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Authors

Kostow, Nora
Welch, Matthew

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Manipulation of host cell plasma membranes by intracellular bacterial pathogens

Nora Kostow[a], **Matthew D. Welch**[a,*]

[a]Department of Molecular & Cell Biology, University of California, Berkeley, Berkeley, CA 94720, USA.

Abstract

Manipulation of the host cell plasma membrane is critical during infection by intracellular bacterial pathogens, particularly during bacterial entry into and exit from host cells. To manipulate host cells, bacteria deploy secreted proteins that modulate or modify host cell components. Here, we review recent advances that suggest common themes by which bacteria manipulate the host cell plasma membrane. One theme is that bacteria use diverse strategies to target or influence host cell plasma membrane composition and shape. A second theme is that bacteria take advantage of host cell plasma membrane-associated pathways such as signal transduction, endocytosis, and exocytosis. Future investigation into how bacterial and host factors contribute to plasma membrane manipulation by bacterial pathogens will reveal new insights into pathogenesis and fundamental principles of plasma membrane biology.

Introduction

The plasma membrane serves the key function of separating a cell's interior from the surrounding environment. Because it serves as a barrier, bacterial pathogens that live inside host cells (intracellular bacterial pathogens) must manipulate the host cell plasma membrane as part of their life cycle [1]. This occurs as bacteria enter into (invade) and exit from the host cell. In this review, we cover recent discoveries of bacterial manipulation of the host cell plasma membrane for several intracellular bacterial pathogens. We discuss stages of invasion including internalization and vacuole scission (reviewed in [2]). We also discuss pathways for exit including cell escape as well as direct cell-to-cell spread by plasma membrane engulfment or plasma membrane fusion [3,4]. Understanding how bacteria manipulate the host cell plasma membrane can teach us about mechanisms of bacterial pathogenesis as well as the features and dynamics of the plasma membrane barrier.

To manipulate the host cell plasma membrane, intracellular bacterial pathogens typically deploy secreted proteins [5,6]. Some secreted proteins are displayed on the bacterial surface whereas others are injected into host cells through bacterial protein secretion systems [6,7]. These include the type-III secretion system (T3SS), which translocates unfolded proteins from bacteria across the host cell plasma membrane into the host cell cytoplasm [8,9]. Once

[*]**Address correspondence to:** Matthew D. Welch, University of California, Berkeley, Department of Molecular & Cell Biology, 301 Weill Hall, Berkeley, CA 94720-3200, (510) 643-9019, welch@berkeley.edu.

secreted, these bacterial proteins carry out their effector functions, for example by binding to or enzymatically modifying host factors to influence their localization or activity [10,11]. It is through effector protein-mediated manipulation of host factors that intracellular bacterial pathogens control host cell structures and functions. Therefore, to reveal how bacterial pathogens manipulate the host cell plasma membrane, it is crucial to understand both the bacterial and host factors that are involved.

Recent work has revealed two common themes of bacterial manipulation of host plasma membranes. One theme is that bacteria manipulate or co-opt host plasma membrane features, such as plasma membrane composition and shape. A second theme is that bacteria manipulate or co-opt plasma membrane-associated host cell pathways including signal transduction, endocytosis, and exocytosis. We explore how these functions are carried out by bacterial proteins and host factors during host cell invasion and exit, focusing only on pathogens for which there have been recent advances, and acknowledging that older contributions reviewed elsewhere are not comprehensively covered here. For some examples covered, the bacterial and host proteins responsible for controlling these features and pathways are unidentified or their roles are only beginning to be understood. Nevertheless, the advances reviewed here give insights into how bacterial pathogens manipulate the host cell plasma membrane to their benefit.

Bacterial invasion of host cells

Host cell invasion is the first step in the intracellular life cycle of bacterial pathogens and involves multiple steps (Figure 1A) [2]. Bacteria must first recognize and bind to components of the host cell plasma membrane, which is often mediated by bacterial proteins as well as host lipids and membrane proteins [2,12]. Bacteria must then transduce a signal across the host plasma membrane to trigger the host cell to internalize the bacterium [2,12]. This process involves cytoskeleton-driven envelopment of the bacterium by the plasma membrane [2,12]. Finally, the invaginated plasma membrane structure containing the bacterium must undergo scission to form an intracellular vacuole [2,12]. Recent work provides examples of bacterial proteins that bind plasma membrane lipids and proteins, change membrane curvature, activate host cell signaling pathways, and alter host protein activities to achieve cell invasion.

Internalization

One common way that bacterial proteins engage with the host cell plasma membrane is by direct binding to lipids or protein receptors on the plasma membrane to initiate a signal transduction cascade. Bacterial lectins have recently been shown to be actively involved in cell invasion [13,14] (Figure 1B). The *Pseudomonas aeruginosa* lectins LecA and LecB are virulence proteins presented on the outer bacterial surface [15]. LecA and LecB form homotetramers that contain four binding sites to either glycosphingolipid globotriaosylceramide (Gb3) for LecA or fucose-bearing lipids for LecB [15]. LecA binding to extracellular Gb3 leads to localization of CD59, flotillin-1 and 2, phosphatidylinositol (3,4,5) trisphosphate ($PI(3,4,5)P_3$), and Src to LecA-bound plasma membrane regions and eventually Rac1 activation [13]. LecA, CD59, and flotillins are all required for efficient

host cell invasion and likely function in the same pathway [13]. The authors propose that LecA recruits these components to promote the cytoskeletal reorganization involved in *P. aeruginosa* internalization [13].

LecB also acts as an invasion protein as it is required for efficient cell invasion and is sufficient to induce the cellular uptake of LecB-coated beads [14] (Figure 1B). Addition of LecB to cells activates a host protein signaling cascade involving Src, PI3 kinase, AKT, and Rac1, which leads to actin rearrangements at LecB-enriched regions [14]. This pathway may mediate LecB-based bacterial uptake into cells. Additionally, LecB alone binds to β1-integrin, which leads to LecB internalization along with β1-integrin binding partners α3-integrin and laminin [16]. Interestingly, LecB induced the formation of membrane invaginations on unilamellar vesicles [16]. The authors propose a mechanism in which membrane invaginations formed by LecB and by crosslinking between glycosphingolipids and integrins causes endocytosis of the lectin-bound plasma membrane region, a mechanism similar to that proposed for internalization of the host lectin galectin-3 [16–18]. β1-integrin is used by other pathogens for host cell invasion [12], but whether integrin binding or internalization participate in *P. aeruginosa* host cell invasion is not known.

Similar to how LecB manipulates host glycosphingolipids for internalization, it was recently found that *Neisseria meningitidis* invasion into host cells involves the glycosphingolipid monosialotetrahexosylganglioside (GM1), with GM1 coating *N. meningitidis* upon host cell invasion [19] (Figure 1B). Unlike for *P. aeruginosa* lectins, this process probably does not involve direct binding of *N. meningitidis* proteins to GM1. Rather, GM1 might be involved in a signaling cascade downstream of type IV pilus-host interactions [19].

Staphylococcus aureus also manipulates host plasma membrane lipid species, including phosphatidylinositol (4,5) bisphosphate ($PI(4,5)P_2$), during invasion [20] (Figure 1B). *S. aureus* uses fibronectin-binding proteins to initiate internalization via binding to fibronectin and integrin downstream of fibronectin [21]. It was recently shown that $PI(4,5)P_2$ also plays a positive role in *S. aureus* internalization along with the kinase PIP5KIγ, which generates $PI(4,5)P_2$ at the plasma membrane [22]. $PI(4,5)P_2$ can help localize integrin-linked proteins such as focal adhesion kinase (FAK) and talin to the plasma membrane [20,23]. The authors find that talin enrichment at bacterial entry sites and FAK activity are reduced upon PIP5KIγ knockdown or perturbation, indicating that $PI(4,5)P_2$ is involved in regulating these components as part of an integrin-mediated cell invasion pathway [22].

Membrane curvature is another important feature of plasma membranes that can be manipulated and used by bacterial pathogens. One example of a protein that induces curvature is LecB which forms membrane invaginations as discussed above [16]. Another recently discovered example is the *Chlamydia pneumoniae* T3SS effector protein SemC, which participates in cell invasion [24] (Figure 1C). SemC is secreted by the T3SS during invasion, binds the inner surface of the plasma membrane of host cells, and induces membrane curvature. SemC directly binds and recruits host sorting nexin 9 (SNX9) which is additionally recruited through the BAR domain of SNX9 sensing the membrane curvature induced by SemC. The interaction with SNX9 is involved in *C. pneumoniae*

internalization, indicating that SemC-recruited SNX9 might facilitate internalization by stabilizing endocytic vesicle curvature.

Vacuole scission

One of the final steps in host cell invasion is scission of the plasma membrane to form what will become the bacterium-containing vacuole. *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) uses the T3SS effectors SopB and SopD during the vacuole scission step of invasion, with SopB recruiting SopD to sites of invasion [25]. It was recently found that SopD functions as a GTPase activating protein (GAP) to inactivate Rab10 [26] (Figure 1D). Rab10 and its downstream effectors MICAL-L1 and EHBP1 function by localizing to membrane invaginations and limiting bacterial host cell invasion, as inactivating these proteins promoted bacterial invasion. SopD inactivation of Rab10 also promotes the recruitment of the endocytosis protein dynamin 2 to invasion sites, which mediates the scission step of *S.* Typhimurium vacuole internalization.

SopB was previously thought to act only as a PI phosphatase [27] to deplete PI(4,5)P_2 at sites of invasion for vacuole scission [28]. However, it was recently shown that SopB can also act as a phosphotransferase to convert PI(4,5)P_2 into PI(3,4)P_2 in the host cell plasma membrane to promote host cell invasion [29] (Figure 1D). This is an unprecedented mechanism of forming PI(3,4)P_2 because it does not use phosphoinositide 3-kinases, which are used by host cells to phosphorylate PI(4)P and produce PI(3,4)P_2. Therefore, SopB is an interesting example of a bacterial effector protein that manipulates plasma membrane phosphoinositides for invasion through a non-canonical mechanism.

Bacterial exit from host cells

Bacteria manipulate the host plasma membrane during bacterial exit, including escape from cells and transfer between cells. During host cell exit, bacteria act from within the cell, necessitating mechanisms of plasma membrane manipulation distinct from those used during cell invasion. Bacteria can escape a host cell in several ways. For example, they can exit a cell through host cell lysis or fusion of a bacterium-containing vacuole with the plasma membrane, among other mechanisms, although most of these will not be discussed in this review (reviewed in [3,4]) (Figure 2A). Some bacteria that live in the host cell cytosol can move directly from one cell to another without leaving the cellular environment, a process called cell-to-cell spread [30,31] (Figure 2A). During cell exit, bacteria use effector proteins and host factors that manipulate plasma membrane shape and composition, cortical tension, and host protein activities.

Escape from host cells

As they exit the host cell, bacteria often manipulate the host plasma membrane in a controlled manner so as not to destroy the host cell or activate the immune system [3]. Some bacteria exit their host cells by escaping the cellular environment and moving into the extracellular space. One recently discovered strategy, used by the cytosolic pathogen *Vibrio parahaemolyticus*, is manipulation of the host cell plasma membrane composition to promote cell exit [32] (Figure 2B). *V. parahaemolyticus* requires the type-II secretion system

(T2SS)-secreted lipase VPA0226 for proper host cell egress. VPA0226 leads to an increase in free, esterified cholesterol and a decrease in cholesterol in the plasma membrane of the host cells. VPA0226 also leads to damage of the plasma membrane. The authors hypothesize that so-called weakening of the plasma membrane aids in bacterial escape from the cell. Determining how plasma membrane cholesterol content participates in the mechanism of escape remains an interesting topic for future investigation.

Cell-to-cell spread by engulfment

Rather than exiting the host cell environment, some bacterial pathogens undergo cell-to-cell spread. Studies of the cytosolic bacterial pathogens *Shigella flexneri* and *Listeria monocytogenes* have established a standard cellular-level pathway of cell-to-cell spread (Figure 2A). In this pathway, bacteria undergo actin-based motility, leading to the bacteria pushing against the “donor” cell plasma membrane and forming a membrane protrusion that extends into the neighboring “recipient” cell. The membrane protrusion is then engulfed by the recipient cell, a double membrane vacuole is formed, and bacteria escape the vacuole to gain access to the recipient cell cytosol. While the cellular level pathway leading to engulfment has been well described [30,31], the molecular details remain largely unknown. Actin-based motility and membrane protrusion formation by a bacterium is sufficient to induce engulfment [33], but bacterial effector proteins unrelated to actin-based motility have been found to contribute to both protrusion formation and engulfment [30,31], suggesting that bacteria have evolved mechanisms to manipulate this process. Similarly, host factors contribute to protrusion formation and engulfment [30,31]. Below are recent insights into bacterial and host factors that contribute to cell-to-cell spread.

As with host cell exit by *V. parahaemolyticus* [32], manipulation of lipid composition from within the host cell is involved in *S. flexneri* cell-to-cell spread [34] (Figure 2C). The T3SS effector IpgD has phosphatidylinositol 4 (PI(4)) phosphatase activity [35] and was recently show to likely also have phosphotransferase activity [29]. IpgD functions to reduce PI(4,5)P_2 levels and acts during protrusion engulfment [34]. Without IpgD, cortical actin accumulates within *S. flexneri* membrane protrusions [34]. The authors suggest that *S. flexneri* secretes IpgD within the cytosol of the protrusion which decreases cortical actin which might aid protrusion engulfment [34]. Determining how reduced levels of PI(4,5)P_2 and cortical actin might contribute to engulfment remains an interesting topic for future investigation.

Beyond manipulating host cell plasma membrane composition, an emerging theme is that cytosolic bacteria manipulate tension at the host cell plasma membrane. Reducing tension functions to either help form membrane protrusions, in the case of *L. monocytogenes* [36], or engulf membrane protrusions, in the case of *Rickettsia parkeri* [37]. It was recently shown that the *S. flexneri* T3SS-secreted effector protein IpaC is required for efficient protrusion formation [38] (Figure 2C). IpaC reduces tension by binding to β-catenin and disrupting adherens junctions at the plasma membrane [38], although precisely how this lowers tension is unclear.

Bacteria also manipulate host cell pathways that involve the plasma membrane, including exocytosis and endocytosis. Recent work explored how cell-to-cell spread of both *S. flexneri* and *L. monocytogenes* involves a specialized exocytosis called polarized exocytosis.

Exocytosis is increased within membrane protrusions and components of the host exocyst complex, which controls the location of polarized exocytosis, are required for efficient spread [39,40] (Figure 2C). The *L. monocytogenes* effector InlC is involved in the increase in polarized exocytosis within protrusions and interacts with the exocyst component Exo70, suggesting that it might recruit the exocyst complex to direct polarized exocytosis to a particular location, for example, to membrane protrusions [39]. *S. flexneri* requires the T3SS for stimulating polarized exocytosis, suggesting the involvement of a T3SS effector protein(s), although an effector protein controlling exocytosis was not identified [40]. For both organisms, polarized exocytosis seems to be required for protrusion formation as well as achieving long protrusions [39,40]. Although several possible hypotheses were proposed, how exocytosis contributes to plasma protrusions remains an interesting open question. Exocyst components have also been shown to be involved in host cell invasion by *L. monocytogenes*, *S. aureus*, and *S.* Typhimurium [41–44], indicating that bacteria target the exocyst to manipulate the plasma membrane during several stages of the infectious lifecycle.

Recent papers also identified host factors involved in clathrin-mediated endocytosis and caveolae-mediated endocytosis as important for cell-to-cell spread [45,46] (Figure 2C). Interestingly, the identified proteins bind or sculpt curved membranes, highlighting that host plasma membrane shape might be a key feature that bacteria manipulate during spread. Caveolins, which form caveolae, were shown to be involved in *L. monocytogenes* cell-to-cell spread [45,46]. Caveolins appear to function during protrusion engulfment, as evidenced by a higher percentage of bacteria in membrane protrusions that are likely paused prior to engulfment upon caveolin knockdown [46]. Another protein involved in caveolae biogenesis, the F-bar protein PACSIN2, was found to be involved in cell-to-cell spread and acts within the receiving cell to promote protrusion engulfment [46]. Epsin-1, a membrane curvature-inducing protein involved in clathrin-mediated endocytosis, is also involved in *L. monocytogenes* cell-to-cell spread [45]. With regards to localization, epsin-1 localizes to membrane protrusions from the receiving cell side [45], but there is conflicting evidence for PACSIN2 and caveolin localization to membrane protrusions [45,46]. Several other proteins were shown to localize to bacterial protrusions formed by *L. monocytogenes*, but their functional involvement in cell-to-cell spread remains to be determined [45]. The involvement of caveolins and PACSIN2 suggests that caveolae-based endocytosis participates in the cell-to-cell spread pathway. Although epsin-1 participates in spread, *L. monocytogenes* spread was previously found to be unaffected by a clathrin-mediated endocytosis inhibitor, indicating that this pathway may not contribute to cell-to-cell spread of *L. monocytogenes* [47]. It was also previously found that *S. flexneri* uses a non-canonical clathrin-mediated endocytosis trafficking pathway, involving epsin-1, for protrusion engulfment [47]. The more recent findings may similarly indicate exploitation of a non-canonical method of protrusion engulfment by *L. monocytogenes*. Precisely how host endocytic proteins function during *L. monocytogenes* cell-to-cell spread is an interesting open question, especially given that the size of the bacteria (2–3 μm) being engulfed is much larger than conventional endocytic vesicles (<0.1 μm) [48]. Other key outstanding questions are whether these host factors simply act in response to membrane protrusion formation, or whether and how bacterial proteins manipulate these host factors.

Cell-to-cell spread by cell-cell fusion

In addition to manipulating plasma membrane features and pathways already discussed in this review, bacterial pathogens can also direct the fusion of plasma membranes. *Burkholderia* species of the pseudomallei group can spread from cell-to-cell by the merging the donor and recipient cell plasma membranes causing cell-cell fusion [49] (Figure 2A). Recently, the cellular-level mechanism of this pathway was described, revealing that *B. thailandensis* forms long membrane protrusions through actin-based motility and that membrane fusion occurs within these protrusions [50] (Figure 2D). Cell-cell fusion requires the bacterial type VI secretion system (T6SS), including the predicted effector protein VgrG5 [51,52]. Furthermore, VgrG5 must be expressed within plasma membrane protrusions to support membrane fusion [50]. It was also recently shown that cell-cell fusion requires the T6SS protein TagD5, which is predicted to interact with VgrG5, suggesting that these proteins may work together [50]. Future work will be needed to reveal whether these bacterial proteins directly induce plasma membrane fusion within membrane protrusions.

Conclusions and future directions

Recent advances have enhanced our understanding of how pathogens manipulate the host cell plasma membrane. However, there remain many open questions and areas for future investigation. For example, it remains unclear how diverse bacteria use distinct mechanisms to transduce a signal across the plasma membrane to induce bacterial invasion. Furthermore, following cell invasion, the vacuole membrane surrounding the bacterium is either manipulated to establish an intracellular niche or disrupted to allow the bacterium to escape into the cytosol. How this occurs is largely unknown for many pathogens. It also remains unclear how diverse pathogens use distinct mechanisms for host cell exit, including using exit pathways not discussed in this review, such as vacuole fusion with the plasma membrane and host cell lysis. Additionally, cell-to-cell spread by engulfment involves non-canonical endocytic pathways, but how these pathways contribute is unknown. Lastly, the molecular mechanisms of bacterially induced cell-cell fusion largely remain a mystery. Future studies should focus on the diverse mechanisms by which secreted bacterial proteins manipulate plasma membrane features, alter host protein activities, and manipulate cell pathways. This will yield a better understanding of mechanisms of pathogenesis and fundamental principles of plasma membrane biology.

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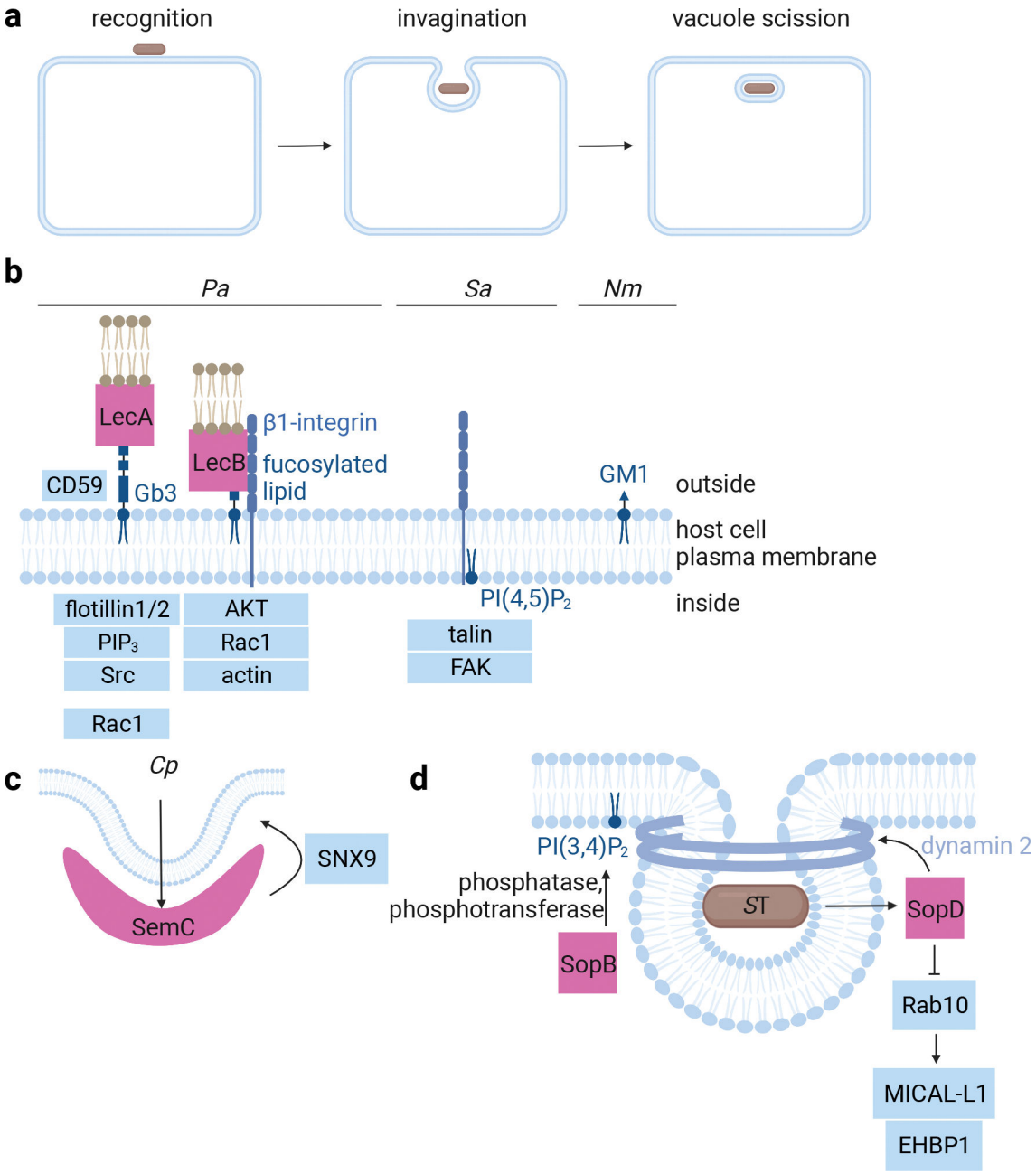

Figure 1.

Plasma membrane manipulation during bacterial invasion of host cells. (A) Three stages through which intracellular bacterial pathogens invade host cells. (B) Bacterial pathogens including *Pseudomonas aeruginosa* (*Pa*), *Staphylococcus aureus* (*Sa*), and *Neisseria meningitidis* (*Nm*), utilize plasma membrane components and induce signal transduction cascades during host cell invasion. (C) *Chlamydia pneumoniae* (*Cp*) secretes effector SemC to manipulate plasma membrane curvature during host cell invasion. (D) *Salmonella enterica* serovar Typhimurium (*ST*) secretes effectors SopB and SopD to manipulate host protein activities and localization as well as plasma membrane components during vacuole scission.

Throughout the figure, bacteria and bacterial membranes are depicted in brown, bacterial proteins in pink, and host components in blue.

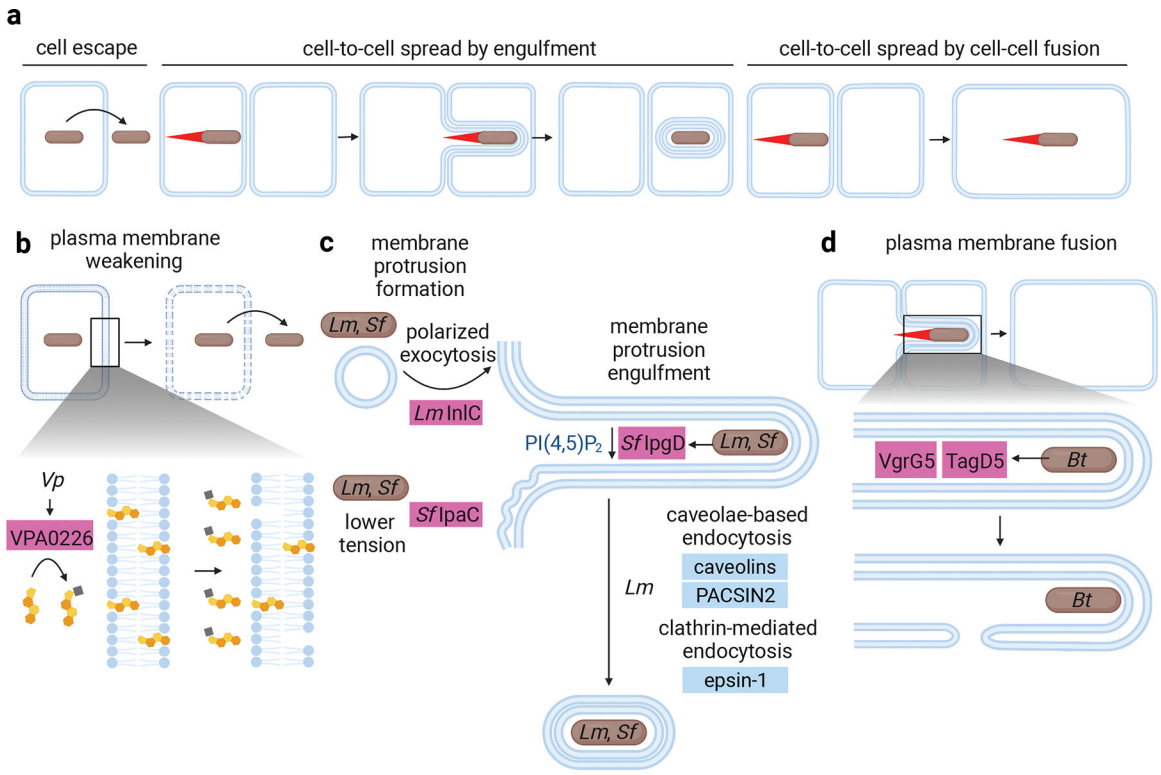

Figure 2.

Plasma membrane manipulation during bacterial exit from host cells. (A) Three pathways through which bacteria exit from host cells, including cell escape, cell-to-cell spread by engulfment, and cell-to-cell spread by cell-cell fusion. The latter two processes involve actin-based motility and the formation of actin comet tails. (B) Depiction of VPA0226 increasing free esterified cholesterol and decreasing cholesterol in the host cell plasma membrane and the resulting hypothesized role of plasma membrane weakening in *Vibrio parahaemolyticus* (*Vp*) host cell exit. (C) Involvement of various bacterial effector proteins, host cell pathways, and host cell plasma membrane features and components during protrusion formation and protrusion engulfment steps of *Shigella flexneri* (*Sf*) and *Listeria monocytogenes* (*Lm*) cell-to-cell spread. (D) Cell-cell fusion by *Burkholderia thailandensis* (*Bt*) requires the T6SS proteins VgrG5 and TagD5, which are required for plasma membrane fusion within membrane protrusions. Throughout the figure, bacteria and bacterial membranes are depicted in brown, actin comet tails in red, bacterial proteins in pink, and host components in blue.