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**David Savage: Design principles of photosynthetic biofuel production**

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Cyanobacteria are photosynthetic gram-negative bacteria that are responsible for nearly 50% of the earth's carbon fixation. Here, I present my results in developing one such cyanobacterium, *Synechococcus elongatus* PCC7942, as a synthetic chassis for probing the molecular organization and constraints of carbon fixation and for the engineering of novel metabolic pathways for the production of biofuels.

Carbon fixation in cyanobacteria takes places within a viral-like capsid termed the carboxysome. The carboxysome is 100 nm in diameter and composed of shell proteins, secondary proteins, and enzymes: in total the carboxysome is greater than 200 MDa in size. It is postulated the carboxysome functions by enclosing RuBisCO, the oxygen-sensitive carboxylase, away from the cellular milieu and in an environment optimal for carbon fixation.

To probe the assembly and function of carboxysomes *in vivo* I screened a small library of carboxysomal proteins fused to YFP and under the induction of various promoters. The large subunit of RuBisCO (RbcL) fused with an N-terminal YFP was found to be the best reporter of carboxysome position (Fig 1). Strikingly, carboxysomes were evenly spaced within the cytoplasm. Furthermore, it was found that regardless of cell length or the number of carboxysomes within a cell, the spacing between carboxysomes remained even. This

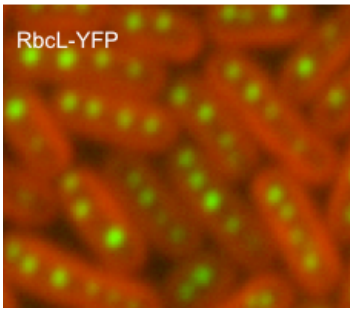


Figure 1. Carboxysomes can be labeled with fluorescent proteins *in vivo*. Thylakoid membranes shown in red.

suggested an underlying mechanism whereby spacing between carboxysomes can be sensed and adjusted for. Single-particle tracking of individual particles showed that carboxysomes essentially do not move with respect to the time scales of cell growth and division. The *Synechococcus* genome contains one actin-like homolog of the bacterial cytoskeleton protein *mreB* and I postulated this protein could mediation spacing. As expected, *mreB* deletion resulted in complete loss of carboxysome organization (Fig 2). These results represent a striking protein organization mechanism never seen before in bacteria, and I am currently readying a manuscript for publication as well as characterizing other proteins we have identified to be involved in carboxysome organization.

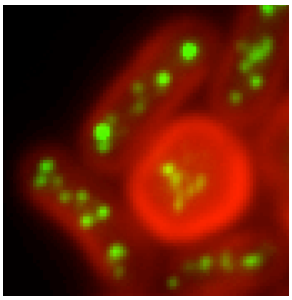

Figure 2. *ΔmreB results in loss of carboxysome organization*

As described above, I am also developing *Synechococcus* as a synthetic biology platform for biofuel production, namely biodiesel. Biodiesel is formed from the esterification of an alcohol to a fatty acid, and I am currently focusing on: i.) the production of a suitable alcohol, ii.). remodeling the fatty acid synthesis pathways of *Synechococcus* for shorter length fatty acids, and iii.) combining these two pathways with the esterifying enzyme wax synthase for the direct production of biodiesel *in vivo*. I have preliminarily shown that expression of plant thioesterases specific for shorter fatty acid length results in the production of caprylic (C8) acid instead of the normal palmitic (C16). I have also shown that incorporation of the alcohol fermentation pathway from *Z. mobilis* results in the production of ethanol. I am currently investigating way in which these pathways can be optimized as well as introducing the wax synthase gene into the combined ethanol/fatty acid producing strain.

Publications resulting from this project:

1) Engineering cyanobacteria to synthesize and export hydrophilic products. Niederholtmeyer H, Wolfstädter BT, Savage DF, Silver PA, Way JC. Appl Environ Microbiol. 2010 Jun;76(11):3462-6. Epub 2010 Apr 2. PMID: 20363793

2) Spatially ordered dynamics of the bacterial carbon fixation machinery. Savage DF, Afonso B, Chen AH, Silver PA. Science. 2010 Mar 5;327(5970):1258-1261. PMID: 20203050

3) Modularity of a carbon-fixing protein organelle. Bonacci, W, Teng, PK, Afonso, B. Niederholtmeyer H, Grob P, Silver PA, Savage DF. Proc Natl Acad Sci U S A 2012 Jan 10;109(2):478-83. Epub 2011 Dec 19. PMC3258634