Published in final edited form as:

Curr Opin Microbiol. 2017 April ; 36: 7–13. doi:10.1016/j.mib.2016.12.001.

Geometric protein localization cues in bacterial cells

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Abstract

Bacterial cells are highly organized at a molecular level. Understanding how specific proteins localize to their proper subcellular address has been a major challenge in bacterial cell biology. One mechanism, which appears to be increasingly more common, is the use of “geometric cues” for protein localization. In this model, certain shape-sensing proteins recognize, and preferentially embed into, either negatively or positively curved (concave or convex, respectively) membranes. Here, we review examples of bacterial proteins that reportedly localize by sensing geometric cues and highlight emerging mechanistic understandings of how proteins may recognize subtle differences in membrane curvature.

1. Introduction

The non-uniform spatial and temporal cellular distribution of macromolecules is a hallmark of living systems. Cellular events such as cytokinesis, differentiation, and development require accurate placement and timing in the assembly of complex protein machinery. Subcellular protein localization requires a beacon that specifies different intracellular sites as destinations for specific proteins. In eukaryotic cells, vesicle-mediated trafficking systems mediate the transport of proteins across tens of microns to subcellular regions of the cell, such as organelles, that are chemically distinct. In bacteria, movement of proteins within the several microns of the cell’s cytosol is mediated largely by diffusion [1]. Diffusing proteins then bind to a beacon at their correct destination- the result is an accumulation of a protein over time at its proper subcellular address [2]. In this “diffusion-and-capture” model, higher order complexes may be formed at a particular site by the sequential recruitment of proteins that recognize a protein that arrived at that destination previously. In such a cascade of recruitment events, the protein that arrives initially must recognize not simply a pre-localized protein, but instead an intrinsic physical feature of the cell upon which to seed the assembly of a higher order complex. In the recent years, several models have been proposed to explain how a founder protein may initially localize to begin a cascade of localization events [3,4]. One proposed cellular feature has been the local curvature of membranes, as defined by the shape of the cell or certain organelles, that may be specifically recognized by certain shape-sensing proteins. Such “geometric cues” have recently been shown to drive the

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proper localization and assembly of macromolecular complexes in both eukaryotes and bacteria [5–7].

Eukaryotic cells, by virtue of harboring membrane-enveloped organelles, display a wide spectrum of differently curved membrane surfaces. Indeed, there are several examples of proteins recruited to either highly positively or negatively curved membranes [8–10]. In a typical bacterial cell, which is usually devoid of organelles, the plasma membrane is entirely negatively curved. However, a rod-shaped bacterium can nonetheless display regions of membranes negatively curved to different degrees, depending on the location (Fig. 1). For example, the hemispherical poles and the point at which a division septum meets the lateral edge of the cell display negative curvature in two dimensions, to differing extents, whereas the lateral edge of the cell is negatively curved in one dimension (Fig. 1A). In rare occasions, certain bacteria may harbor cytosolic organelle-like structures (such as photosynthetic vesicles of *Rhodobacter sphaeroides*, magnetosomes of *Magnetospirillum magneticum*, or forespores elaborated during endospore formation in Firmicutes) [11]. In these instances, positively curved cytosolic membrane surfaces may also be displayed (Fig. 1B).

In this review we will highlight examples of bacterial proteins that preferentially bind to either positively or negatively curved membranes, review proposed mechanisms for how proteins may detect the subtle curvature of cellular membranes, and discuss remaining challenges in the field for elucidating molecular mechanisms of membrane curvature recognition.

1. Recognition of positive membrane curvature

Endospore formation (sporulation) is a stress response initiated by certain Gram-positive bacteria, usually as a result of starvation [12–15]. In *Bacillus subtilis* sporulation initiates by an asymmetric division of a progenitor cell, resulting in two dissimilar sized daughter cells: a larger “mother cell” and a smaller “forespore”. Next, the mother cell engulfs the forespore. As a result, the forespore temporarily resides as a ~1 μ m diameter spherical organelle inside the mother cell (Fig. 1B). As the forespore matures into a spore, the mother cell deposits a protective proteinaceous shell (the “coat”) onto the forespore surface. The coat is composed of over 70 different proteins that are recruited to the forespore in a systematic fashion [16], but the process is seeded by a 26 aa membrane-associated small protein termed SpoVM, wherein SpoVM recruits and anchors the structural component of the basement layer of the coat [17,18]. Several years ago, it was proposed that SpoVM recognizes and preferentially embeds in slightly convex (positively curved) membranes, such as that found on the surface of the forespore [19]. Three lines of evidence supported this hypothesis. First, in *B. subtilis* mutants that failed to engulf the forespore (and therefore did not elaborate convex membranes), SpoVM-GFP promiscuously mis-localized. Second, when produced in yeast or mutant *E. coli*, SpoVM-GFP preferentially localized to the convex surface of forespore-sized vacuoles or vesicle, respectively. Third, when incubated with giant unilamellar vesicles, purified SpoVM-GFP preferentially adsorbed to the surface of smaller, more convex vesicles. Taken together, the in vivo and in vitro data were consistent with the model that

convex membrane curvature could recruit SpoVM, but the structural and mechanistic basis for how SpoVM could recognize this curvature was still unclear.

This quandary was amplified by the observation that, for a single SpoVM molecule, membrane curvature atop a one micron-diameter forespore is so slight that it is largely imperceptible [20]. To test the mechanism of preferential SpoVM adsorption, a more robust in vitro assay was reported that measured SpoVM binding to curved membranes harboring a strictly defined radius of curvature [21••]. This assay employed spherical supported lipid bilayers (SSLBs), composed of tiny silica beads, encased by a phospholipid bilayer [22]. As opposed to using giant unilamellar vesicles, this set up had the advantage of eliminating variability in membrane size and stiffness. By incubating a mixture of differently sized SSLBs with purified SpoVM-GFP at varying concentrations, binding of SpoVM-GFP to either surface could be measured using fluorescence microscopy and quantified. The resulting adsorption isotherms revealed that SpoVM utilizes a combination of increased affinity and a slight increase in apparent cooperativity to preferentially adsorb onto more convex surfaces. Notably, unlike eukaryotic proteins that reportedly exploit a higher density of binding sites present on extremely convex vesicles [23], SpoVM adsorption did not reveal a similar difference in binding site density on differently curved surfaces, suggesting that SpoVM exploits a different mechanism to distinguish slightly curved surfaces. Interestingly, a recent report suggested that the eukaryotic cytoskeletal protein septin Cdc11 in the fungus *Ashbya gossypii* is also recruited to micron-scale positively curved plasma membranes near branch sites by employing an affinity-driven mechanism similar to SpoVM, as opposed to recognizing an increase in binding site density. Thus, not only does it appear that certain non-bacterial proteins may also preferentially embed in membranes slightly curved on micron length scales, but that mechanistic aspects of sensing such curvature may be widely conserved in evolutionarily distant organisms [24•].

To investigate the structural basis for SpoVM's ability to recognize convex membranes, the structure of the protein in the context of a lipid bilayer was solved using NMR [21••]. Unlike other membrane-binding amphipathic helices that shallowly insert into membranes and interact mainly with phospholipid head groups, SpoVM harbored a very short C-terminal α -helical region (residues 15–22) that was buried deep in the membrane, likely precluding any significant interaction with the phospholipid head groups, and instead interacting mainly with the acyl chains of the membrane bilayer. In contrast, the N-terminal residues of SpoVM were flexible and their sidechains could be solvent accessible. Both regions of SpoVM were delineated by a critical Pro residue at position 9 that provided a region of flexibility to the protein (Fig. 2A). Interestingly, in vivo, substitution of Pro9 with Ala resulted in mis-localization of the protein during sporulation in *B. subtilis* [25]. In vitro, SpoVM^{P9A} indiscriminately adsorbed onto membranes regardless of their radius of curvature. Indeed, the NMR structure of SpoVM^{P9A} revealed that its N-terminus was no longer flexible and that the protein was largely helical throughout its entire length (Fig. 2B). Taken together, the structural studies suggested that a flexible N-terminus may mediate the ability of SpoVM to discriminate between differently curved membranes. The deep insertion of SpoVM into the lipid bilayer additionally suggested that SpoVM may sense subtle differences in the packing of acyl chains that are caused by modest changes in membrane curvature and that the secondary structure of SpoVM may be required to detect those packing differences.

A working model for curvature recognition is depicted in Fig. 2C. In this model, SpoVM recognizes slightly positively curved membrane curvature using a mechanism that is distinct from proteins that recognize very highly curved membranes. Instead of detecting packing differences manifested near the lipid head groups, SpoVM likely detects differences in packing of the acyl chains. In vitro studies suggest that this recognition is driven by slight increases in affinity and cooperativity, rather than an increase in the density of protein binding sites on a preferred surface. However, it remains to be determined how slight changes in lipid acyl chain packing due to slight positive curvature initially recruits or stabilizes SpoVM and stimulates cooperative interaction. Indeed, an outstanding question is the molecular nature of the apparent cooperativity between SpoVM molecules. Specifically, do SpoVM molecules directly contact one another in the membrane, or do they entropically cluster in a lipid bilayer? Is this apparent cooperativity mediated by structural features of SpoVM, such as its flexible N-terminus? Additionally, in silico simulations predict that a faster on-rate of SpoVM onto more convex membranes is required to explain the increased affinity of the protein onto more convex surfaces [21••]. Perhaps an in vitro assay that allows for kinetic measurements, not simply steady-state binding, would be able to rigorously test this prediction.

1. Recognition of negative membrane curvature

The first reported example of a bacterial protein proposed to bind negatively curved membranes is the multifunctional structural protein DivIVA in *B. subtilis* [26,27]. DivIVA is a 164 residue protein that is largely α -helical and predicted to contain two major domains that, in the X-ray crystal structure, tetramerized to adopt a coiled-coil arrangement [28]. DivIVA is likely tenuously membrane-associated, perhaps via a single hydrophobic residue that shallowly inserts into the phospholipid bilayer, just past the lipid head groups [28]. During medial cell division, DivIVA localizes near the division septum and recruits cell division inhibitors MinC and MinD [29,30] to prevent aberrant septation events near mid-cell [31,32]. At the onset of sporulation, DivIVA localizes to the cell poles and participates in anchoring newly replicated chromosomes to the poles, thus ensuring each daughter cell receives one chromosome copy [33]. During asymmetric cell division, DivIVA localizes to the polar septum and poles and participates in activating a forespore-specific transcription factor [34,35•]. A common feature of these localization patterns is that DivIVA localization is predominantly coincident to the most negatively curved regions in the cell.

The results of several in vivo tests, which largely relied on manipulating cell shape and monitoring the subsequent localization of DivIVA, were consistent with the hypothesis that DivIVA preferentially embeds in concave membranes. For example, *B. subtilis* cells harboring a deletion in the *mreB* gene contain aberrant protrusions that display highly concave membranes. In these cells, in addition to localizing to division septa, DivIVA-GFP also localized to these aberrant protrusions [26]. In contrast, when cytokinesis was blocked by overproduction of a cell division inhibitor [36], DivIVA-GFP redistributed from the septa to cell poles, which subsequently is the most concaved surface [27]. Similarly, when *B. subtilis* cells were converted to spherical protoplasts that displayed uniform negative membrane curvature, DivIVA-GFP non-specifically localized [27]. Indeed, even in rod-shaped *B. subtilis* devoid of a nucleoid, DivIVA-GFP continued to localize at recent sites of

cell division, suggesting that the presence of the nucleoid did not influence the polar localization of DivIVA. Finally, when produced in mutant *E. coli* that grew as chains (and thus displaying extreme negative curvature on either side of division septa), DivIVA-GFP localized near division septa, suggesting that a *B. subtilis*-specific factor did not drive DivIVA localization [26]. A specific prediction of this hypothesis was that, since membrane invagination during septation produces negative membrane curvature in each of the resulting daughter cells, DivIVA should actually localize to either side of the division septa as adjacent ring-like structures. However, due to the resolution limit of light microscopy, the narrow width of the division septum largely precluded reproducible detection of such adjacent DivIVA rings near division septa [37]. However, by employing structured illumination microscopy, a super-resolution technique, the predicted adjacent rings of DivIVA-GFP were detected, consistent with the model that highly negatively curved membranes could recruit DivIVA [32].

To date, largely due to technical limitations, an in vitro assay using defined components which conclusively demonstrates that membrane curvature is sufficient to drive DivIVA localization has not been reported. In the absence of quantitative measurements of DivIVA binding, it has been difficult to definitively report a mechanism by which DivIVA may recognize negatively curved membranes. Nonetheless, Lenarcic et al. have proposed a model that incorporates several testable parameters [26]. This “molecular bridging” model exploits the observation that DivIVA multimerizes into elongated structures that span several nanometers and combines it with DivIVA’s (albeit slight) affinity for membranes. In this model, adjacent membrane patches, which may occur when a contiguous membrane is highly negatively curved, may both be contacted by a cluster of DivIVA molecules that is large enough to span the distance between the two patches, thereby stabilizing the cluster of DivIVA molecules at that site. Indeed, in a Monte Carlo simulation in which spheres were permitted to interact with one another and membranes spontaneously clustered at highly negatively curved regions of a cylinder [26]. The model predicts a highly cooperative, concentration dependent, mechanism that should be testable in vitro. Recent reports in other systems have suggested controlled methods to generate concave membranes of defined radii, using either supported bilayers or manual deformation of giant unilamellar vesicles [10,38–41]. A remaining challenge would be to incorporate one or more of these strategies to directly measure how a peripheral membrane protein such as DivIVA may detect highly negatively curved membranes.

Recently, several reports have suggested that at least three other bacterial proteins, including one integral membrane protein, may achieve their subcellular localization in a membrane curvature-dependent manner. MreB, which displays some homology to actin, has been implicated in coordinating cell growth with the maintenance of cell shape, especially in rod-shaped bacteria [42]. MreB localizes along the lateral edge of rod-shaped species such as *E. coli* and *B. subtilis* and is thought to participate in incorporating new cell wall material at specific points along the length of the cell during growth [43–45]. How does MreB preferentially associate with the lateral edge of rod-shaped cells? Ursell et al. monitored single cells and correlated cell growth, geometry, and cytoskeletal organization while monitoring MreB localization and dynamics [46••]. The authors observed that in filamentous *E. coli* cells, in addition to the negatively curved cell poles there exists a spectrum of

curvatures along the body of the cell, consisting of outward bulges and inward indentations that generates varying degrees of local positive and negative curvature. Enrichment of fluorescently labeled MreB as a function of cell geometry along the cell periphery showed a clear preference of MreB to regions of negative curvature and depletion to regions of positive curvature along the contour of the cell. Additionally, filamentous cells that were forced into sinusoidal shape (via curved agarose chambers) showed MreB enrichment at negatively curved regions and partial depletion at positively curved regions. Interestingly, it was found that MreB localization to these regions directs proximal cell wall insertion that reduces the extent of negative curvature resulting in cell straightening, thus fulfilling a self-correcting feedback mechanism to help maintain geometric integrity of the cell during active growth. However, there is some evidence to suggest that MreB is excluded from regions that are too negatively curved [47•]. When filamentous *E. coli* cells were trapped in V-shaped microchambers with varying angles at the apex, foci of fluorescently labeled MreB were preferentially associated with more positively curved regions as compared to highly negatively curved membranes. Indeed, the density of MreB foci at the negatively curved region decreased as a function of higher extremes of negative curvature while increasing with higher extremes of positive curvature. This observation was consistent with MreB localizing primarily along the cylindrical, lateral edge of the cell (which is negatively curved in only one dimension) and its large absence at the hemispherical cell poles (which is negatively curved in two dimensions). Taken together, it appears that the subcellular distribution of MreB may be influenced by an interplay between local negatively curved contours along the lateral edge of the cell that recruit the protein and global hemispherical curvature that somehow excludes MreB clusters, whose mechanism of localization is still an outstanding question.

Unlike MreB other bacterial proteins preferentially localize to the highly curved cell poles. For example, the phosphoenolpyruvate-dependent phosphotransferase system (PTS), a central signal transduction system that governs hierarchical uptake of carbohydrates, contains two proteins, termed EI and HPR, that form the “control center” of the PTS, and localize to the cell poles. A series of in vivo experiments suggested that localization of EI is primarily driven by the shape of the poles [48•]. Specifically, EI polar placement was not dependent on lipid composition at the polar regions, the presence of polar localized chemotaxis receptors, or the cell division machinery. Additionally, nucleoid exclusion and localized polar translation of EI-mRNA were ruled out as a mechanism for EI localization. However, induction of artificially negatively curved regions of membranes in a misshapen mutant, resulted in the preferential localization of EI to the negatively curved budding site of intracytoplasmic membrane-bound vesicles. Likewise, in *E. coli* chaining mutants, EI localized near division septa, similar to the ectopic localization pattern of *B. subtilis* DivIVA. Taken together, a simple explanation was that negative membrane curvature may drive EI localization but, as with DivIVA and MreB, a mechanistic understanding of EI localization will likely await the development of a robust in vitro assay to measure EI adsorption to differently curved membranes.

Chemoreceptor sensory complexes in rod shape bacteria localize to the cell poles, and are made up of specific kinases, phosphatases and receptor-modulating proteins that form sensory clusters at cell poles to integrate extracellular signals into unified outputs that

regulate flagellar rotation. A recent report suggested that the chemoreceptor TlpA localizes in a curvature-dependent fashion [49••]. However, it is worth noting that, unlike DivIVA, MreB, or EI, the TlpA is an integral membrane protein, so the molecular mechanism that drives its preferential localization to curved membranes is likely different. In this case, the authors argued that the intrinsically curved shape of chemoreceptor trimer or dimer complexes matches the curvature of membranes at the cell poles and thus confers the specificity needed for targeting negatively polar regions of the cell, similar conceptually to the mechanism posed for the polar localization mechanism of the phospholipid cardiolipin [50]. This idea was tested *in vivo* by abolishing TlpA trimer formation -and thus the curved architecture of the protein- via mutagenizing a conserved residue at the trimerization interface and testing for localization of the mutant protein. While the membrane binding capacity of this mutant remained, its targeting of the septa was severely impaired. This lack of curvature specificity was also observed in mutagenized TlpA that maintained trimer formation but its curved architecture was disrupted by changing a stretch of conserved residues near the membrane-protein interface which results in enhanced torsional flexibility in the dimers. Like DivIVA and EI, TlpA localization was shown to localize to artificially-generated highly concave membranes in a lipid-independent manner *in vivo*.

Thus, DivIVA, MreB, EI, and TlpA represent four candidate proteins reported thus far that assume their proper subcellular localization by exploiting the full spectrum of concave membranes in a rod-shaped bacterial cell. DivIVA and EI appear to preferentially embed into membranes highly curved in two dimensions, such as those present at division septa; TlpA multimers localize to the hemispherically curved membranes at the cell poles of rod-shaped bacteria; and MreB localizes along the long axis of rod-shaped cells, the least concave membranes, negatively curved in only one dimension.

1. Concluding thoughts

Understanding how bacterial proteins achieve their proper subcellular localization remains a central question in the field of bacterial cell biology. Among several mechanisms that have been proposed for different proteins, recognition of geometric localization cues, in which proteins recognize specific membrane topographies, appears to be more widespread than previously thought. As the localization events of more proteins that appear to exploit this strategy are catalogued, the ongoing challenge is to demonstrate the molecular mechanisms underlying these events. This will likely require integrating novel *in vitro* approaches to existing cytology-based techniques to demonstrate the sufficiency of membrane curvature to drive protein localization, and to quantify curvature-driven membrane adsorption so as to formulate mechanistic models for the basis of curvature recognition. Although studies of protein targeting have been traditionally largely restricted to active processes, given the spectrum of membrane shapes available in biology, the spontaneous insertion of proteins into specifically curved membranes may be a widely shared strategy for achieving the proper subcellular organization of macromolecules in a variety of cell types.

Acknowledgments

This work was funded by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

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Highlights

- Bacteria localize proteins to distinct subcellular locations.
- Membrane curvature (convex or concave) can be a beacon to recruit certain proteins.
- In vitro assays using curved membranes can reveal how proteins sense curvature.

Figure 1.

Examples of negative (concave) and positive (convex) membrane curvatures arising in bacterial cells. (A) Depicted are regions of the plasma membrane (blue) in a rod-shaped bacterium that display the highest negative curvature (yellow) at different stages of the cell cycle. In cells that are not dividing, the hemispherical poles display two dimensional negative membrane curvature (top), whereas during cell division, the regions on either side of the division septum where the lateral edge of the cell meets the septum is even more highly negatively curved in two dimensions. (B) During sporulation, the cell first divides asymmetrically, producing negative membrane curvature on either side of the asymmetrically-placed septum (top, yellow). Next, the septum curves as the larger daughter cell engulfs the smaller daughter cell, producing positive membrane curvature (orange) on the surface of the smaller daughter cell.

Figure 2.

Structural and mechanistic basis for positive membrane curvature recognition by SpoVM during sporulation. (A) NMR structure of wild type SpoVM determined in the context of lipid bicelles (PDB ID code 2MVH). Proline at position 9, which delineates the flexible N-terminal region and the α -helical C-terminus is indicated. (B) NMR structure of SpoVM^{P9A}, which mis-localizes *in vivo* and fails to discriminate between differently curved membranes *in vitro* (PDB ID code 2MVJ). N, amino terminus; C, carboxy terminus. (C) Working model for curvature recognition by SpoVM. SpoVM molecules (light blue) synthesized in the mother cell eschew negatively curved membranes (dark blue) and embed preferentially to the convex (orange) surface of the forespore driven by a combination of intrinsic affinity for curved membranes and cooperativity at the properly curved membrane.