Mini Review: Bacterial Membrane Composition and Its Modulation in Response to Stress

Jessica R. Willdigg and John D. Helmann*

Department of Microbiology, Cornell University, Ithaca, NY, United States

Antibiotics and other agents that perturb the synthesis or integrity of the bacterial cell envelope trigger compensatory stress responses. Focusing on Bacillus subtilis as a model system, this mini-review summarizes current views of membrane structure and insights into how cell envelope stress responses remodel and protect the membrane. Altering the composition and properties of the membrane and its associated proteome can protect cells against detergents, antimicrobial peptides, and pore-forming compounds while also, indirectly, contributing to resistance against compounds that affect cell wall synthesis. Many of these regulatory responses are broadly conserved, even where the details of regulation may differ, and can be important in the emergence of antibiotic resistance in clinical settings.

Keywords: bacteria, membrane, lipid, cellular envelope, antimicrobial resistance, metabolism, Bacillus subtilis

INTRODUCTION: MEMBRANE HOMEOSTASIS AND ITS MODULATION IN RESPONSE TO STRESS

The cell envelope is a multilayered outer barrier that protects the cell from a changing environment. Cell envelope stress responses (CESRs) are regulatory pathways that sense threats and mount a protective response, often involving modification of lipopolysaccharides (in Gram-negative bacteria), teichoic acids (Gram-positive bacteria), peptidoglycan, and the inner membrane (Helmann, 2016; Radeck et al., 2017; Mitchell and Silhavy, 2019). Here, we focus on Bacillus subtilis as a Gram-positive model for the role of CESRs in membrane homeostasis.

The cell membrane is a dynamic, fluid mosaic comprising a lipid bilayer and associated proteins (Figure 1). In B. subtilis, the major lipid species are phospholipids, glucolipids, and the lipoteichoic acids (LTA) (Salzberg and Helmann, 2008; Nickels et al., 2017). The membrane proteome includes proteins for transport and signaling, as well as membrane synthesis, remodeling, and protection. As the innermost and last line of defense, the cell membrane is critical for viability. In B. subtilis, for example, collapsing the proton motive force activates autolysins resulting in rapid cell lysis (Jolliffe et al., 1981). Membrane-active compounds such as detergents, antimicrobial peptides, and pore-forming compounds often trigger stress responses that modify the lipidome and membrane proteome to confer resistance. Membrane stress responses can modify the cell membrane, by (i) modulating the length, branching, and saturation of the fatty acid (FA) acyl chains, (ii) altering membrane lipid composition, or (iii) synthesizing proteins that modify or protect the membrane (Table 1).
THE REGULATION OF FA SYNTHESIS DURING GROWTH

Most bacteria utilize a type II FA synthase that catalyzes repeated cycles of acyl chain elongation (Parsons and Rock, 2013). The committed step, catalyzed by acetyl-CoA carboxylase (ACC), generates malonyl-CoA and then malonyl-ACP to serve in FA chain initiation by FabH and elongation by FabF. B. subtilis has two isoforms of FabH, and both preferentially synthesize branched chain FAs (BCFAs) (Choi et al., 2000; Kingston et al., 2011). Acylation of glycerol-3-phosphate by the PIsX/PIsY/PisC acyltransferase system with long chain FAs generates phosphatidic acid, the precursor to all other phospholipids (Yao and Rock, 2013).

FapR is the key transcriptional regulator of membrane lipid synthesis in B. subtilis and clinically relevant pathogens such as Staphylococcus aureus, Bacillus anthracis, and Listeria monocytogenes (Schujman et al., 2003; Fujita et al., 2007; Albanesi et al., 2013; Machinandiarena et al., 2020), and modulates the overall rate of membrane synthesis in response to precursor availability. B. subtilis FapR represses genes for FA and phospholipid synthesis, and this repression is relieved by allosteric interactions with malonyl-CoA or malonyl-ACP (Schujman et al., 2006; Martinez et al., 2010).

As a branchpoint enzyme, ACC is often under complex regulation (Zhang and Rock, 2009; Salie and Thelen, 2016; Machinandiarena et al., 2020). In B. subtilis, ACC is regulated in part by YqhY, a conserved DUF322/Asp23 protein which is highly expressed and often encoded together with ACC subunits as part of an accB-accC-yqhY operon (Todter et al., 2017). The namesake, S. aureus Asp23, is a membrane-associated protein originally linked to alkaline shock (Petersen et al., 2020). Loss of Asp23/YqhY causes cell wall stress and poor growth (Muller et al., 2014; Todter et al., 2017). In B. subtilis, yqhY null mutants acquire suppressors that decrease ACC activity, but this selective pressure is alleviated in medium supplemented with acetate (Todter et al., 2017). We suggest that ACC-dependent depletion of acetyl-CoA may contribute to wall stress by negatively affecting synthesis of UDP-N-acetylglucosamine needed for peptidoglycan synthesis. A key challenge for future research will be to understand the precise role of YqhY/Asp23 proteins and how they control ACC activity to balance FA synthesis with other cellular needs.

MODULATING FA COMPOSITION FOR HOMEOVISCOUS ADAPTATION

Tuning of FA composition provides one way in which the cell can optimize membrane properties in response to a changing environment. Even under non-stressed conditions, B. subtilis membranes contain ~7 distinct FAs varying in length from C14 to C18 (indicating the number of carbon atoms) and include both branched (~24% iso and 66% anteiso) and straight chain (~10%) FAs (Kingston et al., 2011). Since membrane phospholipids and glycolipids each contain 2 FA chains, the lipidome contains a complex mix of species (Figure 1B), with a preponderance containing one C15 and one C17 FA chain (Kingston et al., 2011).

Modifications of FAs are important for regulating membrane fluidity in a process known as homeoviscous adaptation (de Mendoza, 2014; Ernst et al., 2016). In B. subtilis, temperature downshift induces a FA desaturase (Des) controlled by the DesKR two-component system (TCS) (Abriata et al., 2017). Des modifies existing membrane lipids, and is thereby suited for rapid adaptation. DesK is one of the better understood TCS sensors, with both kinase and phosphatase activity (Abriata et al., 2017; Fernandez et al., 2019). DesK lacks an extracellular sensor domain, but has multiple transmembrane segments that sense changes in the membrane physical state. DesK phosphorylates the DesR response regulator, which induces des, encoding a FA Δ5 desaturase (Altabe et al., 2003). The resultant unsaturated FAs increase bilayer fluidity, which restores DesK phosphatase activity in a negative feedback loop (de Mendoza, 2014). Longer term adaptation to low temperatures relies on an isoleucine-dependent switch to primarily anteiso-FAs (Weber et al., 2001). Since anteiso-FAs perturb the lateral interactions between adjacent lipids to a greater extent than iso-FAs (Figure 1B), this shift increases membrane fluidity (Kingston et al., 2011). This shift may result from a cold-dependent change in FabH activity (Beranova et al., 2008; Saunders et al., 2016).

Membranes must also adapt to conditions that increase fluidity. In B. subtilis, the ECF σ factor σW is activated by detergents, antibiotics, and bacteriocins active on the membrane (Cao et al., 2002b; Pietiainen et al., 2005; Butcher and Helmann, 2006; Helmann, 2006, 2016). A σW promoter within the fabHA-fabF operon plays a major role in homeoviscous adaptation (Kingston et al., 2011). Activation of σW leads to a decrease in FabHA levels, resulting in increased reliance on FabHB and an increase in straight chain FAs (from ~10 to 30%). Elevated expression of the FabF elongation enzyme leads to increased FA chain length. The combined effect is a membrane with longer acyl chains and less BCFAs. This increased membrane rigidity serves to protect cells against detergents and antimicrobial peptides (Kingston et al., 2011). The activation of σW is controlled by regulated proteolysis of its membrane-bound anti-σW factor (RsiW) (Schobel et al., 2004; Ellermeier and Losick, 2006; Devkota et al., 2017). However, the mechanisms by which membrane stressors trigger σW activation remain unclear.

To better understand the role of FA heterogeneity in controlling membrane properties, it would be desirable to study bacteria with chemically simple membranes. This has been achieved in B. subtilis by feeding exogenous FAs to cells with de novo FA synthesis blocked by cerulenin and a mutation to inhibit FA degradation (Nickels et al., 2020). Growth can be rescued with only two FA species: a straight-chain C16 FA (high melting) and an anteiso C15 FA (low melting). Even with only these two FA species, four distinct arrangements are possible upon acylation of glycerol-3-phosphate to generate phosphatidic acid. Cells compensate for this reduced FA complexity by altering the distribution of phospholipid headgroups, a modest induction of the DesRK system, apparent downregulation of the σW stress response, and an increase in isoprenoid lipids (Nickels et al., 2020). These results highlight the remarkable adaptability of...
The cell envelope of *B. subtilis* is surrounded by a cell envelope comprised of a thick peptidoglycan (PG) layer and an inner membrane (IM). The membrane-associated lipoteichoic acid (LTA) and PG-linked wall teichoic acid (WTA) are abundant anionic polymers in the envelope (Rajagopal and Walker, 2017). The IM contains lateral microheterogeneity in the form of functional membrane microdomains (FMMs), regions of liquid-ordered (Lo) membrane together with associated proteins such as flotillins (Lopez and Koch, 2017). These are flanked by regions of higher fluidity characterized as liquid-disordered (Ld).

Major membrane lipids: Major membrane lipids include phospholipids and glucolipids (Nickels et al., 2017). Phospholipids (shown) vary in their FA chains, which are largely branched in *B. subtilis*. Shown here are a C_{15} iso-FA and a C_{17} Δ5 (unsaturated) anteiso-FA. Other FA chain lengths (including straight chains), and the positioning of the FA chains on the 1 and 2 positions of glycerol can vary. Variations in the phospholipid headgroups modulate surface charge (red are anionic, blue cationic, and black net neutral). Glucolipids are generally neutral lipids with one or more sugar residues in place of the phosphate shown. Minor membrane lipids: Many of the minor lipids in the membrane are isoprenoids and are derived from the C_{15} intermediate farnesyl-pyrophosphate (FPP). FPP is a precursor for undecaprenyl-PP (for PG synthesis) and for the C_{35} intermediate heptaprenyl-PP. The latter is a precursor for the electron carrier menaquinone (MK-7) and sesquiterpenes including baciterpenol A and its derivatives (sporulenes) (Bosak et al., 2008; Takigawa et al., 2010; Sato et al., 2011; Sato, 2013). Two FPP can also be coupled in a multistep reaction by HpnDCE to generate C_{30} squalene (Pan et al., 2008; van der Donk, 2015), which can be processed into carotenoids (such as staphyloxanthin from *S. aureus*; Garcia-Fernandez et al., 2017; Foster, 2019) or cyclized by squalene-hopene cyclases to generate polycyclic compounds (hopanoids) (Saenz et al., 2015; Belin et al., 2018). In *B. subtilis*, FPP can also be dephosphorylated by YisP to generate the alcohol farnesol (Bell and Chappell, 2014; Feng et al., 2014).
bacterial membranes, and the interconnection between diverse stress responses.

OVERVIEW OF MEMBRANE LIPID COMPOSITION AND SYNTHESIS

One of the persistent challenges in membrane biology is to define the roles of the diverse constituent lipids (Sohlenkamp and Geiger, 2016; Dowhan et al., 2019; Chwastek et al., 2020). Although membranes have a complex and adaptable composition (the lipidome), cells are remarkably resilient to genetic alterations that remove lipid species. Because of its single membrane and ease of genetic manipulation, *B. subtilis* presents an attractive model system (Nickels et al., 2017). The *B. subtilis* lipidome comprises ~70% phospholipids and ~30% neutral glucolipids. The major phospholipids are phosphatidylglycerol (PhG) and phosphatidylethanolamine (PE), with minor contributions from cardiolipin and lysylphosphatidylglycerol (LPG). Variations in phospholipid headgroup size and charge modulate membrane properties (Figure 1B). Membranes also contain LTA anchored to neutral glucolipids, which together with peptidoglycan-linked wall teichoic acid (WTA) can account for up to 60% of the dry weight of the cell wall (Rajagopal and Walker, 2017; Sumrall et al., 2020). However, LTA fractionates with the wall during membrane lipid extraction, and is often not considered in lipidome measurements.

The only essential phospholipid in *B. subtilis* is PhG. Remarkably, the membrane can be simplified to contain close to 100% PhG with no glucolipids. Despite a greatly simplified membrane, such mutants can grow rapidly, albeit with a highly abnormal coiled filament morphology (Salzberg and Helmann, 2008). Genetic perturbations of membrane composition can lead to resistance to cationic antimicrobial peptides (CAMP). For example, gain-of-function mutations in *mprF*, encoding the LPG synthase/flippase, can confer daptomycin resistance possibly by reducing surface charge (Ernst et al., 2018; Ernst and Peschel, 2019). Consistently, *mprF* null mutants have increased daptomycin sensitivity and overexpression decreases sensitivity in *B. subtilis* (Hachmann et al., 2009). Daptomycin resistance also results from *pgsA* mutations that decrease PhG levels (Hachmann et al., 2011; Peleg et al., 2012).

In addition to the dominant phospholipids and glucolipids, membranes contain numerous other lipid species. Most prominent are the isoprenoid lipids synthesized by polymerization of C3 isoprene units (Figure 1C). The key intermediate farnesyl-PP (C15) can be joined (head-to-head) to generate squalene (C30) (Pan et al., 2015; van der Donk, 2015), a precursor of cholesterol and other sterols in eukaryotes and of structurally related hopanoid lipids in many bacteria. One major hopanoid is dipterol (Figure 1C), with five fused rings that can be further modified in a variety of ways (Belin et al., 2018). Farnesyl-PP can also be extended by UppS, which sequentially adds eight isopentenyl units to generate undecaprenyl-PP, the

### TABLE 1 | Representative *B. subtilis* CESRs that modify the lipidome and membrane proteome1.

| CESR Gene(s) | Function | References |
|--------------|----------|------------|
| **Lipidome** |          |            |
| _ov_ fabHA-fabF | Homeoviscous adaptation; increased anteiso FA, decreased straight chain FA | Kingston et al., 2011 |
| DesKR des | Homeoviscous adaptation; Δ-5-FA desaturase | de Mendoza, 2014 |
| _ov_ _ov_ dtABCDE | Surface charge modification; D-α-lanoyltransferase from LTA, WTA; contributes to lantibiotic resistance | Cao and Helmann, 2004; Pietiainen et al., 2005; Kingston et al., 2013 |
| _ov_ _ov_ psaA-ybtM-psd | Surface charge modification; synthesis of PE (wityltionic lipid) from anionic phosphatidylglycerol; upregulated by 1-butanol treatment | Cao and Helmann, 2004; Vinayavekhin et al., 2015 |
| _ov_ ytpAB | YtpA; FA chain hydrolysis to generate lysophospholipids and LPG; initiating enzyme in sesquiterpene synthesis | Tamehiro et al., 2002; Sato et al., 2011 |
| _ov_ ltaSa | Alternative LTA synthase; induced in strains lacking the primary synthase (LtaS) | Eiamphungporn and Helmann, 2008; Wormann et al., 2011; Hashimoto et al., 2013 |
| **Proteome** |          |            |
| _ov_ floA | FloA and FloT flotillins (SPFH family); integral membrane proteins implicated in lipid raft function; induction of yqzA-floA-yqfB operon provides resistance against sublancin. | Butcher and Helmann, 2006; Bramkamp and Lopez, 2015 |
| _ov_ floT | FloA and FloT flotillins (SPFH family); integral membrane proteins implicated in lipid raft function; induction of yqzA-floA-yqfB operon provides resistance against sublancin. | Butcher and Helmann, 2006; Bramkamp and Lopez, 2015 |
| _ov_ pspA | PspA; phage shock protein A (PspA/VIP1/IM30/ESCRT I family), membrane protection and remodeling; contributes to nisin resistance. | Butcher and Helmann, 2006; Lamsa et al., 2012; Yamada et al., 2012; Hoffer et al., 2016 |
| _ov_ yknWXZ | YknWXZ (transporter) and YhLM provide protection against the SdpC “cannibalism toxin.” YhLM is a paralog of the Sdpi immunity protein. | Butcher and Helmann, 2006; Scholz et al., 2014 |
| _ov_ ydbST | YdbST provide protection against Amylovibulin (cylic lipopeptide). | Butcher and Helmann, 2006; Scholz et al., 2014 |
| LiaRS | LiaH, a PspA paralog, anchored by LiaI. Strongly induced by membrane-perturbing antimicrobials; induced by TAT protein export. | Mascher et al., 2004; Radeck et al., 2017; Bernal-Cabas et al., 2020 |
| BceRS | bceAB | Prototype for flux-sensing TCS (BceRS) that integrates signals from the cognate ABC transporter (BceAB). | Fritz et al., 2015; Radeck et al., 2016; Kobras et al., 2020 |
| LnrJK | lnlMN | A flux-sensing system for induction of linearmycin and amphotericin (polypeptide antibiotic) resistance. | Stubbedeck and Straight, 2017; Stubbedeck et al., 2018; Revilla-Guarinos et al., 2020 |

1 This list includes representative systems from *B. subtilis,* but does not include CESRs with related functions from other organisms.
C_{35} carrier lipid that supports cell wall synthesis (Figure 1C). Alternatively, the HepST complex can extend farnesyl-PP to generate heptaprenyl (C_{35})-PP, an isoprenoid used as a lipid anchor for menaquinone (MK-7), the electron carrier for respiration. In B. subtilis, this same precursor can be processed to polyisoprenyl C_{35}-sesquiterpenoids, which may be functionally similar to C_{30} hopanoids (Bosak et al., 2008; Takigawa et al., 2010; Sato et al., 2011; Sato, 2013). This process is initiated by YtpB, which generates tetraprenyl-β-curcumen, and then SqhC (a homolog of squalene-hopene cyclases) to generate the C_{35} tricyclic product known as baciterpenol A (Sato, 2013). Although initially described in spores, and named “sporulenes” (Bosak et al., 2008), these sesquiterpenoids are found in vegetative cells (Takigawa et al., 2010). Finally, heptaprenyl-PP can be coupled to glycerol-1-phosphate by PcrB, and then further processed by an unidentified phosphatase and the YvoF acetyltiranerase to generate an ether linked lipid of unknown function (Linde et al., 2016).

**Lateral Heterogeneity and Functional Membrane Microdomains**

In eukaryotes, cholesterol is associated with the generation of functional membrane microdomains (FMM), also called lipid rafts. These regions have relatively low membrane fluidity (a liquid-ordered, or Lo phase) and are associated with flotillins. B. subtilis also encodes flotillin homologs, regulated by liquid-ordered, or Lo phase) and are associated with flotillins. In eukaryotes, cholesterol is associated with the generation of lateral heterogeneity, including FMMs, which are enriched in flotillins (FloA and FloT) and their associated signaling complexes, with FMM formation apparently stabilized by farnesol (YisP product). No role for the C_{35} isoprenoid lipids has yet been demonstrated in biofilm formation (Lopez and Kolter, 2010) or in FMM formation or function.

Lateral heterogeneity, including FMMs, is likely a feature of most bacterial membranes. However, the lipid species that are required to form FMMs are still poorly understood, but likely include carotenoids, hopanoids, and other polyisoprenoid lipids (Lopez and Koch, 2017). Hopanoids are structurally diverse and fulfill a broad range of functions in bacterial membranes (Belin et al., 2018). The hopanoid diplopterol (Figure 1C) orders saturated lipids and glycolipids in the outer membrane of *Methylobacterium extorquens*, and deficient mutants are impaired in multidrug transport (Saenz et al., 2015). Hopanoids and other polycyclic isoprenoids are present in many Gram-positive bacteria as well, suggestive of a role in the plasma membrane. In methicillin-resistant *S. aureus*, the carotenoid staphyloxanthin (Figure 1C) colocalizes in FMMs with FloA, and disruption of these domains with isoprenoid synthesis inhibitors interferes with the function of the penicillin-binding protein required for β-lactam resistance (PBP2a) (Garcia-Fernandez et al., 2017; Foster, 2019). The formation and function of FMMs, in both the inner (plasma) and outer membrane, remains an important area for future research.

**CELL ENVELOPE STRESS RESPONSES THAT MODULATE LIPID COMPOSITION**

Bacteria generally have a negatively charged membrane, which contributes to their susceptibility to CAMPs, bacteriocins, and antimicrobials. In B. subtilis, membrane composition and properties are regulated by ECF σ factors (Eiamphungporn and Helmann, 2008; Kingston et al., 2013; Helmann, 2016). Because of their overlapping activation and promoter recognition properties, these CESRs are intertwined and referred to as an \( \sigma^{ECF} \) stress response (Mascher et al., 2007). In B. subtilis, activation of \( \sigma^{X} \) reduces the net negative charge of the membrane by increasing zwitterionic PE levels (Cao and Helmann, 2004; Ho and Ellermeier, 2019). The net negative charge of the cell wall can be further reduced by D-alanylation of teichoic acids, activated by \( \sigma^{X} \) (Cao and Helmann, 2004; Ho and Ellermeier, 2019) and \( \sigma^{V} \), a lysozyme-responsive CESR (Guariglia-Oropeza and Helmann, 2011; Ho et al., 2011; Ho and Ellermeier, 2019). In S. aureus, surface membrane charge is modified by the induction of mprF by the GraRS TCS, thereby increasing LPG levels (Falord et al., 2011; Yang et al., 2012). In *B. anthracis*, the membrane-active compound targocil activates the EdsRS TCS, which induces expression of a cardiolipin synthase (Laut et al., 2020). Thus, many different stimuli can trigger changes in the membrane lipidome.

**Bacillus subtilis \( \sigma^{ECF} \) factors also control other membrane-related functions, although the effects are not yet understood. For example, \( \sigma^{M} \) activates the ytpAB operon. The YtpA lysophospholipase cleaves FAs from the 2 position of phospholipids resulting in a lysophospholipid (bacylsocin) suggested to function as an antibiotic (Tamehiro et al., 2002). However, it is unclear if bacylsocin is ever released at levels sufficient to serve as an antibiotic, and it may instead modify membrane properties or be an intermediate in lipid remodeling. As noted above, YtpB initiates synthesis of baciterpenol (Figure 1C; Bosak et al., 2008; Sato et al., 2011; Sato, 2013). Genetic studies have revealed only modest phenotypes for ytpAB mutants, including effects on antibiotic sensitivity, sporulation, and germination (Kingston et al., 2014; Sayer et al., 2019). In the case of ytpB, the observed phenotype (bacitracin sensitivity) was due to the accumulation of the substrate (heptaprenyl-PP) rather than a loss of baciterpenol (Kingston et al., 2014).

Genetic perturbations of membrane composition can also trigger CESRs. For example, deletion of LTA synthases induces...
σECF factors. An ltaS mutation upregulates σM, which then activates expression of the alternate LTA synthase LtaSa. The absence of expression of both ltaS and ltaSa leads to activation of additional σECF factors (Hashimoto et al., 2013). The depletion of PhG, a building block of LTA, also activates σM and to a lesser extent σV (Hashimoto et al., 2009; Seki et al., 2019). The effects of mutations that affect glucolipids have been particularly challenging to understand. Glucolipids produced by UgtP are important membrane lipids and also serve as the lipid anchor of LTA. ugtP mutants lacking glucolipids are shorter and rounder, have abnormal localization of MreB, and altered assembly of FtsZ (Weart et al., 2007). Whether this abnormal morphology is due, in part, to the loss of glucolipids is unclear (Matsuoka, 2018). Mutation of ugtP activates a σECF stress response and can be suppressed by production of monoglycosyldiacylglycerol (MGlcDG) using a heterologous synthase. Since this product does not function as an LTA anchor lipid, this suggests that it is the loss of glucolipids that induces the σECF response (Matsuoka et al., 2016). The mechanistic basis for activation of σECF factors in the absence of glucolipids is unclear, but at least for σV does not require intramembrane proteolysis of the anti-σ factor (Seki et al., 2019). One hypothesis is that glucolipids might regulate folding and function of intramembrane proteins (Matsuoka, 2018).

CELL ENVELOPE STRESS RESPONSES THAT FUNCTION THROUGH MEMBRANE PROTEINS

In addition to modulating lipid composition, CESRs also induce proteins that function in membrane protection and remodeling. In B. subtilis, these proteins include two flotillin homologs (FloA, FloT), two members of the phage shock protein family (LiaH, PspA), as well as antibiotic specific detoxification modules. The roles of these proteins in stabilizing and repairing the membrane are increasingly appreciated, although the precise mechanisms remain controversial.

Flotillins and Modulation of Membrane Fluidity

Flotillins are members of the widely conserved stomatin, prohibitin, flotillin, and HflK/C (SPFH) domain proteins. Flotillins localize to FMMs and are thought to be required for FMM function. In S. aureus, FloA colocalizes with staphyloxanthin in FMMs (Garcia-Fernandez et al., 2017; Foster, 2019). In other systems, flotillins and FMMs are associated with flagellar function and chemotaxis (Padilla-Vaca et al., 2019; Takekawa et al., 2019), type VII secretion (Mielich-Suss et al., 2017), signaling (Wagner et al., 2017), and interaction with the host during infection (Hutton et al., 2017). Ongoing efforts strive to track the mobility, oligomerization state, and interaction partners of flotillins in living cells.

Bacillus subtilis FloA and FloT are oligomeric, integral membrane proteins implicated in the formation of FMMs (Lopez and Kolter, 2016; Bach and Bramkamp, 2013; Bramkamp and Lopez, 2015; Lopez and Koch, 2017), and regulated by σW (Huang et al., 1999; Cao et al., 2002a). FloA and FloT are thought to help partition the membrane into low fluidity FMM regions that are spatially distinct from more fluid regions. A direct role for flotillins in FMM formation has been challenged, however, since B. subtilis FloA and FloT do not always colocalize, and form separated foci of ~100 nm in diameter that appear spatially distinct from FMMs (Dempwoolf et al., 2016). Counter-intuitively, flotillins appear to be required for regions of increased fluidity (RFs), which are the counterpart to the FMMs. A lack of flotillins leads to a decrease in membrane fluidity and a concomitant reduction in activity of the MreB-directed elongosome complex that synthesizes peptidoglycan. This loss of membrane fluidity can be chemically complemented with fluidizing agents such as benzoyl alcohol (Zielinska et al., 2020).

Flotillins also functionally interact with DynA, a constitutively expressed dynamin homolog (Dempwoolf et al., 2012; Dempwoolf and Graumann, 2014). Dynamins are membrane-associated GTPases implicated in membrane remodeling, fusion and fission, and lipid mixing (Guo and Bramkamp, 2019). DynA may help repair damaged membrane regions, and contribute to resistance against antibiotics that bind membrane components, including nisin, bacitracin, and daptomycin (Sawant et al., 2016). Our understanding of flotillins and dynamins, and their roles in bacterial physiology is still incomplete and rapidly evolving.

Phage-Shock Proteins Protect Membrane Integrity

Cell envelope stress responses also support membrane stability through induction of PspA proteins, including two paralogs in B. subtilis: PspA an LiaH. Originally defined as part of the phage shock protein response in Escherichia coli (Kobayashi et al., 2007; Flores-Kim and Darwin, 2016), PspA proteins comprise a conserved family including the vesicle-inducing protein in plastids (VIPP1/IM30) and mammalian ESCRT III (Thurotte et al., 2017; Liu et al., 2020). PspA proteins have a conserved N-terminal amphipathic helix required for membrane binding (McDonald et al., 2015, 2017), which seems to depend on anionic lipid content and regions with unfavorable packing geometries creating stored curvature elastic stress (McDonald et al., 2015). Structural studies reveal that VIPP1 forms oligomeric rings of various symmetries that stack together to form domes (Saur et al., 2017; Gupta et al., 2020). These rings are dynamic, and are thought to stabilize membranes during budding, tubulation, and fusion (Thurotte et al., 2017; Gutu et al., 2018; Junglas and Schneider, 2018). However, the role of these oligomeric structures has been questioned (Siebenaller et al., 2019). An alternative model suggests that these rings dissociate, and the resultant intrinsically disordered monomers interact with the membrane surface to form a protective protein “carpet” to stabilize the membrane and suppress proton leakage (Junglas et al., 2020).

Although PspA proteins are assumed to function in membrane protection and repair, their regulation differs markedly (Manganelli and Gennaro, 2017). B. subtilis PspA is regulated by σW (Wiegert et al., 2001; Cao et al., 2002a), whereas the paralog LiaH is regulated by the LiaRS TCS (Mascher et al., 2004; Jordan et al., 2006). Both paralogs
localize to the membrane in response to stress and protect against membrane-damaging antibiotics (Wolf et al., 2010; Kingston et al., 2013; Domínguez-Escobar et al., 2014; Popp et al., 2020). In the case of LiaH, membrane association is mediated by interaction with the integral membrane protein LiaI (Domínguez-Escobar et al., 2014). LiaH may also protect the membrane against proton leakage during the export of proteins through the twin-arginine translocation (TAT) system (Hou et al., 2018; Bernal-Cabas et al., 2020). While the B. subtilis LiaRS regulon is rather limited in scope (Jordan et al., 2006; Wolf et al., 2010), LiaRS orthologs (e.g., S. aureus VraRS) play an important role in stress resistance in many Gram-positive pathogens, and mutations in these regulators are associated with clinical antibiotic resistance (Tran et al., 2016). In Mycobacterium tuberculosis, the PspA ortholog is also under control of the ECF σ factor ωE (Datta et al., 2015), whereas E. coli psaR requires the σ54 RNAP and PsfP activator (Joly et al., 2010; Flores-Kim and Darwin, 2016). A common theme in these systems is that PspA-like proteins are often regulated by a specific CESR; they can accumulate to high levels in stressed cells, and they seem to protect the membrane against disruptions that can dissipate the proton gradient (Manganelli and Gennaro, 2017).

Antibiotic Specific Detoxification Modules

Bacillus subtilis, like many soil bacteria, can synthesize a wide range of antimicrobial compounds and also encodes diverse resistance mechanisms (Stein, 2005; Caulier et al., 2019). Many antimicrobial peptides induce the B. subtilis LiaRS stress response that protects cells through induction of LiaH. Induction of ωW also leads to expression of the SppA membrane-localized protease and its regulatory protein SppI, which function to clear the membrane of embedded peptides to protect against lantibiotics (Kingston et al., 2013; Henriques et al., 2020). Other antimicrobial peptides induce specific detoxification machinery, often including ABC transporters that either export the peptide antibiotic or disassemble membrane-bound peptide complexes (Staron et al., 2011; Dintner et al., 2014).

A prototype for such systems is the BceRS TCS, which regulates the bacitracin-specific induction of the BceAB ABC transporter (Radeck et al., 2016; Piepenbreier et al., 2020). Bacitracin is a peptide antibiotic made by Bacillus spp. that inhibits cell wall synthesis by binding to undecaprenyl-PP. The BceAB system appears to act in disassembly of bacitracin complexes to confer resistance (Kobras et al., 2020). In addition, BceAB interacts with the BceRS TCS to allow sensing of bacitracin (Ohki et al., 2003; Dintner et al., 2014; Fritz et al., 2015; Koh et al., 2020). The BceRS-AB system provides a first line of defense against bacitracin, with higher levels of antibiotic activating the protective responses mediated by the LiaRS and ωECF regulons (Radeck et al., 2016). The detailed study of the B. subtilis bacitracin stress response has provided lessons relevant to the understanding of other antibiotic detoxification modules. Similar genetic modules, encoding both TCS and ABC transporter/sensors have been described for several other antimicrobial peptides (Revilla-Guarinos et al., 2014). Since induction can be quite specific, these systems provide a basis for antibiotic-inducible gene expression systems (Wolf and Mascher, 2016).

Bacillus subtilis also encodes and responds to many other secondary metabolites that can induce membrane stress (Caulier et al., 2019). For example, the toxic peptide YydF* is encoded by the yydFGHI operon, together with a radical-SAM epimerase (YydG), protease (YydH), and ABC transporter (YydI). Transposon insertions in the presumptive efflux pump lead to the upregulation of the LiaRS stress system (Butcher et al., 2007). Subsequent studies revealed that YydF is post-translationally processed to convert two L-amino acids to D-amino acids (Benjdia et al., 2017). The resulting epipeptide, YydF*, induces LiaRS-regulated LiaH and the FloT flotillin (Popp et al., 2020). The modified YydF* peptide kills B. subtilis cells by dissipating the membrane potential via membrane permeabilization. The associated concomitant decrease in membrane fluidity together with increased membrane permeabilization induces liaH (Popp et al., 2020). YydF* peptides are likely synthesized by a variety of Gram-positive organisms including Enterococcus, Staphylococcus, and Streptococcus spp. as well as members of the human microbiome (Benjdia et al., 2017).

Bacillus subtilis also has CESRs induced by polyketide and polypene-type antimicrobials. For example, Streptomyces spp. produce linear polyketides (linearmycins) that depolarize the membrane (Stubbendieck and Straight, 2015, 2017; Stubbendieck et al., 2018). Linearmycins strongly activate the LnrJK TCS that regulates an ABC transporter, LnrLMN (Stubbendieck and Straight, 2017; Revilla-Guarinos et al., 2020). This ABC transporter also provides resistance against other polycenes, including the anti-fungal amphotericin (Revilla-Guarinos et al., 2020).

OUTLOOK

Here we provide a brief overview of the diverse ways in which CESRs help modify and protect the membrane in response to environmental threats (Table 1). This is a rapidly evolving field, and the impact of membrane composition on cell physiology is still mysterious. We have much to learn about the synthesis and roles of minor lipids (sesquiterpenes, ether lipids, lysophospholipids). There is a growing need to reconcile current models of lipid rafts, and the role that isoprenoid lipids and flotillins play in their formation. The activities of the VIPP1/IM30/PspA family of proteins in membrane repair and protection, and in particular the specific role of different oligomeric states, are still debated. Finally, the mechanisms by which diverse CESRs sense membrane perturbations are largely unknown, although considerable progress has been made in the specific cases of the DesK sensor kinase (Aбриата et al., 2017), flux-sensing by peptide detoxification modules (Koh et al., 2020), and the lysozyme-mediated induction of the ωV protein (Ho and Ellermeier, 2019). The overall picture is of a cell membrane as a complex and adaptable assemblage of many different lipid and protein species that still has many secrets to reveal.
AUTHOR CONTRIBUTIONS
Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING
This work was funded by the National Institutes of Health under award number R35GM122461 to JH. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

ACKNOWLEDGMENTS
We appreciate helpful comments from our Cornell colleagues, Thorsten Mascher, James Saenz, and Diego de Mendoza.

REFERENCES
Abriata, L. A., Albanesi, D., Dal Peraro, M., and de Mendoza, D. (2017). Signal sensing and transduction by histidine kinases as unveiled through studies on a temperature sensor. Acc. Chem. Res. 50, 1359–1366. doi: 10.1021/acs.accounts.6b00593
Albanesi, D., Reh, G., Guerin, M. E., Schaeffer, F., Debarbouille, M., Buschiazzo, A., et al. (2013). Structural basis for feed-forward transcriptional regulation of membrane lipid homeostasis in Staphylococcus aureus. PLoS Pathog. 9.e1003108. doi: 10.1371/journal.ppat.1003108
Altabe, S. G., Aguilar, P., Caballero, G. M., and de Mendoza, D. (2003). The phosphatidylethanolamine-dependent intramembrane protease (ROMA), and transcriptional profiling approaches. J. Mol. Biol. 316, 443–457. doi: 10.1006/jmbi.2001.5372

Cao, M., Wang, T., Ye, R., and Helmann, J. D. (2002b). Antibiotics that inhibit cell wall biosynthesis induce expression of the Bacillus subtilis σW and σH regulons. Mol. Microbiol. 45, 1267–1276. doi: 10.1046/j.1365-2958.2002.03050.x
Caulier, S., Nannan, C., Gillis, A., Liciardi, F., Bragard, C., and Mahillon, J. (2019). Overview of the Antimicrobial Compounds Produced by Members of the Bacillus subtilis Group. Front. Microbiol. 10:302.

Choi, K. H., Heath, R. J., and Rock, C. O. (2000). beta-ketoacyl-acyl carrier protein synthase III (FabH) is a determining factor in branched-chain fatty acid biosynthesis. J. Bacteriol. 182, 365–370. doi: 10.1128/jb.182.2.365-370.2000
Chwastek, G., Surma, M. A., Rizk, S., Grosser, D., Lavrynenko, O., Rucinska, M., et al. (2020). Principles of membrane adaptation revealed through environmentally induced bacterial lipidome remodeling. Cell Rep. 32:108165. doi: 10.1016/j.celrep.2020.108165
Datta, P., Ravì, J., Guerrini, V., Chauhan, R., Neiditch, M. B., Shell, S. S., et al. (2015). The Psp system of Mycobacterium tuberculosis integrates envelope stress-sensing and envelope-preserving functions. Mol. Microbiol. 97, 408–422. doi: 10.1111/mmi.13037

de Mendoza, D. (2014). Temperature sensing by membranes. Annu. Rev. Microbiol. 68, 101–116. doi: 10.1146/annurev-micro-091313-103612

Demwpoloff, F., and Graumann, P. L. (2014). Genetic links between bacterial dynamin and flotillins proteins. Commun. Integr. Biol. 7:e70972. doi: 10.4161/hib.29578

Demwpoloff, 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) paragons provide evidence for defined-sized protein microdomains within the bacterial membrane but absence of clusters containing detergent-resistant proteins. PLoS Genet. 12:e1006116. doi: 10.1371/journal.pgen.1006116

Demwpoloff, F., Wischhusen, H. M., Specht, M., and Graumann, P. L. (2012). The deletion of bacterial dynamin and flotillin genes results in pleiotropic effects on cell division, cell growth and in cell shape maintenance. BMC Microbiol. 12:298. doi: 10.1186/1471-2180-12-298

Devkota, S. R., Kwon, E., Ha, S. C., Chang, H. W., and Kim, D. Y. (2017). Structural insights into the regulation of Bacillus subtilis σW activity by anti-sigma RsiW. PLoS One 12:e0174284. doi: 10.1371/journal.pone.0174284

Dintner, S., Heermann, R., Fang, C., Jung, K., and Gebhard, S. (2014). A sensory complex consisting of an ATP-binding cassette transporter and a two-component regulatory system controls bacitracin resistance in Bacillus subtilis. J. Bacteriol. 196, 716–732. doi: 10.1128/jb.07465-13

Dominguez-Escobar, J., Wolf, D., Fritz, G., Hofler, C., Wedlich-Soldner, R., and Bieber, C. (2014). A protein folding and topogenesis. Protein J. 33, 698–717. doi: 10.1007/s10979-014-9672-x

Eiamphungporn, W., and Helmann, J. D. (2008). The Bacillus subtilis σM regulon and its contribution to cell envelope stress responses. Mol. Microbiol. 67, 830–848. doi: 10.1111/j.1365-2958.2007.06090.x

Ellermeier, C. D., and Losick, R. (2006). Evidence for a novel protease governing regulated intramembrane proteolysis and resistance to antimicrobial peptides in Bacillus subtilis. Genes Dev. 20, 1911–1922. doi: 10.1101/gad.14406
Bacillus subtilis

Ernst, C. M., and Peschel, A. (2019). MprF-mediated daptotypeycin resistance. Int. J. Med. Microbiol. 309, 359–363. doi: 10.1016/j.ijmm.2019.05.010

Ernst, C. M., Slavetinsky, C. J., Kuhn, S., Hauser, J. N., Nega, M., Mishra, N. N., et al. (2018). Gain-of-function mutations in the phospholipid flipase MprF confer specific daptotypeycin resistance. mBio 9(6), e02639-18.

Ernst, R., Eising, C. S., and Antony, B. (2016). Homoeosporic adaptation and the regulation of membrane lipids. J. Mol. Biol. 428, 4776–4791. doi: 10.1016/j.jmb.2016.08.013

Falord, M., Mader, U., Hiron, A., Debarbouille, M., and Msadek, T. (2011). Regulation of fatty acid synthesis in Bacillus subtilis with defects in lipoteichoic acid synthesis. Mol. Microbiol. 81, 191–198. doi: 10.1111/j.1365-2958.2012.08038.x

Feng, X., Hu, Y., Zheng, Y., Zhu, W., Li, K., Huang, C. H., et al. (2014). Structural basis for VIPP1 oligomerization and maintenance of thylakoid membrane integrity. J. Biol. Chem. 293, 7592–7605. doi: 10.1074/jbc.m114.580793

Fonseca, L., and Bramkamp, M. (2019). Bacterial dynamin-like protein DynA forms a membrane protein complex with sppa and inhibits its protease activity in Bacillus subtilis. mSphere 5:e00724-20.

Ho, T. D., and Ellermeier, C. D. (2019). Activation of the extracelltoplasmic function sigma factor σW by lysozyme. Mol. Microbiol. 112, 410–419. doi: 10.1111/mmi.14338

Ho, T. D., Hastie, J. L., Intille, P. J., and Ellermeier, C. D. (2011). The Bacillus subtilis extracelltoplasmic function sigma factor σW is induced by lysozyme and provides resistance to lysozyme. J. Bacteriol. 193, 6215–6222. doi: 10.1128/jb.05467-11

Holley, C., Heckmann, J., Fritsch, A., Popp, P., Gehbard, S., Fritz, G., et al. (2016). Cathombin stress response in Bacillus subtilis. Microbiology (Reading) 162, 164–176. doi: 10.1099/mic.0.001716

Hou, B., Heidrich, E. S., Mehnert-Breitfeld, D., and Bruser, T. (2018). The TatA component of the twin-arginine translocation system locally weakens the cytoplasmic membrane of Escherichia coli upon protein substrate binding. J. Biol. Chem. 293, 7592–7605. doi: 10.1074/jbc.r18.002205

Huang, X., Gallabala, A., Cao, M., and Helmann, J. D. (1999). Identification of target promoters for the Bacillus subtilis extracelltoplasmic function sigma factor, σW. Mol. Microbiol. 31, 361–371. doi: 10.1046/j.1365-2958.1999.01180.x

Hutton, M. L., D’Costa, K., Rossiter, A. E., Wang, L., Turner, L., Steer, D. L., et al. (2017). A Helicobacter pylori homolog of eukaryotic flotillin is involved in cholesterol accumulation, epithelial cell responses and host colonization. Front. Cell Infect. Microbiol. 7:219.

Jolliffe, L. K., Doyle, R. J., and Streips, U. N. (1981). The energized membrane hypothesis of thylakoid metabolism in bacteria. Mol. Microbiol. 66, 829–839. doi: 10.1111/j.1365-2958.2007.05974.x

Guo, L., and Bramkamp, M. (2019). Bacterial dynamin-like protein DynA mediates lipid and content mixing. FASEB J. 33, 11746–11757. doi: 10.1096/fj.201900844r

Gupta, T. K., Klumpe, S., Gries, K., Heinz, S., Wietrzyński, W., Ohnishi, N., et al. (2020). Structural basis for VIPP1 oligomerization and maintenance of thylakoid membrane integrity. BioRxiv [Preprint]. doi: 10.1101/2020.08.11.243204

Gutu, A., Chang, F., and O’Shea, E. K. (2018). Dynamical localization of a thylakoid membrane protein is required for acquisition of photosynthetic competency. Mol. Microbiol. 108, 16–31. doi: 10.1111/mmi.13912

Hachmann, A. B., Angert, E. R., and Helmann, J. D. (2009). Genetic analysis of factors affecting susceptibility of Bacillus subtilis to daptomycin. Antimicrob. Agents Chemother. 53, 1598–1609. doi: 10.1128/aac.01329-08

Hachmann, A. B., Sevim, E., Gaballa, A., Popham, D. L., Antelmo, H., and Helmann, J. D. (2011). Reduction in membrane phosphatidylglycerol content leads to daptomycin resistance in Bacillus subtilis. Antimicrob. Agents Chemother. 55, 4326–4337. doi: 10.1128/aac.01819-10

Hashimoto, M., Seki, T., Matsuoka, S., Hara, H., Asai, K., Sadaie, Y., et al. (2013). Induction of extracelltoplasmic function sigma factors in Bacillus subtilis cells with defects in lipoteichoic acid synthesis. Microbiology (Reading) 159, 23–35. doi: 10.1099/mic.0.063420-0

Hashimoto, M., Takahashi, H., Hara, Y., Hara, H., Asai, K., Sadaie, Y., et al. (2009). Induction of extracelltoplasmic function sigma factors in Bacillus subtilis cells with membranes of reduced phosphatidylglycerol content. Genes. Genet. Syst. 84, 191–198. doi: 10.1266/gss.84.191

Helmann, J. D. (2006). Deciphering a complex genetic regulatory network: the Bacillus subtilis σW protein and intrinsic resistance to antimicrobial compounds. Sci. Prog. 89, 243–266. doi: 10.3184/003685006783234200

Helmann, J. D. (2016). Bacillus subtilis extracelltoplasmic function (ECF) sigma factors and defense of the cell envelope. Curr. Opin. Microbiol. 30, 122–132. doi: 10.1016/j.mib.2016.02.002

Henriques, G., McGovern, S., Neef, J., Antelo-Varela, M., Gotz, F., Otto, A., et al. (2020). SppI forms a membrane protein complex with sppa and inhibits its protease activity in Bacillus subtilis. mSphere 5:e00724-20.

Lamsa, A., Liu, W. T., Dorrestein, P. C., and Pogliano, K. (2012). The Bacillus subtilis cannibalism toxin SDP collapses the proton motive force and induces autolysis in Staphylococcus aureus with depsipeptide resistance. Antimicrob. Agents Chemother. 56, 486–500. doi: 10.1128/AAC.01557-12

Laut, C. L., Perry, W. J., Metzger, A. L., Weiss, A., Stauff, D. L., Walker, S., et al. (2020). Bacillus anthracis responds to targcis-induced envelope damage through EdsRS activation of cardiolipin synthesis. mBio 11:e03375-19.
Liu, J., Tassinari, M., Souza, D. P., Naskar, S., Noel, J. K., Bohuszewicz, O., et al. (2020). Bacterial VipP and PsA are members of the ancient ESCR-III membrane-remodelling superfamily. bioRxiv [Preprint]. doi: 10.1101/2020.08.13.249979

Lopez, D., and Koch, G. (2017). Exploring functional membrane microdomains in bacteria: an overview. *Curr. Opin. Microbiol.* 36, 76–84. doi: 10.1016/j.mib.2017.02.001

Lopez, D., and Kolter, R. (2010). Functional microdomains in bacterial membranes. *Genes Dev.* 24, 1893–1902. doi: 10.1101/gad.1945010

Machinandiarena, F., Nakamatsu, L., Schujman, G. E., de Mendoza, D., and Albani, D. (2020). Revisiting the coupling of fatty acid to phospholipid synthesis in bacteria with FapR regulation. *Mol. Microbiol.* 114:14574.

Manganelli, R., and Gennaro, M. L. (2017). Protecting from envelope stress: variations on the phase-shock-protein theme. *Trends Microbiol.* 25, 205–216. doi: 10.1016/j.tim.2016.10.001

Martinez, M. A., Zamalloa, M. E., Schaeffer, F., Bellinzoni, M., Albañez, D., Schujman, G. E., et al. (2010). A novel role of malonyl-ACP in lipid homeostasis. *Biochemistry* 49, 3161–3167. doi: 10.1021/bi100136n

Mascher, T., Hachmann, A. B., and Helmann, J. D. (2007). Regulatory overlap and functional redundancy among *Bacillus subtilis* extractoplastic function sigma factors. *J. Bacteriol.* 189, 6919–6927. doi: 10.1128/JB.00904-07

Mascher, T., Zimmer, S. L., Smith, T. A., and Helmann, J. D. (2004). Antibiotic-inducible promoter regulated by the cell envelope stress-sensing two-component system LiaRS of *Bacillus subtilis*. *Antimicrob. Agents Chemother.* 48, 2888–2896. doi: 10.1128/AAC.48.8.2888-2896.2004

Matsuoka, S. (2018). Biological functions of glucolipids in *Bacillus subtilis*. *Genes Genet. Syst.* 92, 217–221. doi: 10.1266/ggs-17-00017

Matsuoka, S., Seki, T., Matsumoto, K., and Hara, H. (2016). Suppression of abnormal morphology and extractoplastic function sigma activity in *Bacillus subtilis* ugp mutant cells by expression of heterologous glucolipid synthases from *Acholeplasma laidlawii*. *Biosci. Biotechnol. Biochem.* 80, 2323–2333. doi: 10.1080/09168451.2016.1217147

McDonald, C., Jovanovic, G., Ces, O., and Buck, M. (2015). Membrane stored curvature elastic stress modulates recruitment of maintenance proteins PsP and VipP. *mBio* 6:e001188-15.

McDonald, C., Jovanovic, G., Wallace, B. A., Ces, O., and Buck, M. (2017). Structure and function of PsP and VipP N-terminal peptides: insights into the membrane stress sensing and mitigation. *Biochim. Biophys. Acta Biomembr.* 1859, 28–39. doi: 10.1016/j.bbamem.2016.10.018

Mielich-Suss, B., Wagner, R. M., Mietrach, N., Herltlein, T., Marincola, G., Ohlsen, K., et al. (2017). Flotillin scaffold activity contributes to type VII secretion of the Bacillus subtilis envelope stress response in *Acholeplasma laidlawii*. *Nat. Rev. Microbiol.* 15, 1577–1592. doi: 10.1016/j.vcell.2017.09.001

Mielich-Suss, B., Wagner, R. M., Mietrach, N., Hertlein, T., Marincola, G., Ohlsen, K., et al. (2017). Flotillin scaffold activity contributes to type VII secretion system in *Acholeplasma laidlawii*. *Cell Res.* 27, 668–683. doi: 10.1038/cr.2016.156

Miyashita, Y., and Hattori, M. (2010). From modules to networks: a systems-level analysis of the bacitracin resistance network in *Bacillus subtilis*. *mSystems* 5:e00687-19.

Piettainen, M., Gardemeister, M., Mecklin, M., Leskela, S., Sarvas, M., and Kontinen, V. P. (2005). Cationic antimicrobial peptides elicit a complex stress response in *Bacillus subtilis* that involves ECF-type sigma factors and two-component signal transduction systems. *Microbiology (Reading)* 151, 1577–1592. doi: 10.1099/mic.0.27761-0

Popp, P. F., Bendjida, A., Strahl, H., Bertea, O., and Mascher, T. (2020). The epideptide YydF intrinsically triggers the cell envelope stress response of *Bacillus subtilis* and causes severe membrane perturbations. *Front. Microbiol.* 11:151.

Radeck, J., Fritz, G., and Mascher, T. (2017). The cell envelope stress response of *Bacillus subtilis*: from static signaling devices to dynamic regulatory network. *Curr. Genet.* 63, 79–90. doi: 10.1007/s00294-016-0624-0

Radeck, J., Gehbhard, S., Orchard, P. S., Kirchner, M., Bauer, S., Mascher, T., et al. (2016). Anatomy of the bacitracin resistance network in *Bacillus subtilis*. *Mol. Microbiol.* 100, 607–620. doi: 10.1111/mmi.13336

Rajagopal, M., and Walker, S. (2017). Envelope structures of gram-positive bacteria. *Curr. Top. Microbiol. Immunol.* 404, 1–44. doi: 10.1007/978-2015-5021

Revilla-Guarinos, A., Durr, F., Popp, P. F., Doring, M., and Mascher, T. (2020). Amphotericin B specifically induces the two-component system LmrJK-development of a novel whole-cell biosensor for the detection of amphotericin-like polyenes. *Front. Microbiol.* 11:2022.

Revilla-Guarinos, A., Gehbhard, S., Mascher, T., and Zuniga, M. (2014). Defence against antimicrobial peptides: different strategies in Firmicutes. *Environ. Microbiol.* 16, 1225–1237. doi: 10.1111/1462-2920.12400

Saenz, J. P., Grosser, D., Bradley, A. S., Lagney, T. J., Lavrenenko, O., Broda, M., et al. (2015). Hopanoids as functional analogues of cholesterol in bacterial membranes. *Proc. Natl. Acad. Sci. U.S.A.* 112, 11971–11976. doi: 10.1073/pnas.1516071112

Salie, M. J., and Thelen, J. J. (2016). Regulation and structure of the heteromeric acetyl-CoA carboxylase. *Biochim. Biophys. Acta* 1861, 1207–1213.

Salzberg, L. I., and Helmann, J. D. (2008). Phenotypic and transcriptomic characterization of *Bacillus subtilis* mutants with grossly altered membrane composition. *J. Bacteriol.* 190, 7797–7807. doi: 10.1128/jb.00720-08

Sato, T. (2013). Unique biosynthesis of sesquiterpenes (C35 terpenes). *Biosci. Biotechnol. Biochem.* 77, 1155–1159. doi: 10.1271/bbb.130180

Sato, T., Yoshida, S., Hoshino, H., Tanno, M., Nakajima, M., and Hoshino, T. (2011). Sesquiterpenes (C35 terpenes) biosynthesized via the cyclization of a linear C25 isoprenoid by a tetraprenyl-beta-curcumene synthase and a tetraprenyl-beta-curcumene cyclase: identification of a new terpene cyclase. *J. Am. Chem. Soc.* 133, 9734–9737. doi: 10.1021/ja203779h

Saunders, L. P., Sen, S., Wilkinson, B. J., and Gatto, C. (2016). Insights into the mechanism of homeoviscous adaptation to low temperature in branched-chain fatty acid-containing bacteria through modeling FabH kinetics from the foodborne pathogen *Listeria monocytogenes*. *Front. Microbiol.* 7:1386.

Saur, M., Hennig, R., Young, P., Rusitzka, K., Hellmann, N., Heidrich, J., et al. (2017). A Janus-faced IM30 ring involved in thylokinid membrane fusion is assembled from three IM30 tetramers. *Structure* 25, 1380–1390.e1385.x

Sawant, P., Eissenberger, K., Karier, L., Mascher, T., and Brankamp, M. (2016). A dynamin-like protein involved in bacterial cell membrane surveillance under environmental stress. *Environ. Microbiol.* 18, 2705–2720. doi: 10.1111/1462-2920.13110
