II.I.2 Maximizing Light Utilization Efficiency 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 H_2 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 mass culture.

Technical Barriers

This project addresses the following technical barriers from the Production section of the Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year Research, Development and Demonstration Plan:

(AG) Light Utilization Efficiency

Technical Targets

The Hydrogen, Fuel Cells and Infrastructure Technologies Multiyear Program Plan technical target for 2005 for this project was to reach a 10% utilization efficiency of absorbed light energy (out of a theoretical maximum of 30% possible) in unicellular green. Progress has currently achieved a green alga utilization efficiency of absorbed light energy of about 25%.

Approach

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

Accomplishment

1. Published manuscript on the utility of the *Tla1* gene in conferring a truncated chlorophyll antenna size and on the mechanism by which it maximizes light utilization efficiency and hydrogen production in microalgal cultures.
2. Isolated and characterized new "truncated chlorophyll antenna size" mutants *tla2* and *tlaR*. Properties of these strains are given in Tables 1 and 2.

TABLE 1. *Chlamydomonas reinhardtii* cellular chlorophyll content, photosystem chlorophyll antenna size and energy utilization efficiency in wild type, *tla1, tla2* and *tlaR* mutant mutant strains, as determined by spectrophotometric kinetic analysis (n = 5, ±SD).

	wild type (WT)	*tla1*	*tla2*	*tlaR*	Long-term goal
Chl/cell mol $x10^{-15}$	2.4 ±0.5	0.9 ±0.06	0.93 ±0.1	0.7 ±0.1	
Chl-PSII	222±26	115±36	80±30	50±30	37
Chl-PSI	240±4	160±12	115±10	105±10	95
Light Utilization Efficiency (Solar to Chemical)	~3%	~10%	~15%	~25%	~30%

TABLE 2. Progress achieved *vs* the DOE targets: utilization efficiency of incident solar light energy, E_0xE_1, %.

Year	2000	2003	2005	2008	2010	2015
Program Targets	3%	10%*			15%	20%
Actual Progress Achieved	3% WT	10% tla1	15% tla2	25% tlaR		

* Target adjusted upward to match ahead-of-schedule progress achieved.

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Introduction

The goal of the research is to generate green algal strains with enhanced photosynthetic productivity and H_2 production under mass culture conditions. To achieve this goal, it is necessary to optimize the light absorption and utilization properties of the cells [1-4]. A cost-effective way to achieve this goal is to reduce the number of Chl molecules that function in the photosystems of photosynthesis. Thus, efforts are under way to isolate microalga mu tants with a truncated chlorophyll antenna size.

The rationale for this research 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 H_2 production (Figure 2). It has been shown that a truncated Chl antenna size will enable about 3-4 times greater solar energy conversion efficiency and photosynthetic productivity than could be achieved with fully pigmented cells [5].

Approach

The focal objective of the research is to identify genes that control the Chl antenna size of photosynthesis and, further, to elucidate how such genes 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 Program. This objective is currently being approached through DNA insertional mutagenesis/screening and biochemical/molecular/genetic analyses of *Chlamydomonas reinhardtii* cells.

Results

The *tla2* mutant plasmid insert site has been cloned and a gene of interest has been tentatively identified as causing the *tla2* mutation. This molecular and genetic analysis is currently in progress. Work further described the isolation and biochemical and physiological characterization of a new mutant of *Chlamydomonas reinhardtii*, termed *tlaR*, having a **t**runcated **l**ight-harvesting chlorophyll **a**ntenna size. Properties of the *tla* "truncated Chl antenna size" strains so far isolated are summarized in Tables 1 and 2, Figure 3. The *tlaR* mutant has the smallest yet Chl antenna size known in green algae. State-of-the-art was summarized in a review article by Mitra and Melis [4].

Future efforts will be directed toward the cloning and characterizing the genes responsible for the ***tla*** phenotype in *tla2* and *tlaR* mutants.

Conclusions

- Significant, ahead-of-schedule progress was achieved in terms of acquiring "truncated Chl antenna size" mutants. *This demonstrates feasibility of the approach chosen and success of the methods employed.*

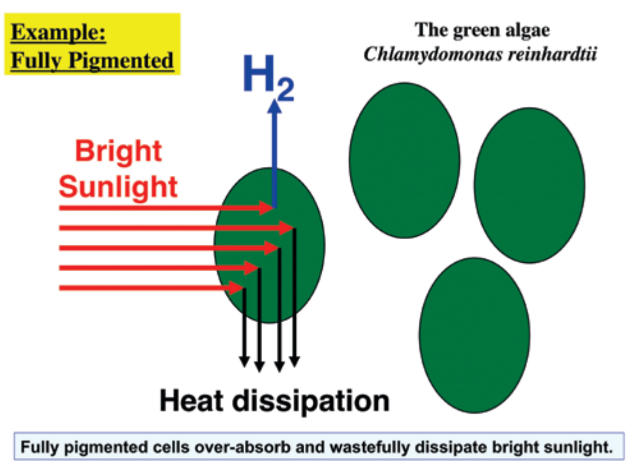

FIGURE 1. Schematic presentation of the fate of absorbed sunlight in fully pigmented (dark green) algae. Individual cells at the surface of the culture over-absorb incoming sunlight (i.e., they absorb more than can be utilized by photosynthesis), and 'heat dissipate' most of it. Note that a high probability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

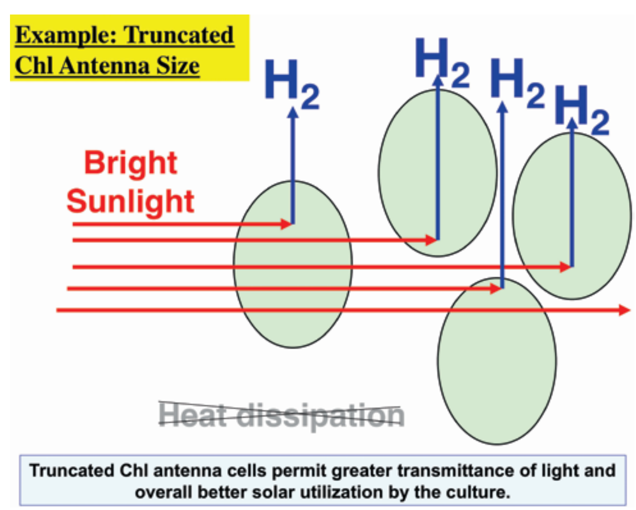

FIGURE 2. Schematic of sunlight penetration through cells with a truncated chlorophyll antenna size. Individual cells have a diminished probability of absorbing sunlight, thereby permitting penetration of irradiance and H_2-production by cells deeper in the culture.

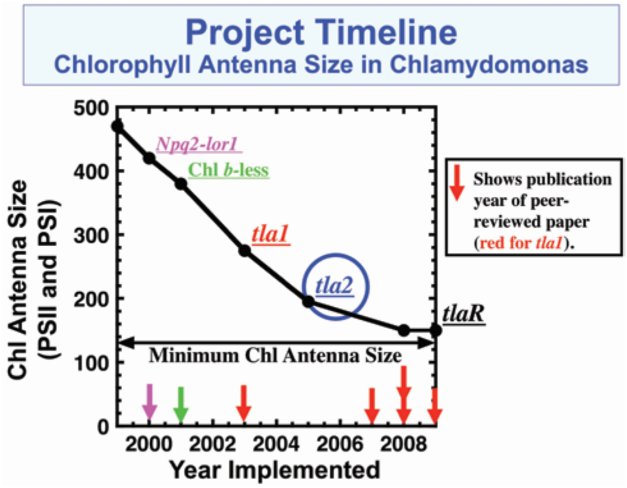

FIGURE 3. Project timeline and publications record on the truncated chlorophyll antenna size project. Arrows show publication year of peer-reviewed paper for each of the truncated Chl antenna size mutants. Note that work with the *tla2* and *tlaR* strains has not yet reached the stage of a peer-reviewed paper.

- A truncated light-harvesting chlorophyll antenna size in the *tla-type* mutants enhanced solar conversion efficiencies and photosynthetic productivity under bright sunlight conditions.
- Insights on the molecular mechanism for the regulation of the Chl antenna size by the *Tla1* gene were obtained (results not shown pending publication of these findings in a peer reviewed journal).

Future Directions

- Advance the biochemical and molecular characterization of the *tla2* and *tlaR* strains.
- Clone the genes that confer the *tla2* and *tlaR* phenotypes.
- Establish transformation (sense and antisense) protocols with *Tla*-type genes to enhance the down-regulation of the Chl antenna size in *Chlamydomonas reinhardtii.*

FY 2009 Publications

1. Berberoglu H, Pilon L, Melis A (2008) Radiation characteristics of *Chlamydomonas reinhardtii* CC125 and its truncated chlorophyll antenna transformants *tla1, tlaX, and tla1-CW*$^{+}$. Intl J Hydrogen Energy 33: 6467-6483.
2. Mitra M, Melis A (2008) Optical properties of microalgae for enhanced biofuels production. Optics Express 16: 21807-21820.

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1. Kok B (1953) Experiments on photosynthesis by *Chlorella* in flashing light. In: Burlew JS (ed), Algal culture: from laboratory to pilot plant. Carnegie Institution of Washington, Washington DC, pp 63-75.
2. Myers J (1957) Algal culture. In: Kirk RE, Othmer DE (eds), Encyclopedia of chemical technology. Interscience, New York, NY, pp 649-668.
3. Radmer R and Kok B (1977) Photosynthesis: Limited yields, unlimited dreams. Bioscience 29: 599-605.
4. Mitra M, Melis A (2008) Optical properties of microalgae for enhanced biofuels production. Optics Express 16: 21807-21820.
5. Melis A, Neidhardt J and Benemann JR (1999) *Dunaliella salina* (Chlorophyta) with small chlorophyll antenna sizes exhibit higher photosynthetic productivities and photon use efficiencies than normally pigmented cells. J. appl. Phycol. 10: 515-525.