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The unsolved problem of how cells sense micron-scale curvature

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Abstract

Membrane curvature is a fundamental feature of cells and their organelles. Much of what we know about how cells sense curved surfaces comes from studies examining nanometer-sized molecules on nanometer-scale curvatures. We are only just beginning to understand how cells recognize curved topologies at the micron scale. In this review, we provide the reader with an overview of our current understanding of how cells sense and respond to micron-scale membrane curvature.

Keywords

Septin; membrane curvature; BAR domain; SpoVM

Cellular Membranes and Curvature

Shape is a common feature used to describe cells. Consider the transformation of a platelet from a smooth disc to a protrusion-rich, activated state; the distinct morphologies of cells such as neurons and podocytes; and the variety of cell shapes found in the microbial world. The ability to make and change shapes is central to specialized cell functions and adaptations of cells to changing environments. This link between form and function is exemplified in a dendritic spine or the dramatic switches between budding and filamentation of pathogenic fungi in hosts. Shape depends on cells spatially controlling networks of proteins and regulating the physical properties of membranes. While it is somewhat understood how cells generate different shapes, comparatively little is known about how cells recognize their own shape and use information about their geometry.

One way cell shape can be described is in terms of membrane curvature. Cell membranes are thin, lipid-protein mosaics that function primarily to compartmentalize the cell and provide a selective barrier to the extracellular environment [1]. Additional functions of membranes include responding to physical forces, serving as signaling platforms, regulating the transport of nutrients and ions into the cell, and the storage of lipids and proteins [2–5]. Membrane composition between cell and organelle membranes can vary extensively, with

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differences arising from the combinations of particular lipid species, fatty acyl chains and saturation. *In vitro* studies have shown that these differences can alter membrane physicochemical characteristics such as thickness, curvature, fluidity, surface charge and what proteins reside in or on specific membranes [6–9]. Ultimately, cell and organelle shape can be influenced by properties of the membrane.

Cellular membrane curvature can span from nanometer to micrometer scales (Figure 1A). Membrane curvature sensing is best understood for nanometer-sized molecules binding nanometer curvatures such as ArfGAP1 [10–12], α-synuclein [13], and BAR proteins [14, 15]. When compared to typical globular proteins, curvatures at the micron scale would have a thousand-fold larger area (Figure 1A). How do nanometer sized proteins perceive membrane curvatures that are several orders of magnitude their scale? In this review, we discuss identified micron-scale curvature sensors and how they may relate to nanometer curvature sensors. We then go on to highlight recent insights into how cells perceive and use micron-scale membrane curvature using nanometer-sized proteins.

Sensing Micron-Scale Curvatures

To date, only SpoVM and the septins have been identified to be able to sense positive micron-scale membrane curvature. SpoVM was identified in the bacterium *Bacillus subtilis* as essential for spore formation [16]. Septins were identified in the budding yeast as essential gene products during cytokinesis, and have since been found to be conserved throughout eukaryotes but missing from land plants [17–20]. For both SpoVM and septins, the ability to sense curvature at this scale is central to their biological function. In the case of SpoVM, curvature sensing is likely essential for spore maturation [21]. For septins, their best-established curvature role is in the morphogenesis checkpoint where they act to sense the presence of a bud in yeast to coordinate cell shape with cell cycle progression [22, 23]. Before elaborating on these cell biological functions, we will discuss experiments that established these proteins as micron-scaled curvature sensors, and the current thinking on how these relatively small proteins recognize such shallow curvatures.

Bacterial Micron-Scale Curvature Sensors: SpoVM

In response to environmental stresses, *B. subtilis* sporulate to generate a dormant endospore [24]. The endospore is metabolically inactive and can withstand harsh environmental conditions [25, 26]. Sporulation is dependent on the formation of an intracellular double membrane structure called the forespore. At 1-micron in diameter, the forespore is the only site of shallow positive membrane curvature in the cell [27] (Figure 1B). SpoVM, a 26 amino acid polypeptide, localizes to the forespore where it recruits SpoIVA [21, 28]. SpoIVA can then recruit a network of forespore proteins necessary for forespore maturation. *spoVM* mutants form very thin, immature spores that are not resistant to environmental stresses [16]. This indicates SpoVM is essential for proper *B. subtilis* sporulation. However, the possibility remained that some other protein is the true curvature sensor, and not SpoVM.

To determine whether SpoVM can distinguish between different curvatures, recombinant SpoVM was incubated with lipid bilayers supported on spherical silica beads of defined curvatures [21, 29]. Saturation binding curves for SpoVM adsorption onto 2 μm and 8 μm diameter surfaces revealed that at low concentrations, SpoVM preferentially binds the 2 μm beads. Increasing the SpoVM concentration showed binding to both bead sizes; however, SpoVM adsorption onto 2 μm beads occurred more rapidly. Saturation binding curves for both bead sizes further revealed that although the number of binding sites for SpoVM were similar on both curvatures, SpoVM had a threefold-higher affinity for the 2 μm-sized beads compared to 8 μm-sized beads. ese current data suggest that SpoVM has intrinsic, micron-scale curvature sensing abilities. Interestingly, replacing the proline as the 9th residue with an alanine ($SpoVM^{P9A}$) abolishes its curvature preference to 2 μm beads, leading to equal binding to both bead sizes, with a two-fold *increase* in affinity when compared to wild-type SpoVM. This raises the question; how does a single amino acid residue contribute to curvature sensitivity?

Analysis of the primary sequence followed by solution state NMR of SpoVM revealed that an amphipathic helix extends from amino acid residues 11 to 23, flanked by flexible N and C termini [21, 29]. Amphipathic helices harbor hydrophobic and polar regions on opposite faces of the helix and are a recurring theme of membrane curvature sensors. Interestingly, in contrast to SpoVM, $SpoVM^{P9A}$ was helical along the entire length of the protein and had an increase in positive charge along its polar face. Probing the interaction of SpoVM with the membrane identified that the helical residues of SpoVM were less accessible to a water-soluble probe than the N or C termini. Molecular dynamics (MD) simulations show that both SpoVM and $SpoVM^{P9A}$ may submerge within the bilayer, well below the phospholipid head groups, oriented parallel to the membrane. Although the wild-type SpoVM N terminus was found to be highly flexible, the $SpoVM^{P9A}$ N terminus was more rigid. It was proposed that the flexible N terminus is important for recruiting additional SpoVM molecules and other forespore proteins to positively curved membranes in a cooperative manner, consistent with the obtained Hill coefficients from saturation binding curves [29]. Cooperativity may emerge from oligomerization and thus it will be important to determine if SpoVM can oligomerize (perhaps mediated by its flexible N-terminus), and if oligomerization is critical for membrane curvature sensing. It will also be important to determine the specificity of SpoVM membrane curvature recognition between nanometer and micrometer-scale curvatures.

Interestingly, similar themes emerge for a sensor of negative membrane curvature from prokaryotes, DivIVA. This protein preferentially assembles at sites of micron-scale, negative membrane curvature and binds membranes using an amphipathic helix. Several prokaryotic proteins, including DivIVA, localize to sites of membrane curvature (Table 1), however, their intrinsic curvature sensing capacity in vitro remains to be determined.

Septins Recognize Micron-Scale Curvature in Eukaryotic Cells

Septins are filament forming, GTP-binding proteins conserved from yeast to humans. Recombinant and immunoprecipitated septins from budding yeast form octameric rod complexes, as seen using negative stain transmission electron microscopy (TEM) [30].

Structural analysis of septins from worms, and mammalian cells show that rod complex formation is an intrinsic feature of septins [31, 32]. The septin rod contains two copies of each septin polypeptide, and depending on the organism, can vary in length from ~ 17 nm in *C. elegans* [31] to ~32 nm in *S. cerevisiae* [30]. Septin rods can associate and diffuse at the membrane, where they can bind end-on to one another to form micron-scaled filaments as demonstrated by total internal reflection fluorescence (TIRF) microscopy [33]. Septin filaments can be arranged to a variety of higher-order structures, some of which are at sites of micron-scale membrane curvature, including at the bud-neck in *S. cerevisiae*, and at the bases of dendritic spines and hyphal branches in neurons and filamentous fungi, respectively [22, 34–36]. At these sites, septins coordinate cell cycle progression [22], influence the organization of actin [37] and microtubules [38], and may function as a lateral diffusion barrier in the membrane [39–41]. The fact that septin structures are often localized to sites of micron-scale membrane curvature suggested that the septins might be able to “sense” these shallow curvatures.

To test if septins could sense curvature, recombinant septins were incubated with supported lipid bilayers on beads of different diameters, similar to experiments done with SpoVM detailed above. Purified septins from *S. cerevisiae* and mammals preferentially bound to membranes with micron-scale curvatures, comparable to what is seen at the bud neck and at the cytokinetic furrow [36]. Specifically, septins were enriched on 1 μm and 3 μm beads, and less so on beads above and below these sizes (Figure 2) [36]. Furthermore, time-lapse microscopy showed that septins accumulated faster onto 1 μm beads than 5 μm beads, suggesting that differences in maximum adsorption alone cannot explain preferential septin binding to different curvatures. Interestingly, septin binding to 5 μm beads had a bimodal distribution such that some beads were coated with septins while other beads were devoid of septins, suggestive of a cooperative mechanism for septin adsorption. These data suggest that membrane affinity and binding cooperativity - rather than the number of available membrane binding sites - are what determine septin binding preference to curved membranes, akin to SpoVM.

How do septins distinguish different curvatures? Is septin filament polymerization required to sense micron-scale curvature? Or can a single septin rod sense shallow membrane curvature? It is tempting to speculate that membrane curvature is recognized at the level of the septin filament which can extend hundreds of nanometers, and not necessarily at the level of the rod. To test this hypothesis, Bridges et al. assessed whether individual, non-polymerizable mutant septin rods preferentially associated to supported lipid bilayers on beads of varying sizes [36]. Non-polymerizable rods did not stably associate to beads of any size, indicating septin association with membranes is likely driven by the collective and cooperative affinity of many subunits of a filament. To bypass this limitation, histidine-tagged, non-polymerizable septin rods were recruited to lipid coated beads containing Ni^{2+}-NTA headgroups. Remarkably, these rods preferentially adsorbed onto 1 μm diameter beads, albeit much less well than the filament-forming septins. These data suggest that at some level a single septin rod can recognize micron scale curvature, possibly through the flexibility of the rod itself [30, 32]. Future work should address how the length scale of septin filaments affects curvature sensing, what surface of the septin binds to curved

surfaces, and whether lateral interactions (bundling) of septin filaments and different membrane curvatures affect septins' cooperative affinity to the membrane.

Sensing micron-scale curvature in the cell: An open question

How might SpoVM or septins "detect" micron-scale membrane curvature at the individual protein level? To address this question, we will discuss mechanisms employed by nanometer-scale curvature sensors and how those mechanisms may relate to micron-scale curvature sensors. Nanometer-scale curvature sensors utilize amphipathic helices [11], tune protein-membrane affinity through electrostatic interactions [42], and formation of polymeric scaffolds [15] to sense curvature (a subset of nanometer sensors summarized in Table 2). Interestingly, some evidence suggests that both SpoVM and septins might also use amphipathic helices and electrostatic interactions.

How are amphipathic helices utilized to "sense" curvature? The expanding leaflet of a membrane in response to high curvature at the nanometer scale can drive phospholipid head groups apart. This reveals open sites for amphipathic helix insertion into the membrane [43]. On the micron-scale, defects in lipid packing are likely to manifest differently, and *may* be at the level of the fatty acyl chain rather than with the phospholipid head group [29]. This could explain why SpoVM penetrates the bilayer and likely localizes well below the phospholipid head group. Altering the charge of an amphipathic helix affects both nanometer and micrometer curvature sensing. Increasing positive charge on the polar face of the SpoVM^{P9A} helix resulted in increased affinity for lipid-coated beads and impaired curvature sensing. Similarly, adding only two lysine residues to the polar face of the otherwise uncharged amphipathic helix of ArfGAP1, a nanometer-scale curvature sensor, increased membrane binding and completely abolished curvature sensitivity [11]. We speculate amphipathic helices may be a conserved feature for both nanometer- and micrometer-scale membrane curvature recognition.

Another common feature among nanometer-scale curvature sensors is their cooperative association with curved membranes. Cooperativity could arise through oligomerization of proteins. For example, the H0 amphipathic helix of endophilin is important in forming lateral contacts with neighboring endophilin molecules to form scaffolds/lattices on lipid tubules in vitro [44]. Alternatively, cooperativity could arise through protein-mediated changes in the membrane, thereby favoring the recruitment of additional proteins. For example, coarse-grained simulations of an N-BAR domain on flat membranes show additional protein recruitment upon the initial adherence and curvature induction via individual proteins (see Box 1) [45]. On the micron scale, both SpoVM and septin adsorption onto micron-scale curvatures seem to be reliant on effective affinity differences for various curved surfaces and cooperativity in binding. Adjusting cooperativity and affinity in Monte Carlo simulations of SpoVM adsorption onto beads highlighted how these two parameters synergistically combine to affect curvature sensitivity. Small differences in affinity combined with modest cooperativity alterations yield clear curvature preferences for SpoVM [29]. For septins, filament polymerization is essential for its stable association to membranes; its bimodal adsorption onto 5 μm beads suggest a cooperative mechanism is particularly important on curved surfaces [36].

Although additional work needs to be done on the molecular basis of cooperativity, it seems central feature of micron-scale curvature recognition. Could the local curvature regulate the recruitment of proteins on the membrane through some cooperative mechanism? It is tempting to speculate that the local curvature could promote the formation of different higher-ordered septin structures. However, this is a hypothesis that remains to be tested. The knowledge of nanometer curvature sensors can serve as a powerful guide for understanding micrometer-scale sensing but further work will be required to determine how the mechanisms established for nanometer perception are also exploited for micrometer sensing. Many questions remain regarding how these mechanisms first described at the nanometer scale transfer to the micron scale, if at all. The depth and breadth of these questions reveal what a ripe problem this is for further study.

How cells use micron scale curvature

Micron scale curvature throughout the cell cycle

Reconstitution of recombinant SpoVM and septins *in vitro* demonstrate these proteins by themselves are sufficient to preferentially associate with micron-scale membrane curvature. How do cells use these micron-scale curvature sensors? One of the best examples of a cell using information about its shape to inform a decision is the morphogenesis checkpoint in budding yeast. The morphogenesis checkpoint effectively monitors whether the cell has bud, delaying nuclear division until after bud emergence [46]. However, this raises the question: how does the cell “know” whether it has a bud? Stresses that prevent bud formation lead to an increase and stabilization in the levels of the inhibitory kinase, Swe1 (wee1 homologue), which phosphorylates the mitotic CDK, delaying the cell cycle [47–49]. After bud emergence, the septin collar at the mother-bud neck recruits a series of effectors (Hsl1, Elm1, and Hsl7) that lead to the hyperphosphorylation and degradation of Swe1 [22, 23, 50–54]. The point at which Hsl1, Elm1, and Hsl7 are localized to the septin collar coincides with when septin filaments are arranged in a parallel array aligned with the positive membrane curvature of the bud neck [23, 55]. In cells that have not properly formed a bud, septins are unable to stably recruit Hsl1, Elm1, and Hsl7, thus allowing Swe1 levels to rise (Figure 3A). Thus, the ability of septins to recognize the positive membrane curvature of the mother-bud neck is critical to the morphogenesis checkpoint.

Septins also localize to the developing forespore membranes during sporulation in both *S. cerevisiae* [56] and *S. pombe* [57]. During sporulation, membranous structures engulf and partition nuclear material into four separate haploid nuclei spores [58, 59]. These spores display curvatures from approximately 1 μm^{-1} – 0.5 μm^{-1} (compared to 2 μm^{-1} at the mother-bud neck, Figure 1C). During spore formation expression of spore specific septins, *SPR3* and *SPR28* increase [60]. An analogous process occurs within *S. pombe* [57, 61]. These spore specific septins are essential for sporulation, as mutants for either of these genes have misshapen forespore membranes, their forespore membranes fail to encapsulate nuclei, and cell walls do not mature properly [57, 62]. Mechanistically, how septins contribute to sporulation, remains unclear, but is presumably related to micron-scale membrane shape changes. It is interesting to note that spore-specific septins and mitotic-specific septins appear recognize different membrane curvatures (the forespore membrane vs. the bud neck),

possibly owing a different composition of the respective septin oligomer. This would suggest that different septin complexes may have different intrinsic curvature preferences, a possibility that is also interesting to consider in mammalian cells where a more diverse suite of septin proteins and isoforms can coexist in the same cell. The problems of how septin curvature sensing is tuned developmentally and via expression of distinct heteromeric complexes is wide open for exploration.

Closely related to budding yeast, the filamentous fungus, *Ashbya gossypii*, has been a powerful, emerging model to examine the varied forms and functions of the septin cytoskeleton. During hyphal growth septins polymerize at the plasma membrane where they can form three distinct higher-ordered structures: 1) interregion rings, which are straight septin bundles organized as bars circumferentially around hyphae, 2.) basal collars that localize to newly emergent hyphae, 3.) thin, flexible filaments that run parallel to the hyphal axis [35] (Figure 3B). Septin accumulation to the base of branches suggested their preferential localization to saddle points or sites of positive curvature. When quantified, it became clear that septins are recruited in proportion to the degree of local positive curvature. In contrast, both the thick bars and thin flexible septin filaments align along the hyphal tube, as to seemingly avoid the negative curvature that exists orthogonal to the hyphal long axis. Interestingly, Hsl7, was found to localize exclusively to the septin bar structures and not curved structures [36]. This suggests that the local geometry of the membrane may dictate the local organization of septin filaments, which in turn could control recruitment of downstream signaling proteins.

Septins are also found at the cleavage furrow of most dividing animal cells [63]. Loss of septins leads to varying degrees of cytokinesis defects, depending on the organism and distinct septin complexes that participate in early cytokinesis and abscission [64]. In many cells, septin enrichment is coincident with ingression, where the membrane curvature is increasing. This raises the possibility that as a cleavage furrow ingresses, septin recruitment is enhanced in a curvature-dependent manner, potentially to stabilize or regulate the actomyosin contractile ring. In this scenario membrane curvature might supply a source of positive feedback in septin recruitment during cytokinesis. Interestingly, there are also F-BAR proteins found to associate with the cytokinetic furrow in a variety of systems [65, 66]. During cytokinesis, some F-BAR proteins are thought to target vesicles from the late secretory pathway, whereas others are thought to participate in membrane curvature induction. It is worth investigating whether F-BAR proteins and septins coordinate the different length scales of curvature changes at the plasma membrane during cytokinesis.

Micron scale curvature in autophagy and lipid droplets

Autophagy is a cellular process where cytoplasmic components are degraded under stress or aging. During autophagy aggregated protein complexes, ribosomes [67], organelles including mitochondria [68], and invading microorganisms including *Listeria monocytogenes* [69] and *Shigella flexneri* [70] can be targeted for degradation suggesting broad size distribution among autophagosomes. Indeed, these structures have been observed to range in curvature from 2.2 μm^{-1} to 4 μm^{-1} in yeast and 1.6 μm^{-1} to 4 μm^{-1} in mammalian cells [71–73]. Recent studies showed that septins assemble into cage-like

structures around intracellular bacteria in a manner promoted by mitochondria [70, 74]. The topology and positive membrane curvature of rod-shaped bacteria and mitochondria are ideal for septins, raising the possibility that a septin-mediated immune response is induced by this ideal curvature.

Another subcellular structure with a wide variety of curvatures are lipid droplets. Lipid droplets (LD), amalgams of neutral lipids surrounded by a phospholipid monolayer, have been shown to contribute to lipid and membrane homeostasis in several organisms and cell types including *S. cerevisiae* [75], adipocytes [76], and hepatocytes [77]. Lipid droplets display curvatures ranging from 0.4 μm^{-1} to 50 μm^{-1} in non-adipocytes [5]. Knockdown of Sept9 in Huh7 cells resulted in a decrease in LD number and size [78]. Additionally, Sept9 was found to colocalize with neutral lipid biosynthetic enzyme, diacylglycerol acyltransferase-1 (DGAT-1), further supporting that septins may be involved in regulating lipid and membrane homeostasis. Further work needs to be done to determine whether septins are in fact directly binding to and sensing the curvature of the surface of lipid droplets, or whether septins are recruited by other factors to the surface of droplets.

Micron scale curvature and lateral compartmentalization and function of membrane-interacting proteins

Biological membranes can be compartmentalized into specific domains with distinct protein compositions. One way to compartmentalize membranes is through membrane diffusion barriers [79–81]. Diffusion barriers have been postulated at the base of cilia [41], dendritic spines [82], and at the tip of mating projections during chemotropism [83], at the mother-bud neck in *S. cerevisiae* [39, 84], and the annulus in spermatozoa [85]. Interestingly, septins localize to each of these structures, and have been proposed to contribute to the membrane diffusion barrier [86].

In the budding yeast, there is even evidence that septins act as a diffusion barrier for membranes that are adjacent to the plasma membrane. A septin-ER tether limits the diffusion of ER transmembrane proteins Pho88 and Ist2 between the mother and bud [40, 84]. One component of the barrier appears to be through a formation of a sphingolipid-dependent compartment at the mother-bud neck. This is thought to create a specialized membrane domain to restrict the mobility of transmembrane proteins [87].

Interestingly, septins were shown to induce local domains of supported lipid bilayers consisting of dimyristol- and dioylphosphatidylcholne (DMPC and DOPC, respectively) and phosphatidylinositol (PI) [88]. It has been postulated that septins can act as a membrane diffusion barrier either by acting as physical hindrance for other membrane associated proteins, through altering the lipid abundance, by affecting lipid localization, or by some combination of the three. Future work is needed to understand the mechanisms by which septins influence mobility in membranes and the degree to which this function is due to the local topology of the membrane.

Regulation of micron-scale curvature sensors

Given the possibility of electrostatics driving curvature recognition, a potential way for cells to tune curvature preferences is through post-translational modifications that modulate the affinity of protein complexes for the membrane. Genetic and biochemistry experiments have revealed numerous potential septin regulatory mechanisms, including direct protein-protein interactions, post-translational modifications (PTMs) and lipid-protein interactions [32, 35, 89–95]. Septin filaments can be assembled into a variety of different organizations and patterns as higher-order structures [35, 55, 89, 96–98]. It is tempting to speculate that septins are tightly regulated to specify different higher-order organizations of septin filaments and possibly even fine tune septin membrane curvature recognition. Although very little is known regarding how septins or other micron-scale curvature sensors are regulated, next we will discuss what is known about septin regulation as it pertains to their ability to sense micron-scale membrane curvature.

Post-translational modifications as a means to regulate protein localization to micron scale membrane curvature

Proteins often undergo dynamic transitions between an insoluble membrane bound state and soluble cytoplasmic state. Post-translational modifications (PTMs) of proteins is an attractive mechanism to spatiotemporally control the localization and functions of proteins, including those that act on micron-scale membrane curvatures. Throughout the cell cycle of *S. cerevisiae*, the septin cytoskeleton is subject to changes in organization [55, 90, 99]. Not surprisingly, all five mitotic septins can be phosphorylated at multiple sites [91, 92]. Septin-associated kinases Gin4, Elm1, and Cla4 have been shown to be important for regulating higher-order septin structures. Cla4 is required for septin collar formation in *S. cerevisiae* [93], whereas loss of either Gin4 or Elm1 in *A. gossypii* results in a loss of thick septin bar structures [35]. Nonetheless, septin localization to sites of positive curvature still persist. This suggests that straight septin bars require phosphorylation whereas assembly on curved membranes does not. However, a direct a link between septin phosphorylation and curvature sensing has yet to be established. Recently, Shen et. al. showed that phosphorylation within the GTP-binding domain of Sept12 abolished septin ring formation at the annulus in sperm [85], further highlighting the importance of septin regulation by phosphorylation. Interestingly, an acetylation/SUMO-ylation switch was found to regulate Cdc11 localization from the bud scar to the new incipient bud site in *S. cerevisiae* [94]. Despite this, it is still unclear whether septin phosphorylation, acetylation, and SUMO-ylation contribute to curvature sensing. We speculate that cells may tune the specific curvature preference of septins using PTMs that change the affinity of septins for different membrane shapes.

The role of the membrane composition in regulating micron scale membrane curvature

Lipid asymmetry across the bilayer is an inherent feature of cellular membranes; phosphatidylcholine and sphingolipids are predominately localized to the outer leaflet of lipid bilayers, whereas phosphatidylserine, phosphatidylethanolamine, phosphoinositols and their phosphoinositide derivatives localize to the inner leaflet [95]. This provides a means to localize membrane binding proteins to particular compartments on the basis of electrostatics, lipid packing, and local geometry. Phosphoinositides (PI) in particular, seem to have an

emerging role in regulating cellular processes where micron-scale curvature is present including forespore membrane extension in *S. pombe* [57], autophagy [100], and micropinocytosis [101] in mammalian cells. Septins may be locally recruited to membranes based on the local lipid composition.

Mammalian septin filaments comprised of, Sept2, Sept6, and Sept7 were found to localize to mature macropinocytic vesicles in a PI(3,5)P2-dependent manner and septins are found in what are thought to be PI(4,5)P2-rich sites in yeast at mating projections and the neck [101, 102]. PI(4,5)P2-containing lipid monolayers were shown to drive septin filament assembly even in high salt conditions where septin polymerization is perturbed in solution [103] These data suggest that phosphoinositides play a key role in regulating protein adsorption onto a wide range of membrane curvatures and may play a role in modulating curvature preferences.

Concluding Remarks

How do SpoVM and septins recognize micron-scale positive membrane curvature? Interestingly, both SpoVM and the septins cooperatively associate membranes with shallow positive curvature; hinting that polymerization may be important. Septins are known to polymerize into filaments hundreds of nanometers to micrometers in length. For septins it is tempting to speculate that membrane curvature is recognized at the level of the filament, and not necessarily at the level of the rod. Nonetheless, even non-polymerizable septin mutants preferentially associate with shallow curved membranes, albeit much weaker than septins that can polymerize. How then might SpoVM and septins sense shallow curvature at the protein level? Many nanometer-scale curvature sensors utilize an amphipathic helix that inserts into the lipid bilayer. SpoVM, and possibly septins, also have amphipathic helixes. Perhaps both insertion of an amphipathic helix to the membrane is conserved mechanism for sensing curvature, even at shallow curvatures.

The fact that individual septin rods can intrinsically detect shallow membrane curvature raises an interesting question. If septin filament polymerization is unnecessary for curvature sensing, why polymerize at all? To answer this question, first consider the kinetics of an individual septin rod on and off the membrane (Figure 1C). Free septin rods that are not incorporated into filaments are favored to dissociate from the membrane (high k_{off} relative to k_{on}). Stable septin association to the membrane depends whether it is incorporated into a polymerized septin filament. This is likely critical for septins to reliably and correctly "sense" *persistent* micron-scale curvature. Otherwise, if individual septin rods could tightly associate with shallow curved membranes without polymerizing, septins would presumably localize to dispersed regions across the cortex, anywhere shallow curvature might incidentally arise (such as a relatively flat, yet slightly undulating membrane). Thus, septin filament polymerization at the membrane ensures that septins curvature recognition is limited only to persistent, shallow membrane curvatures that define cellular shapes at the micron scale.

We are just beginning to understand how cells perceive cellular membrane curvature, and by extension how cells "sense" their shape (see Outstanding Questions). It will be interesting to

determine whether there are analogous proteins to SpoVM that can recognize shallow positive membrane curvature in other prokaryotes. Despite their variety of shapes and forms, both animal and fungal cells utilize the same cytoskeletal proteins, the septins, to sense micron-scale cellular membrane curvature. Oligomeric septin rods can assemble into micron-scaled filaments at the membrane, and these filaments can then assemble into various larger structures depending where they polymerize in cell. Interestingly, the organization of these larger assemblies appear to be dependent on the local membrane curvature. In budding yeast, the ability of septins to "sense" positive curvature at the mother-bud neck is critical to ensure that both the mother and future daughter cell inherit a nucleus before cytokinesis. In many different cell types, septins localize to the cytokinentic furrow and are required for cytokinesis. Further work will hopefully elucidate roles of septins in different cell types, and determine whether the ability of septins to recognize membrane curvature is essential for these cell types.

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Box 1 anometer and micrometer scale curvature induction

Curvature sensing is often linked to curvature induction, however, the degree to which the two are separable can be challenging to study. For the BAR-domain protein amphiphysin, it has been seen that at low protein densities the proteins are generally in a curvature sensing regime and at high protein densities crowding and potentially lateral associations that build a rigid scaffold then induce membrane curvature [104]. Similar density-sensitive behavior has also been seen for dynamin that also can assemble and act in a curvature-sensitive manner [105]. Thus, the role of a protein as a sensor vs an inducer may be highly dependent on the concentration of protein.

Septins can bind and tubulate giant unilamellar vesicles (GUV) to micron-scale tubules [106]. This work suggests that curvature sensing and induction could be tightly connected for septins and potentially sensing the local geometry can be used to recruit more protein and drive membrane bending. However, artificially recruiting high concentrations of his-GFP to Ni^{2+}-NTA containing GUVs also results in tabulation [107], suggesting that protein crowding alone can drive membrane bending. Thus, whether tubulation is a specific function or by-product of crowding is not clear.

The fact that septins induce micron-scale tubules as opposed to highly variable or nanometer-scale tubules would support that induction of curvature may be physiologically relevant but it would be useful to assess septin curvature sensing and induction of a range of protein densities as has been done with amphiphysin and dynamin. Septin recruitment to cell blebs in mammalian cells [108] may be a context where septins both sense and induce curvature changes. In the case of experiments with SpoVM and septins using beads, induction is not possible because the bilayers are supported and similarly in fungal cells, the cell wall limits the role of septins in inducing curvature [36]. In cells without such rigid extracellular domains, further work is required to disentangle the relationship between micron scale-curvature sensing and induction.

Trends:

1. Cells can recognize their own shape and local geometries on the micron scale.
2. SpoVM and the septins have been identified as micron-scale curvature sensors.
3. Modes of protein association with the membrane and oligomerization enable curvature sensing on the micron scale.
4. Cells likely tune the curvature preference of sensors using post-translational modifications and varying local lipid composition.

Outstanding questions:

1. What alternative mechanisms enable micron-scale curvature sensing using nanometer proteins?
2. What is the relationship between curvature sensing and curvature induction?
3. How do cells tune the curvature preference of sensors such as septins so as to sense a wide range of geometries within the same cell or in different cell types?
4. How do membrane composition and organization contribute to micron-scale curvature sensing?
5. What other families of proteins are capable of sensing micron scale-curvature?

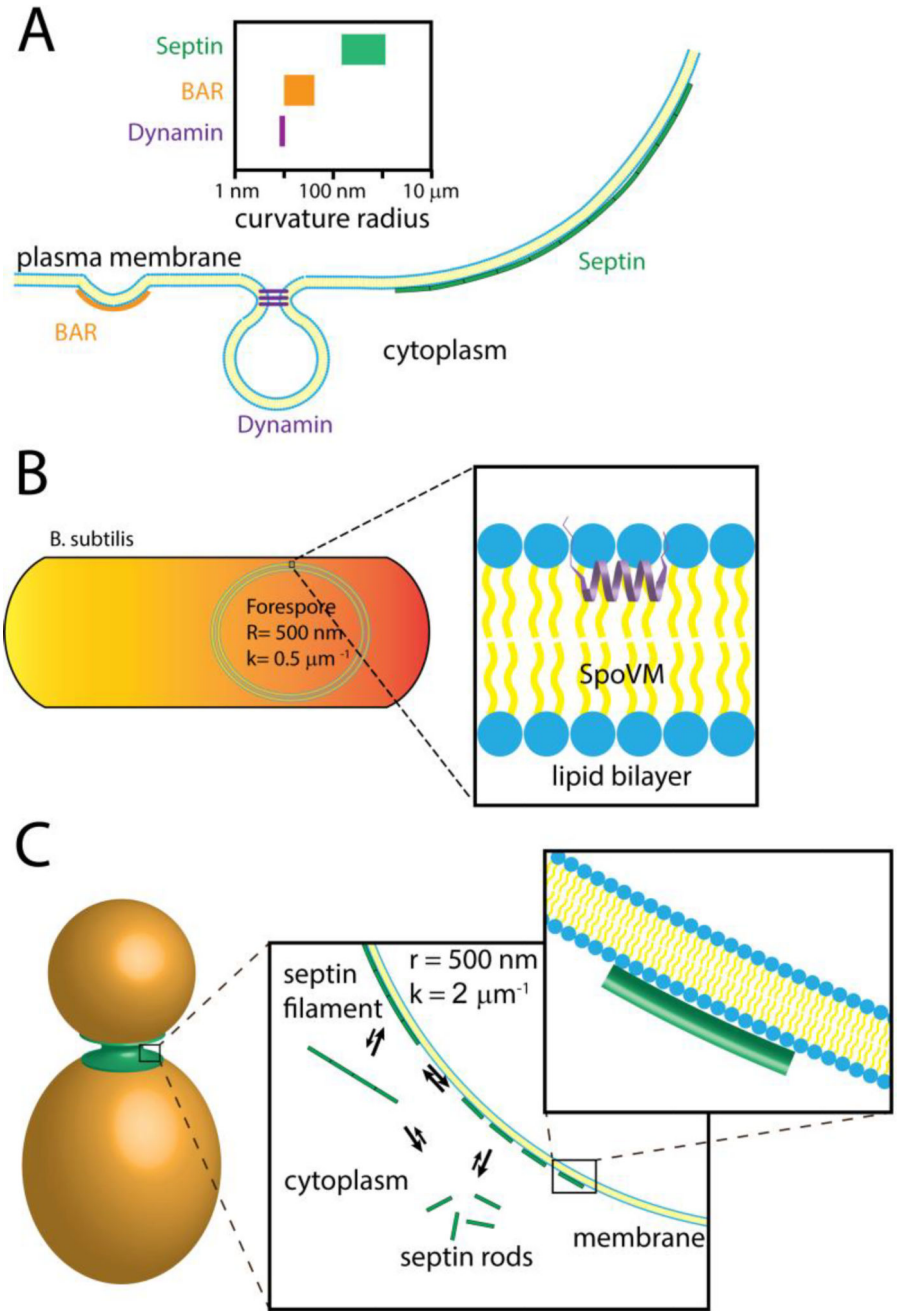

Figure 1. The disparity between nanometer molecules and micron-scale curvature at the membrane

(A) Cells possess a variety of proteins that can bind and recognize curved biological membranes. Some of the best understood membrane curvature "sensors" are BAR domain proteins (left) and dynamin (center), which recognize steep, nanometer-scale curvatures. Comparatively, the septin cytoskeleton (right) recognizes much shallower curvatures at a micron-scale. The range of curvatures recognized by these proteins are depicted above. (B) During sporulation in the bacterium *Bacillus subtilis* SpoVM preferentially binds to the forespore membrane, the only site of positive curvature in the cell, which has a curvature of about 0.5 μm^{-1} (or a radius of 500 nm). The amphipathic helix of SpoVM is thought to

insert into the lipid bilayer of the forespore membrane (inset). Across the length of SpoVM (~ 4 nm), the perceived membrane curvature is nearly flat.

(C) After bud emergence in the budding yeast *Saccharomyces cerevisiaie*, septins are localized to the mother-bud neck, which is the only area of continuous positive curvature at the plasma membrane. Septins are thought to be in an equilibrium between soluble and assembled states at the plasma membrane (first inset). Septins may cooperatively associate with lipid supported bilayers on beads *in vitro* suggesting that septin polymerization into filaments stabilizes their association to the membrane. Across the length of septin rod (17–32 nm), the perceived membrane curvature is nearly flat (second inset).

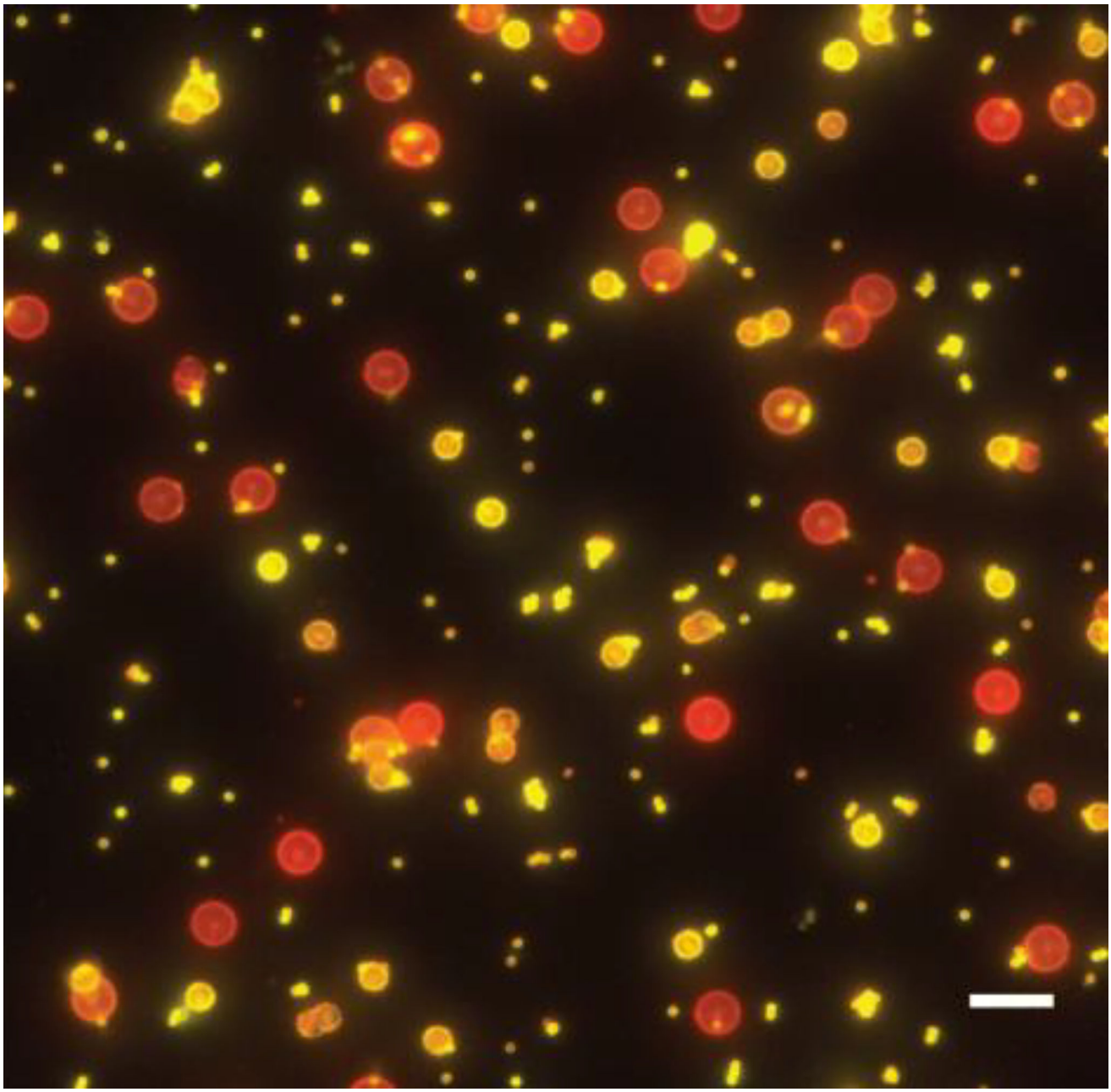

Figure 2. Septins have a preference for micron-scale membrane curvatures

In vitro reconstitution of septins with lipid bilayer-coated beads (red) of various diameters shows septins enrichment on 1 μm and 3 μm beads over 5 μm beads (yellow). Scale bar, 10 μm

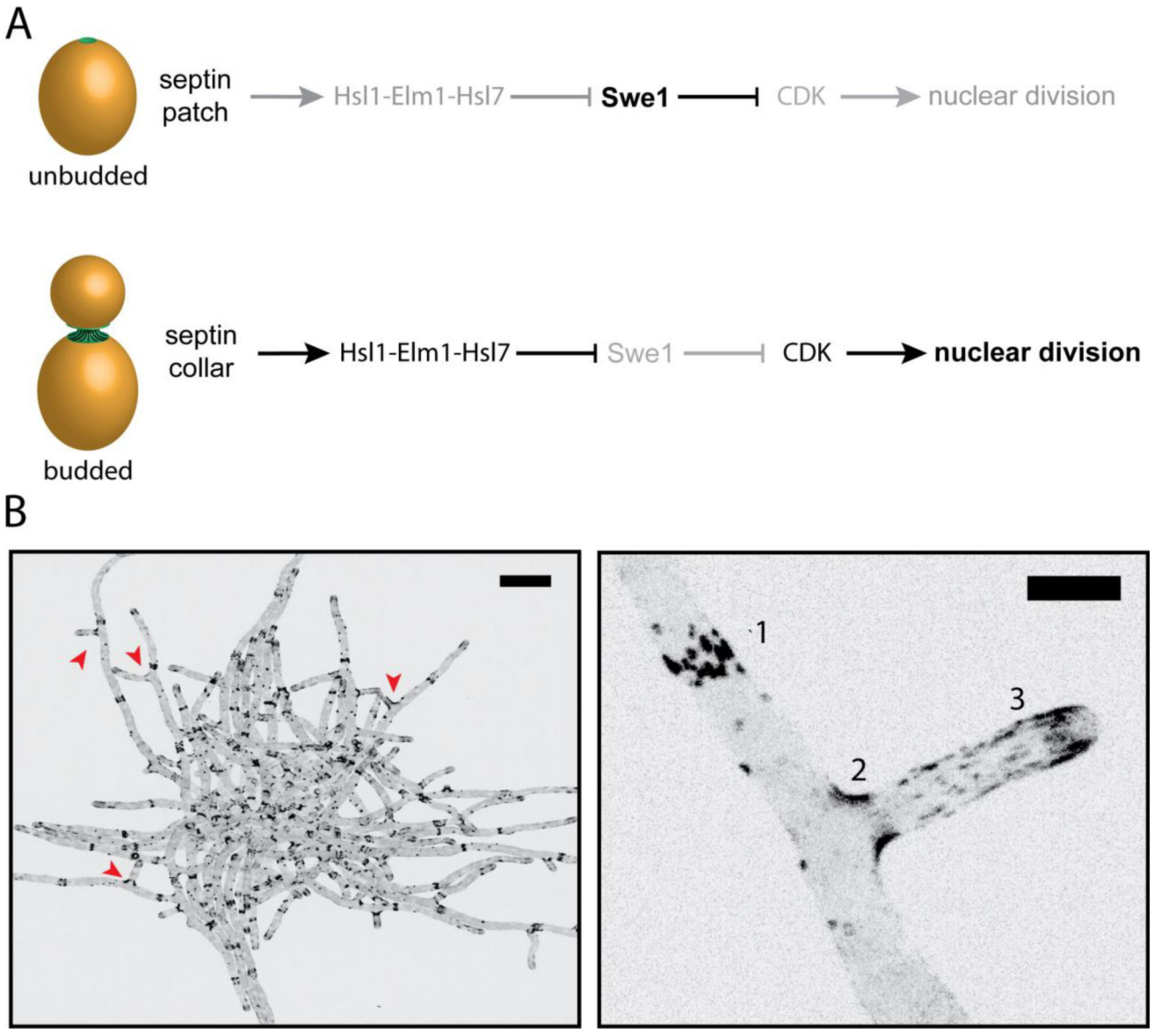

Figure 3. Septin structures and localization in budding yeast and the filamentous fungus *Ashbya gossypii.*

A. Morphogenesis checkpoint in *S. cerevisiae*: Hsl1-Elm1-Hsl7 are not recruited to the septin patch in unbudded cells, thereby allowing the CDK-inhibitor Swe1 levels to rise. As soon as the bud emerges, septin localization transitions to an hourglass shape coincident with the generation of micron-scale curvature. Swe1-regulators are recruited after this transition to initiate the degradation of Swe1 and promote progression through the cell cycle. B. An inverted, maximum z-stack projection of a *A. gossypii* cell expressing Cdc11-GFP. Scale bar, 20 μm. (Bottom right) Zoomed-in image of *A. gossypii* hyphae expressing Cdc11-GFP from above. Septins are organized into various higher-ordered structures in *Ashbya*: [1] Thick bars structures, [2] basal collars at branch points, and [3] thin, flexible filaments. Scale bar, 5 μm.

Table 1

Prokaryotic proteins localizing to sites of membrane curvature.

Protein	Curvature localized to in vivo	Membrane binding motif	Deform liposomes in vitro?	Formation of higher order structures	Primary Function	References
FtsZ	**Negative**	–	**+ when complexed with FtsA* or when fused to a membrane targeting sequence**	+	**Cytokinesis**	[109–111]
FtsA	**Negative**	**Amphipathic helix**	**+ when complexed with FtsZ**	+	**Tether FtsZ to the membrane, Z-ring assembly**	[111–113]
MreB	**Negative and Positive**	**Amphipathic Helix**	+	+	**Cell shape maintenance/ cell wall synthesis**	[114–117]
CrvA	**Low positive to negative**	**Unknown. Data suggests CrvA localizes in the periplasm**	**Unknown. Shown to be required for the curved shape of *V. cholerae***	+	**Cell wall insertion, generates cell curvature**	[118]
CreS	**Positive**	–	**Unknown. Shown to be required for the curved shape in *C. crescentus***	+	**Cell shape maintenance, surface colonization**	[119–122]

Table 2

Nanometer sensors that sense curvature.

Protein	Curvature localized to in vitro	Scale	BAR domain	Insertional amphipathic helix	Deform liposomes in vitro?	Formation of higher ordered structures	Membrane binding sensitive to electrostatics	Membrane binding sensitive to lipid packing defects	Undergo PTM	Primary Function	References
Endophilin	**Positive**	**nm**	**+ N-BAR**	+	+	+	+	+	+	**Endocytosis**	[15, 44, 123–126]
Amphiphysin	**Positive**	**nm**	**+ N-BAR**	+	+	+	+	**Unknown**	**Unknown**	**Endocytosis**	[14, 104, 127]
IRSp53	**Negative**	**nm**	**+ I-BAR**	−	+	**Unknown**	+	**Unknown**	**Unknown**	**Filopodia formation**	[42, 128–131]
ESCRT-III	**Negative**	**nm**	−	+	+	+	+	**Unknown**	**Unknown**	**Multivesicular body formation**	[132–134]
ArfGAP1	**Positive**	**nm**	−	+	**Unknown**	**Unknown**	−	+	+	**Vesicular trafficking**	[10, 12, 135, 136]
SpoVM	**Positive**	**μm**	−	+	**Unknown**	**Unknown**	**Unknown**	**Unknown. Suggested by [29]**	−	**Forespore formation in *B. subtilis***	[21, 29]
DivIVa	**Negative**	**μm**	−	+	−	+	**Unknown**	**Unknown**	−	**Cell division site selection**	[137]
Septin	**Positive**	**μm**	−	**Unknown**	+	+	+	**Unknown**	+	**Cytokinesis**	[30, 33, 36, 91, 103]