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Review

Right Place, Right Time: Focalization of Membrane Proteins in Gram-Positive Bacteria

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Membrane proteins represent a significant proportion of total bacterial proteins and perform vital cellular functions ranging from exchanging metabolites and genetic material, secretion and sorting, sensing signal molecules, and cell division. Many of these functions are carried out at distinct foci on the bacterial membrane, and this subcellular localization can be coordinated by a number of factors, including lipid microdomains, protein–protein interactions, and membrane curvature. Elucidating the mechanisms behind focal protein localization in bacteria informs not only protein structure–function correlation, but also how to disrupt the protein function to limit virulence. Here we review recent advances describing a functional role for subcellular localization of membrane proteins involved in genetic transfer, secretion and sorting, cell division and growth, and signaling.

Trends

Some membrane proteins localize to discrete membrane foci.

Spatial restriction of membrane proteins within subcellular foci can be mediated by protein–protein interactions, lipid domains, and geometric cues.

Focal localization of some membrane proteins is important for their function and can affect the function and/or localization of other (interacting) proteins.

Attention is shifting from single-protein localization studies to understanding the network of interacting proteins.

New molecular and microscopy tools are evolving to address membrane protein focalization.

Localization of Membrane Proteins in Bacteria

Many bacterial proteins involved in a range of cellular functions are subcellularly localized at distinct focal regions on the bacterial membrane [1]. Hereafter, we refer to the phenomenon of localization of proteins at discrete focal sites more concisely as 'focalization'. Importantly, mislocalization of proteins from their native focal positions can disrupt their function, as well as that of colocalized proteins, within vital bacterial processes. As such, targeting and disrupting localized bacterial complexes holds promise for antimicrobial or antivirulence therapies (Box 1). Cellular processes are rarely fulfilled by a single protein but rather by a complex network of various proteins [2], and both transmembrane and membrane-associated proteins are ubiquitous participants within diverse networks [3]. To facilitate membrane targeting, transmembrane proteins possess membrane-targeting domains, hydrophobic membrane-spanning regions (transmembrane helices, or TMH), as well as positively charged cytoplasmic regions [3]. These structural features allow proteins to assemble on the membrane with correct topology and to form functional protein clusters within the membrane. Sites on the membrane in which proteins are focally enriched can be formed via interactions with specific lipids and often depend on protein–protein interactions [4–6]. However, localization of membrane proteins to discrete foci varies between Gram-positive and Gram-negative bacteria due to molecular and structural differences in the cell envelope [7,8]. Since the possible molecular mechanisms governing protein localization in bacteria have been extensively reviewed elsewhere [1,9], in this review, we summarize the current understanding of focally localized membrane proteins (transmembrane and membrane-associated) in Gram-positive bacteria and their roles in genetic exchange, secretion and protein sorting to the cell wall, cell division and growth, and signaling.

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Box 1. Why Study Focalized Proteins?

Understanding the mechanisms underlying focal protein localization in bacteria informs not only protein structure–function correlations but also how to target these proteins to disrupt their function. Targeting a localized protein has a twofold advantage: it aids therapeutic drug design and offers tools for studying the function of other proteins in the same spatiotemporal region. The examples below illustrate the applications of studying protein localization.

As Therapeutic Drug Targets

In light of the growing epidemic of resistance to traditional antibiotics, the discovery of new therapeutic targets and infection intervention points are increasingly important. One target that has received much attention as an antivirulence target is the sortase enzyme responsible for covalent attachment of virulence factors onto the cell wall of Gram-positive bacteria [111,112]. The identification of enzymatic inhibitors of sortase activity holds great promise for their potential as antivirulence drugs [112]. Since genetic studies which mislocalize the pilus-assembling SrtC in *Enterococcus faecalis* also result in reduced piliation [44], molecules that also mislocalize sortases or their associated secretion proteins may serve to limit virulence. To that end, cationic antimicrobial peptides (CAMPs) including α and β defensins which target focal domains in the membrane, can disperse focally localized secretion and sorting enzymes in several Gram-positive pathogens [45,55]. Similar to CAMPs, daptomycin interacts with *E. faecalis* and *Bacillus subtilis* at discrete microdomains [113,114]. In *B. subtilis*, daptomycin exposure results in the formation of membrane patches, recruiting proteins that recognize negative membrane curvature such as DivIVA, leading to altered cell morphology and eventually cell death [113].

As a Tool for Elucidating Localized Protein Function

Small molecules or compounds that specifically interact with focally localized proteins can serve as molecular tools to dissect the mechanisms of protein localization in the cell. For example, exposure of *Streptococcus pneumoniae* to vancomycin, which stops production of peptidoglycan, results in rapid delocalization of septally localized MapZ and demonstrates the requirement of peptidoglycan synthesis for MapZ localization [75]. Vancomycin, as well as ampicillin, was used to study the localization mechanism of StkP in *S. pneumoniae*, and mislocalization upon exposure to these antibiotics demonstrated that StkP requires uncrosslinked peptidoglycan subunits for its localization to the septum and autophosphorylation activity [77].

Genetic Exchange

Bacteria can promiscuously acquire new traits through genetic transfer that enable them to evolve, survive, and propagate in new environments [10,11]. Genetic transfer in bacteria occurs via conjugation, transformation, and transduction. While transduction is mediated by bacteriophages, natural transformation in competent bacteria and conjugation are mediated by chromosomal and conjugative-plasmid-encoded proteins, respectively [12] and are essential for transferring genetic elements, including antibiotic-resistance and virulence genes between bacteria [13,14]. Natural transformation, in which exogenous double-stranded DNA (dsDNA) is converted to single-stranded DNA (ssDNA) and taken up by the cells at specific sites on the cell surface, is widely studied in *B. subtilis* and *S. pneumoniae*, which possess similar transformation machineries [11,15,16]. Localized membrane-associated components that are essential for both *B. subtilis* and *S. pneumoniae* transformation machineries include the ATPase ComGA, polytopic membrane protein ComGB, transmembrane channel ComEC, translocase ComFA, and pseudopilin ComG proteins [15,17–19]. While in *B. subtilis* the dsDNA cleavage protein NucA localizes to the polar membrane, in *S. pneumoniae* the analogous transmembrane deoxyribonuclease EndA localizes to the midcell, indicating no general rule for the subcellular localization of transformation machineries in these two bacteria [20–22]. In cells of *S. pneumoniae* that are not competent for transformation, EndA is distributed throughout the cell membrane; whereas, upon competence induction, it colocalizes with ComEA at distinct midcell foci, even in the absence of exogenous DNA [23]. Similarly, the transformation machinery in *B. subtilis* undergoes dynamic assembly and disassembly at the cell poles and is competence dependent [21].

Conjugation in Gram-positive bacteria results in the transfer of two forms of DNA: ssDNA and circular dsDNA [24]. ssDNA transfer takes place through the type IV secretion system (T4SS) and has been most extensively studied in *Clostridium perfringens* [25,26]