Bacterial Microcompartments

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Annu. Rev. Microbiol. 2010. 64:391–408

The *Annual Review of Microbiology* is online at micro.annualreviews.org

This article's doi: 10.1146/annurev.micro.112408.134211

0066-4227/10/1013-0391$20.00

Key Words

carboxysome, horizontal gene transfer, bacterial organelle, polyhedral bacterial inclusions

Abstract

Bacterial microcompartments (BMCs) are organelles composed entirely of protein. They promote specific metabolic processes by encapsulating and colocalizing enzymes with their substrates and cofactors, by protecting vulnerable enzymes in a defined microenvironment, and by sequestering toxic or volatile intermediates. Prototypes of the BMCs are the carboxysomes of autotrophic bacteria. However, structures of similar polyhedral shape are being discovered in an ever-increasing number of heterotrophic bacteria, where they participate in the utilization of specialty carbon and energy sources. Comparative genomics reveals that the potential for this type of compartmentalization is widespread across bacterial phyla and suggests that genetic modules encoding BMCs are frequently laterally transferred among bacteria. The diverse functions of these BMCs suggest that they contribute to metabolic innovation in bacteria in a broad range of environments.

Contents

BMCs were discovered through electron microscopy. A striking feature of the BMC is its profile in electron micrographs (**Figure 1**), which is suggestive of polyhedral morphology and implies an unusual level of geometric organization. After BMCs were first described in 1956 (23), some investigators assumed they were phages (8). Since then, BMCs have been called polyhedral bodies, polygonal bodies, carboxysomes, enterosomes, and metabolosomes, reflecting the growing understanding of their diverse functions. This review describes our current understanding of BMC function and structure, beginning with genetic and biochemical studies on the most extensively characterized BMC, the carboxysome, and the two relatively well-characterized BMCs of enteric bacteria that are involved in ethanolamine (Eut BMC) and propanediol utilization (Pdu BMC). These have become the focus of X-ray crystallographic studies that have led to an understanding of the structural basis of BMC function. The structural studies have also helped clarify what defines a BMC: two types of shell protein. This, coupled with comparative genomics, has led to our ability to detect the widespread occurrence of BMCs among diverse bacteria, implicating them as frequently laterally transferred metabolic modules important for environmental adaptation.

INTRODUCTION

Bacterial microcompartment (BMC): a general name for self-assembling polyhedral organelles composed entirely of protein

Carboxysome: the BMC of many autotrophic bacteria that enhances the catalytic performance of the encapsulated RubisCO and constitutes the final step of a CO_2-concentrating mechanism

It is increasingly evident that the interior of the bacterial cell is highly organized (29). One of the emerging paradigms for organization within the prokaryotic cell is the bacterial microcompartment (BMC) (**Figure 1**), an organelle composed entirely of protein. BMCs encapsulate functionally related enzymes and auxiliary proteins within a thin protein shell. Concentrating enzymes together enhances catalytic efficiency by enabling the direct transfer of intermediates between enzymes that catalyze sequential reactions. The shell also provides a barrier to loss of metabolites to diffusion and competing pathways; similarly, by sequestering toxic intermediates, it prevents their interference with the bulk of cellular metabolism.

FUNCTIONAL PARADIGMS FOR BACTERIAL MICROCOMPARTMENTS

Carboxysomes

The first to isolate BMCs, from the chemoautotrophic bacterium *Halothiobacillus neapolitanus*, Shively et al. (61) demonstrated that they contain the central enzyme of the Calvin-Bensen-Bassham cycle, ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO), and named them carboxysomes (**Figure 2*a***). Two opposing hypotheses about their biological function centered on the notions that carboxysomes serve as a storage compartment for inactive RubisCO, or that the rather poor

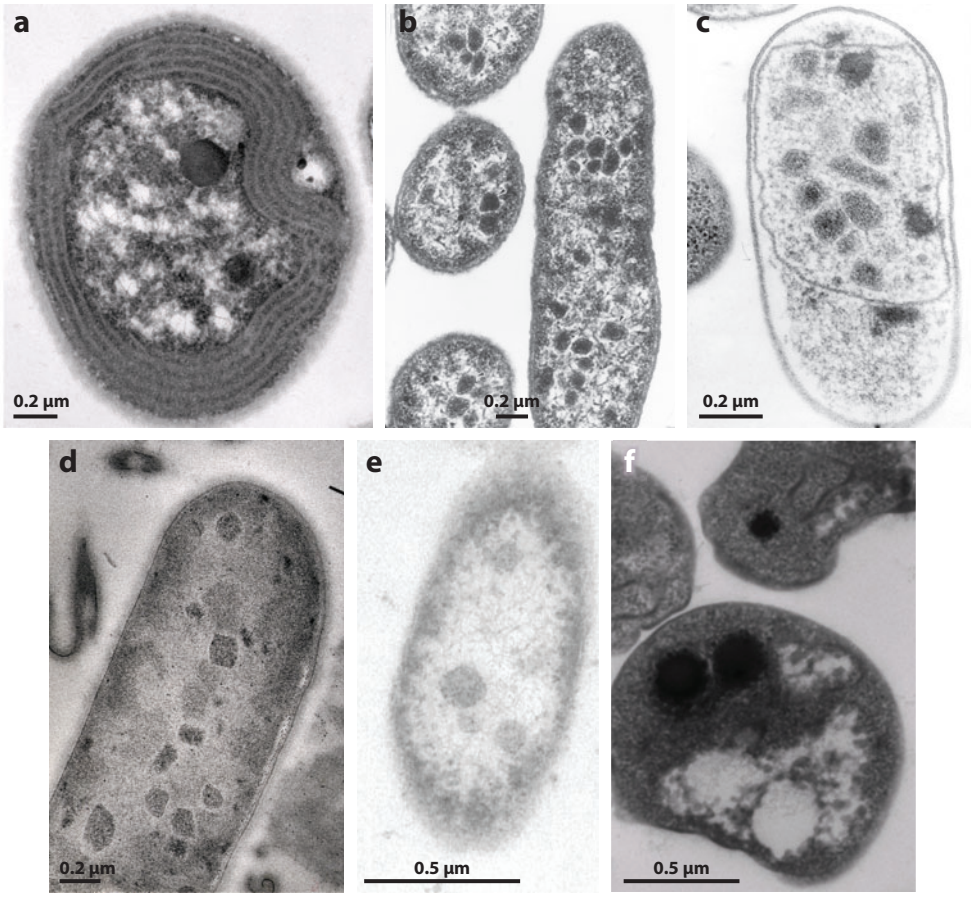


Figure 1

Transmission electron micrographs of bacterial microcompartments (BMCs). (*a*) The cyanobacterium *Synechocystis* PCC6803 with a single carboxysome (P. Shih & C.A. Kerfeld, unpublished data). (*b*) Pdu and (*c*) Eut BMCs in *Salmonella* (courtesy of J. Shively and the late H. Aldrich). (*d*) BMCs in *Clostridium kluyveri* grown on ethanol and acetate (41; courtesy of R. Lurz). (*e*) BMCs in *Clostridium phytofermentans* grown on fucose (courtesy of J. Blanchard). (*f*) Putative BMCs in *Pirellula staleyi* (courtesy of M. Rohde).

kinetic properties of RubisCO are enhanced by packaging the enzyme inside the BMC. It is now well established that carboxysomes are indeed active in CO_2 fixation. This conclusion was reached after studies demonstrated that the isolated particles contain highly active RubisCO (13). In addition, all carboxysomal RubisCO in permeabilized *H. neapolitanus* cells was shown to be in its fully activated state by in situ experiments (14). Physiological evidence supporting an active role of the carboxysome in CO_2 fixation includes the observation in both chemolithotrophic bacteria and cyanobacteria that the number of carboxysomes and the expression of carboxysome genes increase significantly when cells are limited for CO_2 (32). Some mutants, selected from a population of chemically mutagenized cyanobacteria that displays a high CO_2 requiring (*hcr*) phenotype, lack or form aberrantly shaped carboxysomes, suggesting that functional carboxysomes are needed for efficient growth at ambient CO_2 levels (48–50). After the carboxysome (*cso*) operon was identified, targeted mutagenesis of individual genes could be correlated with changes in carboxysome morphology or number per

Enterosome: previously coined term for Pdu and Eut BMCs of enteric bacteria

Metabolosome: alternative term for BMC

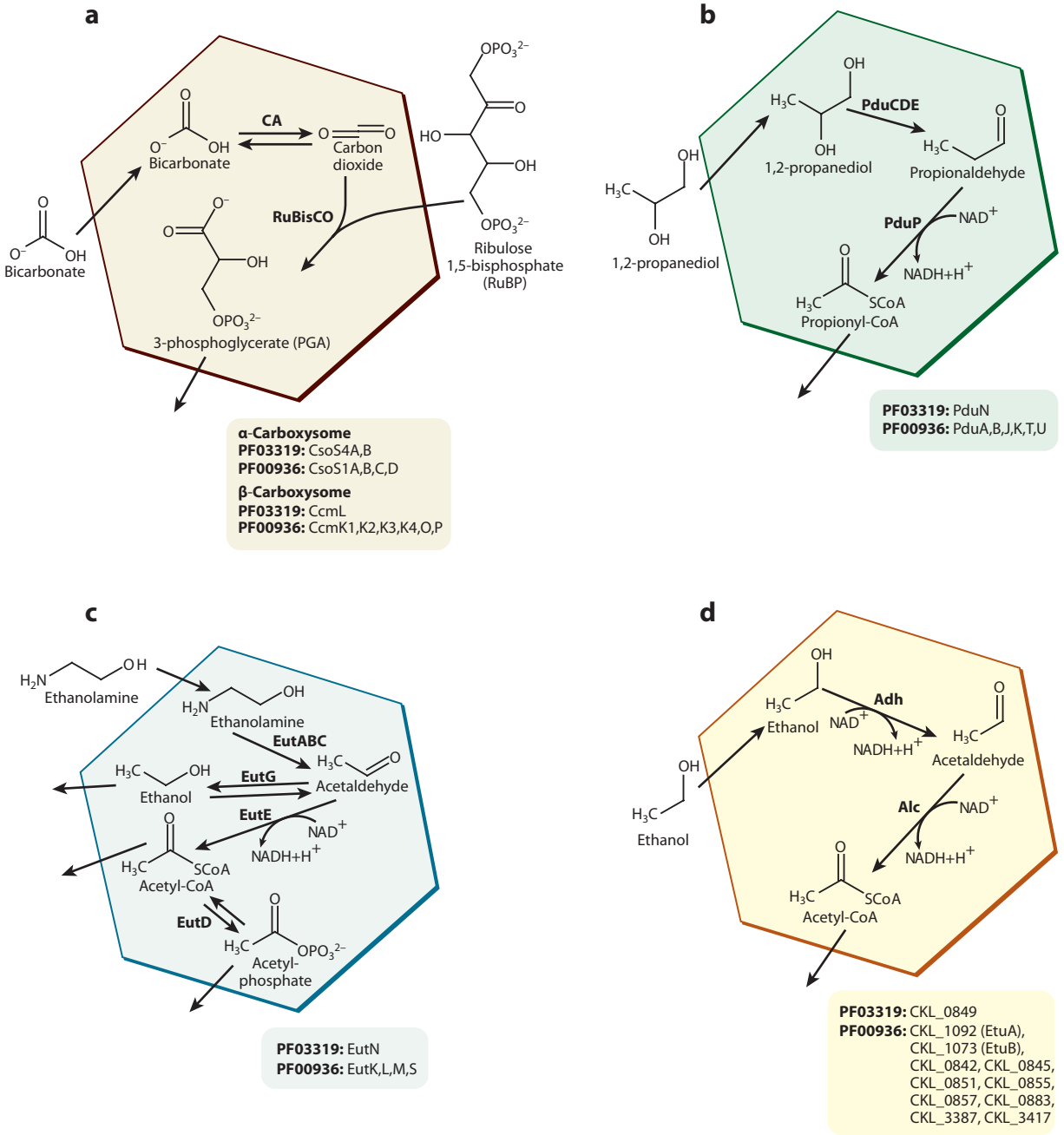


Figure 2

Schematic representation of the function of the (*a*) carboxysome, (*b*) Pdu BMC (adapted from Reference 18), (*c*) Eut BMC (adapted from Reference 47), and (*d*) Etu BMC (adapted from Reference 33). For the Etu BMC, the locus tags of the aldehyde and alcohol dehydrogenase genes are given. Abbreviations: BMC, bacterial microcompartment; Etu, ethanol utilization; Eut, ethanolamine utilization; Pdu, propanediol utilization.

cell, and with the *hcr* phenotype (6, 12, 22, 24, 42).

Most autotrophic bacteria live in environments where dissolved inorganic carbon (DIC) concentrations are well below levels needed to support efficient RubisCO-mediated CO_2 fixation. The carboxysome actively enhances CO_2 fixation by increasing the steady-state concentration of CO_2 in the vicinity of the active site of RubisCO, thereby overcoming the poor affinity of the enzyme for its substrate and allowing it to operate at or near substrate saturation (3–5, 20, 35, 51, 52). DIC is actively accumulated in the cell. The cytoplasmic DIC, which consists largely of bicarbonate, enters the carboxysome, presumably by diffusion, and is rapidly equilibrated with CO_2 by a specialized carbonic anhydrase that is colocalized with RubisCO within the carboxysome (16). The shell of the carboxysome limits the escape of CO_2 out of the BMC and into the cytoplasm (**Figure 2*a***). Quantitative models of CO_2 assimilation and growth of cyanobacteria (1, 54–56), as well as ample experimental data in chemoautotrophs and cyanobacteria, strongly support this mechanism of carboxysome function.

Carboxysomes have been categorized into two types on the basis of the RubisCO ortholog they contain and their shell protein composition. α-carboxysomes are found in chemoautotrophs and in some marine cyanobacteria. They are encoded by *cso* operons of similar organization. By contrast, β-carboxysomes, which are found mainly in freshwater cyanobacteria, are encoded by *ccm* genes distributed throughout the genome (2, 15). As discussed below, two shell protein types are homologous in α- and β-carboxysomes (15). However, although morphologically similar, many β-carboxysomes are larger and tend to be less regularly shaped than α-carboxysomes, which could imply some functional variation. One suggestion that has persisted in the literature is that the carboxysomal shell protects RubisCO from O_2, the competitive inhibitor of the enzyme. The necessity for such a role is difficult to justify in the chemoautotrophs, in which O_2 concentrations are not likely to be very high relative to intracellular DIC concentrations. The oxygenic photosynthesis activity of cyanobacteria, on the other hand, may necessitate that their carboxysomes exclude oxygen from RubisCO in their interior.

Pdu BMCs

Degradation of the common plant cell wall sugars fucose and rhamnose under anaerobic conditions produces 1,2-propanediol, which is used as a carbon and energy source by a number of enteric bacteria and by bacteria that grow in environments such as aquatic sediments, where 1,2-propanediol is readily available due to the breakdown of plant material. Catabolism of 1,2-propanediol requires a complex pathway that is coenzyme-B_{12}-dependent and ultimately provides the cell with propionyl-CoA, an electron sink, and ATP (**Figure 2*b***). The proteins involved in 1,2-propanediol degradation are encoded by the 23 genes of the 1,2-propanediol utilization (*pdu*) operon (34). It was surprising to find that seven of these genes are homologous to carboxysome shell protein genes (17). Subsequent electron microscopic studies showed that, when grown on 1,2-propanediol, *Salmonella enterica* serovar Typhimurium LT2, *Klebsiella oxytoca* (62), and *Lactobacillus reuteri* DSM 20016 (63) contain multiple polyhedral bodies that resemble carboxysomes in size, shape, and electron density (**Figure 1*b***). Immunogold labeling of cell thin sections demonstrated that diol dehydratase (PduCDE) and propionaldehyde dehydrogenase (PduP) are components of the Pdu BMC core (**Figure 2*b***) (7, 40). An inventory of polypeptide constituents of the Pdu BMC established by two-dimensional gel electrophoresis/mass spectrometry analysis of particles isolated from *S. enterica* suggested that additional proteins involved in the Pdu pathway are associated with the BMC (30).

Ethanolamine utilization (Eut) operon: an inducible bacterial operon of 17 individual genes; its protein products mediate the degradation of ethanolamine as a carbon and energy source in a Eut BMC

Propanediol utilization (Pdu) operon: an inducible bacterial operon of 23 individual genes; its protein products mediate the utilization of 1,2-propanediol as a carbon and energy source in a Pdu BMC

RubisCO: ribulose-1,5-bisphosphate carboxylase/oxygenase

***hcr* mutants:** mutants of autotrophic bacteria that are impaired in efficient carbon assimilation and require elevated CO_2 concentrations to support wild-type growth rates

DIC: dissolved inorganic carbon

Carbon dioxide–concentrating mechanism (CCM): a multicomponent CO_2-concentrating mechanism of cyanobacteria and many chemoautotrophs that compensates for low levels of available inorganic carbon and poor CO_2 fixation kinetics

TEM: transmission electron microscopy

Coenzyme B_{12}: a complex organometallic cofactor associated with enzymes central to the degradation pathways of ethanolamine and 1,2-propandiol

Eut BMCs

Ethanolamine is produced in the gastrointestinal tract of mammals as a result of the degradation of the membrane component phosphatidyl ethanolamine. This amino alcohol is an important source of carbon, nitrogen, and energy for many bacteria that inhabit the mammalian gut (65). As in the *pdu* operon, genes for enzymes that catalyze reactions in the ethanolamine degradation pathway are clustered in the *eut* operon with genes encoding homologs of carboxysome shell proteins (65). The presence of polyhedral inclusions in thin-sectioned *S. enterica* cells grown on ethanolamine was shown by transmission electron microscopy (TEM) (10, 38), but to date, a purification procedure for Eut BMCs has not been reported, and therefore their exact protein composition is unknown. However, because the chemistry of the Eut pathway to some extent parallels that of the Pdu pathway, it has been proposed that ethanolamine enters the BMC through the shell and is converted to acetaldehyde and ammonia by coenzyme-B_{12}-dependent ethanolamine ammonia lyase (EutBC) (25) in the interior (**Figure 2*c***); colocalized EutA protein ensures that the enzyme remains in an active state (44, 60).

Models for Pdu BMC and Eut BMC Function

Although the exact roles of the Pdu and Eut BMCs in the metabolism of 1,2-propanediol and ethanolamine, respectively, have yet to be elucidated, three models have been put forth for the biological function of these BMCs. The first model, which is supported by the largest body of experimental evidence for the Pdu BMC, suggests that the BMC shell acts as a diffusional barrier to propionaldehyde and prevents leakage of this intermediate into the cytoplasm, where its relatively high reactivity could have deleterious effects on other cellular components (7, 57). The analogous function of the EutBMCs would be to contain acetaldehyde until it is further metabolized to acetyl-CoA by an acetaldehyde dehydrogenase (EutE; 65) in the BMC interior prior to entry into the central metabolism, or converted to acetyl-phosphate by a phosphotransacetylase (EutD; 9, 10, 64) (**Figure 2*c***). An alternative branch of the pathway, presumed to be contained within the BMC as well, would convert acetaldehyde to ethanol through action of the putative alcohol dehydrogenase EutG (10, 64) (**Figure 2*c***).

Because there does not seem to be an absolute requirement that ethanolamine be metabolized in BMCs in *S. enterica* (10), the second model for the primary role of the Eut BMC in ethanolamine metabolism proposes that BMCs increase the local concentration of key enzymes, their substrates, and their cofactors and thereby ensure optimum metabolic efficiency (10). The advantages of compartmentalizing entire pathways or parts thereof are obvious, and this model would be applicable to all BMC types. However, the Pdu and Eut BMCs are more complex at the molecular level than carboxysomes are; they contain several linked key metabolic enzymes, additional proteins that maintain enzyme activity, and possibly those that provide the necessary coenzyme. Aside from ensuring the proper internal concentration of coenzyme B_{12}, the levels of NAD^+/NADH inside those BMCs must also be maintained and may involve some as yet unknown cycling or transport mechanism across the BMC shell. Although the available evidence supports a role for these BMCs as sophisticated machines for metabolite channeling, to date little work has been published regarding the kinetic parameters of the enzymes they contain. Because Pdu BMCs can be purified (30), detailed studies of individual enzyme activities and of the entire Pdu pathway in intact and disrupted BMCs should be possible and would provide the much needed direct evidence that can help to assign definitive function(s) to the particles.

The third model for BMC function is based on the premise of a unified function for all BMCs. In analogy to the documented role of carboxysomes in concentrating CO_2, Penrod & Roth (47) proposed that the main function of

Eut and Pdu BMCs is to concentrate a valuable yet volatile metabolite by containment within the BMC, which provides a special environment. The authors characterized the phenotypes of a battery of mutants that carry deletions in individual *eut* genes. Those lacking the genes needed for BMC formation or for downstream acetaldehyde metabolism (*eutG* or *eutE*) release more acetaldehyde than the wild type, similar to that observed with a mutant unable to form Pdu BMCs (31). However, contrary to the growth arrest of the *pduA* mutant in the presence of 1,2-propanediol, growth of these *eut* mutants on ethanolamine is not impaired, suggesting that accumulation of acetaldehyde at this level is not toxic to *S. enterica*.

The inability of the mutants that lack Eut BMCs to grow on ethanolamine under conditions that increase loss of acetaldehyde from the cells (high pH and increased gas exchange) suggested that perhaps the inside of the Eut BMC is maintained at a lower pH than the cytoplasm and effectively traps the acetaldehyde. A role for BMCs as a low-pH cage would also explain how carboxysomes enhance the catalytic efficiency of RubisCO by providing an interior environment with an increased concentration of CO_2. The proposed mechanistic commonality for all BMCs is intriguing in light of the shared architectural principles of their shells (see below). However, the multiple reactions and complex milieus required by different enzymes, metabolites, and cofactors within the three BMCs are likely to sustain more than one function and different, well-regulated flux mechanisms across the respective BMC shells. As pointed out by Penrod & Roth (47), there are serious mechanistic problems with the assumption that a protein shell can supplant the functions of a lipid-bilayer-based membrane, as proposed by current models of BMC function. However, recent advances in the structural analysis of shell proteins from all three BMC types have demonstrated a rich assortment of possible paths across the compartment shells, with the potential for regulation of metabolite transfer by gating (37, 69).

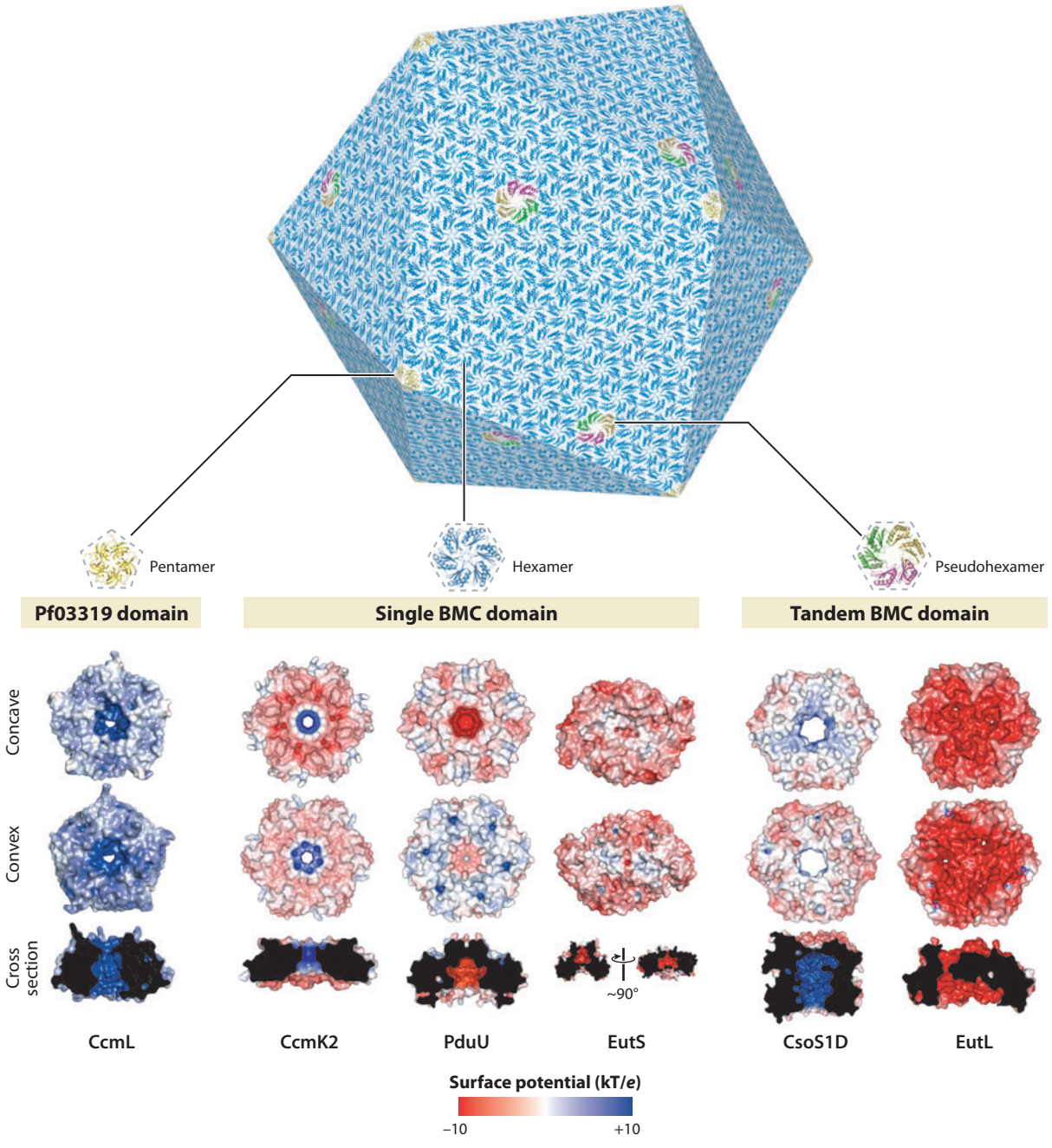
STRUCTURAL STUDIES ON PROTEINS OF THE BACTERIAL MICROCOMPARTMENT SHELL

Single BMC-Domain-Containing Proteins

The defining feature of a BMC is the proteinaceous shell. The shell confines the constituent enzymes and other proteins and likely plays a role in their organization. Like the lipid bilayers that bound other organelles, the structure of the shell establishes a permeability barrier. However, the selectivity properties of this proteinaceous barrier are opposite relative to those of biological membranes, with a preference for free passage of polar instead of nonpolar molecules.

The ~80-residue sequence that was recognized as the common denominator among the carboxysome, Eut, and Pdu microcompartments has become known as the bacterial microcompartment domain (Pf00936). Genes encoding these domains consistently occur in multiple copies in BMC gene clusters. Building a macromolecular assembly from several small, essentially identical proteins requires less genetic material than constructing it from a single protein chain, a strategy seen in viral capsids, flagella, and the cytoskeleton.

Because of the intrinsic relationship between structure and function in proteins involved in subcellular architecture, structural studies of the BMC-domain-containing proteins have been profoundly insightful. In 2005, Kerfeld et al. (36) described the first crystal structures of CcmK2 and CcmK4, two of the paralogs that form the β-carboxysome shell. The BMC domain adopts an α/β-fold; six copies self-assemble into cyclic hexamers ~70 Å in diameter (**Figure 3**). Residues distinctive of specific CcmK paralogs converge at the sixfold axis of symmetry; because these are positively charged side chains, the electrostatic potential of the pore is strongly positive, likely providing an electrostatic attraction to the negatively charged metabolites that must cross the carboxysome shell. The diameter of the pore is relatively restrictive, 4 Å and 7 Å in CcmK4 and



CcmK2, respectively. Compared with pores in transmembrane proteins, the pores in these BMC proteins are shorter and less contoured, suggesting that they are relatively unselective (70). Flux across the shell is presumably driven by the magnitude and the direction of the concentration gradient of the metabolites. For example, relatively high cytosolic concentrations of bicarbonate would support diffusion into the carboxysome (**Figure 2*a***), where the carbonic anhydrase would quickly convert it into CO_2, thereby maintaining the concentration gradient of bicarbonate across the shell. Moreover, the arrangement of the CcmK2 hexamers in the crystal provided further insight into the architecture of the carboxysome shell; the hexamers are arranged as uniformly oriented layers (**Figure 3**) immediately suggestive of how the facets of the carboxysome shell are constructed (36). These interpretations were supported by similar observations in the structural analysis of CsoS1A from the α-carboxysome (70).

The first structure of a shell protein from the Pdu BMC, the minor component PduU (18), confirmed the role of the BMC domain as a hexameric building block of the shell; however, it was a distinct variant (**Figure 3**). In PduU a β-barrel is formed by residues 8–15 of each of the subunits in the hexamer; this caps the pore on one side, presumably precluding metabolite flow. Recently, another variation was described for the PduU homolog EutS (69). The EutS hexamer is twisted about the axis defined by the central β-barrel. The effect is to introduce a bend of ∼40° into the hexamer (**Figure 3**); the edges where two facets of the BMC shell meet could be composed of hexamers of this morphology. However, the lack of a phenotype for a Δ*eutS* mutant in *Salmonella* led to the suggestion that EutS plays only a subtle role in Eut BMC function (47), whereas its presumed structural role would seem to be critical.

RubP: ribulose 1,5-bisphosphate

Tandem BMC-Domain-Containing Proteins

A comparison of the various BMC gene clusters in bacterial genomes shows that many contain genes that encode tandem BMC proteins of ∼180–250 amino acids. The first structure of a tandem BMC protein, the carboxysomal protein CsoS1D, was reported in 2009 by Kerfeld and colleagues (37), revealing that tandem BMC proteins form trimers that structurally mimic the hexamers formed by single BMC domain proteins (**Figure 3**). The structure of CsoS1D also provides a possible solution to a paradox about transport across the BMC shell. Given the small diameter of the pores observed in the BMC shell protein hexamers up to that time, it was unclear how larger metabolites (e.g., RubP) cross the shell. The structure of CsoS1D revealed that shell proteins could be gated; a conserved Arg side chain at the threefold axis of this pseudohexamer appeared in two distinct conformations, one in which the three Arg side chains converge to obstruct the pore and a second in which the side chains extend away from the surface of the protein, leaving an opening 14 Å in diameter (**Figure 3**).

A second observation for a putative two-state pore in a tandem BMC protein trimer

Figure 3

The elements of the architecture of the carboxysome shell, modeled as an icosahedron assembled from hexamers [single bacterial microcompartment (BMC) domain proteins, *blue*], pseudohexamers (tandem BMC domain proteins, *green*, *gold*, and *pink*), and pentamers (quaternary structure of Pf03319 domain in CcmL, *yellow*). For each of the electrostatic surface renderings of representative BMC shell proteins, the concave face is shown uppermost, followed by the relatively convex face and then cross-sectional view showing the (putative) pores. The EutS cross section is shown in two orientations to show the ∼40° distortion in the hexamer. The CsoS1D pseudohexamer is shown in its open form; in the cross section the tightly appressed pair of trimers is shown, one in the open conformation and the other in the closed conformation. Figure prepared with PyMOL (19) and Google SketchUp from PDB codes 3DNC (CcmK2), 3CGI (PduU), 3I87 (EutS), 3F56 (CsoS1D), 3GFH (EutL), and 2QW7 (CcmL). Electrostatics were generated using the Adaptive Poisson-Boltzmann Solver (APBS) plug-in for PyMOL. Surface potentials for electrostatics were visualized with a low value of −10 (*red*) and a high value of +10 (*blue*).

Etu: ethanol utilization

Pf03319 domain: the ~80-amino-acid domain observed to form pentamers, or hexamers

Icosahedral symmetry: an icosahedron is composed of 20 facets and 12 vertices

was described for EutL (58, 69), in which the open form has a negatively charged pore of 11 Å in diameter, and the relatively closed form has three small perforations (2.2 Å diameter at their narrowest point), each formed within a subunit (**Figure 3**). An additional observation of pores formed within a tandem BMC protein, similar to those in the relatively closed form of EutL, was reported for a shell protein from a newly described BMC for ethanol utilization (33). These perforations (less than ~3.8 Å in diameter) are strongly negatively charged and are lined by the side chains of conserved residues. Heldt et al. (33) concluded that they are competent for passage of ethanol, which must cross the Etu BMC shell. The small diameter and the relative asymmetry of these conduits may increase their selectivity. Moreover, as the authors of the first report of the EutL (58) and of the EtuB structures (33) point out, having three pores per unit area in the BMC shell, as opposed to one, has significant implications for increased flux across the shell.

Closing the BMC Shell: Pf03319 Proteins and Assembly of BMCs

The numerous similarities between the emerging picture of the underlying architecture of the carboxysome and that of icosahedral viral capsids suggest there may be a protein that forms pentamers; the consistent presence of an open reading frame encoding the Pf03319 domain in the carboxysome and Pdu and Eut gene clusters made it a likely candidate. In 2008 Kerfeld, Yeates, and their colleagues undertook structural studies of the Pf03319 proteins from the two types of carboxysome, revealing that this domain indeed forms pentamers (67) (**Figure 3**). This also confirmed that the proteins composing the shell could be arranged with icosahedral symmetry. However, several observations have yet to be reconciled with this model; deletion of the Pf03319 domain proteins in the α-carboxysome gene cluster does not appear to affect carboxysome morphology (12). Moreover, two independent structural analyses showed that the single Pf03319 protein of the *eut* operon forms hexamers (27, 67).

Other observations remain to be incorporated to develop a complete picture of BMC shell architecture and function. For example, CsoS1D trimers dimerize to form tightly appressed hexamers (37). Are these structures incorporated into a monolayer shell or do they reflect the physical interaction between two carboxysomes that is sometimes observed in TEMs? Similar, but less tightly associated, dimers of hexamers have been observed for other BMC shell proteins (18, 36, 68). The roles of specific side chains and their conformations underlying the interaction between hexamers in a monolayer have been noted (37, 68), but numerous principles governing BMC assembly await elucidation. The role of the variations at the N and C termini of the shell proteins also will likely prove to be important. As pointed out in the first structural description of the BMC domain (36), the disposition of the C-terminal helix appears to be important for the interactions between hexamers and/or it may be involved in interactions with encapsulated enzymes. Moreover, structural evidence supports the significance of the N terminus in structural plasticity of shell proteins; the PduU structure revealed that its primary structure contains a permutation relative to the canonical BMC domain. The β-barrel formed by this segment of the hexamer is apparently functionally important for EutS and PduU (18, 69). In EtuB (33) and CsoS1D (37), the N-terminal 50–70 amino acids could not be structurally resolved; thus their potential role in the structure of the BMC is unknown.

BACTERIAL MICROCOMPARTMENT DIVERSITY, HORIZONTAL GENE TRANSFER, AND EVOLUTION

Until relatively recently, the diversity and abundance of BMCs among the bacteria were underappreciated, given that detection was dependent on TEM of thin sections of cells serendipitously grown under conditions

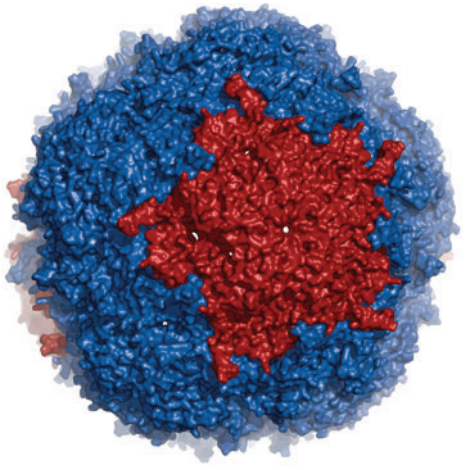


Figure 4

Although evidence for BMCs has not been found in the Archaea, a new type of protein compartment found in both Archaea and the Bacteria was recently characterized from *Thermotoga maritima* (66). With a diameter of only 20–24 nm, this structure is considerably smaller than other BMCs. The shell of this nanocompartment is built from 12 homopentamers (one pentamer is highlighted in red) of the 31-kDa protein encapsulin. The interior is occupied by either an iron-dependent peroxidase or a protein related to the iron carrier ferritin. These proteins, which may play a role in protecting the bacteria from oxidative damage, are targeted to the inside of the encapsulin particle through their C-terminal region, which binds to specific sites on the encapsulin shell. Image courtesy of M. Sutter.

inducing the expression of BMC gene clusters. Now, using the sequences of the BMC shell proteins as probes, comparative genomics approaches provide a new method for detecting the potential to form BMCs. As of December 2009, open reading frames encoding BMC-domain-containing proteins have been found in over 400 of 1962 microbial genomes for which data are available. To date, evidence for BMCs has not been found in the Archaea (**Figure 4**).

The bacterial BMCs are distributed broadly across phyla (**Table 1**). There are many cases in which there are two or three functionally distinct BMC gene clusters within the same organism. Examination of the ever-increasing number of gene clusters detected in genome sequence data permits some generalizations to be made about the requisite building blocks of all BMCs. In terms of their shell protein complement, the presence of two or more BMC domain proteins, in addition to one (but as many as three) copy of the Pf03319 domain, appear to be required. In the few cases that violate these rules (*Lactobacillus hilgardii* and four *Shigella* species), the remaining BMC gene cluster appears to be defunct, containing pseudogenes and/or transposases.

By examining the other open reading frames in proximity to those encoding shell proteins, inferences can be made about the function of the BMC; often, its function appears attributable to the encapsulation of an oxygen-sensitive enzyme and/or because an enzymatic step produces or consumes a toxic or volatile intermediate. The most common of the bioinformatically identified BMCs are those containing a B_{12}-independent glycerol dehydratase (**Table 1**). These enzymes are structurally distinct from the B_{12}-dependent diol dehydratase encapsulated in the Pdu BMC (53). Instead, they resemble pyruvate formate lyase (45) and use an oxygen-sensitive glycyl radical mechanism involving *S*-adenosylmethionine as a cofactor. Differences in the residues of the putative active sites in these homologs suggest that they may bind a range of substrates. A recent study combining bioinformatic-driven hypotheses tested by genome-wide microarray studies, TEM (**Figure 1*e***), and product analyses led to the demonstration that a BMC cluster containing a B_{12}-independent diol dehydratase in *Clostridium phytofermentans* is involved in the fermentation of the plant cell wall components fucose and rhamnose into propanol (J. Blanchard, personal communication). This BMC cluster is one of three in the *C. phytofermentans* genome; the others are a *eut* operon and a second gene cluster containing another homolog of the B_{12}-independent diol dehydratase with an unknown substrate preference.

Additional challenges for inferring BMC function occur in cases in which the BMC appears to be encoded by more than one locus within the genome, as is the case for the

Table 1 Examples of BMC gene clusters in bacterial genomes[a]

Representative organisms	Phylum	Putative key associated enzymes based on annotation[b]
Sebaldella termitidis	*Fusobacteria*	Fusion of *pdu* and *eut* operons
Thermanaerovibrio acidaminovorans DSM 6589 *Dethiosulfovibrio peptidovorans* DSM11002 *Leptotrichia buccalis* DSM 1135*	*Synergistetes* *Fusobacteria*	Eut and Pdu variants
Clostridium phytofermentans ISDg[c]	*Firmicutes*	Fuculose 1-phosphate aldolase B_{12}-independent diol dehydratase Aldehyde dehydrogenase
Clostridium kluyveri DSM555 (Etu BMC)[d]	*Firmicutes*	Aldehyde dehydrogenase Alcohol dehydrogenase
Rhodopseudomonas palustris BisB18 *Ruminococcus obeum* ATCC29174 *Clostridium novyi* NT-2 *Escherichia coli* CFT073 *Shewanella putrefaciens* CN-32	*Alphaproteobacteria* *Firmicutes* *Gammaproteobacteria*	B_{12}-independent diol dehydratase Aldehyde dehydrogenase
Escherichia coli 536 *Klebsiella pneumoniae* 342 *Desulfotalea psychrophila* LSv54 *Desulfovibrio desulfuricans* G20 *Clostridium phytofermentans* ISDg *Anaerococcus hydrogenalis* DSM 7454	*Gammaproteobacteria* *Deltaproteobacteria* *Firmicutes*	Glycyl radical enzyme homolog Aldehyde dehydrogenase
Pirellula staleyi DSM 6068[e] *Opitutus terrae* PB90-1	*Planctomycetes* *Verrucomicrobia*	Aldolase(s) Aldehyde dehydrogenase Malate/lactate dehydrogenase
Mycobacterium smegmatis MC2 155 *Rhodococcus* RHA-1 (plasmid) *Verminephrobacter eiseniae* EF01-2	*Actinobacteria* *Betaproteobacteria*	Aldehyde dehydrogenase Aminotransferase type III
Alkaliphilus metalliredigens QYMF *Carboxydothermus hydrogenoformans* Z-2901	*Firmicutes*	Embedded in a cluster of genes presumed to be involved in purine metabolism
Methylibium petroleiphilum PM1 (plasmid) *Nakamurella multipartite* DSM 44233	*Betaproteobacteria* *Actinobacteria*	Malate/lactate dehydrogenase-like Aldehyde dehydrogenase; glutathione-dependent Formaldehyde dehydrogenase
Haliangium ochraceum SMP-2	*Deltaproteobacteria*	Aldehyde dehydrogenase Aldolase
Chloroherpeton thalassium ATCC 35110	*Chlorobi*	–
Solibacter usitatus Ellin 6076	*Acidobacteria*	Aldehyde dehydrogenase Aldolase Dihydrodipicolinate synthetase

[a]The representative organisms do not constitute a complete list of all observations.
[b]Adapted from Integrated Microbial Genomes (**http://img.jgi.doe.gov/cgi-bin/pub/main.cgi**).
[c]Jeffrey Blanchard, personal communication; see also **Figure 1*e***.
[d]See **Figure 1*d***, **Figure 2*d***, and References 33 and 59.
[e]See **Figure 1*e***.

β-carboxysome. The Etu BMC of *Clostridium kluyveri* (**Figure 2*d***), the subject of the structural analysis (33) noted above, is another example. *C. kluyveri* has long been known to form BMCs; in 1979 (41), ultrastructural evidence was published that demonstrated the presence of BMCs when the organism is grown on ethanol and acetate (**Figure 1*d***). The genome sequence, reported in 2008 (59), revealed a candidate BMC locus encoding the enzymes needed to oxidize ethanol to acetyl-CoA: It contains two shell proteins, EtuA and EtuB; two acetaldehyde dehydrogenase paralogs; and three alcohol dehydrogenase paralogs. Given the paucity of open reading frames encoding shell proteins in this locus, especially the absence of a Pf03319 ortholog in this gene cluster, it was proposed that other constituent shell proteins of the Etu BMC are encoded by a defunct glycerol dehydratase BMC gene cluster elsewhere in the genome (33).

Numerous other types of BMC gene clusters can be identified based on the co-occurrence of the Pf00936 and Pf03319 domains in microbial genomes. In some cases, functions can be suggested on the basis of annotation, but as such they are tenuous (**Table 1**). Some of the less-well-sampled phyla in terms of genome sequencing contain organisms that have multiple clusters of shell proteins scattered in the genome; these include *Solibacter usitatus*, *Haliangium ochraceum*, and *Chloroherpeton thalassium*. This may reflect that operon-based architecture is less common among many bacteria than is generally assumed (26); accordingly, genome context predicts only a relatively small number of functional interactions among proteins (11). Considering this, the widespread distribution of intact BMC-encoding modules in bacterial genomes is remarkable. The same type of BMC can be found across phyla (**Table 1**); for example, the Eut operon is found in the *Firmicutes*, *Fusobacteria*, *Synergistetes*, and *Gammaproteobacteria*. On the other hand closely related organisms can differ in the number of BMC gene clusters. For example, *Klebsiella pneumoniae* 342 contains three (Eut, Pdu, and one encoding a B_{12}-independent diol dehydratase) while *K. pneumoniae* MGH78578 contains only the Pdu and Eut operons.

Collectively, the distribution of BMC gene clusters among the bacteria suggests that the clusters are frequently laterally transferred and supports the concept of the selfish operon (39). That is, the lateral transfer of a unit of function encoded by several physically proximal genes is more likely to be retained because it is more likely than a single gene to be adaptive immediately upon introgression. Moreover, if newly introgressed BMC modules are functionally integrated with the products of genes and gene clusters for transporters and cytoplasmic enzymes, evolutionary innovation arises in a manner analogous to the evolution of proteins through new combinations of existing protein domains (21, 28). The lateral transfer of genetic modules encoding BMCs appears to be a frequent mechanism for adding to the core repertoire of bacterial genomes to expand the metabolic capacity of the recipient and enable adaptation to new ecological niches. As such, BMCs have tremendous potential for bioengineering of metabolic modules in bacteria. The first proof-of-concept for this approach was recently described by Parsons et al. (46); they transferred the Pdu BMC operon from *Citrobacter freundii* to *E. coli* and demonstrated that functional microcompartments were formed.

Given the similarity in ultrastructure and in the underlying design principles in the architecture of their shells (36, 70, 71), what is the relationship between the BMC shell and viral capsids? The structural similarity may simply reflect convergence, driven by the geometric constraints on a self-organizing protein shell (43). Divergence from a common ancestor is yet unsupported; to date, there is no evidence for primary or tertiary structure similarity of the BMC shell proteins to any viral capsid proteins. This might be explained by the relatively small number of viral capsid proteins that have had their primary structure or fold characterized; alternatively, the shell proteins are similar to ancient viral capsid proteins no longer extant. If the BMC shell proteins do prove to be evolutionarily related to capsid proteins,

Pf00936 domain: the ~80-amino-acid sequence known as the BMC domain that is the main building block of the BMC shell

what was the direction of the gene transfer? Were the BMC shell proteins acquired through a phage infection? If bacteriophages are derived from bacterial genomes, is the viral capsid derived from the BMC? Currently available data suggest that most horizontal gene transfer flows from cells to viruses (43); considering this, the BMC shell may have been coopted by viruses for the encapsulation of nucleic acids.

SUMMARY POINTS

1. BMCs are functionally diverse metabolic modules in bacteria; they are defined by their proteinaceous shell and their enzyme contents.
2. The genes for two shell protein families are found in all BMC gene clusters; the assemblies of these proteins (Pf00936 and Pf03319) are the common building blocks of BMC shells.
3. The genes encoding BMCs have been subjected to frequent horizontal gene transfer and are widespread among the bacterial phyla.

FUTURE ISSUES

1. The proposed functions and metabolic capabilities of the various BMCs require direct experimental evidence. Steps toward that goal include expression studies under relevant growth conditions, development of purification procedures that permit the establishment of complete inventories of their protein constituents, kinetic characterization of the encapsulated enzymes, and reconstruction of the metabolic steps that take place in the BMC interior.
2. How do BMCs assemble? Of particular interest are the structural signatures on the encapsulated proteins that guide their incorporation into a BMC and mediate requisite interactions with other BMC components. Likewise, knowledge of strategies that ensure balanced expression of all BMC proteins, apparently crucial for the assembly of BMCs of normal shape, is of importance.
3. How are the additional proteins associated with the BMC shell integrated into or associated with the protein assemblies that form the shell facets and vertices?
4. How dynamic is the BMC shell? Do its composition and permeability properties respond to environmental conditions and to fluctuations in intracellular metabolite ratios? Do they self-repair? Does BMC size change to regulate its internal metabolism in a manner analogous to the way the mitochondrion regulates the Krebs cycle with changes in size?
5. Little experimental evidence exists that addresses the flux of reactants into the BMC or the efflux of products out of the particles. Can mathematical models be developed that predict the flux across the shell and can serve as a guide for further biochemical experiments?
6. What signals trigger biogenesis of BMCs? The sensors, signaling cascade(s), and regulatory mechanisms on the transcriptional and translational levels are currently unknown for most BMCs, as is the question whether organisms express more than one BMC type at a time.
7. What is the evolutionary relationship between the shells of BMCs and viral capsids?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Jeffrey Blanchard and Todd Yeates for providing results prior to publication. We thank Seth Axen for help in figure preparation and Bill Greenleaf and Jay Kinney for helpful discussions. CAK, SH, and GCC acknowledge support of the NSF (MCB 0851094 and MCB 0818680). CAK's work at the U.S. Department of Energy Joint Genome Institute is supported by the Office of Science of the U.S. Department of Energy under contract no. DE-AC02-05CH11231.

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Annual Review of Microbiology
Volume 64, 2010

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