Bacterial membrane dynamics

Bramkamp, Marc; Scheffers, Dirk-Jan

Published in:
Molecular Microbiology

DOI:
10.1111/mmi.15077

Publication date:
2023

Received: 31 March 2023 | Revised: 8 May 2023 | Accepted: 10 May 2023

DOI: 10.1111/mmi.15077

REVIEW ARTICLE

WILEY

Bacterial membrane dynamics: Compartmentalization and repair

Marc Bramkamp [1] | Dirk-Jan Scheffers [2]

[1]Institute for General Microbiology, Christian-Albrechts-University Kiel, Kiel, Germany

[2]Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, the Netherlands

Correspondence
Marc Bramkamp, Institute for General Microbiology, Christian-Albrechts-University Kiel, Kiel, Germany.
Email: bramkamp@ifam.uni-kiel.de

Dirk-Jan Scheffers, Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, the Netherlands.
Email: d.j.scheffers@rug.nl

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: BR2915/7-1; Nederlandse Organisatie voor Wetenschappelijk Onderzoek, Grant/Award Number: 21.401

Abstract

In every bacterial cell, the plasma membrane plays a key role in viability as it forms a selective barrier between the inside of the cell and its environment. This barrier function depends on the physical state of the lipid bilayer and the proteins embedded or associated with the bilayer. Over the past decade or so, it has become apparent that many membrane-organizing proteins and principles, which were described in eukaryote systems, are ubiquitous and play important roles in bacterial cells. In this minireview, we focus on the enigmatic roles of bacterial flotillins in membrane compartmentalization and bacterial dynamins and ESCRT-like systems in membrane repair and remodeling.

KEYWORDS
dynamin, ESCRT, flotillin, functional membrane microdomain, membrane fluidity, membrane repair

1 | INTRODUCTION

Although it is recognized for over 30 years that bacterial membrane proteins can be localized to specific regions such as the cell pole and the division site (Laloux & Jacobs-Wagner, 2014), this has long been considered to happen in a fairly homogeneous mixture of membrane lipids. Nowadays, more and more evidences accumulate that there are membrane domains in the cell that also differ in lipid composition and physical properties, although these domains are poorly defined, and it is still unclear how these domains are linked to protein function. Various cellular functions such as signaling, cell wall synthesis, and division have been described as taking place or requiring domains containing specific lipids and organizing proteins such as flotillins that interact with proteins involved in these functions (Boekema et al., 2013; Bramkamp & Lopez, 2015; Lopez & Koch, 2017; Matsumoto et al., 2006; Strahl & Errington, 2017; Wagner et al., 2017). Other studies argue for a more architectural role of these domains, controlling membrane fluidity (Zielińska et al., 2020). Here, we aim to summarize recent discoveries about bacterial membrane domains and membrane biophysics that have led to new insights in how the membrane is organized, remodeled, and repaired. We also discuss outstanding questions that should be addressed for the field to conceptually move forward. Membrane shaping and curvature, vesicle formation, and lipid asymmetry are (largely) beyond the scope of this short review, but have been excellently described or reviewed in various recent papers (Phillips

et al., 2021; Siebenaller & Schneider, 2023; Strahl & Errington, 2017; Toyofuku, 2019).

2 | MEMBRANE DYNAMICS: REGULATION OF FLUIDITY

Membrane lipids undergo specific interactions with other lipids and proteins. These interactions depend on the nature of the lipid and also on external factors such as temperature or solvents. At low temperatures, the membrane is typically in a solid ordered (S_o) phase (also called gel phase). In this phase, lipids are highly ordered and have little lateral mobility. Upon increasing temperature, the membrane melts, leading to an increase in lateral mobility, or fluidity, of the lipids. This phase is called liquid disordered (Ld) or liquid crystalline phase. Lipids in the Ld phase are characterized by higher diffusion coefficients. The interaction of planar lipids such as cholesterol (mainly in eukaryotes) or hopanoids (in bacteria) organizes the membrane into a liquid ordered (Lo) phase. This phase is usually attributed to be the phase of the so-called lipid rafts. Importantly, biological membrane can exist in more than one phase (usually Ld and Lo phase), and hence, phase separation can help to compartmentalize the membrane (Strahl & Errington, 2017).

The study of the regulation of membrane fluidity, which is critical to correct function, has primarily focused on the adaptation of membrane lipids in organisms to changes in growth temperature (or pH, pressure) with the aim of keeping the membrane in the liquid-crystalline phase—so-called homo-viscous adaptation (Mansilla & de Mendoza, 2019; Siliakus et al., 2017; Sinensky, 1974). This adaptation prevents a phase transition to the gel phase (S_o) at lower temperatures, where the membrane would rigidify and be much less dynamic, and phase transition to a fully fluid phase at higher temperatures, which would result in a loss of the barrier function of the membrane.

A systematic study on *Bacillus subtilis* grown over a range of temperatures showed that the bacterium increases the average length of fatty acid acyl chains, their degree of saturation, and the ratio of iso- and anteiso branched fatty acids with temperature, with the net result of keeping proton permeability of the membrane constant over the range of growth temperatures (van de Vossenberg et al., 1999). Various systems exist to regulate fatty acid synthesis to control fluidity, such as temperature sensitivity of enzymes involved in fatty acid synthesis, as well as a two-component signaling system (Des) that directly senses membrane thickness and controls transcription of desaturases that introduce double bonds in fatty acid chains (Cybulski et al., 2010; Mansilla & de Mendoza, 2019). Recently, an important study described the effect of tuning membrane fluidity by synthetically lowering fluidity in engineered *Escherichia coli* and *B. subtilis* strains, which resulted in the phase separation in the membranes into gel phase and fluid like lipid domains (Gohrbandt et al., 2022). Strikingly, this large-scale phase separation was not lethal, indicating that despite the presence of gel phase domains in the membrane, the permeability function of the membrane remained intact. Nevertheless, many cellular processes were affected due to the crowding of membrane proteins in the fluid like regions, and the membrane potential of these cells was significantly lowered (Gohrbandt et al., 2022).

Although overall fluidity is controlled to keep the membrane in a liquid crystalline phase, recent work has shown that local demixing of membranes into Liquid ordered (Lo) and Liquid disordered (Ld) phases occurs in model systems, provided that the membranes are composed of a mix of fatty acids with saturated and unsaturated acyl chains combined with hopanoids, pentacyclic compounds from bacterial membranes with a function analogous to cholesterol in eukaryotic membranes (Mangiarotti et al., 2019). Evidence for local phase separation in nanoscale Lo domains was also found in live *B. subtilis* cells using neutron scattering, with the domains being up to 40 nm in diameter (Nickels et al., 2017). These domains are too small to image by conventional light microscopy, which has been used to study asymmetric distribution of lipids and membrane fluidity in bacterial cells. One of the earliest dyes to be used to study specific lipid distributions was nonyl acridine orange (NAO). The NAO fluorescence shift toward the red spectrum was thought to be specific for cardiolipin (Mileykovskaya & Dowhan, 2000), but it was later shown that this effect is unspecific upon binding to anionic phospholipids (Oliver et al., 2014; Pogmore et al., 2018). Still, NAO staining identified polar domains in many bacterial species (Strahl & Errington, 2017), and the enrichment of cardiolipin in polar regions was independently confirmed by analysis of the lipid content of *E. coli* minicells (Koppelman et al., 2001), which fits the idea that the conical shape of cardiolipin is a driver for accumulation at the more strongly curved poles of bacterial cells. It is assumed but not proven that the accumulation of cardiolipin and anionic phospholipids at the cell pole serves specific functions, for example, in transertion (below), in chemoreception, or in cell division which generates new cell poles after division.

Staining with DiI-C12, a membrane stain with a preference for fluid areas (Ld domains), showed that there are specific areas in the bacterial membrane distributed along the entire cell surface, where fluidity is increased (Figure 1) (Oswald et al., 2016; Strahl et al., 2014). These areas colocalize with, and are organized by, the peripheral membrane protein MreB and were termed Regions of Increased Fluidity (RIFs) (Strahl et al., 2014). MreB is a critical component of the peptidoglycan synthesis machinery, coordinating the "elongasome" which is a protein complex involved in synthesis of peptidoglycan in the lateral wall of nonspherical bacteria (Egan et al., 2020). These regions are dynamic, and MreB dynamics is directly linked to peptidoglycan synthesis activity (Dominguez-Escobar et al., 2011; Garner et al., 2011; van Teeffelen et al., 2011). Peptidoglycan synthesis requires the presence of Lipid II, the peptidoglycan precursor that is embedded in membranes via an isoprenoid chain and that has been shown to prefer a more fluid membrane environment and in fact induces membrane disorder (Ganchev et al., 2006; Jia et al., 2011). Whether the increased fluidity in RIFs is a direct consequence of the presence of Lipid II, or that RIFs are created to produce a favorable environment for Lipid II and peptidoglycan synthesis, remains to be

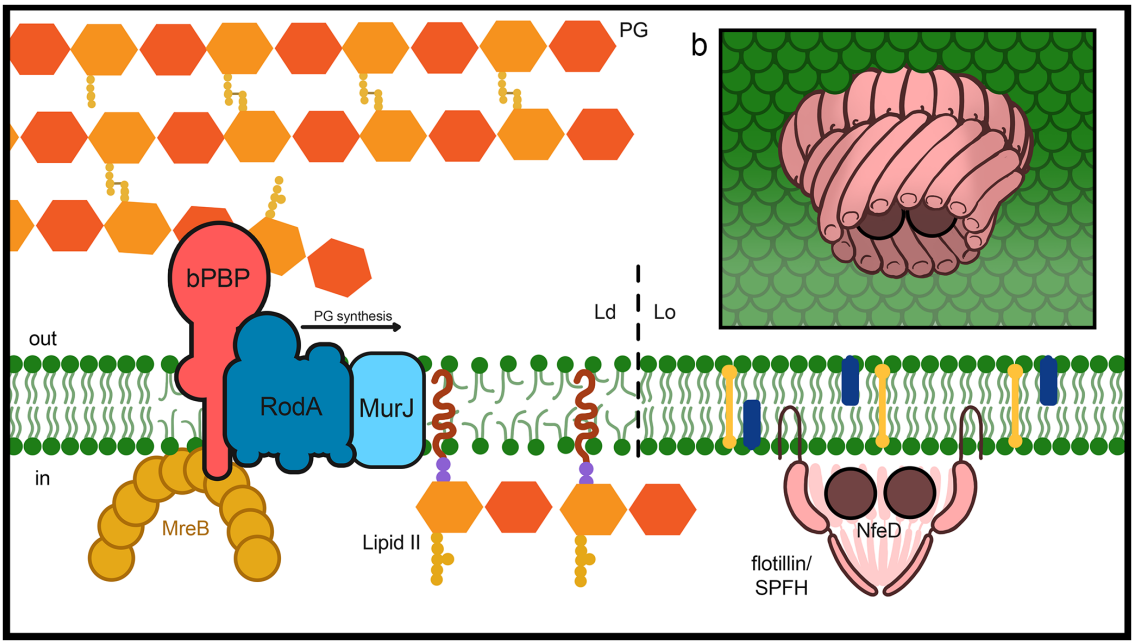

FIGURE 1 Membrane regions with increased and decreased fluidity. Left: Zones of MreB/SEDS/bPBP mediated peptidoglycan synthesis correlate with Liquid disordered (Ld) membranes. Lipid II prefers Ld membrane zones and is translocated by MurJ and inserted into a nascent peptidoglycan strand via the concerted action of a SEDS family glycosyl transferase (RodA) and a Class B Penicillin Binding Protein (bPBP) transpeptidase. Right: Flotillins oligomerize on the membrane, hypothetically forming cage-like structures as seen for the SPFH proteins HflK/C. Putatively, these structures organize Liquid ordered (Lo) domains in the membrane, the so-called Functional Membrane Microdomains (FMMs). Associated with these FMMs are specific lipids such as carotenoids (dark yellow) and hopanoids (dark blue), which could either be on the outside of or enclosed by (or both) the flotillin ring. The SPFH domain cage could provide a space for specific flotillin-associated proteins, such as the NfeD proteases. Insert (b): View of the hypothetical flotillin cage from the cytoplasmic side, with phospholipid headgroups in green.

established. In the absence of MreB and elongasome function, peptidoglycan synthesis in the lateral wall is mediated by class aPBPs, bifunctional peptidoglycan synthases, but the orientation of synthesis by aPBPs is random rather than directional (Dion et al., 2019). It may be that the presence of a more fluid membrane (RIF) allows rapid vectorial movement of the elongasome driven by MreB polymerization (Figure 1).

The nanometer-scale regions of decreased fluidity (Lo domains) observed by neutron scattering in *B. subtilis* are similar in size to membrane patches formed by bacterial flotillin homologs, of which *B. subtilis* has two (FloA and FloT) (Nickels et al., 2017; Schneider et al., 2015). Flotillins, in eukaryotes, are found in membrane domains known as "lipid rafts," which are enriched in sphingolipids and cholesterol. Eukaryotic flotillins are bound to the membrane via palmitoyl- or myristoyl-membrane anchors and contain an N-terminal SPFH (Stromatin, Prohibitin, Flotillin, and HflK/C) domain that associates with the membrane, and a flotillin domain that contains coiled-coil structures that mediate homo-oligomerization. Flotillin homologs are widespread in bacteria, but are anchored to the membrane via an N-terminal hairpin loop or a transmembrane segment, instead of via attached lipids (Bach & Bramkamp, 2015; Bramkamp & Lopez, 2015; Lopez & Koch, 2017). Also, the SPFH domain in bacterial flotillins aids in oligomerization rather than in membrane attachment (Bach & Bramkamp, 2015). Bacterial flotillins, like their eukaryotic counterparts, are enriched in fractions of the membrane that are more resistant to detergent solubilization (detergent resistant membrane, DRM) in fractionation studies and have therefore been associated with a more rigid membrane structure (Lingwood & Simons, 2007). However, it is commonly accepted now that detergent extraction at low temperatures likely induces changes in the membrane organization and DRM fractions thus do not represent lipid and protein assemblies as they occur in the native membrane structure. Analysis of membrane fractions from *B. subtilis* and *Staphylococcus aureus* showed that specific lipids, negatively charged phospholipids and carotenoids, respectively, are enriched in the detergent resistant membrane fraction (DRM) and, in the case of *S. aureus* staphyloxanthin, directly interact with flotillins (Donovan & Bramkamp, 2009; García-Fernández et al., 2017). This indicates that the flotillin oligomers, also known as Functional Membrane Microdomains (FMMs), may interact with specific lipids (Figure 1), as has been shown for several eukaryotic SPFH-domain containing proteins that directly bind cholesterol (Huber et al., 2006). Deletion of flotillins results in an overall increase of membrane rigidity and loss of membrane heterogeneity (Bach & Bramkamp, 2013; Zielińska et al., 2020). Interestingly, similar to the deletion of flotillins, loss of *yisP*, required for the production of farnesol (Feng et al., 2014), leads to an increase in membrane rigidity (Bach & Bramkamp, 2013). Thus, a *yisP* deletion phenocopies a flotillin deletion and both phenotypes can be partially restored by adding carotenoids to cells, for example, staphyloxanthin, which was found to be associated with flotillins in

S. aureus (Bach & Bramkamp, 2013; García-Fernández et al., 2017; Lopez & Kolter, 2010).

3 | CURRENT QUESTIONS SURROUNDING FLOTILLIN FUNCTION AND FMMS

3.1 | What is the relation between DRMs and FMMs?

Following the initial description of bacterial flotillins and the finding that several proteins found in DRMs are no longer found in DRMs when flotillins are absent (Donovan & Bramkamp, 2009; Lopez & Kolter, 2010), the protein composition of DRMs was characterized in various studies, combined with pull-down or native PAGE to enrich proteins physically associated with flotillins (Bach & Bramkamp, 2013; García-Fernández et al., 2017; Schneider et al., 2015; Yepes et al., 2012). This identified a large set of membrane proteins involved in various processes such as signaling, cell division, protein secretion, and peptidoglycan synthesis, which fit the rather pleiotropic phenotypes observed with Flotillin deletions. Careful super-resolution microscopy analysis of membrane protein dynamics revealed that many of the proteins identified as FMM constituents/Flotillin binding partners were in fact not, or only transiently, associated with Flotillins, with the exception of NfeD2 (Dempwolff et al., 2016). Also, when the surface area occupied by FloA and FloT foci is calculated in a *B. subtilis* cell (Schneider et al., 2015), the FMMs occupy less than 2% of the total membrane surface area. It is therefore likely that the DRM fraction contains a much larger fraction of the membrane than only FMMs. Another intriguing question is why deletion of genes involved in the synthesis of carotenoids, which organize Lo domains, leads to the overall *rigidification* of membranes and the reduction (based on protein content) of DRM fractions (Bach & Bramkamp, 2013; Lopez & Kolter, 2010).

3.2 | What is the function of flotillins?

The transient association with a plethora of membrane proteins that function in (homo)oligomeric complexes, many of which function suboptimally when flotillins are absent, led to the hypothesis that flotillins act as oligomerization platforms that ensure correct formation of these membrane protein complexes (Lopez & Koch, 2017). Native PAGE experiments identified such a role in the formation of cell wall synthesis complexes in *S. aureus* (García-Fernández et al., 2017), but this was not confirmed in a similar analysis of cell wall synthesis proteins from *B. subtilis* (Zielińska et al., 2020). Flotillins themselves cluster in high-molecular-weight oligomers (12 to 50 mers), which corresponds with their appearance in distinct patches in the membrane (Bach & Bramkamp, 2013; Dempwolff et al., 2016; Schneider et al., 2015). In vitro, flotillins have been shown to fluidize the membranes, and the membrane rigidification observed in the absence of flotillins in vivo results in a reduced dynamics of MreB, which can be restored when a chemical fluidizer is added to cells (Zielińska et al., 2020). MreB dynamics are directly coupled to elongasome-mediated cell wall synthesis—which, in *B. subtilis*, becomes upregulated in the absence of the aPBP1 (Patel et al., 2020). This explains the observed growth defect of strains that lack both aPBP1 and flotillins: The elongasome, which is now the principal source of peptidoglycan, cannot move fast enough through the membrane (Zielińska et al., 2020). It is not unlikely that the observed rigidification also impacts other membrane processes linked to flotillins (Bramkamp & Lopez, 2015; Lopez & Koch, 2017), such as division, sporulation, signaling, biofilm formation, and protein secretion. Various important questions remain, such as the biophysical mechanism by which flotillins fluidize the membrane and how flotillins interact with other membrane proteins and lipids. An important clue may be found in the recently described structures of the HflK/C-FtsH protease complex (Daumke & Lewin, 2022; Ma et al., 2022; Qiao et al., 2022). HflK and HflC are both proteins with a single transmembrane segment and an SPFH domain, and 12 subunits of each protein together were found to form a large membrane embedded ring that forms a cage like structure on top of the membrane outer leaflet which encloses four FtsH hexamers (Ma et al., 2022; Qiao et al., 2022). The SPFH domains interact at the base of this cage, close to the membrane, with long α-helices forming the barrel of the cage, which is closed on top by a β-barrel formed of β-strands from the individual subunits. It is quite likely that the oligomers formed by flotillins, through interaction of their SPFH domains (Bach & Bramkamp, 2015), can form a similar cage like structure at the inner leaflet of the membrane, anchored to the membrane by hairpin loops or transmembrane segments. Such a ring-like structure would create a circular microdomain, and the extensive interactions could act as a scaffold structure for Liquid–Liquid Phase Separation (Azaldegui et al., 2021; Daumke & Lewin, 2022) to create a microdomain to which, for example, specific lipids, such as hopanoids, could be recruited. Various protein classes containing SPFH domains interact with proteases, such as the HflK/C-FtsH combination (Kihara et al., 1996). Flotillins are often found in genetic organization, but also interacting and colocalizing with a cognate NfeD protein that often contains a serine protease domain and is anchored to the membrane via several transmembrane helices (Dempwolff, Moller, & Graumann, 2012; Hinderhofer et al., 2009). The association with proteases could be a hint that flotillins provide microdomains where membrane-anchored proteases can perform their function in cellular quality control.

Recruitment of specific lipids (such as hopanoids) to these microdomains may facilitate the formation of Ld domains (with Lipid II) elsewhere in the membrane. Interestingly, the effect of flotillins on membrane fluidity was only observed in cells growing fast on rich medium (Zielińska et al., 2020). This suggests that specific lipids, produced when cells grow fast, can be found in these patches—even though the in vitro data strongly suggest that flotillins do not need a specific lipid to fluidize a membrane (Zielińska et al., 2020). Alternatively, the higher rate of Lipid II synthesis at fast growth rates may necessitate a better controlled organization of the membrane to allow efficient peptidoglycan synthesis. A puzzling observation is that expression of flotillin

genes in *B. subtilis* is regulated during growth, with at least one flotillin always present in sufficient amounts and one flotillin upregulated in poor medium, or stress conditions that trigger developmental processes like sporulation and biofilm formation—where at least an effect of flotillins on bulk fluidity cannot be detected (Nicolas et al., 2012; Schneider et al., 2015; Zielińska et al., 2020). Thus, the exact function(s) of flotillins are far from known and may well combine the creation of a favorable membrane environment to allow dynamic processes, as well as controlling protease activity and possibly protein oligomerization. Finally, the limited number of organisms in which flotillin function has been studied so far makes it difficult to make general statements about the role of these proteins.

4 | MEMBRANE COMPARTMENTALIZATION IN MYCOBACTERIA

Recently, a very specific case of membrane compartmentalization was described in *Mycobacteria*, where the synthesis and membrane insertion of the peptidoglycan precursor Lipid II was shown to be spatially separated from the incorporation of Lipid II into peptidoglycan (García-Heredia et al., 2021). The latter process occurs at the tip of the cell, which, like other actinobacteria, grows from the pole. Again, a separation of the membrane in fractions with different biochemical properties, in this case using density centrifugation or solubilization, had revealed that lipid biosynthesis occurs in a distinct membrane fraction and that the two membrane fractions identified had a different proteome and lipidome composition (Morita et al., 2005). The lipid biosynthesis fraction, termed IMD (for intracellular membrane domain—please note that this domain is part of the plasma membrane), is the place where Lipid II synthesis takes place. These IMDs are located just after the tip of the cell where actual peptidoglycan synthesis occurs, and these two domains are clearly separate as demonstrated by microscopy (García-Heredia et al., 2021). The organization of these domains is dependent on the polar organizing protein DivIVA, which localizes to the convex cytoplasmic leaflet of the membrane (Lenarcic et al., 2009). DivIVA is required for polar cell wall synthesis in actinobacteria, such as *mycobacteria*, *corynebacteria*, and *streptomyces* (Howell & Brown, 2016). Again, membrane fluidity seems to play an important role in the separation of these domains as treatment of mycobacteria with a chemical fluidizer delocalized peptidoglycan synthesis from the poles to the sidewall (García-Heredia et al., 2021).

5 | MEMBRANE COMPARTMENTALIZATION IN TRANSERTION ZONES

The coupled transcription and translation at the membrane and the subsequent insertion of membrane proteins into the lipid bilayer at these sites has been termed "*transertion*" (Binenbaum et al., 1999; Matsumoto et al., 2015; Norris, 1995; Norris & Madsen, 1995). The hypothesis of transertion sites as a membrane organizing principle has been formulated about 30 years ago (Norris, 1995; Norris & Madsen, 1995; Woldringh et al., 1995). Cotranslational biogenesis of membrane proteins via the signal recognition particle receptor FtsY that couples the ribosome to the SecYEG/YidC apparatus is a well-known process in bacteria (Rapoport et al., 2017). However, we still have very limited data about transertion processes that also include localization of the respective genes to the membrane. A recent study suggests that the expression and assembly of the type III secretion system (T3SS2) in *Vibrio parahaemolyticus* occurs via a transertion mechanism (Kaval et al., 2023). A two-step transertion mechanism is proposed in which a membrane bound receptor complex composed of VtrA/C is activated by bile salts and recruits the target gene *vtrB* to the membrane. VtrB is a transmembrane protein, acts as transcriptional activator of the T3SS2 genes, and activates transcription and assembly of the T3SS2 needle apparatus (Kaval et al., 2023). Similarly, under inducing conditions, the gene encoding the lactose permease LacY shifts toward the membrane, indicating that LacY could be inserted into the membrane by a transertion mechanism as well (Libby et al., 2012). Negatively charged phospholipids like cardiolipin (CL) and phosphatidylglycerine (PG) have been connected with transertion (Fishov & Norris, 2012; Matsumoto et al., 2015). Cardiolipin enriches at curved membrane regions due to is molecular geometry (Koppelman et al., 2001; Mileykovskaya & Dowhan, 2000; Mukhopadhyay et al., 2008), and hence, several transport proteins have been shown to localize in a CL dependent manner to cell pole regions (Romantsov et al., 2010). However, it remains unclear whether transertion requires specific lipid environments or whether the process itself would induce membrane compartmentalization. Conventional imaging methods do not provide sufficient resolution, and the highly dynamic nature of protein–membrane interactions hampers analysis using nanometer scale resolution with electron microscopy. Recent advances in nanoscopy techniques will likely provide useful insights into these processes.

6 | MEMBRANE DYNAMICS: REPAIR AND REMODELING

The membrane is a vital structure for cellular integrity. Rupture of the plasma membrane in bacteria is usually considered a sign of cell death. Experimentally this is exploited in the classical live/dead staining, using propidium iodine that is not membrane permeable and only intercalates with DNA inside the cell when membrane lesions exist. Ruptures within the membrane, even at small scale, will lead to a rapid loss of ion gradients and de-energize the cell. It is therefore plausible that repair and maintenance systems evolved that help to repair membrane lesions and ensure tight sealing of the cell interior. Interestingly, most of the systems that we currently know have been identified and studied in eukaryotes for a long time. These systems include the membrane fusion and fission catalysts of the dynamin protein family (Bramkamp, 2012; Praefcke & McMahon, 2004) and members of the ESCRT-III/PspA/Vipp protein family (Barisch et al., 2023; Isono, 2021; Jimenez

et al., 2014). We focus here on membrane repair in the sense of sealing of membrane rupture. Phospholipid repair via the LplT/Aas system is not discussed (Lin et al., 2018); however, repair of individual phospholipid molecules contributes to membrane integrity and fluidity control.

7 | FUNCTION OF DYNAMIN-LIKE PROTEINS IN BACTERIA

Membrane remodeling in eukaryotes is often mediated by large GTPases of the dynamin family of proteins (Praefcke & McMahon, 2004). The name-giving dynamin is the paradigm of these enzymes and crucially involved in scission of endocytic vesicles, where it forms a ring-like collar around the neck of the budding vesicle (Perrais, 2022). Assembly stimulated GTP hydrolysis leads to a constriction of the dynamin collar squeezing the membrane until the vesicle pinches off (Schmid & Frolov, 2011). There are many related proteins, sharing a similar domain setup with dynamin, which we collectively call dynamin-like proteins (DLPs) (Praefcke & McMahon, 2004). A hallmark of all DLPs is their low affinity for guanine nucleotides (~10–100 μM) and their relatively high basal activity (~0.4–1 min^{-1}) (Song & Schmid, 2003). They all self-assemble into larger oligomeric structures, bound to membranes (Ford & Chappie, 2019). Consistent with their membrane-binding capacities, DLPs are involved in scission and fusion of membranes. These activities are in particular well studied in membrane dynamics of organelles, such as mitochondria, chloroplasts, and the endoplasmic reticulum (Ford & Chappie, 2019; Praefcke & McMahon, 2004). DLPs share a conserved molecular architecture, a globular, GTP-binding domain (G-domain), a neck (also termed bundle signaling element, BSE), and an extended stalk (or trunk) (Bramkamp, 2012; Praefcke & McMahon, 2004). There are variations in the arrangement of the helical bundle between different DLPs. However, a common theme is that at the tip of the stalk domain, DLPs contain a lipid binding domain. This domain can differ significantly, from a pleckstrin homology domain as in dynamin to a membrane binding loop, merely having amino acids with hydrophobic side chains, poking into one membrane leaflet, usually stabilized by electrostatic interactions coming from positively charged amino acids (Low et al., 2009; Low & Löwe, 2006).

Despite early reports about homologous DLPs in bacteria (Leipe et al., 2002), the function of bacterial dynamin like proteins (BDLPs) has been unclear until very recently. Structures of BDLP reveal their canonical DLP domain architecture (Low et al., 2009, Low & Löwe, 2006). Lipid binding is mediated by a paddle domain. In the *Nostoc punctiforme* BDLP, a phenylalanine and two leucine residues insert into the membrane.

BDLPs have been implicated with several cellular functions, including cytokinesis (Bürmann et al., 2011; Schlimpert et al., 2017), membrane surveillance and repair (Sawant et al., 2016), vesicle formation (Michie et al., 2014), and phage defense (Guo et al., 2021; Sawant et al., 2016). A common theme is that these functions are all associated with cell membrane dynamics. In line with a membrane-associated function of BDLPs, a double deletion of dynamin and flotillin has been shown to impair cell motility in *B. subtilis* (Dempwolff & Graumann, 2014; Dempwolff, Wischhusen, et al., 2012). Consistent with functions of eukaryotic DLPs in membrane fusion and fission, in vitro studies with the *B. subtilis* BDLP DynA revealed that DynA tethers membranes in trans and catalyzes membrane fusion (Bürmann et al., 2011; Guo & Bramkamp, 2019). Lipid mixing, for example, the fusion of the outer leaflet of two membranes, is an intermediate step during both fusion and fission. DynA is capable to mediate content mixing in liposomes, indicating full membrane fusion (Guo & Bramkamp, 2019). In vitro fusion processes were GTP independent; however, they were slow processes and it can be assumed that in vivo membrane fusion is faster (Guo & Bramkamp, 2019). Fusion activity is well in line with function of BDLPs in membrane repair. Indeed, it was shown that BDLPs in various organisms react after treatment of bacteria with antibiotics (Sawant et al., 2016). The membrane surveillance function has been best addressed in the *B. subtilis* DynA protein. In unstressed cells, DynA is mainly cytosolic; however, a significant fraction is associated to the membrane (Sattler & Graumann, 2022; Sawant et al., 2016). The membrane-associated fraction diffuses along the inner leaflet. In response to cell membrane stress, DynA assembles into larger, membrane-associated complexes that are basically immobile (Guo et al., 2021; Sattler & Graumann, 2022; Sawant et al., 2016) (Figure 2). Importantly, DynA reacts exclusively to stresses that induce membrane pores or membrane deformations. This includes pore-forming antimicrobials such as nisin (Sawant et al., 2016) (Figure 2a–c). This is in line with the findings that deletion of *dynA* renders cells more sensitive toward nisin. Nisin induces membrane deformations in *B. subtilis* that can be stained by specific membrane dyes, such as nile red, due to their increase in fluidity. DynA localizes to these areas where it forms stable foci. Similarly, in *Mycobacterium smegmatis*, the BDLP protein IniA was shown to localize to membrane ruffles and is induced by treatment with the anti-tuberculosis drug isoniazide (INH) (Wang et al., 2019). INH inhibits mycolic acid synthesis required for the outer membrane in Mycobacteria and other mycolic acid containing *Corynebacterineae*. A direct effect on the plasma membrane is not reported for INH, but antibiotics interfering with the cell wall or cell envelope in general often cause plasma membrane stress due to the tight coupling of the different envelope layers.

Lack of DynA impairs membrane integrity in *B. subtilis* under membrane stress conditions as shown by live/dead staining techniques (Sawant et al., 2016). The molecular mechanism by which DynA supports membrane integrity in vivo is not entirely clear. Based on the in vitro membrane fusion activity, it is tempting to speculate that DynA fuses membrane lesions or excises defective membrane areas (Figure 2a–c). Additionally, it was shown that DynA directly influences lipid diffusion (Sawant et al., 2016). Cells lacking DynA show a reduced lipid dynamic and, hence, DynA might contribute to membrane repair by fluidizing the membrane that in turn facilitates fusion processes.

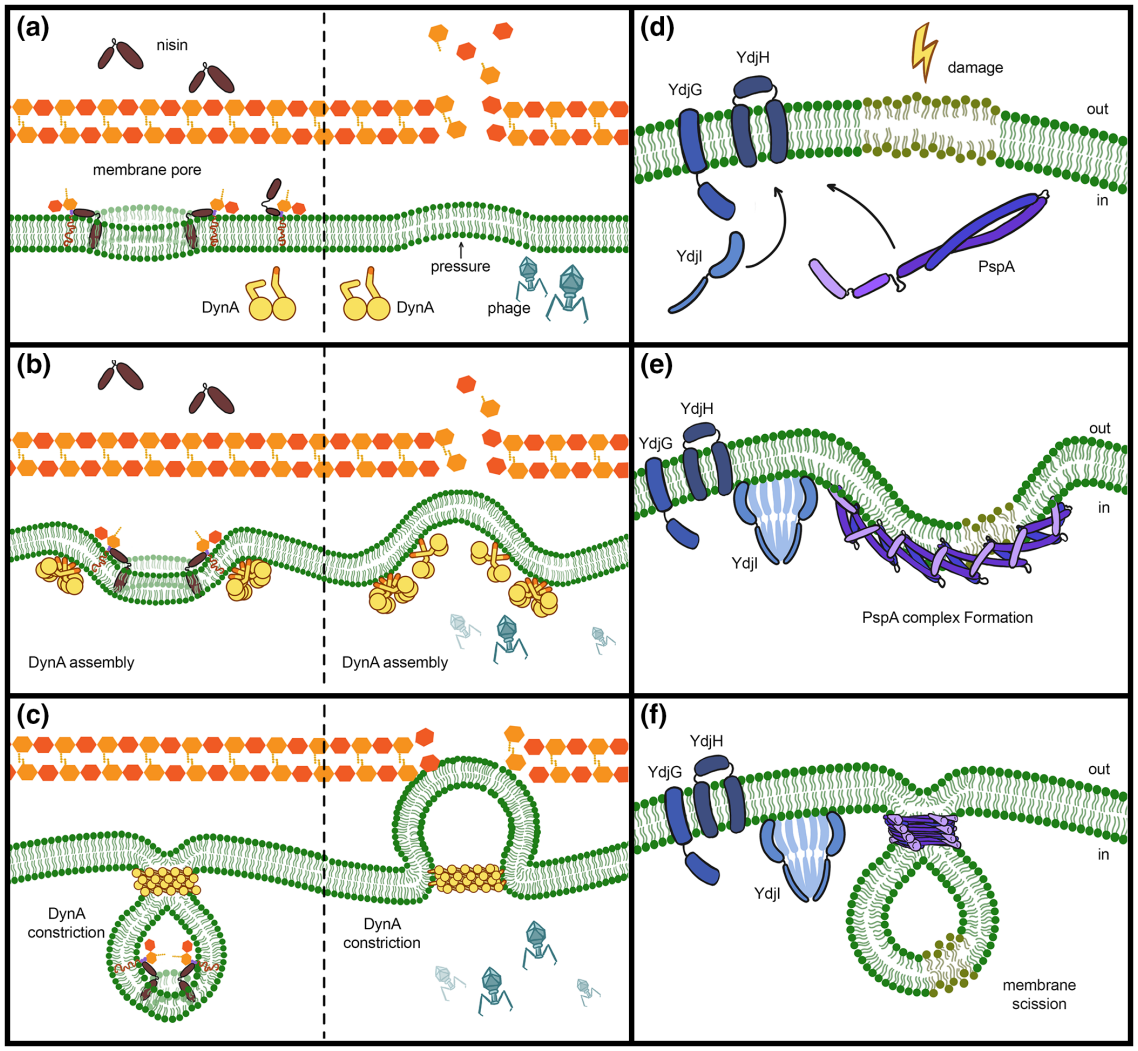

FIGURE 2 Function of dynamin- and ESCRT-III-like proteins in bacterial membrane repair. Left panel: The dynamin-like protein DynA from *B. subtilis* is involved in repair of membrane pores. Exemplarily, pore formation is shown by the antimicrobial compound nisin that forms lipid-II-dependent membrane pores (a, left). DynA accumulates at these sites (b, left) and likely cleaves off parts of damaged cell membrane (c, left). DynA is also involved in prevention of progeny egress upon bacterial infection with phages (a, right). Cell lysis that is triggered by phage encoded murein hydrolases leads to bulging of the plasma membrane through holes in the peptidoglycan (b, right). DynA seems to stabilize these sites and prevents explosive disruption of the membrane and therefore restricts the release of phage progeny (c, right). This results in a protective effect on the population level. Right panel: The ESCRT-III-like protein PspA is recruited to the membrane by the tripartite complex YdjGHI (d). YdjI is a member of the SPFH-domain family of proteins and likely forms oligomeric assemblies like other members of this family. The Ydj complex senses membrane damage and recruits PspA, which in turn assembles at the sites of membrane lesions (e). Oligomerization of PspA likely seals off and cleaves off the damaged membrane area (f).

BDLP-promoted membrane integrity plays also a role in population-wide resistance against phage predation. *B. subtilis* cells infected with phages (or in which prophages have been activated) lyse significantly later and less disruptive, compared to *dynA* mutant strains (Guo et al., 2021). This delays and restricts the spreading of phage progeny, thereby providing a last line of defense in phage infection (Figure 2a–c). Although membrane tethering and fusion by DynA was GTP independent in vitro, phage resistance is strictly dependent on a functional GTPase activity (Guo et al., 2021). Recent phylogenetic data show that genes encoding BDLPs are often in the vicinity of defense islands, gene loci coding for anti-phage systems, further lending support to the notion that BDLPs are involved in phage defense (Millman et al., 2022).

The protein pair DynA/DynA in Streptomces have been implicated with stabilization of the main division protein FtsZ and may also be involved in membrane fusion at the end of cytokinesis (Schlimpert et al., 2017). In vegetative hyphae of *S. coelicolor*, a cell wall deficient, membrane cross wall is formed, independent of FtsZ function (Yague et al., 2016). Currently, it is unclear whether the Streptomyces-specific dynamins are involved in the formation of these membrane structures.

8 | ESCRT-LIKE SYSTEMS

Another conserved family of proteins involved in membrane remodeling is related to the endosomal sorting complex required for transport (ESCRT). ESCRT proteins are ubiquitously distributed in pro- and eukaryotes (Isono, 2021; Siebenaller & Schneider, 2023). The core component of the eukaryotic membrane remodeling complex is the ESCRT-III protein that was shown to have membrane scission activities. The ESCRT-III complex has been implicated with several cellular processes that require membrane dynamics, including cytokinetic abscission (Samson et al., 2008), exosome release (Colombo et al., 2013), vesicle formation (Cruz Camacho et al., 2023), and viral budding (Ju et al., 2021). However, in recent years, it became also clear that ESCRT contributes to cell membrane integrity. In eukaryotic cells, it has been observed that ESCRT-III proteins are specifically recruited to sites of damaged membranes and accumulate there until membrane closure (Jimenez et al., 2014). This process requires calcium ions and several associated proteins, and ESCRT-mediated repair is achieved by membrane budding and subsequent shedding of damaged membranes. Interestingly, ESCRT-III functions in membrane repair also antagonized programmed cell death induced by gasdermins (Ruhl et al., 2018), thereby restricting the inflammatory response. The precise function of ESCRT-III complexes is not entirely clear and a "dome" and "buckling" model are discussed. Similar in both models, the main function is to release budding vesicles away from the cytoplasm in eukaryotes.

In contrast to the ESCRT function in eukaryotes, little is known about the functions in prokaryotic cells. In archaea, ESCRT-III systems are involved in cytokinesis, likely driving the cell membrane dynamics required to separate the daughter cells (Samson et al., 2008). Bacteria encode various ESCRT-III homologs. The best known is the *phage shock protein A* (PspA). PspA was discovered in *E. coli* where large amounts of this protein are produced in response to stresses such as infection by filamentous phages, elevated temperature, ethanol addition, and osmotic shock (Brissette et al., 1990, 1991). A common denominator of all these stresses is their immediate effect on membrane integrity. The formation of PspA carpet-like oligomers has been proposed to seal membrane leakage (Kobayashi et al., 2007). The structures of PspA and its cyanobacterial homolog Vipp1 were recently solved and revealed their canonical helical fold with a helix–loop–helix structure forming a central coiled-coil (Gupta et al., 2021; Junglas et al., 2021; Liu et al., 2021). While PspA functions on the plasma membrane, Vipp1 is required for thylakoid membrane repair. Both proteins form stacked oligomers that bind to membranes via amphipathic helices. Although the overall fold of PspA and Vipp1 is similar to ESCRT-III, their membrane interaction is different (Gupta et al., 2021; Junglas et al., 2021; Liu et al., 2021). While ESCRT-III deforms membranes away from its polymer, PspA and Vipp1 deform membranes toward the polymer, leading to membrane tubulation and eventually to membrane fusion and scission events (Deo & Prinz, 2021).

A recent detailed bioinformatic analysis has shown that PspA homologs are widely distributed in bacteria and archaea (Popp et al., 2022). Among the most conserved proteins of the Psp system is the core subunit PspA, while associated proteins vary drastically in their distribution. This suggests that PspA proteins might interact with so far unknown complex partners in various bacteria. Under nonstressed conditions, *E. coli* PspA forms a complex with PspF, thereby inhibiting PspF-induced transcriptional activation of the *psp* operon (Joly et al., 2009). Genetic regulation of the *pspA-ydjGHI* operon is under control of the extra cytoplasmic function sigma factor σ^W that acts in response to cell envelope stress. Upon membrane stress, the membrane integral components PspB and PspC disassemble the complex and release PspF and initiate PspA oligomerization, likely at sites of membrane damage. In several Gram-negative pathogens, the PspA system has also been linked to virulence and infection, suggesting roles for Psp proteins apart from membrane repair (DeAngelis et al., 2019). Fluorescently labeled PspA forms foci under membrane stress conditions (Scholz et al., 2021).

The *B. subtilis* PspA requires the SPFH domain protein YdjI and the two membrane integral proteins YdjG and YdjH for focus formation (Scholz et al., 2021) (Figure 2d–f). The YdjGHI complex is thought to form a membrane integral sensory complex that reacts to membrane stress. PspA foci do not always co-localize with YdjGHI complexes, suggesting that the Ydj complex does not remain associated with the PspA assemblies during the process of membrane repair. Unlike the other proteins with SPFH domains in bacteria, such as FloT and FloA, YdjI resides in fluid membrane domains and is therefore mutually exclusive with FloT. However, deletion of YdjI also decreases overall membrane fluidity, suggesting a conserved function of SPFH domain proteins in membrane fluidizing (Scholz et al., 2021). Deletion of the membrane integral components YdjG and YdjH results in severe morphology defects, due to mislocalization of the cell wall synthetic machinery, showing how sensitive the cell wall synthesis machinery reacts to impaired cell membrane stress response (Scholz et al., 2021). The genetic link between PspA proteins and SPFH domain proteins (also termed BAND7) is found not only in Firmicutes but also in other phyla (Popp et al., 2022). Since PspA binds membranes via its N-terminal amphipathic helix, it can be speculated that binding of PspA is facilitated in fluid membrane regions.

Similarly, the PspA homolog LiaH from *B. subtilis* is involved in cell envelope stress response induced by specific antibiotics such as nisin, daptomycin, and vancomycin. A weaker induction is also observed in presence of ethanol, detergents, and alkaline shock (Dominguez-Escobar et al., 2014). Although the presence of the *lia* operon does not refer resistance, nor does deletion cause significantly increased sensitivity, it is believed that the system acts in a membrane protective manner. However, LiaH has been linked to auto-resistance against a *B. subtilis* produced antimicrobial peptide, YydF. YydF is a prepro-peptide that gets proteolytically activated and exported, where it acts as a membrane active antimicrobial compound. The Lia system is strongly induced by the active peptide and counteracts membrane pertubations (Popp et al., 2020).

9 | CONCLUDING REMARKS

As is clear, a lot of progress has been made over the past years in the study of various protein families that regulate bacterial membrane dynamics. Many of these families were first described in eukaryotes and there are similarities, but also clear differences, in the way these proteins work in prokaryotes. What is now needed are advanced microscopy techniques that allow the study of the formation of FMMs, PspA carpets, and other events with sufficient temporal and spatial resolution to elucidate their function and regulation. Also, biophysical and structural studies are required to reveal the molecular detail of the protein–protein and protein–lipid interactions involved. Finally, it is becoming more and more clear that physico-chemical properties of the membrane, such as fluidity, regulate various processes, like peptidoglycan synthesis, which were thought to be regulated through protein–protein interactions (almost) exclusively.

AUTHOR CONTRIBUTIONS

Dirk-Jan Scheffers: Conceptualization; funding acquisition; writing – original draft. **Marc Bramkamp:** Conceptualization; funding acquisition; writing – original draft.

ACKNOWLEDGMENTS

The figures for this paper were made by Dr. Lizah van der Aart (https://lizahvanderaart.com/). Work in the Bramkamp laboratory is funded by grants from the Deutsche Forschungsgemeinschaft (BR2915/7-1). Work in the Scheffers laboratory is funded by grants from the Netherlands Organization for Scientific Research (NWO, Merian Fund Sustainable biomass production for a biobased economy 21.401).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

This review does not involve human subjects, patient medical records or animals.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Marc Bramkamp https://orcid.org/0000-0002-7704-3266
Dirk-Jan Scheffers https://orcid.org/0000-0002-9439-9168

REFERENCES

Azaldegui, C.A., Vecchiarelli, A.G. & Biteen, J.S. (2021) The emergence of phase separation as an organizing principle in bacteria. *Biophysical Journal*, *120*, 1123–1138.

Bach, J.N. & Bramkamp, M. (2013) Flotillins functionally organize the bacterial membrane. *Molecular Microbiology*, *88*, 1205–1217.

Bach, J.N. & Bramkamp, M. (2015) Dissecting the molecular properties of prokaryotic flotillins. *PLoS One*, *10*, e0116750.

Barisch, C., Holthuis, J.C.M. & Cosentino, K. (2023) Membrane damage and repair: a thin line between life and death. *Biological Chemistry*, *404*, 467–490.

Binenbaum, Z., Parola, A.H., Zaritsky, A. & Fishov, I. (1999) Transcription- and translation-dependent changes in membrane dynamics in bacteria: testing the transertion model for domain formation. *Molecular Microbiology*, *32*, 1173–1182.

Boekema, E.J., Scheffers, D.J., Van Bezouwen, L.S., Bolhuis, H. & Folea, I.M. (2013) Focus on membrane differentiation and membrane domains in the prokaryotic cell. *Journal of Molecular Microbiology and Biotechnology*, *23*, 345–356.

Bramkamp, M. (2012) Structure and function of bacterial dynamin-like proteins. *Biological Chemistry*, *393*, 1203–1214.

Bramkamp, M. & Lopez, D. (2015) Exploring the existence of lipid rafts in bacteria. *Microbiology and Molecular Biology Reviews*, *79*, 81–100.

Brissette, J.L., Russel, M., Weiner, L. & Model, P. (1990) Phage shock protein, a stress protein of *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 862–866.

Brissette, J.L., Weiner, L., Ripmaster, T.L. & Model, P. (1991) Characterization and sequence of the *Escherichia coli* stress-induced psp operon. *Journal of Molecular Biology*, *220*, 35–48.

Bürmann, F., Ebert, N., van Baarle, S. & Bramkamp, M. (2011) A bacterial dynamin-like protein mediating nucleotide-independent membrane fusion. *Molecular Microbiology*, *79*, 1294–1304.

Colombo, M., Moita, C., Van Niel, G., Kowal, J., Vigneron, J., Benaroch, P. et al. (2013) Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. *Journal of Cell Science*, *126*, 5553–5565.

Cruz Camacho, A., Alfandari, D., Kozela, E. & Regev-Rudzki, N. (2023) Biogenesis of extracellular vesicles in protozoan parasites: the ESCRT complex in the trafficking fast lane? *PLoS Pathogens*, *19*, e1011140.

Cybulski, L.E., Martin, M., Mansilla, M.C., Fernandez, A. & de Mendoza, D. (2010) Membrane thickness cue for cold sensing in a bacterium. *Current Biology*, *20*, 1539–1544.

Daumke, O. & Lewin, G.R. (2022) SPFH protein cage—one ring to rule them all. *Cell Research*, *32*, 117–118.

DeAngelis, C.M., Nag, D., Withey, J.H. & Matson, J.S. (2019) Characterization of the *Vibrio cholerae* phage shock protein response. *Journal of Bacteriology*, *201*, e00761-18.

Dempwolff, F. & Graumann, P.L. (2014) Genetic links between bacterial dynamin and flotillin proteins. *Communicative & Integrative Biology*, *7*(5), e970972.

Dempwolff, F., Moller, H.M. & Graumann, P.L. (2012) Synthetic motility and cell shape defects associated with deletions of flotillin/reggie paralogs in *Bacillus subtilis* and interplay of these proteins with NfeD proteins. *Journal of Bacteriology*, *194*, 4652–4661.

Dempwolff, F., Schmidt, F.K., Hervas, A.B., Stroh, A., Rosch, T.C., Riese, C.N. et al. (2016) Super resolution fluorescence microscopy and tracking of bacterial flotillin (Reggie) paralogs provide evidence for defined-sized protein microdomains within the bacterial membrane but absence of clusters containing detergent-resistant proteins. *PLoS Genetics*, *12*, e1006116.

Dempwolff, F., Wischhusen, H.M., Specht, M. & Graumann, P.L. (2012) The deletion of bacterial dynamin and flotillin genes results in pleiotrophic effects on cell division, cell growth and in cell shape maintenance. *BMC Microbiology*, *12*, 298.

Deo, R.A. & Prinz, W.A. (2021) ESCRTs got your Bac! *Cell*, *184*, 3591–3592.

Dion, M.F., Kapoor, M., Sun, Y., Wilson, S., Ryan, J., Vigouroux, A. et al. (2019) *Bacillus subtilis* cell diameter is determined by the opposing actions of two distinct cell wall synthetic systems. *Nature Microbiology*, *4*(8), 1294–1305.

Dominguez-Escobar, J., Chastanet, A., Crevenna, A.H., Fromion, V., Wedlich-Soldner, R. & Carballido-Lopez, R. (2011) Processive movement of MreB-associated cell wall biosynthetic complexes in bacteria. *Science*, *333*, 225–228.

Dominguez-Escobar, J., Wolf, D., Fritz, G., Hofler, C., Wedlich-Soldner, R. & Mascher, T. (2014) Subcellular localization, interactions and dynamics of the phage-shock protein-like Lia response in *Bacillus subtilis*. *Molecular Microbiology*, *92*, 716–732.

Donovan, C. & Bramkamp, M. (2009) Characterization and subcellular localization of a bacterial flotillin homologue. *Microbiology*, *155*, 1786–1799.

Egan, A.J.F., Errington, J. & Vollmer, W. (2020) Regulation of peptidoglycan synthesis and remodelling. *Nature Reviews. Microbiology*, *18*, 446–460.

Feng, X., Hu, Y., Zheng, Y., Zhu, W., Li, K., Huang, C.H. et al. (2014) Structural and functional analysis of *Bacillus subtilis* YisP reveals a role of its product in biofilm production. *Chemistry & Biology*, *21*, 1557–1563.

Fishov, I. & Norris, V. (2012) Membrane heterogeneity created by transertion is a global regulator in bacteria. *Current Opinion in Microbiology*, *15*, 724–730.

Ford, M.G.J. & Chappie, J.S. (2019) The structural biology of the dynamin-related proteins: new insights into a diverse, multitalented family. *Traffic*, *20*, 717–740.

Ganchev, D.N., Hasper, H.E., Breukink, E. & De Kruijff, B. (2006) Size and orientation of the lipid II headgroup as revealed by AFM imaging. *Biochemistry*, *45*, 6195–6202.

García-Fernández, E., Koch, G., Wagner, R.M., Fekete, A., Stengel, S.T., Schneider, J. et al. (2017) Membrane microdomain disassembly inhibits MRSA antibiotic resistance. *Cell*, *171*, 1354–1367.e20.

García-Heredia, A., Kado, T., Sein, C.E., Puffal, J., Osman, S.H., Judd, J. et al. (2021) Membrane-partitioned cell wall synthesis in mycobacteria. *eLife*, *10*, e60263.

Garner, E.C., Bernard, R., Wang, W., Zhuang, X., Rudner, D.Z. & Mitchison, T. (2011) Coupled, circumferential motions of the cell wall synthesis machinery and MreB filaments in *B. subtilis*. *Science*, *333*, 222–225.

Gohrbandt, M., Lipski, A., Grimshaw, J.W., Buttress, J.A., Baig, Z., Herkenhoff, B. et al. (2022) Low membrane fluidity triggers lipid phase separation and protein segregation in living bacteria. *The EMBO Journal*, *41*, e109800.

Guo, L. & Bramkamp, M. (2019) Bacterial dynamin-like protein DynA mediates lipid and content mixing. *The FASEB Journal*, *33*, 11746–11757.

Guo, L., Sattler, L., Shafqat, S., Graumann, P.L. & Bramkamp, M. (2021) A bacterial dynamin-like protein confers a novel phage resistance strategy on the population level in *Bacillus subtilis*. *mBio*, *13*, e0375321.

Gupta, T.K., Klumpe, S., Gries, K., Heinz, S., Wietrzynski, W., Ohnishi, N. et al. (2021) Structural basis for VIPP1 oligomerization and maintenance of thylakoid membrane integrity. *Cell*, *184*, 3643–3659.e23.

Hinderhofer, M., Walker, C.A., Friemel, A., Stuermer, C.A., Moller, H.M. & Reuter, A. (2009) Evolution of prokaryotic SPFH proteins. *BMC Evolutionary Biology*, *9*, 10.

Howell, M. & Brown, P.J.B. (2016) Building the bacterial cell wall at the pole. *Current Opinion in Microbiology*, *34*, 53–59.

Huber, T.B., Schermer, B., Müller, R.U., Höhne, M., Bartram, M., Calixto, A. et al. (2006) Podocin and MEC-2 bind cholesterol to regulate the activity of associated ion channels. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 17079–17086.

Isono, E. (2021) ESCRT is a great sealer: non-endosomal function of the ESCRT machinery in membrane repair and autophagy. *Plant & Cell Physiology*, *62*, 766–774.

Jia, Z., O'mara, M.L., Zuegg, J., Cooper, M.A. & Mark, A.E. (2011) The effect of environment on the recognition and binding of vancomycin to native and resistant forms of lipid II. *Biophysical Journal*, *101*, 2684–2692.

Jimenez, A.J., Maiuri, P., Lafaurie-Janvore, J., Divoux, S., Piel, M. & Perez, F. (2014) ESCRT machinery is required for plasma membrane repair. *Science*, *343*, 1247136.

Joly, N., Burrows, P.C., Engl, C., Jovanovic, G. & Buck, M. (2009) A lower-order oligomer form of phage shock protein A (PspA) stably associates with the hexameric AAA(+) transcription activator protein PspF for negative regulation. *Journal of Molecular Biology*, *394*, 764–775.

Ju, Y., Bai, H., Ren, L. & Zhang, L. (2021) The role of exosome and the ESCRT pathway on enveloped virus infection. *International Journal of Molecular Sciences*, *22*(16), 9060.

Junglas, B., Huber, S.T., Heidler, T., Schlosser, L., Mann, D., Hennig, R. et al. (2021) PspA adopts an ESCRT-III-like fold and remodels bacterial membranes. *Cell*, *184*, 3674–3688.e18.

Kaval, K.G., Chimalapati, S., Siegel, S.D., Garcia, N., Jaishankar, J., Dalia, A.B. et al. (2023) Membrane-localized expression, production and assembly of *Vibrio parahaemolyticus* T3SS2 provides evidence for transertion. *Nature Communications*, *14*, 1178.

Kihara, A., Akiyama, Y. & Ito, K. (1996) A protease complex in the *Escherichia coli* plasma membrane: HflKC (HflA) forms a complex with FtsH (HflB), regulating its proteolytic activity against SecY. *The EMBO Journal*, *15*, 6122–6131.

Kobayashi, R., Suzuki, T. & Yoshida, M. (2007) *Escherichia coli* phage-shock protein A (PspA) binds to membrane phospholipids and repairs proton leakage of the damaged membranes. *Molecular Microbiology*, *66*, 100–109.

Koppelman, C.M., Den Blaauwen, T., Duursma, M.C., Heeren, R.M. & Nanninga, N. (2001) *Escherichia coli* minicell membranes are enriched in cardiolipin. *Journal of Bacteriology*, *183*, 6144–6147.

Laloux, G. & Jacobs-Wagner, C. (2014) How do bacteria localize proteins to the cell pole? *Journal of Cell Science*, *127*, 11–19.

Leipe, D.D., Wolf, Y.I., Koonin, E.V. & Aravind, L. (2002) Classification and evolution of P-loop GTPases and related ATPases. *Journal of Molecular Biology*, *317*, 41–72.

Lenarcic, R., Halbedel, S., Visser, L., Shaw, M., Wu, L.J., Errington, J. et al. (2009) Localisation of DivIVA by targeting to negatively curved membranes. *The EMBO Journal*, *28*, 2272–2282.

Libby, E.A., Roggiani, M. & Goulian, M. (2012) Membrane protein expression triggers chromosomal locus repositioning in bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 7445–7450.

Lin, Y., Bogdanov, M., Lu, S., Guan, Z., Margolin, W., Weiss, J. et al. (2018) The phospholipid-repair system LplT/Aas in gram-negative bacteria protects the bacterial membrane envelope from host phospholipase A(2) attack. *The Journal of Biological Chemistry*, *293*, 3386–3398.

Lingwood, D. & Simons, K. (2007) Detergent resistance as a tool in membrane research. *Nature Protocols*, *2*, 2159–2165.

Liu, J., Tassinari, M., Souza, D.P., Naskar, S., Noel, J.K., Bohuszewicz, O. et al. (2021) Bacterial Vipp1 and PspA are members of the ancient ESCRT-III membrane-remodeling superfamily. *Cell*, *184*, 3660–3673.e18.

Lopez, D. & Koch, G. (2017) Exploring functional membrane microdomains in bacteria: an overview. *Current Opinion in Microbiology*, *36*, 76–84.

Lopez, D. & Kolter, R. (2010) Functional microdomains in bacterial membranes. *Genes & Development*, *24*, 1893–1902.

Low, H.H. & Löwe, J. (2006) A bacterial dynamin-like protein. *Nature*, *444*, 766–769.

Low, H.H., Sachse, C., Amos, L.A. & Löwe, J. (2009) Structure of a bacterial dynamin-like protein lipid tube provides a mechanism for assembly and membrane curving. *Cell*, *139*, 1342–1352.

Ma, C., Wang, C., Luo, D., Yan, L., Yang, W., Li, N. et al. (2022) Structural insights into the membrane microdomain organization by SPFH family proteins. *Cell Research*, *32*, 176–189.

Mangiarotti, A., Genovese, D.M., Naumann, C.A., Monti, M.R. & Wilke, N. (2019) Hopanoids, like sterols, modulate dynamics, compaction, phase segregation and permeability of membranes. *Biochimica et Biophysica Acta (BBA)—Biomembranes*, *1861*, 183060.

Mansilla, M.C. & de Mendoza, D. (2019) Regulation of membrane lipid homeostasis in bacteria upon temperature change. In: Geiger, O. (Ed.) *Biogenesis of fatty acids, lipids and membranes*. Handbook of Hydrocarbon and Lipid Microbiology. Springer, Cham.

Matsumoto, K., Hara, H., Fishov, I., Mileykovskaya, E. & Norris, V. (2015) The membrane: transertion as an organizing principle in membrane heterogeneity. *Frontiers in Microbiology*, *6*, 572.

Matsumoto, K., Kusaka, J., Nishibori, A. & Hara, H. (2006) Lipid domains in bacterial membranes. *Molecular Microbiology*, *61*, 1110–1117.

Michie, K.A., Boysen, A., Low, H.H., Møller-Jensen, J. & Löwe, J. (2014) LeoA, B and C from enterotoxigenic *Escherichia coli* (ETEC) are bacterial dynamins. *PLoS One*, *9*, e107211.

Mileykovskaya, E. & Dowhan, W. (2000) Visualization of phospholipid domains in *Escherichia coli* by using the cardiolipin-specific fluorescent dye 10-N-nonyl acridine orange. *Journal of Bacteriology*, *182*, 1172–1175.

Millman, A., Melamed, S., Leavitt, A., Doron, S., Bernheim, A., Hor, J. et al. (2022) An expanded arsenal of immune systems that protect bacteria from phages. *Cell Host & Microbe*, *30*, 1556–1569.e5.

Morita, Y.S., Velasquez, R., Taig, E., Waller, R.F., Patterson, J.H., Tull, D. et al. (2005) Compartmentalization of lipid biosynthesis in mycobacteria *. *Journal of Biological Chemistry*, *280*, 21645–21652.

Mukhopadhyay, R., Huang, K.C. & Wingreen, N.S. (2008) Lipid localization in bacterial cells through curvature-mediated microphase separation. *Biophysical Journal*, *95*, 1034–1049.

Nickels, J.D., Chatterjee, S., Stanley, C.B., Qian, S., Cheng, X., Myles, D.A.A. et al. (2017) The in vivo structure of biological membranes and evidence for lipid domains. *PLoS Biology*, *15*, e2002214.

Nicolas, P., Mader, U., Dervyn, E., Rochat, T., Leduc, A., Pigeonneau, N. et al. (2012) Condition-dependent transcriptome reveals high-level regulatory architecture in *Bacillus subtilis*. *Science*, *335*, 1103–1106.

Norris, V. (1995) Hypothesis: chromosome separation in *Escherichia coli* involves autocatalytic gene expression, transertion and membrane-domain formation. *Molecular Microbiology*, *16*, 1051–1057.

Norris, V. & Madsen, M.S. (1995) Autocatalytic gene expression occurs via transertion and membrane domain formation and underlies differentiation in bacteria: a model. *Journal of Molecular Biology*, *253*, 739–748.

Oliver, P.M., Crooks, J.A., Leidl, M., Yoon, E.J., Saghatelian, A. & Weibel, D.B. (2014) Localization of anionic phospholipids in *Escherichia coli* cells. *Journal of Bacteriology*, *196*, 3386–3398.

Oswald, F., Varadarajan, A., Lill, H., Peterman, E.J. & Bollen, Y.J. (2016) MreB-dependent organization of the *E. coli* cytoplasmic membrane controls membrane protein diffusion. *Biophysical Journal*, *110*, 1139–1149.

Patel, Y., Zhao, H. & Helmann, J.D. (2020) A regulatory pathway that selectively up-regulates elongasome function in the absence of class A PBPs. *eLife*, *9*, e57902.

Perrais, D. (2022) Cellular and structural insight into dynamin function during endocytic vesicle formation: a tale of 50 years of investigation. *Bioscience Reports*, *42*(11), BSR20211227.

Phillips, D.A., Zacharoff, L.A., Hampton, C.M., Chong, G.W., Malanoski, A.P., Metskas, L.A. et al. (2021) A bacterial membrane sculpting protein with BAR domain-like activity. *eLife*, *10*, e60049.

Pogmore, A.R., Seistrup, K.H. & Strahl, H. (2018) The gram-positive model organism *Bacillus subtilis* does not form microscopically detectable cardiolipin-specific lipid domains. *Microbiology (Reading)*, *164*, 475–482.

Popp, P.F., Benjdia, A., Strahl, H., Berteau, O. & Mascher, T. (2020) The epipeptide YydF intrinsically triggers the cell envelope stress response of *Bacillus subtilis* and causes severe membrane perturbations. *Frontiers in Microbiology*, *11*, 151.

Popp, P.F., Gumerov, V.M., Andrianova, E.P., Bewersdorf, L., Mascher, T., Zhulin, I.B. et al. (2022) Phyletic distribution and diversification of the phage shock protein stress response system in bacteria and archaea. *mSystems*, *7*, e0134821.

Praefcke, G.J. & McMahon, H.T. (2004) The dynamin superfamily: universal membrane tubulation and fission molecules? *Nature Reviews. Molecular Cell Biology*, *5*, 133–147.

Qiao, Z., Yokoyama, T., Yan, X.F., Beh, I.T., Shi, J., Basak, S. et al. (2022) Cryo-EM structure of the entire FtsH-HflKC AAA protease complex. *Cell Reports*, *39*, 110890.

Rapoport, T.A., Li, L. & Park, E. (2017) Structural and mechanistic insights into protein translocation. *Annual Review of Cell and Developmental Biology*, *33*, 369–390.

Romantsov, T., Battle, A.R., Hendel, J.L., Martinac, B. & Wood, J.M. (2010) Protein localization in *Escherichia coli* cells: comparison of the cytoplasmic membrane proteins ProP, LacY, ProW, AqpZ, MscS, and MscL. *Journal of Bacteriology*, *192*, 912–924.

Ruhl, S., Shkarina, K., Demarco, B., Heilig, R., Santos, J.C. & Broz, P. (2018) ESCRT-dependent membrane repair negatively regulates pyroptosis downstream of GSDMD activation. *Science*, *362*, 956–960.

Samson, R.Y., Obita, T., Freund, S.M., Williams, R.L. & Bell, S.D. (2008) A role for the ESCRT system in cell division in archaea. *Science*, *322*, 1710–1713.

Sattler, L. & Graumann, P.L. (2022) Assembly of *Bacillus subtilis* dynamin into membrane-protective structures in response to environmental stress is mediated by moderate changes in dynamics at a single molecule level. *Microbial Physiology*, *32*, 57–70.

Sawant, P., Eissenberger, K., Karier, L., Mascher, T. & Bramkamp, M. (2016) A dynamin-like protein involved in bacterial cell membrane surveillance under environmental stress. *Environmental Microbiology*, *18*, 2705–2720.

Schlimpert, S., Wasserstrom, S., Chandra, G., Bibb, M.J., Findlay, K.C., Flardh, K. et al. (2017) Two dynamin-like proteins stabilize FtsZ rings during Streptomyces sporulation. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, E6176–E6183.

Schmid, S.L. & Frolov, V.A. (2011) Dynamin: functional design of a membrane fission catalyst. *Annual Review of Cell and Developmental Biology*, *27*, 79–105.

Schneider, J., Klein, T., Mielich-Suss, B., Koch, G., Franke, C., Kuipers, O.P. et al. (2015) Spatio-temporal remodeling of functional membrane microdomains organizes the signaling networks of a bacterium. *PLoS Genetics*, *11*, e1005140.

Scholz, A.S., Baur, S.S.M., Wolf, D. & Bramkamp, M. (2021) An stomatin, prohibitin, flotillin, and HflK/C-domain protein required to link the phage-shock protein to the membrane in *Bacillus subtilis*. *Frontiers in Microbiology*, *12*, 754924.

Siebenaller, C. & Schneider, D. (2023) Cyanobacterial membrane dynamics in the light of eukaryotic principles. *Bioscience Reports*, *43*(2), BSR20221269.

Siliakus, M.F., Van Der Oost, J. & Kengen, S.W.M. (2017) Adaptations of archaeal and bacterial membranes to variations in temperature, pH and pressure. *Extremophiles*, *21*, 651–670.

Sinensky, M. (1974) Homeoviscous adaptation—a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, *71*, 522–525.

Song, B.D. & Schmid, S.L. (2003) A molecular motor or a regulator? Dynamin's in a class of its own. *Biochemistry*, *42*, 1369–1376.

Strahl, H., Burmann, F. & Hamoen, L.W. (2014) The actin homologue MreB organizes the bacterial cell membrane. *Nature Communications*, *5*, 3442.

Strahl, H. & Errington, J. (2017) Bacterial membranes: structure, domains, and function. *Annual Review of Microbiology*, *71*, 519–538.

Toyofuku, M. (2019) Bacterial communication through membrane vesicles. *Bioscience, Biotechnology, and Biochemistry*, *83*, 1599–1605.

van de Vossenberg, J.L., Driessen, A.J., da Costa, M.S. & Konings, W.N. (1999) Homeostasis of the membrane proton permeability in *Bacillus subtilis* grown at different temperatures. *Biochimica et Biophysica Acta*, *1419*, 97–104.

van Teeffelen, S., Wang, S., Furchtgott, L., Huang, K.C., Wingreen, N.S., Shaevitz, J.W. et al. (2011) The bacterial actin MreB rotates, and rotation depends on cell-wall assembly. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 15822–15827.

Wagner, R.M., Kricks, L. & Lopez, D. (2017) Functional membrane microdomains organize signaling networks in bacteria. *The Journal of Membrane Biology*, *250*, 367–378.

Wang, M., Guo, X., Yang, X., Zhang, B., Ren, J., Liu, A. et al. (2019) Mycobacterial dynamin-like protein IniA mediates membrane fission. *Nature Communications*, *10*, 3906.

Woldringh, C.L., Jensen, P.R. & Westerhoff, H.V. (1995) Structure and partitioning of bacterial DNA: determined by a balance of compaction and expansion forces? *FEMS Microbiology Letters*, *131*, 235–242.

Yague, P., Willemse, J., Koning, R.I., Rioseras, B., Lopez-Garcia, M.T., Gonzalez-Quinonez, N. et al. (2016) Subcompartmentalization by cross-membranes during early growth of Streptomyces hyphae. *Nature Communications*, *7*, 12467.

Yepes, A., Schneider, J., Mielich, B., Koch, G., Garcia-Betancur, J.C., Ramamurthi, K.S. et al. (2012) The biofilm formation defect of a *Bacillus subtilis* flotillin-defective mutant involves the protease FtsH. *Molecular Microbiology*, *86*, 457–471.

Zielińska, A., Savietto, A., De Sousa Borges, A., Martinez, D., Berbon, M., Roelofsen, J.R. et al. (2020) Flotillin-mediated membrane fluidity controls peptidoglycan synthesis and MreB movement. *eLife*, *9*, e57179.

How to cite this article: Bramkamp, M. & Scheffers, D-J. (2023). Bacterial membrane dynamics: Compartmentalization and repair. *Molecular Microbiology*, *120*, 490–501. https://doi.org/10.1111/mmi.15077