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Compartmentalization and Organelle Formation in Bacteria

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Abstract

A number of bacterial species rely on compartmentalization to gain specific functionalities that will provide them with a selective advantage. Here, we will highlight several of these modes of bacterial compartmentalization with an eye towards describing the mechanisms of their formation and their evolutionary origins. Spore formation in *Bacillus subtilis*, outer membrane biogenesis in Gram-negative bacteria and protein diffusion barriers of *Caulobacter crescentus* will be used to demonstrate the physical, chemical and compositional remodeling events that lead to compartmentalization. In addition, magnetosomes and carboxysomes will serve as models to examine the interplay between cytoskeletal systems and the subcellular positioning of organelles.

Introduction

The cell is crowded place and subcellular organization is vital to regulating its many metabolic processes [1]. In eukaryotes, such organization is prominently achieved through the compartmentalization of biochemical reactions in various intracellular organelles. By limiting diffusion to a confined space, concentrations of enzymes and substrates can be optimized to promote specific enzymatic reactions. In turn, sequestration of activities within compartments protects the cell from toxic byproducts of such reactions. While historically considered to be simple cells with a low degree of subcellular differentiation, compartmentalization in the form of organelles is also a widespread phenomenon amongst bacterial cells [2]. Unlike the eukaryotic endomembrane system, bacterial species are not equipped with a standard set of organelles. Instead, varying combinations of organelles provide unique capabilities to individual bacterial species. One notable class is the protein-bounded bacterial microcompartments, exemplified by the carbon-fixing carboxysomes of cyanobacteria [3]. Lipid-bounded organelles, including the dazzling varieties of photosynthetic membranes found in heterotrophic bacteria and cyanobacteria, constitute another set of bacterial compartments [4–6]. In addition to organelles, other modes of compartmentalization, such as the creation of spores and protein diffusion barriers to subdivide the cytoplasmic space, have also been described in bacterial cells [7,8••].

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Despite the impressive list of bacterial compartments and their cytological characterization, the molecular mechanisms that govern their formation, function and segregation are still a major question in bacterial cell biology. Here, we will focus on recent discoveries on the physical, chemical and compositional remodeling of membranes during compartmentalization as well as the mechanisms leading to the spacing and positioning of organelles within the cell. Those interested in acquiring a more in depth knowledge of this fascinating topic, are encouraged to read several recent review articles on the function, diversity and evolution of bacterial organelles [2,9].

Membrane remodeling

A fundamental aspect of organelle formation in any organism is the remodeling of cellular membranes during the compartmentalization process. Remodeling can be physical in nature such as the bending, migration and fusion of lipid bilayers to produce and stabilize organelles. Chemical remodeling of lipids can also produce distinct compartments within the cell. Additionally, compositional remodeling of membrane domains, through protein targeting and/or exclusion, can subdivide a continuous structure into distinct compartments. These types of membrane remodeling have been described in numerous eukaryotic systems. In contrast, almost nothing is known about the methods and molecules used by bacteria to remodel their lipids into a compartment. Here we highlight three cases where mechanisms and evolution of bacterial membrane remodeling events have been recently elucidated.

Physical remodeling: spore formation—Perhaps the most thoroughly studied example of membrane remodeling in bacteria is the engulfment of forespore during the sporulation process of *Bacillus subtilis*. When growth is challenged by harsh conditions, *B. subtilis* cells undergo a unique developmental program to form a highly durable and dormant endospore. During the early stages of sporulation, an asymmetric division event creates a larger "mother" cell that proceeds to engulf the smaller "forespore" cell to form an internal, double-membraned compartment (Figure 1a). A number of mechanisms have been implicated to drive the mother cell membrane around the forespore and have been recently reviewed [10]. These include cell wall synthesis, cell wall degradation and specific protein interaction between SpoIIQ and SpoIIIAH that bridge across the mother and forespore membrane to prevent membrane retraction (Figure 1a)[10–13].

The final step of the engulfment process is the joining of the two ends of the migrating membranes to create a completely internalized endospore. Genetic analysis had implicated one protein, SpoIIIE, in membrane fission. However, *spoIIIE* null mutants also have defects in DNA translocation and septum morphology at earlier stages of sporulation raising the possibility that impaired membrane fission may be an indirect effect [14–16••]. By examining a set of mother cell genes that are expressed at the early stages of engulfment that when mutated, fail to release the forespore into the mother cell cytoplasm, Doan and colleagues recently identified fission protein B (FisB) as a direct catalyst of membrane fission in *B. subtilis* [16••] (Figure 1a). Fluorescent protein fusions to FisB localize primarily to the edges of the migrating mother cell membrane that serve as the sites of membrane fission. Furthermore, purified FisB promotes membrane fusion *in vitro* via a specific interaction involving the extracellular domain of FisB and cardiolipin, an anionic

phospholipid that is enriched in highly curved membranes including the forespore of *B. subtilis* [16••–18]. The authors propose that FisB may concentrate cardiolipin and create localized defects in the lipid bilayer that would favor the fission reaction. While these results highlight the importance of FisB in membrane fission, a small percentage of *fisB* mutants eventually complete engulfment, suggesting that alternative mechanisms may function at a slower rate to complete engulfment [16]. A recent report identified core set of genes shared amongst sporulating bacteria, including three novel genes involved in endospore formation [19]. This genomic signature may be a useful tool to identify additional mechanisms for membrane remodeling.

Chemical remodeling: Gram-negative outer membrane—Membrane remodeling is not limited to the physical movement and bending of lipid bilayers. Often, extensive modification of the chemical composition of cellular membranes is required for compartment formation and function. In bacteria, the construction of the Gram-negative outer membrane (OM) provides a model for understanding the mechanisms of chemical remodeling in biological membranes. As should be familiar to most students of biology, the Gram stain, developed by Hans Christian Gram in 1884, is a histological method for identifying and classifying bacterial species. Gram-positive bacteria have a single cell membrane that is surrounded by a thick, multi-layered cell wall. Gram-negative bacteria have two membranes separated by a thin cell wall [20] (Figure 1b). The OM displays a number of characteristics that define it as a bacterial compartment. It is an asymmetric lipid bilayer with lipopolysaccharide (LPS), a glycolipid, on its outer leaflet and phospholipids on its inner leaflet. Beta-barrel proteins in the OM form pores that allow for non-specific exchange of small molecules with the extracellular environment while limiting diffusion of many cytoplasmically secreted proteins. Furthermore, the tight packing of LPS on the outer leaflet provides an impermeable barrier to small hydrophobic and hydrophilic molecules that can be harmful to the cell [20]. The OM does not just function as barrier but also creates a chemically distinct periplasmic compartment where important processes, such as certain types of respiration, can occur.

While tremendous progress has been made in defining the chemical nature of the OM and uncovering the molecular mechanisms of its biogenesis [21,22], the evolutionary origins of this structure have remained mysterious. A recent study has searched for the answer to this question by examining the curious mode by which some bacterial cells produce and germinate spores [23••]. Phylogenetically, most endospore forming bacteria fall under the *Bacilli* and *Clostridia* classes of the Gram-positive *Firmicutes* phylum [24]. Interestingly, some members of the *Clostridia*, such as *Acetonema longum*, can form spores but are decidedly Gram-negative in their vegetative state, as they possess an inner membrane and an OM that contains LPS. Through high-resolution electron cryo-tomographic (ECT) imaging of *A. longum* and *B. subtilis* cells at different stages of sporulation and spore germination, Tocheva and colleagues were able to show that the inner membrane of the mother cell engulfs the forespore and forms the outer spore membrane [23••,25]. During germination in *A. longum*, however, this membrane acquires the morphological and chemical signatures of an OM, including LPS (Figure 1b). Sequencing of the *A. longum* genome revealed the presence of homologs of genes known to encode for OM proteins in Gram-negative bacteria

as well as those involved in sporulation in Gram-positives. Phylogenetic analysis showed that the OM proteins of *A. longum* do not cluster with those of other Gram-negatives whereas its sporulation proteins group with those of other Gram-positives. A parsimonious explanation for these results is that *A. longum* did not acquire the ability to produce an OM due to a recent horizontal gene transfer event, in which case its OM proteins would have been in tighter phylogenetic groups with homologs from Gram-negative organisms. Similarly, sporulation also seems to be an ancient characteristic of this organism. These findings lead to the tantalizing hypothesis that ancestors of Gram-positive and Gram-negative bacteria were able to link spore germination to construction of an OM. Subsequently, the majority of Gram-positives lost the ability to form an OM while the loss of sporulation genes gave rise to present-day Gram-negative organisms.

Compositional remodeling: protein diffusion barriers—Diffusion gradients are used by cells to control the timing and location of essential cellular processes [26]. Protein[EC1] diffusion barriers had only been described as a feature of eukaryotic cells until their recent discovery by Schlimpert and colleagues in the Gram-negative bacterium, *Caulobacter crescentus* [8••,27]. At a specific point during its cell cycle, *C. crescentus* physically remodels its cell envelope to form a polar stalk that functions as a holdfast for attachment to surfaces. During phosphorus limiting growth periods the stalk undergoes a dramatic elongation leading to the hypothesis that it may have a role in phosphorus uptake from the environment. Even though the stalk is a protrusion of the cell envelope, Schlimpert and colleagues found that periplasmic proteins do not move between the stalk and the cell body. Furthermore, they found that the stalk itself was compartmentalized into several segments that restricted protein movement. Early electron [EC2] microscopic examination had found that curious cross band structures were spread through out the *C. crescentus* stalk. Using a combination of genetic and biochemical approaches, the authors identified a protein complex consisting of four proteins (StpA, StpB, StpC and StpD) that localize to these conspicuous crossband structures. Deletions of the genes encoding the Stp factors result in a loss of cross band structures visualized by ECT and absence of compartmentalization within the stalk (Figure 2a). By preventing the accumulation of newly synthesized proteins into the stalk, these cross band structures essentially lead to a compositional remodeling of the compartment. One consequence of the loss of stalk compartmentalization is that proteins synthesized after stalk formation are no longer restricted to the cell body and are less concentrated in locales where their activity might be needed. This is especially of concern in phosphate starvation conditions in which the stalk elongates to the point that its surface area exceeds that of the cell body. In agreement with this model, the authors demonstrate that mutants missing the *stp* genes, take longer to accumulate proteins within their cell body and are outcompeted by wild-type cells in co-culturing experiments. Thus, in the case of the *C. crescentus* stalk, compositional remodeling partially acts to prevent the dilution of important cellular proteins (Figure 2b). Additionally, the authors hypothesize that the compositional remodeling of the stalk may also allow for localized activities that may be used to shuttle phosphate into the cell.

Organelle positioning

The subcellular positioning of compartments plays a key role in the function of many bacterial organelles. Recent studies have uncovered some of the molecular mechanisms that lead to the organization and positioning of two unique bacterial organelles, the magnetosome compartments of magnetotactic bacteria and the carboxysomes of cyanobacteria [28].

Compartmentalized through an invagination of the inner cell membrane, the magnetosome creates a controlled chemical environment optimal for formation and maturation of magnetite or greigite both of which are iron-based magnetic crystals. Magnetosome formation requires multiple steps utilizing genes that form membrane invaginations, localize proteins, nucleate crystals and align structures into a chain [29–34]. The end product of these processes is a well-aligned magnetosome chain (Figure 3a), which is thought to provide navigational capability towards preferred oxygen concentrations in a stratified water column, a process termed magnetoaerotaxis [35].

Subcellular organization of magnetosomes into a chain is dependent on MamK, a bacterial actin homolog that is present in all magnetotactic bacteria. High resolution imaging of *Magnetospirillum magneticum* AMB-1 by ECT revealed that organized chains of magnetosomes are flanked by filaments that disappear in a *mamK* deletion mutant (Figure 3a)[34]. Much like other actin-like proteins, MamK forms filaments that are dynamic *in vivo* [36•] and *in vitro* [37,38] in a manner that is dependent on the ATPase activity of the protein. The dynamic behavior of MamK *in vivo* also relies on the redundant action of acidic proteins MamJ and LimJ (Figure 3a)[EC3][36•]. In their absence, MamK filaments are static as seen in a fluorescence recovery after photobleaching assay. In addition, the loss of *mamJ* and *limJ* results in multiple gaps within the magnetosome chain and a clustering of putative MamK filaments within these spaces. One interpretation of these results is that MamK dynamics may be acting to pull magnetosomes together. Alternatively, the loss of MamJ and LimJ could change the localization of MamK in the cell leading to altered dynamics and an inability to form a coherent chain. [EC4]

Despite the clear advances in understanding the behavior and properties of MamK as an actin-like protein, the specific mechanism by which it acts on the magnetosome chain remains unknown. The phenotypes of the *mamK* deletion strains are consistent with its action in either establishing or maintaining the magnetosome chain. Furthermore, in a related species, *Magnetospirillum gryphiswaldense* MSR-1, deletion of *mamK* results in ectopic chain localization and failure of partitioning the chain between daughter cells [39,40]. It has even been suggested that MamK acts to link physical forces on the magnetosome chain into changes in cell motility [41]. Regardless of the specific mechanism of its action, MamK is clearly important for proper navigation in magnetic fields [42]. A recent study using an agar plate magnetoaerotaxis assay found that *mamK* mutants had a significantly wider path around a magnetic field as compared to the wild type strain. In the environment, such a difference could translate into a longer travel period, and subsequently higher energy expenditure, for reaching a desired locale.

Similar to magnetosomes, the carboxysome organelles of cyanobacteria are also arranged linearly within a cell with a consistent spacing pattern. This arrangement relies on the action of the cytoskeletal protein, ParA, without which carboxysomes are unequally distributed amongst daughter cells (Figure 3b)[43••]. ParA filaments are also highly dynamic within the cell and undergo pole-to-pole oscillations that may be important in carboxysome positioning (Figure 3b). Given its crucial role in carbon fixation, the failure to acquire carboxysomes results in a significantly longer doubling time. ParA and its homologs belong to the Walker type family of ATPases and are often associated with segregation of plasmid or chromosomal DNA in diverse bacterial species. However, recent work has shown that a ParA homolog, PpfA, can also be used to segregate chemotaxis protein clusters during cell division in *Rhodobacter sphaeroides* [44]. In this case, nonspecific interactions of PpfA with DNA as well as specific interactions with its chemotaxis protein cargo provide the means for separating the protein complexes [45]. These findings leave open the possibility that in cyanobacteria, carboxysomes interact with chromosomes and that ParA is primarily directing the localization and segregation of DNA. However, deletion of *parA* does not affect the spacing of chromosomal DNA in cyanobacteria while disrupting the organization of carboxysomes [46]. Thus, while DNAParA interactions may be important, specific interactions of this cytoskeletal protein with the carboxysome are likely needed for the proper positioning of this bacterial organelle.

Conclusion

The discovery of cytoskeletal elements, organelles and means of sophisticated spatio-temporal regulation in bacteria have transformed the simplistic views of this complex and metabolically diverse domain of life. The most fundamental roadblock in understanding compartmentalization comprehensively in bacteria is the scarcity of reliable model systems that can help to illuminate specific facets of the organelle formation process. As such, isolated examples of compartmentalization in genetically tractable organisms have carried the bulk of the weight in defining the mechanistic basis of membrane remodeling, protein localization and organelle segregation in bacteria. The diversity of these organelles and the specifications in their morphology and function makes it unlikely that a universal mechanism for compartmentalization exists amongst all bacteria. For instance, membrane remodeling in sporulation relies in part on the use of peptidoglycan as a guide while no such cues will be available during magnetosome formation. Likewise, while magnetosomes and carboxysomes rely on cytoskeletal elements for their subcellular positioning, the proteins involved are not related hinting at distinct molecular activities in these two systems. Clearly, an expansion of available model organisms and a more thorough cataloging of bacterial organelles are needed to truly revolutionize this field. If successful, such endeavors will open new avenues of research in microbiology and uncover novel mechanisms of cell organization and compartmentalization.

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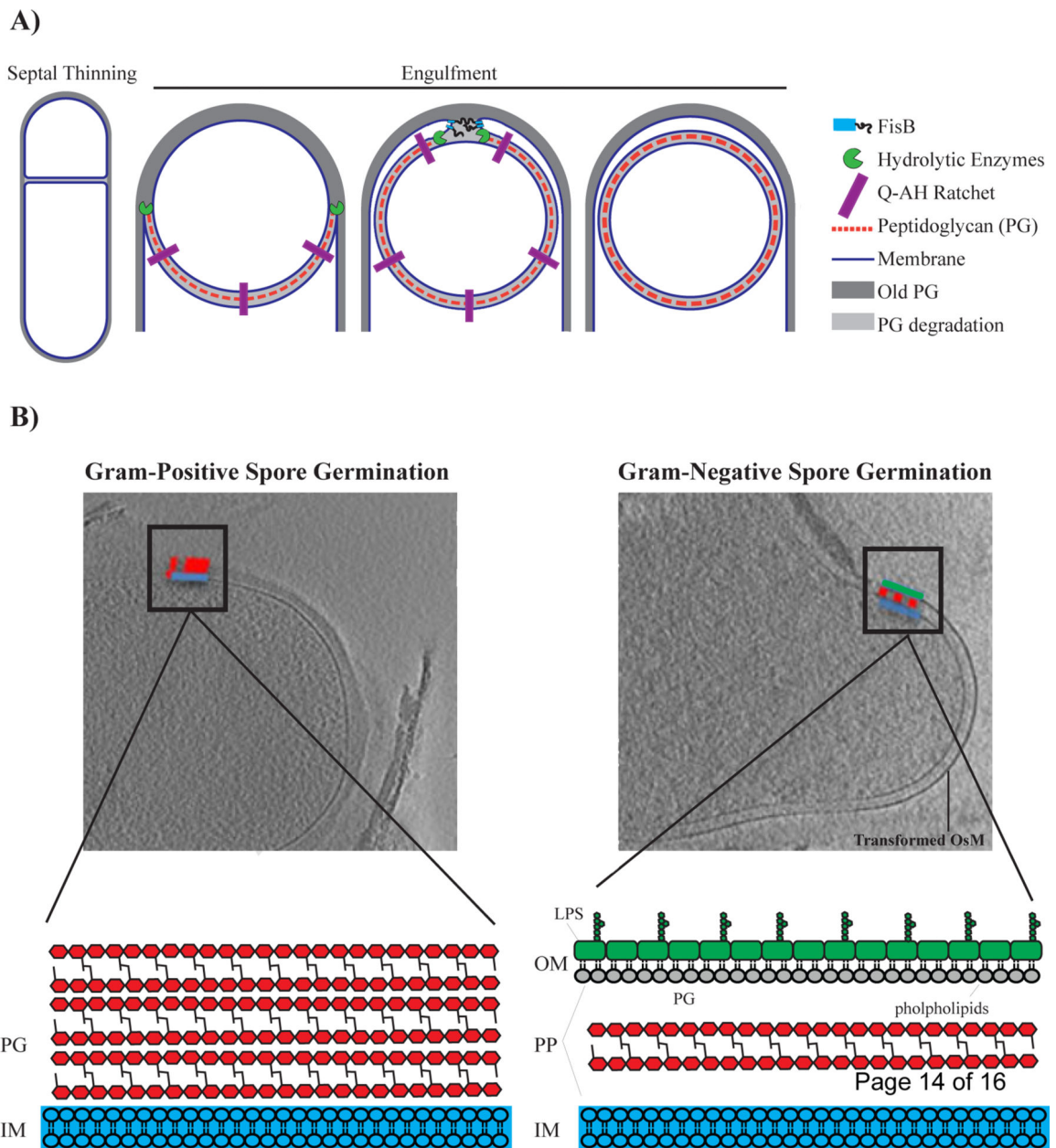
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Highlights

1. Bacterial cells contain lipid- and protein-bounded organelles.

2. FisB mediates membrane fission during spore formation in *Bacillus subtilis*.

3. Outer membrane of Gram-negatives may have originated in a sporulating bacterium.

4. A protein complex forms a diffusion barrier in the stalks of *Caulobacter crescentus*.

5. Magnetosomes and carboxysomes are positioned within the cell by cytoskeletal proteins.

A)

Septal Thinning   Engulfment

Legend:
- FisB
- Hydrolytic Enzymes
- Q-AH Ratchet
- Peptidoglycan (PG)
- Membrane
- Old PG
- PG degradation

B)

Gram-Positive Spore Germination

Gram-Negative Spore Germination

Transformed OsM

LPS

OM

PG

PP

pholpholipids

Page 14 of 16

PG

IM

IM

Figure 1.
A) Mechanisms of membrane remodeling during the different stages of engulfment.
Engulfment initiates with degradation of septal peptidoglycan, commonly referred to as
septal thinning. Peptidoglycan synthesis, peptidoglycan degradation and a specific "ratchet-
like" mechanism that is mediated by proteins SpoIIQ and SpoIIIAH (QAH) are all factors
that have been shown to be important for driving the mother cell membrane around the
forespore. To be released into the mother cell, migrating membranes meet at the cell pole
and undergo a fission event that is mediated by FisB. Note that the outer spore membrane
(OsM) is derived from the cytoplasmic membrane of the mother cell. B) Spore germination
of Gram-positive *Bacillus subtilis* (left) as compared to Gram-negative *Acetonema longum*
(right). Upon germination, Gram-positive *Bacillus subtilis* (left) sheds its OsM whereas

Gram-negative *Acetonema longum* (right) retains its OsM. Furthermore, *A. longum* transforms its OsM into a canonical Gram-negative outer membrane (OM). Cell envelope of Gram-negative vs. Gram-positive bacteria are very different. Gram-positive bacteria have a thick cell wall that is made up of multiple layers of peptidoglycan (PG) that surrounds an inner membrane (IM). On the other hand, Gram-negative bacteria maintain a thin layer of cell wall in-between two membranes. The OM of Gram-negative bacteria is compositionally different from the IM and creates a compartment, the periplasm (PP), that chemically distinct from the cytoplasm. The OM is an asymmetric bilayer with lipopolysaccharide (LPS) distributed in the outer leaflet and phospholipids distributed in the inner leaflet. Copyright (2013) Wiley. Used with permission from [Tocheva et al., *Mol Micro*, 2013].
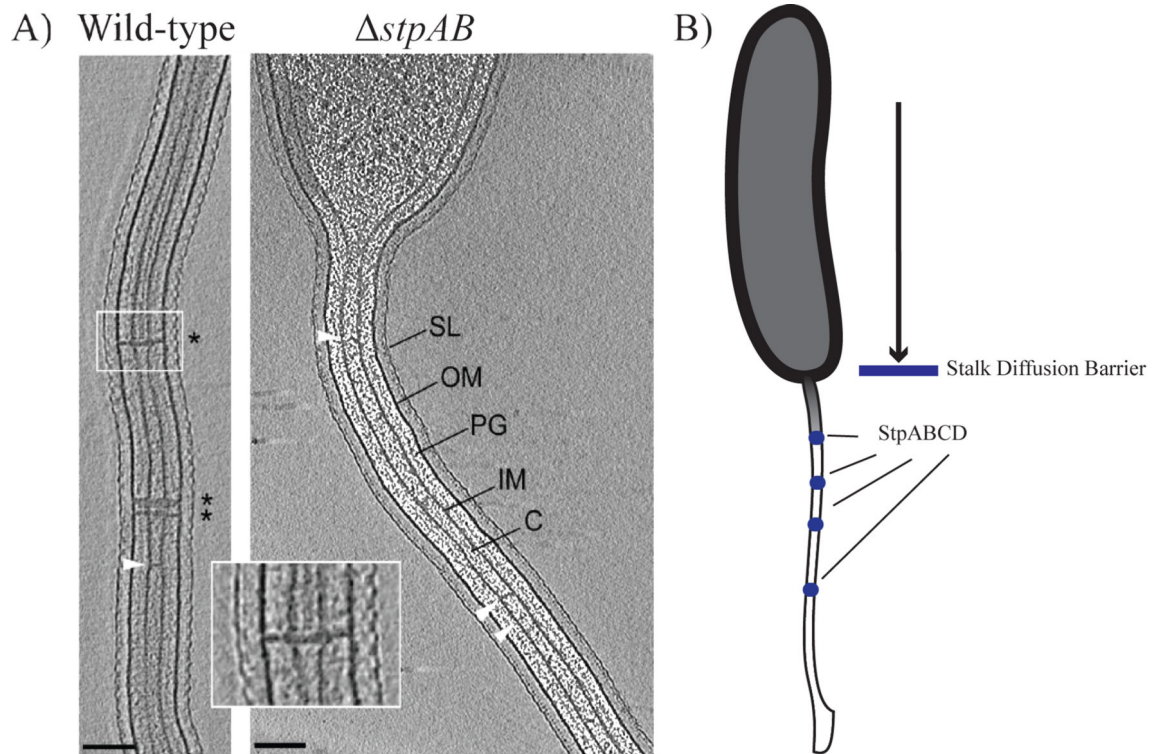
Figure 2.
A) Electron cryotomography images of wild-type *Caulobacter crescentus* cells containing stalk diffusion barriers (* and inset). stpAB cells lack these structures. White arrows indicate unidentified structures that span the interior of the stalk (SL= S layer, OM= outer membrane, PG= peptidoglycan, IM= inner membrane, C= cytoplasm). Scale bar 100 nm. Reprinted from [Schlimpert et al., *Cell*, 2011] with permission from Elsevier. B) Stalk diffusion barriers: Comprising a protein complex of StpA, StpB, StpC and StpD (blue circles), diffusion barriers limit soluble and membrane protein diffusion into the stalk of *C. crescentus*.
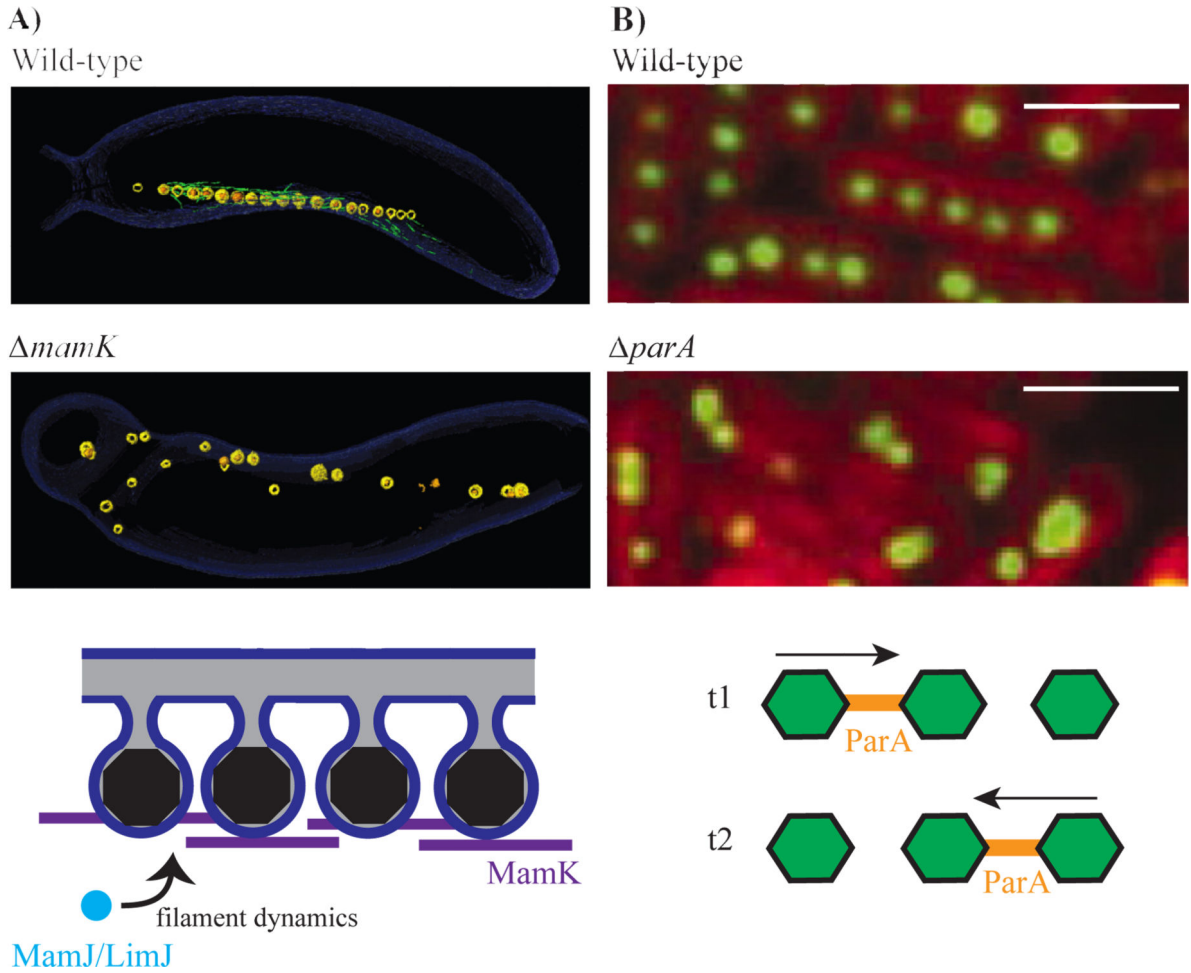
Figure 3.
Organization of bacterial organelles by cytoskeletal filaments. A) The bacterial actin protein MamK is responsible for alignment of magnetosomes into a chain in Magentotactic Bacteria. Reconstructed electron cryotomography images of wild-type *Magnetospirillum magneticum* AMB-1 with an aligned magnetosome chain (top panel) and *mamK* cells with disorganized magnetosomes (middle panel). The magnetosome (yellow) chain contains iron-based crystals (orange) and is flanked by filamentous structures (green) that disappear in the *mamK* mutant. MamK dynamic filament behavior is influenced by the presence of MamJ and LimJ (bottome panel). The precise mechanisms that govern this process remain elusive. From [Komeili et al. Sceince, 2006]. Reprinted with permission from AAAS. B) Alignment and segregation of carboxysomes in cyanobacteria is dependent on ParA, another bacterial cytoskeletal protein. Carboxysomes are evenly distributed throughout the cell in wild-type *Synechococcus elongatus* PCC 7942 (top panel), but not in a mutant lacking *parA* (middle panel). Rubisco protein (RbcL) fused to YFP indicates the localization of carboxysomes (green) and thylakoid membrane fluorescence is shown in red. ParA oscillates from pole to pole and is found distributed in between carboxysomes (bottom panel). T1 and T2 represent time points that that are roughly 30 minutes apart. From [Savage et al., *Science*, 2010]. Reprinted with permission from AAAS. Scale bar 2 μm.