Modularity of a carbon-fixing protein organelle

Bonacci, Teng, Afonso, Niederholtmeyer, Grob, Silver, Savage

PNAS - November 17, 2011

Shelley Ackerman & Donnie Turlington

20.385 Journal Club I

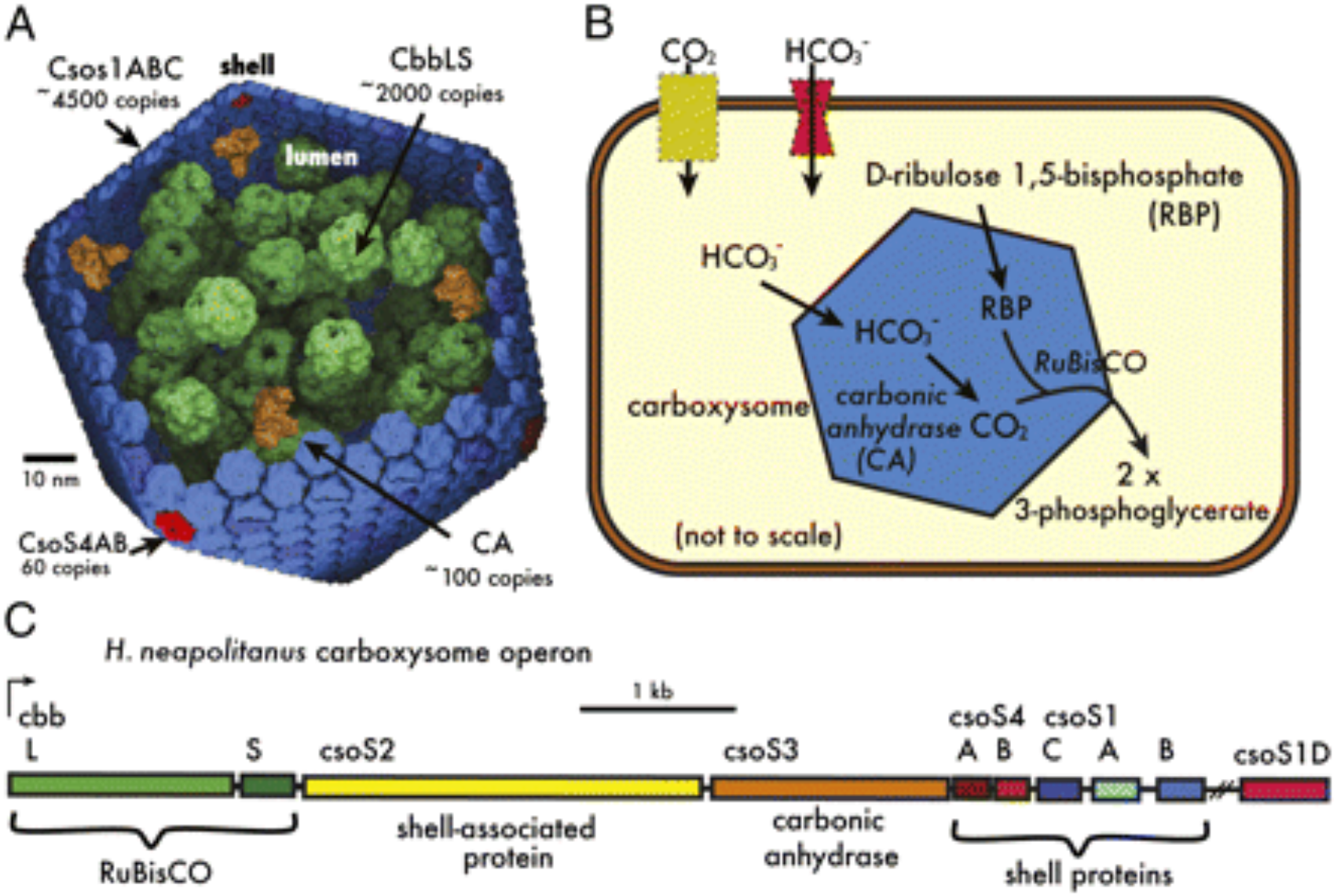
Types of Bacterial Microcompartments (BMCs)

- CB – CO_2 fixing carboxysomes
- PDU – 1,2-propanediol utilization microcompartment
- Ethanol-amine utilization microcompartment

• BMC protein domains appear in about 20% of bacteria

  •Possibly a large and diverse group of BMCs we have not yet accounted for

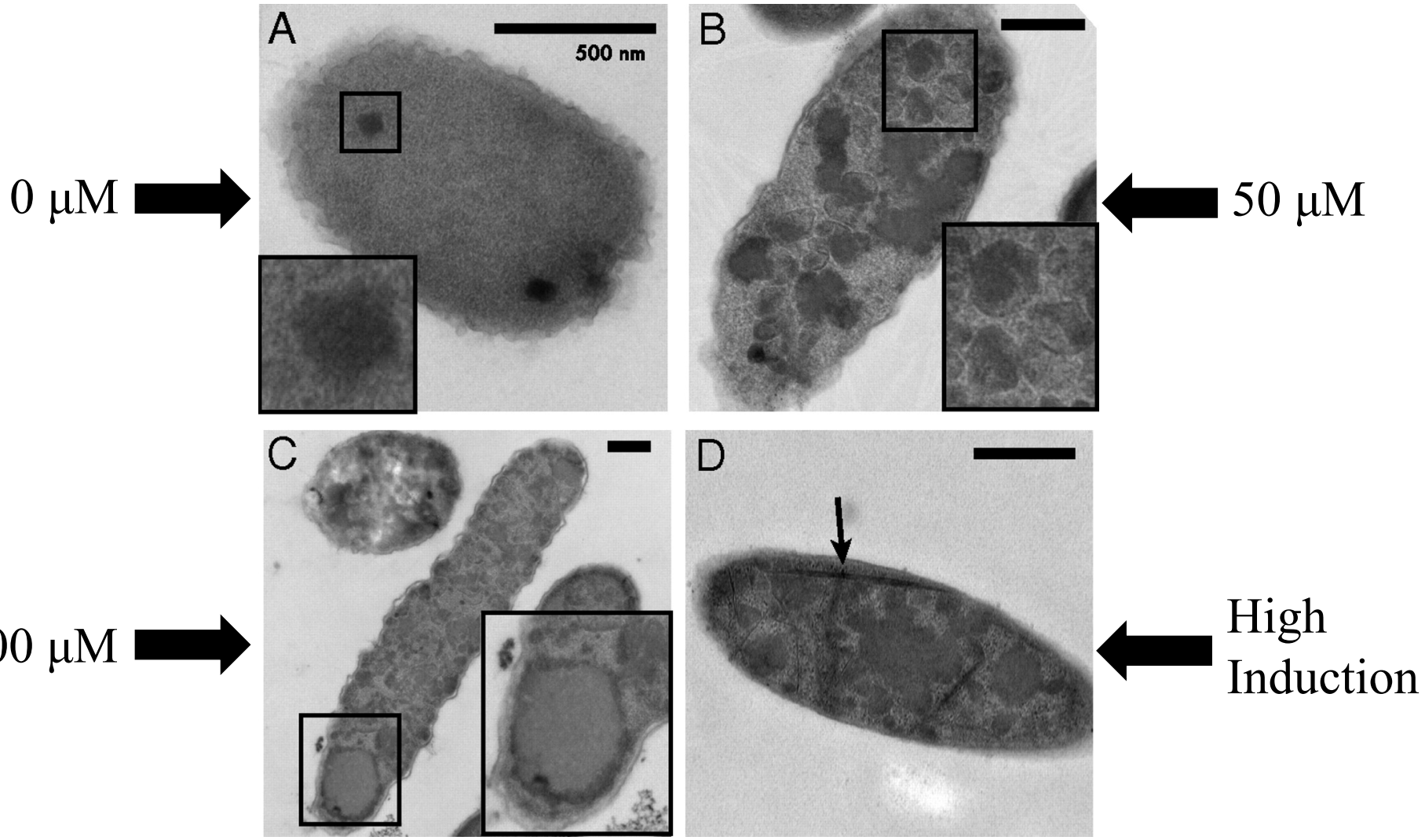
Wild Type System



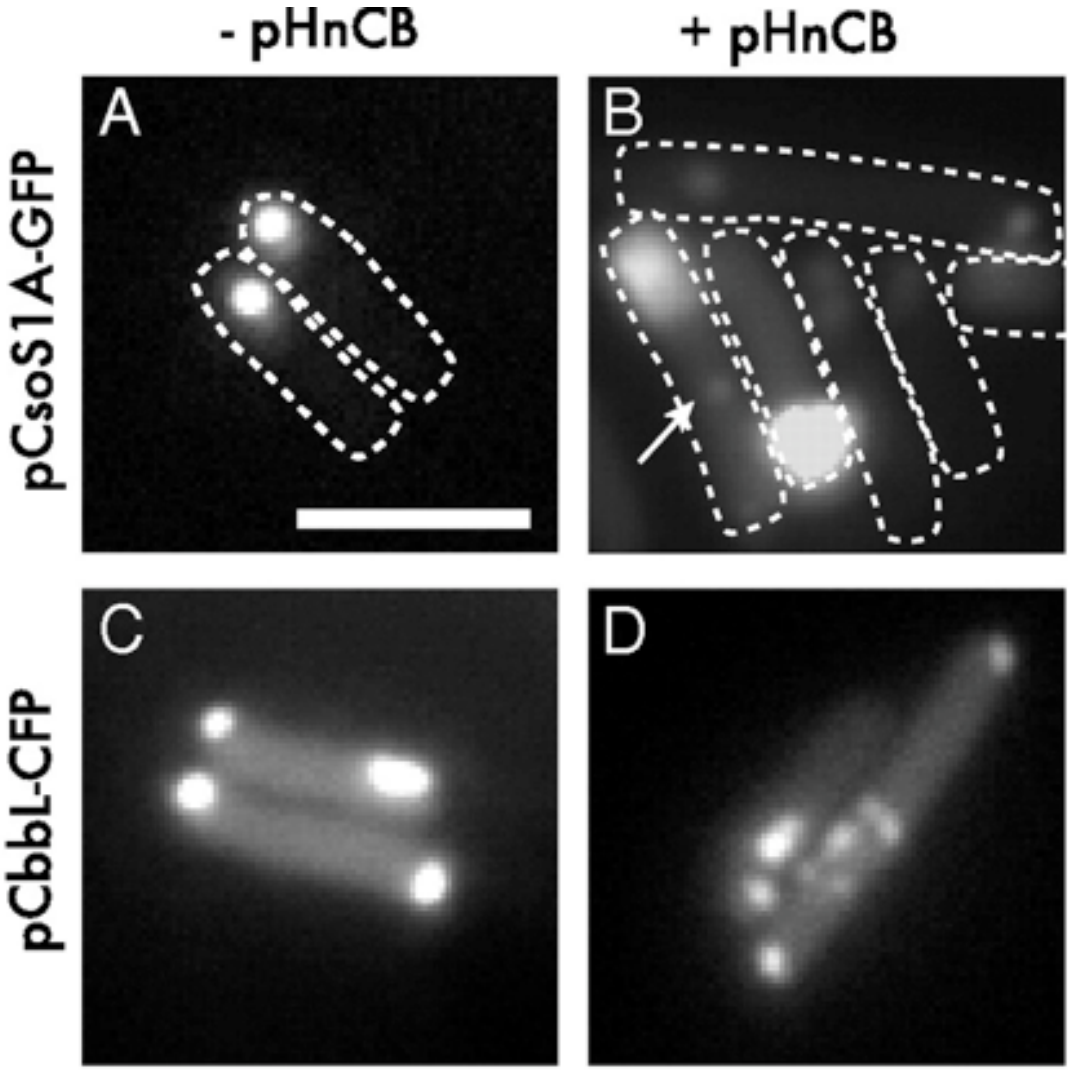
Their Original System

- Goal: to present a genetic system for the expression of functional BMCs.
  - create CBs in E. Coli from *H. neapolitanus*
  - Show that synthetic CBs contain proper proteins
  - Modularize a CB system
- 9 genes from *H. neapolitanus* and placed into E. Coli
- Used IPTG inducible plasmid (pNS3)

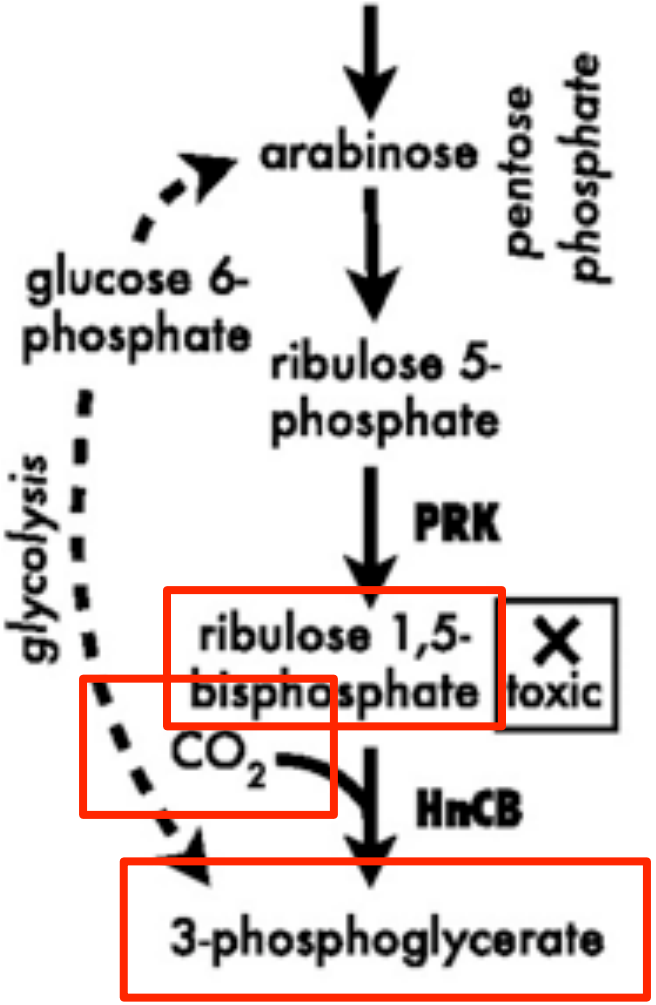
HnCB Expression Increases with Higher IPTG Concentrations



Functional Carboxysomes Assemble Correctly *in vivo*

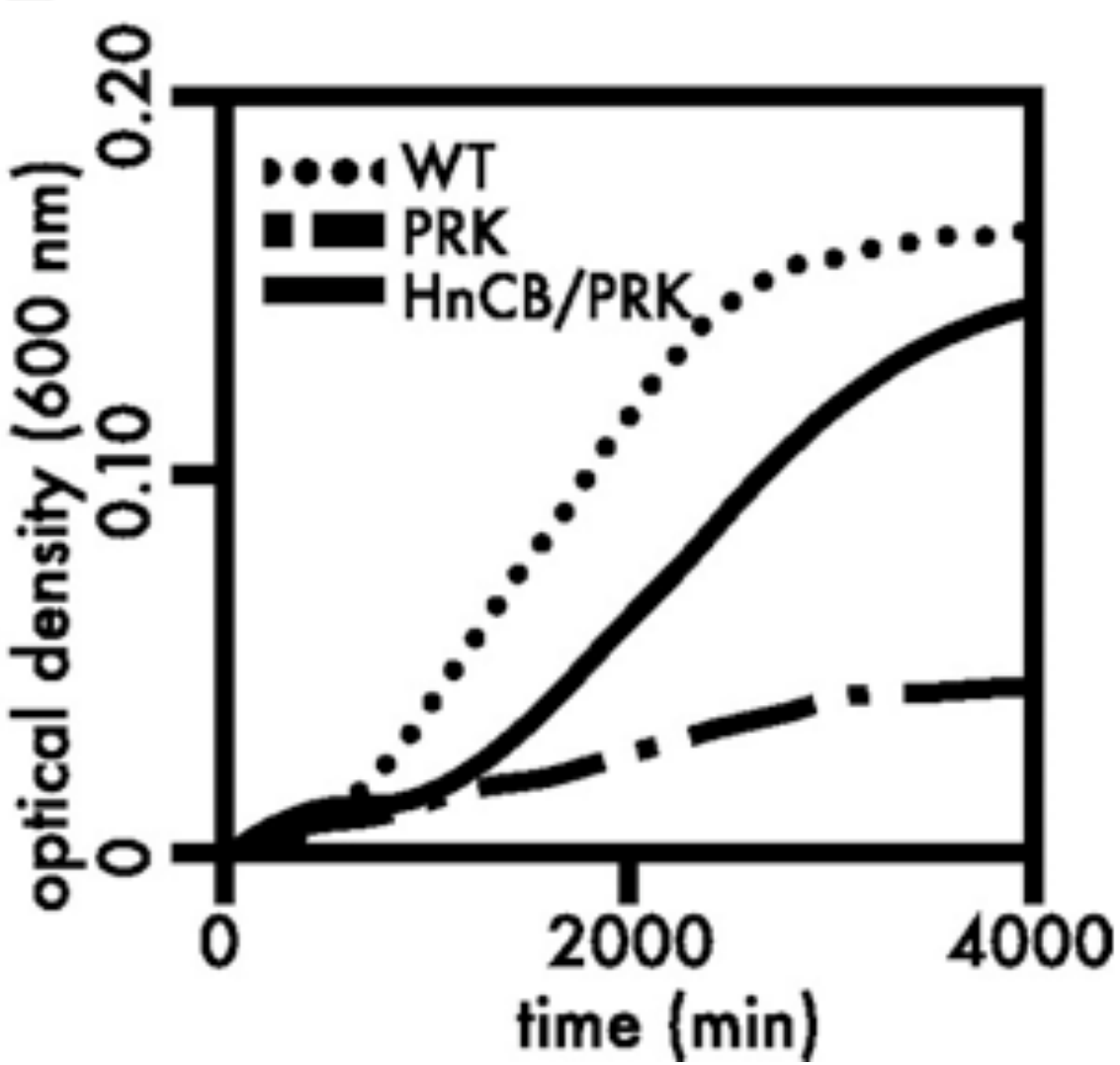


Carbon Fixation measured in 9-Gene System using Pentose Phosphate Pathway



- Key Metabolites:
  - Ribulose 1,5-bisphosphate
  - Carbon dioxide
  - 3-phosphoglycerate

HnCB Expression Results in Creation of Carbon Fixing Functional CBs



Assessing & Redesigning the System

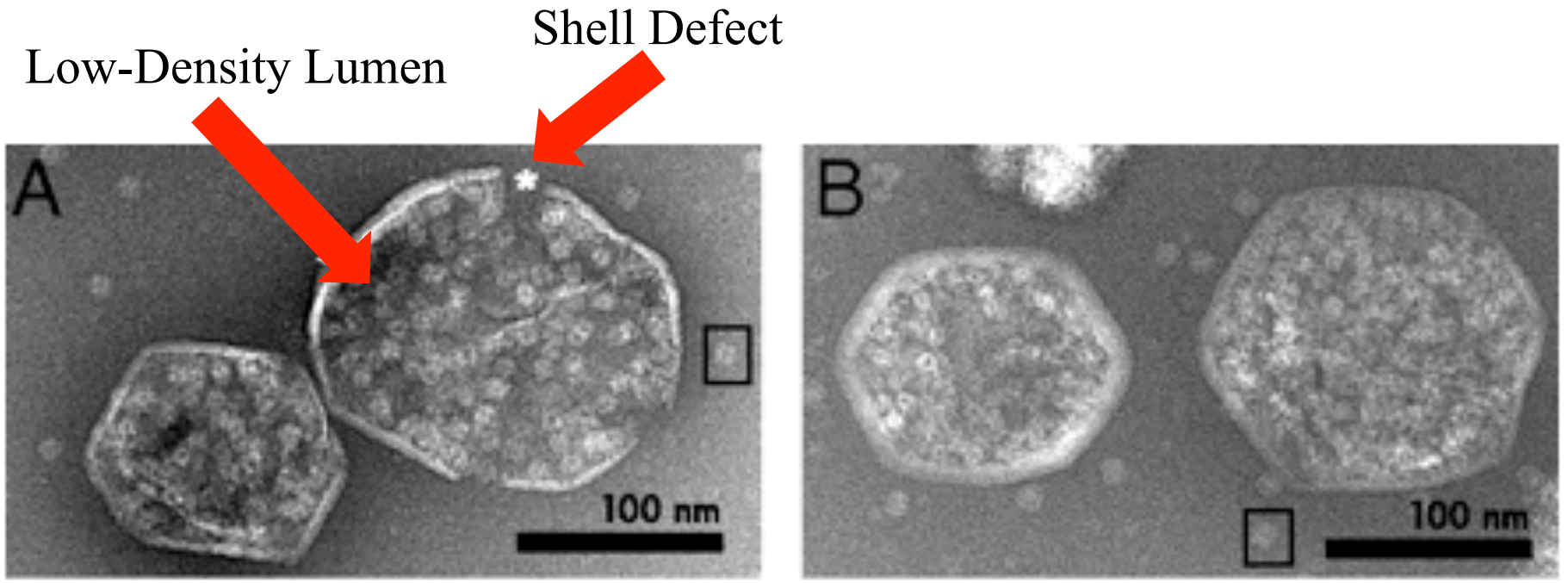
Problems with 9-Gene System:

- Error Rate in Assembly
- Heavy IPTG Dependence

What's Next? Bring in 10th Gene: CsoS1D

- Goal: See how addition of protein affects CB function and structure

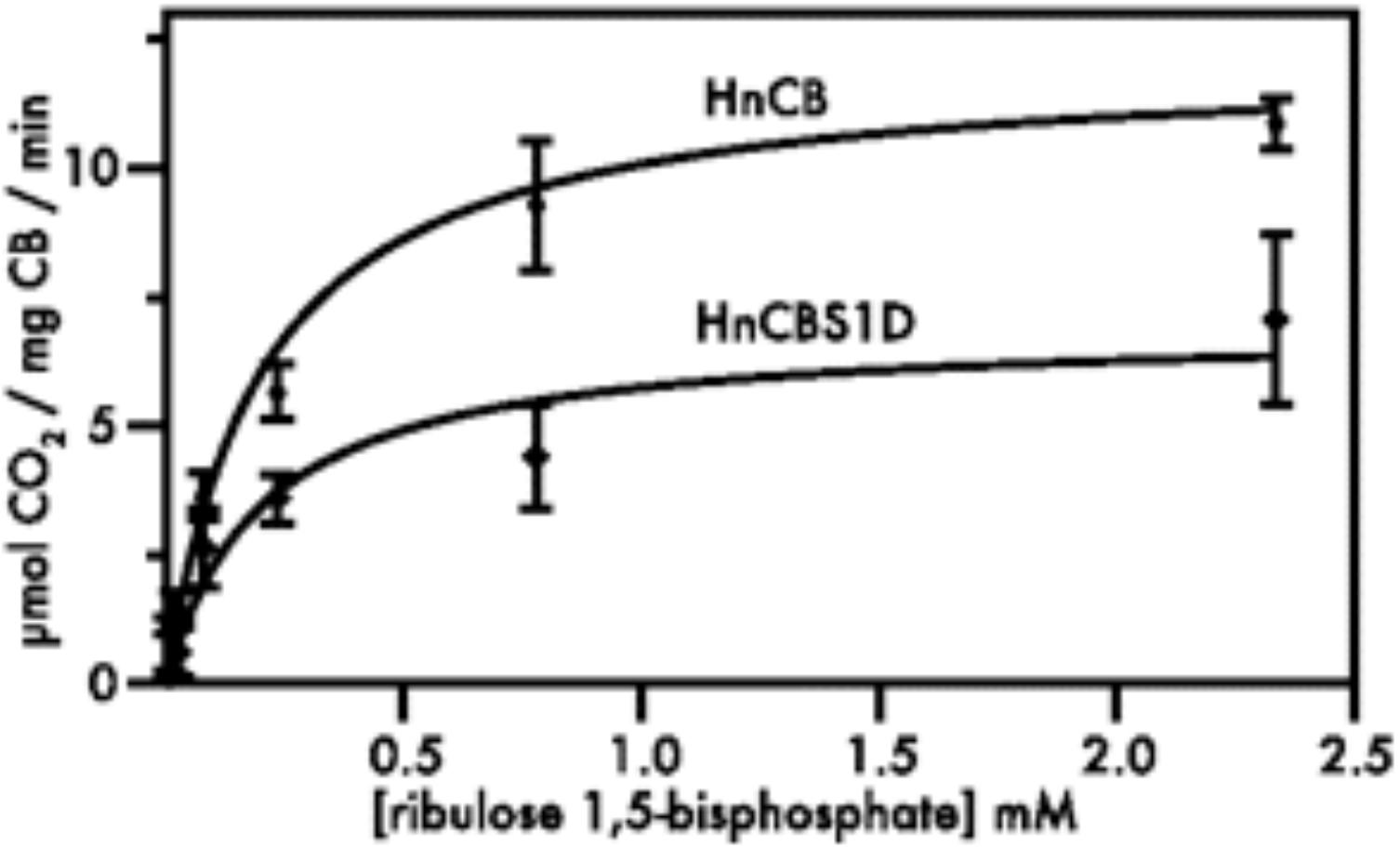
pHnCBS12D Results in Smaller Error in Assembly than pHnCB



pHnCB Plasmid

pHnCBS1D Plasmid

HnCBS1D results in faster and higher levels of carbon fixation



Carbon Fixation in 10-Gene System Verified *in vitro*

- Recorded 2-phosphoglycerate dependent NADH oxidation in coupled enzyme reaction system

- Created Michaelis-Menten Model
  - Similar K_M values for pHnCB & pHnCBS1D
  - V_{Max} two fold higher for pHnCB CB's

What was accomplished?

- Successfully moved a CB
- Proved that the CBs were still functioning
- Modified and heavily improved operon
  - adding tenth gene, CsoS1D
  - This was only tested *in vitro*, not *in vivo*

Significance

- Modularity
  - standardized parts
  - compartmentalization
- Importance of Carbon Fixation
- Future applications
  - targeting different assembly systems
- Improvements:
  - Optimization of the operon for specific hosts
  - Shell structure and permeability research
    - CsoS1D a minor component of shell structure
      - Possible Importance

Future Applications

- Plant adaptations
- Biofuels
- Molecular chassis
- Nanotechnology
- Future Research:
  - Identification of necessary genes
  - Improved understanding of shell formation
  - Development of cargo targeting system

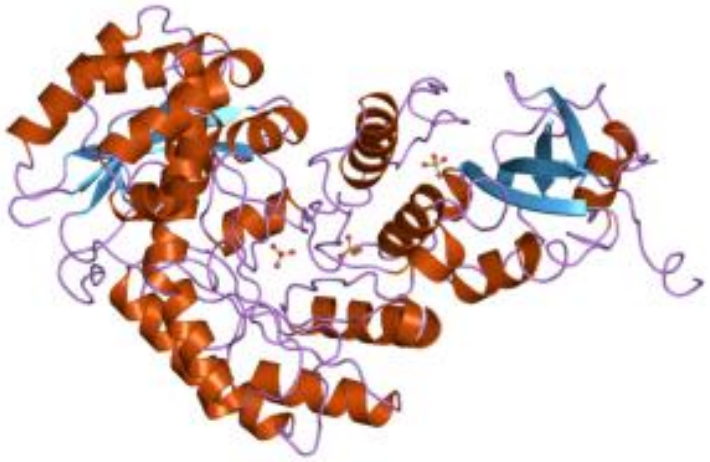
http://www.news.stanford.edu

Works Cited

- Talaro, Kathleen P., *Foundations in Microbiology*, sixth edition. 2008 McGraw-Hill Companies.
- Bonacci, Walter, Teng, Pho K., Afonso, Bruno, Niederholtmeyer, Henrike, Grob, Patricia, Silver, Pamela A., Savage, David F. Modularity of a carbon-fixing protein organelle, *PNAS*,.Jan 10, 2012, Vol. 109, no.2.

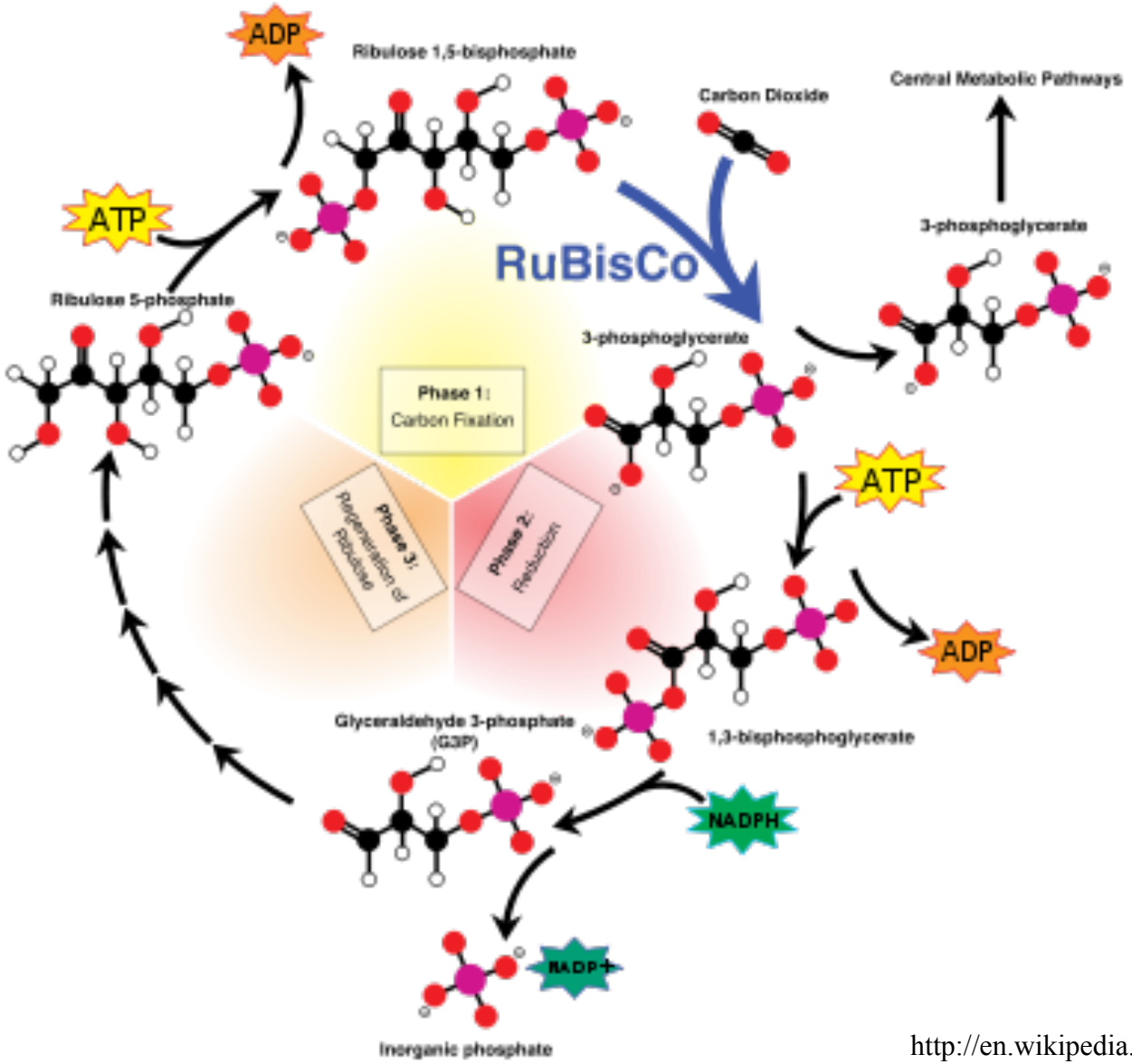
RuBisCo

- Ribulose-1,5-bisphosphate carboxylase oxygenase
- Major part of the Calvin cycle
- The rate of RuBisCO increases with increases in CO_2 concentration (also affected by temperature, water, nitrogen availability, ect.)
- Poor specificity for CO_2
- Photosynthesis is single largest natural regulator of CO_2 in our atmosphere, a good understanding is necessary for any climate impact studies.



http://upload.wikimedia.org/wikipedia/commons/7/79/PDB_3rub_EBI.jpg

Calvin-Benson-Basham cycle



http://en.wikipedia.org/wiki/Calvin_cycle