Overexpression of Two Carbonic Anhydrase Enzymes to Amplify Algal Cell Growth

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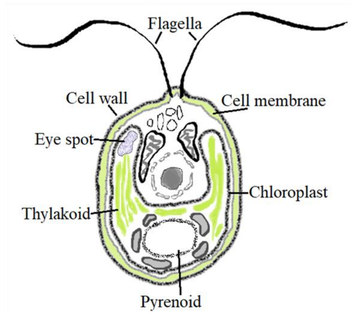
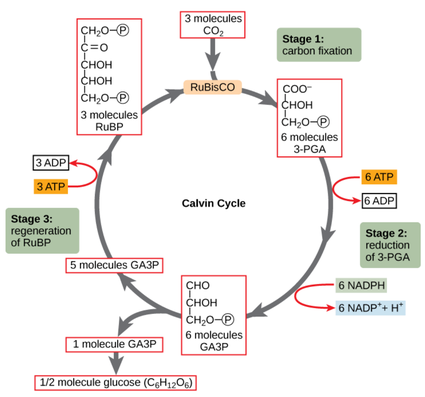
Introduction

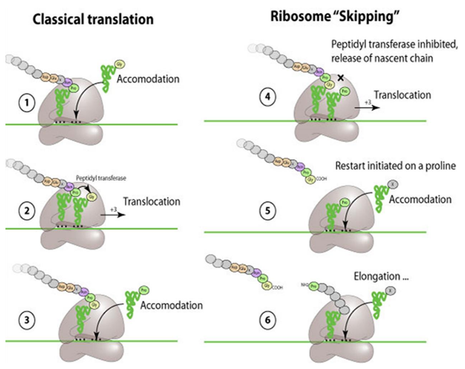
Green algae are photosynthetic plant-like organisms with great promise as a source of sustainable biofuels. Production of biofuels from algae is a sustainable alternative to fossil fuels. *Chlamydomonas reinhardtii* (Fig 1.), a single celled microalga, is the most widely used model organism for algal biofuel production research . Many tools are available for molecular genetic manipulation of *C. reinhardtii*; it is easy to culture, making it an excellent platform for biotechnology research. The limiting factor for algal growth, carbon dioxide (CO_2), is the focus of our algal biofuel research. Certain enzymes play a role in intracellular CO_2 uptake, a crucial part of the Calvin Cycle (Fig 2.), as well as conversion into carbohydrates and eventually lipids. These enzymes can be manipulated using *Ble* (*Bleomycin + viral 2A peptide fragment*). (Fig 3.) to improve photosynthesis and growth. The focus of this study are two genes that encode enzymes involved in CO_2 uptake. Carbonic anhydrase 6 (*CAH6*) (Fig 4.) converts CO_2 into carbonate in the chloroplast stroma, increasing carbon flow into the pyrenoid, where CO_2 fixation into carbohydrates takes place. Carbonic anhydrase 3 (*CAH3*) (Fig 4.) converts carbonate back into CO_2 in the pyrenoid. Over expression of these enzymes should improve photosynthesis and thus cell growth.



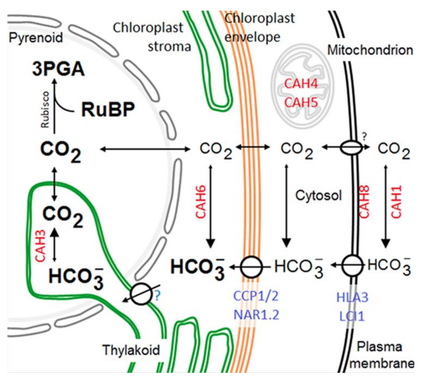
Chlamydomonas reinhardtii
Figure 1. Cell model of *Chlamydomonas reinhardtii*, a green microalga with two flagella, showing the chloroplast and its components, as well as the cell wall and membrane. Ensor, C., Carpe, S.. August 2nd, 2017.



The Calvin Cycle
Figure 2. 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.



Classical Translation Vs. Ribosome "Skipping"
Figure 3. 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



Carbon Concentrating Mechanisms in Chlamy
Figure 4. 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.

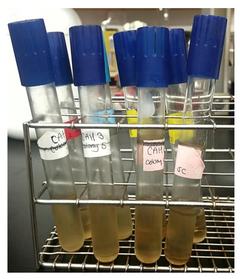
Methods

Cell Culture

Using aseptic technique we plated competent cells containing plasmids onto LB Amp plates. The plasmids have either *CAH3, CAH6,* or *Ble* coding regions. Plates were at 37°C for 12-18 hours.

Inoculation

Autoclaved toothpicks were used to pick up individual colonies. Colonies were introduced to 7.5mL of LB amp media via toothpick. Tubes were allowed to grow overnight at 37°C in shaker incubator.



DNA Purification

DNA was purified using a plasmid cleanup kit. The samples were further purified using ethanol precipitation technique, then nanodropped to check DNA concentration (ng/µl). Each sample was digested using restriction enzyme Xho1, then a diagnostic gel was run.

In-Gel Ligation

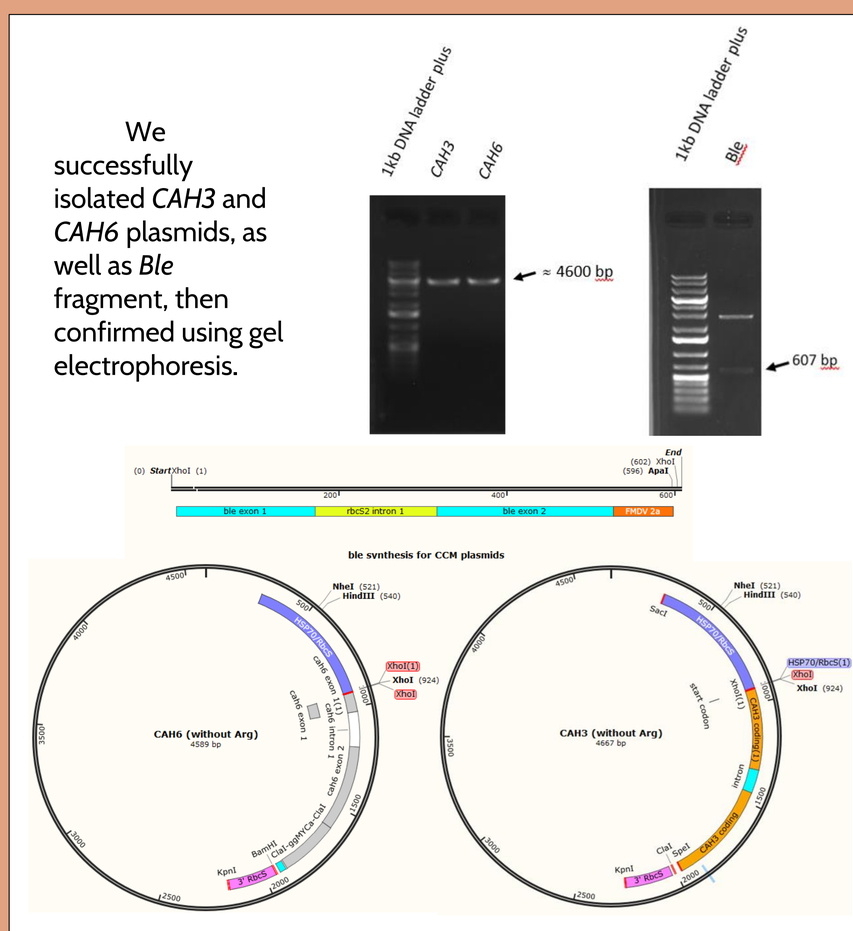
A low-melt agarose gel was used to isolate *Ble*, *CAH3*, and *CAH6* fragments after XhoI digestion. Isolated *Ble* and *CAH3*, or isolated *Ble* and *CAH6*, were incubated with DNA ligase.

Transformation

Ligation products will be transformed into chemically competent cells. Cells will be plated and selected for ampicillin resistance, then DNA from surviving colonies will be analyzed.

Results

We successfully isolated *CAH3* and *CAH6* plasmids, as well as *Ble* fragment, then confirmed using gel electrophoresis.



Future Directions

Utilizing recombinant DNA technology, we will take generated vectors with *CAH3* or *CAH6* coding regions and connect them to an insert *Ble* gene sequence with the hope of increasing expression of these proteins. The *Ble* sequence encodes a selectable marker protein; if expressed it will indicate successful transformation. Using a pulse of electrical current to momentarily open the pores of the cell membrane, a technique called electroporation, we will introduce these vectors into *C. reinhardtii*. We will use western-blot analysis to determine transgenic protein expression. Finally, we will use an algal multicultivator to observe and compare growth of our transformants to a control strain using optical density measurements. If overexpression of *CAH3* or *CAH6* improves growth in *C. reinhardtii*, then our methods can be applied to other algal species, such as *Chlorella vulgaris*, a biotechnology production organism.

Conclusion

Algal biofuel production is a sustainable alternative to fossil fuels and has more potential than ethanol produced from corn, another alternative fuel source. Transformation of these genes into *Chlamydomons reinhardtii* would bring scientists closer to successfully developing a more economic sustainable energy source.

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