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The protein shells of bacterial microcompartment organelles

Todd O Yeates^{1,2}, Michael C Thompson¹ and Thomas A Bobik³

Details are emerging on the structure and function of a remarkable class of capsid-like protein assemblies that serve as simple metabolic organelles in many bacteria. These bacterial microcompartments consist of a few thousand shell proteins, which encapsulate two or more sequentially acting enzymes in order to enhance or sequester certain metabolic pathways, particularly those involving toxic or volatile intermediates. Genomic data indicate that bacterial microcompartment shell proteins are present in a wide range of bacterial species, where they encapsulate varied reactions. Crystal structures of numerous shell proteins from distinct types of microcompartments have provided keys for understanding how the shells are assembled and how they conduct molecular transport into and out of microcompartments. The structural data emphasize a high level of mechanistic sophistication in the protein shell, and point the way for further studies on this fascinating but poorly appreciated class of subcellular structures.

Addresses

¹ UCLA Department of Chemistry and Biochemistry, Los Angeles, CA, United States

² UCLA Molecular Biology Institute, Los Angeles, CA, United States

³ Department of Biochemistry, Biophysics & Molecular Biology, Iowa State University, Ames, IA, United States

Corresponding author: Yeates, Todd O (yeates@mbi.ucla.edu)

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Introduction

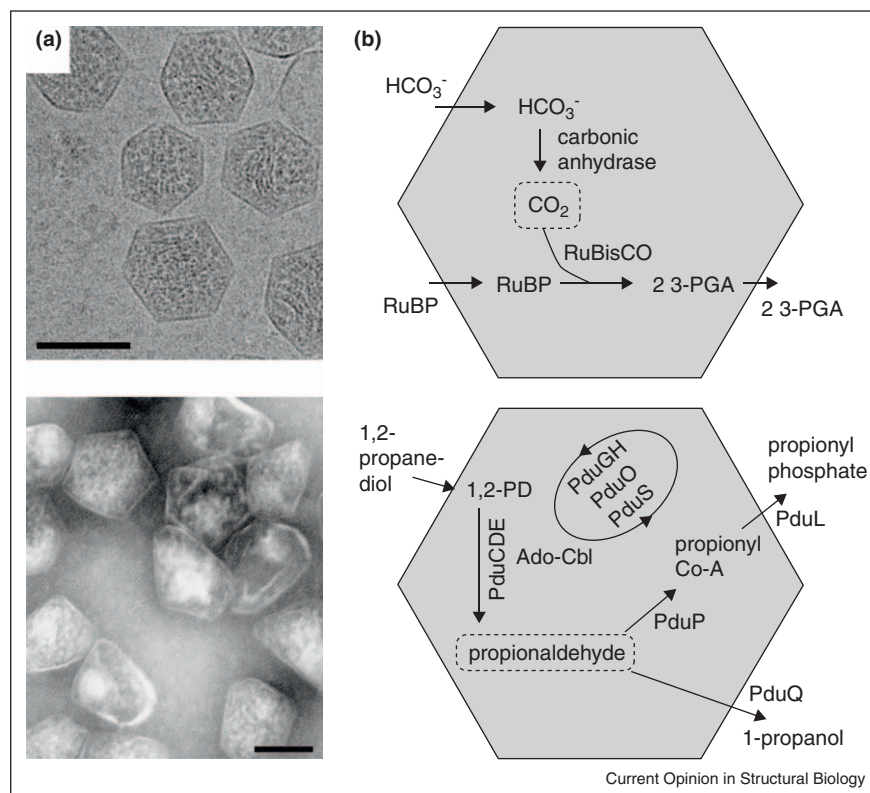
Many bacteria contain large, polyhedral, protein-based organelles referred to as bacterial microcompartments (reviewed in [1–4]). Polyhedral inclusions that had been visualized by electron microscopy in cyanobacteria and some chemoautotrophs were first isolated in 1973 and determined to contain the CO₂-fixing enzyme RuBisCO [5^{••}]; they were therefore named carboxysomes, and are now recognized as the founding member of a diverse group of microcompartments (Figure 1). Subsequent genetic and physiological studies on carboxysomes indicated that they played an active role in CO₂ fixation [6–9]. As part of a mechanism for enhancing CO₂ fixation,

carbonic anhydrase, which dehydrates bicarbonate to CO₂, is encapsulated in the carboxysome together with RuBisCO [10[•],11,12]. This arrangement is believed to provide a high concentration of CO₂ for RuBisCO under growth conditions where inorganic carbon is limiting, and may confer other advantages as well [13]. Besides the carboxysome, several other types of bacterial microcompartments carrying out more complex reactions have been either studied directly or inferred from genomic data.

A unifying feature of diverse bacterial microcompartments is a thin shell composed primarily of a few thousand small protein subunits belonging to a family of homologous so-called BMC (for bacterial microcompartment) shell proteins. This outer shell encapsulates the interior enzymes while allowing transport of substrates and products. BMC shell proteins, which were first identified in carboxysomes [14,15[•],16], were subsequently identified in the propanediol utilization operon (*pdu*) of *Salmonella* (a heterotroph) [17,18^{••}]. Genetic and biochemical studies demonstrated that the Pdu microcompartment encapsulates a series of enzymes that metabolize 1,2-propanediol (1,2-PD) [19[•],20] (Figure 1). Sequestering that metabolic pathway prevents exposure of the cytosol to propionaldehyde — an intermediate in the reaction pathway — which is toxic to the cell at high concentrations [20,21]. Likewise, genes for BMC shell proteins are found in an operon for metabolizing ethanolamine in enteric bacteria [22,23], including *Salmonella* and *Escherichia coli*. The ethanolamine utilization (or Eut) microcompartment system shares similar chemistry and analogous enzymes with the Pdu system. Experiments on the Eut system in *Salmonella* indicate that the sequestered metabolism of ethanolamine prevents exposure of the cytosol to the reactive acetaldehyde intermediate [24], while also preventing the detrimental evaporative loss of that compound from the cell [25]. Compared to the carboxysome, the latter two types of microcompartments are more complex. Besides encapsulating more enzymes, the interior reactions involve numerous cofactors, including adenosyl cobalamin (B₁₂), NAD⁺/NADH, acetyl-CoA, ATP, and [Fe–S] clusters (reviewed in [1,3]).

Motivated by the discovery of BMC shell proteins in diverse bacteria, searches for homologous shell proteins across the known protein sequence databases have emphasized the widespread occurrence of microcompartments across the bacterial kingdom, and their likely spread by horizontal gene transfer [2–4,26,27^{••}]. Approximately 1700 unique proteins containing BMC domains can be identified at present, covering at least 10 different

Figure 1



Examples of bacterial microcompartments. (a) Electron micrographs of purified carboxysomes (top) from *Halothiobacillus neapolitanus* and purified propanediol utilization (Pdu) microcompartments (bottom) from *Salmonella enterica*. The scale bars are 100 nm. Under conditions where they are produced, several microcompartments are typically found within each bacterial cell. (b) Diagram of CO₂ fixation in the carboxysome (top) and a simplified diagram of propanediol metabolism in the Pdu microcompartment (bottom). RuBP is 1,5-ribulose biphosphate and 3-PGA is 3-phosphoglycerate. Reactions in the Pdu microcompartment involve multiple cofactors (not shown), including NAD⁺/NADH, ATP, coenzyme-A, and a B₁₂ cofactor, with reactivation and replacement of the latter cofactor being carried out by the PduGH, PduS, and PduO enzymes. In each microcompartment, the key encapsulated intermediate is boxed. Carboxysomes were provided by Gordon Cannon and Sabine Heinhorst and imaged by Kelly Dryden and Mark Yeager.

bacterial phyla. Multiple paralogs of the shell proteins are essentially always found together. Experimental studies on these wide ranging systems are limited, though inferences regarding their likely functions can be made in some cases from the genomic contexts in which the BMC shell proteins are found [2,3]. Enzymes found to occur often in chromosomal proximity to BMC shell proteins have been tabulated [3]. These represent avenues for exploratory experimental work.

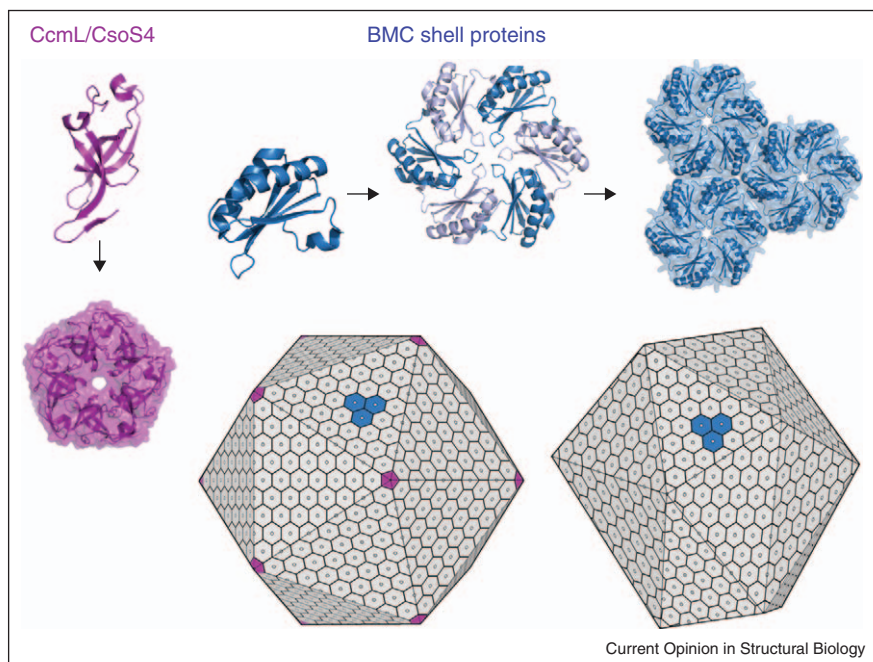
Until recently, the structure and mechanisms of bacterial microcompartment shells were unclear. At the architectural level, early electron microscopy studies on carboxysomes had not provided a definitive shape. Likewise, at the atomic level, three-dimensional details were lacking. How the shell proteins assembled to form a semi-permeable layer, and why multiple distinct paralogs are involved, were unknown. Crystal structures of numerous BMC shell proteins, first from the carboxysome [27^{••},28^{••},29^{••},30,31] and then from the Pdu [32[•],33[•]],

Eut [34[•],35,36^{••}], and other systems [37[•]], have clarified how BMC shell proteins assemble to form a shell, how they might facilitate molecular transport through their pores, and how distinct paralogs play specialized roles in microcompartment organelles.

Elements of BMC shell protein assembly

The typical BMC domain is about 90 amino acids in length and adopts an alpha/beta fold ([27^{••}], reviewed in [3]). Individual BMC proteins self-assemble to form cyclic, disc-shaped hexamers that constitute the basic building blocks of the shell (Figure 2). Each hexamer typically presents a narrow pore through the middle, along the six-fold axis of symmetry. Although individual BMC proteins differ, as described subsequently, in most cases the two sides of the disc reveal dramatically different shape properties. One side, described as concave, generally bears a bowl-shaped depression with a relatively hydrophobic surface. The N-termini and C-termini, which typically reside on this concave side, tend to diverge in length

Figure 2



Structure and assembly of microcompartment shells. BMC shell proteins (top) form hexamers that constitute the main building blocks of the shell. These assemble further to form tightly packed layers with pores in the middle of the hexamers, some of which have gated openings. BMC shell proteins are found widely across the bacteria. Where they occur, they provide a signature for the presence of a microcompartment organelle. In the carboxysome, pentameric proteins (CcmL and CsoS4) likely form the vertices of the shell, which is nearly icosahedral in shape (lower left). Pdu microcompartments and some other types form less geometrically regular shells (lower right) from hexameric BMC proteins; the presence of pentameric shell proteins in those cases has not been established.

and structure between BMC paralogs, and often appear flexible or disordered in crystal structures. This side of the shell proteins has been argued to be the side facing towards the microcompartment interior [38].

Crystal structures show that individual BMC hexamers are tailored to further assemble side-by-side, thereby forming a tightly packed molecular layer [27^{••},30,35,36^{••},38] (Figure 2). Multiple lines of evidence indicate that the molecular layers visualized within the context of three-dimensional crystals represent the arrangement of proteins in the facets of natural biological shells. The specific mode of packing is conserved in at least six of the crystal structures reported to date, and key intermolecular hydrogen bonds are conserved at the interfaces in cases where hexamers pack tightly together. Furthermore, true two-dimensional layers of BMC proteins can be formed at the air-water interface of a liquid droplet, where they recapitulate the packing seen in three-dimensional crystals [39]. From these extended arrays, a picture of the shell emerges as a tightly packed molecular layer perforated by narrow protein pores spaced just less than 70 Å apart (Figure 2).

The question of how an otherwise flat layer of BMC shell protein hexamers bends or folds up to make a closed shell

was answered in part by structural studies on another family of conserved proteins that, along with the BMC protein family, also appears to be present in all microcompartments. Homologous proteins CcmL and CsoS4A from two different types of carboxysomes were both shown to be pentamers whose size and shape are compatible with their placement at the vertices of an icosahedral shell built from many hexamers and 12 pentamers [29^{••}] (Figure 2). An icosahedral structure for the carboxysome was anticipated by the hexameric structure of the BMC shell proteins [27^{••}], and confirmed by EM cryotomography studies [40[•],41[•]]. A scenario in which the CcmL or CsoS4 pentamers occupy icosahedral vertices is consistent with the low abundance of these proteins in the shell, and with mutational experiments in which deletion of the genes (or their homologs in other systems) led to extended assemblies that generally failed to close up [42[•],43]. However, recent mutagenesis experiments on the carboxysome suggest that the situation may be more complicated [44]. A further complication is presented by homologs of this protein family from other types of microcompartments. The homologous protein in the Eut microcompartment is EutN, which forms hexamers instead of pentamers when expressed and crystallized by itself [29^{••}]; whether EutN and other members of this protein family from different microcompartment types

are pentamers in the context of an intact shell is unknown. It is notable however that the Pdu and Eut microcompartments do not resemble a regular icosahedron as closely as the carboxysome does; this may correlate with the different behaviors of the CcmL/CsoS4/EutN/PduN family of proteins in different systems.

The roles of BMC protein fold variations

Crystal structures of numerous BMC proteins have revealed a surprising array of conformational and topological variations within a small protein domain (reviewed in [3]) (Table 1). Some BMC proteins are related to the canonical type by a circular permutation, so that a similar tertiary structure arises from secondary structures occurring in a different order [28^{••},32[•],33[•],34[•],36^{••},37[•]]. This produces N-termini and C-termini at different spatial locations, which may be an important feature. In permuted BMC proteins PduU and EutS, the occurrence of the N-terminus on the other side of the disk makes it possible for an extended tail to create a 6-stranded beta barrel that blocks the pore [33[•],36^{••}].

Numerous BMC proteins, including at least one from all the microcompartments that have been analyzed to date, contain tandem-BMC domains. In such cases, three copies

of the protein assemble to make a symmetric trimer with pseudo six-fold symmetry [28^{••},32[•],34[•],36^{••},37[•]]. Examples of such structures have been obtained from four types of microcompartments. In two of these cases (CsoS1D and EutL), crystal structures have been visualized of two alternate forms related by dramatic conformational changes that open and nearly close the central pore [28^{••},35,36^{••}]. The symmetry breaking that follows from domain duplication appears to facilitate conformational variation and flexibility [3,37[•]]. Also notable is the finding that different tandem-BMC domain proteins can have their consecutive domains arranged in opposite orders in the pseudo-hexamers [36^{••}]. Furthermore, some tandem domain proteins are built from two circularly permuted domains (CsoS1D, EutL and EutB), while some are built from two canonical BMC domains (PduT) [32[•]], further highlighting the evolution of structural variety in this protein family.

Another form of symmetry breaking was observed in the structure of the permuted, single-BMC domain protein, EutS. Here, six chemically identical subunits form a hexagonal disc that is bent or creased by about 40° [36^{••}]. Mutagenesis and sequence comparisons identify a glycine substitution as critical for the formation of the

Table 1

Known structures of shell proteins from bacterial microcompartments.

Protein	Structure	Properties
<i>α</i> -Carboxysome (e.g. <i>Halothiobacillus neapolitanus</i>)		
CsoS1A	Single-BMC domain hexamer	Major shell component.
CsoS1C	Single-BMC domain hexamer	Major shell component.
CsoS1D	Tandem-BMC domain (permuted) trimer	Trimers stack to form a hexamer with D3 symmetry; open and closed conformations suggest gated transport.
CsoS4A/B	Pentamer (non-BMC domain)	Minor shell component; believed to form icosahedral vertices.
<i>β</i> -Carboxysome (e.g. <i>Synechocystis PCC6803</i>)		
CcmK1	Single-BMC domain hexamer	Major shell component.
CcmK2	Single-BMC domain hexamer	Major shell component.
CcmK4	Single-BMC domain hexamer	Minor shell component.
CcmL	Pentamer (non-BMC domain)	Minor shell component; believed to form icosahedral vertices.
<i>Pdu</i> Microcompartment (e.g. <i>Salmonella enterica</i>)		
PduA	Single-BMC domain hexamer	Major shell component.
PduT	Tandem-BMC domain trimer	Pore likely involved in electron transport or Fe-S cluster transport.
PduU	Single-BMC domain hexamer (permuted)	Minor shell component; pore is blocked by 6-stranded β-barrel in crystal structure.
<i>Eut</i> Microcompartment (e.g. <i>S. enterica</i> and <i>E. coli</i>)		
EutK	Single-BMC domain fused to helix-turn-helix (HTH) domain	HTH motif with positively charged surface suggests role in nucleic acid binding.
EutL	Tandem-BMC domain trimer	Pore observed in open and closed conformations; probable role in gated transport.
EutM	Single-BMC domain hexamer	Probable major shell component.
EutN	Non-BMC domain hexamer	Crystallizes as a hexamer despite homology to pentameric CcmL/CsoS4 family; role unclear.
EutS	Single-BMC domain (permuted) hexamer	Hexamer forms bent structure with C2 symmetry; possible role in edge formation within shell.
<i>Other microcompartments</i>		
EtuB	Tandem-BMC domain (permuted) trimer	Part of presumptive ethanol utilization microcompartment in <i>Clostridium kluyveri</i> .
PduT homolog	Tandem-BMC domain trimer	Part of microcompartment of uncharacterized function in <i>Desulfitobacterium halfniense</i> .

bent structure. Such deviations from symmetry are rare in nature, and often have functional significance ([45,46], reviewed in [47]). This particular BMC paralog could form edges in the context of an intact shell, or generally promote curvature of the BMC protein layer.

Further structural variation appears in the form of double disk structures, which have been seen in one carboxysome protein (CsoS1D) [28**] and one protein (homologous to PduT) from *Desulfitobacterium hafniense* (PDBID 3NWG). CsoS1D displays open and nearly closed conformations, which could allow alternating access to the chamber formed by two opposing discs [28**]. Alternatively, it was suggested that the observed two-tier structure could either indicate that shells are actually composed of double protein layers, or represent the way adjacent microcompartment shells might be bound together. However, the presence of a relative twist between the two discs appears incompatible with formation of two layers in an extended register, which weighs against the latter two scenarios.

Finally, numerous proteins contain BMC domains as fusions with other domains. The full range of fused domains has not been characterized, and only one structure has been elucidated to date. In the Eut microcompartment, the EutK protein contains a C-terminal domain of approximately 60 amino acids. A crystal structure of the fusion domain revealed a helix-turn-helix motif, which is common in nucleic acid binding proteins, and a positively charged surface consistent with that putative function [36**]. The unexpected prediction of a connection between BMC proteins and nucleic acids presents an open line for investigation.

Protein pores and molecular transport

The small pores visualized in the centers of typical BMC shell proteins are presumed to be the routes of transport for the substrates, products, and cofactors for varied microcompartments [27**,30,32*]. Size, electrostatic and hydrogen bonding features of the pores have been identified as potentially important in facilitating the transport of substrates and products more readily than the sequestered metabolic intermediates (Figure 3). BMC proteins that represent major components of their respective shells all appear to possess these pores, whereas some other minor BMC shell components (*e.g.* PduU) have occluded pores. This trend suggests that the porosity conferred by the system of small pores is an important property of the shells [30]. Considerably larger pores in BMC shell proteins have been visualized in two cases so far [28**,34*,36**]. In both cases, the observation of alternate, nearly closed conformations, suggests the likelihood of gated transport, possibly as a mechanism to allow the transport of larger substrates or cofactors while somehow limiting the escape of sequestered intermediates.

A recent structure of the PduT shell protein from *Salmonella* provides the first indication of how the redox state inside the Pdu microcompartment might be balanced [32*]. Spectroscopic data indicated that PduT binds a [4Fe-4S] cluster [48], which was determined by crystallography to be bound in the center of the pore by cysteine residues contributed by three protein subunits [32*]. It has been proposed that the metal cluster in the pore either first, facilitates electron flow, presumably out of the microcompartment in order to regenerate NAD⁺ required to oxidize propanediol, or second provides a route for transporting intact iron-sulfur clusters into the microcompartment to replace damaged metal clusters in interior enzymes, including PduS. The latter hypothesis is supported by the recent structure of a PduT homolog (PDBID 3NWG) with an alternate pore conformation lacking a metal cluster.

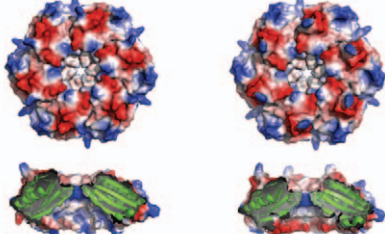
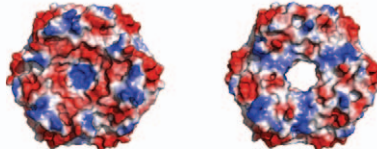
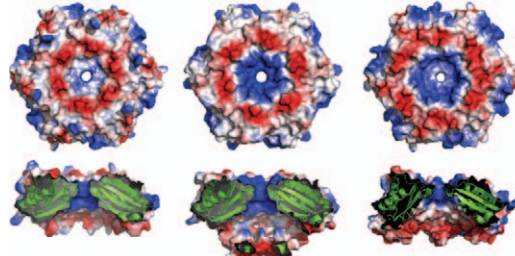
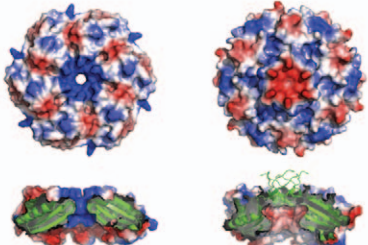
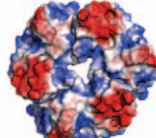
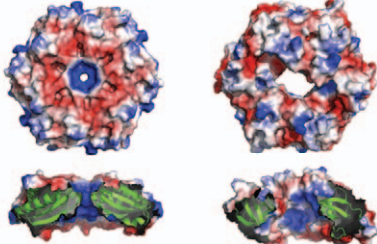
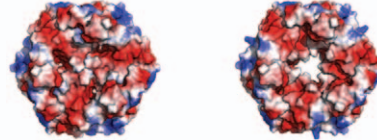
Despite the tantalizing clues regarding molecular transport offered by the structural findings, experimental data are lacking. In some crystal structures, electron density features in the central pores have suggested the presence of substrates or products, but the evidence has been inconclusive [30,32*,38]. Those observations could reflect weak affinity for molecules that need to move through the pores. The structural data do however provide a framework for investigating the proposed transport phenomena by mutagenesis and physiological studies. Those experiments will be vital for advancing our understanding of molecular transport.

Targeting enzymes to the shell interior

Some of the details concerning how enzymes are targeted to the interior surfaces of their respective microcompartments are beginning to emerge. Interestingly, at least two different kinds of mechanisms appear to operate in different microcompartments. In carboxysomes belonging to the so-called beta type, a highly unusual protein, CcmM, appears to serve as a scaffold for establishing interactions to both shell proteins and enzymes [49,50]. CcmM bears an N-terminal domain that carries a redox-sensitive carbonic anhydrase activity [51] and a C-terminal region that carries multiple tandem domains with homology to the small subunit of RuBisCO [43,52]; these presumably mimic the native interactions of the small subunit with large subunits of RuBisCO inside the carboxysome.

In the Pdu microcompartment, and likely the Eut and other closely related types, recent experiments have demonstrated that some of the internal enzymes are directed to the interior by special N-terminal targeting sequences [42*,53*]. The simplicity of this system makes it attractive for engineering studies aimed at encapsulating distinct enzymes in order to create novel reaction chambers. The feasibility of such a goal is supported by the demonstration that the Pdu shell can be assembled

Figure 3

	Homohexamer (Single BMC Domain)	Pseudo-hexameric Trimer (Tandem BMC Domains)
Alpha-Type Carboxysome	<p>CsoS1A CsoS1C</p> 	<p>CsoS1D</p>  <p>Closed Open</p>
Beta-Type Carboxysome	<p>CcmK1 CcmK2 CcmK4</p> 	<p>No structures of this type have been reported to date.</p>
Pdu Microcompartment	<p>PduA PduU</p> 	<p>PduT</p>  <p>PduT contains a triad of cysteine residues in its triangular pore, which form a presumptive Fe-S cluster binding site.</p>
Eut Microcompartment	<p>EutM EutN</p> 	<p>EutL</p>  <p>Closed Open</p>

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BMC pore variations and transitions. Diagrams illustrate the structural diversity of several different pores in BMC shell proteins from experimentally characterized microcompartments (carboxysomes, Pdu, and Eut). Varied shell proteins exhibit differences in size, shape and electrostatic properties (blue = positive, red = negative). Some shell proteins that have tandem-BMC domains are able to alter their porosity through conformational changes that open and close the pore. In addition to the presence of gated pores, other interesting pore features include: a beta barrel that plugs an otherwise wide and negatively charged pore in PduU; a 4Fe-4S cluster binding site at the center of the PduT pore, and a distorted pore at the center of EutN, which is not made up of BMC proteins, but is instead a hexamer of CcmL/CsoS4-type protein subunits. The diverse properties illustrated are responsible for the ability of BMC shells to transport a wide range of substrates, products, and cofactors, while simultaneously limiting the efflux of metabolic intermediates. In each panel, the shell proteins are oriented so that the top surface represents the side predicted to represent the outside of the microcompartment (i.e. the side facing the cytosol). Known shell protein structures not illustrated here include CcmL, CsoS4A, EutS and EutB.

heterologously in the absence of native interior enzymes [42^{*}]; similarly, carboxysomes have been shown to assemble *in vivo* when RuBisCO has been deleted [54]. The timing of events in the formation of a shell and the encapsulation of enzymes has been partially clarified by examination of electron microscopy images of carboxysomes in various stages of apparent assembly, which suggest that enzymes (i.e. RuBisCO) form layers together with the shell proteins early in the process of forming facets of the shell, so that the shell and its interior materialize simultaneously [55].

Open questions and future directions

Myriad questions remain concerning the function and evolution of bacterial microcompartments. Recent studies indicate that active cellular mechanisms may control the arrangement and movement of microcompartments in the cell [42^{*},56], and that microcompartments may interact with other cellular components and inclusions [55]. Further studies are required to understand these cellular interactions. Likewise, little is known about how various enzymes are organized within microcompartments. Those details will be critical to understand how metabolic flux is enhanced.

Despite the obvious architectural similarity, no evolutionary linkage can be drawn at the present time between bacterial microcompartments and viruses; the BMC shell proteins do not resemble known viral capsid proteins. This is notable in contrast to the recent discovery in archaea of encapsulins, 60-subunit protein shells that encapsulate enzymes using a shell protein whose structure reveals a common ancestry with the capsid protein from the HK97 family of viruses [57]. With a single known exception, BMC shell proteins have been reported only in bacteria. They are not detected in plants or even primitive algae [58], which is notable given the origin of the chloroplast via endosymbiosis of an ancestral cyanobacterium that may have contained carboxysomes. Among eukaryotes, carboxysome shell proteins have only been reported in the unusual amoeboid, *Paulinella chromatophora* [59], which acquired a photosynthetic inclusion via a relatively recent endosymbiosis of a cyanobacterium.

The varied proteinaceous organelles described here all possess shells that were derived from a common ancestral shell gene. Their appearance therefore represents a singular evolutionary development in the bacterial lineage. On the other hand, a genomic context study hints that other analogous systems might await discovery [60].

Conflict of interest

The authors declare no competing financial interests.

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