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Protein Localization by Recognition of Membrane Curvature

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Abstract

Bacteria often sort proteins to specific subcellular locations, but many of the chemical beacons that specify those sites and subsequently recruit proteins have not been identified. Recent reports suggest that some bacterial proteins localize to specific subcellular sites by recognizing either convex or concave membrane curvature. Thus, degrees of membrane curvature, dictated by the shape of the cell, can define a geometric cue for the recruitment of curvature-sensing proteins.

Introduction

The proper subcellular localization of proteins is an ultimate step in developmental programs. After replication of a cell's genetic material, cellular differentiation typically proceeds by the transcriptional activation of specific genes in daughter cells which leads to the production of proteins that dictate the eventual developmental destiny of those cells [1]. Often times, though, the particular activities of such proteins need to be manifested not only at specific times during development, but at specific places within a cell as well [2]. As such, the sorting of proteins to their proper subcellular address is a culminating event of several energy-intensive steps during morphogenesis.

Advances in light microscopy within the last two decades have led to the rapid accumulation of evidence that microbes are highly organized at the molecular level. Observations of live cells harboring proteins of interest fused to fluorescent tags have revealed that many proteins accumulate in specific locations within a cell, sometimes even in a dynamic manner [3–5]. Preferred sites of localization are usually architecturally distinct regions of a cell, such as recent sites of cell division, at the extreme poles of rod-shaped bacteria, on the surface of organelles, or near supramolecular structures [4–6]. Understanding the mechanisms that govern the intracellular sorting of proteins has therefore been a major challenge in the field of bacterial cell biology.

A general model that has developed from these studies is that, in the confines of micron-sized bacterial cells, the movement of proteins towards their destination is typically driven simply by diffusion: either in three dimensions in the cytosol, in the case of soluble proteins, or laterally in the plasma membrane, in the case of transmembrane proteins [7,8]. Upon randomly arriving at its proper destination, a protein of interest is captured by some feature that is unique to that destination, thereby restricting its further diffusion, and eventually resulting in the accumulation of the protein at that site over time [7,8]. Specificity of

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localization, then, is achieved by the presence of a motif on the localizing protein that recognizes the unique feature at the destination [9]. How, though, is the destination itself defined? Historically, a reliable answer to this problem has been that a pre-localized factor, usually a protein, serves as a beacon that recruits other proteins to that site. Thus, localized supramolecular complexes may be built in an ordered fashion when their individual components arrive at a location by recognizing a protein that arrived at that site earlier. Although definitive, this solution immediately raises the conundrum of how the very first protein in such a localization cascade initially arrives at a destination. This review will address the localization of two different proteins that employ one recently described solution to this first-to-arrive problem, whereby membrane curvature, delineated by the cell's shape itself, provides a geometric (rather than chemical) beacon for the initial recruitment of proteins to a subcellular site.

SpoVM: recognition of convex membranes during development

The notion that slightly curved cellular membranes could provide a subcellular protein localization cue came from studies of a protein whose proper localization is required during the developmental program of endospore formation in *Bacillus subtilis*. Upon perceiving the imminent onset of starvation, cells of *B. subtilis* begin to sporulate by dividing asymmetrically, producing two cells: a larger “mother cell” and a smaller “forespore” ([10]; Figure 1A). Next, the asymmetric division septum curves as it migrates around the forespore. Eventually, the mother cell entirely engulfs the forespore; as a result, the forespore resides as an organelle in the mother cell cytosol as it develops into a mature spore. A key step in spore maturation is the deposition of a thick protein shell, called the coat, onto the surface of the forespore [11,12]. Coat proteins are synthesized in the mother cell and individually localize to the forespore surface, largely by recognizing a previously localized coat protein [13,14]. Among the first, if not the first, coat protein to localize is a small 26 amino-acid-long peptide called SpoVM [13,15], which anchors the basement layer of the coat onto the forespore surface [16]. SpoVM forms an amphipathic alpha helix [17] and inserts into the membrane such that its hydrophobic face is buried into the membrane bilayer [16]. Fusion of green fluorescent protein to SpoVM revealed that the protein almost exclusively localized to the mother cell face of the asymmetric division septum shortly after it began to curve, and tracked the engulfing membrane until it eventually uniformly surrounded the forespore ([18]; Figure 1A). Unlike studies of other coat proteins, genetic and biochemical approaches failed to identify a protein that was solely responsible for the proper localization of SpoVM. This led to the hypothesis that SpoVM may recognize a non-proteinaceous cue to drive its localization: specifically, the convex (positive) membrane curvature which is present only on the surface of the forespore and nowhere else in the mother cell cytosol.

Three sets of observations were consistent with this hypothesis [19*]. First, in mutant cells in which the asymmetric septum remained straight during sporulation and failed to curve (and therefore failed to display a convex membrane surface), SpoVM-GFP promiscuously localized to all available membrane surfaces, suggesting that elaboration of a convex surface is required for proper localization of SpoVM. Such mutant cells, though, at a small frequency, elaborated spurious membrane bulges that presented an artificial convex surface. In these cells, SpoVM-GFP localized to these newly created convex surfaces, suggesting that any positively curved surface formed during sporulation, not just the forespore, could recruit SpoVM. Second, SpoVM-GFP produced in mutant *E. coli* that elaborated internal vesicles or in mutant *S. cerevisiae* that harbored fragmented organelles called vacuoles, localized almost exclusively to the convex surface of these internal structures. However, a mutant variant of SpoVM that was shown to mis-localize in *B. subtilis* during sporulation [18], mis-localized to both convex and concave membranes in these heterologous cells.

Taken together, the data suggested that a *Bacillus*-specific factor was not required for the proper localization of SpoVM and that any convex membrane could efficiently recruit the peptide. Third, in an *in vitro* experiment in which purified SpoVM-GFP was incubated with a heterogenously-sized population of lipid vesicles, the protein preferentially adsorbed onto the surface of the smallest (and therefore most convex) vesicles and bound poorly to the largest vesicles. Moreover, the mutant variant of SpoVM that mis-localized *in vivo* adsorbed more promiscuously onto both small and large (and therefore less convex) vesicles in the *in vitro* assay [19*]. These experiments indicated that (although other cellular factors may contribute to the fidelity of SpoVM localization) SpoVM is able to recognize positively curved membrane surfaces and that this preference for convex membranes could drive the localization of the protein onto the surface of the forespore during sporulation.

DivIVA: recognition of concave membranes during cell division

A common preferred site for the localization of many proteins is the extreme poles of rod-shaped bacteria [4,5]. The degree of cellular curvature at hemisphere-shaped poles is of course similar to that of the forespore surface, only it is concave (negative), rather than convex. Could negative membrane curvature drive the subcellular localization of some proteins as well? An excellent candidate to test this hypothesis was the well-studied cell division protein DivIVA which has been reported to localize to the extreme poles of *B. subtilis* cells [20]. Like SpoVM, DivIVA is a relatively small protein, only 164 amino acids long [21]. A recently solved crystal structure of DivIVA suggested an unusual membrane binding motif, wherein, upon dimerization, a single hydrophobic amino acid from each DivIVA monomer was exposed in a sharply turned loop that crossed over the corresponding loop of the other monomer [22*]. The authors of the study proposed that these two hydrophobic residues could insert partially into the phospholipid bilayer, and that the presence of several neighboring positively charged residues could stabilize this interaction with the membrane by interacting with the negatively charged phospholipid head groups. Thus, like SpoVM, DivIVA appears to be peripherally associated with the plasma membrane via hydrophobic residues that insert partway into the bilayer. DivIVA recruits the cell division proteins MinC and MinD [23,24], which inhibit polymerization of the tubulin homolog FtsZ, the protein responsible for membrane constriction at division septa [25–27]. As a result, localization of DivIVA spatially restricts where division septa are able to form [28,29]. At the onset of sporulation, DivIVA additionally participates in anchoring the origins of the newly replicated chromosomes to the extreme poles of the sporangium to ensure that both the mother cell and the forespore get exactly one copy of the chromosome [30,31].

During exponential growth, *B. subtilis* grows largely as chains of cells that have undergone cytokinesis but have not separated into individual motile cells [32]. As a result, the presence of regularly spaced division septa creates regions of membrane that are highly negatively curved in two dimensions where the septum meets the lateral edge of the cell. At the ends of these chains are cells that display a hemispherical pole which also displays concave curvature in two dimensions, but to a lesser degree than that found at division septa (Fig. 1B). Finally, the lateral edges of the rod-shaped cell represent the least concave surfaces, being negatively curved in only one dimension. Examination of mutant cells ($\Delta sinI$) that grow as separated, motile cells during exponential growth (and therefore harbor two hemispherical poles at either end and a single division septum at mid-cell; [33]) revealed that DivIVA fused to GFP localized preferentially to nascent division septa, the structure harboring the greatest degree of negative membrane curvature [34*]. In single cells that were not actively dividing, or in filamenting cells in which division septa were dismantled by artificial overexpression of a cytokinesis inhibitor, DivIVA-GFP instead accumulated at the hemisphere-shaped poles: less negatively curved than division septa and apparently a

second-choice site for DivIVA localization [34*]. In order to test if differing degrees of negative curvature are required for distinct subcellular localization of DivIVA, examination of DivIVA-GFP in spherical *B. subtilis* protoplasts (in which the cell wall had been enzymatically removed, producing cells displaying uniformly negative membrane curvature) revealed a uniform distribution of the protein [34*]. On the other hand, expression of DivIVA-GFP in mis-shapen *B. subtilis* cells that displayed aberrant negatively curved membranes (mutants in which the actin homolog *mreB* was deleted [35]) resulted in the accumulation of the protein in these artificially produced areas of negative membrane curvature [36,37*]. The membrane curvature model is also consistent with the old observation that DivIVA-GFP, when expressed in heterologous rod-shaped cells like the bacterium *E. coli* or the yeast *S. pombe*, accumulated at the extreme poles, the most negatively curved regions available [38], suggesting that concave membrane curvature alone is sufficient for proper localization of DivIVA. Interestingly, the recent observation that MinC (whose localization depends on DivIVA) primarily localizes to sharply negatively curved division septa [29], and not to the less curved hemispherical poles as previously thought, is also consistent with the membrane curvature-dependent localization pattern of DivIVA. Taken together, the results suggested that a concave surface is sufficient for the recruitment of DivIVA, and that DivIVA can distinguish between degrees of negative membrane curvature and displays a hierarchical preference for the most concave surface available.

Perspectives and concluding remarks

In the past few years, several reports have presented solutions to the “first-to-arrive” protein localization problem in bacteria [39–43*]. These solutions have proposed that proximity to an adjacent cell or the relative ages of cell poles may function as subcellular localization landmarks, or invoke the capacity of certain proteins to spontaneously cluster as a mechanism for preferential polar localization. Many other protein localization mechanisms likely await discovery.

A recent addition to this list is the hypothesis that membrane curvature, defined by cellular architecture, can recruit proteins to distinct cellular locales. This mechanism appears to be true for at least two different proteins as described above, during two different cellular processes, but is the strategy widespread? Recently, experiments in the rod-shaped fission yeast *S. pombe* revealed that a dose-dependent inhibitor of mitotic entry, Pom1, localizes to the poles of the cell, resulting in decreased levels of Pom1 at midcell [44*]. The mechanism by which Pom1 localizes to the poles of *S. pombe* is not known, but the unique geometry of this environment raises the possibility that Pom1 is a eukaryotic example of a protein that recognizes membrane curvature on a cellular scale [45]. Perhaps employing membrane curvature to drive the subcellular localization of proteins is a strategy that is shared by widely divergent cell types.

Beyond identifying additional proteins that localize by recognizing membrane curvature, a significant remaining challenge is to understand the molecular mechanisms by which such proteins are able to detect the ever-so-slight curvature that exists at cell poles and the surface of large organelles (for a discussion of scale, see [46]). Exploiting newly developed *in vitro* systems that allow for the manipulation of membrane bilayers [47–50] and imaging technologies that deliver ever-higher levels of resolution *in vivo* may help reveal how cells use physical cues like geometry to organize themselves.

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Figure 1. Subcellular localization of SpoVM and DivIVA

(A) A *Bacillus subtilis* sporangium is depicted, in which asymmetric division (top) creates a mother cell and a forespore. The mother cell then engulfs forespore (middle panels). Eventually, the forespore is pinched off as a double-membrane-bound organelle (bottom). The site of SpoVM localization, corresponding to the region of highest positive (convex) membrane curvature, is indicated in red. (B) Schematic representation of a chain of unseparated *B. subtilis* cells during vegetative growth. The most negatively curved (concave) membrane is found where division septa meet the lateral edge of the cell. Lesser concave curvature is found at the hemispherical poles of cells at the ends of a chain. DivIVA preferentially localizes to regions of highest concave curvature (green circles), and less extensively (green patches) to the extreme poles, where the degree of negative curvature is less.