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Geometric protein localization cues in bacterial cells

Taylor B Updegrove and Kumaran S Ramamurthi

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.

Address
Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, United States

Corresponding author: Ramamurthi, Kumaran S (ramamurthiks@mail.nih.gov)

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

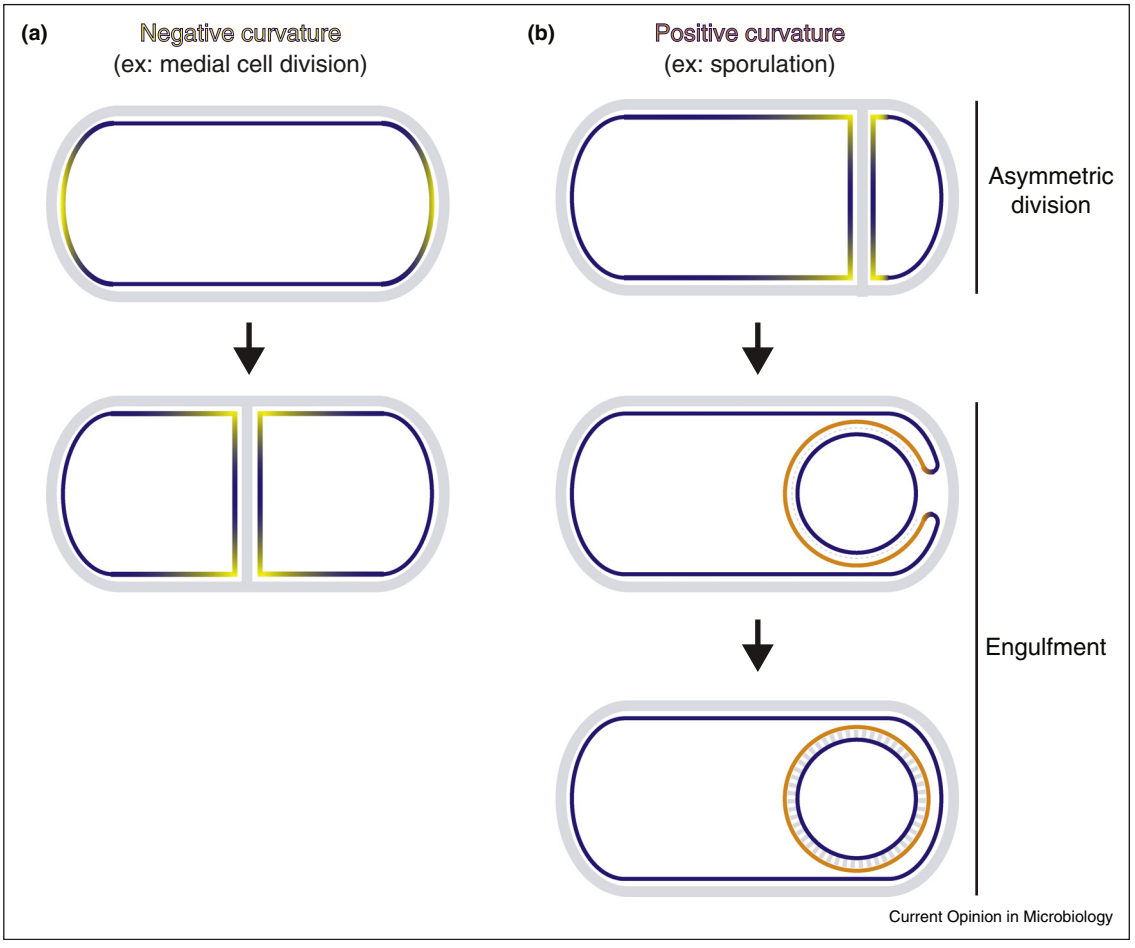
Eukaryotic cells, by virtue of harboring membrane-enveloped organelles, display a wide spectrum of differently curved membrane surfaces. Indeed, there are several examples of proteins recruited to either highly positively or negatively curved membranes [8–10]. In a typical bacterial cell, which is usually 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 (Figure 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 (Figure 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 (Figure 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.

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,

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.

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 μm diameter spherical organelle inside the mother cell (Figure 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.