Formation and function of bacterial organelles

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Abstract | Advances in imaging technologies have revealed that many bacteria possess organelles with a proteomically defined lumen and a macromolecular boundary. Some are bound by a lipid bilayer (such as thylakoids, magnetosomes and anammoxosomes), whereas others are defined by a lipid monolayer (such as lipid bodies), a proteinaceous coat (such as carboxysomes) or have a phase-defined boundary (such as nucleolus-like compartments). These diverse organelles have various metabolic and physiological functions, facilitating adaptation to different environments and driving the evolution of cellular complexity. This Review highlights that, despite the diversity of reported organelles, some unifying concepts underlie their formation, structure and function. Bacteria have fundamental mechanisms of organelle formation, through which conserved processes can form distinct organelles in different species depending on the proteins recruited to the luminal space and the boundary of the organelle. These complex subcellular compartments provide evolutionary advantages as well as enabling metabolic specialization, biogeochemical processes and biotechnological advances. Growing evidence suggests that the presence of organelles is the rule, rather than the exception, in bacterial cells.

Phase-defined
In cases of high concentration of nucleic acids (or specific proteins) a phase separation can be observed in the cytoplasm that results in a partitioning of specific proteins that either prefer or disfavour the conditions of each phase. A notable example is the nucleolus in eukaryotes and the ribonucleoprotein granules found in bacteria such as *Caulobacter crescentus*.

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Bacterial cells were once presumed to be 'bags of enzymes' with minimal oganization[1]. Yet, in the past 10 years, numerous studies have demonstrated that bacteria compartmentalize many cellular processes into subcellular structures, including organelles. Whole metabolic pathways were shown to occur without substantial diffusion of metabolites owing to the clustering of enzymes into metabolosomes in discrete locations within the cytoplasm[2–4]. DNA and DNA-associated proteins are confined to discrete territories in the cytoplasm[5–8], and the segregation of RNA transcripts into subcellular territories provides spatial control over their translation into polypeptides[9–13]. In several bacterial lineages, distinct membrane districts are packed with photosynthetic machinery to maximize the efficiency with which bacteria can harvest energy from light[14–18].

What is an organelle? Previous definitions have reflected the knowledge available at the time, with one definition being that an organelle is a subcellular location where a specific biological process takes place. This would encapsulate all recognized organelles but could also include large protein oligomers like ribosomes. However, it is now clear that, in the case of ribosomes, flagella and other such structures, a better mechanistic definition is that these are molecular machines: numerous components coming together to drive a single (albeit highly complicated and highly regulated) process. A more recent definition of an organelle insisted on enclosure by a phospholipid membrane; however, with advances in imaging technologies, an updated definition is needed to embrace newly seen organelles with a protein coat that cordons a distinct luminal compartment to enact metabolic pathways or physical functions as well as phase-defined membraneless subcellular compartments. Therefore, in this Review, we define an organelle as a subcellular structure containing a proteomically distinct interior and a defined boundary layer (whether lipid membrane, lipid monolayer, proteinaceous or phase-defined) that affects cellular physiology. In turn, organelle formation requires two processes: the production of an organellar boundary (for example, by involution and/or membrane fission) and the packaging of luminal contents into the organelle.

Just as they do for eukaryotes, organelles confer various physiological advantages for bacteria. Broadly, they enable the more resolved and sophisticated spatiotemporal regulation of cellular processes[19,20]. Depending on the organelle, they also have a range of more specific functions: (1) enhancing the efficiency of metabolic processes by increasing local concentrations of metabolites and enzymes (for example, carboxysomes), (2) increasing the surface area for energy transduction (for example, thylakoids), (3) sequestration of toxic or volatile intermediates from other cellular components (for example, metabolosomes), and (4) providing microenvironments

Carboxysomes
Anabolic bacterial microcompartments that have a polyhedral protein shell and a lumen filled with the CO_2-fixing enzyme RuBisCO. Examples include the β-carboxysomes of the phototroph *Synechococcus elongatus* and the α-carboxysomes of the lithotroph *Acidithiobacillus* spp.

Thylakoids
Membrane-bound organelle that mediates light-harvesting and energy transduction in photosynthetic cyanobacteria. Through evolutionary links, thylakoids are also one of the three membrane systems found in the chloroplasts of eukaryotes.

Metabolosomes
Catabolic bacterial microcompartments bound by polyhedral protein shells and housing enzymes to oxidize a specific metabolite. Notable examples include the organelles that metabolize propanediol or ethanolamine in various bacteria and archaea, including *Salmonella enterica*.

Acidocalcisomes
First identified in single-celled eukaryotes, these organelles have a single membrane boundary. A pH gradient across the membrane is generated by a proton-translocating pyrophosphatase, providing an acidic, phosphate-rich lumen that can accommodate excess cellular calcium. Typical examples are found in Alphaproteobacteria such as *Agrobacterium tumefaciens*.

Mitochondria
Eukaryotic organelles with two membrane systems that primarily mediate ATP synthesis through aerobic respiration. Derived through endosymbiosis of an alphaproteobacterial cell.

Chloroplasts
Plastids are organelles with three membrane systems found in eukaryotes, with the chloroplast being defined as a type of plastid mediating oxygenic photosynthesis in plants and algae. Derived through endosymbiosis of a cyanobacterial cell.

with distinct pH and/or redox states compared to the rest of the cell (for example, acidocalcisomes)[19–21] (FIG. 1).

Several factors have contributed to our awareness of organelles in bacteria lagging behind that in eukaryotes. First, given the small size of bacterial cells, the development of nanoscale imaging technologies over the past decades has enabled an increasingly robust assessment of bacterial cell ultrastructure. Pioneering studies by Sergei Winogradsky using early techniques in light microscopy saw sulfur globules in bacteria and raised interest in bacterial cell ultrastructure[22], but developments in transmission electron microscopy and, ultimately, in cryo-electron tomography were needed to bring clarity and appreciation of the organelles in bacterial cells. Furthermore, a given species of bacteria might have only one seemingly species-specific organelle and perhaps produce it only under some environmental conditions. Together, these issues have required that we consider the formation of each potential organelle as a distinct and unrelated aspect of bacterial cell biology, leaving unclear the extent to which the formation of at least some of these organelles might reflect fundamental processes in bacterial cell biology. New approaches in comparative genomics and systems biology, together with revolutionary imaging technologies such as super-resolution imaging and electron cryo-tomography, now make this exciting goal achievable. In this Review, we discuss the rich insights provided by such studies into the structure, function and formation of bacterial organelles.

Types of membrane-bound organelles

A classic definition of an organelle is a membrane-bound compartment in which the luminal content is distinct from the proteome of the surrounding milieu[23,24]. Functionally, most bacterial membrane-bound organelles were discovered in quests to understand the molecular basis of photosynthesis and other aspects of bioenergetics. All together, these research lines have led to what sometimes seem to be unrelated ultrastructural features of bacterial membranes but which can be considered comparable organelles with some conserved and fundamental properties (FIG. 1). Some of these fundamental properties have been carried forward into the evolution of eukaryotes: the involution of the bacterial inner membrane in Alphaproteobacteria is similar to cristae in mitochondria[25–27] and the stacked layers of photosynthetic membranes in Cyanobacteria gave rise to the thylakoid membranes of chloroplasts[16,18,28]. These organelles have important roles in eukaryotic cell biology. Equivalent involution of the bacterial inner membrane also drives the production of magnetosomes and, potentially, of many other cytoplasmic organelles in diverse bacterial lineages[24,29,30].

Alphaproteobacteria have enigmatic compartments called intracytoplasmic membranes (ICMs), and some lineages possess organelles derived from them. These ICMs mediate biogeochemically important functions in physiologically diverse bacteria and were likely antecedents of the cristae membranes of eukaryotic mitochondria (derived from an alphaproteobacterial endosymbiont)[27,31,32]. In common with mitochondria, ICMs enhance ATP generation in two key ways: maximizing surface area for energy transduction and enhancing local control over proton gradients and metabolite concentrations. At least three physiologically distinct alphaproteobacterial groups (anoxygenic phototrophs, aerobic methanotrophs and nitrifiers) have tailored ICMs to perform their specialist bioenergetic functions; they achieve this specialization by packaging the central enzymes of these pathways (that is, photosystems, particulate methane monooxygenases and nitrite oxidoreductases, respectively) into the membranes at high density (upwards of 50% of the total membrane protein content)[17,27,33]. Although many ICMs remain contiguous with the cell membrane, the photosynthetic chromatophores of purple non-sulfur bacteria such as *Rhodobacter sphaeroides* have been shown to be completely segregated and form spherical organelles[14,34,35] (FIG. 1). Cyanobacteria and the chloroplasts derived from them possess a distinct internal membrane system called thylakoids that drives oxygenic photosynthesis. The major distinction of this photosynthetic organelle, as seen by electron microscopy and electron cryo-tomography, is that it forms stacked layers of membrane-bound compartments pervasive throughout the bacterial cell[36–39].

A specialist group of autotrophic bacteria, the anaerobic ammonium oxidizers (anammox bacteria), conserve energy using elaborate organelles called anammoxosomes[40,41] (FIG. 1). These organelles possess luminal enzymes that convert ammonium and nitrite to dinitrogen gas, with nitric oxide and hydrazines as intermediates, in a process central for both biogeochemical nitrogen cycling and wastewater treatment. Electron microscopy studies suggest that anammoxosomes can be 600 nm in size and occupy 60% of the cell[41–47]. These organelles confer several physiological advantages to anammox Planctomycetes: a greater cellular space dedicated to energy conversion, sequestration of the reactive intermediate hydrazine and increased efficiency of coupling. A unique and dominant component of anammoxosome membranes are ladderane phospholipids containing concatenated cyclobutane rings[46,48]; by increasing membrane density, these lipids prevent proton leakage (that is, uncoupling) and in turn enhance energetic efficiency[49,50]. It should be noted that other Planctomycetes also exhibit extensive compartmentalisation, with some, such as *Gemmata obscuriglobus*, even possessing membranes surrounding the nucleoid[41,51–54]. However, 3D imaging suggests that these compartments are complex interconnected invaginations of the cell membrane[55] rather than closed membrane-bound organelles as in anammoxosomes[46].

Other bacteria use various organelles to sequester metal ions for a range of purposes. Magnetotactic bacteria such as the alphaproteobacterium *Magnetospirillum gryphiswaldense* produce magnetosomes (FIG. 1). The lumen of these membrane-bound organelles contain magnetite (Fe_3O_4) or greigite (Fe_3S_4) crystals, together with specific membrane-bound and luminal proteins that mediate iron transport and regulate crystal nucleation[24,29,56–59]. Current studies address whether these luminal proteins are packed into the developing magnetosome during membrane involution or are

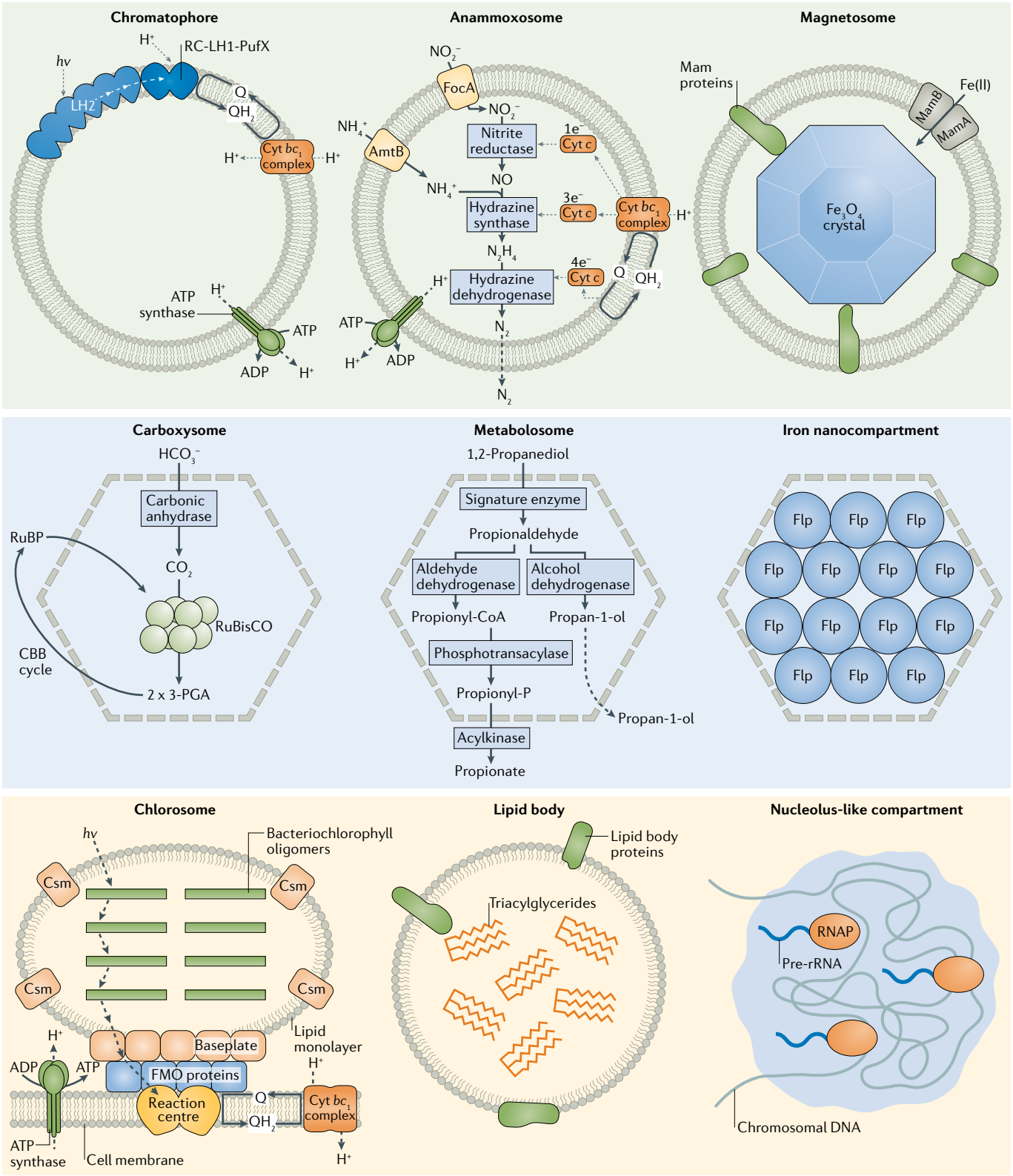

Fig. 1 | **Structural and functional diversity of bacterial organelles.** Bacteria have four broad classes of organelles: membrane bound (for example, chromatophores, anammoxosomes and magnetosomes; green shading), protein bound (for example, carboxysomes, metabolosomes and iron nanocompartments; blue shading), lipid monolayer (for example, chlorosomes and lipid bodies; orange shading) and phase-defined (for example, nucleolus-like compartment; orange shading, far right). All of these organelles have common roles in increasing metabolic efficiency and/or sequestering specific compounds. Several structurally distinct organelles, for example, chromatophores and chlorosomes, have equivalent or analogous physiological roles. 3-PGA, 3-Phosphoglyceric acid; CBB cycle, Calvin–Benson–Bassham cycle; Cyt, cytochrome; FMO protein, Fenna-Matthews-Olson protein; Q, quinone (for example, ubiquinone); RNAP, RNA polymerase; RuBP, ribulose 1,5-bisphosphate.

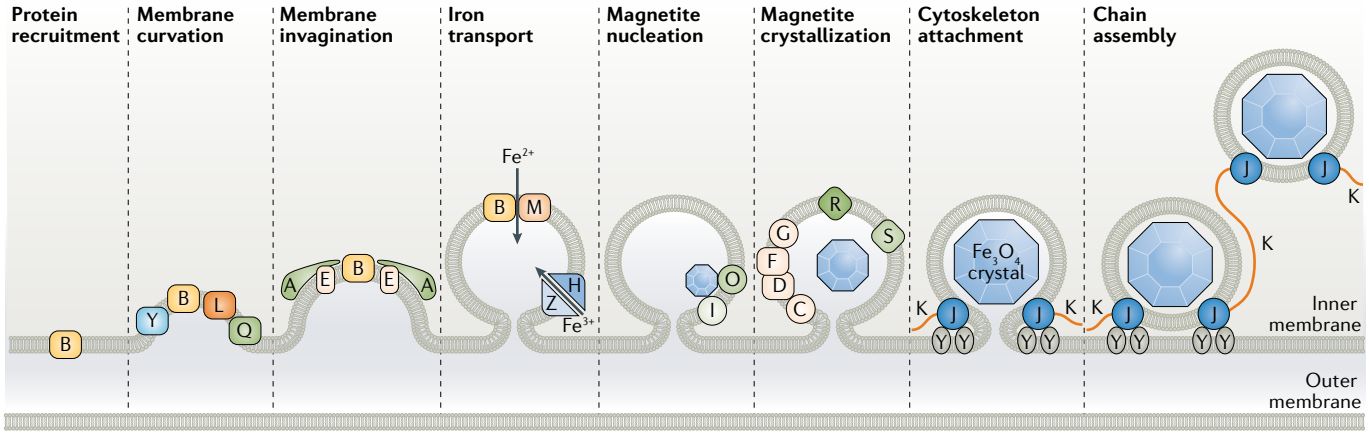

Fig. 2 | **Formation of membrane-bound organelles.** The schematic shows a model pathway for magnetosome formation based on REF.[29]. In common with other membrane-bound organelles, magnetosomes are formed in two separable stages: membrane involution and luminal protein loading. Key proteins proposed to mediate each step are shown based on studies in *Magnetospirillum magneticum* (Mam proteins, shown with abbreviated name, that is, MamA are labelled as A and so on).

Magnetosomes
Membrane-bound organelles that contain a lumen filled with magnetic iron oxide or iron sulfide crystals, which enable bacteria, such as *Magnetospirillum gryphiswaldense*, to orient towards magnetic fields and, in turn, mediate aerotaxis.

Chromatophores
Membrane-bound organelles that function in light harvesting and energy transduction during anoxygenic photosynthesis. Examples are found in Alphaproteobacteria such as *Rhodobacter sphaeroides*.

Anammoxosomes
Large membrane-bound organelles containing enzymes for anaerobic ammonium oxidation in the lumen and associated energy transduction in the membrane. Exclusively found in anammox Planctomycetes such as *Kuenenia stuttgartiensis*.

Nucleoid
Region within bacterial cells defined by the compacted genome. These regions are often phase-separated from the surrounding cytoplasm and therefore contain a distinct population of proteins.

Nitrate vacuoles
Giant membrane-bound organelles that store the anaerobic electron acceptor nitrate and found in various sulfur-oxidizing, nitrate-reducing Gammaproteobacteria such as *Thioploca araucae*.

translocated across the membrane. In other organelles, these two processes — cargo packaging[60] and protein translocation[61] — have distinct requirements for cargo-receptors or protein translocases, respectively. In magnetosome formation, membrane involution and formation of the luminal contents seem to be separate processes: magnetosomes form constitutively by involution of the inner membrane but the protein-mediated loading with iron only occurs when sufficient iron is present in the environment[62] (FIG. 2). A characteristic feature of the magnetosomes is their alignment by cytoskeletal filaments; this enables magnetotactic cells to orient themselves according to the Earth's magnetic field and, through aerotaxis, move to environments with desired oxygen tension[24,29]. The ferrosome is a functionally and structurally distinct iron-accumulating organelle. These membrane-bound, electron-dense compartments, 20 nm in diameter, are thought to enable iron storage in dissimilatory iron reducers such as *Shewanella putrefaciens* and *Desulfovibrio magneticus*[24,63–67]. Recent phylogenetic analyses suggest that diverse bacteria and some methanogenic archaea might also synthesize these organelles[67]. Two other membrane-bound storage organelles have been characterized: the large nitrate vacuoles of filamentous sulfur bacteria that accumulate nitrate for anaerobic respiration (reviewed in REF.[68]) and the acidocalcisomes of various alphaproteobacteria that store calcium and polyphosphates (reviewed in REF.[69]); the latter is a rare example of an organelle conserved in both bacteria and eukaryotes[69,70].

Advances in electron cryo-tomography suggest more membrane-bound organelles await characterization. An analysis of more than 15,000 cryo-tomograms from 90 different bacterial species identified a collection of structures that currently defy explanation and yet point to a fundamental ability of diverse bacteria to form membrane-bound organelles within their cytoplasmic compartment[30]. Most of the species of bacteria studied were shown to have membrane-bound compartments of the same size as magnetosomes and ferrosomes but that are not electron dense.

Formation of membrane-bound organelles

Although the structure and function of mature bacterial organelles are diverse, at least some common steps may occur in their formation. Indeed, in eukaryotes, defining organelle biogenesis pathways (BOX 1) was a key stage in elucidating that apparently different organelles, such as mitochondria, mitosomes and hydrogenosomes, are evolutionarily diversified versions of the same organelle[71]. Similarly peroxisomes, glycosomes and glyoxysomes are the same organelles, which are simply packaged with different luminal contents in animals, parasites and plants, respectively[72,73]. In the case of bacteria, a common feature of many membrane-bound compartments is that they seem to be derived from involution of the bacterial inner membrane; involutions observed during magnetosome formation[24,29], for example, are analogous to the membrane involutions that form ICMs. Even for cyanobacterial thylakoids, electron cryo-tomography studies suggest that spherical vesicles budding from the inner membrane are the initial membrane form; dynamins are then proposed to remodel these into intricately shaped and flattened mature thylakoid membranes[18,28,37,74,75]. Anammoxosomes are an important exception. There is acceptance that these organelles may have derived from the invagination of the inner membrane during the early phase of their evolutionary history; however, they are now produced by organelle division during binary fission, thereby ensuring inheritance of anammoxosomes through each round of cell division[76]. It is now yet known which proteins mediate the elongation and curvature of the anammoxosome membrane during this fission event.

The production of membrane-bound organelles through involution of the cell membrane requires two key steps: (1) induction of membrane curvature and budding from the inner membrane and (2) population of the luminal space with a proteome specific for the function of the organelle (FIG. 2). A common feature is that incorporation of curvature-inducing proteins drives membrane invagination (FIG. 2). In the case of eukaryotic organelles, it is known that curvature-inducing proteins

Ferrosomes
A recently discovered group of small membrane-bound organelles that store iron, including in iron-reducing bacteria such as *Shewanella putrefaciens*.

Mitosomes
Organelles with two membrane systems found in some single-celled eukaryotes, notably in the genera *Giardia* and *Entamoeba*, containing luminal enzymes required for the biosynthesis of iron–sulfur clusters. These organelles are functionally specialized derivatives of mitochondria.

Hydrogenosomes
Organelles with two membrane systems found in some anaerobic protists, fungi and animals. They contain luminal enzymes that produce ATP via fermentation, resulting in the production of end products such as hydrogen gas. These organelles are functionally specialized derivatives of mitochondria.

Peroxisomes
Organelles found in many animals and fungi, containing luminal enzymes required for oxidative reactions such as lipid biosynthesis and detoxification reactions. These single-membrane-bound organelles are evolutionarily related to glycosomes and glyoxysomes.

can act either directly to physically curve the membrane or indirectly by the recruitment or modification of lipid components[77,78]. Current evidence from studies on diverse bacterial species suggests that lineage-specific sets of protein components drive membrane curvature, even in the case of seemingly similar organelles[66]. In the case of chromatophores, the same core proteins that mediate light-harvesting and photosynthetic electron transfer (that is, LH2 complexes and RC-LH1-PufX complexes) have intrinsic curvature properties. In localized regions where these complexes coalesce, the curvature that they induce initiates membrane invagination[79–81]. Whether a further cause or a consequence of membrane curvature, other proteins are sorted into the membrane or luminal compartment of the organelle[82] and, ultimately, the chromatophores bud away from the inner membrane[35]. The formation of ICMs has been addressed from an evolutionary angle given that, in mitochondria, the formation of cristae membranes depends on machinery called MICOS (mitochondrial contact site and cristae organizing systems). A study using a comparative genomics approach demonstrated that Alphaproteobacteria have a conserved 'alphaMICOS' to induce and stabilize membrane curvature[27,83,84]. Morphologically similar ICMs can be induced even in *Escherichia coli* by the overexpression of the *b* subunit of ATP synthase[85], consistent with the role of ATP synthase proteins in curving other membranes[86]. In a further demonstration of this principle, the expression of a single membrane curvature protein (human caveolin) in *E. coli* generates membrane compartments of similar size to magnetosomes and other organelles[87]. Membrane curvature is stabilized by the recruitment of cardiolipin and other lipids[88]. Altogether, these findings suggest that curvature-inducing proteins are sufficient for organelle production. Moreover, the results also suggest that a gene expression programme switching on the expression of key membrane curvature proteins would initiate the formation of an organelle (FIG. 2) and could potentially be regulated to respond to environmental cues.

A second unifying concept is the packaging of luminal contents into the organelle. The functionality of organelles depends on the proteins sorted into the membranes and packaged into the luminal space. Depending on the organelle, proteins can be targeted during membrane involution or following organelle abscission[89–91]. However, the mechanisms responsible for the selective targeting of proteins have remained elusive. One possibility is that one or more of the membrane proteins in the nascent organelle is a translocase selective for the subset of proteins that must be transported into the luminal compartment (FIG. 2). Mitochondria and plastids have translocases whose evolutionary origins are not entirely clear but which were probably derived from a bacterial ancestor[18,92]. Nevertheless, we are unaware of any evidence yet for specific protein import pathways in bacterial organelles. An alternative way to populate the luminal compartment would be to use the Sec or Tat protein translocases, as has been suggested in the formation of anammoxosomes[41,47] and thylakoids[16,18,90]. Consistently, Sec and Tat translocons have been identified in thylakoid membranes, and signal peptides for both translocases have been detected in thylakoid-targeted proteins. However, given that identical Sec and Tat translocases are present in both the cell and thylakoid membranes, it remains unclear how proteins are differentially sorted between compartments[16,93]. One possibility is that thylakoid-targeted proteins are sorted prior to translocation, for example, through subtle variations in their signal peptides[16,94], although sorting post-translocation cannot be ruled out[16]. In the case of anammoxosomes, bioinformatics predictions suggest that only the anammoxosome contains substrates with Tat signal peptides; this has led to the suggestion that Tat may be exclusively localized in the organellar membrane, providing a mechanism for the selective targeting of key anammox enzymes[90]. A further hypothesis would be that differential targeting of mRNAs to the two distinct membrane locations segregates where the proteins are translated and translocated (BOX 2). Distinct local populations of membrane proteins can be formed in the inner membrane by this process: transcripts encoding membrane proteins can be selectively targeted to the inner membrane before ribosomes engage to produce the polypeptide chain, thus dictating its translocation through the nearest translocon[10,11,13,95]. This targeting process, referred to as transertion, has been invoked in other aspects of bacterial cell biology for which subsets of proteins need to be targeted to discrete submembrane sites to create membrane heterogeneity[12,96–99].

Beyond these unifying principles, the molecular processes leading to organelle formation are highly complex and vary between species. This variation is best reflected in the detailed studies of magnetosome formation in *Magnetospirillum* species (FIG. 2). At least 30 specific genes contribute to magnetosome assembly, most of them organized into five operons across a 130 kb genomic island[100,101]. Extensive genetic dissection has gradually resolved their roles, revealing that some genes are essential and others are dispensable[102–105]. In the first

Box 1 | **Unifying concepts in organelle biogenesis: insights from eukaryotes**

In eukaryotes, studies aimed at understanding biogenesis have provided unequivocal evidence that a single organelle can evolve to have very different metabolic capacity in different lineages. For example, animals have an organelle called the peroxisome that houses enzymes such as catalase for oxidative detoxification reactions. Plants have an organelle, called the glyoxysome, housing the enzymes for the glyoxylate cycle for two-carbon metabolism. Single-celled eukaryotes, such as the trypanosomes, have an organelle called the glycosome, which houses many of the enzymes for glycolysis[72]. Peroxisomes, glyoxysomes and glycosomes are all derived from the same ancestral organelle. This common ancestry is illustrated by the evidence that the cargo proteins within these organelles are imported by a conserved pathway using a conserved protein import machinery and controlled by the cargo proteins all carrying the same C-terminal 'peroxisomal targeting sequence'[72,73,210].

Mitochondria are the 'powerhouse' compartment housing oxidative phosphorylation reactions in many lineages of eukaryotes. However, some single-celled microorganisms, such as the genera *Giardia* and *Trichomonas*, were textbook examples of eukaryotes that have no mitochondria. Studies aimed at understanding the formation of these different organelles showed that mitosomes, which are involved in iron–sulfur cluster biogenesis in *Giardia*, and the hydrogenosomes, which enable fermentative ATP production in anaerobes such as *Trichomonas*, are evolutionarily diversified versions of mitochondria. All of these organelles are built through a conserved membrane biogenesis pathway and populated with cargo through a 'mitochondrial' protein import pathway[71,211].

Box 2 | **Transertion as a mechanism for organelle formation in bacteria**

The protein components embedded in the bacterial inner membrane are not uniformly distributed. Rather, a high degree of membrane heterogeneity is observed, whereby distinct functional precincts can be created[9,212]. Membrane precincts dedicated to membrane biogenesis events have recently been characterized in the outer membrane of *Escherichia coli*[213]. Although no equivalent assembly precincts have been detected to date in the inner membrane, several lines of evidence suggest that membrane heterogeneity in the inner membrane could enable the budding of subcellular membranes with a distinct membrane proteome.

The concept of transertion is built from evidence that membrane protein localization relies on three processes that are each subject to spatial control in bacterial cells. Thus, transcription–translation–insertion (transertion) must all be considered in order to understand how a membrane protein precinct is created. There is good evidence from many sources that mRNA transcripts encoding membrane proteins are targeted to the membrane for highly localized translation of the encoded polypeptide[9,97,214]. Single-cell measurements show that multiple translation events from a single mRNA thereby result in a given translocon inserting several copies of a given protein, creating a high local concentration of that protein[97,98,212]. For a protein that induces membrane curvature, this local concentration would favour the initial steps in the formation of a membrane-bound compartment.

major step, several proteins induce membrane curvature, protein sorting and vesicle formation. The most important of these proteins seems to be MamB, with its synthesis triggering de novo magnetosome formation[91,102]. Another key assembly factor, MamA, forms a globular scaffold around the surface of the emerging vesicle and is thought to recruit other proteins through protein–protein interactions[106–108]. In the second major step, the lumen contents are gradually packaged through the nucleation and maturation of magnetite crystals. Two members of the cation diffusion facilitator family (MamB and MamM) mediate Fe^{2+} uptake[109], whereas a network of other proteins mediate crystal nucleation, expansion and contraction as well as redox control[102,110,111]. Finally, proteins are specifically involved in attaching the magnetosome to the cytoskeleton and aligning the mobility axis. This depends on extensive interactions between the actin-like MamK, the adaptor MamJ and membrane-bound scaffold-generating MamY[112–116]. Further details on the formation of magnetosomes are provided in a recent review[29].

Membrane-free organelles

Bacteria also have numerous subcellular compartments that, despite having no phospholipid bilayer, have a distinct luminal proteome that generates a distinct compartment-specific metabolome. In several cases, membrane-free organelles are functionally analogous to membrane-bound compartments in other bacterial lineages. In the absence of a phospholipid bilayer, these organelles have either (1) a phase-transition boundary created by the differences in the luminal contents compared to the external proteome, (2) a proteinaceous boundary formed by the multimerisation of shell proteins such as encapsulins or (3) a lipid monolayer supported by the protein components. Similar to membrane-bound compartments, their synthesis depends on two steps: formation of an outer coat and targeting of cargo macromolecules. However, these steps can occur simultaneously or sequentially (in either order) depending on the specific organelle. The first step in the formation of many of these organelles is the local cytosolic accumulation of cargo macromolecules, which drives a phase transition.

Phase-derived membraneless organelles. Over the past decade, it has been increasingly recognized that liquid–liquid phase separation contributes to the compartmentalization of eukaryotic and bacterial cells alike[117–120]. Cellular processes that create high local concentrations of nucleic acids can induce a phase transition, thereby isolating a proteome associated with those nucleic acids from the surrounding milieu. The resulting compartments are variably described as 'phase-defined membraneless organelles' or 'biomolecular condensates'[118,120,121]. In bacteria, a seminal study in *Caulobacter crescentus* revealed that ribonucleoprotein granules (RNP bodies) form during mRNA degradation; the key enzyme mediating mRNA processing, RNase E, also contains an intrinsically disordered C-terminal domain that is necessary and sufficient to cause phase separation[122]. DEAD-box ATPases (such as DeaD, SrmB and RhlE) are also central regulators of phase separation and are conserved across all three domains of life[121]. Most recently, it has been observed in *E. coli* that RNA polymerase itself is subject to liquid–liquid phase separation during rapid cell division[120].

Remarkably, bacteria also form a phase-derived membraneless organelle that is structurally and functionally equivalent to the nucleolus of eukaryotic cells[123,124]. The luminal space of the nucleolus-like compartment supports the assembly of ribosomes and other ribonucleoprotein complexes[121,123]. In rapidly growing bacterial cells, the chromosomal loci corresponding to the rRNA genes produce massive amounts of ribosomal RNA in a small territory within the cytoplasm, inducing a phase-transition boundary[124]. A key factor facilitating the formation of the compartment is the organization of bacterial chromosomes into territories[5–7], with the compacted nucleic acid environment and the concentration of protein components featuring elements of native disorder, resulting in a localized phase transition[117,125] (FIG. 1).

Further blurring the boundaries between bacterial and eukaryotic cells, the extraordinary levels of DNA and RNA production associated with bacteriophage replication can yield a phase-derived compartment resembling a nucleus[8,126,127]. Analysis of *Pseudomonas chlororaphis* cells infected by bacteriophage 201φ2-1 by cryo-electron tomography demonstrated that this central compartment is rich in DNA and RNA and that it excludes ribosomes and other cytoplasmic protein features, including green fluorescent protein reporters. A phage-encoded microtubule-like spindle maintains this nucleus-like structure at a mid-cell position, assisting maturing phage to leave the nucleus-like structure and find the inner membrane to initiate egress from the bacterium[128]. Two new studies have revealed that the formation of nucleus-like structures enables bacteriophages to evade CRISPR-associated nucleases[126,127]. In combination, these findings suggest that the formation of phase-derived organelles is a fundamental feature across bacteria and other forms of life.

Glycosomes
Organelles found in trypanosomes and other single-celled eukaryotes, containing enzymes required for glycolysis in their lumen. These single-membrane-bound organelles are functionally specialized derivatives of peroxisomes.

Glyoxysomes
Organelles found in many plants and some other eukaryotes, containing in the lumen the enzymes required for the glyoxylate cycle. These single-membrane-bound organelles are functionally specialized derivatives of peroxisomes.

Protein-bound microcompartments and nanocompartments. Various organelles have been described in which luminal contents are surrounded by a protein coat (FIG. 3). One major class, the bacterial microcompartments, enable diverse organisms to optimize their metabolic processes[129,130]. The proteinaceous shell of these organelles is made from three core proteins that first oligomerize into hexamers and pentamers and then 'tile' together to form a 200–300 nm pseudo-icosahedron[131–134] (FIG. 3a). Members of at least 23 bacterial phyla are predicted to encode microcompartment shell proteins[135]. The best-studied microcompartments are carboxysomes, which increase the efficiency of carbon fixation in photoautotrophs and chemoautotrophs. These compartments contain a higher concentration of CO_2 than the rest of the cell owing to the activity of carbonic anhydrase and the selective permeability of the protein shell, which in turn increases both the rates and specificity of the encapsulated carbon-fixing enzyme RuBisCO (ribulose 1,5-bisphosphate carboxylase/oxygenase) (FIG. 1). Another class, the metabolosomes, sequester and degrade reactive aldehyde intermediates formed during the oxidation of alternative carbon sources (such as propanediol, ethanolamine and choline)[130,136,137]. Such organelles are proposed to facilitate niche expansion of environmental and pathogenic bacteria by enabling efficient catabolism of alternative growth substrates[129].

Bacterial microcompartments are self-assembling organelles and, as such, are being explored for uses in biotechnological applications (BOX 3). Super-resolution imaging can distinguish the proteins localized to the outer protein shell from the luminal proteins within the organelle and is gradually providing detailed information on the sequence of events for organelle formation[138]. Detailed genetic and structural studies suggest that the β-carboxysome of *Synechococcus elongatus* can be assembled in a defined, stepwise manner. The core of the carboxysome forms first, as a result of RuBisCO aggregation facilitated by CcmM, and shell components then encapsulate the phase-separated aggregate[139,140]. Aggregation is facilitated by dynamic interactions between the RuBisCO large subunit and the CcmL modules, which are homologous to the RuBisCO small subunit[140,141] (FIG. 3c,d). Whereas metabolosomes seem

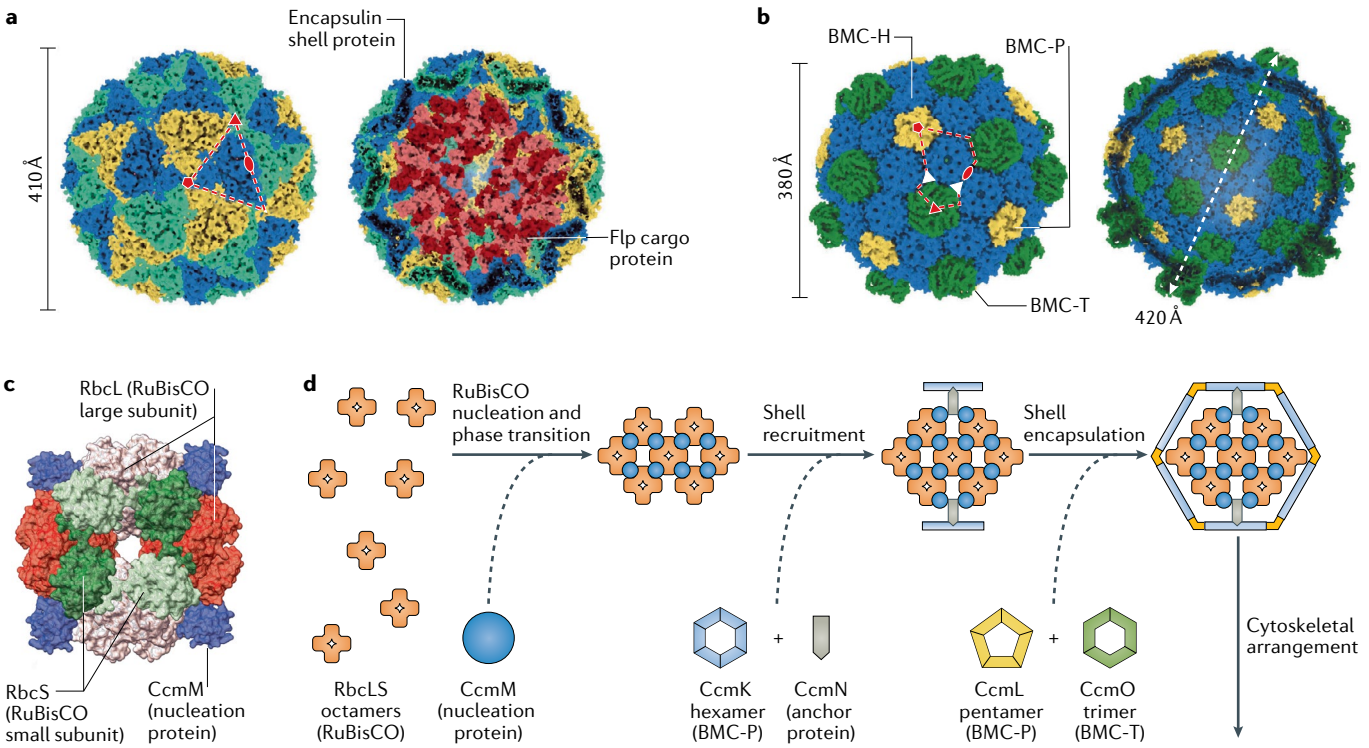

Fig. 3 | **Structure and formation of protein-bound organelles. a** | Structure and assembly of the iron-sequestering nanocompartment from *Quasibacillus thermotolerans* (PDB 6NJ8). The icosahedral shell forms through tiling of 240 identical encapsulin protomers (left; differentially shaded for contrast in blue, yellow and green). Cross-section (right) shows that iron-bound ferritin-like cargo proteins (Flp; red and pink) bind the interior face of the encapsulin protomers via their C-terminal peptides. **b** | Structure of microcompartment shells based on a *Haliangium ochraceum* organelle of unknown function (PDB 5V74). The icosahedral shell forms through the multimerisation and assembly of three proteins, the hexameric BMC-H (blue), trimeric BMC-T (green) and pentameric BMC-P (yellow). On the right, the cross-section of the hollow organelle is shown. For parts **a** and **b**, the red dotted lines show the icosahedral asymmetric unit and associated shapes show symmetry operators (hexagon for fivefold, triangle for threefold, oval for twofold and a hollow triangle for pseudo-threefold). **c** | Structure of the protein complex formed during the initiation of assembly of the *Synechococcus elongatus* β-carboxysome (PDB 6HBC). The structure shows the octamerization of ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) large subunit RbcL (light and dark red) and small subunit RbcS (light and dark green). Interactions between RbcL and the inner carboxysome protein CcmM (blue) trigger RuBisCO nucleation and lead to the recruitment of shell proteins. **d** | Schematic model of the assembly of microcompartments based on the *S. elongatus* β-carboxysome[139]. Following the nucleation of RuBisCO as a result of the interaction between RbcL and CcmM, the shell is gradually assembled by the recruitment and multimerisation of other shell proteins through protein–protein interactions.

Box 3 | Engineering bacterial organelles for biotechnological applications

Researchers are taking advantage of our increased understanding of the structure, function and formation of bacterial organelles to engineer them for diverse applications. A wide range of applications for bacterial organelles have been proposed, some of which have considerable industrial potential. For example, there have been several remarkable reports of the engineering of protein-bound bacterial organelles for the development of orthogonal biocatalytic cascades[215], targeted delivery of therapeutics[216] and even high-resolution ultrasound imaging[217]. Similarly broad applications have been proposed for membrane-bound organelles, especially magnetosomes, spanning from pollutant bioremediation to biomedical imaging[218,219].

Most biotechnological applications rely on the common ability of organelles to sequester molecules into isolated compartments and/or increase surface area for processes to occur. These functions are particularly useful for industrial biocatalysis[215]. By concentrating enzymes and metabolites into organelles, it is possible to favourably modulate the kinetics, thermodynamics and stability of metabolic processes (both natural and novel). Moreover, spatially restricting these processes can reduce metabolic crosstalk, the accumulation of toxic metabolites and the disruption of native processes. Thus, engineered organelles can dramatically enhance processes and drive increases in complexity just as they do in natural systems.

As recently reviewed[220], nanocompartments are particularly adaptable. These organelles can be readily engineered into tractable non-native hosts (including eukaryotes) or, alternatively, their encapsulins can spontaneously assemble for cell-free applications. Moreover, a range of non-native proteins can be specifically targeted to these organelles by fusing them with targeting peptides.

Nanocompartments
Minimalistic organelles containing a polyhedral shell made of the protein encapsulin and a lumen containing targeted cargo proteins. These organelles are widespread in bacteria and archaea, for example, supporting oxidative stress responses in *Mycobacterium tuberculosis*.

Gas vesicles
Protein-bound compartments that function in buoyancy for bacterial and archaeal cells, for example, to position photosynthetic bacteria in a water column to access optimal light levels; also known as gas vacuoles.

Carbonosomes
A recently defined organelle that stores polyhydroxyalkanoates and potentially other carbon storage compounds; thought to be bound by proteins rather than phospholipids.

Chlorosomes
Efficient light-harvesting organelles found in green sulfur bacteria and some other anoxygenic phototrophs. They contain a protein-coated lipid monolayer boundary and are attached to the inner membrane through a proteinaceous baseplate. Chlorosomes have been extensively studied in *Chlorobaculum tepidum*.

to assemble in an analogous manner[134], it is proposed that the core and shell components of the structurally unrelated α-carboxysomes assemble simultaneously[142]. Nevertheless, in a remarkable example of convergent evolution, the interaction between RuBisCO and a unique intrinsically disordered RuBisCO-binding protein (CcoS2) drives the formation of phase-separated aggregates and leads to the recruitment of additional subunits[143]. Common to some membrane-bound organelles, bacterial microcompartments are spatially ordered through the cell and this order ensures segregation during cell division. However, this interaction appears to be mediated through self-organizing ParA-like ATPases rather than through direct cytoskeletal interactions[144–146].

A minimalistic type of protein-bound organelle, the encapsulin nanocompartments, contain multiple copies of a single curved protomer, called encapsulin, that assemble into a 18–42 nm icosahedral coat[147]. Encapsulins have common folds, but no sequence homology, with HK97 capsid proteins from tailed bacteriophages and herpesviruses[148]. In the interior, the encapsulins form binding sites for C-terminal targeting peptides of cargo proteins[147,149,150]. Encapsulin genes are distributed across 15 bacterial phyla, suggesting that encapsulin nanocompartments are among the most widespread organelles. These nanocompartments typically contain cargo proteins implicated in oxidative and nitrosative stress resistance[151]. For example, various bacteria encase iron-binding proteins in these nanocompartments, just as others use membrane-bound magnetosomes to perform this function[147,148,152] (FIG. 1). This function is illustrated by the recent structure of the 42 nm iron storage nanocompartment from the Gram-positive bacterium *Quasibacillus thermotolerans*[149]. Each particle contains 240 encapsulin protomers, which encase ferritin-like proteins (Flp) and over 23,000 iron atoms (FIG. 3b). Various other cargo proteins are also targeted to these nanocompartments[150,153]; for example, Flp, DyP-type peroxidase and a folate biosynthesis enzyme are all targeted to nanocompartments in *Mycobacterium tuberculosis* as a possible strategy to coordinate oxidative stress responses[153]. Moreover, preliminary evidence suggests that anammox bacteria synthesize nanocompartments in addition to membrane-bound anammoxosomes[151,154]. Although the mechanisms of nanocompartment assembly are incompletely understood, in vitro and in vivo studies have shown that encapsulins can self-sort cargo proteins and spontaneously assemble[155–157].

Two other classes of protein-bound organelles have been described: gas vesicles and carbonosomes. In contrast to the protein-encapsulating organelles previously discussed, gas vesicles have hollow gas-filled interiors, which provide buoyancy to diverse planktonic bacteria and archaea. Details on the distribution, structure and assembly of gas vesicles have been reviewed[158]. Carbonosomes mediate and regulate the storage of polyhydroxyalkanoates, a widespread class of energy storage polymers[159,160]. As recently reviewed[161], contrary to classical models[162], these organelles in fact lack phospholipids and are instead coated with a layer of structural and functional proteins[159–161,163].

Organelles bound by lipid monolayers and associated proteins. Thylakoids and chromatophores are two types of membrane-bound organelles conducting photosynthesis. However, distinct photosynthetic antennae, called chlorosomes, are found in all members of the Chlorobi (green sulfur bacteria) as well as in photosynthetic bacteria in Chloroflexi and Acidobacteria[15,164–167]. Chlorosomes are fascinating in many respects, including how they enable bacterial cells to harvest photons at extremely low light intensities such as sunlight at water depths below 100 m and potentially even infrared rays produced at geothermal vents[168–170]. Structurally, they are also remarkable given that they hybridize features of protein-bound and membrane-bound organelles. Appressed to the cytosolic face of the cytoplasmic membrane, chlorosomes form ellipsoids of variable size and volume[15,171]. These organelles are separated from the cell membrane by a proteinaceous baseplate[172–174] and from the cytosol by a boundary membrane with properties consistent with a monolayer of phospholipids, glycolipids and associated proteins[175–178] (FIG. 1). The extreme efficiency of light harvesting by chlorosomes reflects the dense packing of thousands of pigments; the large chlorosomes of *Chlorobaculum tepidum* (133×57×36 nm)[15,179] each contain up to 250,000 bacteriochlorophyll pigments organized into concentric rod-shaped oligomers[180,181]. Excitation energy is sequentially transferred from the bacteriochlorophylls in the rods, baseplate and FMO complex to the proton-translocating reaction centre in the cell membrane[174,178,181]. The synthesis and size of chlorosomes is regulated by environmental conditions such as light intensity[182,183].

Similarly to microcompartments and nanocompartments[129], the self-assembly of luminal contents contributes to chlorosome assembly. The bacteriochlorophylls of Chlorobi and Chloroflexi self-aggregate in the absence of proteins into rod-shaped oligomers,

Lipid bodies
Organelles found in eukaryotes and bacteria that function in the storage and hydrolysis of triacylglycerols, wax esters and other neutral lipids. They have a boundary formed by a phospholipid monolayer that also includes protein components. Notable examples are found in *Mycobacterium tuberculosis* and *Rhodococcus opacus*; also known as lipid droplets or oil bodies.

similar to those found in chlorosomes[184,185]. It has also been hypothesized that pigment accumulation triggers the assembly of the proteinaceous and lipid components of chlorosomes[186], although this notion is not consistent with observations that carotenoid-containing chlorosomes are still formed even in bacteriochlorophyll biosynthesis mutants[187]. Likewise, it remains unresolved which mechanisms ensure that chlorosome assembly occurs in association with the inner membrane and results in the generation of the proteinaceous boundary. A further enigma is the role of the proteins associated with the lipid monolayer. In addition to the baseplate protein CsmA[174], *C. tepidum* chlorosomes contain nine other Csm proteins[188–191], some of which are conserved in Chloroflexi and Acidobacteria phototrophs[190,192]. Mutagenesis studies show that these Csm proteins are individually dispensable[187] but variably influence the size, shape, composition and function of chlorosomes[193,194].

Finally, a more widespread and potentially related[186] group of protein–lipid monolayer organelles are the lipid bodies, also known as lipid droplets[195]. These large

Table 1 | **Types, functions and distribution of organelles in bacteria**

Organelle	Primary function	Reported occurrence	Key references
Membrane bound			
Thylakoids	Light harvesting and energy transduction during oxygenic photosynthesis	Cyanobacteria (for example, *Synechococcus elongatus*)	28,38
Chromatophores	Light harvesting and energy transduction during anoxygenic photosynthesis	Alphaproteobacteria (for example, *Rhodobacter sphaeroides*)	34,35
Anammoxosomes	Energy transduction during anaerobic ammonium oxidation	Planctomycetes (for example, *Kuenenia stuttgartiensis*)	44,54
Ferrosomes	Iron storage in iron-reducing bacteria	Proteobacteria (for example, *Shewanella putrefaciens*), likely others	63,67
Magnetosomes	Magnetoreception through orientation of iron oxides	Proteobacteria, Nitrospirae (for example, *Magnetospirillum gryphiswaldense*)	29,102
Acidocalcisomes	Calcium and polyphosphate storage in acidic compartments	Proteobacteria (for example, *Agrobacterium tumefaciens*), likely others	69,70
Nitrate vacuoles	Nitrate storage in giant compartments	Gammaproteobacteria (for example, *Thioploca araucae*)	68,209
Protein bound			
β-Carboxysomes	Carbon concentration and fixation in phototrophs	Cyanobacteria (for example, *S. elongatus*)	139,140
α-Carboxysomes	Carbon concentration and fixation in phototrophs and lithotrophs	Proteobacteria, Cyanobacteria (for example, *Halothiobacillus neapolitanus*)	142,143
Metabolosomes	Sequestration of catabolic pathways that produce reactive aldehydes	Multiple bacterial and archaeal phyla	130,133
Nanocompartments	Sequestration of iron and oxidative stress enzymes	Multiple bacterial and archaeal phyla	149,151
Gas vesicles	Hollow gas-filled compartments that enable buoyancy	Multiple bacterial and archaeal phyla	158
Carbonosomes	Storage of polyhydroxyalkanoates as energy reserves	Proteobacteria (for example, *Ralstonia eutropha*), likely others	159,161
Protein–lipid monolayer			
Lipid bodies	Storage of lipid compounds as energy reserves	Multiple bacterial and archaeal phyla	195,198
Chlorosomes	Light harvesting during anoxygenic photosynthesis	Chlorobi, Chloroflexi, Acidobacteria (for example, *Chlorobaculum tepidum*)	170,186
Phase-defined			
Nucleolus-like compartment	Transcription of ribosomal RNAs	Proteobacteria, Firmicutes (for example, *Escherichia coli*), likely others	6,119
RNP bodies and degradosomes	Localized sites of mRNA degradation	Proteobacteria (for example, *Caulobacter crescentus*), likely others	119,122
RNAP clusters	Localized sites of transcription containing RNA polymerase	Proteobacteria (for example, *E. coli*), likely others	120
Nucleus-like compartment	Nucleic acid-rich compartment formed during bacteriophage replication	Infected Proteobacteria (for example, *Pseudomonas chlororaphis*), likely others	8,128

globular organelles contain a neutral core of energy storage lipids, typically triacylglycerols or wax esters, surrounded by a phospholipid monolayer and various associated proteins[195–198]. In some organisms, these organelles can comprise over 80% of cellular dry weight, for example, in the biotechnologically important lipid producer *Rhodococcus opacus*[199,200]. These abundant energy reserves have been shown to sustain the long-term survival of both environmental and pathogenic actinobacteria[201–203]; for example, in a remarkable subversion of host defences, *M. tuberculosis* acquires and stores triacylglycerols derived from host macrophages during its transition to dormancy[201,204]. Although lipid bodies are among the oldest known organelles, microbiologists underestimated their complexity until recently, just as they did for bacterial cells as a whole[198,205]. There is increasing evidence that lipid accumulation is tightly regulated and depends on a specific organellar proteome, including lipid synthesis as well as dynamin-like and SNARE-like proteins[197,198,206]. On this basis, it has been hypothesized that bacterial lipid body formation may be mediated at the cell membrane by dynamin-like proteins[198], although this hypothesis requires empirical testing and alternative models have been proposed[207]. Analogous storage organelles are also found in eukaryotes, although they are proposed to be produced through distinct mechanisms[208].

Conclusions

Morphological and genomic studies suggest that diverse organelles are widely distributed across bacteria. These organelles can be broadly classified into three types: membrane-bound, protein-bound and phase-derived organelles (TABLE 1). Although bacterial organelles mediate diverse specific functions, a common feature is that they enhance the control of physiological processes and increase metabolic efficiency. Many described organelles enable specialist metabolic processes, including various forms of photoautotrophy and lithoautotrophy. Some structurally unrelated organelles have common roles, for example, in photosynthetic light reactions and iron accumulation.

Given that uncharacterized membrane-bound and protein-bound organelles are common in bacteria, a key priority is to define their luminal proteomes and infer their physiological roles. It is increasingly possible to synergize imaging, structural and genomic approaches to investigate organelles in non-model systems.

Although mature organelles can look very different and have been historically considered quite distinct, the hypothesis that common processes are at play should be tested. Whether organelles are formed by membrane invagination (for example, magnetosomes) or by fission of a parental organelle (for example, anammoxosomes), two concepts unify the formation of membrane-bound organelles. First, membrane curvature-inducing proteins initiate invagination; this curvature is stabilized by lipids and is mediated by specific protein factors on the membrane. Second, the packaging of specific proteins into the lumen of a membrane compartment will determine its function. The mechanism underlying protein translocation is currently unknown. Diverse pathways result in the formation of phase-derived, protein-bound and lipid monolayer organelles. One common, though not universal, concept is that the cytosolic accumulation of cargo macromolecules (for example, RuBisCO in carboxysomes, bacteriochlorophylls in chlorosomes or nucleic acids in nucleolus-like compartments) induces a phase transition and leads to the recruitment of coat components.

Further investigations are needed to understand how environmental cues and developmental processes regulate the formation of organelles. A promising approach is to use genetic and physiological approaches in model systems to understand which signals are read and how they drive organelle formation.

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