Magnifying Algal Cell Growth Through Overexpression of a Carbonic Anhydrase Enzyme

Sarah Carpe[2], Charity Ensor[1], Lian Jackson[2], Amrita Madabushi[1], Stephen M. Miller[2]

[1]Arts and Sciences, Biotechnology Program, Baltimore City Community College, 2901 Liberty Heights Ave, Baltimore, MD 21215

[2]Department of Biological Sciences, UMBC, 1000 Hilltop Circle, Baltimore, MD 21250

Introduction

Green algae have exciting potential as an alternative to fossil fuels because they can produce large amounts of neutral lipids that can then be modified into biodiesel that is potentially more economic than ethanol from corn. *Chlamydomonas reinhardtii* (Fig 1.), a single celled microalga characterized by two flagella, is the model organism for algal biofuel *production* research. Many tools are available in its genetic manipulation and it is easy to culture, making it an excellent platform for biotechnology research. The limiting factor for algal growth, carbon dioxide availability (CO_2), is the focus of our algal biofuel research. Improving the rate of CO_2 fixation in *C. reinhardtii* should improve growth and lipid production. The enzyme Carbonic Anhydrase 6 is the focus of this study, represented by the *CAH6* gene. *CAH6* utilizes changes in osmotic pressure gradients to increase CO_2 availability in carbon fixation by converting some CO_2 into HCO_3^- (Fig 3.). Overexpression may be achieved by inserting a *Ble* fragment (*Bleomycin + viral 2A peptide fragment* upstream of the *CAH6* coding region (Fig 4.). to improve photosynthesis and growth. Over expression of these enzymes should improve photosynthesis, cell growth, and lipid content.

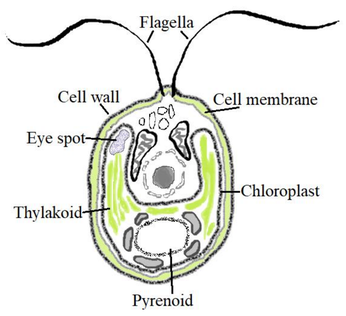


Figure 1: Chlamydomonas reinhardtii
Cell model of *Chlamydomonas reinhardtii*, a green microalga with two flagella, showing the chloroplast and its components, as well as the cell wall and membrane. Ensor, C., Carpe, S.. August 2nd, 2017.

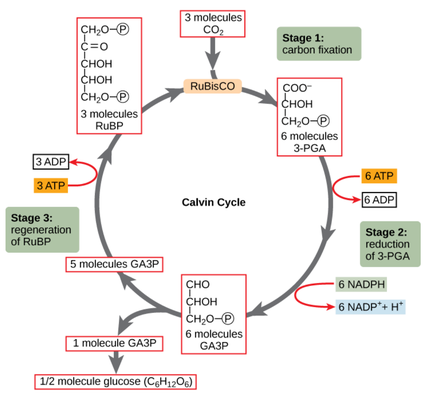


Figure 2 : **The Calvin Cycle**
A Diagram of the Calvin Cycle; a set of chemical reactions that takes place in the chloroplast during photosynthesis resulting in production of Glyceraldehyde 3-phosphate, a carbohydrate used as an immediate nutrient that can be converted into sugar or lipids. OpenStax, Biology. OpenStax CNX. May 27, 2016 http://cnx.org/contents/185cbf87-c72e-48f5-b51e-f14f21b5eabd@10.53.

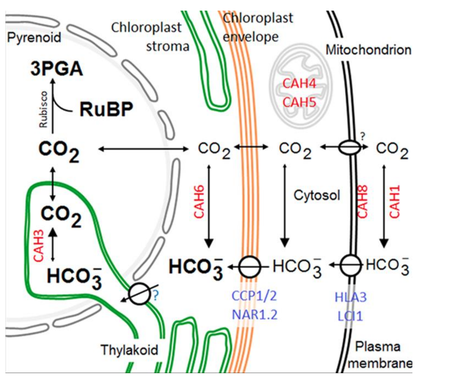


Figure 3 : **Carbon Concentrating Mechanisms in Chlamy**
Illustration of the CO_2 concentrating mechanism of *Chlamydomonas reinhardtii,* showing inorganic carbon transport *proteins LCI1, HLA3, CCP1/2 and NAR1.2*, and carbonic anhydrases *CAH1, CAH3, CAH4/5, CAH6* and *CAH8.* Jungnick, N., Ma, Y., Mukherjee, B. et al. Photosynth Res (2014) 121: 159.

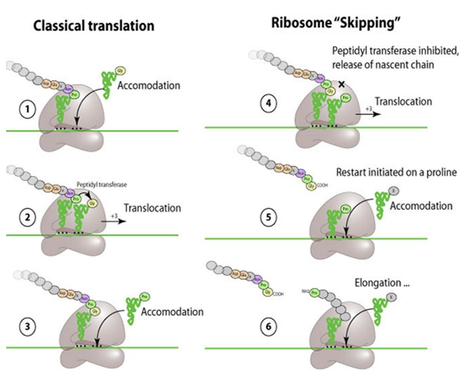
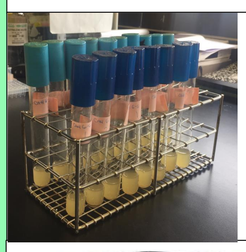


Figure 4 : **Classical Translation Vs. Ribosome "Skipping"**
A comparison of classical translation and ribosome "skipping"; peptidyl transferase inhibited following translation of 2A peptide fragment. Ribosomal Skipping. Retrieved August 2nd, 2017 from http://viralzone.expasy.org/914?outline=all_by_species

Methods

p-ARG CAH6
- Variant *CAH6* plasmid transformed
- Protein expression was not observed
  - *ARG* gene section of plasmid cut
  - Plasmid was allowed to religate.



Cell Culture & Inoculation
- *CAH6* plasmid and *Ble* fragment
- heat shocked into competent cells
  - incubated overnight at 37°C
- Plucked individual colonies were plucked
  - grown in LB media overnight at 37 °

DNA Purification
- DNA purified with plasmid cleanup kit/boiling miniprep.
  - Samples ethanol precipitated
- DNA concentration (ng/µl) measured
  - Samples digested with Xho1
    - Diagnostic gel run.

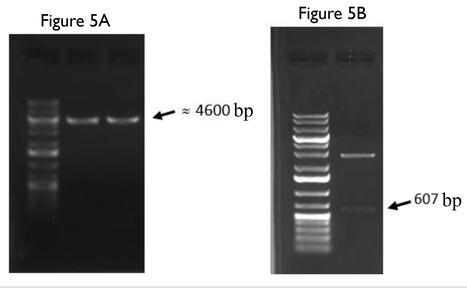
In-Gel Ligation
- *Ble* and *CAH6* isolated from low melt gel
  - Samples incubated with DNA ligase
- Ligation products were heat shocked into competent cells and plated.
- Colony DNA to be be checked for competency

Transformation
- *CAH6 + Ble* plasmid DNA to be verified Isolation of plasmid
- Transformation into *Chlamydomonas reinhardtii*

Results

Figure 5A: Vector Isolates
Lane 1 > 1Kb Geneladder
Lane 2 > Linearized *CAH3* isolate
Lane 3 > Linearized *CAH6* Isolate

Figure 5B: Insert Isolate
Lane 1 > 1Kb Geneladder
Lane 2 > Ble fragment



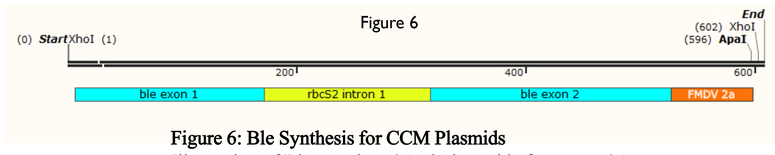


Figure 6: Ble Synthesis for CCM Plasmids
Illustration of Bleomycin + 2A viral peptide fragment. 2A viral peptide fragment is responsible for ribosome skipping.

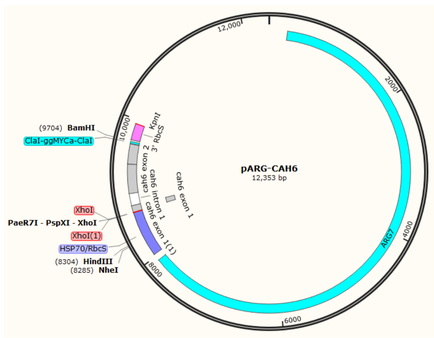


Figure 7A: *p-ARG CAH6*
Illustration of plasmid containing *CAH6* coding region. This plasmid variant has an *ARG* coding region.

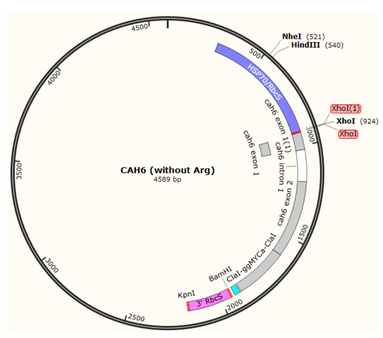
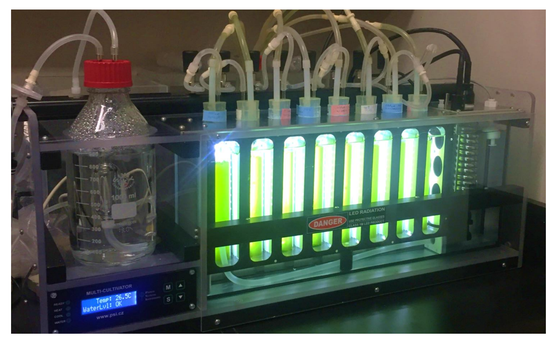


Figure 7B: *CAH6*
Illustration of plasmid containing *CAH6* coding region. This plasmid variant has had the *ARG* coding region cut out by ECORv. Xho1 sites are illustrated, this is where *Ble* fragment will be inserted.

Future Directions

- *Ble* fragment to be inserted upstream of *CAH6* coding region
  - ➢ selectable marker protein expression indicates successful transformation
- Plasmid to be inserted into *C. reinhardtii* with Electroporation
- Western-blot analysis will be used to confirm transgenic protein expression
- Use of an algal multicultivator to observe and compare growth
  - ➢ Compare *CAH6 + Ble* transformant to a control wild type
  - ➢ OD measurements
- Application of methods to *Chlorella vulgaris*, biotechnology production organism, upon improved growth



Conclusion

Algal biofuel production is a sustainable alternative to fossil fuels that has more potential than ethanol produced from corn, another alternative fuel source. Although biodiesel does give off carbon dioxide when burned, the same amount carbon is taken out from the atmosphere in its growth; therefore, the burning of biodiesel is carbon neutral. Carbon dioxide is the limiting factor of algal growth. carbon fixation can be modified in the favor of producing more lipids per algal cell. Transformation of these genes into *Chlamydomonas reinhardtii* could increase carbon availability. These methods could be implemented in biotechnology production organisms, thereby bringing scientists closer to a more economic sustainable energy source.

Acknowledgements

These results were obtained as part of the Research Experience and Mentoring (REM) program in the Department of Biological Sciences at the University of Maryland Baltimore County. This program is funded by a grant (REM supplement to NSF-EFRI-1332344) from the National Foundation (NSF) Directorate for Engineering (ENG) Office of Emerging Frontiers in Research and Innovation (EFRI). We also thank Jose Ortega, Rudolph Park, Erin Pueblo, Jacqueline Rivera, Holly Asbury, Jihye Yeon, Newton Bosire, and Kimuel Villanova for technical assistance and helpful discussions.