Maximizing Photosynthetic Efficiencies and Hydrogen Production in Microalgal Cultures

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Objectives

- Minimize, or truncate, the chlorophyll (Chl) antenna size in green algae to maximize photobiological solar conversion efficiency and hydrogen production.
- Demonstrate that a truncated Chl antenna size would minimize absorption and wasteful dissipation of sunlight by individual cells, resulting in better light utilization efficiency and greater photosynthetic productivity by the green alga culture.

Technical Barriers

This project addresses the following technical barrier from the Hydrogen Production section of the Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year R,D&D Plan:

- I. Light Utilization Efficiency

Approach

- Employ DNA insertional mutagenesis, screening, biochemical and molecular genetic analyses for the isolation of "truncated Chl antenna size" strains in the green alga *Chlamydomonas reinhardtii.*
- Clone and characterize the gene(s) that affect the "Chl antenna size" in *Chlamydomonas reinhardtii.*
- Apply such genes to generate a "truncated Chl antenna size" in this green alga.

Accomplishments

- First-time cloning of *Tla1*, a 'Chl antenna size regulatory gene' (30-year breakthrough).
- DNA, mRNA and protein sequences were deposited in the GenBank (GenBank Accession No. AF534570 and AF534571).
- Manuscript on this gene and its application to this project was published in the peer-reviewed international journal 'Planta'.
- The DOE's Joint Genome Institute validated the sequence information on the Tla1 gene and credited our work regarding the function of this gene.
- Results from this work apply directly to green alga hydrogen production, biomass accumulation, and carbon sequestration efforts.

Future Directions

- Identify additional *Tla*-type genes.
- Functionally characterize these genes (how do they work?).
- Perform genetic crosses to combine different tla-type properties and phenotypes.
- Establish transformation protocols with Tla-type genes to downregulate the Chl antenna size in *Chlamydomonas reinhardtii.*

Introduction

The goal of the research is to generate green algal strains with enhanced photosynthetic productivity and hydrogen production under mass culture conditions. To achieve this goal, it is necessary to optimize the light absorption and utilization properties of the cells (Kok 1953; Myers 1957; Radmer and Kok 1977). A cost-effective way to achieve this goal is via a reduction in the number of chlorophyll (Chl) molecules that function in photosynthesis. Thus, efforts are under way to isolate microalga mutants with a **truncated chlorophyll antenna size**.

The rationale for this R&D is that a truncated light-harvesting Chl antenna size in green algae will prevent individual cells at the surface of the culture from over-absorbing sunlight and wastefully dissipating most of it (Figure 1). A truncated Chl antenna size will permit sunlight to penetrate deeper into the culture, thus enabling many more cells to contribute to useful photosynthesis and hydrogen production (Figure 2). It has been shown that a truncated Chl antenna size will enable up to ~3 times greater solar energy conversion efficiency and photosynthetic productivity than could be achieved with fully pigmented cells (Melis et al. 1999). The research seeks to develop *Chlamydomonas reinhardtii* strains having a permanently truncated Chl antenna size, and to isolate and characterize the genes and proteins that confer this property to the green algae. This information will find direct application in hydrogen production, biomass accumulation and carbon sequestration.

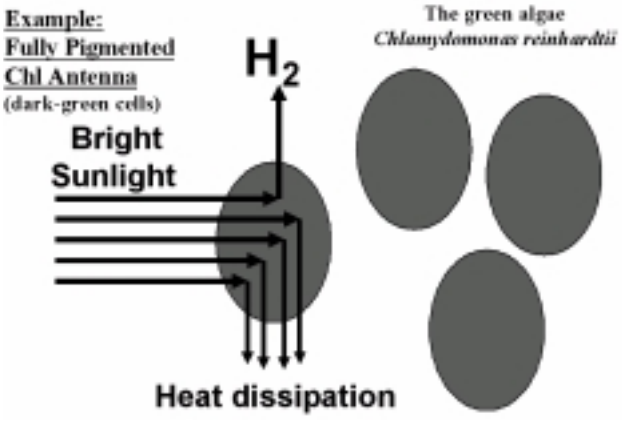

Figure 1. Schematic of the fate of absorbed sunlight in fully pigmented (dark green) algae. Individual cells at the surface of the culture over-absorb incoming sunlight (i.e., they absorb more than can be utilized by photosynthesis), and 'heat dissipate' most of it. Note that a high probability of absorption by the first layer of cells would cause shading, i.e., would prevent cells deeper in the culture from being exposed to sunlight.

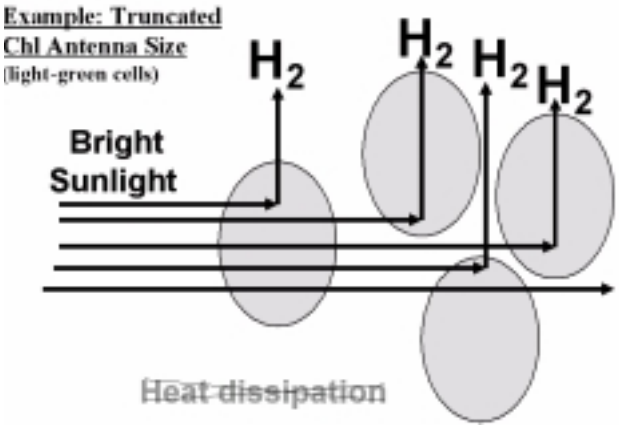

Figure 2. Schematic of sunlight penetration through cells with a truncated chlorophyll antenna size. Individual cells have a diminished probability of absorbing sunlight, thereby permitting deeper penetration of irradiance and hydrogen production by cells deeper in the culture.

Approach

The immediate objective of the research is to identify genes that control the Chl antenna size of photosynthesis, and further, to manipulate such genes so as to confer a truncated Chl antenna size in the model green alga *Chlamydomonas reinhardtii.* Identification of such genes in *Chlamydomonas* will permit a subsequent transfer of this property, i.e., "truncated Chl antenna size", to other microalgae of interest to the DOE Hydrogen, Fuel Cells and Infrastructure Technologies Program.

This objective is currently being approached through DNA insertional mutagenesis/screening and biochemical/molecular/genetic analyses of *Chlamydomonas reinhardtii* cells.

Results

In FY 2003, work described the molecular, genetic and functional properties of tla1, a *Chlamydomonas reinhardtii* DNA insertional transformant having a **t**runcated light-harvesting chlorophyll **a**ntenna size. The plasmid insertion into the nuclear DNA of this transformant interrupted *Tla1*, a newly identified gene, which is apparently responsible for the regulation of the Chl antenna size in green algae (Polle et al. 2003). To the best of our knowledge, this is a first-time cloning and genomic-proteomic characterization of a Chl antenna size regulatory gene in photosynthesis. A summary of the results obtained is given in bullet form, below:

- 6,500 DNA insertional transformants were generated and screened (Polle et al. 2003). Only one mutant with a 'truncated Chl antenna size' could be identified.
- Genetic crosses and mapping of the DNA around the insertion site confirmed that the exogenous plasmid interfered with a novel gene, termed by us as *Tla1*.
- DNA, mRNA and protein sequences of *Tla1* were elucidated and deposited in the GenBank (GenBank Accession No. AF534570 and AF534571).
- Absorbance-difference kinetic spectrophotometry (Dspec) revealed that interference with the *Tla1* gene expression resulted in a truncated photosystem II (PSII) Chl antenna size, down to 50%, and a photosystem I (PSI) Chl antenna size, down to 67% (Table 1).

Table 1. C. *reinhardtii* cellular chlorophyll content and photosystem chlorophyll antenna size in wild type and tla1 mutant as determined by Dspec analysis (n = 5, ±SD).

	wild type	*tla1*	% change	Long-term goal
Chl/cell-mol x10^{-15}	2.4 ±0.5	0.9 ±0.06	38%	
Chl-PSII	222±26	114±36	51%	37
Chl-PSI	240±4	159±12	66%	95

- Sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis revealed lack of specific light-harvesting complex proteins from the antenna of the *tla1* (Polle et al. 2003).
- A light-saturation curve of photosynthesis measurements revealed a greater light intensity for the saturation of photosynthesis in the *tla1* mutant than in the wild type, revealing a minimized ability to absorb light and less wasteful dissipation of excitation energy as heat.
- The *tla1* mutant showed enhanced light utilization efficiency and greater photosynthetic productivity under mass culture conditions (Figure 3).

Conclusions

- The partially truncated chlorophyll antenna size of the *tla1* mutant alleviates the over-absorption of incident sunlight by individual cells and the wasteful dissipation of over-absorbed irradiance.
- A truncated light-harvesting chlorophyll antenna size in the *tla1* mutant diminishes the severe cell shading that occurs in normally pigmented wild type and permits a more uniform illumination of the cells in a mass culture.
- A truncated light-harvesting chlorophyll antenna size in the tla1 mutant leads to better solar conversion efficiencies and greater photosynthetic productivity of the algae under bright sunlight conditions.

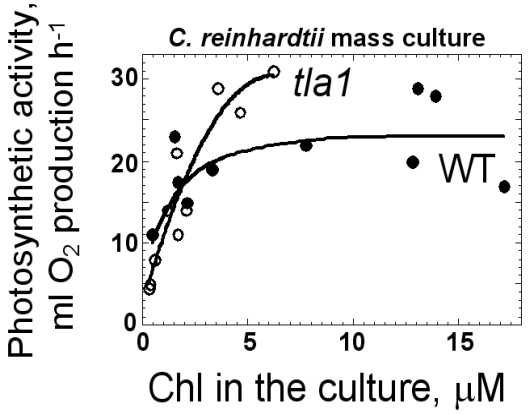

Figure 3. Photosynthetic productivity measurements were conducted with wild type and the *tla1* mutant of *Chlamydomonas reinhardtii* as a function of chlorophyll concentration in a mass culture (Polle et al. 2003). Measurements were conducted in the greenhouse at a solar incident intensity (photosynthetically active radiation) of about 1,500 μmol photons m^{-2} s^{-1}.

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4. Polle JEW, Kanakagiri S and Melis A (2003) tla1, a DNA insertional transformant of the green alga Chlamydomonas reinhardtii with a truncated light-harvesting chlorophyll antenna size. Planta 217: 49-59

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3. Melis A (2002) Green alga hydrogen production: progress, challenges and prospects. Intl. J. Hydrogen Energy 27: 1217-1228

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5. Masuda T, Tanaka A and Melis A (2003) Chlorophyll antenna size adjustments by irradiance in *Dunaliella salina* involve coordinate regulation of chlorophyll *a* oxygenase (*CAO*) and *Lhcb* gene expression. Plant Mol. Biol. 51: 757-771

6. Polle JEW, Kanakagiri S and Melis A (2003) *tla1,* a DNA insertional transformant of the green alga *Chlamydomonas reinhardtii* with a truncated light-harvesting chlorophyll antenna size. Planta 217: 49-59, DOI: 10.1007/s00425-002-0968-1