Spatially Ordered Dynamics of the Bacterial Carbon Fixation Machinery

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Cyanobacterial carbon fixation is a major component of the global carbon cycle. This process requires the carboxysome, an organelle-like proteinaceous microcompartment that sequesters the enzymes of carbon fixation from the cytoplasm. Here, fluorescently tagged carboxysomes were found to be spatially ordered in a linear fashion. As a consequence, cells undergoing division evenly segregated carboxysomes in a nonrandom process. Mutations of the cytoskeletal protein ParA specifically disrupted carboxysome order, promoted random carboxysome segregation during cell division, and impaired carbon fixation after disparate partitioning. Thus, cyanobacteria use the cytoskeleton to control the spatial arrangement of carboxysomes and to optimize the metabolic process of carbon fixation.

Efficient cellular metabolism relies on the compartmentalization of enzymatic reactions. Prokaryotes achieve this organization by using capsidlike protein microcompartments to isolate metabolic pathways from the cellular milieu (Fig. 1). The best-characterized microcompartment, the carboxysome, is found in cyanobacteria and chemoautotrophs and is responsible for catalyzing more than 40% of Earth’s carbon fixation (2–4). Structurally, the carboxysome consists of an icosahedral proteinaceous shell that encloses the enzymes carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) (5–8). The shell may act as a semipermeable barrier, allowing the passive import of the negatively charged reactants, HCO3−, and ribulose-1,5-bisphosphate, and excluding the competing substrate O2. Within the carboxysome, carbonic anhydrase catalyzes the production of CO2, where it is fixed by RuBisCO into 3-phosphoglycerate. Carbon fixation is the basis of biosynthesis in cyanobacteria, and genetic disruption of the carboxysome is lethal (9, 10). Thus, the proper assembly and function of carboxysomes is fundamental to carbon fixation and cellular fitness.

We developed methods to visualize carboxysomes and to investigate their dynamical behavior in living cells. The carboxysome consists of ~5000 monomers of the shell protein CcmK (~2000 monomers of RuBisCO (5)). Expression of these proteins in the cyanobacterium Synechococcus elongatus PCC7942 (hereafter Synechococcus) fuses green, yellow, or cyan fluorescent protein (GFP, YFP, or CFP) yielded fluorescent particles, and the proteins colocalized when coexpressed in the same cell, which indicated assembled carboxysomes (Fig. 1A). The labeled carboxysomes also contained endogenous RuBisCO (Fig. 1B). Electron microscopy showed that all carboxysomes contain RbcL-GFP and that all RbcL-GFP was in carboxysomes (Fig. 1C). Carboxysome morphology and cellular growth rates were unaffected by YFP fusions (Fig. S1). Carboxysomes were evenly spaced along the long axis of Synechococcus (Fig. 2A). On average, there were 3.7 ± 1.2 carboxysomes per cell under log phase growth (Fig. 2B). We calculated the pairwise distances between carboxysomes in cells (n = 2508) with four carboxysomes (Fig. 2C). The average spacing between adjacent carboxysomes was 0.66 μm but was proportional to cell length (Fig. 2D). Thus, normalizing by cell length sharpened the pair-
wise distance probability distribution (Fig. 2E). This suggested that the spacing of carboxysomes could be actively controlled.

The diffusive dynamics of individual carboxysomes over time was constrained (Fig. 3A). Carboxysomes ($n = 350$) had an average diffusion coefficient $D$ of $4.58 \times 10^{-5} \pm 4.50 \times 10^{-5}$ $\mu m^2/s$ (Fig. 3A). Using cytoplasmic parameters (12) and a mean particle radius of 50 nm, we estimate a theoretical value of $D = 3.85 \times 10^{-12}$ $\mu m^2/s$, four orders of magnitude greater than the observed. The measured value of $D$ suggests that a carboxysome will diffuse an average distance $r = \sqrt{4 \times 4.58 \times 10^{-5} \mu m^2/s \times 64,800 s} = 3.45 \mu m$ during the cell cycle. Considering the displacement occurring from cell growth, the carboxysomes do not appear to be diffusing randomly.

Carboxysomes are known to associate with cellular structures, including unidentified filaments (1, 13, 14). Deletions of the five ($mreB$,

![Fig. 2. Carboxysomes are spatially organized in vivo. (A) RbcL-YFP (green) expression shows organized carboxysomes. Thylakoid membrane fluorescence is shown in red. (B) Average number of carboxysomes per cell. (C) Pairwise distance probability distribution. (D) Carboxysome spacing is proportional to cell length. (E) Same as (C) except carboxysome distances were normalized for cell length.](image)

![Fig. 3. Carboxysomes are organized by the cytoskeleton. (A) Diffusion of four tracked carboxysomes. Trajectories were used to compute $D$ in the two-dimensional diffusion equation $<r^2> = 4Dt$, where $t$ is time. (B) Rbcl-CFP (green) in $mreB$-deficient cells showing spherical cells and loss of carboxysome spatial organization. Thylakoid membrane fluorescence is shown in red. (C) RbcL-CFP (green) in $\Delta parA$ cells showing the loss of carboxysome spatial organization with no change in morphology. (D) Fluorescence image of ParA-GFP overlaid onto phase-microscopy image of cells showing the filament-like nature of ParA. Image is the deconvolved middle focal plane of a z stack. (E) Kymograph of the oscillatory behavior of ParA-GFP showing polymer dynamics. (F) ParA oscillation in relation to carboxysomes. Arrows denote increased ParA-GFP (green) between carboxysomes (Rbcl-CFP, red). Scale bars, 2 $\mu m$.](image)
ftsZ, minD, and two parA-like genes) annotated cytoskeletal genes present in the *Synechococcus* genome were constructed (11). Mutations in *mreB* and the parA-like gene Synpc7942_1833 (hereafter, parA) disrupted carboxysome organization (Fig. 3, B and C). As deletion of *mreB* was pleiotropic, we investigated *parA* (hereafter, parA) disruption carboxysome organization (Fig. 3, B and C). ParA proteins are thought to partition randomly (17), but segregation of carboxysomes was highly nonrandom (Pearson’s chi-square test, \( P = 3.9 \times 10^{-11} \)). Because of the loss of equal spacing, resolving individual carboxysomes was not possible; instead, we quantified the intensity of carboxysomes partitioning to daughter cells. The *parA* deletion strain exhibited much lower fidelity in partitioning carboxysomes than did wild-type cells (two-tailed Kolmogorov-Smirnov test, \( P = 4.4 \times 10^{-5} \)) (Fig. 4B).

The loss of carboxysome organization was responsible for reduced fitness. We observed reductions in which one *ΔparA* daughter cell received no carboxysomes. Lineage tracking revealed that these cells divided 2.8 ± 2.6 (\( n = 39 \)) hours later than their corresponding sister cells (Fig. 4C and movies S3 to S5). Populations of newly divided *ΔparA* cells with low or high numbers of carboxysomes were isolated by cell sorting (Fig. 4D). Cells with more carboxysomes fixed ∼50% more carbon per unit of time than cells with fewer did (Fig. 4E). Thus, disruption of carboxysome spatial organization compromised the fidelity of carboxysome partitioning and impaired daughter-cell fitness.

The regular spacing of cellular machinery is emerging as a fundamental aspect of bacterial physiology. Vesicular magnetosomes and plasmids distribute regularly along the long axis of the cell (16, 18). Here, an enzymatic complex was observed to behave in a similar manner. The organization of carboxysomes depends on cytoskeletal components, including ParA and MreB. Because MreB is located underneath the inner membrane (19), we favor a model in which MreB defines a structure that is used to organize carboxysomes. ParA proteins evenly space plasmids in the cell and mediate the positioning of a pole-localized protein in *Rhodobacter sphaeroides* (20). Here, mutation of *parA* affected carboxysome organization, and ParA filaments connected adjacent carboxysomes. Thus, ParA is a specific mediator of carboxysome spacing.

**Fig. 4.** Carboxysome organization mediates partitioning and cellular fitness. (A) Distribution for carboxysome segregation superimposed onto that expected for a binomial distribution. (B) The observed errors in segregation of carboxysome intensity between wild type and the *ΔparA* mutant as a function of the initial total intensity (\( I \)). Errors expected for an all-or-none or binomial (\( <\Delta I > = \sqrt{I} \)) process are also shown. (C) Phylogenetic tree associated with an all-or-none carboxysome segregation (movie S3). Tree width represents carboxysome number; length is division time. A cell receiving no carboxysomes requires an extra 9 hours to divide. (D) Histogram of GFP intensity in *ΔparA* cells expressing Rbl1-GFP. The upper (orange) and lower (cyan) 10% of intensities were sorted and verified by microscopy (inset). (E) 14C carbon fixation rates (with standard deviation, \( n = 3 \)) of the populations sorted in (D). The populations are different as judged by an unpaired \( t \) test (\( P = 2.9 \times 10^{-2} \)).
Retromer Is Required for Apoptotic Cell Clearance by Phagocytic Receptor Recycling

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The cell surface receptor CED-1 mediates apoptotic cell recognition by phagocytic cells, enabling cell corpse clearance in Caenorhabditis elegans. Here, we found that the C. elegans intracellular protein sorting complex, retromer, was required for cell corpse clearance by mediating the recycling of CED-1. Retromer was recruited to the surfaces of phagosomes containing cell corpses, and its loss of function caused defective cell corpse removal. The retromer probably acted through direct interaction with CED-1 in the cell corpse recognition pathway. In the absence of retromer function, CED-1 associated with lysosomes and failed to recycle from phagosomes and cytosol to the plasma membrane. Thus, retromer is an essential mediator of apoptotic cell clearance by regulating phagocytic receptor(s) during cell corpse engulfment.

In Caenorhabditis elegans, cell corpse engulfment is controlled by two parallel pathways, one that recognizes and transduces engulfing signals, and the other that induces cytoskeleton reorganization (1). However, how components of these pathways are regulated and what other factors are involved remain unclear. To identify additional regulators of these pathways we performed genome-wide and candidate-based RNA interference (RNAi) screens (2) for genes whose inactivation greatly increased cell corpse numbers in the C. elegans germ line. Three genes, snx-1, snx-6, and lst-4, encoding homologs of mammalian sorting nexins 1/2, 5/6, and 9/18/33 regulate endocytosis (3). The deletion mutants snx-1(tm847) (4) and snx-6(tm847) (5) affect clearance of both RNAi- and vps-26(RNAi) cell corpses. (6) Germ cell corpse phenotypes of control RNAI- and vps-26(RNAi) treated N2 and snx-1(tm847) mutants. Thirty germ cell corpses were recorded for each strain. (C) Four-dimensional microscopy analysis of germ cell corpse duration in N2, snx-1(tm847), and snx-6(tm3790) mutants. Thirty germ cell corpses were recorded for each strain. (D) Quantification of germ cell corpses in vps-26(RNAI)- and vps-29(RNAI)-treated N2 and snx-1(tm847) mutants. The retromer affects clearance of apoptotic cells. (A) Quantification of germ cell corpses in N2 (wild type), snx-1(tm847), snx-6(tm3790), and snx-6(tm847);snx-1(tm847) mutants. Thirty germ cell corpses were recorded for each strain. (B) Quantification of germ cell corpses in all strains. Comparisons were performed using unpaired t tests. **P < 0.001.

References

11. Materials and methods are available as supporting material on Science Online.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/327/5970/1258/DC1

Materials and Methods

SOM Text

Figs. S1 to S4

Tables S1 to S3

References

Movies S1 to S5

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