Streamlined Construction of the Carboxysome for the Development of Modular Protein-Based Nanoreactors

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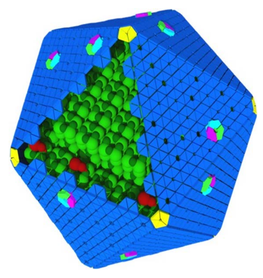
Introduction

The carboxysome is a self-assembling cyanobacterial organelle that encapsulates the enzymes ribulose-1,5-bisphosphate carboxylase/ oxygenase (RuBisCO) and carbonic anhydrase (CA) within a proteinaceous shell. Carboxysomes increase the local concentration of CO_2 in the vicinity of RuBisCO, thereby favoring its carboxylation activity.



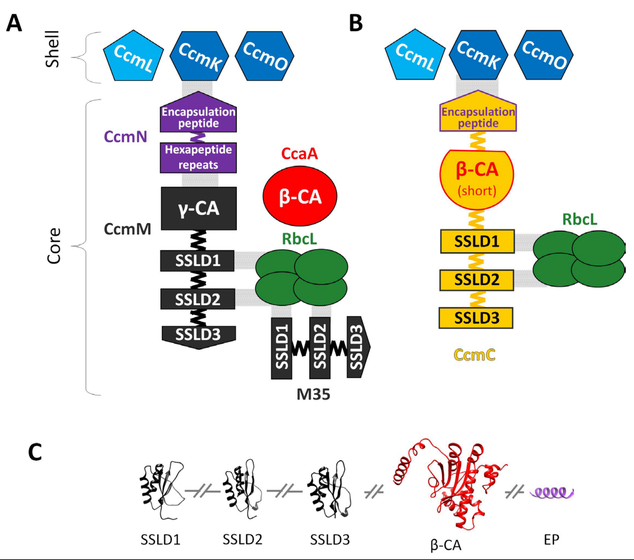
Carboxysomes have captured the interest of bioengineers interested in photosynthetic organisms because of their proposed potential for enhancing CO_2 fixation in photosynthetic organisms besides cyanobacteria. More broadly, carboxysome-based architectures may be redesigned as novel nanoreactors for the production of renewable chemicals (Kerfeld and Erbilgin, 2015). Potential uses of engineered carboxysomes include metabolic compartmentalization to increase pathway efficiency, segregation of toxic or volatile intermediates, etc.

However, carboxysomes are complex machines comprised of thousands of copies of at least 6 gene products; this creates significant challenges for the redesign of carboxysomes and for their transfer, regulation, assembly into heterologous systems. Hence, to overcome this bottleneck, a redesign of the carboxysome is necessary.

We designed a chimeric protein (CcmC) that structurally and functionally replaces four gene products required for carboxysome formation in the cyanobacterium *Synechococcus elongatus* PCC 7942.

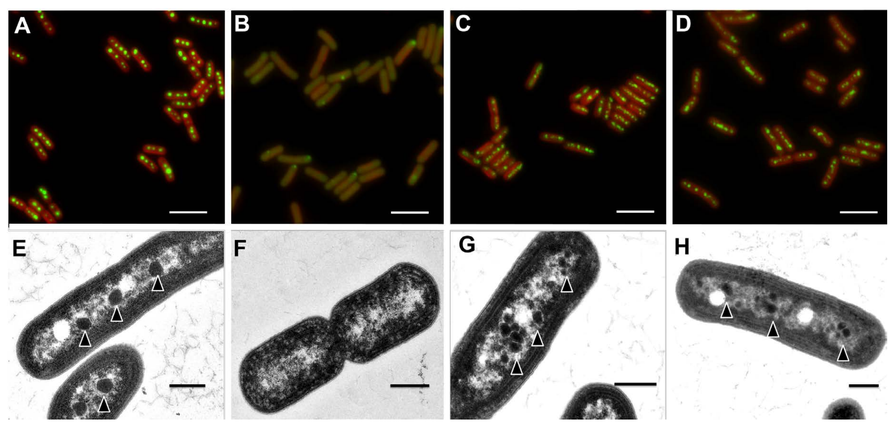
The chimeric protein CcmC

Assembly of the native carboxysome occurs from the inside out (Cameron et. al, 2013) through the protein-protein interactions shown in (A). By fusing the essential domains from the core proteins into a single chimeric protein that mimics the native assembly (C), we are able to form functional genetically and structurally streamlined carboxysomes (B).



CcmC can structurally replace the core proteins CcmM, M35, CcmN and CcaA

We confirmed the assembly of the chimeric carboxysomes through fluorescence and transmission electron microscopy in deletion strains expressing CcmC (COREΔ2: deletion of CcmM and CcmN and COREΔ3: deletion of CcmM, CcmN and CcaA).

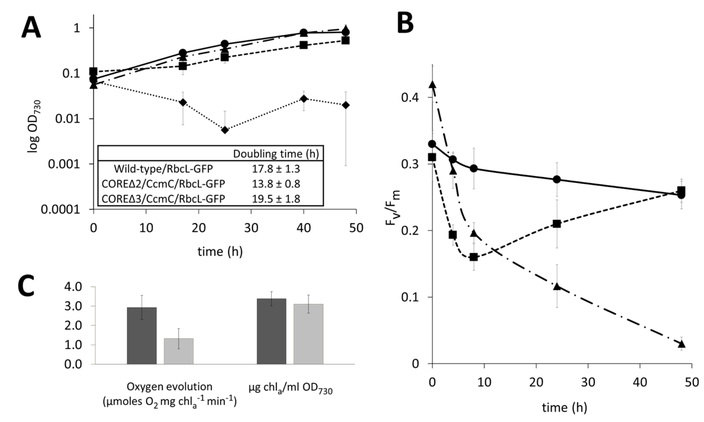


Top panel: Carboxysome visualization by fluorescence microscopy. The background of the strains is as follows: A: Wild-type/RbcL-GFP; B: COREΔ2/RbcL-GFP; C: COREΔ2/CcmC/RbcL-GFP; D: COREΔ3/CcmC/RbcL-GFP. Scale bar: 5 µm.

Bottom panel: Electron micrographs of the same strains from the top panel. E: Wild-type; B: COREΔ2/RbcL-GFP; C: COREΔ2/CcmC/RbcL-GFP; D: COREΔ3/CcmC/RbcL-GFP. Arrowhead: carboxysomes. Scale bar: 500 nm.

A streamlined carboxysome supports photosynthesis in cyanobacteria

The success of the fusion strategy comes at the expense of regulatory flexibility. However, differences in the physiology of the strain with chimeric carboxysome cores and the Wild type/RbcL-GFP strain (B and C) do not have a major effect on the capacity for photosynthetic growth of the cyanobacterial strain (A).



A: Optical density (730 nm) over time of independent cultures grown in air. n=3; Error bars= SD.
B: Change of Fv/Fm in cultures grown at 3% CO_2 and transferred to air at time= 0 h. n=3; Error bars= SD.
C: Oxygen evolution rates (normalized to chlorophyll *a)*: n≤5. Error bars= SD.

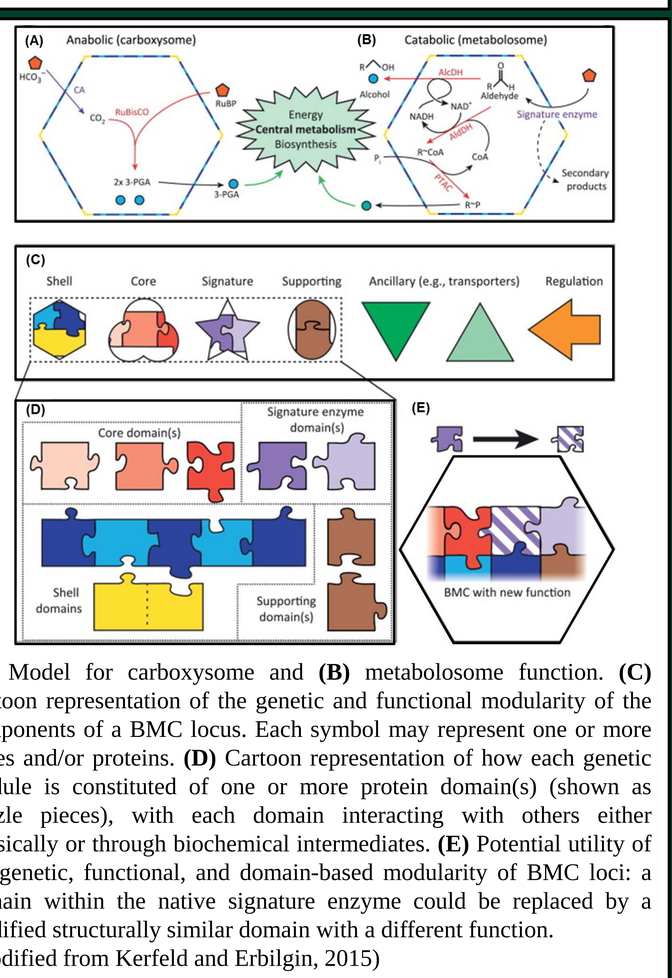
- Wild type/RbcL-GFP: Solid line and dark grey bar.
- COREΔ2/RbcL-GFP: Dotted line.
- COREΔ2/CcmC/RbcL-GFP: Dash-dotted line.
- COREΔ3/CcmC/RbcL-GFP: Dash line and light grey bar.

Conclusion

CcmC acts as a multi-protein carboxysomal core that nucleates RuBisCO, around which the shell assembles. Streamlined carboxysomes can support photosynthesis. Our domain-fusion approach makes the design of synthetic bacterial microcompartment (BMC) cores readily tractable. We propose that (re)engineering carboxysomes by focusing on domain structures and interactions can be used to build new subcellular architectures.

Implications

Carboxysomes play the key role of carbon fixation in cyanobacteria. As such, they hold great potential for industrial carbon capture. Our strategy for repurposing carboxysomal architectures opens new possibilities in the realm of synthetic biology: the carboxysome shell with a redesigned core could increase the efficiency of an encapsulated pathway, sequester volatile intermediates or separate toxic intermediates from the cell lumen, etc. for diverse engineered biosynthetic applications. (Kerfeld and Erbilgin, 2015). Carboxysome-based nanoreactors will be specially valuable for the efforts in renewable chemical production from photosynthetic microbes.



(A) Model for carboxysome and **(B)** metabolosome function. **(C)** Cartoon representation of the genetic and functional modularity of the components of a BMC locus. Each symbol may represent one or more genes and/or proteins. **(D)** Cartoon representation of how each genetic module is constituted of one or more protein domain(s) (shown as puzzle pieces), with each domain interacting with others either physically or through biochemical intermediates. **(E)** Potential utility of the genetic, functional, and domain-based modularity of BMC loci: a domain within the native signature enzyme could be replaced by a modified structurally similar domain with a different function.
(Modified from Kerfeld and Erbilgin, 2015)

Methods

Strain generation. *Synechococcus elongatus* PCC 7942 (Syn 7942) cells were transformed by double homologous recombination with plasmids design to replace CcmM. CcmN and CcaA with antibiotic resistance markers. Afterwards, a plasmid containing the fusion gene *ccmC* was transformed into the deletion strains, removing the selection marker using growth in air as positive selection. Finally, cells were transformed with a plasmid containing *rbcL-GFP*.

Microscopy. Syn 7942 carboxysomes were visualized from cultures at exponential phase. GFP fluorescence was used for fluorescence microscopy in a Zeiss Axio Observer.D1 inverted microscope. Electron microscopy was performed on cells embedded in Spur's resin and sectioned (70 nm thick), stained with uranyl acetate/lead citrate and imaged on a JEM 100CX II transmission electron microscope.

Physiological measurements. Growth was measured by the change in optical density at 730 nm; total chlorophyll as optical density measurement at 663 nm from methanol extracts; Fv/Fm through fluorescence measurement of chlorophyll (dark adapted for 3 min) using an Aquapen-C instrument and oxygen evolution in culture aliquots supplemented with 10 mM bicarbonate using a Clark-type oxygen electrode (Oxygraph plus) at 950 µmoles photons m^{-2} s^{-1}.

References

Cameron Jeffrey C, Wilson Steven C, Bernstein Susan L, Kerfeld Cheryl A (2013) Biogenesis of a Bacterial Organelle: The Carboxysome Assembly Pathway. Cell 155 (5):1131-1140.

Kerfeld CA, Erbilgin O (2015) Bacterial microcompartments and the modular construction of microbial metabolism. Trends in Microbiology 23 (1):22-34. doi:http://dx.doi.org/10.1016/j.tim.2014.10.003

Detailed data and methods from this poster can be found at:
Gonzalez-Esquer CR, Shubitowsk, TB, Kerfeld CA (2015) Streamlined construction of the cyanobacterial CO2-fixing organelle via protein domain fusions for Use in Plant Synthetic Biology. The Plant Cell, *DOI:* 0.1105/tpc.15.00329.

Acknowledgments

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