

Chapter 2

Cell Compartmentalization and Endocytosis in Planctomycetes: Structure and Function in Complex Bacteria

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Abbreviations

Anammox	Anaerobic ammonium oxidation
PVC	<i>Planctomycetes</i> , <i>Verrucomicrobia</i> , and <i>Chlamydiae</i>
TEM	Transmission electron microscopy
CM	Cytoplasmic membrane
CW	Cell wall
N	Nucleoid
MC	Membrane-coating

2.1 Introduction

When planctomycetes were first investigated, electron microscopy was not available and cell compartments were not suggested, though since organisms such as *Planctomyces bekefi* were mistaken as fungi (see Chap. 1 and (Fuerst 1995)), this might have been so. However, the main reasoning for fungal relationship was based on what we now know are noncellular stalks of these freshwater bacteria, not mycelia.

In the 1970s and 1980s planctomycetes were first examined via electron microscopy by Jean Schmidt and Mortimer Starr, often from environmental water samples or enrichments, but even when pure cultures were examined (of a *Pirellula*-like “morphotype IV” strain) chemical fixation methods applied did not clearly reveal internal cell compartments (Schmidt and Starr 1980), though various “parallel stacked structures,” amorphous regions, and inclusions were observed (Schmidt and Starr 1982). This was one of the reasons for placing these organisms with the bacteria and prokaryotes, though there was no molecular data at that time (Schmidt and Starr 1982). At the same time when *Pirellula staley* was first described as “*Pasteuria ramosa*” (Staley 1973) and *Planctomyces maris* first described, the chemical fixation used for preparation of cells for TEM of thin sections was not sufficient to reveal compartments in cells. The first indication that there may be more complex organization of the cell in planctomycetes was the original description by Peter Franzmann and VBD Skerman (1984) of the freshwater planctomycete *Gemmata obscuriglobus* where a “packaged DNA” was noted from electron micrographs and variation in phase-darkness of different regions of the cell observed from phase contrast light microscopy. They noted “considerable intracellular membrane development that may give an unwarranted appearance of membrane involvement in the DNA package,” but a membrane-bounded nucleoid was not clearly indicated from their chemically fixed cells (all grown on low-nutrient media such as lake water agar or soil extract agar or “Staley’s maintenance medium”). In 1991 Fuerst and Webb (Fuerst and Webb 1991) applied cryosubstitution involving freeze-fixation to *Gemmata obscuriglobus* and found that much less distortion of cell morphology and shape resulted than in the case of chemical fixation of the same strain.

It was later found that if the chemical fixative was too high in osmolarity (e.g., 3 mM cacodylate buffer), cells would shrink and distort asymmetrically giving rise to a crescent morphology not observed in cryosubstituted cells (Lindsay et al. 1995).

This also is relevant to the question of the cytoplasmic membrane, since absence of plasmolysis under high osmolar conditions suggests shrinkage of a cell wall closely apposed to a cytoplasmic membrane in this organism. Of interest are early reports of autolysis in at least some freshwater planctomycetes under conditions poor in divalent cations (Schmidt 1978); this does not seem to apply to all such freshwater planctomycetes however, e.g., *Gemmata obscuriglobus*.

In 1997, the first ultrastructure study of *Pirellula* group species *Pirellula staleyi* and *Blastopirellula marina* was published, and a simple single intracytoplasmic membrane discovered separating a ribosome-free outer region from a major compartment containing the ribosomes and nucleoid (Lindsay et al. 1997). The major ribosome-containing compartment was termed a pirellulosome.

In 2001, a study of several available pure cultured species representing genera *Gemmata*, *Pirellula*, *Blastopirellula*, *Planctomyces*, and *Isosphaera* as well as the non-pure cultured anammox “*Candidatus Kuenenia stuttgartiensis*”—a shared cell plan (described below)—was discovered to be common to all planctomycetes examined (Lindsay et al. 2001). Variation occurred as further membrane-bounded structures within the pirellulosome major compartment, as in *Gemmata* with its membrane-bounded nuclear body and anammox planctomycete species with their anammoxosomes. Recent advances include confirmation of extensive internal membranes and the condensed nature of the nucleoid in *Gemmata obscuriglobus* (Lieber et al. 2009b) and its correlation with radiation resistance in this species.

Tomography of cryosubstituted cells of anammox species demonstrated an intriguing cell division ring (van Niftrik et al. 2009) and also the occurrence of ATP synthase in all the membranes of “*Candidatus Kuenenia stuttgartiensis*” including those of the membrane underlying the cell wall (consistent with its identification as cytoplasmic membrane) and the anammoxosome (consistent with biochemical models for anammox physiology postulating a pmf generation across the anammoxosome membrane) (van Niftrik et al. 2010) (see Chap. 4 in this volume).

Functionally, one of the most dramatic findings has been that of protein uptake by *Gemmata obscuriglobus* (Lonhienne et al. 2010), a process similar in many features to receptor-mediated endocytosis of eukaryotes, involving internal vesicle formation and association of ligand protein with such vesicles. These functional results are consistent with the bioinformatic prediction in planctomycetes (and other members of the PVC superphylum) of proteins in a family of membrane-coating (MC) proteins of eukaryotes, some of which are necessary for receptor-mediated (clathrin-mediated) endocytosis (Santarella-Mellwig et al. 2010). Significantly in relation to the unique occurrence of the protein endocytosis only in planctomycetes within the domain Bacteria, bioinformatics analysis initially revealed MC protein homologs only within the PVC superphylum members among the bacteria (recent analysis indicates that there may also be MC proteins in at least one other phylum, Bacteroidetes—see Chap. 3 and (McInerney et al. 2011)).

2.2 Shared Compartments and Components of the Planctomycete Cell

2.2.1 Introduction

All planctomycetes share a common cell plan involving internal compartments separated from other regions of the cell via membranes (Fuerst 2005; Fuerst and Sagulenko 2011). Within this plan, there are then more complex variations involving further compartments and membranes (Fig. 2.1). The simplest, shared plan is one

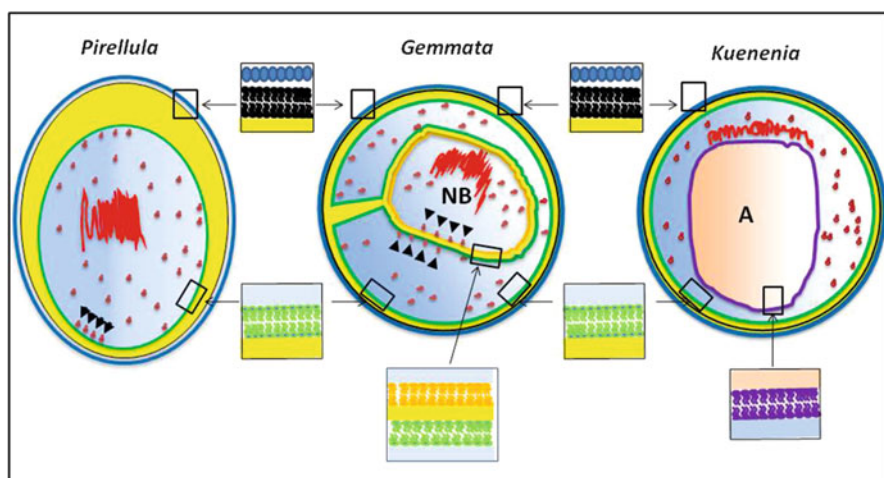


Fig. 2.1 Schematic diagram of planctomycete cell plans for members of genera *Pirellula*, *Gemmata*, and *Kuenenia*, displaying the shared features of the planctomycete cell plans as well as the modifications beyond this shared cell plan in *Gemmata* and *Kuenenia*. The cell compartments characteristic for these plans are defined by internal membranes. All internal cytoplasm is surrounded by a cytoplasmic membrane—a single bilayer membrane (black)—which is closely apposed to the cell wall (dark blue). A single bilayer intracytoplasmic membrane (ICM) (green) is present in all the example cells shown, separates the ribosome-free paryphoplasm (yellow) from the ribosome-containing pirellulosome (light shaded blue), and thus surrounds the pirellulosome. Pirellulosome can also be termed riboplasm due to its content of ribosomes. These ribosomes in some places can form linear arrays along internal membranes, in *Pirellula* along the inner side of the ICM, and in *Gemmata* along both membranes and thus both sides of the nuclear envelope. In *Gemmata* the ICM is continuous with the outer membrane of a double-membrane envelope composed of this outer membrane and a closely apposed inner membrane (orange). The “pericisternal” space between the two membranes of the nuclear envelope is continuous with the paryphoplasm. This envelope surrounds the nucleoid DNA (red) and some riboplasm, forming the nuclear body (NB). In *Kuenenia* and other anammox planctomycetes, a single bilayer membrane (purple) surrounds an internal organelle situated within the pirellulosome, the anammoxosome (light shaded brown). In summary, all three plans have two compartments in common, the paryphoplasm and pirellulosome, and in *Gemmata* and *Kuenenia* there is a third compartment situated within the pirellulosome. Insets show enlarged views of particular (boxed) regions of membrane significant for understanding the nature of the membranes in relation to associated compartments

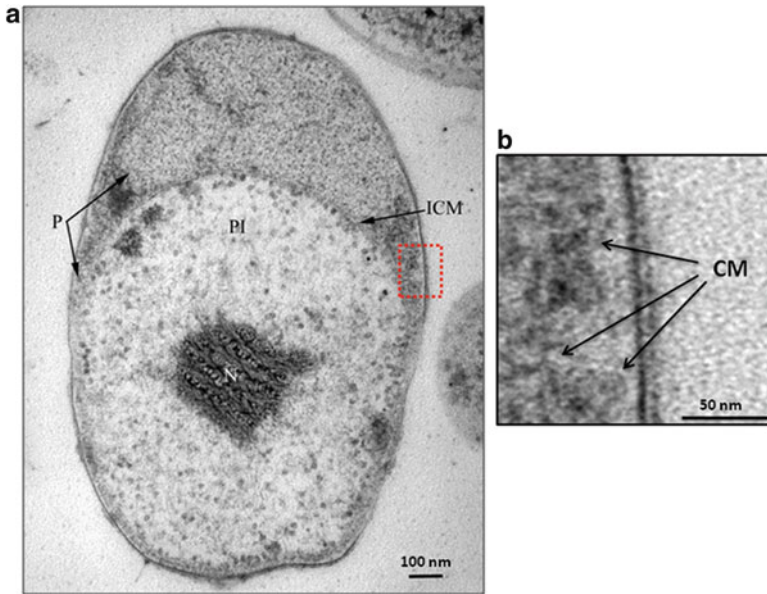


Fig. 2.2 (a) Transmission electron micrograph of thin-sectioned cell of *Blastopirellula marina*, processed by cryosubstitution. Two cell compartments are seen within the cell, a ribosome-free paryphoplasm (P) forming a polar cap region at one pole of the cell, and a major pirellulosome region (PI) containing a condensed fibrillar nucleoid (N) and ribosomes. These compartments are separated by an intracytoplasmic membrane (ICM). Bar marker, 100 nm. Modified from, (b) (enlargement of red box in (a)) shows enlarged view of the paryphoplasm area. Arrows indicate invagination of cytoplasmic membrane (CM). Bar marker, 50 nm

illustrated by organisms such as *Pirellula staleyi*, *Blastopirellula marina* (Lindsay et al. 1997), *Planctomyces limnophilus* (Jogler et al. 2011), and *Schlesneria paludicola* (Kulichevskaya et al. 2007). In this plan (Fig. 2.2) the cell is divided into two compartments, a ribosome- and nucleoid-containing major compartment termed the “pirellulosome” and, surrounding this pirellulosome, a ribosome-free region, the paryphoplasm (Lindsay et al. 1997). This plan has also been found to apply to *Planctomyces limnophilus* (Jogler et al. 2011). There are other planctomycetes such as *Isosphaera* which seem to preserve the basic topology of this plan even though they appear superficially distinct (thus, the paryphoplasm may invade even the center of the cell yet still connect with its outer origin so that the cell is still divided into just two regions) (Lindsay et al. 2001). More complexity is observed however in the case of *Gemmata* species and the anammox planctomycetes, where there appears to be a third membrane-bounded cell compartment, the effectively double-membrane-bounded nuclear body in the case of *Gemmata* (Figs. 2.3 and 2.4) and the single-membrane-bounded anammoxosome in the case of anammox planctomycetes such as *Kuenenia* (Lindsay et al. 2001).

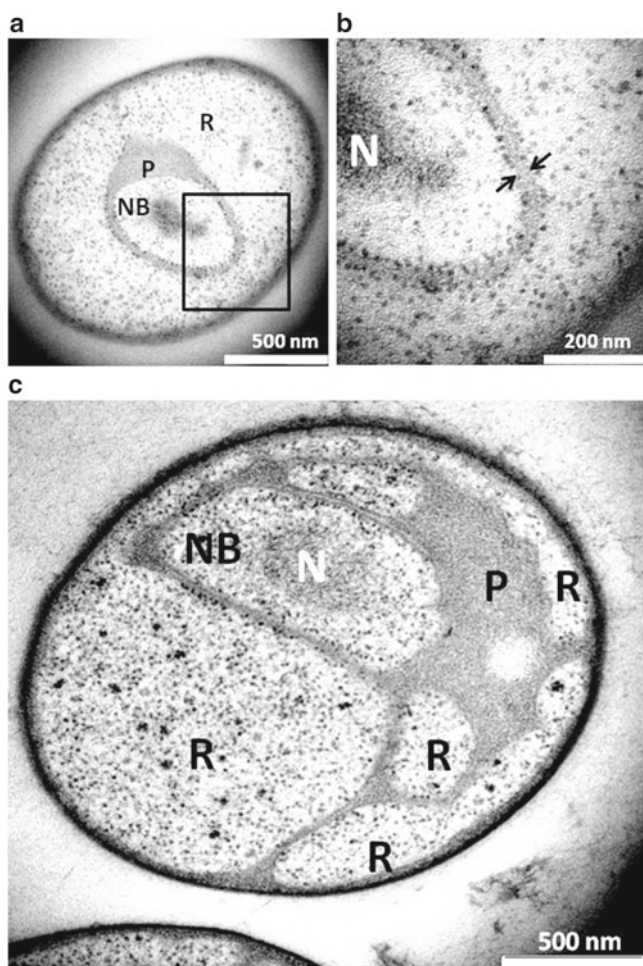
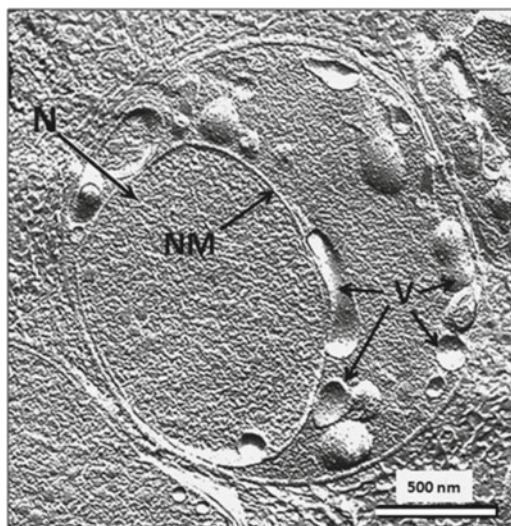


Fig. 2.3 Transmission electron micrographs of thin-sectioned cells of *Gemmata obscuriglobus* prepared by cryosubstitution (a) section of whole cell showing a single nuclear body (NB) surrounded by a nuclear envelope and connected to a portion of paryphoplasm (P) continuous with the pericisternal space between the membranes of the nuclear envelope. Riboplasm (R) of the pirellulosome surrounds the nuclear body, which also contains ribosomes. Bar marker, 500 nm. *Inset box* is enlarged in (b) where the nuclear envelope can be seen to consist of two membranes (arrows), and the fibrillar structure of the condensed nucleoid (N) is displayed. Ribosomes can be seen to be bound to both inner and outer membranes of the nuclear body. Bar marker, 200 nm. (c) An example of a more complex internal cell structure in *G. obscuriglobus*. As in (a) and (b) the nuclear body possesses an envelope of two membranes, but the space between the nuclear membranes is continuous with a complex paryphoplasm (P) connecting both to paryphoplasm at the cell rim and with other regions, appearing to divide the pirellulosome riboplasm (R) into distinct vesicle-like regions, each surrounded by ICM. The appearance of a complex endomembrane system is given in such cells. Ribosomes are bound in linear array only to the nuclear envelope membranes surrounding the nucleoid. Bar marker, 500 nm

Fig. 2.4 Transmission electron micrograph of freeze-fracture replica of a cross-fractured *Gemmata obscuriglobus* cell showing the separate compartment nature of the nuclear body containing the nucleoid (N) and completely surrounded by a nuclear envelope (NM). The region of the cell cytoplasm surrounding the nuclear body contains numerous vesicles (V), while the nuclear body is mostly devoid of vesicles. Bar marker, 500 nm



Following some perspectives used in synthetic biology, the structure and nature of the unique components and compartments of the planctomycete cell as now understood will be approached first as a “parts list.” These can then be integrated after appreciating the nature of each part, for discussion of their potential interrelations and inter-part communication mechanisms.

2.2.2 *The Parts of the Planctomycete Cell: Cell Wall and Cytoplasmic Membrane*

In a synthetic biology project, how would we go about constructing a planctomycete? One of the first components we would need would be a cell wall composed largely of protein, in contrast to all other known bacterial walls. The unusual indeed unique nature of planctomycetes was really indicated firstly by chemical analysis of cell wall composition (König et al. 1984; Liesack et al. 1986), which revealed no evidence of either muramic acid or meso-diaminopimelic acid, components characteristic of peptidoglycan, the polymer essential for cell wall structural strength and integrity in all the known bacteria with cell walls with the exception of chlamydia (which in light of suggestions of a planctomycete relationship to chlamydia may be an evolutionarily expected exception). Consistent with the absence of peptidoglycan analytically, early reports of planctomycete resistance to penicillin G and cycloserine, antibiotics targeting peptidoglycan synthesis (Schmidt 1978) were followed by confirmation of the resistance of many planctomycetes to peptidoglycan synthesis-targeting antibiotics such as beta-lactams and cycloserine, antibiotics inhibiting peptidoglycan synthesis at different stages in that synthesis (König et al. 1984). Such

resistance has been confirmed for wider selections of planctomycete species (Cayrou et al. 2010; Jogler et al. 2012). Although some *dcw* operon genes (e.g., *murD*) needed for peptidoglycan synthesis as well as cell division in other bacteria are present in planctomycetes (Pilhofer et al. 2008), they are distributed among different operons in different species or are not organized at all (Jogler et al. 2012). It has been suggested that an ancestral planctomycete or even a PVC superphylum ancestor may have contained all *dcw* operon genes including all for peptidoglycan synthesis, that an FtsZ-dependent divisome may have been present, and that these have been lost differentially in different members (Pilhofer et al. 2008). Even accepting that, planctomycetes seem to have replaced this divisome with one not depending on FtsZ or connected with peptidoglycan synthesis, so the function of the *dcw* operon genes in planctomycetes is not clear, as is the case to a lesser degree with chlamydiae (which have most of the peptidoglycan synthesis genes but no FtsZ).

The dominance of protein in the wall is indicated by the chemical analysis of several planctomycetes including *Gemmata obscuriglobus* (Stackebrandt et al. 1986), *Isosphaera pallida* (Giovannoni et al. 1987a), and a number of organisms in the *Pirellula* and *Planctomyces* groups such as *Planctomyces maris* and *Pirellula staleyi* (studied as “*Pasteuria ramosa*”) and strains related to *Pirellula staleyi* or the *Pirellula* group (König et al. 1984; Liesack et al. 1986). In *I. pallida*, 99 % of the dry weight of the rigid detergent-resistant wall layer was recovered as amino acids. In one study, percentages of protein versus total cell dry weight range from 51 % for *Gemmata obscuriglobus* to 80 % for *Planctomyces maris* and 82 % for *Pirellula staleyi* (Stackebrandt et al. 1986) while in another in 7/8 strains studied the protein of the wall comprised 75–82 % of the 10 % SDS-resistant envelope dry weight, with only *Pl. maris* having a significantly lower amount at 62.6 % (Liesack et al. 1986). The amino acid composition is also of interest since cystine was a significant component in walls of *Pirellula* and *Planctomyces* strains indicating that significant cross-linking via disulfide bonds might occur in intact walls (Liesack et al. 1986). However this may not be universal as *G. obscuriglobus* displayed low cystine content relative to that in *P. staleyi* and *Pl. maris* walls (Stackebrandt et al. 1986).

Proteinaceous walls of planctomycetes have the remarkable property of resisting prolonged (e.g., 30 min) boiling in 10 % SDS detergent (Liesack et al. 1986; Stackebrandt et al. 1986), an approach which would normally be used to isolate peptidoglycan from Gram-negative bacteria, e.g., from *Neisseria* (Dougherty 1985), and eliminate any typical Gram-negative outer membrane. This has been explained for some species on the basis of a high content of cystine in the wall protein suggesting disulfide bridge stabilization (Liesack et al. 1986). The resulting rigid sacculi seem to retain the cell shape, as do isolated peptidoglycan wall sacculi of other bacteria, so they represent structurally functional walls (Giovannoni et al. 1987a). A characteristic property of planctomycete cell surfaces is the presence of pit-like structures called crateriform structures (Fig. 2.5), and these seem also to be preserved in the wall when these are isolated after SDS treatment (Giovannoni et al. 1987a; Liesack et al. 1986; Stackebrandt et al. 1986). The nature of these structures is not clear but they do not seem to be simple holes or pits as judged by negative stained preparations of whole cells (but regularly spaced perforations can be seen in isolated

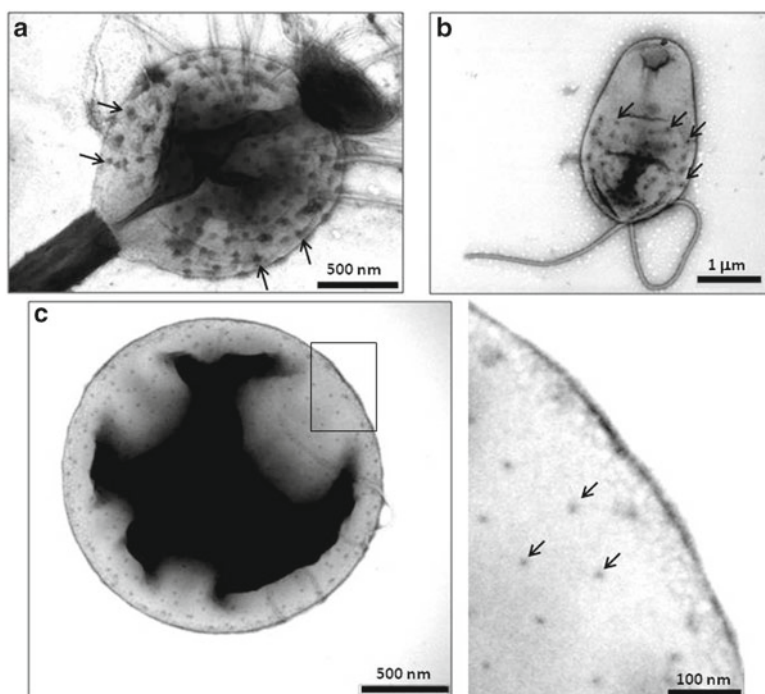


Fig. 2.5 Transmission electron micrograph of negatively stained planctomycete cells showing crateriform structures (a) *Planctomyces bekefii* morphotype cell from a freshwater lake showing numerous uniformly distributed large crateriform structures (arrows). A stalk consisting of many closely packed fibrils can be seen extending from one side of the cell—this stalk connected the cell to the center of a rosette of several radiating stalks with cells at their tips. Bar marker, 500 nm. (b) Transmission electron micrograph of negatively stained cell *Blastopirellula* group planctomycete isolated from giant tiger prawn *Penaeus monodon* showing large crateriform structures (arrows) only at the broad lower pole (much smaller crateriform structures are clustered at the upper pole). Bar marker, 1 μ m. (c) Transmission electron micrograph of a negatively stained *Gemmata obscuriglobus* cell showing uniformly distributed crateriform structures. Bar marker, 500 nm. *Inset* shows an enlarged view of the boxed region on the whole cell illustrating more detailed structure and uniform spacing of these crateriform structures (arrows) on the cell surface. Bar marker, 100 nm

walls—e.g., see Giovannoni et al. (1987a)). Their distribution varies with the taxonomic position of the planctomycete—members of the *Pirellula* group have polar distribution while *Gemmata* species have uniform distribution of these structures over the cell surface. Different classes of crateriform structures based on size can be distinguished—in a freshwater *Pirellula staleyi* strain 12 and 5–7 nm classes can be discerned in more mature buds (Tekniepe et al. 1981), with the former's large form at the pole of the bud attached to the mother cell, while the small form clustered at the opposite pole distal to the mother cell. In the mature mother cell, the large forms can enlarge to a maximum diameter of 30 nm, while the smaller structures become less visible. *Isosphaera pallida* also possesses large (27 nm) diameter ringlike

crateriform structures (Giovannoni et al. 1987b). In *Planctomyces limnophilus* swarmer (swimmer) cells, two size classes of crateriform structures also occur—a large form is uniformly distributed while the small form is again limited to a flagellum-containing pole (Jogler et al. 2011). Such observations strongly suggest that polar localization of proteins is likely to be correlated with the cell cycle in planctomycetes, as extensively documented in other bacteria with sessile cell-dispersal cell life cycles and cell differentiation (Ebersbach and Jacobs-Wagner 2007; Iniesta and Shapiro 2008).

Via electron microscopy, cell walls of planctomycetes appear similar overall to those of a *Pirellula staleyi* strain where they appear to consist in chemically fixed cells of inner and outer electron-dense layers separated by a narrow electron-transparent layer, with the inner layer observed as wider and more electron dense than the outer layer (Schmidt and Starr 1982) but without any indication of a clear dense peptidoglycan layer. Similar studies on *Planctomyces* and *Pirellula* group strains also indicated no peptidoglycan layer but identified the other structures as an “outer membrane” (König et al. 1984). In *Isosphaera pallida* the width of the wall “trilaminar structure” resembling a unit membrane was at 11 nm noted as thicker than the width of classical unit membranes of 7–8 nm (Giovannoni et al. 1987a). Planctomycete walls of several genera when isolated after harsh detergent treatment retain the cell shape (Giovannoni et al. 1987a; Liesack et al. 1986), suggesting that they are not typical lipid unit membranes, but also that they are covalently bounded rigid macromolecules. No peptidoglycan-consistent layer similar to one seen in sectioned Gram-negative bacterial cells is seen. There are some suggestions or impressions that planctomycete walls are Gram-negative in structure (Cavalier-Smith 2010), but the nature of the rigid protein wall and absence of peptidoglycan typical of Gram-negative bacteria walls are not consistent with this perspective. Some planctomycetes may contain hydroxy fatty acids consistent with lipid A of Gram-negative bacterial LPS, and genes for lipid A synthesis and modification via dephosphorylation have also been annotated (Jogler et al. 2012; Sutcliffe 2010), as well as markers for outer membrane biogenesis and homologs to outer membrane proteins of Gram-negative bacteria. It is not clear yet where these molecules are localized within the cell envelope and whether they are localized in the wall or the membranes internal to the wall (see below). It is possible that planctomycetes could be described as “diderm” but without lipid A or LPS in the wall, as occurs in some other bacterial phyla such as *Spirochaetes*, *Deinococcus-Thermus*, and *Thermotogae* (Sutcliffe 2010), but data are insufficient to decide this at present. However, all of those phyla lack lipid A synthesis genes, unlike planctomycetes. The walls of some planctomycetes seem to have prostheca-like projections positioned in a distinct polar orientation, as in the “humps” of *Pirellula staleyi* (Butler et al. 2002) and the “spurs” of *Schlesneria paludicola* (Kulichevskaya et al. 2007), *Planctomyces brasiliensis*, and *Planctomyces* sp. AGA/M18 (Fuerst et al. 1997), and other prosthecate planctomycetes have been observed among environmental isolates (Schlesner 1994). This suggests some structural differences within either the wall or the internal cytoskeletal proteins maintaining a polar projection of wall.

Homologs to some genes normally needed for peptidoglycan synthesis seem to occur in planctomycetes (e.g., *Rhodopirellula* (Glockner et al. 2003)) as they do in chlamydiae where peptidoglycan also seems undemonstrable chemically. As with chlamydiae they may perform some function other than peptidoglycan synthesis. The latter exception may not be an accidental one, as chlamydiae were later suggested to be related phylogenetically to planctomycetes as members of the PVC superphylum. However, it may be that peptidoglycan is synthesized during particular phases of chlamydial growth, a hypothesis not yet confirmed, and chlamydiae have many homologs of peptidoglycan synthesis genes (McCoy and Maurelli 2006). It is known that among other PVC superphylum bacteria, at least *Verrucomicrobium spinosum* possesses enzymes typical of synthesis of the Gram-negative bacterial type of peptidoglycan containing diaminopimelate in its tetrapeptide side chain (Nachar et al. 2012).

Recent proteomic studies suggested that there may be planctomycete-specific wall proteins with characteristic motifs such as YTV (Hieu et al. 2008), but further work has suggested that such proteins may be part of a surfaceome not necessarily solely part of the cell wall itself (Voigt et al. 2012). It is not clear however that the YTV proteins detected in the surfaceome via surface biotinylation and recovery of biotinylated proteins are not after all connected to the structural wall, as might occur if similar methods were applied to teichoic acids of Gram-positive bacteria.

The question of the existence and nature of the cytoplasmic membrane (or plasma membrane when discussing cell structure comparison with eukaryotes) in planctomycete cells is a significant one, since it is central to recognizing the unique nature of the paryphoplasm compartment. Evidence for the existence of a cytoplasmic membrane in planctomycetes comes from several sources, starting with electron microscopy of sectioned cryosubstituted cells of the *Pirellula* group (*Pirellula staleyi* and *Blastopirellula marina*), in which the cytoplasmic membrane can be seen clearly in some regions of partial plasmolysis where the membrane has retracted in very limited regions from the cell wall (Lindsay et al. 1997) (Fig. 2.2). Freeze fracture replicas of *Gemmata obscuriglobus* confirmed its existence just below the cell wall (Lindsay et al. 2001), and cytochemistry of ATP synthase distribution in the anammox planctomycete “*Candidatus Kuenenia stuttgartiensis*” demonstrated that such an enzyme consistent with generation of pmf to be expected across a cytoplasmic membrane was present in the wall-associated cytoplasmic membrane of that planctomycete (van Niftrik et al. 2010). Shrinkage of wall in hyperosmolar conditions is consistent with a membrane that may be attached tightly to wall preventing significant plasmolysis in native living cells (Lindsay et al. 1995), but the invagination of cytoplasmic membrane during the endocytotic protein uptake phenomenon (see below) suggests that the membrane must be free to invaginate at some point away from wall. A significant difference between planctomycete CM and that of other bacteria is that ribosomes are never found attached to planctomycete CMs so that co-translational protein secretion into periplasm and external milieu clearly does not occur in planctomycetes with the same topology as occurs in other bacteria. The absence of ribosomes in contact with CM is analogous to the exclusively internal location of membrane-bounded ribosomes in the eukaryote cell, where they are

attached to rough endoplasmic reticulum. This has been considered by some to be a fundamental distinction between prokaryotes and eukaryotes.

Recent BLAST-based analysis of planctomycete genomes has identified putatively annotated marker genes for lipopolysaccharide insertion, outer membrane biogenesis, and outer membrane proteins (Speth et al. 2012) and on this alone it has been suggested that what was previously considered the proteinaceous wall-associated cytoplasmic membrane of planctomycetes is actually an asymmetric bilayer analogous to the outer membrane of classical Gram-negative bacteria. At least one other group seems to follow a similar scheme seeing no difference between Gram-negative bacterial walls and those of planctomycetes other than invagination of the intracytoplasmic membrane, which is considered by them as the genuine cytoplasmic membrane (Reynaud and Devos 2011). There is no experimental or electron microscopic data supporting this interpretation of planctomycete wall and membranes, and localization of ATP synthase in the anammox planctomycete “*Candidatus Kuenenia stuttgartiensis*” in the wall-associated outermost membrane is in conflict with this view. There seems no reason to center on the cytoplasmic membrane as the location of proteins homologous with Gram-negative outer membrane proteins. These OMPs could be embedded in the proteinaceous wall itself, in a similar manner to the way in which porins are embedded in the peptidoglycan walls of the Gram-positive *Mycobacterium* (Faller et al. 2004; Trias et al. 1992). Until experimental evidence is forthcoming confirming the location of outer membrane proteins or lipids within the outermost wall-associated membrane, we believe that this planctomycete cell membrane should continue to be called “cytoplasmic membrane.”

2.2.3 Paryphoplasm

The paryphoplasm (from Greek *paryphe*, meaning border woven along a robe) is a region between the cytoplasmic membrane and the intracytoplasmic membrane which appears in sectioned cryosubstituted cells to be completely free of ribosomes (Fig. 2.2) though not necessarily of RNA. It varies in electron density of its contents depending on the species; e.g., in *G. obscuriglobus* it is relatively electron dense, while in anammox “*Candidatus Kuenenia stuttgartiensis*” it can appear relatively electron transparent in sections of cryosubstituted cells (van Niftrik et al. 2009). It can appear to be mainly concentrated around the rim of the cell or it can occupy extensive areas within the cell while still connected to the paryphoplasm at the rim and still bounded at its inner edge by intracytoplasmic membrane. In members of the *Pirellula* group such as *Pirellula staleyi* and *Blastopirellula marina* it tends to form polar regions (“polar caps”) such that one pole has more paryphoplasm than the other and such polar regions can be quite extensive relative to the area of cells seen in thin section, implying that they occupy a large volume of the cell of possibly up to 45 % of the whole cell (Lindsay et al. 1997). In *Rhodopirellula baltica*, the paryphoplasm contains vesicles similar in structure to small versions of the pirellosome but without nucleoids (Schlesner et al. 2004). In *Pirellula* group members

including *Rhodopirellula baltica* rosettes formed from several cells suggest that the paryphoplasm may be associated with the cell pole involved with attachment to other cells or perhaps multiple budding events (Lindsay et al. 1997; Schlesner et al. 2004), but the budding pole of cells is not invariably the one with a polar cap (Lindsay et al. 1997). In *Planctomyces limnophilus*, the cell plan is similar to that in *Blastopirellula*, but the paryphoplasm shape changes with different cells, and changes along the z-axis of individual cells in serial sections, and it is thus not rotationally symmetrical (Jogler et al. 2011). Nevertheless, the identification of planctomycete paryphoplasm with a periplasm homologous with that of Gram-negative bacteria is assumed in some literature—see Reynaud and Devos (2011). However, its position underneath a confirmed cytoplasmic membrane is one argument against its being some form of periplasm and its small but established content of some form of RNA is a second (Lindsay et al. 1997). A third argument is that during protein uptake in *Gemmata obscuriglobus*, the cytoplasmic membrane invaginates into the paryphoplasmic space forming vesicles in that space containing incorporated protein (see below) (Lonhienne et al. 2010). The contents of such vesicles are topologically equivalent to the external medium or the periplasm in contact with it, but the paryphoplasm itself forms a space distinct from the vesicle contents and periplasm, one in which the vesicles lie and which is separated from their contents by a vesicle membrane. In this sense it is similar to the cytoplasm in which endosomes form in eukaryotes, not to a periplasm outside the cell contents topologically. A fourth argument is that in planctomycetes such as *Pirellula* and *Blastopirellula*, the paryphoplasm is distributed asymmetrically such that a polar accumulation occurs rather than an even distribution only around the cell rim—suggesting an asymmetry incompatible with a periplasm closely following the line of the cell wall where turgor pressure applies under hypotonic conditions pressing cytoplasmic membrane to the wall. There may well be a periplasm in planctomycetes, but it is more likely to be placed between the proteinaceous wall and the closely apposed cytoplasmic membrane, as proposed for some Gram-positive bacteria (Matias and Beveridge 2006), or trapped between the columns of a surface protein wall as proposed for some Archaea (Engelhardt 2007; Nickell et al. 2003). The paryphoplasm was first defined in planctomycetes of the *Pirellula* group, but was found to be a shared feature of all planctomycete species in pure culture examined (Lindsay et al. 2001) as well as of the anammox planctomycetes, a group yet to be isolated in pure culture (van Niftrik et al. 2008a).

Cytochemical methods applied to anammox planctomycetes are also consistent with the concept of the paryphoplasm in planctomycetes as a genuine cytoplasmic compartment. The paryphoplasm compartment of anammox planctomycete *Kuenenia* does not display peroxidase staining, and this is consistent with this compartment being a cytoplasmic rather than a periplasmic compartment (van Niftrik et al. 2008a). This indirectly supports the existence of a true cytoplasmic membrane bounding this paryphoplasm cytoplasm at its outer boundary.

Consistent with the concept of a cytoplasmic paryphoplasm compartment inside the cytoplasmic membrane, in *G. obscuriglobus*, the endocytotic uptake of protein (see below) apparently occurs via infolding of the cytoplasmic membrane to form

vesicles within the paryphoplasm—protein taken up from external milieu was bound to membranes forming such vesicles.

In the anammox planctomycete *Candidatus Kuenenia*, the occurrence of a cell division ring and its component non-FtsZ kustu1438 protein in the paryphoplasm (van Niftrik et al. 2009) is also consistent with the cytoplasmic nature of paryphoplasm, as the analogous Z ring in bacteria such as *E. coli* and *B. subtilis* forms in the cytoplasm during cell division. However, homologs of kustu1438 have not been detected in non-anammox planctomycetes (Jogler et al. 2012). In budding planctomycetes like *G. obscuriglobus*, the paryphoplasm may play a role in the budding division process at the reproductive pole (see Chap. 1), since the paryphoplasm is the only compartment clearly involved when budding first commences (Lee et al. 2009). Cell wall components such as the major YTV proteins (Hieu et al. 2008) must also accumulate in the paryphoplasm before transport through the cytoplasmic membrane and assembly into new wall, and thus via techniques such as immunogold should in future experiments be identifiable in the paryphoplasm as well as wall. It appears that several cell biology activities may be performed in the paryphoplasm, e.g., endocytotic protein trafficking and budding initiation, making it an active compartment of the planctomycete cytoplasm despite its apparent absence of ribosomes. Ruthenium red staining of sections in *Pirellula* and *Blastopirellula* indicates presence in the paryphoplasm of either polysaccharide or glycoprotein (Lindsay et al. 1997), and RNase-gold cytochemistry in both the *Pirellula* group species and in *G. obscuriglobus* indicates the presence of ribonuclease-degradable RNA significantly above background levels despite the absence of ribosomes but the identity of such RNA remains a mystery (Lindsay et al. 1997, 2001). Paryphoplasm appears to be the site of accumulation of protein incorporated from the external medium via an endocytosis-like mechanism, which appears to accumulate this protein within vesicles in the paryphoplasm. Paryphoplasm composition remains to be investigated in detail, especially regarding its proteome, but is important for understanding the functions of internal membranes and compartmentalization in planctomycetes, including the cell biological process of endocytosis previously unknown outside the eukaryotes.

2.2.4 Intracytoplasmic Membrane

The intracytoplasmic membrane (ICM) forms the inner border of the paryphoplasm. It is a single trilaminar membrane often much more clearly visible than the cytoplasmic membrane in electron micrographs of sectioned cryosubstituted cells (see Fig. 2.2). In *Planctomyces limnophilus* it is a bilayer ca. 6 nm wide, the same width as the cytoplasmic membrane in these cells (Jogler et al. 2011). However, the ICM never appears in direct contact with the cytoplasmic membrane or to display continuity with the cytoplasmic membrane so that it does not appear to directly derive from cytoplasmic membrane in the way which has been reported for other bacterial intracytoplasmic membranes (e.g., cyanobacterial thylakoids, magnetosome membranes). However, since the cytoplasmic membrane appears to infold during

endocytotic protein uptake and form vesicles within the paryphoplasm (Lonhienne et al. 2010), it may be that there is via such vesicles some membrane trafficking of components of the cytoplasmic membrane with those of the ICM, though there is no compositional evidence of this yet.

2.2.5 *Pirellulosome*

The pirellulosome is a major compartment of all planctomycetes, containing all the ribosomes and nucleoid DNA of the cell, and bounded by the ICM. In the case of *G. obscuriglobus*, the ICM seen in section actually invaginates into the pirellulosome to form the outer membrane of the envelope of the nuclear body region containing the nucleoid, but in planctomycetes such as those of the Pirellula group the ICM always forms a continuous boundary to the ribosome- and nucleoid-containing pirellulosome compartment, and the compartment was named for its discovery in the Pirellula group (Lindsay et al. 1997). It was identified in the species *Pirellula staleyii* and what is now known as *Blastopirellula marina* as a region containing a condensed fibrillar nucleoid and electron-dense ribosome-like particles ca. 9–18 nm in diameter, and bounded by a single ICM membrane up to 5 nm wide in *B. marina* but as thin as 3.2 nm wide in *Pi. staleyii*. (Lindsay et al. 1997). As expected from the ribosome-like particles, the pirellulosome in these species contains RNA as assayed via RNase-gold labeling of sectioned cells. A second region of the cell surrounding the pirellulosome was termed initially a polar cap region but was later known as the paryphoplasm after it was found to be a type of compartment shared by all planctomycetes examined.

In *Rhodopirellula baltica*, however, also a member of the Pirellula group, what seem to be several smaller versions of membrane-bounded pirellulosomes are enclosed within the paryphoplasm, probably containing ribosomes but apparently without nucleoids (Schlesner et al. 2004). This needs reexamination with tomography to exclude a complex 3D shape of a single pirellulosome resulting in this 2D appearance—the underlying cell plan may be identical to that of other Pirellula group members. *Planctomyces limnophilus* appears to have the same type of pirellulosome and pirellulosome contents as other members of the Pirellula group, but with complex shapes and organization giving rise to possible similarities to the smaller versions of pirellulosome seen in *Rhodopirellula baltica* (Jogler et al. 2011). In *Isosphaera pallida* the ICM can invaginate to form a large lobe of the paryphoplasm so that the pirellulosome forms a crescent-shaped region at the cell margins, with a similar phenomenon also observed in *Planctomyces maris* (Lindsay et al. 2001). In the Pirellula group, ribosomes of the pirellulosome can be seen in some regions to line the inner boundary of the ICM, suggesting that co-translational secretion of newly synthesized proteins into the paryphoplasm may occur.

In *G. obscuriglobus*, however, ribosomes appear to be arrayed linearly along both the inner and outer membranes of the nuclear envelope, at least in the case of sectioned cells prepared via high-pressure freezing followed by cryosubstitution.

This gives an appearance to the nuclear envelope of a eukaryotic endoplasmic reticulum. One assumes that the pirellulosome performs many of the essential metabolic functions of the cell such as glycolysis and because of the presence of DNA and ribosomes is almost certainly the location of all mRNA transcription and protein translation. However, future experiments locating for example glycolytic and other metabolic enzymes, DNA-dependent RNA polymerase, and the more abundant mRNA species and ribosomal proteins via immunogold techniques combined with transmission electron microscopy of sectioned cells are needed to confirm this.

2.2.6 Condensed Nucleoid

All planctomycete species so far examined by TEM of sectioned cells which have been prepared by cryofixation followed by cryosubstitution possess condensed fibrillar nucleoids residing within the pirellulosome or in the case of *G. obscuriglobus*, the membrane-bounded nuclear region within the pirellulosome. Such nucleoids display various types of folding of the fibrils within the nucleoid, suggesting a high degree of condensation even relative to nucleoids of bacteria such as *E. coli* where a “coralline” nucleoid extends throughout the cell volume. In the species *G. obscuriglobus*, at least where cell division for a planctomycete has been more extensively examined than for other planctomycetes, the nucleoid appears to remain condensed throughout the cell cycle, even during passage of the nucleoid through the bud neck into the new bud where the fibrils appear to unfold to some extent but remain associated (see below). Electron tomographic reconstruction of the *G. obscuriglobus* nucleoid from sectioned cryosubstituted cells demonstrates a liquid crystalline cholesteric organization (where molecules such as DNA filaments are ordered in each of a series of helically arranged layers where molecules in each layer are rotated relative to each other), resulting in the DNA arranged in a series of nested arcs visible in TEM of sectioned chromosomes similar to that found on the chromosomes of eukaryotic dinoflagellates (Yee et al. 2012). The condensation of nucleoids in planctomycetes has implications for how transcription and DNA replication may be organized in these organisms, and the location of RNA polymerases via immunogold electron microscopy may help to illuminate this arrangement. There is some indication that folded nucleoids in *G. obscuriglobus* may divide as a unit (Yee 2012), and the occurrence of multiple nucleoids in *G. obscuriglobus* suggests that segregation of the chromosomes of nucleoids may occur within cells before or during budding division.

It has been suggested that the condensation of the nucleoids of *G. obscuriglobus* may be one mechanism of its pronounced resistance to both UV and gamma radiation (Lieber et al. 2009a). It is not known whether other planctomycetes also display such resistance, but this would be predicted if condensation is a major radiation resistance mechanism.

2.3 Planctomycete Russian Dolls: Cells with Three Compartments

The simplest compartmentalized cell plan in planctomycetes is that there are only two compartments, the paryphoplasm and pirellulosome, separated by the major internal ICM membrane (Fig. 2.1). However, at least two groups of planctomycetes contain within their pirellulosome a third compartment bounded by a membrane or membranes. So viewed in three dimensions, these cells consist of at least three nested containers, each defined by a membrane separating one compartment from another—the paryphoplasm, the pirellulosome, and a third compartment within the pirellulosome. In anammox planctomycetes, there is a metabolic organelle, the anammoxosome, bounded by a single membrane, the structure and function of which are outlined in Chap. 4 of this volume. It contains enzymes of significance to the unique anaerobic ammonium-oxidizing ability of anammox planctomycetes, and the anammoxosome membrane contains ATP synthase (van Niftrik et al. 2010) consistent with the development of proton motive force across the membrane, making it a unique energy-generating internal organelle for bacteria. We will emphasize here the second example, occurring throughout strains within the *Gemmata* clade of planctomycetes, including the model *Gemmata obscuriglobus*. In this organism, the nucleoid is surrounded by an envelope which in many regions consists of two membranes closely apposed, in a manner analogous to that observed for the nuclear envelope of eukaryotes (Fig. 2.3). Serial sectioning of cryosubstituted cells (Lindsay et al. 2001) and more recently cryotomography (in preparation) confirm that the nucleoid and associated cytoplasm surrounded by membranes form a “nuclear body” region analogous to a eukaryotic nucleus. TEM of freeze-fracture replica is also consistent with a bounded nuclear region (Fig. 2.4). The analogy to a nucleus occurs in at least two ways—the envelope commonly consists of two closely associated membranes, and all the cell’s DNA appears to be enclosed by a membranous envelope. In addition there are often ribosomes lining the outer membrane (Fig. 2.3b), as in the outer membrane of eukaryote nuclei. However, there also appear to be ribosomes or at least ribosome-like particles inside the nuclear body and lining the inner side of the inner membrane of the double-membraned envelope (Fig. 2.3b), thus differing from the situation in eukaryotic nuclear envelopes where only the outer nuclear membrane has bound arrays of ribosomes. Significantly, the *Gemmata* three-compartment cell plan including nuclear envelope with two membranes is conserved within at least six strains clustering in the *Gemmata* clade via 16S rRNA-based phylogenetics (Wang et al. 2002). This applied to strains both relatively distant and relatively close to *G. obscuriglobus*, as long as they clustered within a *Gemmata* group significantly supported as a separate clade within a phylogenetic tree of planctomycetes, suggesting that the ancestor of the *Gemmata* group also possessed this cell plan. Cell plan types may be conserved within planctomycetes—the *Pirellula* group members all have the relatively simple two-compartment plan, as do members of *Planctomyces* and *Isosphaera* groups. *Zavarzinella*, a genus related to the *Gemmata* group (Kulichevskaya et al. 2009), also appears to possess

a somewhat similar plan (Lee 2010), but though there is a double-membrane nuclear envelope, whether there is a fully enclosed nuclear body compartment needs to be tested by serial sectioning or tomography of sectioned cells.

It should be noted that at least under some growth conditions, e.g., high nutrient levels, the internal membranes of *G. obscuriglobus* can be quite complex and the nuclear body or bodies are difficult to delineate, especially if only the view from individual sections is considered, but networks of double membranes with attached ribosomes are usually seen even in these cases. In very old cultures, only a single membrane is sometimes seen to bound a large region containing condensed nucleoid and ribosomes, with large vesicles containing ribosomes through the paryphoplasm at the cell rim. In some studies, networks of double membranes have been seen associated with condensed nucleoids but not necessarily forming a bounding envelope in 3D (Lieber et al. 2009b).

Where enclosed nuclear bodies are seen in cells processed from young cultures (displaying a high proportion of motile cells), the outer membrane of the nuclear envelope is often seen to be continuous in one or more places with the ICM, sometimes forming a large region of paryphoplasm. In regions where this occurs, which may sometimes be extensive, only a single membrane (continuous with the inner nuclear envelope membrane) surrounds the nucleoid and associated ribosomes of the nuclear body. This is exactly equivalent topologically to the regions of the nuclear envelope of eukaryotes where endoplasmic reticulum is continuous with the outer nuclear membrane, but in the latter cases the region of single membrane is very limited. From this perspective, the paryphoplasm and especially the pericisternal space are equivalent to the lumen of the RER of eukaryotes. The “pericisternal space” between the inner and outer membranes of the nuclear envelope is clearly continuous topologically with the paryphoplasm, though it may not be functionally identical.

The apparently sealed nature of the nuclear envelope in *G. obscuriglobus* has implications for molecular biology processes in these cells. Transcription and DNA replication must occur in the nuclear body and be restricted to it, though this requires confirmation by techniques such as autoradiography or bromodeoxyuridine-based methods. We do know from in situ hybridization experiments on sectioned *G. obscuriglobus* cells via EM using labeled oligonucleotides and immunogold methods (Butler 2007) that 16S rRNA appears within the nuclear body and in the pirellulosome outside the body (but not within the ribosome-free paryphoplasm, as we would of course predict), compatible with the idea that translation might occur in both regions where ribosome-like particles are identified via EM.

As in eukaryote cells, there must be some provision for communication of macromolecules such as protein and RNA between the nuclear body interior where the genomic DNA resides and the rest of the ribosome-containing pirellulosome cytoplasm, assuming that these separated ribosomes are active in translation. Ribosomes bound to both sides of the nuclear envelope suggest secretion of some proteins across the envelope as they are synthesized, but the ribosomes bound to the outer nuclear membrane as well as the free ribosomes in the rest of the pirellulosomes must use mRNA transported across the nuclear envelope. Proteins translated on free ribosomes of the pirellulosome, if destined for the nucleus, must also be transported

across the envelope, but it is possible that these ribosomes are specialized to non-nuclear proteins of course. There are indications that the “double”-nuclear envelope is in fact a series of “folded single-membrane” flattened vesicles where the outer and inner nuclear membranes meet in continuity at certain points around the nucleus (Fuerst 2005). This immediately suggests that these cells may possess a mechanism of nucleocytoplasmic transport depending on some form of nuclear pore structure interrupting the envelope at points where inner and outer nuclear membranes meet. There are indeed preliminary indications of nuclear pore structures, and extensive proteomic studies on fractionated envelope membranes containing such pores are under way in the authors’ laboratory to investigate the possibility of their containing homologous proteins to those of eukaryotic nuclear pore complexes.

The possible evolutionary pressures which might give rise to origins of nuclear structures in a bacterium or an ancestor of a bacterium are considered in Chap. 11. Clearly the occurrence of these “nested box” cell plans in bacteria is of great significance for understanding possible reasons for how and why a eukaryote nucleus may have originated and been sustained through later evolution. This is regardless of whether any evolutionary homology or relationship by descent exists between planctomycetes and eukaryotes. Even an analogous structure may help us understand why such a structure could have evolved and been of adaptive value to the organism in which it originated. In any case, *G. obscuriglobus* is likely to teach us a lot of new molecular biology and cell biology. When considering planctomycetes, it is advisable to understand also the only example of integrated endomembrane systems and membrane-bounded organelles we are able to study, that of the eukaryote cell. The discovery of endocytosis ability in *G. obscuriglobus*, possessing also such a fascinating membrane-bounded nuclear structure, may prove to be only the first such phenomenon of integrated cell biology with astonishing resemblance to similarly integrated eukaryote cell biology.

2.4 Cell Division in Planctomycetes and the Model of *Gemmata obscuriglobus*

The dominant form of division in planctomycetes is budding, in which a “mirror image” of a much smaller daughter cell is formed from a large mother cell. Members of genera such as *Pirellula*, *Blastopirellula*, *Rhodopirellula*, *Planctomyces*, *Gemmata*, *Isosphaera*, and *Aquisphaera* use this form of reproduction, but in *Isosphaera* intercalary buds are formed along a filament of cells (Giovannoni et al. 1987b) and they do not separate from the mother cell as they do in other budding planctomycetes. Binary fission occurs in a minority of planctomycetes, including the anammox planctomycete “*Candidatus Kuenenia stuttgartiensis*” (van Niftrik et al. 2008b, 2009)—see Chap. 4—and the marine genus *Phycisphaera*, proposed as a member of a distinct class *Phycisphaerae* of the planctomycete phylum (Fukunaga et al. 2009). In the anammox planctomycete “*Candidatus Kuenenia stuttgartiensis*,” not only does the cell constrict at a midpoint during division, but there is also apparent division of the

anammoxosome organelle within the cell. A cell division ring is observed within the paryphoplasm (rather than in cytoplasm as in bacteria with FtsZ rings) and composed of a protein with no clear homology to the FtsZ protein of the divisome of other bacteria (van Niftrik et al. 2009). So even where budding is not found in a planctomycete, cell division can have unique features not found in other bacteria.

The life cycle of an organism now known to be a *Pirellula staleyi* strain, known initially as Morphotype IV of the “Blastocaulis-Planctomyces group,” was early established to involve a motile swarmer cell phase (Tekniepe et al. 1981), and *Rhodopirellula baltica* and *Planctomyces limnophilus* appear also to have a similar cycle (Gade et al. 2005; Jogler et al. 2011; Wecker et al. 2010). In the *Pi. staleyi* strain (Tekniepe et al. 1981), after a swarmer (or more accurately “swimmer”) cell matures into a mother cell (phase A) budding (phase B) over *ca.* 3 h involves formation of a protuberance on the mother cell gradually developing into a daughter cell increasing to about half the mother cell diameter and synthesizing a flagellum while attached. A motile “swarmer” (i.e., swimmer) cell is eventually detached. There are significant phases in the cycle in addition to budding itself involving lags (during which events such as DNA replication might occur)—detached daughter buds mature (phase A) and gradually enlarge until *ca.* 30 h after budding, they synthesize fimbriae but lose their flagellum—as mother cells they then enter budding taking 3 h (phase B). Multiple budding from a mother cell is possible, up to four times from the same pole, with significant “resting phase” lags (phase C) of 7–9 h before new bud formation, suggesting cycles similar to DNA replication S phases of eukaryote and budding *Caulobacter* bacteria cell cycles. In this *Pirellula* group organism, elegant immunoferritin labeling of cell surfaces of mother cells allowed to bud, combined with whole-cell TEM, has shown that *de novo* synthesis of surface antigens occurs over the bud, and that some intercalation of newly synthesized surface occurs also on the mother cell surface as it matures (Tekniepe et al. 1982).

However, it is not clear that all planctomycetes have a motile swarmer phase, and acidic wetland strains of at least two genera, *Singulisphaera* and *Telmatocola*, have only nonmotile cells (Kulichevskaya et al. 2008, 2012). *Isosphaera pallida* displays gliding motility over surfaces but not swimming motility. Mother cells as well as swarmer cells may have flagella in *Gemmata*.

In *Rhodopirellula baltica*, pear-shaped mother (“adult”) cells are nonmotile and by phase contrast microscopy a polar organization is seen (consistent with electron microscopy of thin sections showing the *Pirellula*-type polar cap region); crateriform structures have a polar distribution at the broader cell pole and fimbriae originate from them (Gade et al. 2005). Rosettes can arise from attachment of the smaller nonreproductive cell pole via a holdfast substance. Budding occurs from the broader, reproductive cell pole and produces a smaller “mirror image” of the mother cell; buds have a uniform crateriform structure distribution and a subpolar flagellum so that they are released as a “swarmer” cell. Particular morphotypes could be correlated with distinct growth phases, swimmers decreased in later phases, and rosettes of several cells were which dominated stationary phase. Proteomics suggested that such changes could be correlated with protein regulation.

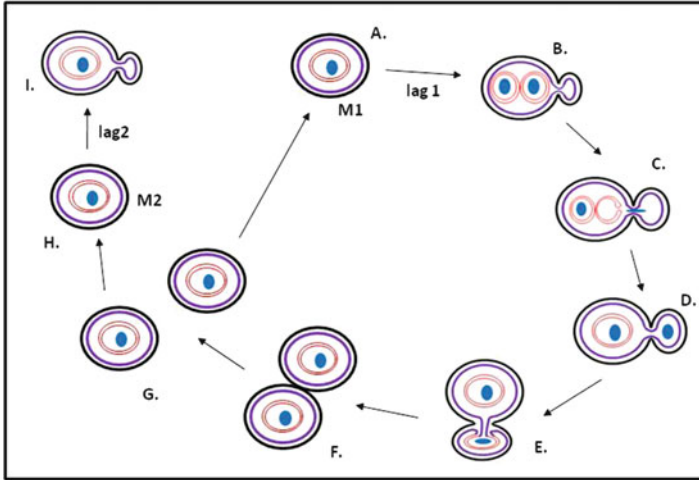


Fig. 2.6 Schematic diagram of the cell cycle and budding division of *Gemmata obscuriglobus*. In (A) and (B), a bud appears as a protuberance from one point on the mother cell M1, which possesses a nucleoid (blue) surrounded by a double-membrane nucleoid envelope (red). The mother cell ICM (dark purple) is continuous with the ICM of the bud (also dark purple). The bud initially does not possess a nucleoid. In stage C, the nuclear body double-membrane envelope opens up at the bud neck and the naked nucleoid passes through to the bud via the bud neck. (D) shows the result of that process where a single nucleoid is now present in the bud but without a membranous envelope at this stage. In (E), the bud nucleoid becomes enveloped by two membranes, an inner membrane (red) continuous with mother cell ICM and an outer membrane (also red) continuous with the bud ICM (dark purple). In (F), the bud nucleoid is completely surrounded by the two closely apposed membranes of the matured nuclear envelope (shown in red) where membrane fusion and pinching off have resulted in a double-membrane nucleoid envelope completely separated from ICM membranes. Incomplete separation is however commonly observed in micrographs. (F) Is the end-point of the mechanism where the bud reaches similar cell size to that of mother cell M1. (G) Shows the separation of the mother cell and the matured bud. The mother cell M1 initiates the next budding cycle after a 2–4-h lag (lag 1) while the matured bud M2 originating from M1 starts its first budding cycle (H and I) after a 3–5.5-h lag (lag 2). Courtesy of Kuo-Chang Lee (modified from (Lee et al. 2009))

In most of the above studies, the fate of the internal membranes critical to compartmentalization in planctomycetes has not been determined. It is clearly of interest to determine what happens concerning the internal structure during the process of division, and especially so in the case of *Gemmata obscuriglobus* with its membrane-bounded nucleoid and three compartments. Cells of *G. obscuriglobus* divide by budding over *ca.* 12 h until new bud separation and a mother cell can bud repeatedly as in other planctomycetes, taking several hours to start new bud formation (Fig. 2.6) (Lee et al. 2009). Both fluorescence microscopy with DNA stains and TEM of thin-sectioned cryofixed cells show that initially the new immature bud is devoid of nucleoid, but that when the nucleoid does appear in the bud it is initially naked and not surrounded by membranes. It appears that there is a stage in which a nucleoid is passed to the bud through the bud neck (Fig. 2.7a) so that the mother cell nuclear envelope must be open at some point of their extent. Later on membranes

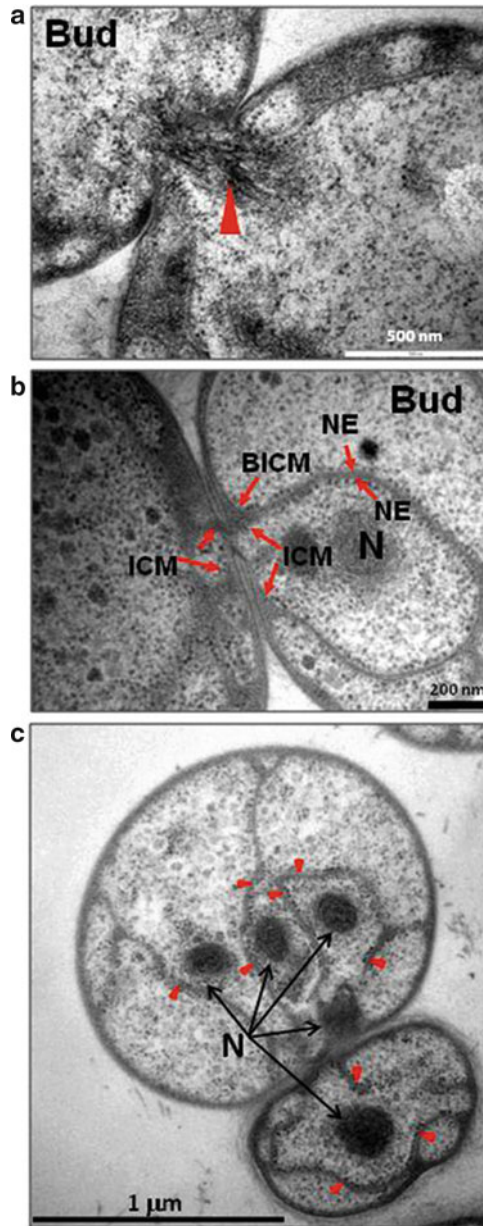


Fig. 2.7 Transmission electron micrographs of sectioned cells of *Gemmata obscuriglobus* processed by cryosubstitution, illustrating selected stages in cell division. (a) Fibrillar nucleoid material (red arrow) is shown passing through the neck of a newly formed bud on the left. Bar 500 nm. (b) A later stage where the nucleoid (N) has passed into the bud, and the process of formation of a new nuclear envelope around a naked nucleoid is nearing completion. The new nuclear envelope (NE) is now formed of two membranes, each with bound ribosomes—the outer membrane of the NE is continuous with the intracytoplasmic membrane of the bud (BICM), while the inner membrane of the nuclear envelope is continuous with the ICM of the mother cell (ICM). Bar marker 200 nm. (c) Budding cell of

appear to enclose the bud nucleoid until a complete double-membrane envelope is generated, and this seems to occur by an intriguing process perhaps unique within living organisms where the ICMs of both mother cell and bud contribute to the double membrane of the bud's nuclear envelope. At one late stage in formation of the mature bud, the inner membrane of the bud nuclear envelope shows continuity with the ICM of the mother cell, while the outer membrane of the bud nuclear envelope is continuous with the ICM of the bud (Fig. 2.7b). This suggests that the mode of distribution of nucleoid and nuclear body is not analogous to either closed mitosis as occurs in yeast or open mitosis of animal cells where nucleoid envelope disassembles. However, there may be some similarities with some aspects of open mitosis where new nuclear envelope formation is derived from existing endoplasmic reticulum membrane (Anderson and Hetzer 2007), which planctomycete nuclear envelope and its outer membrane resemble in some ways (e.g., regarding ribosome binding). There is no evidence however of any microtubules which could form mitotic spindles, so such analogies are limited.

Both fluorescence microscopy (Lee et al. 2009) and EM of thin-sectioned cells suggest that at least under some growth conditions the mother cell may possess multiple nucleoids and multiple nuclear bodies, perhaps up to four (Fig. 2.7c). Flow cytometry estimating DNA content of cells from an actively dividing culture is consistent with this (unpublished results, Fuerst lab; (Lee 2010)). Mother cells may replicate DNA and prepare nucleoids for transfer to a new bud on the next division, and this would be consistent with the lag observed between budding events.

Concerning molecular mechanisms underlying division in planctomycetes the key bacterial cytoskeletal protein and divisome component FtsZ appears to be absent from the available genomes examined. This implies another type of division system, as occurs in some Archaea where homologs of the eukaryotic ESCRT proteins function in division. One of the few components of the FtsZ-linked divisome in planctomycetes appears to be FtsK. In *G. obscuriglobus*, there is some evidence using antibodies against it in conjunction with immunogold that it is associated with the chromosomal DNA of the nucleoid, even as the nucleoid passes into the bud during division (Lee 2010). This is consistent with the known role of FtsK in chromosome segregation but is not informative concerning processes of constriction at the bud neck. Since this process seems not to involve peptidoglycan one expects it to be distinct from septation in other bacteria.

Allowing for limitations of primary structural bioinformatics homology searches, a comparative genomics study of planctomycetes suggests that many genes homologous with cell division genes of other bacteria may be present in all non-anammox

Fig. 2.7 (continued) *G. obscuriglobus*, the mother cell, half of which contains multiple nucleoids (N) and complex nuclear envelope double membranes (indicated by red arrowheads) surrounding the nucleoids. These nucleoids appear to be in separate nuclear body compartments suggesting possible division of nuclear bodies as a whole during the DNA replication S phase of the cell cycle preceding budding. The bud has one nucleoid but the nuclear envelope is not fully closed at this point. Ribosomes line nuclear envelope membranes in both mother cell and bud. Bar marker 1 μm

planctomycetes for which genomes are available including homologs of genes for ClpP, CpaE, ddl, FtsE, FtsK, ParA, MraW, and MraY (Jogler et al. 2012), while homologs of for example MraZ were only found in *Planctomyces* and *Blastopirellula*, of FtsW only in *Planctomyces*, and other genes for proteins such as homologs of the significant shape-determining actin-like cytoskeletal protein MreB also only seem present in some genera (e.g., *Planctomyces* and *Blastopirellula* but not *Gemmata*). Some species have homologs of the FtsZ-like FtsZl-1 proteins, which do not in any case seem likely to form Z-rings such as those composed of FtsZ. Since planctomycetes do not synthesize peptidoglycan, and FtsZ-based divisomes are largely based on interaction with peptidoglycan synthesis enzyme systems, we might not expect many division gene homologs related to peptidoglycan. The alternative ESCRT system of Archaea homologous to eukaryote ESCRT-III proteins does not seem to be present in planctomycetes, but some homology with VPS4 components of such a system is found, albeit modified in such a way as to throw doubt on homology of their function with the archaeal system.

2.5 Endocytosis in Planctomycetes as a Functional Correlate of Cell Compartments

If we had all the genes and the architectural cell plan for a synthetic planctomycete, with all its unique internal membrane and compartment features, we might expect such a cell to also have abilities unique to planctomycetes among the bacteria. One of the characteristics we would expect for a cell based on the plan of *Gemmata obscuriglobus* is the ability to take up proteins from the external medium. This ability is not found elsewhere in the bacteria but is expected of a eukaryote capable of the process called endocytosis. *G. obscuriglobus* can incorporate proteins including green fluorescent protein (GFP), immunoglobulin, and bovine serum albumin, and competition between uptake of such proteins is consistent with a receptor-mediated process and one capable of saturation of what must be a rather nonspecific protein receptor (Lonhienne et al. 2010). Such proteins as bovine serum albumin appear to be degraded once inside the cell, suggesting a nutritional function for the protein uptake. Evidence from fluorescence microscopy for GFP and immunogold electron microscopy of sectioned cryosubstituted cells (Fig. 2.8) shows that the protein is taken up only into the paryphoplasm compartment and does not appear elsewhere in the cell. It does not appear in the pericisternal space between the two nuclear envelope membranes even though this space is topologically continuous with the paryphoplasm. It appears to be taken up via small (*ca.* 50 nm diameter) vesicles within the paryphoplasm resulting from an initial invagination of cytoplasmic membrane to which the protein cargo binds, presumably via receptors on the external leaf of the cytoplasmic membrane bilayer (see Fig. 2.9 and Fuerst and Sagulenko (2010); Lonhienne et al. (2010)). Such vesicles appear to possess associated proteins homologous to MC proteins of eukaryotes such as the clathrins known to be necessary for receptor-mediated endocytosis in eukaryotes.

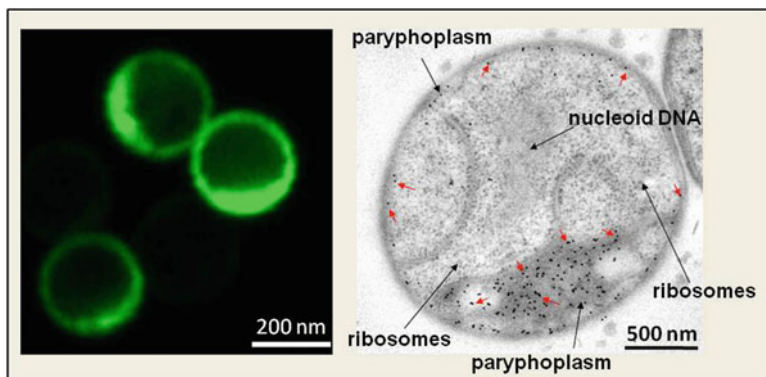


Fig 2.8 (a) Fluorescence micrograph illustrating endocytosis-like uptake of green fluorescent protein (GFP) by *Gemmata obscuriglobus*. Note that GFP (green) is localized to a distinct outer region of each cell consistent with a localization in the paryphoplasm. Bar: 2 μ m. (b) Transmission electron micrograph of sectioned cell of cryosubstituted *G. obscuriglobus* illustrating internal GFP protein that has been incorporated by an endocytosis-like process. The 10 nm colloidal gold particles (red arrowheads) result from immunogold labeling using anti-GFP antibody, and are localized only in ribosome-free electron-dense paryphoplasm regions. Ribosomes are confined to the pirellulosome including the nuclear body containing the single nucleoid. Bar: 500 nm

The process of protein uptake in *G. obscuriglobus* bears a number of similarities to receptor-mediated, clathrin-dependent endocytosis in eukaryotes. Uptake of individual specific proteins appears to compete with the uptake of other proteins (e.g. uptake of bovine serum albumin or a mouse immunoglobulin inhibits uptake of green fluorescent protein, and uptake can reach saturation with increasing concentration of the external protein). The mechanism of uptake via vesicles budded from plasma membrane is also consistent with this type of endocytosis.

Finally, the presence in the genome of *G. obscuriglobus* of homologs of the eukaryote MC protein family, and association of one of the proteins with vesicles within sectioned whole cells of *G. obscuriglobus* (Santarella-Mellwig et al. 2010), is consistent with this mechanism. The significance of MC proteins for planctomycetes is indicated by their being a component of the core genome of eight different planctomycete species defined relative to *E. coli* and *Bacillus* genomes (Jogler et al. 2012).

The uptake of proteins in *G. obscuriglobus* occurs only via the paryphoplasm or vesicles in the paryphoplasm—other cell compartments are not labeled in the process. This provides strong confirmation of the functional reality of compartmentalization in this planctomycete and is consistent with the integrity of the paryphoplasm as a compartment separated from other cell compartments in this organism. Protein uptake also involves degradation of the protein, which seems to also be confined to the paryphoplasm, so that the function of the endocytosis process may depend on compartmentalization, confining processing of external proteins such that proteins in other compartments are protected. It seems likely that either specialized lysosome-like vesicles will be found or the paryphoplasm itself is something like a lysosome,

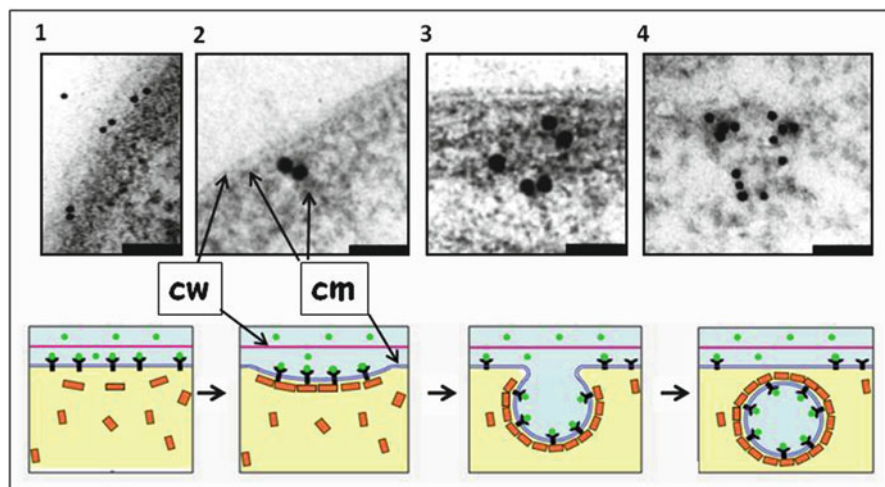


Fig. 2.9 TEM of sectioned high-pressure frozen cryosubstituted *Gemmata obscuriglobus* cell preincubated with GFP (*upper row of panels*), and a proposed model of the events involved in uptake of extracellular proteins (*lower row of panels*). Micrographs in the *top row* show enlarged views of the potential different stages of GFP uptake at peripheral cytoplasmic membrane and paryphoplasm in cells of *G. obscuriglobus*. Cells were incubated with GFP, immunolabeled with anti-GFP antibody and then cryofixed, processed via cryosubstitution, and sectioned for TEM. Gold particles indicating GFP can be seen associated with cytoplasmic membrane (1 and 2) and then with the membrane of vesicles inside the paryphoplasm region in the cell interior (3 and 4). The schematic series of diagrams in the *bottom row* show corresponding suggested stages for the GFP uptake process and its mechanism, consistent with the evidence from micrographs; stage 1 shows binding of GFP to plasma membrane, and stages 2 and 3 show initial steps of invagination of plasma membrane and association of GFP with vesicles in the process of being generated. At the final stage (stage 4) the vesicle formation is complete. In this model GFP ligand in the external milieu binds to receptors in the cytoplasmic membrane, MC-like (clathrin-like) proteins coat the outside of the vesicle, possibly in association with other ancillary adaptin proteins (not shown), and ligand becomes oriented to the inside membrane surface of the vesicle during its formation due to the effect of infolding. Infolding and formation of vesicles occur in the paryphoplasm. Arrows indicate cytoplasmic membrane (cm) indicated in blue and cell wall (cw) is indicated in magenta; green circles in the model indicate GFP; orange rectangles, MC-like proteins; black Y's, protein receptor. Paryphoplasm is indicated in yellow. Bars, 200 nm (micrograph 1), 50 nm (micrographs 2 and 3), and 100 nm (micrograph 4). Modified from Fig. 1b of Fuerst and Sagulenko (2010)

though the former is more likely. If so vesicles with acid pH containing specialized degradative enzymes operating at such pH might be found. Consistent with the nature of the cytoplasmic membrane is that vesicles can be seen to be budding into the paryphoplasm from that membrane (Fig. 2.9) so that it is indeed the outermost membrane of the planctomycete cell, external to the ICM bounding the inner side of the paryphoplasm, as mentioned above (Sect. 2.2.2).

Homologs of MC proteins of eukaryotes occur in *G. obscuriglobus* as well as other planctomycetes and at least some other members of the related PVC superphylum (see Chap. 3). In eukaryotes such MC proteins include clathrin forming the “cages” around vesicles formed during receptor-mediated endocytosis. The MC protein gp4978 which has been found associated with vesicles of *G. obscuriglobus* including those associated with endocytosed protein not only bears structural

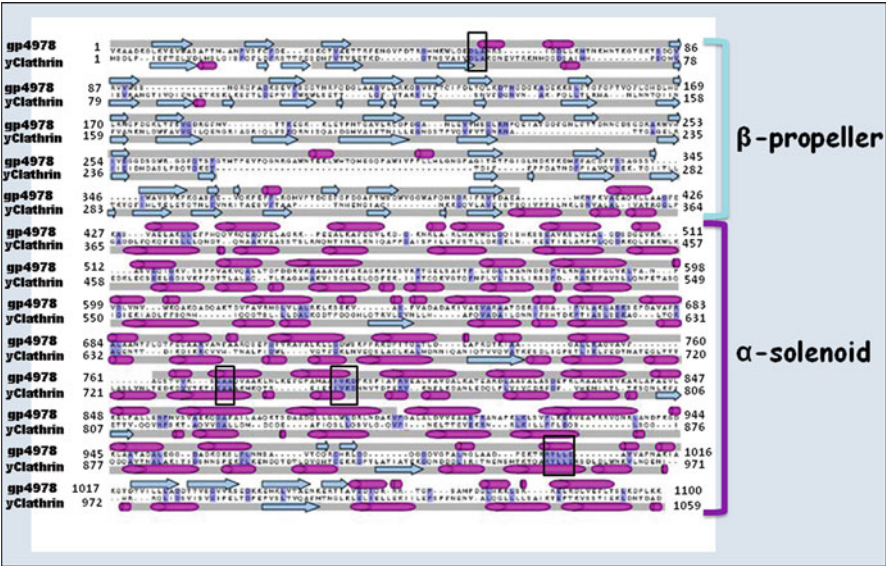


Fig. 2.10 Alignment and secondary structure comparison of gp4978 MC protein homolog from *Gemmata obscuriglobus* and yeast clathrin heavy chain (Uniprot ID: P22137- CLH_YEAST) from *Saccharomyces cerevisiae*. Protein profiles were aligned with the “hhalign” program. Secondary structures (alpha-helices and beta-strands) predicted with the PSI-PRED program are represented as pink cylinders (alpha-helices) and cyan arrows (beta-strands) above and below the aligned sequences, respectively. Where regions containing either beta-sheets or alpha-helices conform to domains corresponding to beta-propeller or alpha-solenoid domains, the predicted (for gp4978) and known (for clathrin) domains of these beta-propellers and alpha-solenoids are highlighted by a grey shading of the secondary structures. Amino acids in the alignment where identities occur in three or more consecutive amino acids are indicated by boxes outlined in black

similarity to eukaryotic MC proteins in its possession of alpha-solenoid and beta-propeller domains but even at the primary amino acid sequence level shows some remarkable identities with a protein such as clathrin heavy chain of yeast, scattered through the sequence (Fig. 2.10).

2.6 Molecular Aspects and Implications of a Compartmentalized Cell Plan

2.6.1 The Potential Role of Sterols in Membrane Rigidity Regulation and Compartment/Vesicle Formation

Two strains of at least one genus of planctomycete, *Gemmata*, including the model organism *G. obscuriglobus*, as well as soil strain Wa1-1, synthesize simple sterols such as lanosterol and parkeol (Pearson et al. 2003). Lanosterol is an intermediate in the synthesis of compounds such as ergosterol and cholesterol by eukaryotes, but

Gemmata strains stop at the lanosterol stage. Amounts (e.g. 20 mg/g of cell biomass) are some ten times of those found in cells of another bacterial sterol producer, *Methylococcus capsulatus*. In *Gemmata*, parkeol is actually produced in even higher amounts relative to lanosterol, a unique synthetic distribution for any organism. Interestingly, extractable lipid fractionation largely misses these sterols, which require direct hydrolysis to be released. Although some other groups of bacteria synthesize sterols, the pathway present in *G. obscuriglobus* may be the simplest known (Pearson et al. 2003). There appears to be both a putative squalene monooxygenase and an oxidosqualene cyclase, a minimum needed for lanosterol and parkeol synthesis, but the WPV motif in the oxidosqualene cyclase indicates a nondiscriminate enzyme similar to that observed in some laboratory mutations of plant cycloartenol synthase studied in yeast (Hart et al. 1999). There is some evidence that the enzymes may have homology with those of eukaryotes (Pearson et al. 2003; Summons et al. 2006) but they are not related closely to any contemporary group of eukaryotes, and this suggests that if lateral gene transfer from eukaryotes is to account for sterol synthesis genes in bacteria, then the transfer must have been quite ancient (Chen et al. 2007; Desmond and Gribaldo 2009). The oxidosqualene cyclase of *G. obscuriglobus* differs from all other such enzymes in particular amino acid position substitutions at F444L and S445G close to T381 and C449 and it has been suggested that they may be significant in biasing the particular sterol production yield towards the unusual parkeol “protosterol” (Summons et al. 2006). Since sterols may enable deformation of membranes more easily than other lipid components, the ability to synthesize sterols may be relevant to the ability of planctomycetes to form compartments via internal membranes and to form endomembrane vesicles during endocytosis (Summons et al. 2006). Sterol synthesis may have assisted compartment and vesicle formation by enabling greater membrane deformation ability (Bacia et al. 2005). Planctomycetes also synthesize hopanoids (Damste et al. 2004) more typical of bacteria and also thought to alter membrane properties, so planctomycetes may use both mechanisms for modifying membrane properties. It is conceivable that sterols are important for endocytosis and if so there may be a need for regulation of sterol synthesis by elements similar to the sterol regulatory element-binding proteins of eukaryotes (Castoreno et al. 2005).

2.6.2 Protein Secretion, Signal Peptides, and Signal Transduction Systems

The existence of internal compartments separated from other compartments of the cell by membranes, in some cases single membranes such as the ICM and anammoxosome membranes, suggests that mechanisms for protein transport across such membranes must exist, and perhaps also unusual features of signal transduction connected with the existence of the compartments and the difficulty of transmission of signals across compartments, for example for factors acting via transcription to yield signal output. Unusual signal peptides have been predicted for many planctomycete proteins of *Rhodopirellula baltica*, the species with the first complete genome

sequence among the planctomycetes (Studholme et al. 2004). There may be signal transduction systems especially suited to transmission of signals across internal membranes, as in the extracytoplasmic function (ECF) sigma factor system (Jogler et al. 2012), and these seem to have special properties in the planctomycetes.

A bioinformatic study of the genome of the anammox planctomycete “*Candidatus Kuenenia stuttgartiensis*” is highly relevant to both the potential systems for transport of proteins across membranes in a three-compartment planctomycete and the function of both cytoplasmic membrane and the membrane of the anammoxosome organelle (Medema et al. 2010). The Tat translocation system appears to be exclusively located in the organellar membrane, while the Sec system seems to occur on both cytoplasmic and anammoxosome membrane. Remarkably, the cytoplasmic membrane was predicted to be mainly a transport membrane rather than a membrane essential to metabolism containing cytochromes and the electron transport systems. Such metabolic functions were confined to the anammoxosome membrane, an internal organelle membrane, so that a situation analogous to a typical eukaryote with a mitochondrion seems to apply. This needs confirmation experimentally, but is consistent with some cytochemical data on cytochrome distribution in anammox planctomycetes (van Niftrik et al. 2008a).

Comparative genomics studies of non-anammox planctomycetes including *Rhodopirellula baltica* and seven species of *Pirellula*, *Blastopirellula*, *Planctomyces*, *Isosphaera*, and *Gemmata* have yielded some insights into the unusual features of the signal transduction systems of planctomycetes (Jogler et al. 2012). Planctomycetes possess all three known signal transduction systems operating in bacteria, one-component systems (ICSs), two-component systems (2CSs), and the ECF system.

However, planctomycetes show significant variations within these systems from other bacteria. Serine/threonine protein kinases (STPKs), common in eukaryotes, form a much more significant proportion of the ICS proteins than in other bacteria, where DNA-binding proteins dominate. It may be that the STPKs acting via direct protein phosphorylation rather than DNA-binding reflect the compartmentalization and separation of nucleoid from much of the cytoplasm. The 2CS system proteins of planctomycetes also display unusual features, many consisting of multiple potential input domains that represent hybrid complex histidine kinases, consistent with regulation via response regulators in complex phosphorelays needed for integrating different stimuli as found for the decision to sporulate in *Bacillus* for example. The 2CS response regulators include a novel type that appears unique to planctomycetes.

The ECF system involves alternative σ factors for RNA polymerase working in conjunction with negative regulation via anti-sigma factors, which may be transmembrane proteins able to release sigma factor for interaction with corresponding promoters on receiving a signal external to the membrane (Helmann 2002). *G. obscuriglobus* ECFs have substantial C-terminal extensions, which can be much longer than the core sequence of this protein (Jogler et al. 2012). The extensions of many proteins in the family distinguished as ECF01-Gob contain multiple WD40 repeats conceivably important for assembling multi-protein complexes. Most ECF01-Gob proteins are membrane anchored, harboring 1–3 putative transmembrane helices between the ECF core and the C-terminal extension and it has been

proposed that these transmembrane ECFs may enable signal transduction across internal membranes such as across the nuclear envelope. If this is so we should find evidence for this type of signaling protein in future *Gemmata* genomes when sequenced. This study also identified ECF groups functionally linked to STPKs, suggesting a novel signaling mechanism. All these bioinformatics deductions from genomic analysis need experimental verification, but the results are what we might predict if planctomycete cells must use novel signaling mechanisms necessitated by their internal compartmentalization.

2.6.3 Ribosome Binding to Membranes and Co-translational Secretion

In planctomycetes including members of the *Pirellula* group and especially *Gemmata obscuriglobus*, ribosomes can be seen lining internal membranes, in the case of *G. obscuriglobus* those of the nuclear envelope itself. In bacteria like *E. coli*, the signal recognition particle receptor FtsY is needed for targeting of ribosomes to membranes. However, in constructs depleted in signal recognition particle (via Ffh depletion) or translocons (via SecE depletion), intracellular membrane structures can be formed in *E. coli* cells which display ribosomes bound to these membranes (Herskovits et al. 2002). It is conceivable that accumulation of FtsY–ribosome complexes in such may even induce formation of intracellular membranes to which they become bound. Ribosome targeting to membranes and formation of an intracellular membrane could be linked in this model. If this applies to planctomycetes, it may not be very difficult or involve many genetic changes to evolve from a classical bacterium the complex wild-type state for planctomycete cells with intracellular membranes and the ribosomes bound to them. The phylogenetics and regulation of proteins such as FtsY, SecE, and Ffh in planctomycetes may be especially interesting for study as a key to the mechanism of internal membrane formation and internal occurrence of membrane-bounded ribosomes in planctomycetes. The selective pressure for retaining such internal compartments and protein secretion is not obvious, but could be linked to the need for separation of functional compartments connected with endocytosis and protein degradation (see Chap. 11).

2.6.4 Transcription and Translation Separation and Consequences

Transcription and DNA replication and thus RNA polymerase and DNA topoisomerase distribution are predicted to be predominantly restricted to the nuclear body. However with respect to association with the nucleoid DNA, this is not necessarily vastly different distribution to that reported for a bacterium with a free-floating nucleoid such as *Bacillus subtilis*—even without a nuclear membrane transcription and translation this can be separately located in bacterial cells (Lewis et al. 2000). The presence of the nuclear envelope has implications for RNA transport if we

assume that the ribosomes in the pirellulosome outside the nuclear body are active translationally, since mRNA must be transported across the envelope to those extra-nuclear ribosomes. This is so even if some translation actually occurs on the ribosomes in the nuclear body, the nature of which in relation to activity is not clear.

2.6.5 Endocytosis and Related Processes

We would expect that if the receptor-mediated endocytotic protein uptake in *G. obscuriglobus* is mediated via the MC protein clathrin homologs, homologs of other elements of clathrin-mediated endocytosis in eukaryotes might also be found and function in the mechanism. These might include homologs to the adaptins, membrane-curving proteins such as epsins and amphiphysins and Rab GTPases, and also the actins and myosins (Galletta and Cooper 2009). Affinity pull-downs using MC proteins combined with proteomics are one way to retrieve and identify such proteins beyond bioinformatic prediction.

In addition to receptor-mediated or MC (clathrin)-dependent endocytosis we would expect that fluid-phase endocytosis perhaps for transport of macromolecules other than proteins would occur and there is some preliminary evidence for this in a wider range of planctomycetes than the *Gemmata* strains exhibiting protein uptake (Sagulenko et al. in preparation). There is no evidence so far for any caveolae or caveolin-dependent process of cytosol.

Endocytosis in *G. obscuriglobus* implies some form of exocytosis as well, by which cytoplasmic membrane captured during protein uptake can be returned to the membrane. There is a need for turnover of membrane via an endocytic–exocytic cycle similar to that occurring in eukaryote cells. This is regardless of any homology of the process at the molecular level with eukaryotic processes. Secretory vesicles might occur in paryphoplasm for this process, and be involved in secretion of material such as cell wall proteins destined for the exterior of the cytoplasmic membrane and enlargement of cytoplasmic membrane as well as wall during budding division. Transcription must occur in the nuclear compartment and protein synthesis is unlikely to occur in the ribosome-free paryphoplasm, so proteins for the cell membrane and wall must be transported across the intracytoplasmic membrane—vesicles moving from ICM to cytoplasmic membrane and trafficking macromolecules via such transport may be a way to do this. Regulatory pathways for governing exocytosis relative to endocytosis can be predicted, perhaps even being controlled spatially in terms of membrane sites for secretion (Chamberlain et al. 2001).

2.6.6 Condensed Nucleoids: Is a Histone-Like Protein Needed for Nucleoid Folding in Planctomycetes?

It is striking that in sectioned cryosubstituted cells of named planctomycete species that have been so far examined by TEM, the nucleoid is highly condensed, such condensation occurs throughout the cell cycle, and the organization of DNA fibrils

displays a cholesteric liquid crystal organization similar to that seen for eukaryotic dinoflagellate chromosomes (Yee et al. 2012). Such condensation could conceivably involve homologs to eukaryote DNA-binding proteins such as histone-like proteins. So far bioinformatics searches for such proteins have not yielded clear candidate homologs of eukaryotic histones, though there is some evidence for homologs of the HU proteins occurring in other bacteria such as *E. coli*, unusual for the occurrence of two different versions in the same *G. obscuriglobus* species, one with an N-terminal and one with a C-terminal extension, unknown to occur together in other bacteria (Yee 2012; Yee et al. 2011). Condensation of nucleoid DNA has been proposed as a possible mechanism for resistance to both UV and gamma radiation displayed by *G. obscuriglobus* (Lieber et al. 2009a). It would be useful to test other planctomycetes for such radiation resistance, as correlation with nucleoid condensation follows from the hypothesis of that resistance mechanism. Proteomics of isolated nucleoid DNA may yield data concerning DNA-binding proteins responsible for condensation, but it is conceivable that such condensation is not due to protein but due to divalent cation interactions as suggested for dinoflagellate condensed chromosomes (Levi-Setti et al. 2008).

2.7 Conclusions

We have seen that there is both unity and diversity in the structural plans of planctomycete cells involving compartments defined by internal membranes. The universal occurrence of the major paryphoplasm and pirellulosome (riboplasm) compartments defined by the universal ICM endomembrane provides unity, while occurrence of a third compartment within the cell plan of *Gemmata* strains and anammox planctomycetes such as “*Candidatus Kuenenia*” is an example of the major type of variation within the shared cell plan. In both *Gemmata* and *Kuenenia*, the structural plans are correlated with function—endocytotic uptake of proteins into the paryphoplasm in the case of *Gemmata* and metabolic reactions for the biochemistry of ammonium oxidation in the case of *Kuenenia*. In the latter case, the anammoxosome organelle within the pirellulosome seems to be a case where the anammoxosome membrane is the site of proton motive force generation via an ATP synthase, analogous in some ways to the pmf generation across a eukaryotic mitochondrion membrane but unlike the mitochondrial biochemistry completely anaerobically. In the former case of the nucleated *Gemmata obscuriglobus*, protein uptake is via a mechanism bearing startling resemblance to receptor- and clathrin-mediated endocytosis of eukaryotic cells, down to the involvement of vesicles and associated clathrin-like proteins. Clearly the planctomycetes are significant for our understanding of the evolution of cell complexity, endomembranes, and the nucleus itself (see Chap. 11). Recent comparative genomic analysis suggests that even though we might expect sets of genes in addition to MC protein homologs shared by compartmentalized PVC superphylum members, we may have to experimentally determine their functions clearly (Kamneva et al. 2012). Future experimentation as well as comparative genomics

should yield some interesting answers and perhaps even more evolutionary questions about some of the major transitions in the history of the cell. Planctomycetes may also form a model for the simplest possible compartmentalized cell, perhaps forming the basis for a future synthetic biology construction of a complex cell with some characteristic functional features of eukaryote cell biology.

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References

- Anderson DJ, Hetzer MW (2007) Nuclear envelope formation by chromatin-mediated reorganization of the endoplasmic reticulum. *Nat Cell Biol* 9:1160–1166
- Bacia K, Schwille P, Kurzchalia T (2005) Sterol structure determines the separation of phases and the curvature of the liquid-ordered phase in model membranes. *Proc Natl Acad Sci U S A* 102:3272–3277
- Butler MK (2007) Planctomycete diversity and cell biology: perspectives from the molecular, cellular and organism levels. School of Molecular and Microbial Sciences, The University of Queensland, St. Lucia, Queensland
- Butler MK, Wang J, Webb RI, Fuerst JA (2002) Molecular and ultrastructural confirmation of classification of ATCC 35122 as a strain of *Pirellula staleyi*. *Int J Syst Evol Microbiol* 52:1663–1667
- Castoreno AB, Wang Y, Stockinger W, Jarzylo LA, Du H, Pagnon JC, Shieh EC, Nohturfft A (2005) Transcriptional regulation of phagocytosis-induced membrane biogenesis by sterol regulatory element binding proteins. *Proc Natl Acad Sci U S A* 102:13129–13134
- Cavalier-Smith T (2010) Origin of the cell nucleus, mitosis and sex: roles of intracellular coevolution. *Biol Direct* 5:7
- Cayrou C, Raoult D, Drancourt M (2010) Broad-spectrum antibiotic resistance of Planctomycetes organisms determined by Etest. *J Antimicrob Chemother* 65:2119–2122
- Chamberlain LH, Burgoyne RD, Gould GW (2001) SNARE proteins are highly enriched in lipid rafts in PC12 cells: implications for the spatial control of exocytosis. *Proc Natl Acad Sci U S A* 98:5619–5624
- Chen LL, Wang GZ, Zhang HY (2007) Sterol biosynthesis and prokaryotes-to-eukaryotes evolution. *Biochem Biophys Res Commun* 363:885–888
- Damste JSS, Rijpstra WIC, Schouten S, Fuerst JA, Jetten MSM, Strous M (2004) The occurrence of hopanoids in planctomycetes: implications for the sedimentary biomarker record. *Org Geochem* 35:561–566
- Desmond E, Gribaldo S (2009) Phylogenomics of sterol synthesis: insights into the origin, evolution, and diversity of a key eukaryotic feature. *Genome Biol Evol* 1:364–381
- Dougherty TJ (1985) Analysis of *Neisseria gonorrhoeae* peptidoglycan by reverse phase, high-pressure liquid chromatography. *J Bacteriol* 163:69–74
- Ebersbach G, Jacobs-Wagner C (2007) Exploration into the spatial and temporal mechanisms of bacterial polarity. *Trends Microbiol* 15:101–108
- Engelhardt H (2007) Are S-layers exoskeletons? The basic function of protein surface layers revisited. *J Struct Biol* 160:115–124
- Faller M, Niederweis M, Schulz GE (2004) The structure of a mycobacterial outer-membrane channel. *Science* 303:1189–1192
- Franzmann PD, Skerman VB (1984) *Gemmata obscuriglobus*, a new genus and species of the budding bacteria. *Antonie Van Leeuwenhoek* 50:261–268

- Fuerst JA (1995) The planctomycetes: emerging models for microbial ecology, evolution and cell biology. *Microbiology* 141(Pt 7):1493–1506
- Fuerst JA (2005) Intracellular compartmentation in planctomycetes. *Annu Rev Microbiol* 59: 299–328
- Fuerst JA, Sagulenko E (2010) Protein uptake by bacteria: an endocytosis-like process in the planctomycete *Gemmata obscuriglobus*. *Commun Integr Biol* 3:572–575
- Fuerst JA, Sagulenko E (2011) Beyond the bacterium: planctomycetes challenge our concepts of microbial structure and function. *Nat Rev Microbiol* 9:403–413
- Fuerst JA, Webb RI (1991) Membrane-bounded nucleoid in the eubacterium *Gemmata obscuriglobus*. *Proc Natl Acad Sci U S A* 88:8184–8188
- Fuerst JA, Gwilliam HG, Lindsay M, Lichanska A, Belcher C, Vickers JE, Hugenholtz P (1997) Isolation and molecular identification of planctomycete bacteria from postlarvae of the giant tiger prawn, *Penaeus monodon*. *Appl Environ Microbiol* 63:254–262
- Fukunaga Y, Kurahashi M, Sakiyama Y, Ohuchi M, Yokota A, Harayama S (2009) *Phycisphaera mikurensis* gen. nov., sp nov., isolated from a marine alga, and proposal of *Phycisphaeraceae* fam. nov., *Phycisphaerales* ord. nov and *Phycisphaerae* classis nov in the phylum Planctomycetes. *J Gen Appl Microbiol* 55:267–275
- Gade D, Stuhmann T, Reinhardt R, Rabus R (2005) Growth phase dependent regulation of protein composition in *Rhodopirellula baltica*. *Environ Microbiol* 7:1074–1084
- Galletta BJ, Cooper JA (2009) Actin and endocytosis: mechanisms and phylogeny. *Curr Opin Cell Biol* 21:20–27
- Giovannoni SJ, Godchaux W, Schabtach E, Castenholz RW (1987a) Cell wall and lipid composition of *Isosphaera pallida*, a budding eubacterium from hot springs. *J Bacteriol* 169: 2702–2707
- Giovannoni SJ, Schabtach E, Castenholz RW (1987b) *Isosphaera pallida*, gen. and comb. nov., a gliding, budding eubacterium from hot springs. *Arch Microbiol* 147:276–284
- Glockner FO, Kube M, Bauer M, Teeling H, Lombardot T, Ludwig W, Gade D, Beck A, Borzym K, Heitmann K, Rabus R, Schlesner H, Amann R, Reinhardt R (2003) Complete genome sequence of the marine planctomycete *Pirellula* sp. strain 1. *Proc Natl Acad Sci U S A* 100:8298–8303
- Hart EA, Hua L, Darr LB, Wilson WK, Pang JH, Matsuda SPT (1999) Directed evolution to investigate steric control of enzymatic oxidosqualene cyclization. An isoleucine-to-valine mutation in cycloartenol synthase allows lanosterol and parkeol biosynthesis. *J Am Chem Soc* 121:9887–9888
- Helmann JD (2002) The extracytoplasmic function (ECF) sigma factors. *Adv Microb Physiol* 46:47–110
- Herskovits AA, Shimoni E, Minsky A, Bibi E (2002) Accumulation of endoplasmic membranes and novel membrane-bound ribosome-signal recognition particle receptor complexes in *Escherichia coli*. *J Cell Biol* 159:403–410
- Hieu CX, Voigt B, Albrecht D, Becher D, Lombardot T, Glockner FO, Amann R, Hecker M, Schweder T (2008) Detailed proteome analysis of growing cells of the planctomycete *Rhodopirellula baltica* SH1T. *Proteomics* 8:1608–1623
- Iniesta AA, Shapiro L (2008) A bacterial control circuit integrates polar localization and proteolysis of key regulatory proteins with a phospho-signaling cascade. *Proc Natl Acad Sci U S A* 105:16602–16607
- Jogler C, Glockner FO, Kolter R (2011) Characterization of *Planctomyces limnophilus* and development of genetic tools for its manipulation establish it as a model species for the phylum Planctomycetes. *Appl Environ Microbiol* 77:5826–5829
- Jogler C, Waldmann J, Huang X, Jogler M, Glockner FO, Mascher T, Kolter R (2012) Planctomycetes comparative genomics: identification of proteins likely involved in morphogenesis, cell division and signal transduction. *J Bacteriol* 194(23):6419–6430
- Kamneva OK, Knight SJ, Liberles DA, Ward NL (2012) Analysis of genome content evolution in PVC bacterial super-phylum: assessment of candidate genes associated with cellular organization and life-style. *Genome Biol Evol*. doi:10.1093/gbe/evs1113
- König E, Schlesner H, Hirsch P (1984) Cell-wall studies on budding bacteria of the *Planctomyces/Pasteuria* group and on a *Prosthecomicrobium* sp. *Arch Microbiol* 138:200–205

- Kulichevskaya IS, Ivanova AO, Belova SE, Baulina OI, Bodelier PL, Rijpstra WI, Sinninghe Damste JS, Zavarzin GA, Dedysh SN (2007) *Schlesneria paludicola* gen. nov., sp. nov., the first acidophilic member of the order Planctomycetales, from Sphagnum-dominated boreal wetlands. *Int J Syst Evol Microbiol* 57:2680–2687
- Kulichevskaya IS, Ivanova AO, Baulina OI, Bodelier PL, Damste JS, Dedysh SN (2008) *Singulisphaera acidiphila* gen. nov., sp. nov., a non-filamentous, *Isosphaera*-like planctomycete from acidic northern wetlands. *Int J Syst Evol Microbiol* 58:1186–1193
- Kulichevskaya IS, Baulina OI, Bodelier PL, Rijpstra WI, Damste JS, Dedysh SN (2009) *Zavarzinella formosa* gen. nov., sp. nov., a novel stalked, Gemmata-like planctomycete from a Siberian peat bog. *Int J Syst Evol Microbiol* 59:357–364
- Kulichevskaya IS, Serkebaeva YM, Kim Y, Rijpstra WI, Damste JS, Liesack W, Dedysh SN (2012) *Telmatocola sphagniphila* gen. nov., sp. nov., a novel dendriform planctomycete from northern wetlands. *Front Microbiol* 3:146
- Lee K-C (2010) Cell compartmentalization and cell division in phyla Planctomycetes and Verrucomicrobia. School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane
- 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
- Levi-Setti R, Gavrilov KL, Rizzo PJ (2008) Divalent cation distribution in dinoflagellate chromosomes imaged by high-resolution ion probe mass spectrometry. *Eur J Cell Biol* 87:963–976
- Lewis PJ, Thaker SD, Errington J (2000) Compartmentalization of transcription and translation in *Bacillus subtilis*. *EMBO J* 19:710–718
- Lieber A, Leis A, Kushmaro A, Minsky A, Medalia O (2009) Chromatin organization and radio resistance in the bacterium *Gemmata obscuriglobus*. *J Bacteriol* 191:1439–1445
- Liesack W, König H, Schlesner H, Hirsch P (1986) Chemical composition of the peptidoglycan-free cell envelopes of budding bacteria of the *Pirella/Planctomyces* group. *Arch Microbiol* 145:361–366
- Lindsay MR, Webb RI, Hosmer HM, Fuerst JA (1995) Effects of fixative and buffer on morphology and ultrastructure of a fresh-water planctomycete, *Gemmata obscuriglobus*. *J Microbiol Methods* 21:45–54
- Lindsay MR, Webb RI, Fuerst JA (1997) Pirellulosomes: a new type of membrane-bounded cell compartment in planctomycete bacteria of the genus *Pirellula*. *Microbiology* 143:739–748
- Lindsay MR, Webb RI, Strous M, Jetten MS, Butler MK, Forde RJ, Fuerst JA (2001) Cell compartmentalisation in planctomycetes: novel types of structural organisation for the bacterial cell. *Arch Microbiol* 175:413–429
- Lonhienne TG, Sagulenko E, Webb RI, Lee KC, Franke J, Devos DP, Nouwens A, Carroll BJ, Fuerst JA (2010) Endocytosis-like protein uptake in the bacterium *Gemmata obscuriglobus*. *Proc Natl Acad Sci U S A* 107:12883–12888
- Matias VR, Beveridge TJ (2006) Native cell wall organization shown by cryo-electron microscopy confirms the existence of a periplasmic space in *Staphylococcus aureus*. *J Bacteriol* 188:1011–1021
- McCoy AJ, Maurelli AT (2006) Building the invisible wall: updating the chlamydial peptidoglycan anomaly. *Trends Microbiol* 14:70–77
- McInerney JO, Martin WF, Koonin EV, Allen JF, Galperin MY, Lane N, Archibald JM, Embley TM (2011) Planctomycetes and eukaryotes: a case of analogy not homology. *Bioessays* 33:810–817
- Medema MH, Zhou M, van Hijum SA, Gloerich J, Wessels HJ, Siezen RJ, Strous M (2010) A predicted physicochemically distinct sub-proteome associated with the intracellular organelle of the anammox bacterium *Kuenenia stuttgartiensis*. *BMC Genomics* 11:299
- Nachar VR, Savka FC, McGroty SE, Donovan KA, North RA, Dobson RC, Buckley LJ, Hudson AO (2012) Genomic and biochemical analysis of the diaminopimelate and lysine biosynthesis pathway in *Verrucomicrobium spinosum*: identification and partial characterization of L, L-diaminopimelate aminotransferase and UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-meso-diaminopimelate Ligase. *Front Microbiol* 3:183

- Nickell S, Hegerl R, Baumeister W, Rachel R (2003) Pyrodictium cannulae enter the periplasmic space but do not enter the cytoplasm, as revealed by cryo-electron tomography. *J Struct Biol* 141:34–42
- Pearson A, Budin M, Brocks JJ (2003) Phylogenetic and biochemical evidence for sterol synthesis in the bacterium *Gemmata obscuriglobus*. *Proc Natl Acad Sci U S A* 100:15352–15357
- Pilhofer M, Rappl K, Eckl C, Bauer AP, Ludwig W, Schleifer KH, Petroni G (2008) Characterization and evolution of cell division and cell wall synthesis genes in the bacterial phyla Verrucomicrobia, Lentisphaerae, Chlamydiae, and Planctomycetes and phylogenetic comparison with rRNA genes. *J Bacteriol* 190:3192–3202
- Reynaude EG, Devos DP (2011) Transitional forms between the three domains of life and evolutionary implications. *Proc Biol Sci* 278:3321–3328
- Santarella-Mellwig R, Franke J, Jaedicke A, Gorjanacz M, Bauer U, Budd A, Mattaj IW, Devos DP (2010) The compartmentalized bacteria of the planctomycetes-verrucomicrobia-chlamydiae superphylum have membrane coat-like proteins. *PLoS Biol* 8:e1000281
- Schlesner H (1994) The development of media suitable for the microorganisms morphologically resembling *Planctomyces* spp, *Pirellula* spp, and other *Planctomycetales* from various aquatic habitats using dilute media. *Syst Appl Microbiol* 17:135–145
- Schlesner H, Rensmann C, Tindall BJ, Gade D, Rabus R, Pfeiffer S, Hirsch P (2004) Taxonomic heterogeneity within the Planctomycetales as derived by DNA-DNA hybridization, description of *Rhodopirellula baltica* gen. nov., sp. nov., transfer of *Pirellula marina* to the genus *Blastopirellula* gen. nov. as *Blastopirellula marina* comb. nov. and emended description of the genus *Pirellula*. *Int J Syst Evol Microbiol* 54:1567–1580
- Schmidt JM (1978) Isolation and ultrastructure of freshwater strains of *Planctomyces*. *Curr Microbiol* 1:65–70
- Schmidt JM, Starr MP (1980) Some ultrastructural features of *Planctomyces bekefi*, Morphotype I of the *Blastocaulis-Planctomyces* group of budding and appendaged bacteria. *Curr Microbiol* 4:189–194
- Schmidt JM, Starr MP (1982) Ultrastructural features of budding cells in a prokaryote belonging to Morphotype-IV of the *Blastocaulis-Planctomyces* group. *Curr Microbiol* 7:7–11
- Speth DR, van Teeseling MC, Jetten MS (2012) Genomic analysis indicates the presence of an asymmetric bilayer outer membrane in planctomycetes and verrucomicrobia. *Front Microbiol* 3:304
- Stackebrandt E, Wehmeyer U, Liesack W (1986) 16S ribosomal RNA- and cell wall analysis of *Gemmata obscuriglobus*, a new member of the order Planctomycetales. *FEMS Microbiol Lett* 37:289–292
- Staley JT (1973) Budding bacteria of the Pasteuria-Blastobacter group. *Can J Microbiol* 19:609–614
- Studholme DJ, Fuerst JA, Bateman A (2004) Novel protein domains and motifs in the marine planctomycete *Rhodopirellula baltica*. *FEMS Microbiol Lett* 236:333–340
- Summons RE, Bradley AS, Jahnke LL, Waldbauer JR (2006) Steroids, triterpenoids and molecular oxygen. *Philos Trans R Soc Lond B Biol Sci* 361:951–968
- Sutcliffe IC (2010) A phylum level perspective on bacterial cell envelope architecture. *Trends Microbiol* 18:464–470
- Tekniepe BL, Schmidt JM, Starr MP (1981) Life-cycle of a budding and appendaged bacterium belonging to Morphotype-IV of the *Blastocaulis-Planctomyces* Group. *Curr Microbiol* 5:1–6
- Tekniepe BL, Schmidt JM, Starr MP (1982) Immunoferritin labeling shows denovo synthesis of surface components in buds of a prokaryote belonging to morphotype-IV of the *Blastocaulis-Planctomyces* Group. *Curr Microbiol* 7:1–6
- Trias J, Jarlier V, Benz R (1992) Porins in the cell wall of mycobacteria. *Science* 258:1479–1481
- van Niftrik L, Geerts WJ, van Donselaar EG, Humbel BM, Webb RI, Fuerst JA, Verkleij AJ, Jetten MS, 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. *J Bacteriol* 190:708–717

- van Niftrik L, Geerts WJ, van Donselaar EG, Humbel BM, Yakushevskaya A, Verkleij AJ, Jetten MS, Strous M (2008b) Combined structural and chemical analysis of the anammoxosome: a membrane-bounded intracytoplasmic compartment in anammox bacteria. *J Struct Biol* 161: 401–410
- van Niftrik L, Geerts WJ, van Donselaar EG, Humbel BM, Webb RI, Harhangi HR, Camp HJ, Fuerst JA, Verkleij AJ, Jetten MS, Strous M (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, van Helden M, Kirchen S, van Donselaar EG, Harhangi HR, Webb RI, Fuerst JA, Op den Camp HJ, Jetten MS, Strous M (2010) Intracellular localization of membrane-bound ATPases in the compartmentalized anammox bacterium ‘*Candidatus Kuenenia stuttgartiensis*’. *Mol Microbiol* 77:701–715
- Voigt B, Hieu CX, Hempel K, Becher D, Schluter R, Teeling H, Glockner FO, Amann R, Hecker M, Schweder T (2012) Cell surface proteome of the marine planctomycete *Rhodopirellula baltica*. *Proteomics* 12:1781–1791
- Wang J, Jenkins C, Webb RI, Fuerst JA (2002) Isolation of Gemmata-like and Isosphaera-like planctomycete bacteria from soil and freshwater. *Appl Environ Microbiol* 68:417–422
- Wecker P, Klockow C, Schuler M, Dabin J, Michel G, Glockner FO (2010) Life cycle analysis of the model organism *Rhodopirellula baltica* SH 1(T) by transcriptome studies. *Microb Biotechnol* 3:583–594
- Yee B (2012) The diversity and cell biology of Planctomycetes. School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, Queensland
- Yee B, Sagulenko E, Fuerst JA (2011) Making heads or tails of the HU proteins in the planctomycete *Gemmata obscuriglobus*. *Microbiology* 157:2012–2021
- Yee B, Sagulenko E, Morgan GP, Webb RI, Fuerst JA (2012) Electron tomography of the nucleoid of *Gemmata obscuriglobus* reveals complex liquid crystalline cholesteric structure. *Front Microbiol* 3:326



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