

## Regulation of Carbon Dioxide Usage in *Halothiobacillus neapolitanus* and *Thiomonas intermedia*



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## Global Warming...

- What causes Global Warming?
- What can be done to prevent further damage?



<http://www.spacetoday.org/Satellites/TerraAqua/TerraStory.html>

## *H. neapolitanus* and *T. intermedia*

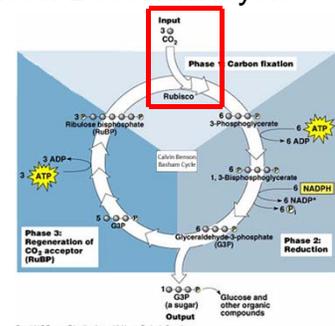
- Why these bacteria:
  - Easy to access, grow, and maintain
  - Naturally remove CO<sub>2</sub> via the Cbb cycle.
  - Model structure for cyanobacteria



<http://genome.jgi-psf.org/halne/halne.home.html>

## Calvin-Benson-Bassham Cycle

- Same cycle occurs in plants
- Initiated by RuBisCO
- Sugar is final product



<http://www.bio.miami.edu/~cmallery/150/phts/c10x17calvin-cycle.jpg>

## CbbR & RuBisCO

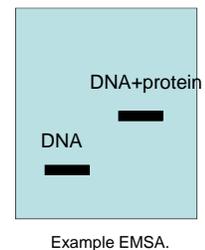
- Protein
- Produced with form II RuBisCO
- Possible regulator?

Hypothesis: CbbR will bind to form I.



## Overview of Procedures

1. Isolate and label DNA
2. Express desired protein in *E. coli*
3. Add protein to DNA
4. Determine binding using Electrophoretic Mobility Shift Assay (EMSA)



### Isolation of Labeled DNA Fragment

Ti form I and II DNA.

Hn form I and II DNA.

- Desired DNA was copied and labeled with biotin.

### *T. intermedia* CbbR Protein

Ti CbbR protein and vector.

Ti CbbR protein in *E. coli*.

- The DNA for CbbR was inserted into *E. coli*.
- As *E. coli* grew, CbbR was produced within it.

### *H. neapolitanus* CbbR Protein

Hn CbbR protein and vector.

Hn CbbR protein in *E. coli*.

### *T. intermedia* EMSA

Ti form I EMSA.

Hn form I EMSA.

- Samples migrate through gel based on weight; lighter samples migrate farther down.

### Conclusions

Ti form I EMSA.

Hn form I EMSA.

- CbbR protein binds to regulatory DNA of form I and form II RuBisCO.
- Decrease in protein shifts confirm binding.

### Future Research

- Competition Assay
- Create a *cbbR* knockout mutant.

Example Competition Assay. Percentage of labeled DNA plus protein.

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