# Bacterial Microcompartments

These intracellular structures, formed by polypeptides, confine and help to retain nonpolar and volatile metabolites

**Thomas A. Bobik**

Although the carboxysome was for nearly 30 years the only microcompartment recognized within microbial cells, additional microcompartments, including some that are involved in metabolic processes such as degrading carbon sources, are known. Cluster analysis of homologues of microbial microcompartment-specific proteins suggests that such enclosures could be involved in as many as seven different metabolic processes in various bacterial species. Although such microcompartments appear to provide barriers for molecules that are poorly retained by lipid-based membranes, their other functions, if any, and how they operate remain unknown.

The carboxysome and similar microcompartments contain 5–10 different polypeptides that form a polyhedral shell 100–200 nm across and whose volume is more than 1,000 times greater than that of a ribosome. Their size helps to explain why early investigators mistook them for bacteriophage (Fig. 1). The building blocks of bacterial microcompartments are exclusively proteins and glycoproteins; there is no published evidence that they contain DNA, RNA, or lipids. Indeed, electron microscopy shows no lipid monolayer or bilayer surrounding such microcompartments, making them the only known protein-based metabolic compartments in living cells. An important unifying property of microcompartments appears to be that they retain volatile compounds, according to Joe Penrod and John Roth at the University of California, Davis.


### Summary

- Some bacterial cells have microcompartments, including the carboxysome, which is involved in fixing carbon dioxide, and others that apparently help to sequester volatile or toxic metabolites.
- Although about 25%, or 85 of 337 bacterial genomes whose sequences are available contain gene homologues suggesting that they can make microcompartments, no such homologues are found in *Archaea* or *Eucarya*.
- The distinguishing feature of bacterial microompartments is their structure, which consists of a complex protein shell that encases metabolic enzymes


## Other Microcompartments, Carboxysomes Share Features

Carboxysomes were identified during the late 1960s, first through electron microscopy studies of autotrophic bacteria, but soon they were also found in cyanobacteria and in chemoautotrophs. The shell of a carboxysome contains multiple copies of 6 to 10 different polypeptides, while the lumen contains many molecules of ribulose bisphosphate carboxylase/oxygenase (RuBisCO), the $CO_2$-fixing enzyme of the Calvin cycle. The carboxysome apparently concentrates $CO_2$ for RuBisCO, which converts it and ribulose bisphosphate into two molecules of 3-phosphoglycerate (Fig. 2).

Similar microcompartments are also found in nonphotosynthetic bacterial species where they are involved in other metabolic processes. Two distinct microcompartments (Fig. 3), found in the heterotrophic enteric bacterium *Salmonella enterica*, contain the components, including coenzyme $B_{12}$, needed for degrading 1,2-propanediol

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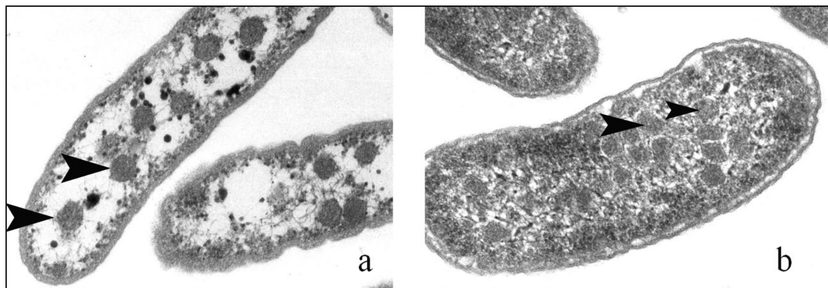
FIGURE 1




Electron micrograph of polyhedral microcompartments: a. The carboxysomes of *H. neapolitanus*; b. The microcompartments formed during growth of *Salmonella enterica* on 1,2-propanediol. Arrowheads point to the microcompartments.

or ethanolamine. They are important carbon sources for such cells in natural environments. Members of other bacterial genera, including *Escherichia*, *Klebsiella*, *Clostridium*, *Fusobacterium*, Shigella, *Listeria*, and *Yersinia*, also can degrade one or both of these compounds and appear to use microcompartments for this purpose.

These catabolic microcompartments were long overlooked because they do not form when cells are grown on standard laboratory media. Moreover, electron microscopy is required to observe them. They were discovered after researchers realized that the operons specifically involved in degrading 1,2-propanediol and ethanolamine include genes that encode multiple homologues of carboxysome shell proteins.

My collaborators and I purified and analyzed the microcompartment involved in degrading 1,2-propanediol in 2003 (Fig. 3). It is composed of at least 14 different polypeptides, including four enzymes and seven different putative shell polypeptides (Fig. 4). Although the ethanolamine microcompartment has not been purified, it is unlikely to differ dramatically in numbers of protein components from those found in the other two microcompartments.

Some investigators suspect that the 1,2-propanediol and ethanolamine microcompartments help to retain the two volatile aldehydes, propionaldehyde and acetaldehyde, respectively, that are degradative intermediates in these two pathways (Fig. 4). Retaining them confers two benefits: protecting cytoplasmic components against their toxic effects, and preventing their loss through lipid-based membranes.

## The Microcompartment Phenomenon Seems To Be Restricted to Bacteria

The discovery of microcompartments in cyanobacteria, *Salmonella*, and other bacteria raised questions about their distribution and diversity. How widespread is the microcompartment phenomenon and how diverse is microcompartment function?

To address these questions, we recently searched GenBank for microcompartment shell genes and analyzed gene clusters within which they reside. About 25% (85 of 337) of bacterial genomes whose sequences are available in this database contain shell gene homologues. However, no homologues were found among genomic sequences for archaea or eukaryotes.

In most of those 25% of bacterial genomes that carry those homologue genes, the shell genes cluster with other genes encoding microcompartment-associated enzymes. Based on this conserved arrangement, we define a microcompartment genomic signature (MGS) as a gene cluster that includes multiple shell genes interspersed with structural genes encoding enzymes. Further, we assign microcompartment types based on the enzymes that are encoded within an MGS. For example, signatures that encode shell proteins as well as $B_{12}$-dependent diol dehydratase are typed as a microcompartment for degrading 1,2-propanediol.

Based on the diversity of enzymes within MGS, we estimate that microcompartments are involved in seven or more different metabolic processes. Those involved in fixing $CO_2$ or in degrading 1,2-propanediol and ethanolamine can be considered well-established. The remaining four are putative, of unknown function, and might underestimate the total because our analysis would miss microcompartments containing shell proteins that diverge substantially from those that are known. Perhaps, as with viral capsids, there is a great diversity of microcompartment shells, serving an array of metabolic partitioning needs of other microbes.

## Probing Microcompartment Structural Components

The structure of the microcompartment—a complex protein shell encasing metabolic en-

## Bobik: Dig Deeply into Scientific Problems To Find Knowledge

Thomas Aquinas Bobik wanted to work, not attend college, after high school. However, his mother persuaded him to try college for one year, which "turned into four, and then into five more in graduate school," he says. "My high school chemistry teacher, Mr. Motts, got me interested in science, and later I went to Indiana University at Bloomington where there was an excellent undergraduate program in micro," he recalls. "I liked the blend of biology and chemistry, and I majored in microbiology."

Since 2004, Bobik has been an associate professor in the Department of Biochemistry, Biophysics and Molecular Biology, at Iowa State University, where he focuses on vitamin $B_{12}$ metabolism in bacteria. "My research focuses on understanding bacterial metabolism. I chose this area because organic chem was one of my favorite subjects," he says. "I like the order it brings to chemical reactions." Bobik also is investigating microbial production of fuels, "a hot topic these days," he says. "Petroleum reserves are limited. Available energy is important to the quality of human life, so an alternative to fossil fuels will be needed in the coming years."

Bobik grew up in South Bend, Ind., where his father teaches philosophy at the University of Notre Dame. "Of course, I am an 'Irish' [Notre Dame] football fan," he says. As a child, he enjoyed taking things apart "to find out how they worked, and then trying to use the parts to build new things—although this latter goal was never fully realized," Bobik says. His mother called him "Doubting Thomas" because "I questioned what I was told, and what people said." He has one brother and three sisters. Although one sister is a medical technician, "my other sibs did not choose a science-based career," he says.

Bobik received a B.S. degree in microbiology from Indiana University, then moved to the University of Illinois where he completed an M.S. in 1986, then in 1990 a Ph.D., both in microbiology. After doing postdoctoral research at the University of Utah in Salt Lake City, he became an assistant professor in the department of microbiology and cell science at the University of Florida in Gainesville, where he remained until moving to Iowa in 2004, conducting genetic studies of vitamin $B_{12}$ metabolism in *Salmonella* and in higher organisms, and also working on bacterial biosensors.

Bobik credits several mentors with enriching his appreciation—and respect—for research. "My graduate advisor, Ralph Wolfe, taught me the importance of scientific rigor," he says. "He would say that the foundation must be strong to support the weight of future advances. He would also say that a stool needs at least three legs to stand up—and that three independent lines of evidence are needed to support any scientific discovery."

His postdoctoral advisor, John Roth, taught him important lessons about the nature of bacterial genetics. "Bacterial genetics is, in part, a puzzle where we try to put together enough pieces so that we can make scientific sense out of phenotypic observations," Bobik says. "He would say that 'there's a pony in there somewhere,' meaning that if we dig deep into a problem, we can find new scientific knowledge."

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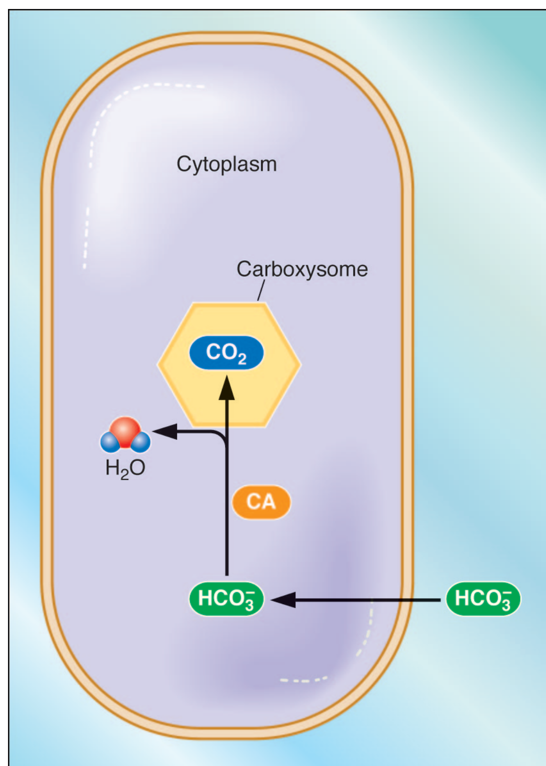
zymes—is unusual and distinguishes bacterial microcompartments from all other metabolic multiprotein complexes.

The microcompartment shell somehow influences the activity of enzymes within the lumen. For instance, mutants that cannot properly form the carboxysome shell require high $CO_2$ for autotrophic growth, indicating that the shell plays a role in increasing the overall catalytic efficiency of RuBisCO. The 1,2-propanediol and ethanolamine microcompartments contain catabolic enzymes involved in producing and consuming aldehydes. In these cases, shell mutants release massive amounts of aldehydes into the growth medium, suggesting that these microcompartments are important for retaining aldehydes and possibly for regulating aldehdye production.

Microcompartments also may influence metabolic reactions by acting as containers for enzyme substrates. For instance, the carboxysome shell seems to be a barrier to $CO_2$, concentrating it in the immediate vicinity of RuBisCO and thus enhancing its rate of fixation. Similarly, the 1,2-

FIGURE 2




Simplified model for the carboxysome. RuBisCO is the $CO_2$-fixing enzyme of the Calvin cycle. It catalyses the conversion of $CO_2$ and ribulose bisphosphate into two molecules of 3-phosphoglycerate. The carboxysome is proposed to be an essential part of a carbon dioxide concentrating mechanism (CCM) that improves the efficiency of $CO_2$ fixation by RuBisCO. The CCM starts with the concentration of $HCO_3^-$ in the cytoplasm of the cell by active transport. Equilibrium with $CO_2$ is not reached due to a lack of carbonic anhydrase (CA). Carboxysomal CA converts $HCO_3^-$ to $CO_2$ and releases it within the microcompartment. The protein shell of the microcompartment impedes $CO_2$ diffusion. Consequently, $CO_2$ is concentrated in the immediate vicinity of RuBisCO which fills the lumen of the microcompartment. The result is an increase in $CO_2$ fixation and suppression of photorespiration (a nonproductive process in which $O_2$ replaces $CO_2$ as a substrate RuBisCO competitively inhibiting carboxylation).

propanediol and ethanolamine microcompartments apparently sequester aldehydes, preventing toxicity and carbon loss (Fig. 4). Although these microcompartments respectively restrict the flow of aldehydes and $CO_2$, they somehow remain selectively permeable to other substrates, products, and cofactors.

Some microcompartment shells appear to incorporate transport systems and selective pores. For example, the enzyme carbonic anhydrase is a component of the carboxysome shell in *Halothiobacillus*, according to Gordon Cannon and his collaborators at the University of Southern Mississippi in Hattiesburg. Their finding suggests that $CO_2$ molecules enter microcompartments by means of anhydrase-catalyzed transport.

In some cases, positive or negative charges may be used to control the flow of molecules between the cytosol and lumen of microcompartments. Todd Yeates and his collaborators at the University of California, Los Angeles, recently determined the crystal structures of the carboxysome shell proteins CcmK2 and CcmK4. Both are wedge-shaped monomers that pack tightly to form a hexamer with a central pore. These hexamers have nearly flat sides and form tight sheets that could form the outer face of these microcompartment shells. The central pores and the gaps between hexamers are 4–7 Å wide. Because the hexamers have a large net positive electrostatic potential, the pores might accommodate only charged molecules and thus would not allow passage of uncharged compounds such as $CO_2$.

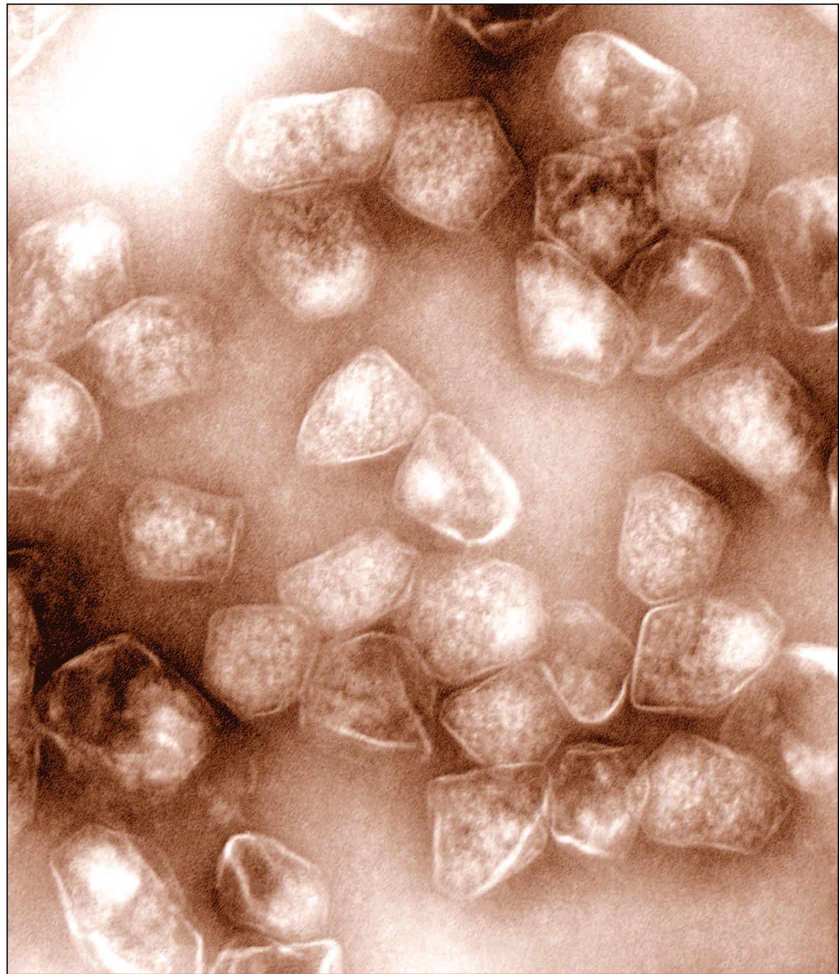
### Much about Microcompartment Functions Remains Uncertain

Precisely how microcompartments work is uncertain. Of the several different types of microcompartments, the carboxysome is best understood. Their main function seems to be enhancing $CO_2$ fixation through a $CO_2$-concentrating mechanism (CCM) (Fig. 2). The CCM includes an active transport system for concentrating dissolved $CO_2$ in the form of $HCO_3^-$ in the cell cytoplasm. However, $HCO_3^-$ and $CO_2$ do not equilibrate in the cytoplasm because CA is not present there. Instead, a carboxysome-associated CA converts $HCO_3^-$ to $CO_2$, which is released into the microcompartment lumen, building to higher levels until it equilibrates with cytoplasmic $HCO_3^-$. Thus, high $CO_2$ levels are maintained in the immediate vicinity of RuBisCO within the microcompartment lumen, increasing the $CO_2$-fixation rate and suppressing photorespiration (a nonproductive process in which $O_2$ competitively inhibits RuBisCO-catalyzed carboxylation).

According to the CCM model, carboxysomes are permeable to both ribulose bisphosphate and 3-phosphoglycerate, the substrate and product of the RuBisCO-catalyzed, $CO_2$–fixing reaction. Importantly, the carboxysomes restrict the movement of $CO_2$. Their charged pores could provide a conduit for ribulose bisphosphate and 3-phosphoglycerate, while restricting the movement of uncharged compounds such as $CO_2$. According to this model, $CO_2$ could be brought into the lumen by carbonic anhydrase. Although the 1,2-propanediol microcompartments are believed to retain the pathway intermediate propionaldehyde selectively, its retention during 1,2-propanediol degradation involves selection among a complex mix of reactants. The substrate for diol dehydratase (1,2-propanediol) is uncharged. Moreover, the lumen enzymes within the 1,2-propanediol microcompartment require several cofactors, including ATP, NAD, coenzyme $B_{12}$, and coenzyme A, all of which are too large to fit through small pores. An analogous situation exists for the ethanolamine microcompartments.

A possible alternative to the selective permeability hypothesis is that the ethanolamine microcompartments concentrate the metabolic enzymes and their substrates, thereby improving growth, minimizing acetaldehyde toxicity, and maintaining coenzyme A balances, suggests Jorge Escalante-Semerena and his collaborators at the University of Wisconsin, Madison.

FIGURE 3



Purified bacterial microcompartments from *S. enterica* grown on 1,2-propanediol.

## Microcompartments Raise Structural Issues

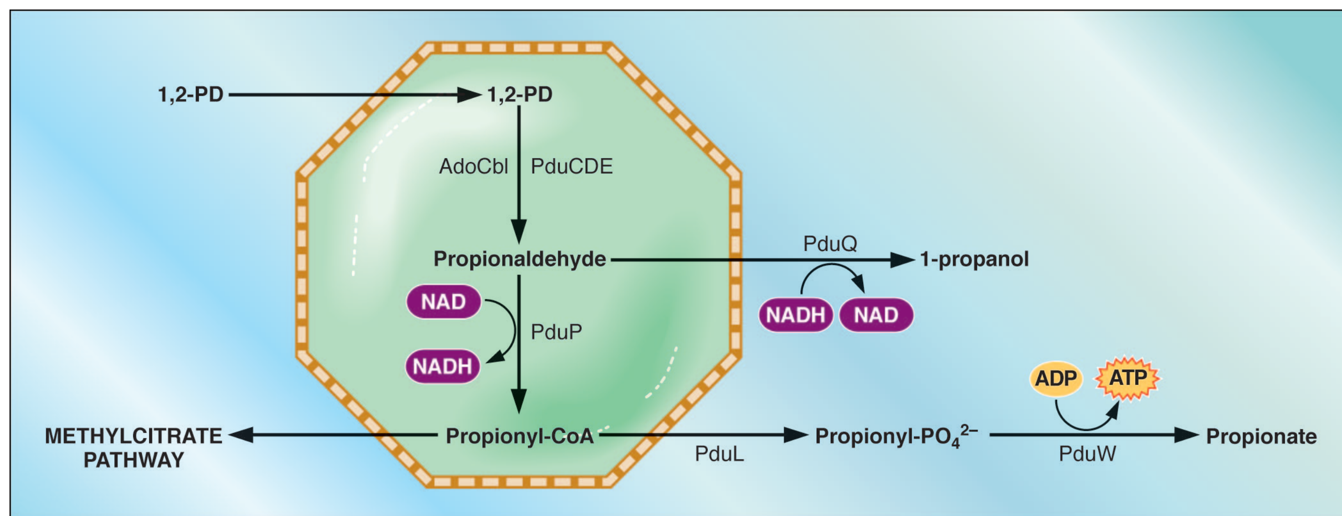
How are microcompartments constructed? One possibility is that they self-assemble with aid from chaperone proteins and other assembly factors. For instance, the ethanolamine microcompartment *eut* operon includes genes that encode two auxiliary proteins, EutA and EutJ. They appear to be distantly related to the DnaK-family of chaperonins. Although EutA is involved in reactivating ethanolamine ammonia lyase, the function of EutJ is unknown, meaning it remains a candidate microcompartment-assembly chaperone.

Similarly, two proteins required for carboxysome function, OrfA and OrfB, cannot be found in the final assembly. Thus, they might be minor (and thus undetectable) components that help to assemble these microcompartments.

Not all microcompartment gene clusters encode proteins that appear to be related to chaperones. However, other chaperones in cells or other factors might assist in forming microcompartment structures, even though no such assembly factors have been identified.

Another puzzle is how proteins are targeted and find their way to the lumen of these protein-based compartments. Perhaps the shell forms

FIGURE 4




Model for 1,2-propanediol degradation by *S. enterica*. The dashed line indicates the shell of the microcompartment. The first two steps of 1,2-PD degradation (conversion of 1,2-PD to propionyl-CoA) are thought to occur in the lumen. The proposed function of this microcompartment is to retain propionaldehyde formed in the first reaction in order to minimize toxicity and prevent carbon loss. The degradation of ethanolamine occurs via an analogous pathway that begins with acetaldehyde formation. Abbreviations: 1,2-propanediol (1,2-PD), coenzyme $B_{12}$ (AdoCbl), coenzyme $B_{12}$-dependent diol dehydratase (PduCDE), propionaldehyde dehydrogenase (PduP), phosphotransacylase (PduL), propionate kinase (PduW), 1-propanol dehydrogenase (PduQ). Two additional enzymes associated with the microcompartment (but not shown in the figure) are a putative diol dehydratase reactivase (PduGH) and an ATP:cob(I)alamin adenosyltransferase (PduO) involved in $B_{12}$ recycling.

around the lumen enzymes, and maybe they act as a scaffold for that shell. Although this explanation is plausible for carboxysomes in which the small subunit of RuBisCO is required for proper assembly, it does not seem to fit the case of the 1,2-propanediol microcompartments. For them, deleting the genes encoding each lumen enzyme one by one does not prevent the shell from forming. Thus, 1,2-propanediol microcompartments are unlikely to depend on a highly structured scaffold composed of lumen enzymes.

Several proteins found in the lumen of microcompartments have N- or C-terminal extensions. For example, two of the three subunits of $B_{12}$-dependent diol dehydratase within the lumen of the 1,2-propanediol microcompartments have short N-terminal extensions compared to their homologues that are confined to the cytoplasm. Deleting these extensions dramatically increases the solubility of this diol dehydratase, but does not appreciably affect its catalytic activity, according to Tetsuo Toraya and his collaborators at Okayama University in Japan. They speculate that these sequences are involved in assembling microcompartments, presumably through protein-protein interactions.

Although microcompartment shells resemble viral capsids, electron microscopy reconstructions indicate that carboxysomes are not icosahedra but are D6 polyhedra, a symmetry that is not found among known viral capsids. Moreover, Blast, PSI-Blast, and direct structural searches uncover no significant similarity between viral capsid and microcompartment shell proteins. Thus, microcompartment shells and viral capsids appear to be unrelated to one another.

However, bacterial microcompartment shells are not the only protein sheets known in biology. Viral capsids and cell envelopes of prokaryotes and eukaryotes in many cases are partly composed of protein sheets that help to protect the cell or viral nucleic acid and provide receptors for substances binding to those cells. Bacterial microcompartments may indicate that such protein sheets are more functionally sophisticated than is appreciated. If so, the principles of microcompartment design and function could have broad relevance in biology.

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