example, replacement of a cylindrical lipid (e.g. PC), with a conical shaped, charged phospholipid (e.g. PS) in the inner leaflet, causes the plasma membrane to attain an overall outward curvature. In contrast to PC, sphingomyelin forms a taller and narrower cylinder, which along with the configuration of its acyl chains, allowing this lipid to pack more tightly than phospholipids (Cebecauer et al., 2018). The tight packing, along with the preference of cholesterol to intercalate the acyl chains of sphingomyelin creates the liquid-ordered membrane domains, such as the so-called "lipid rafts" (Van Meer et al., 2008) (Figure 2C). These domains decrease the local fluidity in the membrane relative to the regions comprised primarily of phospholipids. This is especially important for membrane signaling functions as the liquid-ordered domains often serve to aggregate membrane-associated proteins (Cebecauer et al., 2018). Lipids also interact with plasma membrane localized proteins to regulate membrane tension and fluidity. For example, the cytoskeletal proteins interact with membrane lipids to supply the cortical tension that regulates the global shape of the plasma membrane and produces cell movement (Cebecauer et al., 2018; Sezgin et al., 2017). Membrane proteins also help locally shape and provide rigidity to the plasma membrane by interacting with specific lipids or lipid domains (Cebecauer et al., 2018). All of the above mechanisms for regulating the physical properties of the membrane play important roles in determining how a cell responds to plasma membrane injury and undergoes successful repair.

3.1.   Plasma membrane tension and dynamics are interrelated after injury

In response to an injury, a sudden change in hydrostatic pressure and local disassembly of the cortical cytoskeleton causes a rapid drop in membrane tension (Jaiswal et al., 2014; Miyake, McNeil, Suzuki, Tsunoda, & Sugai, 2001; Togo et al., 2000). Further, the addition of new membrane by increased calcium-triggered exocytosis also decreases membrane tension and is required for plasma membrane repair (Togo et al., 2000). However, to successfully repair the cell also needs to restore the barrier function of the resealed membrane. This process is itself facilitated by mechanisms that regulate membrane tension, in particular the re-establishment of the actin cortex. While initial loss of cortical actin aids in repair by allowing physical access for vesicles to fuse with the plasma membrane, the accumulation of F-actin at the injury site in the minutes following injury, is also required to provide structural support to the repairing/repaired membrane and prevent additional injury due to membrane fragility (Demonbreun et al., 2016; Horn et al., 2017; Jaiswal et al., 2014; McDade, Archambeau, & Michele, 2014; Miyake et al., 2001). Quantification of membrane tension after injury to fibroblasts demonstrated that a minimum tension force was reached by approximately 30 seconds post-injury, but tension was fully restored by 80 seconds post-injury (Togo et al., 2000). These observations align well with the observed kinetics of membrane resealing, which suggests that plasma membrane wounds can be resealed within

30 seconds; however, restoration of membrane tension shortly afterward is required for successful membrane remodeling and completion of the repair process.

While reassembly of the cortical cytoskeleton in the minutes following injury is known to restore membrane tension (described in Section 3.3), membrane remodeling also contributes to increasing tension. In order to balance any decrease in membrane tension, such as that caused by exocytosis, cells routinely utilize endocytosis, which reduces the membrane area and results in increased tension (Dai & Sheetz, 1995). Endocytosis also occurs in response to plasma membrane injury and has been described as a mechanism for membrane resealing (Idone et al., 2008). However, in addition to this role, restoring membrane tension is another role that has been proposed for endocytosis after the membrane is resealed following a pore forming toxin injury (Skalman, Holst, Larsson, & Lundmark, 2018). This study showed that endocytosis occurs after membrane resealing and is mediated by the CLIC/GEEC pathway effector GRAF1, which is implicated in repair of muscle fiber plasma membrane injury (Lenhart et al., 2015). Accumulation of GRAF1 at the repair site occurs 2 minutes after injury, supporting its potential role in membrane remodeling following resealing. Such a role of CLIC/GEEC-mediated endocytosis in membrane remodeling is also supported by the observation that CLIC/GEEC is immediately activated in response to decreased membrane tension and has the capacity to turn over large amounts of plasma membrane (Thottacherry et al., 2018). The observation, that endocytosis may not be required for resealing through the removal of pore-forming toxins but instead acts as a mechanosensor to buffer membrane tension, may also explain how endocytosis participates in the repair of mechanical damage, where no membrane-spanning pores need to be removed.

Endocytosis can also occur at sites of membrane invagination known as caveolae, and caveolae internalization has been implicated in membrane repair (Corrotte et al., 2013). In response to increased stress on the plasma membrane, caveolae have been proposed to act as mechanosensors, buffering membrane tension in response to mechanical stress (Cheng et al., 2015; Sinha et al., 2011). These membrane invaginations require the presence of caveolin proteins, loss of which can result in poor membrane repair and muscular dystrophy (Gazzerro, Sotgia, Bruno, Lisanti, & Minetti, 2010; Minetti et al., 1998). However, whether caveolae facilitate repair by buffering membrane tension remains unclear because, unlike the capacity of CLIC/GEEC endocytosis, caveolae make up a small portion (as small as 0.03%) of the membrane area and are not found ubiquitously in all cells (Gauthier et al., 2012; Sinha et al., 2011). It remains possible that caveolar endocytosis does help partially balance membrane tension, but another possibility is that they serve as mechanosensitive platforms during membrane repair. For example, caveolae are the site for assembling membrane repair proteins such as EHD2 and MG53 (Cai et al., 2009; Daumke et al., 2007; Marg et al., 2012). Thus, caveolae may serve a signaling function during membrane repair (see Section 4).

3.2. Regulation of plasma membrane lipid mobility and oxidation is required for repair

Similar to the changes in tension described above, the fluidity of the plasma membrane is also dynamic after injury. Plasma membrane damage increases the fluidity of individual lipids, allowing them more freedom to migrate laterally, rotate, or even flip – appearing in the opposite leaflet of the membrane. This phenomenon may be explained by the kinetic

energy imparted on the membrane lipids by mechanical disruption, which can result in lipid mixing without relying on membrane tension (Petersen, Chung, Nayebosadri, & Hansen, 2016). Indeed, mechanical injury of healthy cells transiently increases the mobile fraction of plasma membrane lipids by as much as 9% (Sreetama et al., 2018). However, uninjured muscle cells from Limb Girdle Muscular Dystrophy 2B (LGMD2B) patients show increased membrane fluidity, which is associated with their poor membrane repair ability (Sreetama et al., 2018). The poor repair of these patient cells can be mirrored in healthy cells by increasing their membrane lipid mobility through the removal of cholesterol or by addition of a glucocorticoid (prednisone) (Heier et al., 2013; Sreetama et al., 2018). Thus, while increase in lipid mobility following plasma membrane injury is associated with successful repair, excessive lipid mobility leading to membrane instability is detrimental to the repair process. This Goldilocks principle applies to several aspects of lipid involvement in the repair process. In the case of lipid mobility, this principle is exemplified by the observation that decreasing the excessive lipid mobility in LGMD2B patient cells using a membrane permeant modified glucocorticoid improves repair (Sreetama et al., 2018). Similar benefits have been attributed to membrane stabilizing copolymers such as poloxamer 188, that improved repair after physiological mechanical injury (Plataki, Lee, Rasmussen, & Hubmayr, 2011), and injury to dystrophic cells (Houang et al., 2015; Yasuda et al., 2005). While membrane stabilization at the time of injury appears to improve membrane repair, a chronically rigid plasma membrane may inhibit the beneficial effects of transient lipid mobility after injury. Copolymers such as poloxamer 188 avoid this potential issue by only stabilizing the plasma membrane once lipid packing density is sufficiently reduced, such as after an injury. This is due to their lack of integration into the membrane under normal lipid packing conditions. Once membrane tension is restored, the poloxamer compound is forced out of the outer membrane leaflet (Houang, Sham, Bates, & Metzger, 2018). These examples illustrate the far-reaching consequence of lipid movement on structural stability of the plasma membrane and its ability to successfully repair.

Transient change in lipid mobility can have many effects on plasma membrane function related to both structure and signaling, and deciphering which of these are beneficial for repair requires further studies. One of the roles of lipid mobility may be to allow for the movement of individual lipids to form microdomains near the site of injury, which has been shown to be important for repair (Vaughan et al., 2014), perhaps by facilitating lipid-mediated signaling. For example, shear force on the plasma membrane, such as that experienced during a mechanical injury, results in lipid mixing, which increases the mobility of signaling lipids and proteins residing in stable lipid microdomains (Petersen et al., 2016). This may allow these lipids and proteins to interact with new partners that were unavailable due to spatial segregation prior to injury. Furthermore, exposure of the plasma membrane hydrophobic core as a result of reduced lipid packing provides the opportunity for injury-triggered lipid signaling through the binding of cholesterol (see Section 4).

Endogenous mechanisms of repair in healthy cells appear to mimic the beneficial effect provided by poloxamer 188 by allowing for transient increases in lipid mobility while restricting fluidity increase to a relatively small spatial or temporal window. One such mechanism for this may be mediated by the protein MG53. While intracellular MG53 is a redox-sensitive protein capable of facilitating vesicle aggregation, it is proposed to have

multiple functions during membrane repair. It also works extracellularly to improve repair of injured muscle and other cells (Gushchina et al., 2017; Weisleder et al., 2012). Repair of muscle fibers lacking the dysferlin protein, which results in reduced membrane stability, is improved by the presence of extracellular (oxidized) MG53 protein, suggesting that this protein can act on the outer leaflet of the plasma membrane to improve plasma membrane in diseased cells. This raises the intriguing possibility that MG53 may affect plasma membrane structural properties. As MG53 interacts with the plasma membrane in a cholesterol-dependent manner ((Zhu et al., 2012); see Section 4), it's extracellular role may involve a mode of action similar to poloxamer 188, where it inserts within membranes in a disordered lipid environment. This mechanism is particularly intriguing in light of the redox-sensitive nature of MG53. Local oxidation at the site of membrane injury activates MG53 oligomerization (Cai et al., 2009), which may locally increase membrane rigidity. This is notable because it is hypothesized that a beneficial role of decreasing membrane fluidity is preventing the spread of harmful lipid hydroperoxides, which likely form in the oxidative environment near the site of injury (Braughler & Hall, 1992; Hall, Wang, Miller, Cebak, & Hill, 2018). Transient and local lipid peroxidation may affect membrane stabilization as peroxidation of polyunsaturated fatty acids changes head group separation and acyl chain interdigitation, increasing their molecular volume and causing formation of distinct cholesterol domains in the membrane, which increases the membrane rigidity (Jacob & Mason, 2005; Mason, Walter, & Mason, 1997). Thus, local lipid peroxidation may provide transient membrane stabilization, while mechanisms such as redox-dependent MG53 binding may limit the spread of lipid peroxides. Limiting the spread of lipid hydroperoxides may also explain the beneficial effect of vitamin E treatment on plasma membrane repair (Howard, McNeil, & McNeil, 2011; Labazi et al., 2015). Compared with cytosolic antioxidants, which can be detrimental to repair (Spaeth et al., 2012), vitamin E is membrane-localized and could therefore allow for the local buildup of oxidized lipids at the site of injury while preventing the global spread of lipid oxidation. Another endogenous mechanism of acutely decreasing membrane lipid mobility after injury involves the lipid species ceramide, the formation of which causes endocytosis in response to membrane injury through the activity of extracellular acid sphingomyelinase (Tam et al., 2010). This process, facilitated by the dysferlin-mediated release of acid sphingomyelinase (Defour et al., 2014) creates microdomains of ceramide from sphingomyelin. However, ceramide formation also changes membrane dynamics by forming rigid microdomains and potentially by displacing cholesterol from lipid rafts (London, 2004).

3.3.   Lipid-protein interactions shape the repairing membrane

The discussion above illustrates several roles for lipids in changing biophysical properties of the injured plasma membrane during repair. Additional structural changes the plasma membrane must adopt for repair include molding the shape of the membrane to limit wound expansion as well as pulling in the membrane edges to close the wound. In addition to working on their own, lipids also interact with proteins to coordinate these processes. Annexins are a class of calcium-sensitive proteins that rapidly accumulate at the site of a membrane injury (Potez et al., 2011). Their localization and function in response to injury is controlled by the patterning of anionic lipids such as PS, which is present at the wound edge and is a known binding partner of annexins (Gerke, Creutz, & Moss, 2005). Annexins can

physically manipulate the injured plasma membrane by stabilizing, folding, and contracting in order to facilitate repair (Gerke et al., 2005; Jaiswal & Nylandsted, 2015). With their calcium and lipid affinities spanning a wide spectrum, annexins accumulate slightly differently from each other at the site of injury and perform different functions to facilitate repair of the wounded plasma membrane (see Section 4.1). For example, Annexin 5 accumulates first to form a lattice structure, which provides a force opposing the tension at the wound edge (Bouter et al., 2011). This can limit the expansion of the wound area itself. Following this, accumulation of Annexin 4 and Annexin 6 results in folding and inward contraction of the injured membrane, respectively (Boye et al., 2017). In contrast, Annexin 1, one of the earliest responders to plasma membrane injury (Leikina et al., 2015; McNeil, Rescher, Gerke, & McNeil, 2006), does not appear to be essential for plasma membrane repair (Leikina et al., 2015; McNeil et al., 2006). Along with the annexins, which act quickly to physically manipulate the membrane itself, reassembly of the cytoskeleton plays a crucial role in restoring membrane tension in the later stages of repair. This cytoskeletal assembly is regulated by Rho GTPase activity, which triggers F-actin accumulation at the site of injury. Spatial arrangement of lipids is also known to regulate Rho family GTPase activity (see Section 4.3).

4. Signaling role for lipids during plasma membrane repair

For plasma membrane repair to occur successfully, the cell must possess a means to sense that injury has occurred, coordinate the change in activity and localization of repair machinery, and ultimately close the wounded area. To achieve these tasks, cells employ signaling networks, which respond to the changing microenvironment after injury and activate the diverse plasma membrane repair mechanisms with precise control in time and space. The structural role of lipids described above illustrates how their ability to modify the biophysical properties of the plasma membrane is beneficial for resealing. Lipids also react to the changing biochemical environment to become signaling molecules that determine the spatiotemporal dynamics of protein activation (Eyster, 2007) (Figure 1B). This is in part achieved through the activity of lipid modifying enzymes, such as kinases, phosphatases, and phospholipases. Influx of calcium, increase in oxidation, and change in tension on the plasma membrane due to injury all trigger changes in lipid structure and behavior to initiate signaling. Therefore, injury-triggered protein-lipid interactions that result in modified lipids generates signaling that allows for changes in activity and localization of plasma membrane repair machinery. Below we describe the biochemical signaling role of lipids in facilitating plasma membrane repair.

Lipids act as signaling molecules through their ability to directly bind or otherwise alter the activity or localization of target proteins (Figure 2B, C). Lipids may serve as ligands for specific proteins, or act as a scaffold to bring cytosolic proteins to the plasma membrane. Direct lipid modification of proteins, such as GTPases, provides another method to regulate protein localization and activity (Casey, 1995). While a common thread in lipid signaling is the modification of a target protein, either directly or indirectly, the numerous mechanisms lipids use to achieve this outcome allows for a great diversity of signaling. For example, the dynamic arrangement of lipids in the plasma membrane as discussed above, and the electrostatic or chemical changes in lipids due to enzymatic activity of lipid modifying

enzymes rapidly affect lipids themselves as well as the target proteins in the plasma membrane (Figure 2B, C). Collectively, these changes enable lipids to initiate/regulate local signaling allowing precise spatial and temporal control over downstream plasma membrane repair pathways. The goal of signaling during plasma membrane repair is to generate a polarized response such that the repair machinery can be spatially and temporally localized and activated at the repair site. Plasma membrane lipids help with successful repair by being part of the affected entity that also works to sense membrane injury, providing spatial and temporal cues to trigger signaling for downstream repair pathways, and ultimately being the benefactor of the successful wound repair response.

4.1   Sensing and containing the membrane damage

One signaling function of lipids is the recruitment of peripheral membrane proteins to the plasma membrane. As described above, damage to the plasma membrane transiently increases the mobility of individual lipids. This allows for the movement and patterning of lipids into signaling domains, changing the spatial arrangement of proteins that selectively interact with a particular lipid species. In this way, lipids play an important role in polarizing the cellular response to an injury.

The signaling lipid PS is interesting in this regard due to its localization to the wound edge after injury. Accumulation of PS at the site of membrane injury allows it to act as a damage sensor, marking a key site for the recruitment of repair proteins such as annexins (Boye et al., 2017). Unlike Annexin 5 that forms a lattice structure at the wound edge (Bouter et al., 2011), Annexins A4 and A6 work together to mediate constriction of the wounded edges of the plasma membrane, aiding in the closure of the wound (Boye et al., 2017). Presence of PS at the free membrane wound edge helps directs the annexin proteins to this site in a calcium-dependent manner, where they perform vital functions required for stabilization and shaping of the repairing membrane (see Section 3.3). In a similar manner, PS signaling may be involved in the early recruitment of other membrane repair proteins such as dysferlin (McDade et al., 2014; Middel et al., 2016) and MG53 (Cai et al., 2009). In addition to allowing for the lateral translocation of membrane lipids, injury-triggered increase in membrane fluidity also results in reduced lipid packing. This causes the exposure of the membrane hydrophobic core and allows proteins to interact with cholesterol. The MG53 binding protein PTRF (cavin-1) binds cholesterol in response to membrane injury (Zhu et al., 2012). PTRF presumably contributes to the translocation of MG53 to the injury-site, where MG53 is also able to bind PS and become activated in an oxidation-dependent manner (Cai et al., 2009).

While the local accumulation of proteins such as annexins acts to stabilize the structurally unstable membrane after injury, remodeling of the membrane itself can achieve a similar outcome. For example, membrane shedding is a known mechanism of plasma membrane repair, which functions to limit the lateral expansion of the wound area (Andrews, Almeida, & Corrotte, 2014; Horn & Jaiswal, 2018). Formation of these outward budding vesicles at the plasma membrane is associated with an increase in cytosolic calcium and oxidation, as well as the disruption of the actin cytoskeleton–plasma membrane interface (Pollet, Conrard, Cloos, & Tyteca, 2018), and each of these occurs locally in the immediate aftermath of

membrane injury (Andrews et al., 2014; Horn & Jaiswal, 2018). Mechanistically, the process of membrane shedding is mediated by the endosomal sorting complexes required for transport (ESCRT) proteins (Jimenez et al., 2014; Scheffer et al., 2014). ESCRT assembly for membrane shedding is activated by the calcium-dependent ALG-2 protein, which then recruits its interacting partner ALIX leading to subsequent recruitment of the remaining complex members (Scheffer et al., 2014). While this mechanism outlines how ESCRT proteins assemble, the lipid signaling that determines the appropriate spatial localization relative to the membrane injury in order to limit wound expansion has not been elucidated. Of potential interest in this regard is the unconventional phospholipid lysobisphosphatidic acid (LBPA), which is found on endolysosomes. This lipid binds ALIX and is capable of budding vesicles away from the cytosol (Matsuo et al., 2004). Presence of LBPA at the plasma membrane through vesicle fusion could allow for ALIX recruitment and ESCRT assembly (Bissig et al., 2013); however, the presence of LBPA at membrane wound sites has not been directly observed. Following injury, lysosomes are known to fuse with the damaged membrane and may deposit LBPA at the site of injury, which would in turn facilitate ALIX-mediated vesicle shedding. Lysosome fusion is required for the process of repair (Reddy, Caler, & Andrews, 2001). Delivery of membrane (i.e. lipids) is one of the roles proposed for lysosome-mediated repair (Andrews et al., 2014; McNeil, 2002). Another role identified for lysosomal fusion in membrane repair is the secretion of the lipid modifying enzyme acid sphingomyelinase (ASM) (Defour et al., 2014; Tam et al., 2010). ASM activity at the plasma membrane has been shown to facilitate microvesicle shedding (Bianco et al., 2009). Furthermore, ASM activity at the injury site would locally produce ceramide from sphingomyelin. Structurally, the conical ceramide molecule results in negative membrane curvature associated with rapid nucleation of ESCRT complex proteins (Lee, Kai, Carlson, Groves, & Hurley, 2015). Ceramide microdomains formed by the activity of extracellular acid sphingomyelinase near the site of injury could appear on the extracellular leaflet (resulting in endocytosis) or the inner leaflet either by selective flipping across the membrane bilayer (Pollet et al., 2018) or through diffusion of sphingomyelinase through the wounded area to act on inner leaflet sphingomyelin found proximal to the wound edge. In either case, the outward budding of the membrane produced by these ceramide-rich microdomains is thought to assist in microvesicle shedding.

Another role of plasma membrane lipids in wound detection is that of mechanosensing provided by lipid microdomains, such as caveolae, by way of sensing injury-triggered alteration in membrane stress (see Section 3.1). While it is unclear whether caveolae can provide structural assistance to the damaged membrane in the context of a large membrane injury (as opposed to sub-injury threshold stress, (Sinha et al., 2011)), caveolae may still provide a signaling function during the repair process. This signaling function may occur due to the activity of the repair protein EHD2, which accumulates at the site of membrane injury and is thought to play a role in shaping membrane dynamics (Daumke et al., 2007; Demonbreun et al., 2016; Marg et al., 2012). EHD2 localization at caveolae results from its affinity for phosphatidylinositol 4,5-bisphosphate (PIP$_2$), which is enriched around the caveolae boundary (Parton & Del Pozo, 2013; Simone, Caplan, & Naslavsky, 2013). PIP$_2$ also accumulates at the site of membrane injury (Demonbreun et al., 2016; Vaughan et al., 2014). The precise role of EHD2 in membrane repair is not known, although it is known as a

membrane shaping protein that may be involved in membrane fusion (Campelo, Fabrikant, McMahon, & Kozlov, 2010). Interestingly, as a result of caveolae flattening, EHD2 dissociates from the plasma membrane and translocates to the nucleus where it alters gene transcription (Torrino et al., 2018). This suggests the possibility that caveolae could act as mechanosensors that facilitate adaptation to membrane injury through gene transcription, although this remains to be explored.

4.2   Initiating and coordinating downstream repair pathways

Translocation of existing lipid species to the site of injury provides a mechanism to recruit and activate protein machinery. This is especially important in sensing membrane damage. However, lipid signaling also helps to coordinate downstream repair pathways that result in the membrane trafficking and cytoskeletal reorganization required for repair. This is achieved in part through the activity of lipid modifying enzymes, such as phospholipases, which are activated by the changing biochemical environment after injury. These enzymes initiate signaling through the generation of new lipid species, providing an added spatial, as well as a temporal component to lipid signaling, helping to more precisely coordinate the repair response.

Calcium entry after plasma membrane injury is required for repair. Calcium can activate proteins directly, and ultimately is the initiator of many downstream repair pathways. Calcium also initiates lipid signaling after plasma membrane injury by activating phospholipases, such as phospholipase C (PLC). While PLC is able to cleave PC, the classic substrate of PLC is PIP_2, which upon cleavage generates the membrane bound DAG and the cytosolic inositol trisphosphate (IP_3) – both of which are increased after injury (Lamb, Harper, McKinney, Rzigalinski, & Ellis, 1997; Vaughan et al., 2014). Similar to PS, DAG directly binds proteins to provide its signaling function; however, because it is generated de novo after injury, an additional temporal component to signaling is achieved. Additionally, in contrast to PS, which recruits proteins directly involved in repair, DAG appears to recruit signaling proteins such as protein kinase C (PKC) (Vaughan et al., 2014; Zuzek, Fan, Spaeth, & Bittner, 2013). PKC, in turn, influences a wide variety of downstream pathways known to facilitate repair including vesicle fusion, Rho GTPase patterning, as well as repair potentiation (Togo, 2004; Vaughan et al., 2014; Zuzek et al., 2013). Thus, DAG acts as a scaffold that initiates and coordinates downstream signaling pathways within a tight spatial and temporal window rather than as a marker of membrane injury for fast-acting structural proteins. Along with DAG, IP_3 is also generated by the activity of PLC. It is unclear what role, if any, that IP_3 may have in repair, but its role in calcium signaling and the fact that injured cells secrete IP_3 for hours post-injury (Lamb et al., 1997) suggest a possible signaling role in repair that may extend beyond the process of membrane resealing, which needs further investigation.

Similar to PLC, phospholipase D (PLD) activity is also increased after injury and is required for repair (Arun et al., 2013). While PLD is also activated by calcium, recent findings have provided insight into the role of mechanical stress on initiating lipid signaling regulated by PLD (Petersen et al., 2016). This study showed that shear force on the membrane, as experienced by cells during mechanical injury, facilitated increased PLD activity through the

process of lipid mixing. Prior to increase in shear force, PLD associates with lipid rafts, physically segregated from its activator PIP_2 and its substrate PC (Petersen et al., 2016). However, shear force on the membrane increases the kinetic energy required for lipid mixing (i.e. increased lipid fluidity described in Section 3) and allows for the membrane associated PLD to diffuse outward from lipid rafts and interact with PIP_2 in order to metabolize PC. Importantly, sequestration of cholesterol alone increased PLD activity, supporting the idea that transient increase in lipid fluidity after membrane injury may be required for PLD-mediated signaling. The product of PC metabolism by PLD is the signaling lipid PA (Cazzolli, Shemon, Fang, & Hughes, 2006). While no defined roles for PA after membrane injury are known, PA has been observed to rapidly appear at the wound edge (Vaughan et al., 2014), which fits with the fast (650 μs) timescale of PA generation by PLD (Petersen et al., 2016). Further, PA is implicated in processes critical to the success of membrane repair such as vesicle fusion with the plasma membrane and GTPase signaling (Cazzolli et al., 2006; Zhang & Du, 2009). In addition to PA, PG is another lipid product of PLD activity relevant to membrane repair, and its synthesis has been implicated in the process of membrane repair and repair of epithelial wounds (Arun et al., 2013; Piazza & Marmer, 2007).

4.3 Closing the wound and membrane remodeling

Wound closure is mediated in part by the interactions of the cortical actin cytoskeleton with the plasma membrane. While initial depolymerization of the local F-actin network is thought to assist with vesicle fusion and membrane shedding, delayed accumulation of F-actin may facilitate repair either by working in coordination with myosin to pull the wounded membrane edges toward each other or by providing a barrier and stabilizing function for the newly formed membrane. F-actin accumulation is also responsible for providing support to the newly resealed membrane, restoring tension, and preventing subsequent injury. F-actin reorganization during repair is coordinated by the activity of lipids through their regulation of signaling proteins (reviewed in (Horn & Jaiswal, 2018)), as well as through direct interaction with actin-binding proteins such as F-actin bundling by the Annexin A2-S100 A11 complex (Jaiswal et al., 2014).

Small GTPases, such as Rho family members, associate with biological membranes via lipid modifications (ten Klooster & Hordijk, 2007); however, selectivity for the plasma membrane is regulated through the polybasic domain comprised of a cluster of positively charged amino acids (Do Heo et al., 2006; Maxwell, Zhou, & Hancock, 2018). This allows small GTPases, such as Rac1 and Cdc42 to preferentially bind the negatively charged PIP_2 and PIP_3 (Johnson, Erickson, & Cerione, 2012; Maxwell et al., 2018; Remorino et al., 2017). Thus, electrostatic interaction with signaling lipids helps to facilitate GTPase localization, which is a critical feature that determines the efficiency and magnitude of the downstream response (Das et al., 2015; Yogurtcu & Johnson, 2018). These lipids also exist at the boundary of lipid-ordered domains, such as lipid rafts, indicating that GTPases are targeted to these regions where protein accumulation at the membrane is common (Moissoglu et al., 2014), increasing their relative signaling capacity. Rac1, a Rho family GTPase required for repair (Verboon & Parkhurst, 2015), forms nanoclusters at sites enriched in PA and PIP_3, whose roles in regulating Rac1 appear to be non-overlapping (Maxwell et al., 2018). While

PA mediates targeting to the membrane, Rac1 activity depends on PIP_3, suggesting multiple roles for signaling lipids in GTPase activity after repair.

The spatial arrangement of lipids at the plasma membrane is not only important for GTPase recruitment, but also for their activity. GTPases are molecular switches that require the cycling of nucleotides to remain active. This process is more efficient when GTPases and their regulatory proteins (which are themselves regulated by lipids) are clustered (Ligeti, Dagher, Hernandez, Koleske, & Settleman, 2004). The nanoclusters appear to form specifically at the boundary of ordered raft domains and disordered domains where signaling lipids such as PIP_3 and PIP_2 are found. Shear stress on the plasma membrane also results in the dissociation of the negative regulator RhoGDI and its binding partner Rho (Shao et al., 2018). This dissociation has the effect of allowing Rho GTPase translocation to the membrane where it can interact with signaling lipids. Intriguingly, PA preferentially binds the Rho family member Rac1, resulting in nanocluster formation (Maxwell et al., 2018).

In addition to regulating the patterning and activity of Rho family GTPases, lipids also have a more direct role in regulating F-actin association with the plasma membrane. The membrane phosphoinositides, and PIP_2 in particular, play an important role in regulating the interaction of F-actin with the plasma membrane (Kapus & Janmey, 2013; Saarikangas, Zhao, & Lappalainen, 2010). In general, PIP_2 is a positive regulator of F-actin polymerization and the presence of PIP_2 increases the stability of the actin cytoskeleton–plasma membrane interface. This occurs primarily through the direct interaction of PIP_2 with actin-binding proteins, and change in PIP_2 distribution has been shown to precede actin build-up at the plasma membrane (Nebl, Oh, & Luna, 2000; Senju & Lappalainen, 2019; Tran, Masedunskas, Weigert, & Ten Hagen, 2015). PIP_2 dynamics after plasma membrane injury support a role for PIP_2 in actin assembly during repair as its accumulation near the site of injury is generally delayed. Myofiber injury in zebrafish was found to cause rapid loss of PIP_2, followed by restoration to pre-injury levels by 30 seconds post-injury (Middel et al., 2016). Similarly, peak PIP_2 accumulation at the injury site occurred 45 seconds post-injury in *Xenopus* oocytes (Vaughan et al., 2014). PIP_2 accumulation was observed as early as 4 seconds in mouse myofibers; however, it continued to accumulate even 1 minute post-injury suggesting a role in the later stages of repair (Demonbreun et al., 2016). These observations on PIP_2 kinetics line up remarkably well with the accumulation of F-actin at the injury site, which begins around 30 seconds after injury and extends for several minutes (Godin, Vergen, Prakash, Pagano, & Hubmayr, 2011; Horn et al., 2017). Regulation of actin binding proteins by PIP_2 occurs in part through electrostatic interactions (Senju et al., 2017), suggesting that clusters of PIP_2 molecules may be necessary to achieve build-up of F-actin itself. Interestingly, several methods for PIP_2 micro-domain formation may allow for this to occur after plasma membrane injury. The primary method for PIP_2 formation in cells is by the activity of PI(4)P-5 kinase (PI5K) (Kolay, Basu, & Raghu, 2016). PI5K activity is itself driven by regulators of membrane repair including Rho GTPases (Gilmore & Burridge, 1996) and PLD (Roach et al., 2012). PLD-mediated activation of PI5K relies on the formation of PA, which itself is able to determine the spatial localization of PI5K as well as cause its activation (Roach et al., 2012). Intriguingly, PIP_2 is needed for PLD activity suggesting the possibility that a feed-forward loop leads to increasing PIP_2 concentrations as repair progresses ultimately facilitating the necessary build-up of F-actin (Figure 1B).

The lipid-mediated cytoskeletal rearrangement described above provides the cell with a mechanism to close the wounded site and add structural support to the newly resealed membrane. This phase represents restoration of the barrier function of the plasma membrane; however, cells must still undergo a membrane remodeling phase due to the presence of cytoskeletal as well as other repair proteins and lipids that accumulate during the repair process. Without adequate remodeling, the plasma membrane protein and lipid composition would change dramatically, particularly after repeat injuries, and no longer function as in its pre-injury state. This remodeling is likely provided by processes described previously: membrane endocytosis (Section 3.1) and membrane shedding (Section 4.1). These remodeling events actively promote plasma membrane repair; however, they also act as extensions of the repair response and may continue long after successful resealing in order to restore the plasma membrane to its pre-injury state.

5. Lipids as a mediator of tissue repair

Failure of injured cells to repair results in cell death and activates a tissue repair response. Despite the many different types of tissue, there is a common repair program involved in tissue repair. This involves a series of distinct, but mutually dependent stages including inflammation, regeneration, and remodeling of the tissue (Gurtner, Werner, Barrandon, & Longaker, 2008). Use of lipidomics during epidermal wound repair identified that several of the plasma membrane lipids discussed above are enriched during wound repair. They include glycerophosphocholines, glycerophosphoglycerols, glycerophosphoinositols, as well as triacylglycerols (Taverna, Nanney, Pollins, Sindona, & Caprioli, 2011). By direct imaging of skeletal myofibers during the course of repair from a focal injury, exposure of PS on the injured myofiber surface was found to attract macrophages to the injury site. This helps to remodel the newly repaired plasma membrane (Middel et al., 2016), but may also serve to potentiate tissue inflammation.

Inflammatory exudates provide insights into the regulation of the first stage of wound repair – inflammation. They consist of a variety of lipid mediators derived from the omega-3 essential fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and include lipoxins, resolvins and protectins. These mediators promote wound resolution by acting upon the immune and endothelial cells and help to control the duration and nature of the immune and phagocytic cell infiltration at the injury site (Serhan, 2014). Alteration in this response inhibits the subsequent stages, tissue regeneration and remodeling, leading to increased tissue scarring. The second stage of tissue repair, regeneration, makes use of signaling by different lipids, one of which is sphingolipid. As discussed above, sphingolipids in the plasma membrane are an important regulator of plasma membrane repair. During regeneration, sphingolipids such as sphingosine 1-phosphate (S1P) are known for their growth promoting effect on tissue resident stem cells (Calise et al., 2012; Nojima, Freeman, Gulbins, & Lentsch, 2015). Another broad group of lipid carriers that are recognized for their role in activating stem cells are extracellular vesicles (EVs), which are released locally at the site of injury or from a distant site and through their lipid and other cargoes regulate regeneration of injured tissues by way of stem cell activation (Riazifar, Pone, Lötvall, & Zhao, 2017). EVs are known to be generated in response to plasma membrane injury of single cells, and this is required for successful repair (Jimenez et al., 2014; Scheffer et al.,

2014). Given their ability to traverse away from the site of synthesis (Verweij et al., 2019), the EVs generated during plasma membrane injury may activate both local and distant tissue regeneration responses needed for repair. This method of extracellular communication may allow for the proper execution of inflammatory and regenerative responses needed for appropriate tissue remodeling required to restore organ function. Failure or delay in these processes, as in chronic inflammatory conditions and conditions of regenerative deficit would lead to aberrant tissue remodeling resulting in fibrotic or adipogenic replacement of the lost tissue.

6. Conclusion

While often considered to be a passive resident of the plasma membrane, there is ample evidence to support a more active role of lipids in the process of plasma membrane repair as well as tissue repair. In this review, we have discussed how lipids working at the individual as well as at the population level facilitate the proper orchestration of the repair response. These roles of lipids in plasma membrane repair include both a structural role and a signaling role. Rather than these roles being separated from each other, they overlap significantly such that one can easily be an extension of the other. The structural role of lipids may extend into the signaling role, which can then impact back on the structural characteristics of the repairing membrane by changing the composition or distribution of individual lipids. Thus, the signaling role of lipids affects membrane structure and how the wound is eventually resealed and remodeled, returning the plasma membrane to homeostasis. We have briefly discussed how such an integral role of lipids in plasma membrane repair also extends into tissue-level repair and restoration of organ function. This role of lipids extends further by way of regulating the response of proteins during the repair process as well as long-term gene expression-based adaptations required for the recovery of injured cells and tissues. Thus, it is clear that there is a need to acknowledge the role of lipids as an active participant in the processes of both cell and tissue repair.

Acknowledgements

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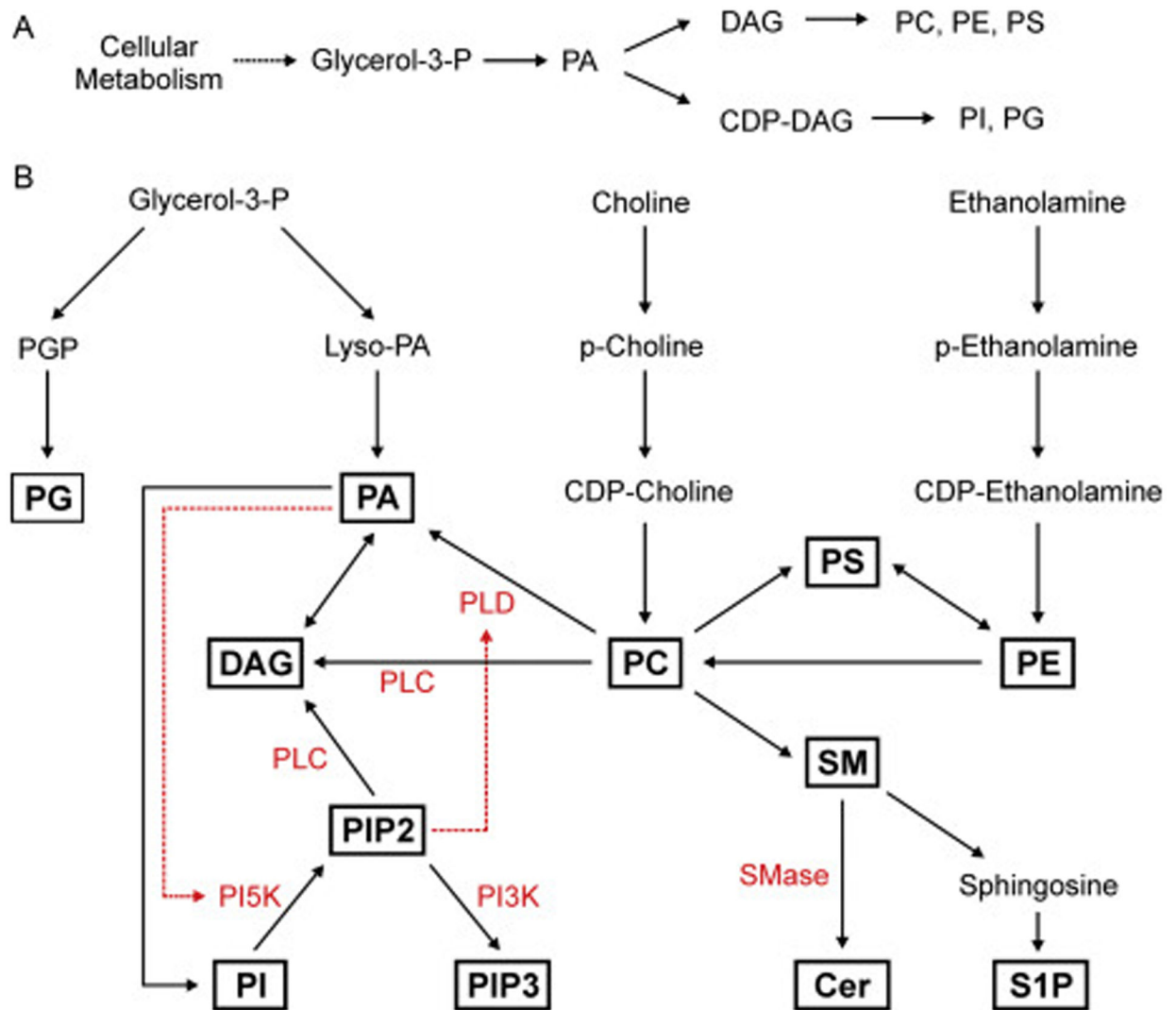
Figure 1: Lipid biosynthesis and signaling interactions.
(A) Plasma membrane phospholipids are generated from biosynthetic pathways, which rely on formation of the molecule diacylglycerol (DAG) or cytidine diphosphate diacylglycerol (CDP-DAG) from phosphatidic acid (PA). PA is itself generated primarily from glycerol-3-phosphate, which is a product of glycolysis. Phospholipid biosynthetic pathways require the addition of different head groups to the DAG backbone. **(B)** Phospholipid biosynthesis depends on multiple inputs from metabolism (glycerol-3-phosphate) and extracellular import (choline and ethanolamine). Glycerol-3-phosphate is used to generate PA as well as PG through the intermediate phosphatidylglycerol phosphate (PGP). PA is then used to create other phospholipids including PI, PC, PS, and PE, through the use of the DAG backbone. Phospholipids and sphingolipids are connected by the head group choline, found on both PC and sphingomyelin (SM). SM utilizes the ceramide (Cer) backbone and can be used to generate the signaling lipid sphingosine-1-phosphate (S1P). Plasma membrane lipids are routinely interconverted as a means to maintain lipid homeostasis and for lipid-mediated signaling. Enzymes that generate lipid signaling after plasma membrane injury (in red) include the phospholipases C and D (PLC and PLD), phosphatidyloinositol kinases (PI5K

and PI3K), and sphingomyelinase (SMase). Each of these provides another mechanism for generating specific membrane lipids acutely in parallel to biosynthetic inputs.
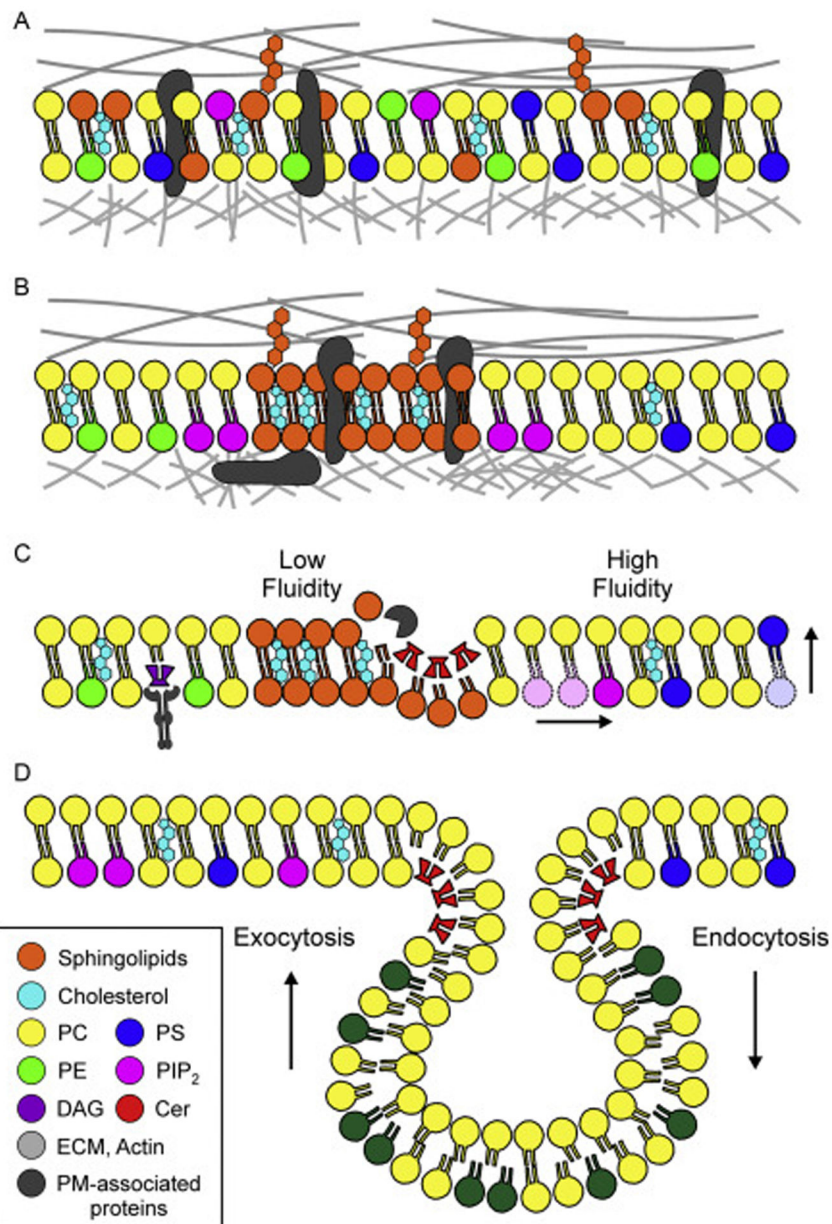
Figure 2: Plasma membrane structure and dynamics.

(A) The plasma membrane is a fluid-mosaic of lipids, including glycerophospholipids, sphingolipids, and cholesterol, as well as plasma membrane-associated proteins. Plasma membrane lipids and proteins interact with the extracellular matrix (ECM) and the cortical actin network, both of which provide sources of tension that support the structure of the membrane. **(B)** The plasma membrane is not a homogenous mixture of proteins and lipids (as in A). Instead, distinct inter- and intra-leaflet heterogeneity exists. Charged phospholipids such as PIP_2, PS, and PE are almost exclusively found on the inner leaflet, while the glycosphingolipids are only found on the outer leaflet. Within a single leaflet, lateral heterogeneity is found in the form of lipid microdomains, such as the cholesterol and sphingolipid-rich domains that serve as signaling platforms to accumulate plasma

membrane-associated proteins. The organization of lipids within the membrane also affects the structure of underlying cortical cytoskeleton. For example, PIP$_2$ interacts with actin-associated proteins, resulting in a local strengthening of the plasma membrane-cytoskeleton interface. **(C)** Plasma membrane is dynamic and individual lipids have the capacity to move laterally within a leaflet (shown in pink) and between the leaflets (shown in blue). The fluidity of the membrane is determined in part by its composition, with cholesterol and sphingolipid-rich regions being less fluid than those areas comprised primarily of phospholipids. Furthermore, individual lipids may be modified by proteins, which generate new lipid species that can change membrane structural properties (red, Cer) or be used for signaling (purple, DAG). **(D)** The lipid make-up of the plasma membrane constantly changes. To regulate the composition of plasma membrane lipids, cells utilize vesicle trafficking, which can add lipids (by exocytosis) or remove lipids (by endocytosis) from the plasma membrane. This form of membrane remodeling maintains lipid homeostasis and plasma membrane functionality.