ANNUAL REVIEWS

Annual Review of Microbiology

Bacterial Membranes: Structure, Domains, and Function

Henrik Strahl and Jeff Errington

Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle Upon Tyne, NE2 4AX United Kingdom; email: h.strahl@ncl.ac.uk, jeff.errington@ncl.ac.uk

Annu. Rev. Microbiol. 2017. 71:519–38

First published as a Review in Advance on July 11, 2017

The *Annual Review of Microbiology* is online at micro.annualreviews.org

https://doi.org/10.1146/annurev-micro-102215-095630

Keywords

membrane domain, lipid domain, membrane structure, membrane curvature, bacterial lipids

Abstract

The bacterial cytoplasmic membrane is composed of roughly equal proportions of lipids and proteins. The main lipid components are phospholipids, which vary in acyl chain length, saturation, and branching and carry head groups that vary in size and charge. Phospholipid variants determine membrane properties such as fluidity and charge that in turn modulate interactions with membrane-associated proteins. We summarize recent advances in understanding bacterial membrane structure and function, focusing particularly on the possible existence and significance of specialized membrane domains. We review the role of membrane curvature as a spatial cue for recruitment and regulation of proteins involved in morphogenic functions, especially elongation and division. Finally, we examine the role of the membrane, especially regulation of synthesis and fluid properties, in the life cycle of cell wall–deficient L-form bacteria.

Contents

INTRODUCTION

Bacterial cytoplasmic membranes, composed of roughly equal proportions of proteins and lipids, have a multitude of important or essential functions. The membrane comprises the major boundary outlining the cell cytoplasm, and it controls the transport and diffusion of myriads of small molecules and secreted proteins between the intracellular and extracellular space. The transmembrane electrochemical gradient powers many crucial cell functions. In principle, membrane surfaces can act as topological landmarks used by morphogenic systems to mold the shape and division of the cell. Expansion of the membrane must at least keep pace with overall cell growth to enable all of the above to work.

Lipids in the bacterial cytoplasmic membrane form a classical bilayer structure. The lipids fall into a relatively small number of general chemical classes, but within most of these classes there are molecular variants differing in size and isomeric form (122). The precursor molecules are derived mainly from central metabolism, although some pathogens and commensals can acquire fully formed fatty acids or lipids from the environment (146). In general, the genes for lipid synthesis are widely conserved, suggesting that they emerged early in evolution. The key synthetic reactions are well established and have been studied in detail in several organisms (95).

Although the biochemical basis of lipid synthesis is relatively well understood, much less is known about how synthesis is regulated, particularly how different classes and isoforms are made in order to adapt to changes in internal and external conditions. Different lipid components are assumed to contribute to specialization of functions in membranes, but again, we know little about the molecular details of these effects. Finally, there has been much speculation and discussion about the existence of structurally and functionally specialized lipid domains in the membranes of higher organisms and to a lesser extent bacteria. This review summarizes and discusses recent developments in our understanding of specialization in bacterial membrane structure, particularly in relation to bacterial morphogenesis.

INTERNAL ORGANIZATION OF THE BACTERIAL CYTOPLASMIC MEMBRANE

Our understanding of the structure and function of biological membranes has been guided by the classical fluid mosaic model of Singer & Nicolson (121). In this early model, the cell membrane was defined as a two-dimensional liquid in which lipids and embedded proteins are free to diffuse laterally, thus resulting in a largely homogeneous structure. The original model has been refined over the years to incorporate an increasing level of complexity and organization imposed by cytoskeletal protein structures that limit and compartmentalize the diffusion of lipids and proteins, and the presence of membrane areas that differ in their composition and physicochemical properties (lipid domains) (86, 142). Advances in our understanding of the organization of biological membranes have been driven predominantly by research on eukaryotic cells and in vitro model systems. From these studies, three closely interconnected properties of lipid bilayers have emerged as key parameters determining their ability to demix and form lipid domains: (*a*) the lipid head group and the associated charge and physical shape of the lipid species (33, 77), (*b*) the lipid fatty acid moieties and the associated differences in the fluidity and packing of the lipid bilayers (95, 141), and (*c*) the phase behavior of lipid bilayers (99). These phospholipid-driven demixing processes are further modulated by either membrane-associated proteins (12, 69) or membrane-intercalating molecules such as cholesterol (57, 109).

Although less studied, bacterial cytoplasmic membranes also exhibit lateral heterogeneity and can form specific lipid domains (6, 65). The first indications that bacterial cytoplasmic membranes are not entirely homogeneous emerged from studies demonstrating that membranes can be separated into fractions that differ in protein composition (14, 41, 64). These early studies were later supported by both spectroscopic and microscopic methods demonstrating that phospholipids also exhibit lateral heterogeneity in bacterial cytoplasmic membranes (31, 140, 141, 144). In the following sections, we summarize current evidence for the existence of lipid domains in bacteria and discuss postulated explanations for their formation and biological functions.

Domains Determined by Lipid Head Groups

The major bacterial lipid species are the anionic phosphatidylglycerol (PG) and diphosphatidylglycerol (cardiolipin, CL) and the zwitterionic phosphatidylethanolamine (PE). Minor quantities of anionic phosphatidic acid (PA), positively charged lysyl phosphatidylglycerol (lysyl-PG), or glucolipids (mono- and diglucosyl diacylglycerol) are also commonly present (36, 56, 95). In most cases, we lack methods to localize specific lipid species within the cell. CL forms a prominent exception to this difficulty, and our understanding of cellular localization is significantly more comprehensive for CL than it is for other phospholipid species. Nonyl acridine orange (NAO) is a fluorescent, largely nontoxic membrane dye that binds anionic phospholipids (**Table 1**). Upon association with CL, NAO undergoes a fluorescence emission red-shift, which allows the microscopic detection of CL-rich domains (**Figure 1**).

In their seminal paper, Mileykovskaya & Dowhan (75) demonstrated that CL forms polar localized domains in *Escherichia coli* membranes. Independent evidence for polar CL domains was provided by analysis of the lipid composition of *E. coli* minicells. These small anucleate cells formed by a misplaced polar division turned out to be enriched in CL at the expense of PG, thus confirming the polar preference of CL (52). Following these findings, CL domains were later shown to exist in numerous other bacterial species, including *Bacillus subtilis*, *Pseudomonas putida*, *Enterococcus faecalis*, and *Streptococcus pyogenes* (8, 65, 108, 133). Importantly, the polar and septal CL domains modulate the localization and activity of membrane proteins, including the compatible solute transporter ProP and magnesium transporter MgtA (106, 107, 128). CL domains have been suggested to

Table 1 Lipid specificities of agents used to visualize membrane structure

Dye	Charge	Lipid specificity	Notable characteristics	Ref.
FM 4-64	Positive	Potential preference toward anionic lipids	Does not translocate across bacterial membranes	7, 117
FM 5-95	Positive	Potential preference toward anionic lipids	Slightly less lipophilic variant of FM 4-64	132
Nile Red	Neutral	No known lipid preference	Some preference toward fluid membrane areas	125
MitoTracker Green	Positive	Potential preference toward anionic lipids	Translocates across bacterial membranes	117
NAO	Positive	Preference toward anionic lipids	Cardiolipin/anionic lipid–dependent fluorescence emission spectrum	75, 89
Ro09–0198	N/A	Specific for phosphatidylethanolamine	Nonfluorescent peptide	87
Laurdan	Neutral	No known lipid preference	Membrane fluidity–dependent fluorescence emission spectrum	5, 125
DiI-C12	Positive	Potential preference toward anionic lipids	Strong preference toward fluid membrane areas	84, 125

play a role in cell division and division site selection processes (76, 87). However, the observed effects of anionic phospholipid–deficient strains on the cell division process are relatively minor and potentially reflect a general requirement for anionic phospholipids in membrane anchoring of the division machinery (21, 78, 79, 97). Whether specific (negatively charged) lipid domains are required for the cell division process remains to be demonstrated.

The negative curvature of polar membranes has been proposed as the reason why CL accumulates at the cell poles. Indeed, both computational simulations (42, 82) and experimental data (105) support a model in which the intrinsic conical shape of CL (43), matching the negative membrane curvature present at the cell division sites and cell poles, drives the localization and clustering of CL molecules. In the absence of both CL and PG, two anionic phospholipids, PA and *N*-acylphosphatidylethanolamine, which *E. coli* accumulates under these conditions, also form

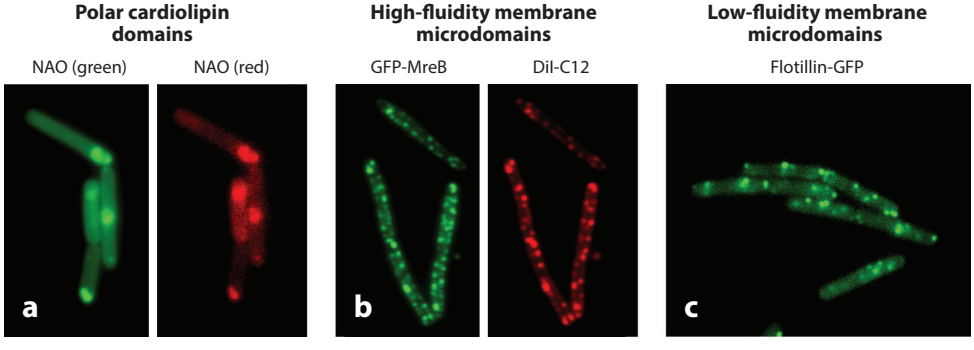

Figure 1

Lipid domains in bacterial cytoplasmic membranes. (*a*) The localized green and red fluorescence of *Bacillus subtilis* cells stained with NAO following the protocol described by Kawai et al. (46), and the fluorescence emission captured in green- and red-wavelength ranges, respectively. (*b*) *B. subtilis* cells expressing GFP-MreB and stained with the fluid-membrane-preferring dye DiI-C12 (125). (*c*) The focalized localization pattern of flotillin (FloT-GFP) in *B. subtilis* (18). Abbreviations: GFP, green fluorescent protein; NAO, nonyl acridine orange.

polar domains (79). PA, at least, shares a conical shape with CL (51). However, Oliver et al. (89) demonstrated a polar accumulation and clustering of PG only in the absence of CL. In contrast to CL and PA, PG has little intrinsic curvature, arguing for an additional shape-independent mechanism that recruits anionic phospholipids to the cell poles.

In their article, Oliver et al. (89) also rigorously analyzed the labeling specificity of NAO. On the basis of binding assays and spectroscopic analyses, a more general preference of NAO for anionic phospholipids, even in the case of the red-shifted fluorescence previously thought to be CL specific, is suggested. Thus, rather than relying on NAO exclusively, the cellular localization patterns, and other phenomena linked to polar CL domains, should be verified by different approaches, such as through use of the available phospholipid synthase deletion mutants.

Septal membranes of *B. subtilis* (but not of *E. coli*) are proposed to be enriched in PE in addition to CL (87). In this case, labeling was based on the cyclic peptide Ro09–0198, which specifically binds PE. This staining method relies on immunofluorescence and thus chemical fixation of the cells, followed by lysozyme treatment. These treatments can disturb the distribution of fluorescent dyes such as FM 5-95 and Nile Red in *B. subtilis* (H. Strahl, unpublished), highlighting the need for careful controls. Unfortunately, the pattern of Ro09–0198 staining was compared only against NAO. A clear colocalization was observed, although NAO should not stain PE (89). For these reasons, and in the absence of confirmatory studies, the existence of PE-enriched lipid domains in *B. subtilis* membranes remains debatable.

Lipid Domains Determined by High Membrane Fluidity

PG, the third major phospholipid species in bacteria, has also been postulated to form domains in membranes. This hypothesis was based on the observation that, under certain conditions, the positively charged fluorescent membrane dye FM 4-64 stains the membranes of *B. subtilis* in a nonhomogeneous manner, producing intricate patterns with a helix-like appearance (7). These putative lipid structures are disrupted by depletion of PG and colocalize with the cell division site regulator MinD (7). Crucially, the membrane association of MinD relies on anionic phospholipids such as PG in vitro (78). On the basis of the cationic nature of FM 4-64, the disruption caused by depletion of PG, the difference in staining pattern compared to NAO, and the recruitment of MinD, these domains were postulated to be enriched in PG (7). Later, the authors showed that depletion of the essential glycosyltransferase MurG, which catalyzes the last step of the cell wall precursor lipid II biosynthesis, dissolves these lipid domains, pointing to an intriguing connection between domain structure and the process of cell wall synthesis (81).

In the course of analyzing the consequences of membrane depolarization on protein localization in *B. subtilis* (126), we noticed that depolarization is accompanied by the appearance of membrane foci that are preferentially stained by both the cationic dyes FM 4-64 and FM 5-95 and an uncharged membrane dye, Nile Red. These depolarization-triggered membrane foci turned out to be associated with delocalization of the lateral cell wall synthetic machinery and depend on actin homologs of the MreB type (125). Because these domains were also stained with uncharged Nile Red, it was initially unclear whether they were a facet of the domain network reported by Barak et al. (7) or a separate phenomenon. The solution to this puzzle emerged from two experiments. First, depletion of the essential phospholipid PG results in membrane depolarization and, consequently, in MreB-dependent changes in the staining pattern of both FM 4-64 and Nile Red, thus questioning the specific involvement of PG (125). Second, use of the fluidity-sensitive dyes laurdan and DiI-C12 showed that the domains were instead characterized by increased local membrane fluidity (**Figure 1**). Finally, the fluid domain organization of the membrane was also shown to have a general impact on the localization and diffusion of membrane proteins. The existence of

an MreB-associated membrane domain organization was later endorsed by Oswald et al. (91). By conducting single-molecule tracking experiments with DiI-C12 and integral membrane proteins, the authors demonstrated for the first time in bacteria that lateral mobility of membrane proteins is subject to a temporal confinement caused by MreB-dependent membrane microdomains. Importantly, this study also demonstrated that the lipid domain network associated with the cell wall elongation machinery is conserved in the gram-negative model organism *E. coli*.

Local membrane fluidity can be increased by several mechanisms, including the binding of charged proteins (69) and the recruitment of specific lipids that increase local membrane disorder (149). The isoprenoid lipid carrier for cell wall precursor synthesis, lipid II, both favors a more fluid membrane surrounding and promotes local membrane disorder in its vicinity (34, 44). Because the MreB cytoskeleton spatially organizes the lateral cell wall synthesis machinery and directly interacts with proteins involved in lipid II synthesis (30, 80, 134), lipid II emerges as a prime candidate responsible for the fluid lipid domains. It is not yet clear whether lipid II is indeed the domain-inducing agent or whether the fluid lipid network has indeed a specific biological function. Are these lipid domains a simple consequence of lipid II clustering, or does the local high fluidity restrict the diffusion of lipid II away from the cell wall synthetic sites, thereby enabling high processivity of the lateral wall synthesis machinery?

Irrespective of their biological purpose, these fluid domains have a surprising relevance to our understanding of the mode of action of the membrane-targeting antibiotic daptomycin. It turned out that daptomycin has a clear preference for the lipid domains associated with the cell wall synthetic machinery. Upon incorporation, daptomycin releases the lipid II synthesis protein MurG into the cytoplasm. The resulting inhibition of cell wall synthesis represents the main mechanism through which low concentrations of daptomycin inhibit *B. subtilis* (84).

Lipid Domains Determined by Low Membrane Fluidity

The lipid rafts found in eukaryotic membranes are arguably the best-studied examples of lipid domains (40, 55, 57). At the core of lipid raft formation lies the ability of lipid bilayers to undergo phase separation, leading to spatially separated membrane areas with distinct composition and physicochemical properties (120). Most lipid bilayers can undergo phase separation between a fluid liquid-disordered phase and a more rigid and tightly packed gel phase. This process is dictated largely by temperature and intrinsic properties of the lipids involved, such as the head group species and the degree of fatty acid unsaturation or branching. Specific membrane intercalating molecules can induce a third phase termed the liquid-ordered phase, which lies between the liquid-disordered and gel phases in terms of fluidity and lipid packing. Lipid rafts are cholesterol-induced liquid-ordered phase domains found in the cytoplasmic membranes of eukaryotic cells (57). These low-fluidity domains are also enriched in sphingolipids and specific proteins and play an important role in various cellular processes, including membrane trafficking and signaling (40, 55, 57).

The membranes of most bacteria and archaea do not contain cholesterol, and lipid rafts were thus assumed to be limited to eukaryotes. The first indication that this might not be true came from the discovery that KinC, a sensory kinase involved in the regulation of *B. subtilis* biofilm formation, is not functional in the absence of farnesyl diphosphate phosphatase, YisP (58). The product of YisP, farnesol, is required for the synthesis of various important terpenoids, such as squalene, carotenoids, and hopanoids. These molecules increase lipid bilayer order and are therefore prime candidates to functionally replace cholesterol in lipid domain formation (39, 109, 110, 123). Owing to the more ordered structure, lipid rafts are more resistant toward nonionic detergents, allowing the isolation of detergent-resistant membrane (DRM) fractions enriched in lipid raft components (11, 116). When this analysis was carried out with *B. subtilis*, KinC was indeed

found exclusively in the DRM fraction. Crucially, this enrichment relied on the YisP-dependent synthesis of terpenoids (58). These findings led to the hypothesis that lipid-raft-like ordered lipid domains involved in signaling also exist in bacterial membranes (58). Because these domains are not cholesterol dependent and thus not lipid rafts in the strict sense, the term functional membrane microdomains was suggested.

A more detailed analysis of the DRM fractions identified additional proteins involved in signaling, and also others involved in protein secretion and proteolysis. Importantly, DRM fractions are highly enriched in proteins homologous to eukaryotic flotillins (18, 58, 95, 147). In eukaryotes, flotillins localize to lipid rafts and play an essential role in their cellular function (148). Demonstrating a functional connection between flotillins and the terpenoid-dependent signaling, the *B. subtilis* flotillin homologs FloT and FloA are essential for the proper function of KinC (58). In addition to biofilm formation (147), the deletion of flotillin homologs also impairs motility, competence, protein secretion, and cell morphology (5, 15, 16, 74). In agreement with a role in regulation of membrane order, overexpression of FloT reduces membrane fluidity upon membrane stress (53). The absence of flotillins in turn disturbs membrane fluidity and causes low-fluidity domains, most likely representing liquid-ordered-phase membrane areas, to coalesce (5). Pull-down experiments using flotillins identified various proteins, including components of the protein secretion machinery, transporters, and proteins involved in cell wall turnover and respiration (5). Thus, there is convincing evidence for a functional connection between the fluidity-modulating flotillins and various cellular processes.

In agreement with the concept of membrane microdomains characterized by flotillins and specific lipids forming a platform for certain membrane-associated processes, the flotillin homologs were shown to localize to distinct foci in the membranes of both *B. subtilis* and *Staphylococcus aureus* (**Figure 1**) (18, 58). However, it is not entirely clear how the localization of the two flotillin homologs relates to the proteins linked to the postulated raft-like domains such as KinC, FtsH, and SecY. Originally, the flotillin homologs FloT and FloA and the sensory kinase KinC were postulated to colocalize in a terpenoid-dependent manner (58). Later, however, FloT and FloA were shown to form separate clusters, thus arguing for two distinct membrane microdomains associated with the individual flotillin homologs (114). The spatially separate nature of the two flotillin clusters was later confirmed by Dempwolff et al. (16). By providing compelling evidence that flotillins neither colocalize nor codiffuse with proteins postulated to form a part of the membrane microdomains, such as KinC, FtsH, and SecA, this study casts significant doubt on the idea of a relatively static raft-like microdomain composed of flotillins, so far unidentified terpenoids, and other proteins (16). Thus, more work is needed to unify these findings and to decipher the detailed role of the flotillin homologs in the organization of bacterial membranes.

DYNAMIC PROTEIN MEMBRANE INTERACTIONS IN MORPHOGENESIS

The cytoplasmic membrane serves as the central scaffold for cellular machineries driving and regulating bacterial morphogenesis. Rather than establishing a permanent membrane tether by use of transmembrane domains, essential membrane interactions in morphogenesis are frequently established by amphitropic proteins that bind the membrane surface in a reversible and regulatable manner. In bacteria, the association of peripheral proteins with the cytoplasmic membrane is commonly based on direct electrostatic and hydrophobic interactions with the lipid bilayer or on lipid anchors introduced by posttranslational modification. In the following sections, we discuss the basic biochemical factors underlying these membrane binding processes and review the morphogenetic proteins that associate with membranes via peripheral binding.

Lipoproteins

Bacterial lipoproteins are anchored to cellular membranes by covalent binding to a lipid via a conserved N-terminal cysteine residue. The lipidation process is initiated by secretion of a prelipoprotein to the extracellular face of the cytoplasmic membrane, followed by covalent attachment of commonly mono-, di-, or triacylglycerols derived from regular anionic phospholipids (85, 151). Prominent examples of lipoproteins involved in cell morphogenesis are the gram-negative outer membrane proteins LpoA and LpoB, which regulate the activity of penicillin-binding proteins (93, 135), and EnvC and NlpD, which regulate the activity of cell wall–degrading amidases (136).

Membrane Association Based on Electrostatic and Hydrophobic Interactions

The biosynthetically complex targeting mechanism based on lipidation provides robust membrane association but leaves little room for regulation or reversion of the membrane association or for the adjustment of membrane affinity. More commonly, the peripheral membrane association is achieved by direct interactions between specific surface-exposed amino acid residues and the lipid bilayer (145). Owing to the composition dominated by anionic phospholipids PG and CL, and the zwitterionic phospholipid PE, electrostatic interactions between anionic phospholipids and cationic amino acid side chains provide significant membrane affinity (83, 95). Similarly, large hydrophobic residues such as leucine, isoleucine, and phenylalanine favor the hydrophobic interior of lipid bilayers, whereas the slightly more polar tyrosine and tryptophan prefer the interface between the hydrophobic membrane interior and the lipid head group layer (62, 98). The resulting hydrophobic interactions thus promote membrane association. This type of membrane interaction, established by amino acids either clustered at the membrane-facing surface of the protein or localized in a specific membrane-interacting loop, is responsible for the membrane binding of the polar landmark protein DivIVA (88); the bacterial homolog of the membrane-remodeling GTPase dynamin (59); and the actin homolog MreB, involved in spatial regulation of cell wall synthesis (111). In gram-negative bacteria, the membrane association of MreB is further supported by a membrane-binding amphipathic helix (111).

Membrane Association Based on Amphipathic Helices

The arguably best-studied mechanism establishing reversible membrane association, and also the mechanism most frequently utilized by proteins involved in morphogenesis, is based on membrane-binding amphipathic helices. These abundant membrane-binding modules can be found in the gram-negative MreB homologs (111); in the cell division proteins FtsA and SepF, responsible for tethering of the cytokinetic FtsZ ring to the membrane (21, 97); in the cell division site regulators MinD, MinE, and Noc (1, 118, 130, 150); and as membrane anchors for certain penicillin-binding proteins (25). Other prominent examples not directly involved in morphogenesis are the membrane stress protein PspA (45), the ribonucleases RNase E and RNase II (49, 60), the flagellar basal body protein FlhG (115), and the signal recognition particle receptor FtsY (94).

Conceptually, amphipathic helices combine the electrostatic and hydrophobic membrane interactions of individual amino acids into a functional module. Although amphipathic helices can be found embedded within protein structures, as for RNase E (49), they are most commonly associated with the C or N termini of proteins. The transplantable nature of several amphipathic helices has been demonstrated, and it is tempting to speculate that these small, modular, and independently functioning domains allow membrane association to evolve with relative ease via domain swaps and insertions, thus potentially explaining their wide distribution. Typically, a membrane-binding amphipathic helix is a 10- to 30-amino-acid-long polypeptide that is commonly unfolded

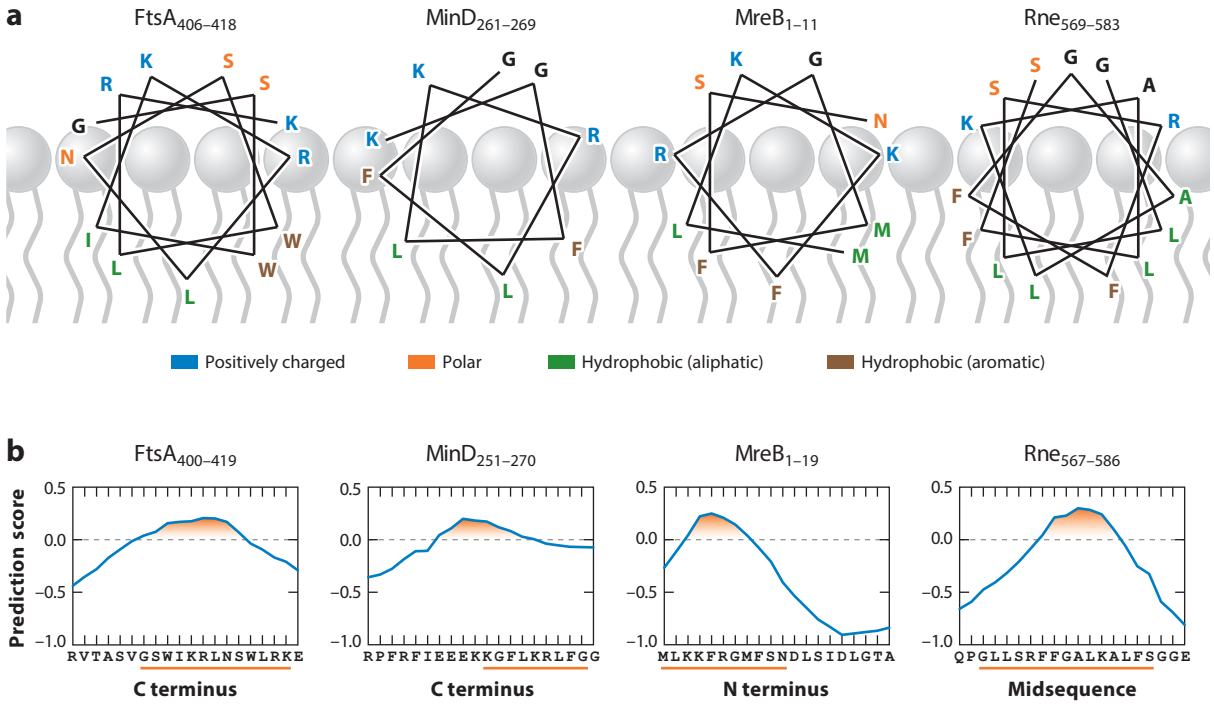

Figure 2

Membrane-binding amphipathic helices. (*a*) Helical wheel representation and the predicted orientation with respect to the lipid bilayer of the amphipathic helices of *Escherichia coli*, FtsA, MinD, MreB, and Rne (49, 97, 111, 130, 150). (*b*) Sequence-based AmphipaSeek prediction of the amphipathic helices depicted in panel *a* (113). A prediction score higher than 0 indicates a high likelihood for an α-helical region with an amphipathic character. The amino acid ranges depicted in the corresponding helical wheel representations are underlined.

in solution but adopts an α-helical configuration upon membrane binding (13, 20). In a folded state, amphipathic helices have characteristic opposing hydrophobic and polar faces, resulting in a hydrophobic moment driving membrane intercalation. Frequently, cationic side chains establishing electrostatic interactions with anionic phospholipids, and aromatic side chains favoring the lipid head group layer, are found flanking the hydrophobic face of the helix (**Figure 2*a***). The membrane affinity is thus determined by the hydrophobic moment, the bulkiness of the side chains determining the displacement of lipids required for bilayer intercalation, and the more specific interactions formed by cationic and aromatic residues (13, 20). These small helices thus represent compact membrane-binding modules that can be relatively easily adopted and tuned by evolution for use in a range of membrane-associated processes.

As a consequence of the binding mode, amphipathic helices are not merely static membrane tethers providing attachment: Both environmental factors and properties of the membrane can crucially influence the membrane binding process. The ion composition of the aqueous environment has a complex effect on the membrane binding of amphipathic helices. Monovalent or especially divalent cations can reduce membrane affinity by quenching electrostatic interactions, but higher ionic strength also promotes binding by increasing the hydrophobic moment. Owing to their reliance on electrostatic interactions between cationic residues and the negatively charged membrane surface, bacterial amphipathic helices commonly require anionic phospholipids, PG

or CL, for efficient binding. Accordingly, a higher affinity for anionic phospholipid–enriched lipid domains, such as the polar CL domains, has been reported (76, 78). Because the membrane binding process requires lateral displacement of phospholipids, the more relaxed lipid packing density associated with both high membrane fluidity and high positive membrane curvature stimulates the binding of amphipathic helices (9, 20). A higher affinity toward lipid bilayers segregated into domains, a phenomenon likely linked to reduced line tension associated with lipid domain boundaries, has also been reported (9, 68). Finally, transmembrane potential stimulates membrane binding of relatively weak amphipathic helices, such as those found in MinD and FtsA, via a so far unresolved mechanism (126). In addition to properties of the membrane modulating the binding, amphipathic helices can also directly influence membranes, upon binding, by inducing and stabilizing lipid domains (13, 67) and by locally deforming the lipid bilayer (4, 13, 20).

Because the function is based on the amphipathic character of a relatively short polypeptide rather than on a conserved primary sequence, and because these domains are commonly unresolved in crystal structures, it was relatively difficult in the past to identify and to distinguish membrane-binding amphipathic helices from non-membrane-binding amphipathic helices found on protein surfaces. However, this difficulty has been effectively removed by the development of a specific prediction tool, AmphipaSeek (113). This algorithm, based on an SVM (support vector machine) classifier trained with a set of 21 experimentally verified amphipathic helices, has contributed to the identification of MreB, SepF, Noc, and FtsY amphipathic helices (1, 21, 94, 111) and can retrospectively reliably identify known bacterial helices, such as those found in MinD, MinE, and FtsA (**Figure 2*b***).

MEMBRANE CURVATURE AS A SPATIAL CUE

In addition to internal domain organization, the shape of the cytoplasmic membrane also plays a crucial role in establishing bacterial cell architecture. Four distinct types of membrane topologies can be found in rod-shaped bacteria: the cylindrical part of the cell (zero Gaussian curvature), the curved tip of the septum (negative Gaussian curvature), the concave membrane surface found at the inner surface of the cell poles (positive Gaussian curvature), and the convex membranes found at the outer surface of the cell poles and endospores (positive Gaussian curvature). In the following sections, we discuss how topological features of the cytoplasmic membrane are used as spatial cues to localize membrane proteins.

Protein Recruitment to Convex Membrane Surfaces

Currently, the only bacterial protein known to utilize convex membrane surfaces as a spatial cue is the *B. subtilis* sporulation protein SpoVM. This small α-helical and amphipathic membrane protein localizes to the outer surface of the developing prespore and plays an important role in spore coat assembly (**Figure 3**) (70, 100, 138). Recruitment to the prespore surface relies on the convex geometry of the prespore membranes (101). Importantly, Ramamurthi et al. (101) could demonstrate that SpoVM is recruited to the prespore membranes only if they are curved and that SpoVM efficiently recognizes nonspore membranes of comparable curvature. In agreement with a curvature-dependent binding mechanism, SpoVM preferentially binds liposomes with smaller diameter in vitro (101). Amphipathic helices exhibit a general preference toward highly convex membranes owing to the associated lipid packing defects. This phenomenon, which increases the binding capacity of highly curved membranes, is common for amphipathic helices that insert relatively shallowly into the lipid bilayer (20). In contrast, the curvature preference of the deeper intercalating SpoVM protein is established through a cooperative binding process resulting in an

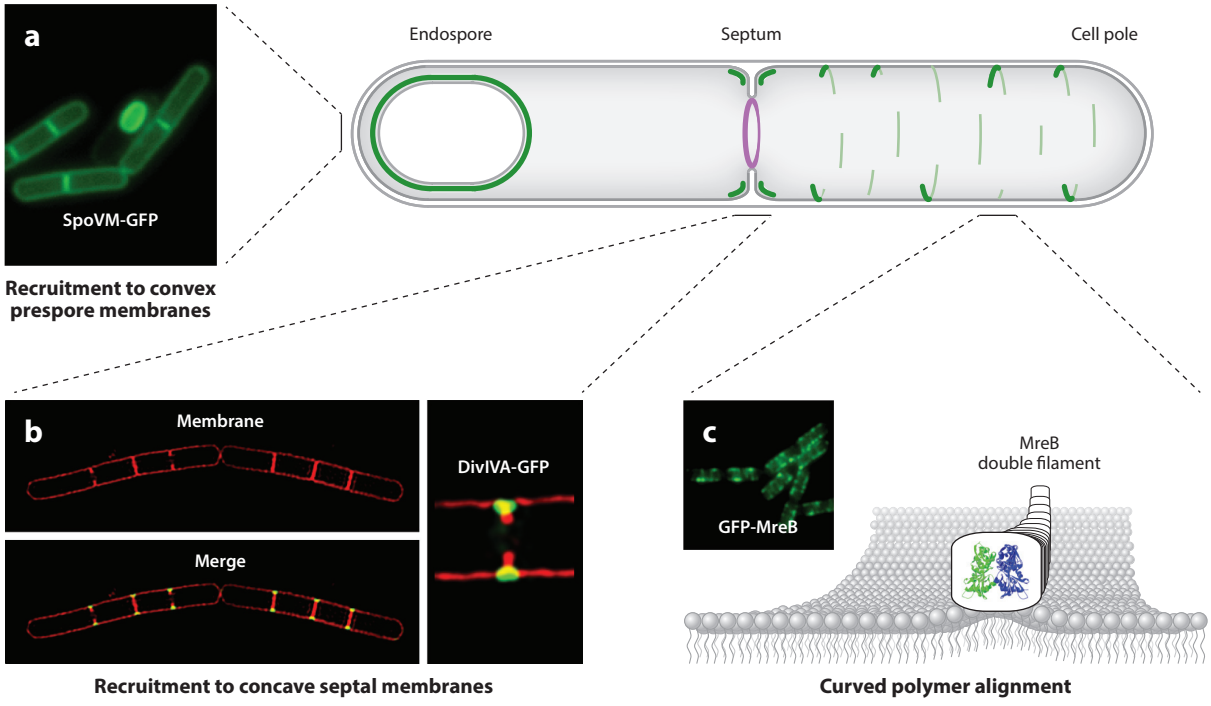

Figure 3

Membrane-curvature-dependent cellular localization. Schematic representation of a *Bacillus subtilis* cell indicating the cellular localization of SpoVM recruited to the curved prespore membranes (*a*), the localization of DivIVA recruited to the highly curved base of the septum (*b*), and MreB filaments recruited to inward bent areas of the cytoplasmic membrane (*c*). Abbreviation: GFP, green fluorescent protein.

increased binding affinity, which allows SpoVM to sense a significantly shallower curvature, thus arguing for a different mechanism (37, 143). Whereas the case for the curvature-dependency of SpoVM is solid, the curvatures associated with the prespore membranes are extremely shallow and a mechanism based on direct curvature sensing by SpoVM seems unlikely (37). The exact feature of shallow-curved convex membranes sensed by SpoVM thus remains to be identified.

Protein Recruitment to Concave Membrane Surfaces

Arguably the best-characterized example of a bacterial protein recognizing and localizing to concave membranes is the polar landmark protein DivIVA (22). This versatile scaffold protein directly binds cytoplasmic membranes via its N-terminal domain (88) and, depending on the cellular context and species, recruits other proteins such as components involved in cell division, division site determination, chromosome segregation, and cell wall synthesis (29, 32, 61, 132). The involvement of DivIVA homologs in morphogenesis was recognized because of DivIVA's role in division site determination in *B. subtilis* and because it localizes to the cell division sites (22). Later, *B. subtilis* DivIVA was shown to localize in a division- or pole-dependent manner also in *E. coli* and in *Schizosaccharomyces pombe*, thus demonstrating that DivIVA can independently recognize an unknown but conserved feature of cell division sites (23). This feature is the high membrane curvature found at the base of the septum (**Figure 3**) (28, 54, 102). The exact molecular mechanism through which DivIVA exhibits its strong preference toward concave membranes is still

enigmatic. However, DivIVA can form complex oligomeric structures that are potentially large enough to perceive the high local membrane curvature (124). A mechanism based on large DivIVA oligomers forming higher-order assemblies that are stabilized by increased membrane interactions occurring at highly curved membranes has been postulated. This molecular bridging model was indeed sufficient to localize DivIVA to highly curved membranes in computational simulations (54). However, more work is needed to test this model experimentally.

Bacterial membrane chemoreceptors form large sensory arrays that typically localize to the cell poles in rod-shaped bacteria (66). On the basis of studies of *E. coli* chemoreceptors, several polar targeting mechanisms, including preference for CL domains (119), pattern formation based on stochastic clustering (131), interaction with the cell division machinery (112), and localization based on polar membrane curvature (24), have been postulated. Recently, we showed that the polar localization pattern in *B. subtilis* is indeed based on a curvature-dependent mechanism (127). However, instead of recognizing the shallow curvature at the cell poles, chemoreceptor complexes cluster at the same highly curved membranes formed by the cell division process as DivIVA. This local enrichment appears to stimulate the formation of large and immobile chemosensory arrays that remain polar localized upon completion of the cell division process. On the basis of mutagenesis, we could show that the localizing unit is a trimeric complex of chemoreceptor dimers, and the preference toward highly concave membranes is likely due to the rigid tripod-like shape of the protein complex matching the high membrane curvature found at the base of the septum (127). Very recently, the mechanism of polar chemoreceptor targeting based on membrane curvature was shown to be conserved in *E. coli* as well (19).

Finally, components of the phosphotransferase system localize to the cell poles and cell division sites both in *E. coli* and in *B. subtilis* (38). This localization appears to rely on concave membrane areas; however, the components of the phosphotransferase system are soluble and do not directly interact with the cytoplasmic membrane. Thus, again, the mechanism underlying close proximity to the curved membrane remains to be discovered.

Membrane Curvature in Determination of Rod Shape

In virtually all bacteria the cell shape is determined by the spatially regulated synthesis of the cell wall, which lies immediately outside the cytoplasmic membrane. The wall is composed of an elastic mesh-like material, peptidoglycan, and is made by a complex constellation of synthases and hydrolases acting in concert (134). The key regulatory component of the cell elongation machinery in most rod-shaped bacteria is the actin homolog MreB, which localizes in the form of filaments oriented roughly perpendicular to the cell axis, which move processively in a cell wall synthesis–dependent manner (17, 26, 35, 103, 139). In the absence of the MreB cytoskeleton, cells lose their normal shape and adopt a largely coccoid morphology. How the MreB cytoskeleton guides cell wall synthesis and thus cell morphology remains poorly understood.

Due partly to artefacts caused by the use of nonfunctional GFP fusions, a considerable debate with respect to the importance and even existence of MreB filaments has been ongoing (26). The most recent studies, however, provide convincing evidence that MreB forms dynamic filamentous structures that can vary in length and are oriented approximately perpendicular to the cell length axis (90, 92, 103, 137). Intriguingly, by following the localization of MreB in artificially bent *E. coli* cells (104), and by correlating the localization of MreB with naturally occurring undulations in cell shape (137), Renner et al. and Ursell et al. showed that MreB filaments are guided by the curvature of the cytoplasmic membrane. In contrast to the previously discussed recruitment to simple convex and concave membranes, MreB filaments appear to show preferential enrichment at membrane areas that exhibit low mean negative Gaussian curvature. In the cellular context,

these are membrane areas with saddle-like topology formed by a bend in the regular rod shape. As a consequence, MreB filaments and the associated cell wall synthesis machinery are recruited to cellular areas with an inward bend, thereby triggering faster local cell wall expansion, which in principle can straighten the bend. The rod shape is thus postulated to be maintained by a self-correcting feedback mechanism based on a curvature-dependent recruitment of MreB filaments guiding the cell wall synthesis to areas deviating from a regular rod shape (137). The detailed mechanism through which MreB filaments can sense the negative Gaussian curvature remains to be established, but it likely involves the ability of MreB to form extensive polymers at the membrane surface (111).

The above-described mechanism provides a model of how a regular rod shape can be maintained. However, how the rod shape is established in the first instance, and what determines its diameter, remains unanswered. An important cue was provided by the ability of both *B. subtilis* (47) and *E. coli* (10) to transition between a regular walled state and a cell wall–free L-form state (25). These findings crucially demonstrate that no preexisting template structure is needed for morphogenesis. Intriguingly, MreB not only is required for the restoration of the cell shape but also appears to direct de novo cell wall synthesis to inward-curved areas of the undulating L-form surface. As a consequence of this bias, the cell undergoing reversion to the walled state gradually adopts an increasingly elongated shape (10). The curvature preference of MreB filaments thus appears to be crucial also in establishing the rod shape morphology. Finally, in line with the emerging picture that intrinsic physical properties of MreB polymers play a key role in establishing and maintaining a rod shape, the diameter of *E. coli* tightly correlates with the orientation of the MreB filaments (92). On the basis of these findings, a model was postulated in which the cell diameter is determined by the helical pitch angle of the MreB filaments guiding the chiral growth of the cell wall.

MEMBRANE ALTERATIONS IN CELL WALL–DEFICIENT BACTERIA

As mentioned above, many bacteria can switch to a cell wall–deficient, L-form state (3, 27). The cell wall is normally essential because loss of wall integrity results in catastrophic osmotic rupture of the membrane and loss of cell contents. However, this liability can be overcome by the use of osmoprotective culture media. L-forms have a flexible pleiomorphic shape and are highly variable in size because the cell wall normally plays a critical role in defining shape and division. Many bacteria can undergo the switch from walled growth to L-form state growth in response to treatment with antibiotics or by acquiring relatively simple genetic changes (73). The membrane plays a critical role in L-form growth, and the key physiological change required for L-form proliferation lies in upregulation of membrane synthesis (72). In *B. subtilis*, a mutation upregulating synthesis of the rate-limiting enzyme in fatty acid synthesis (FAS), acetyl-CoA carboxylase (ACC), is sufficient to drive L-form proliferation (72). Increased synthesis of malonyl-CoA, the product of ACC, induces the FAS II enzymes that drive fatty acid synthesis (via a regulator called FadR) (2, 63), presumably leading to increased lipid synthesis and thus membrane surface area. The increased ratio of cell surface area to volume seems to be sufficient to drive L-form proliferation (72)—a model that has support from both in vitro (96) and theoretical approaches (129).

Although it is clear how ACC upregulation can lead directly to L-form proliferation, it is less easy to explain how antibiotics or mutations that interfere with cell wall (peptidoglycan) synthesis also seem to drive L-form proliferation by increased fatty acid synthesis. The simplest explanation is that these treatments or mutations reduce the utilization of sugars derived from the glycolytic pathway. This reduction leads to increased flux through glycolysis and thereby increased production of acetyl-CoA, which in turn drives the fatty acid synthetic pathway. In support of the

increased flux idea, L-form-promoting mutations frequently seem to generate oxidative stress (48): Acetyl-CoA also drives the tricarboxylic acid cycle, leading to enhanced electron transport chain activity, the main cellular source of reactive oxygen species. This model makes the slightly surprising prediction that flux through glycolysis is not subject to a feedback mechanism that senses the excess fatty acid synthesis. Moreover, because inhibition of peptidoglycan precursor synthesis (e.g., by phosphomycin treatment) leads to L-form growth in a wide range of bacteria (73), it seems that this form of regulation is absent in many or most bacteria. Perhaps the coupling of increased membrane synthesis in response to inhibition of cell wall synthesis is an evolutionarily selected metabolic feature that enables bacteria to respond to cell wall inhibition by effecting an L-form (cell wall–independent) mode of proliferation.

One final feature of L-forms worthy of comment emerged from a genetic screen for mutations that prevent L-form but not walled growth (71). The strongest mutation obtained inactivated a gene called *lpd*, which is required for synthesis of the precursors for branched-chain fatty acids, which are normally approximately 90% of *B. subtilis* fatty acids (50, 125). It emerged that the defect arose from a specific deficiency in *anteiso* branched-chain fatty acids and that the mutation worked by reducing membrane fluidity. The mutant L-forms were able to grow and undergo spontaneous shape changes similar to those of wild-type L-forms, but they failed to undergo the final scission event that produces separated sister cells (71). Studies of L-forms have therefore unexpectedly provided an interesting new window on the structure, function, and regulation of bacterial membranes.

CONCLUDING REMARKS

For many years the bacterial cytoplasmic membrane was regarded as intrinsically unstructured and diffusion dominated, as in the original fluid mosaic model. However, during the past 10–15 years a more complex domain organization, reminiscent of that of eukaryotes, has emerged for bacteria. In addition to lipid domains, topological features of the membrane are used extensively to establish cellular organization. Thus, the cytoplasmic membrane, together with the nucleoid, is emerging as a central cellular scaffold that both governs the cell's inner architecture and guides its morphogenesis. We hope to have illustrated that membrane structure is a neglected and often overlooked facet of pivotal importance to bacterial cell biology.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Work in the Strahl lab is supported by Wellcome Trust Institutional Strategic Support Funds (ISSF) grant 105617/Z/14/Z for Newcastle University. Work in the Errington lab is funded by grants from the Wellcome Trust (WT098374AIA) and the European Research Council (BH141574; ELFBAD).

LITERATURE CITED

1. Adams DW, Wu LJ, Errington J. 2015. Nucleoid occlusion protein Noc recruits DNA to the bacterial cell membrane. *EMBO J.* 34:491–501

2. Albanesi D, de Mendoza D. 2016. FapR: from control of membrane lipid homeostasis to a biotechnological tool. *Front. Mol. Biosci.* 3:64
3. Allan EJ, Hoischen C, Gumpert J. 2009. Bacterial L-forms. *Adv. Appl. Microbiol.* 68:1–39
4. Antonny B. 2006. Membrane deformation by protein coats. *Curr. Opin. Cell Biol.* 18:386–94
5. Bach JN, Bramkamp M. 2013. Flotillins functionally organize the bacterial membrane. *Mol. Microbiol.* 88:1205–17
6. Barak I, Muchova K. 2013. The role of lipid domains in bacterial cell processes. *Int. J. Mol. Sci.* 14:4050–65
7. Barak I, Muchova K, Wilkinson AJ, O'Toole PJ, Pavlendova N. 2008. Lipid spirals in *Bacillus subtilis* and their role in cell division. *Mol. Microbiol.* 68:1315–27
8. Bernal P, Muñoz-Rojas J, Hurtado A, Ramos JL, Segura A. 2007. A *Pseudomonas putida* cardiolipin synthesis mutant exhibits increased sensitivity to drugs related to transport functionality. *Environ. Microbiol.* 9:1135–45
9. Bigay J, Antonny B. 2012. Curvature, lipid packing, and electrostatics of membrane organelles: defining cellular territories in determining specificity. *Dev. Cell* 23:886–95
10. Billings G, Ouzounov N, Ursell T, Desmarais SM, Shaevitz J, et al. 2014. *De novo* morphogenesis in L-forms via geometric control of cell growth. *Mol. Microbiol.* 93:883–96
11. Brown DA. 2002. Isolation and use of rafts. *Curr. Protoc. Immunol.* Suppl. 51:11.10. 1–23. **https://doi.org/10.1002/0471142735.im1110s51**
12. Chichili GR, Rodgers W. 2009. Cytoskeleton-membrane interactions in membrane raft structure. *Cell. Mol. Life Sci.* 66:2319–28
13. Cornell RB, Taneva SG. 2006. Amphipathic helices as mediators of the membrane interaction of amphitropic proteins, and as modulators of bilayer physical properties. *Curr. Protein Pept. Sci.* 7:539–52
14. De Leij L, Witholt B. 1977. Structural heterogeneity of the cytoplasmic and outer membranes of *Escherichia coli*. *Biochim. Biophys. Acta* 471:92–104
15. Dempwolff F, Moller HM, Graumann PL. 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. *J. Bacteriol.* 194:4652–61
16. Dempwolff F, Wischhusen HM, Specht M, Graumann PL. 2012. The deletion of bacterial dynamin and flotillin genes results in pleiotrophic effects on cell division, cell growth and in cell shape maintenance. *BMC Microbiol.* 12:298
17. Domínguez-Escobar J, Chastanet A, Crevenna AH, Fromion V, Wedlich-Söldner R, Carballido-López R. 2011. Processive movement of MreB-associated cell wall biosynthetic complexes in bacteria. *Science* 333:225–28
18. Donovan C, Bramkamp M. 2009. Characterization and subcellular localization of a bacterial flotillin homologue. *Microbiology* 155:1786–99
19. Draper W, Liphardt J. 2017. Origins of chemoreceptor curvature sorting in *Escherichia coli*. *Nat. Commun.* 8:14838
20. Drin G, Antonny B. 2010. Amphipathic helices and membrane curvature. *FEBS Lett.* 584:1840–47
21. Duman R, Ishikawa S, Celik I, Strahl H, Ogasawara N, et al. 2013. Structural and genetic analyses reveal the protein SepF as a new membrane anchor for the Z ring. *PNAS* 110:E4601–10
22. Edwards DH, Errington J. 1997. The *Bacillus subtilis* DivIVA protein targets to the division septum and controls the site specificity of cell division. *Mol. Microbiol.* 24:905–15
23. Edwards DH, Thomaides HB, Errington J. 2000. Promiscuous targeting of *Bacillus subtilis* cell division protein DivIVA to division sites in *Escherichia coli* and fission yeast. *EMBO J.* 19:2719–27
24. Endres RG. 2009. Polar chemoreceptor clustering by coupled trimers of dimers. *Biophys. J.* 96:453–63
25. Errington J. 2013. L-form bacteria, cell walls and the origins of life. *Open Biol.* 3:120143
26. Errington J. 2015. Bacterial morphogenesis and the enigmatic MreB helix. *Nat. Rev. Microbiol.* 13:241–48
27. Errington J, Mickiewicz K, Kawai Y, Wu LJ. 2016. L-form bacteria, chronic diseases and the origins of life. *Phil. Trans. R. Soc. B* 371:20150494
28. Eswaramoorthy P, Erb ML, Gregory JA, Silverman J, Pogliano K, et al. 2011. Cellular architecture mediates DivIVA ultrastructure and regulates *min* activity in *Bacillus subtilis*. *mBio* 2:e00257-11

29. Fadda D, Santona A, D'Ulisse V, Ghelardini P, Ennas MG, et al. 2007. *Streptococcus pneumoniae* DivIVA: localization and interactions in a MinCD-free context. *J. Bacteriol.* 189:1288–98
30. Favini-Stabile S, Contreras-Martel C, Thielens N, Dessen A. 2013. MreB and MurG as scaffolds for the cytoplasmic steps of peptidoglycan biosynthesis. *Environ. Microbiol.* 15:3218–28
31. Fishov I, Woldringh CL. 1999. Visualization of membrane domains in *Escherichia coli*. *Mol. Microbiol.* 32:1166–72
32. Flardh K, Richards DM, Hempel AM, Howard M, Buttner MJ. 2012. Regulation of apical growth and hyphal branching in *Streptomyces*. *Curr. Opin. Microbiol.* 15:737–43
33. Frolov VA, Shnyrova AV, Zimmerberg J. 2011. Lipid polymorphisms and membrane shape. *Cold Spring Harb. Perspect. Biol.* 3:a004747
34. Ganchev DN, Hasper HE, Breukink E, de Kruijff B. 2006. Size and orientation of the lipid II headgroup as revealed by AFM imaging. *Biochemistry* 45:6195–202
35. Garner EC, Bernard R, Wang W, Zhuang X, Rudner DZ, Mitchison T. 2011. Coupled, circumferential motions of the cell wall synthesis machinery and MreB filaments in *B. subtilis*. *Science* 333:222–25
36. Gidden J, Denson J, Liyanage R, Ivey DM, Lay JO. 2009. Lipid compositions in *Escherichia coli* and *Bacillus subtilis* during growth as determined by MALDI-TOF and TOF/TOF mass spectrometry. *Int. J. Mass Spectrom*. 283:178–84
37. Gill RL Jr., Castaing JP, Hsin J, Tan IS, Wang X, et al. 2015. Structural basis for the geometry-driven localization of a small protein. *PNAS* 112:E1908–15
38. Govindarajan S, Elisha Y, Nevo-Dinur K, Amster-Choder O. 2013. The general phosphotransferase system proteins localize to sites of strong negative curvature in bacterial cells. *mBio* 4:e00443-13
39. Gruszecki WI, Strzalka K. 2005. Carotenoids as modulators of lipid membrane physical properties. *Biochim. Biophys. Acta* 1740:108–15
40. Honigmann A, Pralle A. 2016. Compartmentalization of the cell membrane. *J. Mol. Biol.* 428:4739–48
41. Horiuchi S, Marty-Mazars D, Tai PC, Davis BD. 1983. Localization and quantitation of proteins characteristic of the complexed membrane of *Bacillus subtilis*. *J. Bacteriol.* 154:1215–21
42. Huang KC, Mukhopadhyay R, Wingreen NS. 2006. A curvature-mediated mechanism for localization of lipids to bacterial poles. *PLOS Comput. Biol.* 2:e151
43. Huang KC, Ramamurthi KS. 2010. Macromolecules that prefer their membranes curvy. *Mol. Microbiol.* 76:822–32
44. Jia Z, O'Mara ML, Zuegg J, Cooper MA, Mark AE. 2011. The effect of environment on the recognition and binding of vancomycin to native and resistant forms of lipid II. *Biophys. J.* 101:2684–92
45. Jovanovic G, Mehta P, McDonald C, Davidson AC, Uzdavinys P, et al. 2014. The N-terminal amphipathic helices determine regulatory and effector functions of phage shock protein A (PspA) in *Escherichia coli*. *J. Mol. Biol.* 426:1498–511
46. Kawai F, Shoda M, Harashima R, Sadaie Y, Hara H, Matsumoto K. 2004. Cardiolipin domains in *Bacillus subtilis* Marburg membranes. *J. Bacteriol.* 186:1475–83
47. Kawai Y, Mercier R, Errington J. 2014. Bacterial cell morphogenesis does not require a preexisting template structure. *Curr. Biol.* 24:863–67
48. Kawai Y, Mercier R, Wu LJ, Domínguez-Cuevas P, Oshima T, Errington J. 2015. Cell growth of wall-free L-form bacteria is limited by oxidative damage. *Curr. Biol.* 25:1613–18
49. Khemici V, Poljak L, Luisi BF, Carpousis AJ. 2008. The RNase E of *Escherichia coli* is a membrane-binding protein. *Mol. Microbiol.* 70:799–813
50. Klein W, Weber MH, Marahiel MA. 1999. Cold shock response of *Bacillus subtilis*: isoleucine-dependent switch in the fatty acid branching pattern for membrane adaptation to low temperatures. *J. Bacteriol.* 181:5341–49
51. Kooijman EE, Carter KM, van Laar EG, Chupin V, Burger KN, de Kruijff B. 2005. What makes the bioactive lipids phosphatidic acid and lysophosphatidic acid so special? *Biochemistry* 44:17007–15
52. Koppelman CM, Den Blaauwen T, Duursma MC, Heeren RM, Nanninga N. 2001. *Escherichia coli* minicell membranes are enriched in cardiolipin. *J. Bacteriol.* 183:6144–47
53. Lee YH, Kingston AW, Helmann JD. 2012. Glutamate dehydrogenase affects resistance to cell wall antibiotics in *Bacillus subtilis*. *J. Bacteriol.* 194:993–1001

54. Lenarcic R, Halbedel S, Visser L, Shaw M, Wu LJ, et al. 2009. Localisation of DivIVA by targeting to negatively curved membranes. *EMBO J.* 28:2272–82
55. Lewis RNAH, Mannock DA, McElhaney RN. 1997. Membrane lipid molecular structure and polymorphism. *Curr. Top. Membr.* 44:25–102
56. Lin TY, Weibel DB. 2016. Organization and function of anionic phospholipids in bacteria. *Appl. Microbiol. Biotechnol.* 100:4255–67
57. Lingwood D, Simons K. 2010. Lipid rafts as a membrane-organizing principle. *Science* 327:46–50
58. López D, Kolter R. 2010. Functional microdomains in bacterial membranes. *Genes Dev.* 24:1893–902
59. Low HH, Sachse C, Amos LA, Löwe J. 2009. Structure of a bacterial dynamin-like protein lipid tube provides a mechanism for assembly and membrane curving. *Cell* 139:1342–52
60. Lu F, Taghbalout A. 2013. Membrane association via an amino-terminal amphipathic helix is required for the cellular organization and function of RNase II. *J. Biol. Chem.* 288:7241–51
61. Lutkenhaus J. 2007. Assembly dynamics of the bacterial MinCDE system and spatial regulation of the Z ring. *Annu. Rev. Biochem.* 76:539–62
62. MacCallum JL, Bennett WFD, Tieleman DP. 2008. Distribution of amino acids in a lipid bilayer from computer simulations. *Biophys. J.* 94:3393–404
63. Martinez MA, Zaballa ME, Schaeffer F, Bellinzoni M, Albanesi D, et al. 2010. A novel role of malonyl-ACP in lipid homeostasis. *Biochemistry* 49:3161–67
64. Marty-Mazars D, Horiuchi S, Tai PC, Davis BD. 1983. Proteins of ribosome-bearing and free-membrane domains in *Bacillus subtilis*. *J. Bacteriol.* 154:1381–88
65. Matsumoto K, Kusaka J, Nishibori A, Hara H. 2006. Lipid domains in bacterial membranes. *Mol. Microbiol.* 61:1110–17
66. Mauriello EM. 2013. Cell biology of bacterial sensory modules. *Front. Biosci.* 18:928–43
67. Mazor S, Regev T, Mileykovskaya E, Margolin W, Dowhan W, Fishov I. 2008. Mutual effects of MinD-membrane interaction: I. Changes in the membrane properties induced by MinD binding. *Biochim. Biophys. Acta* 1778:2496–504
68. Mazor S, Regev T, Mileykovskaya E, Margolin W, Dowhan W, Fishov I. 2008. Mutual effects of MinD-membrane interaction: II. Domain structure of the membrane enhances MinD binding. *Biochim. Biophys. Acta* 1778:2505–11
69. Mbamala EC, Ben-Shaul A, May S. 2005. Domain formation induced by the adsorption of charged proteins on mixed lipid membranes. *Biophys. J.* 88:1702–14
70. McKenney PT, Eichenberger P. 2012. Dynamics of spore coat morphogenesis in *Bacillus subtilis*. *Mol. Microbiol.* 83:245–60
71. Mercier R, Domínguez-Cuevas P, Errington J. 2012. Crucial role for membrane fluidity in proliferation of primitive cells. *Cell Rep.* 1:417–23
72. Mercier R, Kawai Y, Errington J. 2013. Excess membrane synthesis drives a primitive mode of cell proliferation. *Cell* 152:997–1007
73. Mercier R, Kawai Y, Errington J. 2014. General principles for the formation and proliferation of a wall-free (L-form) state in bacteria. *eLife* 3:e04629
74. Mielich-Süss B, Schneider J, Lopez D. 2013. Overproduction of flotillin influences cell differentiation and shape in *Bacillus subtilis*. *mBio* 4:e00719-13
75. Mileykovskaya E, Dowhan W. 2000. Visualization of phospholipid domains in *Escherichia coli* by using the cardiolipin-specific fluorescent dye 10-*N*-nonyl acridine orange. *J. Bacteriol.* 182:1172–75
76. Mileykovskaya E, Dowhan W. 2005. Role of membrane lipids in bacterial division-site selection. *Curr. Opin. Microbiol.* 8:135–42
77. Mileykovskaya E, Dowhan W. 2009. Cardiolipin membrane domains in prokaryotes and eukaryotes. *Biochim. Biophys. Acta* 1788:2084–91
78. Mileykovskaya E, Fishov I, Fu X, Corbin BD, Margolin W, Dowhan W. 2003. Effects of phospholipid composition on MinD-membrane interactions *in vitro* and *in vivo*. *J. Biol. Chem.* 278:22193–98
79. Mileykovskaya E, Ryan AC, Mo X, Lin CC, Khalaf KI, et al. 2009. Phosphatidic acid and *N*-acylphosphatidylethanolamine form membrane domains in *Escherichia coli* mutant lacking cardiolipin and phosphatidylglycerol. *J. Biol. Chem.* 284:2990–3000

80. Mohammadi T, Karczmarek A, Crouvoisier M, Bouhss A, Mengin-Lecreulx D, den Blaauwen T. 2007. The essential peptidoglycan glycosyltransferase MurG forms a complex with proteins involved in lateral envelope growth as well as with proteins involved in cell division in *Escherichia coli*. *Mol. Microbiol.* 65:1106–21
81. Muchova K, Wilkinson AJ, Barak I. 2011. Changes of lipid domains in *Bacillus subtilis* cells with disrupted cell wall peptidoglycan. *FEMS Microbiol. Lett.* 325:92–98
82. Mukhopadhyay R, Huang KC, Wingreen NS. 2008. Lipid localization in bacterial cells through curvature-mediated microphase separation. *Biophys. J.* 95:1034–49
83. Mulgrew-Nesbitt A, Diraviyam K, Wang J, Singh S, Murray P, et al. 2006. The role of electrostatics in protein–membrane interactions. *Biochim. Biophys. Acta* 1761:812–26
84. Müller A, Wenzel M, Strahl H, Grein F, Saaki TNV, et al. 2016. Daptomycin inhibits cell envelope synthesis by interfering with fluid membrane microdomains. *PNAS* 113(45):E7077–86
85. Nakayama H, Kurokawa K, Lee BL. 2012. Lipoproteins in bacteria: structures and biosynthetic pathways. *FEBS J.* 279:4247–68
86. Nicolson GL. 2014. The fluid-mosaic model of membrane structure: still relevant to understanding the structure, function and dynamics of biological membranes after more than 40 years. *Biochim. Biophys. Acta* 1838:1451–66
87. Nishibori A, Kusaka J, Hara H, Umeda M, Matsumoto K. 2005. Phosphatidylethanolamine domains and localization of phospholipid synthases in *Bacillus subtilis* membranes. *J. Bacteriol.* 187:2163–74
88. Oliva MA, Halbedel S, Freund SM, Dutow P, Leonard TA, et al. 2010. Features critical for membrane binding revealed by DivIVA crystal structure. *EMBO J.* 29:1988–2001
89. Oliver PM, Crooks JA, Leidl M, Yoon EJ, Saghatelian A, Weibel DB. 2014. Localization of anionic phospholipids in *Escherichia coli* cells. *J. Bacteriol.* 196:3386–98
90. Olshausen PV, Defeu Soufo HJ, Wicker K, Heintzmann R, Graumann PL, Rohrbach A. 2013. Superresolution imaging of dynamic MreB filaments in *B. subtilis*—a multiple-motor-driven transport? *Biophys. J.* 105:1171–81
91. Oswald F, Varadarajan A, Lill H, Peterman EJ, Bollen YJ. 2016. MreB-dependent organization of the *E. coli* cytoplasmic membrane controls membrane protein diffusion. *Biophys. J.* 110:1139–49
92. Ouzounov N, Nguyen JP, Bratton BP, Jacobowitz D, Gitai Z, Shaevitz JW. 2016. MreB orientation correlates with cell diameter in *Escherichia coli*. *Biophys. J.* 111:1035–43
93. Paradis-Bleau C, Markovski M, Uehara T, Lupoli TJ, Walker S, et al. 2010. Lipoprotein cofactors located in the outer membrane activate bacterial cell wall polymerases. *Cell* 143:1110–20
94. Parlitz R, Eitan A, Stjepanovic G, Bahari L, Bange G, et al. 2007. *Escherichia coli* signal recognition particle receptor FtsY contains an essential and autonomous membrane-binding amphipathic helix. *J. Biol. Chem.* 282:32176–84
95. Parsons JB, Rock CO. 2013. Bacterial lipids: metabolism and membrane homeostasis. *Prog. Lipid Res.* 52:249–76
96. Peterlin P, Arrigler V, Kogej K, Svetina S, Walde P. 2009. Growth and shape transformations of giant phospholipid vesicles upon interaction with an aqueous oleic acid suspension. *Chem. Phys. Lipids* 159:67–76
97. Pichoff S, Lutkenhaus J. 2005. Tethering the Z ring to the membrane through a conserved membrane targeting sequence in FtsA. *Mol. Microbiol.* 55:1722–34
98. Pilpel Y, Ben-Tal N, Lancet D. 1999. kPROT: a knowledge-based scale for the propensity of residue orientation in transmembrane segments. Application to membrane protein structure prediction. *J. Mol. Biol.* 294:921–35
99. Quinn PJ. 2012. Lipid-lipid interactions in bilayer membranes: married couples and casual liaisons. *Prog. Lipid Res.* 51:179–98
100. Ramamurthi KS, Clapham KR, Losick R. 2006. Peptide anchoring spore coat assembly to the outer forespore membrane in *Bacillus subtilis*. *Mol. Microbiol.* 62:1547–57
101. Ramamurthi KS, Lecuyer S, Stone HA, Losick R. 2009. Geometric cue for protein localization in a bacterium. *Science* 323:1354–57
102. Ramamurthi KS, Losick R. 2009. Negative membrane curvature as a cue for subcellular localization of a bacterial protein. *PNAS* 106:13541–45

103. Reimold C, Defeu Soufo HJ, Dempwolff F, Graumann PL. 2013. Motion of variable-length MreB filaments at the bacterial cell membrane influences cell morphology. *Mol. Biol. Cell* 24:2340–49
104. Renner LD, Eswaramoorthy P, Ramamurthi KS, Weibel DB. 2013. Studying biomolecule localization by engineering bacterial cell wall curvature. *PLOS ONE* 8:e84143
105. Renner LD, Weibel DB. 2011. Cardiolipin microdomains localize to negatively curved regions of *Escherichia coli* membranes. *PNAS* 108:6264–69
106. Romantsov T, Battle AR, Hendel JL, Martinac B, Wood JM. 2010. Protein localization in *Escherichia coli* cells: comparison of the cytoplasmic membrane proteins ProP, LacY, ProW, AqpZ, MscS, and MscL. *J. Bacteriol.* 192:912–24
107. Romantsov T, Stalker L, Culham DE, Wood JM. 2008. Cardiolipin controls the osmotic stress response and the subcellular location of transporter ProP in *Escherichia coli*. *J. Biol. Chem.* 283:12314–23
108. Rosch JW, Hsu FF, Caparon MG. 2007. Anionic lipids enriched at the ExPortal of *Streptococcus pyogenes*. *J. Bacteriol.* 189:801–6
109. Saenz JP, Grosser D, Bradley AS, Lagny TJ, Lavrynenko O, et al. 2015. Hopanoids as functional analogues of cholesterol in bacterial membranes. *PNAS* 112:11971–76
110. Saenz JP, Sezgin E, Schwille P, Simons K. 2012. Functional convergence of hopanoids and sterols in membrane ordering. *PNAS* 109:14236–40
111. Salje J, van den Ent F, de Boer P, Lowe J. 2011. Direct membrane binding by bacterial actin MreB. *Mol. Cell* 43:478–87
112. Santos TMA, Lin TY, Rajendran M, Anderson SM, Weibel DB. 2014. Polar localization of *Escherichia coli* chemoreceptors requires an intact Tol-Pal complex. *Mol. Microbiol.* 92:985–1004
113. Sapay N, Guermeur Y, Deleage G. 2006. Prediction of amphipathic in-plane membrane anchors in monotopic proteins using a SVM classifier. *BMC Bioinform.* 7:255
114. Schneider J, Klein T, Mielich-Süss B, Koch G, Franke C, et al. 2015. Spatio-temporal remodeling of functional membrane microdomains organizes the signaling networks of a bacterium. *PLOS Genet.* 11:e1005140
115. Schuhmacher JS, Rossmann F, Dempwolff F, Knauer C, Altegoer F, et al. 2015. MinD-like ATPase FlhG effects location and number of bacterial flagella during C-ring assembly. *PNAS* 112:3092–97
116. Shah MB, Sehgal PB. 2007. Nondetergent isolation of rafts. *Methods Mol. Biol.* 398:21–28
117. Sharp MD, Pogliano K. 1999. An *in vivo* membrane fusion assay implicates SpoIIIE in the final stages of engulfment during *Bacillus subtilis* sporulation. *PNAS* 96:14553–58
118. Shih YL, Huang KF, Lai HM, Liao JH, Lee CS, et al. 2011. The N-terminal amphipathic helix of the topological specificity factor MinE is associated with shaping membrane curvature. *PLOS ONE* 6:e21425
119. Shiomi D, Yoshimoto M, Homma M, Kawagishi I. 2006. Helical distribution of the bacterial chemoreceptor via colocalization with the Sec protein translocation machinery. *Mol. Microbiol.* 60:894–906
120. Simons K, Ikonen E. 1997. Functional rafts in cell membranes. *Nature* 387:569–72
121. Singer SJ, Nicolson GL. 1972. The fluid mosaic model of the structure of cell membranes. *Science* 175:720–31
122. Sohlenkamp C, Geiger O. 2016. Bacterial membrane lipids: diversity in structures and pathways. *FEMS Microbiol. Rev.* 40:133–59
123. Spanova M, Zweytick D, Lohner K, Klug L, Leitner E, et al. 2012. Influence of squalene on lipid particle/droplet and membrane organization in the yeast *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* 1821:647–53
124. Stahlberg H, Kutejova E, Muchova K, Gregorini M, Lustig A, et al. 2004. Oligomeric structure of the *Bacillus subtilis* cell division protein DivIVA determined by transmission electron microscopy. *Mol. Microbiol.* 52:1281–90
125. Strahl H, Bürmann F, Hamoen LW. 2014. The actin homologue MreB organizes the bacterial cell membrane. *Nat. Commun.* 5:3442
126. Strahl H, Hamoen LW. 2010. Membrane potential is important for bacterial cell division. *PNAS* 107:12281–86
127. Strahl H, Ronneau S, Gonzalez BS, Klutsch D, Schaffner-Barbero C, Hamoen LW. 2015. Transmembrane protein sorting driven by membrane curvature. *Nat. Commun.* 6:8728

128. Subramani S, Perdreau-Dahl H, Morth JP. 2016. The magnesium transporter A is activated by cardiolipin and is highly sensitive to free magnesium in vitro. *eLife* 5:e11407
129. Svetina S. 2009. Vesicle budding and the origin of cellular life. *ChemPhysChem* 10:2769–76
130. Szeto TH, Rowland SL, Rothfield LI, King GF. 2002. Membrane localization of MinD is mediated by a C-terminal motif that is conserved across eubacteria, archaea, and chloroplasts. *PNAS* 99:15693–98
131. Thiem S, Sourjik V. 2008. Stochastic assembly of chemoreceptor clusters in *Escherichia coli*. *Mol. Microbiol.* 68:1228–36
132. Thomaides HB, Freeman M, El Karoui M, Errington J. 2001. Division site selection protein DivIVA of *Bacillus subtilis* has a second distinct function in chromosome segregation during sporulation. *Genes Dev.* 15:1662–73
133. Tran TT, Panesso D, Mishra NN, Mileykovskaya E, Guan Z, et al. 2013. Daptomycin-resistant *Enterococcus faecalis* diverts the antibiotic molecule from the division septum and remodels cell membrane phospholipids. *mBio* 4:e00281-13
134. Typas A, Banzhaf M, Gross CA, Vollmer W. 2012. From the regulation of peptidoglycan synthesis to bacterial growth and morphology. *Nat. Rev. Microbiol.* 10:123–36
135. Typas A, Banzhaf M, van den Berg van Saparoea B, Verheul J, Biboy J, et al. 2010. Regulation of peptidoglycan synthesis by outer-membrane proteins. *Cell* 143:1097–109
136. Uehara T, Parzych KR, Dinh T, Bernhardt TG. 2010. Daughter cell separation is controlled by cytokinetic ring-activated cell wall hydrolysis. *EMBO J.* 29:1412–22
137. Ursell TS, Nguyen J, Monds RD, Colavin A, Billings G, et al. 2014. Rod-like bacterial shape is maintained by feedback between cell curvature and cytoskeletal localization. *PNAS* 111:E1025–34
138. van Ooij C, Losick R. 2003. Subcellular localization of a small sporulation protein in *Bacillus subtilis*. *J. Bacteriol.* 185:1391–98
139. van Teeffelen S, Wang S, Furchtgott L, Huang KC, Wingreen NS, et al. 2011. The bacterial actin MreB rotates, and rotation depends on cell-wall assembly. *PNAS* 108:15822–27
140. Vanounou S, Parola AH, Fishov I. 2003. Phosphatidylethanolamine and phosphatidylglycerol are segregated into different domains in bacterial membrane. A study with pyrene-labelled phospholipids. *Mol. Microbiol.* 49:1067–79
141. Vanounou S, Pines D, Pines E, Parola AH, Fishov I. 2002. Coexistence of domains with distinct order and polarity in fluid bacterial membranes. *Photochem. Photobiol.* 76:1–11
142. Vereb G, Szollosi J, Matko J, Nagy P, Farkas T, et al. 2003. Dynamic, yet structured: the cell membrane three decades after the Singer-Nicolson model. *PNAS* 100:8053–58
143. Wasnik V, Wingreen NS, Mukhopadhyay R. 2015. Modeling curvature-dependent subcellular localization of the small sporulation protein SpoVM in *Bacillus subtilis*. *PLOS ONE* 10:e0111971
144. Welby M, Poquet Y, Tocanne JF. 1996. The spatial distribution of phospholipids and glycolipids in the membrane of the bacterium *Micrococcus luteus* varies during the cell cycle. *FEBS Lett.* 384:107–11
145. Whited AM, Johs A. 2015. The interactions of peripheral membrane proteins with biological membranes. *Chem. Phys. Lipids* 192:51–59
146. Yao J, Rock CO. 2015. How bacterial pathogens eat host lipids: implications for the development of fatty acid synthesis therapeutics. *J. Biol. Chem.* 290:5940–46
147. Yepes A, Schneider J, Mielich B, Koch G, García-Betancur JC, et al. 2012. The biofilm formation defect of a *Bacillus subtilis* flotillin-defective mutant involves the protease FtsH. *Mol. Microbiol.* 86:457–71
148. Zhao F, Zhang J, Liu YS, Li L, He YL. 2011. Research advances on flotillins. *Virol. J.* 8:479
149. Zhao J, Wu J, Veatch SL. 2013. Adhesion stabilizes robust lipid heterogeneity in supercritical membranes at physiological temperature. *Biophys. J.* 104:825–34
150. Zhou H, Lutkenhaus J. 2003. Membrane binding by MinD involves insertion of hydrophobic residues within the C-terminal amphipathic helix into the bilayer. *J. Bacteriol.* 185:4326–35
151. Zuckert WR. 2014. Secretion of bacterial lipoproteins: through the cytoplasmic membrane, the periplasm and beyond. *Biochim. Biophys. Acta* 1843:1509–16