Cell Biology of Prokaryotic Organelles

Dorothee Murat, Meghan Byrne, and Arash Komeili

Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, California 94720-3102

Correspondence: komeili@berkeley.edu

Mounting evidence in recent years has challenged the dogma that prokaryotes are simple and undefined cells devoid of an organized subcellular architecture. In fact, proteins once thought to be the purely eukaryotic inventions, including relatives of actin and tubulin control prokaryotic cell shape, DNA segregation, and cytokinesis. Similarly, compartmentalization, commonly noted as a distinguishing feature of eukaryotic cells, is also prevalent in the prokaryotic world in the form of protein-bounded and lipid-bounded organelles. In this article we highlight some of these prokaryotic organelles and discuss the current knowledge on their ultrastructure and the molecular mechanisms of their biogenesis and maintenance.

The emergence of eukaryotes in a world dominated by prokaryotes is one of the defining moments in the evolution of modern day organisms. Although it is clear that the central metabolic and information processing machineries of eukaryotes and prokaryotes share a common ancestry, the origins of the complex eukaryotic cell plan remain mysterious. Eukaryotic cells are typified by the presence of intracellular organelles that compartmentalize essential biochemical reactions whereas their prokaryotic counterparts generally lack such sophisticated subspecialization of the cytoplasmic space. In most cases, this textbook categorization of eukaryotes and prokaryotes holds true. However, decades of research have shown that a number of unique and diverse organelles can be found in the prokaryotic world raising the possibility that the ability to form organelles may have existed before the divergence of eukaryotes from prokaryotes (Shively 2006).

Skeptical readers might wonder if a prokaryotic structure can really be defined as an organelle. Here we categorize any compartment bounded by a biological membrane with a dedicated biochemical function as an organelle. This simple and broad definition presents cells, be they eukaryotes or prokaryotes, with a similar set of challenges that need to be addressed to successfully build an intracellular compartment. First, an organism needs to mold a cellular membrane into a desired shape and size. Next, the compartment must be populated with the proper set of proteins that carry out the activity of the organelle. Finally, the cell must ensure the proper localization, maintenance and segregation of these compartments across the cell cycle. Eukaryotic cells perform these difficult mechanistic steps using dedicated molecular pathways. Thus, if connections exist between prokaryotic and eukaryotic organelles it seems likely that relatives of these molecules may be involved in

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the biogenesis and maintenance of prokaryotic organelles as well.

Prokaryotic organelles can be generally divided into two major groups based on the composition of the membrane layer surrounding them. First are the cellular structures bounded by a nonunit membrane such a protein shell or a lipid monolayer (Shively 2006). Well-known examples of these compartments include lipid bodies, polyhydroxy butyrate granules, carboxysomes, and gas vacuoles. The second class consists of those organelles that are surrounded by a lipid-bilayer membrane, an arrangement that is reminiscent of the canonical organelles of the eukaryotic endomembrane system. Therefore, this article is dedicated to a detailed exploration of three prokaryotic lipid-bilayer bounded organelle systems: the magnetosomes of magnetotactic bacteria, photosynthetic membranes, and the internal membrane structures of the *Planctomycetes*. In each case, we present the most recent findings on the ultrastructure of these organelles and highlight the molecular mechanisms that control their formation, dynamics, and segregation. We also highlight some protein-bounded compartments to present the reader with a more complete view of prokaryotic compartmentalization.

Magnetosomes: Bacterial Compasses

The **magnetosomes** of magnetotactic bacteria (MB) are one of the most fascinating prokaryotic compartments (Fig. 1). MB are a phylogenetically diverse group of microorganisms with the ability to use geomagnetic field lines as guides in their search for their preferred redox conditions (Bazylinski and Frankel 2004; Komeili 2007). This behavior is achieved through the use of a unique magnetic organelle termed the magnetosome. A magnetosome consists of a lipid bilayer membrane that houses an approximately 50-nanometer crystal of the magnetic mineral magnetite (Fe_3O_4) or greigite (Fe_3S_4). Individual magnetosomes are arranged into one or more chains within the cell where they act passively to orient the bacterium within a magnetic field. The unusual properties of these magnetic minerals and their potential to be exploited

in a variety of applied settings have made them center of most studies on magnetosomes (Bazylinski and Frankel 2004).

From a cell biological perspective, however, it is the often-neglected magnetosome membrane that may hold the key to understanding fundamental properties of prokaryotic organelles. Detailed electron microscopic (EM) work and biochemical studies have shown that the magnetosome membrane has the cytological and chemical properties of a lipid bilayer membrane (Gorby et al. 1988; Grünberg et al. 2004). Additionally, numerous proteomic studies have shown that this compartment contains a unique mix of soluble and transmembrane domain-containing proteins, implying the existence of a dedicated protein sorting pathway (Okuda et al. 1996; Grünberg et al. 2001; Grünberg et al. 2004; Tanaka et al. 2006). The magnetosome membrane loaded with its protein cohort is present before crystal formation and serves as the site of biomineralization further confirming that it is an independent organelle (Komeili et al. 2004). The organization of magnetosomes into one or multiple chains also suggests that mechanisms must exist for the proper localization and division of this structure within the cell. This already detailed view of the magnetosome has been pushed to the next level with two recent imaging studies that describe the use of cryo-electron tomography (CET) to obtain high resolution three-dimensional images of MB (Komeili et al. 2006; Scheffel et al. 2006). In CET a series of two-dimensional images of a specimen, taken by tilting the stage of an electron microscope at various angles relative to the electron beam, is translated into a three-dimensional image using a specific algorithm. This technique provides such a detailed view of a cell that disruptive fixing and staining treatments common in other EM techniques are not needed. As a result one can prepare a sample by a simple rapid freezing method and subsequently image a cell at high resolution in a near-native state (Milne and Subramaniam 2009). This combination of rapid preservation, minimal disruption of cellular features, and nanometer scale resolution revealed features of magnetosomes that had not been visualized in

Figure 1. Magnetosomes can be easily visualized with various forms of electron microscopy. The electron-dense magnetite crystals are seen as a chain running through the cell in (A). Cryo-electron tomography was instrumental in demonstrating that the magnetosome membrane is an invagination of the inner cell membrane (B) and cytoskeletal filaments surround the magnetosome chain (C). (A, Reprinted, with permission from Komeili et al. 2004 [© National Academy of Sciences]; B, reprinted with permission from Komeili et al. 2006 [© AAAS]; C, image courtesy of Zhuo Li and Grant Jensen.)

more than 30 years of work on MB. Most striking was the finding that in *Magnetospirillum magneticum* AMB-1, individual magnetosomes are not separated into vesicles and are instead invaginations of the inner cell membrane (Fig. 1B). This state was observed in empty magnetosomes as well as those that contained fully formed

crystals implying that this organelle is an invagination of the inner membrane at all times (Komeili et al. 2006). Although such an organization might seem puzzling at first it does make sense in the context of magnetosome function and magnetite biomineralization. Because the primary job of the magnetosome chain is to

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orient the cell in external magnetic fields the organelle must be attached to the rest of the cell and by integrating the magnetosome into the cell membrane no additional machinery is needed to achieve proper orientation in magnetic fields. It has also been hypothesized that the biomineralization of magnetite may involve the formation of precursor minerals such as ferrihydrite in the periplasmic space (Frankel et al. 1983). In such a case the small opening between the magnetosome lumen and the periplasm would provide a simple path for the transport of these precursor minerals. The CET imaging of *Magnetospirillum gryphiswaldense* MSR-1, an organism closely related to AMB-1, did not specifically explore the existence of any connections between the magnetosome membrane and the inner cell membrane (Scheffel et al. 2006). However, in this organism the magnetosomes were found juxtaposed against the cell membrane consistent with the possibility that they are also invaginations of the inner cell membrane (Scheffel et al. 2006). These tremendous imaging studies have revealed the organization and ultrastructure of the magnetosome at nanometer scales and recent studies are beginning to define the molecular basis of magnetosome formation and organization.

MB are fastidious and slow growing organisms but offer multiple advantages as model systems for the molecular study of organelle formation in prokaryotes. Multiple MB genomes have been sequenced in the past few years, magnetosomes can be readily purified from cell extracts using simple magnetic columns and most importantly, magnetosomes are not essential for cell survival under laboratory growth conditions, opening the door to the use of genetics as a tool for uncovering the steps involved in magnetosome formation. A combination of these approaches has led to the identification of a large list of genes thought to be involved in the formation and function of magnetosomes. Surprisingly, most of these genes are organized into a coherent and unstable genomic region whose core components are conserved across multiple species of magnetotactic bacteria (Ullrich et al. 2005; Fukuda et al. 2006; Richter et al. 2007; Jogler et al. 2009). This region, termed the

magnetosome island or MAI, carries signature features of other genomic islands found in bacteria and encompasses a substantial portion of the genome. For instance, in AMB-1 the MAI is predicted to contain over one hundred genes accounting for approximately 2% of the organism's gene content (Fukuda et al. 2006). From an evolutionary perspective the organization of core magnetosome genes into an unstable genomic segment implies that the appearance of this organelle in diverse bacterial species was accomplished through lateral transfer of the MAI (Jogler et al. 2009). What makes the MAI intriguing to cell biologists is the possibility that it contains the unique functions required to build a magnetosome. Biochemical and genetic studies have shown that a number of MAI genes encode proteins that can influence the size and morphology of magnetite crystals (Arakaki et al. 2003; Scheffel et al. 2008; Murat et al. 2010). Other factors, such as the MamA protein, appear to function in activating or priming preformed magnetosomes for biomineralization (Komeili et al. 2004). And, as described later, one core region of the MAI is essential for the formation of the magnetosome membrane, protein sorting to this organelle and its specific localization within the cell (Komeili et al. 2006; Scheffel et al. 2006; Murat et al. 2010).

At the heart of the MAI is the *mamABE* operon, a gene cluster conserved in multiple species of MB. A comprehensive genetic analysis of the MAI showed that in the absence of the *mamABE* operon, AMB-1 is nonmagnetic and fails to even form empty magnetosome membranes (Murat et al. 2010). An analysis of individual deletions of each of the 18 genes of this cluster revealed a range of mutant phenotypes with defects at every step of magnetosome formation. Interestingly, four genes, *mamI*, *mamL*, *mamQ* and *mamB*, seem to be essential for the formation of the magnetosome membrane (Murat et al. 2010). None of these genes encodes for proteins with homology to known membrane deformation factors found in eukaryotes. However, they contain intriguing features that may hint at a potential mechanism for magnetosome formation. MamB and MamQ share homology with large families of membrane

proteins whereas MamI and MamL are unique to MB. These two latter proteins are small (~70 amino acid) polypeptides with two predicted transmembrane domains. MamI does not possess any distinguishing structural features but MamL contains a cytoplasmic tail that is rich in positively charged residues. One potential model for membrane deformation is that this tail interacts with one leaflet of the inner cell membrane creating an asymmetry that favors the bending of the membrane. Another finding of this work is that membrane formation can be decoupled from the sorting of at least a subset of magnetosome proteins. When the putative protease, MamE, is absent, empty magnetosome membranes are still formed although a number of magnetosome proteins are mislocalized arguing for a step-wise assembly of this organelle (Murat et al. 2010).

One of the central genes of the *mamABE* operon, *mamK*, is homologous to the large and diverse family of bacterial actin-like proteins discovered in the last decade (Carballido-López 2006). When *mamK* is deleted in AMB-1, the resulting mutants are not defective in magnetosome membrane formation or biomineralization of magnetite. Instead, magnetosomes are no longer organized into chains and are spread out across the cell membrane (Komeili et al. 2006). The CET imaging studies of AMB-1 and MSR-1 had also discovered that the magnetosome chain is surrounded by a network of cytoskeletal filaments with dimensions similar to bacterial actin-like filaments (Komeili et al. 2006; Scheffel et al. 2006) (Fig. 1C). Interestingly, these filaments are no longer present when *mamK* is deleted (Komeili et al. 2006). Together with recent observations that MamK can form filaments in heterologous systems and in vitro, these results suggest that this actin-like protein constitutes the structural component of the magnetosome-specific cytoskeleton (Pradel et al. 2006; Taoka et al. 2007). MamJ, a highly acidic protein encoded by a gene directly upstream of *mamK*, seems to play a crucial role in the organization of magnetosome chain as well. When *mamJ* is deleted in MSR-1, the magnetosome chain collapses into a ball within the cell (Scheffel et al. 2006). In this mutant,

structures similar to the magnetosome-specific cytoskeleton can still be seen by CET but they are no longer associated with magnetosomes. Given that MamJ can associate with MamK in a bacterial two-hybrid system a simple and attractive model has been proposed whereby MamJ can anchor MamK to the magnetosome membrane allowing it to organize individual organelles into a chain (Scheffel et al. 2006; Scheffel and Schüller 2007). Taken together these results suggest that similar to eukaryotic cells, prokaryotes can take advantage of cytoskeletal elements to position and organize subcellular compartments.

As can be seen, progress in the study of magnetosomes has been rapid in the last few years and the incredible gains made from the ultrastructural characterization of this organelle are beginning to be matched with molecular studies. Yet, much work remains to be performed. Although the discovery and genetic analysis of the MAI provides a potential “parts list” for the magnetosome formation machinery, the specific mechanisms that control membrane biogenesis and protein sorting have yet to be defined. Moreover, even though the discovery of the MamK/MamJ system for chain formation is a breakthrough advance in this field, its mechanism of action remains elusive. A resolution of these key issues is necessary before evolutionary comparisons can be drawn between eukaryotic organelles and magnetosomes. The elucidation of these key cellular mechanisms will also provide new modes for exploitation of magnetosomes in a variety of applied settings.

Photosynthetic Membranes: Variations on a Theme

Photosynthetic membranes are perhaps the most thoroughly studied of all prokaryotic organelles (Fig. 2). They fall into three general categories and each has unique and intriguing characteristics that make it ideal for the study of membrane dynamics and intracellular organization in prokaryotes. The first category, historically referred to as **chromatophores**, contains the various **intracytoplasmic membrane** (ICM)

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Figure 2. Photosynthetic membranes were the first of bacterial organelles to be imaged with electron microscopy. (A) is an image from a 1967 imaging study of *Rhodospseudomonas palustris*. The photosynthetic membranes (Th) are arranged as ribbon-like structures that are clearly continuous with the inner cell membrane (CM) at the point indicated by the arrow. These features are revealed in three dimensions in a surface rendered reconstruction of *Rhodospseudomonas viridis* in (B). Thylakoid membranes of cyanobacteria (C) are arranged in several circular layers and display species-specific morphologies. In contrast with photosynthetic membranes of purple bacteria, thylakoids appear to be fully separated from the inner cell membrane. (A, Reprinted, with permission, from Tauschel and Drews 1967 [© Springer]; B, reprinted, with permission, from Konorty et al. 2008 [© Elsevier]; C, reprinted, with permission, from Nevo et al. 2007 [© Nature Publishing Group].)

structures that house the photosynthetic protein complexes of the purple photosynthetic bacteria. The second category consists of the numerous examples of **thylakoid** membrane compartments found in cyanobacteria. The **chlorosome** compartments of green photosynthetic bacteria constitute the third major category of bacterial photosynthetic compartment. All of these organelle systems act to maximize

the efficiency of photosynthesis by increasing the number of available photosynthetic protein complexes, maximizing the size of the light-exposed membrane surface and by providing an idealized subcellular environment for this vital reaction. However, despite their functional relatedness these organelles differ in fundamental ways that impact the mechanisms by which they are formed and maintained.

Chromatophores were first studied biochemically where it was shown that a defined fraction of cellular extract was capable of carrying out certain light-dependent reactions *in vitro*. Elegant EM imaging of different species of purple phototrophic bacteria showed the presence of extensive intracellular membranes with distinct and species-specific morphological characteristics (Oelze and Drews 1972). For instance, the photosynthetic membranes of *Rhodospseudomonas palustris* appear as neatly folded membrane stacks that are continuous with the cell membrane (Tauschel and Drews 1967) (Fig. 2A). In contrast, in the *Rhodobacteri* species these structures are spherical invaginations of the inner membrane where multiple bubbles are connected to one another. These early ultrastructural studies have recently been augmented with the CET imaging of *Rhodospseudomonas viridis*, an organism that forms membranes that are similar in morphology to those observed in *R. palustris* (Konorty et al. 2008) (Fig. 2B). The near-native state preserved by cryofixation reveals much of the same features observed in traditional electron microscopic imaging of the same organism. Membranes are folded in an accordion-like structure and they are invaginations of the inner membrane with a distinct 128 nm wide opening to the periplasmic space (Konorty et al. 2008). In many organisms, including *R. viridis*, chromatophores are produced only under photosynthetic growth conditions. The CET imaging of *R. viridis* at early time points after switch to photosynthetic growth reveals the presence of small vesicular structures adjacent to the cell membrane (Konorty et al. 2008). Presumably these early compartments eventually mature into the membrane stacks seen in cells grown under continuous photosynthetic conditions.

How are these exquisite and species-specific membrane morphologies generated? The answer appears to be a simple and elegant mechanism in which the inherent properties of photosynthetic protein complexes determine the resulting shape of the membrane. The chromatophores of purple bacteria such as *R. sphaeroides* house the major components of the photosynthetic machinery (Tavano and

Donohue 2006). These protein complexes include the light harvesting 2 (LH2) protein complex and the “core” complex consisting of the multimeric light harvesting 1 (LH1) and reaction center (RC) polypeptides. In some species, dimers of core complexes are formed through a linkage with the PufX protein. These protein complexes are first assembled in the inner cell membrane at sites that will invaginate to form chromatophores (Tavano and Donohue 2006). Genetic studies with *R. sphaeroides* have shown that in the absence of LH2 the chromatophore membranes lose their characteristic stacked spherical shape and instead turn into long tubules within the cell (Tavano and Donohue 2006). Interestingly, when *pufX* is deleted in these LH2-deficient strains the membrane tubules disappear and instead large spherical internal membranes are observed (Tavano and Donohue 2006). Thus, the major protein components of chromatophores play a decisive role in determining the morphology of the membrane. Recent structural and biophysical modeling studies of photosynthetic membrane proteins have built on these functional studies to provide a mechanistic basis for the remodeling of the cell membrane into chromatophores. When chromatophores are imaged by atomic force microscopy (AFM) ordered arrays of photosynthetic protein complexes are seen to densely pack the membrane surface (Sturgis et al. 2009). Based on the known structures of these complexes distinct domains containing dimers of the RC-LH1-PufX complex as well as rings of LH2 can be placed in the AFM images (Sturgis et al. 2009). Interestingly, three-dimensional electron microscopic reconstruction of negatively stained single particles reveals that the dimers of RC-LH1-PufX form a complex that is bent toward the lumen of the chromatophore (Qian et al. 2008). An *in silico* model of chromatophores based on this bent structure of the core complex predicts the formation of long membrane tubules with dimensions similar to that observed in mutants lacking LH2 (Qian et al. 2008). Using these functional and structural results as a guide, other modeling studies have also supported the hypothesis that the biophysical properties of photosynthetic

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proteins and their long-range interactions with each other would be sufficient to produce curved membrane structures in vivo (Chandler et al. 2008). Despite these convincing arguments in favor of a self-assembly model for chromatophore formation, it is possible that other factors may be involved in the process. For instance, a recent proteomic analysis of *R. sphaeroides* has shown that a number of proteins outside the major photosynthetic complexes may also be present within chromatophores raising the prospects that novel factors may have roles in the development of this organelle (Zeng et al. 2007).

The thylakoid membranes of cyanobacteria are the evolutionary precursors of chloroplasts. As with chromatophores these organelles are responsible for some of the central light-dependent reactions of photosynthesis. However, the morphology and subcellular arrangement of thylakoids is markedly different than that of chromatophores (Fig. 2C). Several recent CET studies have corroborated earlier EM studies of thylakoids and provided additional insights into the organization and species-specific diversity of this fascinating organelle (van de Meene et al. 2006; Nevo et al. 2007; Ting et al. 2007). In most cases thylakoids appear as several flattened and stacked layers of lipid-bilayer membrane that encircle the cell. The number of layers and the spacing between them follows a species-specific arrangement (Nevo et al. 2007). Although these layers cover much of the cytoplasmic space there is still substantial flow of cellular components in between the thylakoid stacks. This is because of the presence of numerous perforations within the thylakoid membrane and in CET images a number of macromolecules such as ribosomes and storage granules are seen within these openings (Nevo et al. 2007). The three-dimensional images provided by CET also reveal numerous bridges and fusions formed by membranes that traverse the different stacks of thylakoids (Nevo et al. 2007). Finally, large cytoplasmic vesicles are seen near and at times fused to the thylakoids. The highly networked nature of this membrane system suggests that long-range communication and transport may occur throughout the whole organelle

(Nevo et al. 2007). CET studies as well as other attempts to reconstruct the cellular arrangement of thylakoids have also revealed that, in contrast with the chromatophore membrane, the inner cell membrane and the thylakoid membrane are not continuous with each other (Liberton et al. 2006; van de Meene et al. 2006; Nevo et al. 2007; Ting et al. 2007). The lack of connections between thylakoids and the cell membranes has also been shown through the use of various fluorescent membrane dyes (Schneider et al. 2007). FM1-43 is a hydrophobic dye that fluoresces once incorporated into membranes and is thought to be incapable of diffusing past the inner cell membrane. When it is used to stain the cyanobacterium *Synechocystis* sp. PCC 6803 the inner membrane and outer membrane are labeled but the thylakoids do not incorporate the dye indicating that these membrane systems are separate entities or that a physical barrier prevents the migration of the dye to the thylakoids. In contrast when Mitotracker, a membrane dye that can diffuse past cellular membranes, is used as a marker all membranes including the thylakoids are stained in this organism. Long incubations with FM1-43 initially stain intracellular structures resembling vesicles and eventually highlight the thylakoid membranes indicating a mode for transfer of lipids and proteins from the cell membrane to this organelle (Schneider et al. 2007). Thus, similar to eukaryotes, cyanobacteria form membrane structures that are discontinuous from the cell membrane implying the presence of mechanisms for bending and fission of cellular membranes.

Given their evolutionary connections, one clue to the mechanisms of thylakoid membrane formation has come from examining the pathways of chloroplast biogenesis in plants. The vesicular inducing protein in plastid 1, Vipp1, is a protein implicated in membrane remodeling and vesicular trafficking in chloroplasts in *Arabidopsis* (Kroll et al. 2001). Cyanobacteria contain homologs of Vipp1 and its absence in *Synechocystis* results in the loss of stacks of thylakoid membranes (Westphal et al. 2001). These findings had suggested a possible role for Vipp1 in the biogenesis of thylakoid

membranes but a recent study suggests that this defect may have less to do with membrane biogenesis than it does with the assembly of photosynthetic complexes (Gao and Xu 2009). Using a repressible promoter, *Vipp1* was depleted to levels in which cells could no longer perform photosynthesis. Under these conditions the thylakoid membranes had a wild-type appearance suggesting that *Vipp1* may function at a step downstream of membrane biogenesis (Gao and Xu 2009). Another fascinating possibility has come from the observation that homologs of eukaryotic dynamin can be found in several species of cyanobacteria (Low and Lowe 2006). In eukaryotes dynamin and dynamin-like proteins are important for membrane fission and tubulation in processes ranging from endocytosis to cytokinesis (Praefcke and McMahon 2004). As with eukaryotic dynamins, the putative dynamin homolog found in cyanobacteria is also a GTPase, can bind liposomes *in vitro*, and localizes to cellular membranes *in vivo* (Low and Lowe 2006). More strikingly, the three-dimensional structure of prokaryotic dynamin is remarkably similar to that of eukaryotic dynamin (Low and Lowe 2006). Given these similarities in structure and biochemical activity it has been postulated that cyanobacterial dynamins may play a role in establishing the complex assemblies of thylakoid membranes (Low and Lowe 2006). However, dynamin-like proteins are not found in all cyanobacteria and in the strains where they do exist, no functional data exists to suggest that they have a dedicated role in thylakoid membrane biogenesis.

Chlorosomes are the largest light-harvesting systems found thus far in photosynthetic organisms, and they have been shown to allow cells to harvest light energy at extremely low light intensities (Frigaard and Bryant 2006). A striking example of this is a chlorosome-containing obligate phototroph that was found 2391 meters below the surface of the Pacific Ocean and thought to extract the energy necessary for growth from the infrared radiation of a geothermal vent (Beatty et al. 2005). Chlorosomes are found in all *Chlorobi* or green sulfur bacteria and some *Chloroflexi* or green filamentous anoxygenic phototrophs. Recently, chlorosomes

were discovered in an acidobacterium isolated from a microbial mat community in Yellowstone National Park making it the first photosynthetic bacterium that has been identified in the phylum *Acidobacteria* (Bryant et al. 2007).

Chlorosomes are flattened, ellipsoidal structures that are connected to the cytoplasmic membranes by a relatively thick baseplate (Fig. 4A). The chlorosome envelope is 3–5 nm thick and electron opaque, as seen by thin-layer transmission electron microscopy (Cohen-Bazire et al. 1964; Staehelin et al. 1980). This layer is thinner than the cytoplasmic membrane (8 nm), indicating it is not a lipid bilayer. However, lipids have been identified in purified chlorosomes, and the chlorosome envelope fractures in freeze-fracture electron microscopy in a manner characteristic of lipids, suggesting that the envelope is a lipid monolayer (Staehelin et al. 1980; Frigaard and Bryant 2006).

Chlorosomes primarily contain bacteriochlorophyll (BChl) c, d, or e, which can number 150,000–300,000 molecules in a single organelle. Ten proteins have been purified from *Chlorobium tepidum* chlorosomes, and all of them have been shown to be susceptible to cleavage by proteases, suggesting they are surface exposed. Antisera to these proteins can precipitate chlorosomes, further supporting the model that these proteins are in the chlorosome envelope (Chung and Bryant 1996; Vassilieva et al. 2002). A number of these envelope proteins show similarity with each other leading to the hypothesis that they perform redundant functions. This idea is supported by genetic studies in which individual deletions of 9 of the 10 chlorosome genes had virtually no effect on chlorosome structure or function (Frigaard et al. 2004). However, when double, triple and quadruple mutants were created in which combinations of genes predicted to be in the same family were deleted, dramatic phenotypes in the size and morphology of chlorosomes were uncovered suggesting that the protein content of the organelle determines its ultrastructural properties (Li and Bryant 2009). The 10th gene, *csmA*, has been proposed to act in the flow of energy from the antenna to the reaction center. Interestingly, in the aforementioned study *csmA*

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could not be deleted, suggesting that it is essential to the cells (Frigaard et al. 2004).

The discovery of chlorosome proteins and the directed functional studies detailed earlier are important steps in understanding the mechanism of chlorosome formation. The unique arrangement of lipids and envelope proteins suggests that this mechanism will be different than the one used to form other lipid-bounded organelles. To account for their architecture and composition a recent hypothesis suggests that a self-assembly process is responsible for the formation of chlorosomes (Hohmann-Marriott and Blankenship 2007). According to this model, bacteriochlorophylls and other pigment molecules accumulate in between the two leaflets of the inner membrane creating a growing bubble surrounded by a single lipid layer. In fact, when the gene encoding for bacteriochlorophyll synthase *c* was deleted in *Chlorobium tepidum* normal chlorosomes were not formed and instead smaller deflated structures containing other pigments were seen within the cell (Frigaard et al. 2002). Within this monolayer, glycosyl diacylglycerides are enriched because of their preferred interactions with the accumulated pigments. Finally, chlorosome proteins are recruited because of their preference for these chlorosome components. A combination of genetic and biochemical studies are now needed to directly test this simple self-assembly model for chlorosome biogenesis.

Planctomycete Membrane Compartments: True Ancestors of Eukaryotic Organelles?

The examples discussed thus far represent the broad spectrum of intracellular compartmentalization that can be found in the prokaryotic world. These structures, however, do not resemble the characteristic organelles that define the endomembrane system of eukaryotes making it difficult to draw any evolutionary parallels. The members of the *Planctomycetes*, a deep branching phylum of the Bacteria, however, may contain the bacterial ancestors of eukaryotic organelles. Most species of this phylum are characterized by extensive and truly unique compartmentalization of their cytoplasmic space

(Fuerst 2005). The simplest configuration is found in organisms such as those of the genus *Pirellula* in which a large lipid-bilayer bounded compartment contains and separates the chromosome and ribosomes from other cellular components. This organelle, termed the **pirel-lulosome**, is surrounded by a small area of cytoplasmic space known as the **paryphoplasm** (Fig. 3B). Unlike the periplasmic space of Gram-negative bacteria macromolecules such as RNA can be found in the paryphoplasm (Lindsay et al. 1997).

In some *Planctomycetes*, more complicated forms of compartmentalization have been observed in which the pirellosome is further subdivided into smaller and more specialized compartments. The most dramatic example is found in species such as *Gemmata obscuriglobus* in which a compacted chromosome is surrounded by a double lipid-bilayer membrane to form a nuclear body (Lindsay et al. 2001) (Fig. 3A). Ribosomes are found both within the nuclear body and throughout the rest of the pirellosome indicating that some translational activity may be separated from transcription. The unusual membrane architecture and the partial separation of transcription from translation are reminiscent of the eukaryotic nucleus thus raising the possibility that the *Planctomycetes* may represent the early forms of compartmentalization that has come to define the eukaryotes. This arrangement also implies that communication and transport of macromolecules must occur between the various compartments of *G. obscuriglobus*. Although molecular pathways and evidence for such transport have not been found, microscopic examination has revealed that the folding of the lipid bilayer membrane surrounding the nuclear body creates a small opening that may be a portal for transport of macromolecules (Lindsay et al. 2001). Time-lapse microscopy experiments have also helped to elucidate the steps involved in the segregation of nuclei and biogenesis of organelles during cell division. Many of the *Planctomycetes*, including *G. obscuriglobus*, divide by budding rather than the binary fission mechanism often seen in bacteria (Lee et al. 2009). During early

Figure 3. The nucleus-like organelle of *Gemmata obscuriglobus* is shown in (A). The nuclear envelope (E) is a double lipid-bilayer membrane containing the chromosome (N). The inset highlights the intracytoplasmic membrane (ICM) that separates the riboplasm from the paryphoplasm (P) compartment. A simpler organization is seen in organisms such as *Pirellula marina* in which the intracytoplasmic membrane (ICM) differentiates the pir-ellulosome (PI) from the paryphoplasm (P) (B). Many of the *Planctomycetes* contain another unique organelle called the anammoxosome (C). Here a CET reconstruction of *Brocadia fulgida* is shown. The anammoxosome is the central compartment of this cell and iron particles (red) are found within it. (A, B, Reprinted, with permission, from Lindsay et al. 2001 [© Springer]; C, reprinted, with permission, from Niftrik et al. 2008a [© ASM].)

stages of the budding process the newly divided nucleoid unbound by any membranes can be seen in a relatively young bud. As the bud grows a complex migration of the mother cell inner membrane and the daughter cell inner membrane are followed by membrane fusion events

to create the new nuclear envelope (Lee et al. 2009). At present little is known about the molecular mechanisms of organelle formation in these organisms and the studies of this fascinating topic are hampered by a lack of robust genetic tools. However, recent sequencing of

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several *Planctomycete* genomes may help in identification of novel gene products with a unique role in organelle assembly and dynamics (Studholme et al. 2004; Staley et al. 2005). One such clue has emerged from the genome of *Gemmata* Wa-1 in which a homolog of the eukaryotic Gle2 protein, a component of the nuclear pore complex, has been discovered (Staley et al. 2005). A recent study conducted a more directed search for bacterial proteins that contain signatures of eukaryotic membrane coat proteins, which play key roles in vesicle trafficking and organelle maintenance in eukaryotes (Santarella-Mellwig et al. 2010). These proteins are typified by an unusual combination of structural domains where a specialized arrangement of β -sheets, called a β -propeller, is followed by an α -helical structure termed an α solenoid. These proteins are ubiquitous among the eukaryotes but when the genomes of all sequenced bacteria were queried only species within the *Planctomycete-Verrucomicrobia-Chlamydiae* phyla contained genes encoding for eukaryotic coatlike proteins. Interestingly one of these candidates found in *G. obscuriglobus* was seen to localize to the organism's internal membrane structures (Santarella-Mellwig et al. 2010). These results provide molecular evidence for the possible ancestral link between *Planctomycete* compartments and eukaryotic organelles.

Other species of the *Planctomycetes* have an additional membrane-bound compartment called the **anammoxosome** capable of anaerobic ammonium oxidation (Strous et al. 1999) (Fig. 3C). For decades, this anammox reaction had been hypothesized to exist based on thermodynamic calculations but had never been associated with a living organism (Broda 1977). The anammoxosome is located within the pirellulosome and it is the only *Planctomycete* organelle that can be purified, which has facilitated its study (Lindsay et al. 2001). Among the proteins found in the anammoxosome membrane is hydroxylamine oxidoreductase, a unique enzyme that catalyzes ammonium oxidation (Schalk et al. 2000). Analysis of the anammoxosome composition has also revealed that its membrane is enriched in an unusual type of concatenated lipids, never before found in

nature (Sinninghe Damsté et al. 2002). These molecules, termed ladderane lipids form a denser and more impermeable barrier than regular biological membranes that may prevent the diffusion of the toxic intermediates produced during the anammox reaction. The diffusion barrier provided by this organelle is also thought to help in retaining the intermediates of the slow anammox reaction within the cell (Sinninghe Damsté et al. 2002). A recent study of this organelle by CET has revealed that its membrane is highly curved leading to the proposal that the curvature could optimize the membrane surface and thus the membrane-associated metabolic processes that happen in the anammoxosome (van Niftrik et al. 2008a; van Niftrik et al. 2008b). Some anammox bacteria are also distinguished by their unique mode of cell and organelle division. In *Kuenenia stuttgartiensis* cell division follows the typical binary fission mode observed in other bacteria (van Niftrik et al. 2009). As a result, the anammoxosome is divided in half during each division cycle and segregated equally among the two daughter cells. EM and CET imaging reveal the presence of a distinct cytokinetic ring apparatus in the outermost compartment of this organism. Most bacteria use the tubulin-like protein FtsZ to form a division ring but the genome of *K. stuttgartiensis* is devoid of any homolog to *ftsZ*. Instead, another GTPase, named *kustd1438*, was found to specifically localize to the cytokinetic ring of this organism (van Niftrik et al. 2009). The observation that *kust1438* homologs are not found outside of the anammox bacteria also hints at its unique and important function in this process. However, further functional studies are required to determine a direct role for this protein in cell division and organelle partitioning. Ultimately, development of robust genetic systems will help to further define the molecular mechanisms of organelle formation in the *Planctomycetes*.

PROTEIN-BOUNDED COMPARTMENTS

Carboxysomes are one of the best-known examples of protein-bounded organelles in bacteria (Yeates et al. 2008). They occur in all

cyanobacteria as well as chemoautolithotrophs where they serve as the site for the first step of the Calvin cycle. The major catalytic components of carboxysomes are the enzymes Ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) and carbonic anhydrase. RuBisCO catalyzes the reaction of CO₂ with ribulose biphosphate to two molecules of 3-phosphoglyceric acid (3PGA) and carbonic anhydrase catalyzes the conversion of bicarbonate to CO₂. By increasing the local concentration of RuBisCO and the CO₂ substrate, carboxysomes are likely increasing the efficiency of the productive carbon fixation reaction (Yeates et al. 2008). This idea is supported by recent electron cryotomography studies, which show that each carboxysome (measuring 80 to 150 nm) contains over 200 RuBisCO enzyme complexes arranged in concentric layers (Schmid et al. 2006; Iancu et al. 2007) (Fig. 4B).

Only a few genes, found in one or more operons, are involved in the formation of carboxysomes. In *Halothiobacillus neopolitans*, the carboxysome genes encode for the large and small RuBisCO subunits, three small shell proteins that share high homology, a large shell protein, carbonic anhydrase, and two unknown proteins that seem to have a regulatory function. Other bacteria that form carboxysomes have slightly different genes in their operons, but all contain homologs of the small shell protein genes and genes that encode for the RuBisCO subunits. Recently, small shell proteins from both a cyanobacterium and a chemolithoautotrophic bacterium have been crystallized, which has provided valuable insights into how the protein shell of the carboxysomes may assemble (Kerfeld et al. 2005; Tsai et al. 2007). These crystal structures reveal that the proteins, purified individually, self-assemble into hexamers that bind edge-to-edge to form monolayer sheets. These sheets of protein have been proposed to make up the walls of the carboxysome. The crystal structures also revealed a positively charged pore at the center of the hexamers. This pore could allow for the passage of negatively charged molecules such as bicarbonate while blocking the entrance of O₂ creating another way in which the carboxysome could

increase the efficiency of RuBisCO and the fixation of carbon.

The defined set of proteins found in these operons is likely to be the minimal components required to build a carboxysome. However, recent results show that the proper organization and segregation of carboxysomes across the cell cycle require it to interface with other cellular components (Savage et al. 2010). Fluorescent protein fusions to either a shell protein or to a RuBisCO component revealed that carboxysomes are linearly spaced throughout the cell. The most relevant consequence of this arrangement is that during cell division approximately equal numbers of carboxysomes will be partitioned to each daughter cell (Savage et al. 2010). This arrangement relies on cytoskeletal systems as disruptions of either *mreB* (a bacterial actin-like protein) or *parA* lead to a disorganization of carboxysomes within the cell. In the *parA* mutants, some daughter cells do not receive any carboxysomes meaning that they have to build their carbon fixation machinery de novo, which in turn causes a significant lengthening of their doubling times (Savage et al. 2010). This fascinating study establishes a clear link between the cytoskeleton and carboxysome organization. However, the specific connections between this organelle and ParA, as well as the mechanisms by which the proper spacing of carboxysomes is achieved remain to be elucidated.

Carboxysomes are actually part of a larger family of protein-bounded compartments, which are all related through homology between their shell proteins. One such organelle is the **1,2-propanediol use (Pdu) compartment** found in *Salmonella enterica*. Similar to carboxysomes, Pdu compartments house specific enzymes that are important for their cellular function. Interestingly, a recent report has shown that these enzymes all share a 20 amino acid amino-terminal sequence that is necessary for their packaging within the Pdu compartment (Fan et al. 2010). Furthermore, these amino-terminal sequences are also sufficient to target heterologous proteins such as GFP to Pdu compartments. Such amino-terminal sequence extensions were also detected in enzymes thought to be associated with other

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Figure 4. Chlorosomes of *Chlorobium tepidum* appear as flattened ovals arranged around the cell periphery (A). A representation of a single carboxysome based on CET imaging. The interior of the carboxysome appears to be packed with RuBisCO based on similarities between the known crystal structure of the enzyme and electron-dense entities seen in CET reconstructions (B). A TEM image of a cyanobacterial cell reveals that the cytoplasmic space is filled with gas vesicles sectioned in two different orientations (C). (A, Reprinted, with permission, from Frigaard et al. 2002 [© ASM]; B, reprinted, with permission, from Iancu et al. 2007 [© Elsevier]; C, reprinted, with permission, from Walsby 1994 [© ASM].)

microcompartments making it likely that this mode of protein localization is universal among protein-bounded organelles (Fan et al. 2010). Beyond their relevance to understanding the cell biology of organelles, this finding also provides a method for engineering protein compartments in bacteria through the specific targeting of heterologous enzymes.

Another unique protein-bounded organelle in bacteria is the **gas vesicle** (Fig. 4C). Gas vesicles are gas-filled, protein-bound organelles that function to modulate the buoyancy of cells (Walsby 1994). They are found in a number of bacteria and archaea including halophilic and methanogenic archaea and phototrophic and heterotrophic bacteria. Most bacteria and archaea that have been shown to form gas vesicles are found in aqueous environments and are non-motile. The proteinaceous walls of gas vesicles are freely permeable to gas molecules. Water is also able to enter the gas vesicles but cannot form droplets on the inner surface because of its highly hydrophobic nature. Thus, any water that enters the gas vesicles evaporates (Walsby 1994), and the gas-filled vesicles decrease the overall density of the cells, allowing them to float upward. By controlling the formation of the gas vesicles, these organisms can specify their position in the water column to regulate

their exposure to light, salt, nutrients and other environmental stimuli. Gas vesicles are cylindrical or spindle-shaped and the size of gas vesicles varies between species. Cells that grow at greater depths have gas vesicles that are narrower in width and are able to withstand greater hydrostatic pressure.

Ten to fourteen gas vesicle protein (*gvp*) genes, depending on the species, have been identified as being involved in gas vesicle formation. In *Halobacterium halobium*, at least ten *gvp* genes were found to be required for gas vesicle formation (DasSarma et al. 1994), and eight *gvp* genes in the halophilic archaeon *Halobacterium salinarum* are necessary and sufficient for gas vesicle formation (Offner et al. 2000). One of the essential genes encodes GvpA, the main vesicle wall component and one of the most hydrophobic proteins known. The crystal structure of GvpA has not been solved, mainly because GvpA aggregates and cannot be dissolved without denaturation. Nonetheless, the structure of gas vesicles has been investigated by X-ray analysis and atomic force microscopy (Blaurock and Walsby 1976; Blaurock and Wober 1976; McMaster et al. 1996), which revealed that the proteins form very ordered ribs and that the protein subunits align at an angle of 54° to the rib axis. Interestingly, 54° is

close to the angle at which transverse and longitudinal stresses are equal in the wall of a cylindrical structure (Walsby 1994).

Much work has been performed to understand the physical properties of gas vesicles including their structure, their ability to withstand hydrostatic pressure, their ability to exclude water, and their permeability to gas. However, how the gas vesicle proteins interact to form the gas vesicles, and how gas vesicle formation is regulated in response to environmental cues remains largely unknown. Finally, it is possible that gas vesicle-like structures are found in other bacteria that exist in nonaqueous environments. Homologs of gas vesicle genes have also been found in actinomycetes that live in the soil yet no gas vesicle-like structures and no buoyancy phenotype has been seen (van Keulen et al. 2005).

CONCLUDING REMARKS

In conclusion, compartmentalization is not a feature limited to the eukaryotic world and numerous examples of highly complex and dynamic organelle systems can be found among the prokaryotes. The limited knowledge of the molecular mechanisms that control the biogenesis of these prokaryotic organelles does not allow for a direct mechanistic and evolutionary comparison to their well-studied eukaryotic counterparts. In many cases attempts to study prokaryotic organelles are hampered by their small size and a lack of molecular and genetic tools. With the advent of high-resolution imaging systems such as CET and the availability of numerous genome sequences, some of the barriers to the study of prokaryotic organelle biology are beginning to fade. Although the molecular understanding of organelle formation in prokaryotes is still at a relatively immature stage some general rules can be seen in the recent findings detailed in this article. First, it is clear that the proteins that can influence organelle formation are unique to each of the organelle systems discussed here. MamI and MamL are found only within the magnetotactic bacteria, the putative eukaryotic-like proteins found in the *Planctomycetes* are also unique to

a limited group of bacteria and photosynthetic membranes are formed through a self-assembly mechanism using the photosynthetic proteins. These findings may imply that among bacteria membrane-bounded organelles evolved multiple times independently. Second, self-assembly may be a common mode of organelle biogenesis in both lipid-bounded organelles such as photosynthetic membranes and chlorosomes and protein-bounded compartments such as carboxysomes. At the moment, the restrictions placed on the cell by this mode of organelle formation remain unknown. For instance, can newly synthesized enzymes still be targeted to carboxysomes after the shell has closed? There are clear exceptions to this rule as well. In the case of magnetosomes, a large number of magnetosome proteins can be eliminated and yet the initial stages of membrane formation can still occur. Finally, cytoskeletal elements are used in multiple divergent systems as a means to organize and divide organelles. The magnetosomes of magnetotactic bacteria and the protein-bounded carboxysomes both require cytoskeletal proteins for accurate placement in the cell, which aids in proper function and segregation of these organelles during division.

Beyond the establishment of model systems and robust tools, a change in perspective may also be needed to move the understanding of these organelles to the next level. For decades the major focus of research in the study of prokaryotic organelles has been to uncover the enzymatic basis of their function and to take advantage of the biochemical products of these reactions for applied purposes. We would like to suggest that a dedicated focus on the cell biology of these organelles is needed to move forward. The approach should be similar to that taken by cell biologists studying organelle formation in eukaryotes in which experiments are focused on understanding the mechanisms that allow for membrane bending, protein sorting, and organelle division. By defining the cellular basis for organelle formation in prokaryotes we may then be able to directly tackle the evolutionary basis of compartmentalization across the various domains of life. Furthermore, this avenue of research will shed light on the general

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mechanisms used by prokaryotes to build large macromolecular assemblies and organize their cytoplasmic space. Finally, understanding the cell biology of prokaryotic organelles will allow for a more rational approach to their re-engineering in biotechnological and biomedical applications.

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REFERENCES

- Arakaki A, Webb J, Matsunaga T. 2003. A novel protein tightly bound to bacterial magnetic particles in *Magnetospirillum magneticum* strain AMB-1. *J Biol Chem* **278**: 8745–8750.
- Bazylinski DA, Frankel RB. 2004. Magnetosome formation in prokaryotes. *Nat Rev Micro* **2**: 217–230.
- Beatty JT, Overmann J, Lince MT, Manske AK, Lang AS, Blankenship RE, Van Dover CL, Martinson TA, Plumley FG. 2005. An obligately photosynthetic bacterial anaerobe from a deep-sea hydrothermal vent. *Proc Natl Acad Sci* **102**: 9306–9310.
- Blaurock AE, Walsby AE. 1976. Crystalline structure of the gas vesicle wall from *Anabaena flos-aquae*. *J Mol Biol* **105**: 183–199.
- Blaurock AE, Wöber W. 1976. Structure of the wall of *Halobacterium halobium* gas vesicles. *J Mol Biol* **106**: 871–878.
- Broda E. 1977. Two kinds of lithotrophs missing in nature. *Zeitschrift für allgemeine Mikrobiologie* **17**: 491–493.
- Bryant DA, Costas AM, Maresca JA, Chew AG, Klatt CG, Bateson MM, Tallon LJ, Hostettler J, Nelson WC, Heidelberg JF, et al. 2007. *Candidatus Chloracidobacterium thermophilum*: An aerobic phototrophic *Acidobacterium*. *Science* **317**: 523–526.
- Carballido-López R. 2006. The bacterial actin-like cytoskeleton. *Microbiol Mol Biol Rev* **70**: 888–909.
- Chandler DE, Hsin J, Harrison CB, Gumbart J, Schulten K. 2008. Intrinsic curvature properties of photosynthetic proteins in chromatophores. *Biophys J* **95**: 2822–2836.
- Chung S, Bryant DA. 1996. Characterization of csmB genes, encoding a 7.5-kDa protein of the chlorosome envelope, from the green sulfur bacteria *Chlorobium vibrioforme* 8327D and *Chlorobium tepidum*. *Arch Microbiol* **166**: 234–244.
- Cohen-Bazire G, Pfennig N, Kunisawa R. 1964. The fine structure of green bacteria. *J Cell Biol* **22**: 207–225.
- DasSarma S, Arora P, Lin F, Molinari E, Yin LR. 1994. Wild-type gas vesicle formation requires at least ten genes in the gvp gene cluster of *Halobacterium halobium* plasmid pNRC100. *J Bacteriol* **176**: 7646–7652.

- Fan C, Cheng S, Liu Y, Escobar CM, Crowley CS, Jefferson RE, Yeates TO, Bobik TA. 2010. Short N-terminal sequences package proteins into bacterial microcompartments. *Proc Natl Acad Sci* **107**: 7509–7514.
- Frankel RB, Papaefthymiou GC, Blakemore RP, Obrien W. 1983. Fe₃O₄ precipitation in magnetotactic bacteria. *Biochim Biophys Acta* **763**: 147–159.
- Frigaard NU, Bryant DA. 2006. Chlorosomes: antenna organelles in green photosynthetic bacteria. In *Complex intracellular structures in prokaryotes (Microbiology Monographs)* (ed. JM Shively), pp. 79–114. Springer.
- Frigaard NU, Voigt GD, Bryant DA. 2002. Chlorobium tepidum mutant lacking bacteriochlorophyll c made by inactivation of the bchK gene, encoding bacteriochlorophyll c synthase. *J Bacteriol* **184**: 3368–3376.
- Frigaard NU, Li H, Milks KJ, Bryant DA. 2004. Nine mutants of *Chlorobium tepidum* each unable to synthesize a different chlorosome protein still assemble functional chlorosomes. *J Bacteriol* **186**: 646–653.
- Fuerst JA. 2005. Intracellular compartmentation in planctomycetes. *Annu Rev Microbiol* **59**: 299–328.
- Fukuda Y, Okamura Y, Takeyama H, Matsunaga T. 2006. Dynamic analysis of a genomic island in *Magnetospirillum* sp. strain AMB-1 reveals how magnetosome synthesis developed. *FEBS Lett* **580**: 801–812.
- Gao H, Xu X. 2009. Depletion of Vipp1 in *Synechocystis* sp. PCC 6803 affects photosynthetic activity before the loss of thylakoid membranes. *FEMS Microbiol Lett* **292**: 63–70.
- Gorby YA, Beveridge TJ, Blakemore RP. 1988. Characterization of the bacterial magnetosome membrane. *J Bacteriol* **170**: 834–841.
- Grünberg K, Müller EC, Otto A, Reszka R, Linder D, Kube M, Reinhardt R, Schüler D. 2004. Biochemical and proteomic analysis of the magnetosome membrane in *Magnetospirillum gryphiswaldense*. *Appl Environ Microbiol* **70**: 1040–1050.
- Grünberg K, Wawer C, Tebo BM, Schüler D. 2001. A large gene cluster encoding several magnetosome proteins is conserved in different species of magnetotactic bacteria. *Appl Environ Microbiol* **67**: 4573–4582.
- Hohmann-Marriott MF, Blankenship RE. 2007. Hypothesis on chlorosome biogenesis in green photosynthetic bacteria. *Febs Letters* **581**: 800–803.
- Iancu CV, Ding HJ, Morris DM, Dias DP, Gonzales AD, Martino A, Jensen GJ. 2007. The structure of isolated *Synechococcus* strain WH8102 carboxysomes as revealed by electron cryotomography. *J Mol Biol* **372**: 764–773.
- Jogler C, Kube M, Schübbe S, Ullrich S, Teeling H, Bazylinski DA, Reinhardt R, Schüler D. 2009. Comparative analysis of magnetosome gene clusters in magnetotactic bacteria provides further evidence for horizontal gene transfer. *Environ Microbiol* **11**: 1267–1277.
- Kerfeld CA, Sawaya MR, Tanaka S, Nguyen CV, Phillips M, Beeby M, Yeates TO. 2005. Protein structures forming the shell of primitive bacterial organelles. *Science* **309**: 936–938.
- Komeili A. 2007. Molecular mechanisms of magnetosome formation. *Annu Rev Biochem* **76**: 351–366.

- Komeili A, Li Z, Newman DK, Jensen GJ. 2006. Magnetosomes are cell membrane invaginations organized by the actin-like protein MamK. *Science* **311**: 242–245.
- Komeili A, Vali H, Beveridge TJ, Newman DK. 2004. Magnetosome vesicles are present before magnetite formation, and MamA is required for their activation. *Proc Natl Acad Sci* **101**: 3839–3844.
- Konorty M, Kahana N, Linaroudis A, Minsky A, Medalia O. 2008. Structural analysis of photosynthetic membranes by cryo-electron tomography of intact *Rhodospseudomonas viridis* cells. *J Struct Biol* **161**: 393–400.
- Kroll D, Meierhoff K, Bechtold N, Kinoshita M, Westphal S, Voithknecht UC, Soll J, Westhoff P. 2001. VIPP1, a nuclear gene of *Arabidopsis thaliana* essential for thylakoid membrane formation. *Proc Natl Acad Sci* **98**: 4238–4242.
- Lee KC, Webb RI, Fuerst JA. 2009. The cell cycle of the planctomycete *Gemmata obscuriglobus* with respect to cell compartmentalization. *Bmc Cell Biol* **10**: 4.
- Li H, Bryant DA. 2009. Envelope proteins of the CsmB/CsmF and CsmC/CsmD motif families influence the size, shape, and composition of chlorosomes in *Chlorobaculum tepidum*. *J Bacteriol* **191**: 7109–7120.
- Liberton M, Howard Berg R, Heuser J, Roth R, Pakrasi H. 2006. Ultrastructure of the membrane systems in the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803. *Protoplasma* **227**: 129–138.
- Lindsay MR, Webb RI, Fuerst JA. 1997. Pirellosomes: A new type of membrane-bounded cell compartment in planctomycete bacteria of the genus *Pirellula*. *Microbiology-Uk* **143**: 739–748.
- Lindsay M, Webb R, Strous M, Jetten M, Butler M, Forde R, Fuerst J. 2001. Cell compartmentalisation in planctomycetes: Novel types of structural organisation for the bacterial cell. *Arch Microbiol* **175**: 413–429.
- Low HH, Lowe J. 2006. A bacterial dynamin-like protein. *Nature* **444**: 766–769.
- McMaster TJ, Miles MJ, Walsby AE. 1996. Direct observation of protein secondary structure in gas vesicles by atomic force microscopy. *Biophys J* **70**: 2432–2436.
- Milne JLS, Subramaniam S. 2009. Cryo-electron tomography of bacteria: Progress, challenges and future prospects. *Nat Rev Microbiol* **7**: 666–675.
- Murat D, Quinlan A, Vali H, Komeili A. 2010. Comprehensive genetic dissection of the magnetosome gene island reveals the step-wise assembly of a prokaryotic organelle. *Proc Natl Acad Sci* **107**: 5593–5598.
- Nevo R, Charuvi D, Shimon E, Schwarz R, Kaplan A, Ohad I, Reich Z. 2007. Thylakoid membrane perforations and connectivity enable intracellular traffic in cyanobacteria. *EMBO J* **26**: 1467–1473.
- Oelze J, Drews G. 1972. Membranes of photosynthetic bacteria. *Biochim Biophys Acta* **265**: 209–239.
- Offner S, Hofacker A, Wanner G, Pfeifer F. 2000. Eight of fourteen gvp genes are sufficient for formation of gas vesicles in halophilic archaea. *J Bacteriol* **182**: 4328–4336.
- Okuda Y, Denda K, Fukumori Y. 1996. Cloning and sequencing of a gene encoding a new member of the tetra-ricopeptide protein family from magnetosomes of *Magnetospirillum magnetotacticum*. *Gene* **171**: 99–102.
- Pradel N, Santini CL, Bernadac A, Fukumori Y, Wu LF. 2006. Biogenesis of actin-like bacterial cytoskeletal filaments destined for positioning prokaryotic magnetic organelles. *Proc Natl Acad Sci* **103**: 17485–17489.
- Praefcke GJK, McMahon HT. 2004. The dynamin superfamily: Universal membrane tubulation and fission molecules? *Nat Rev Mol Cell Biol* **5**: 133–147.
- Qian P, Bullough PA, Hunter CN. 2008. Three-dimensional reconstruction of a membrane-bending complex: the RC-LH1-PufX core dimer of *Rhodobacter sphaeroides*. *J Biol Chem* **283**: 14002–14011.
- Richter M, Kube M, Bazylnski DA, Lombardot T, Glöckner FO, Reinhardt R, Schüler D. 2007. Comparative genome analysis of four magnetotactic bacteria reveals a complex set of group-specific genes implicated in magnetosome biomineralization and function. *J Bacteriol* **189**: 4899–4910.
- Santarella-Mellwig R, Franke J, Jaedicke A, Gorjanacz M, Bauer U, Budd A, Mattaj JW, Devos DP. 2010. The compartmentalized bacteria of the planctomycetes-verrucomicrobia-chlamydiae superphylum have membrane coat-like proteins. *Plos Biol* **8**: e1000281.
- Savage DF, Afonso B, Chen AH, Silver PA. 2010. Spatially ordered dynamics of the bacterial carbon fixation machinery. *Science* **327**: 1258–1261.
- Schalk J, de Vries S, Kuenen J, Jetten M. 2000. Involvement of a novel hydroxylamine oxidoreductase in anaerobic ammonium oxidation. *Biochemistry* **9**: 635–642.
- Scheffel A, Schüler D. 2007. The acidic repetitive domain of the *Magnetospirillum gryphiswaldense* MamJ protein displays hypervariability but is not required for magnetosome chain assembly. *J Bacteriol* **189**: 6437–6446.
- Scheffel A, Gruska M, Faivre D, Linaroudis A, Plitzko JM, Schüler D. 2006. An acidic protein aligns magnetosomes along a filamentous structure in magnetotactic bacteria. *Nature* **440**: 110–114.
- Scheffel A, Gärdes A, Grünberg K, Wanner G, Schüler D. 2008. The major magnetosome proteins MamGFDC are not essential for magnetite biomineralization in *Magnetospirillum gryphiswaldense* but regulate the size of magnetosome crystals. *J Bacteriol* **190**: 377–386.
- Schmid MF, Paredes AM, Khant HA, Soyer F, Aldrich HC, Chiu W, Shively JM. 2006. Structure of *Halothiobacillus neapolitanus* carboxysomes by cryo-electron tomography. *J Mol Biol* **364**: 526–535.
- Schneider D, Fuhrmann E, Scholz I, Hess WR, Graumann PL. 2007. Fluorescence staining of live cyanobacterial cells suggest non-stringent chromosome segregation and absence of a connection between cytoplasmic and thylakoid membranes. *BMC Cell Biol* **8**: 39.
- Shively J. 2006. Complex intracellular structures in prokaryotes. Springer-Verlag, Berlin Heidelberg, Germany.
- Sinninghe Damsté JS, Strous M, Rijpstra WIC, Hopmans EC, Geenevasen JAJ, van Duin ACT, van Niftrik LA, Jetten MSM. 2002. Linearly concatenated cyclobutane lipids form a dense bacterial membrane. *Nature* **419**: 708–712.
- Staehelin LA, Golecki JR, Drews G. 1980. Supramolecular organization of chlorosomes (chlorobium vesicles) and of their membrane attachment sites in *Chlorobium limicola*. *Biochim Biophys Acta* **589**: 30–45.
- Staley JT, Bouzek H, Jenkins C. 2005. Eukaryotic signature proteins of *Prostheco bacter de jongeei* and *Gemmata* sp

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- Wa-1 as revealed by in silico analysis. *Fems Microbiology Letters* **243**: 9–14.
- Strous M, Fuerst JA, Kramer EH, Logemann S, Muyzer G, van de Pas-Schoonen KT, Webb R, Kuenen JG, Jetten MS. 1999. Missing lithotroph identified as new planctomycete. *Nature* **400**: 446–449.
- Studholme DJ, Fuerst JA, Bateman A. 2004. Novel protein domains and motifs in the marine planctomycete *Rhodopirellula baltica*. *Fems Microbiology Letters* **236**: 333–340.
- Sturgis JN, Tucker JD, Olsen JD, Hunter CN, Niederman RA. 2009. Atomic force microscopy studies of native photosynthetic membranes. *Biochemistry* **48**: 3679–3698.
- Tanaka M, Okamura Y, Arakaki A, Tanaka T, Takeyama H, Matsunaga T. 2006. Origin of magnetosome membrane: Proteomic analysis of magnetosome membrane and comparison with cytoplasmic membrane. *Proteomics* **6**: 5234–5247.
- Taoka A, Asada R, Wu L, Fukumori Y. 2007. Polymerization of the actin-like protein mamK, which is associated with magnetosomes. *J Bacteriol* **189**: 8737–8740.
- Tauschel HD, Drews G. 1967. [Morphogenesis of thylakoids in *Rhodospseudomonas palustris*]. *Archiv für Mikrobiologie* **59**: 381–404.
- Tavano CL, Donohue TJ. 2006. Development of the bacterial photosynthetic apparatus. *Curr Opin Microbiol* **9**: 625–631.
- Ting CS, Hsieh C, Sundararaman S, Mannella C, Marko M. 2007. Cryo-electron tomography reveals the comparative three-dimensional architecture of *Prochlorococcus*, a globally important marine cyanobacterium. *J Bacteriol* **189**: 4485–4493.
- Tsai Y, Sawaya MR, Cannon GC, Cai F, Williams EB, Heinrichs S, Kerfeld CA, Yeates TO. 2007. Structural analysis of CsoS1A and the protein shell of the *Halothiobacillus neapolitanus* carboxysome. *Plos Biol* **5**: e144.
- Ullrich S, Kube M, Schübbe S, Reinhardt R, Schüler D. 2005. A hypervariable 130-kilobase genomic region of *Magnetospirillum gryphiswaldense* comprises a magnetosome island which undergoes frequent rearrangements during stationary growth. *Journal of Bacteriology* **187**: 7176–7184.
- van de Meene AML, Hohmann-Marriott MF, Vermaas WFJ, Roberson RW. 2006. The three-dimensional structure of the cyanobacterium *Synechocystis* sp. PCC 6803. *Arch Microbiol* **184**: 259–270.
- van Keulen G, Hopwood DA, Dijkhuizen L, Sawers RG. 2005. Gas vesicles in actinomycetes: old buoys in novel habitats? *Trends Microbiol* **13**: 350–354.
- van Niftrik L, Geerts WJC, van Donselaar EG, Humbel BM, Webb RI, Fuerst JA, Verkleij AJ, Jetten MSM, Strous M. 2008a. Linking ultrastructure and function in four genera of anaerobic ammonium-oxidizing bacteria: Cell plan, glycogen storage, and localization of cytochrome c proteins. *Journal of Bacteriology* **190**: 708–717.
- van Niftrik L, Geerts WJC, van Donselaar EG, Humbel BM, Webb RI, Harhangi HR, Camp HJMOd, Fuerst JA, Verkleij AJ, Jetten MSM, et al. 2009. Cell division ring, a new cell division protein and vertical inheritance of a bacterial organelle in *Anammox planctomycetes*. *Mol Microbiol* **73**: 1009–1019.
- van Niftrik L, Geerts WJC, van Donselaar EG, Humbel BM, Yakushevska A, Verkleij AJ, Jetten MSM, Strous M. 2008b. Combined structural and chemical analysis of the anammoxosome: A membrane-bounded intracytoplasmic compartment in anammox bacteria. *J Structural Biol* **161**: 401–410.
- Vassilieva EV, Stirewalt VL, Jakobs CU, Frigaard NU, Inoue-Sakamoto K, Baker MA, Sotak A, Bryant DA. 2002. Subcellular localization of chlorosome proteins in *Chlorobium tepidum* and characterization of three new chlorosome proteins: CsmE, CsmH, and CsmX. *Biochemistry* **41**: 4358–4370.
- Walsby AE. 1994. Gas Vesicles. *Microbiol Rev* **58**: 94–144.
- Westphal S, Heins L, Soll J, Vothknecht UC. 2001. Vipp1 deletion mutant of *Synechocystis*: A connection between bacterial phage shock and thylakoid biogenesis? *Proc Natl Acad Sci* **98**: 4243–4248.
- Yeates TO, Kerfeld CA, Heinrichs S, Cannon GC, Shively JM. 2008. Protein-based organelles in bacteria: Carboxysomes and related microcompartments. *Nature Reviews Microbiology* **6**: 681–691.
- Zeng X, Zeng X, Roh JH, Roh JH, Callister SJ, Callister SJ, Tavano CL, Tavano CL, Donohue TJ, Donohue TJ, et al. 2007. Proteomic characterization of the *Rhodobacter sphaeroides* 2.4.1 photosynthetic membrane: Identification of new proteins. *J Bacteriol* **189**: 7464.

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