

II.D Photobiological Production

II.D.1 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 H₂ 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 Hydrogen Production section of the Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year Research, Development and Demonstration Plan:

- I. Light Utilization Efficiency

Approach

- Employ DNA insertional mutagenesis, screening, and 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” in *Chlamydomonas reinhardtii*.
- Apply such genes to generate a “truncated Chl antenna size” in this green alga.

2004 Accomplishments

- Isolated a new “truncated chlorophyll antenna size” mutant (*tlaX*) with 195 Chl molecules in its photosynthetic apparatus (42% of the wild type). The specific photosystem Chl antenna size measurements of the *tlaX* strain were PSII=80 and PSI=115 Chl molecules.
- A photon use efficiency of ~30% was measured for the *tlaX* photosynthesis. This translated into ~15% utilization efficiency of absorbed light energy. Such achievement surpassed this year’s 7.5% energy utilization efficiency target.

Future Directions

- Advance the biochemical and molecular characterization of the *tlaX* strain. Publish *tla1*- and *tlaX*-related analyses.

- Functionally characterize the corresponding *Tla1* and *TlaX* genes (how do they work?).
- Establish transformation (sense and antisense) protocols with *Tla*-type genes to enhance the down-regulation of the Chl antenna size in *Chlamydomonas reinhardtii*.
- Perform comparative green-alga light utilization efficiency and photosynthetic (H_2) productivity measurements under mass culture conditions in wild type and *tla*-type mutants.
- Perform genetic crosses to combine different *tla*-type properties.

Introduction

Microalgae growing under direct sunlight have low photon use efficiencies (<0.05) because the rate of photon absorption by the chlorophyll (Chl) antennae far exceeds the maximal rate of photosynthesis (Kok 1953; Myers 1957; Radmer and Kok 1977). The absorbed excess photons are dissipated as fluorescence or heat. The goal of this research is to alleviate such low light utilization efficiency in photosynthesis and hydrogen production by reducing the number of Chl molecules in the photosystems, thereby limiting the absorption of sunlight by individual cells and thus enabling enhanced photosynthetic productivity and H_2 production under mass culture conditions.

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 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 (Melis et al. 1999).

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 Program. This

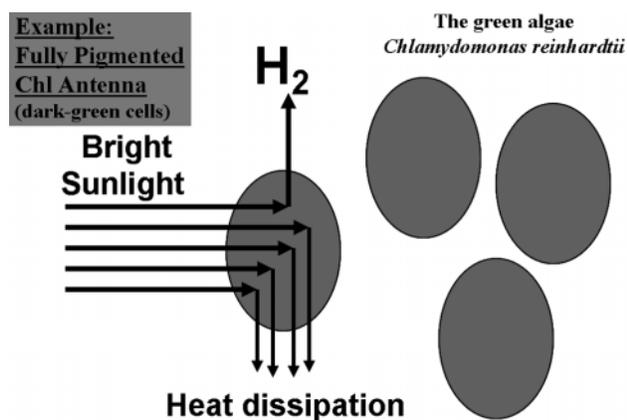


Figure 1. Schematic presentation of the fate of absorbed sunlight in fully pigmented (dark green) algae. Individual cells at the surface of the culture over-absorb incoming sunlight (i.e., they absorb more than can be utilized by photosynthesis) and ‘heat dissipate’ most of it. Note that a high probability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

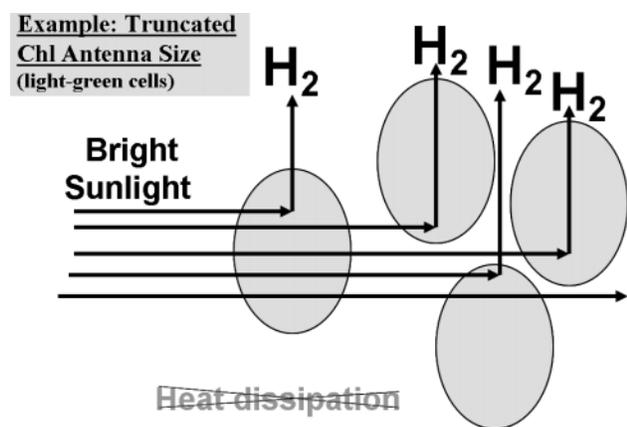


Figure 2. Schematic of sunlight penetration through cells with a truncated chlorophyll antenna size. Individual cells have a diminished probability of absorbing sunlight, thereby permitting penetration of irradiance and H_2 production by cells deeper in the culture.

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

Results

In FY 2004, work described the isolation and biochemical and physiological characterization of a new mutant of *Chlamydomonas reinhardtii*, termed *tlaX*, having a truncated light-harvesting chlorophyll antenna size. This mutant has the smallest yet Chl antenna size known in green algae. This was achieved upon generating and screening an additional 4,500 transformants, following the protocol of Polle et al. (2003). Properties of the *tlaX* putative “truncated Chl antenna size” strain are summarized in Table 1.

Figure 3 shows the timeline of this research and the position of the *tlaX* truncated Chl antenna mutant in relation to the wild type and to some earlier isolates having a smaller Chl antenna size. Photosynthetic productivity measurements were conducted with the wild type, *tla1* and *tlaX* strains as a function of chlorophyll concentration in a mass culture according to Polle et al. (2003). Strains were grown under ambient conditions in 2.5-liter bottles

Table 1. *Chlamydomonas reinhardtii* Cellular Chlorophyll Content and Photosystem Chlorophyll Antenna Size in Wild Type and *tlaX* Mutant as Determined by Spectrophotometric Kinetic Analysis (n=5, +/-SD).

	Wild Type	<i>tlaX</i>	Long-term goal
Chl/Cell mol x 10 ⁻¹⁵	2.4 +/- 0.5	0.93 +/- 0.1	
Chl-PSII	222 +/- 26	80 +/- 36	37
Chl-PSI	240 +/- 4	115 +/- 12	95
Energy Utilization Efficiency	~5%	~15%	~30%

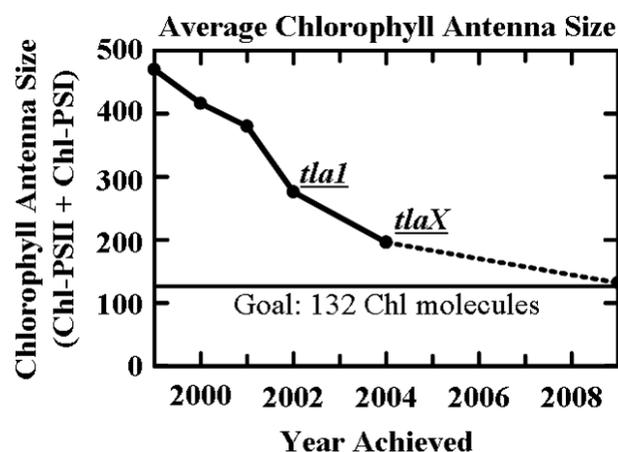


Figure 3. Graphic presentation of the progression of this R&D effort. The Chl antenna size, estimated as the sum of the functional Chl antenna size of photosystem-II (PSII) and photosystem-I (PSI) in the photosynthetic apparatus of *Chlamydomonas reinhardtii*, is plotted as a function of time when the respective strain was identified (FY 2002 for *tla1* and FY 2004 for *tlaX*). It is projected (dashed line) that the smallest possible chlorophyll antenna size of 132 Chl molecules will be attained in FY 2009.

having an internal diameter of about 15 cm (~6 inches). Measurements were conducted for several hours at an incident intensity (photosynthetically active radiation) of about 1,800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Figure 4 shows the comparative productivity in such mass cultures of the wild type, *tla1* and *tlaX* mutants. It is seen that the *tlaX* strain outperformed both the *tla1* mutant and the wild type.

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.
- A truncated light-harvesting chlorophyll antenna size in the *tla*-type mutants leads to enhanced solar conversion efficiencies and greater photosynthetic productivity of the algae under bright sunlight conditions.
- Results from this work apply directly to green alga H₂ production, biomass accumulation, and carbon sequestration efforts.

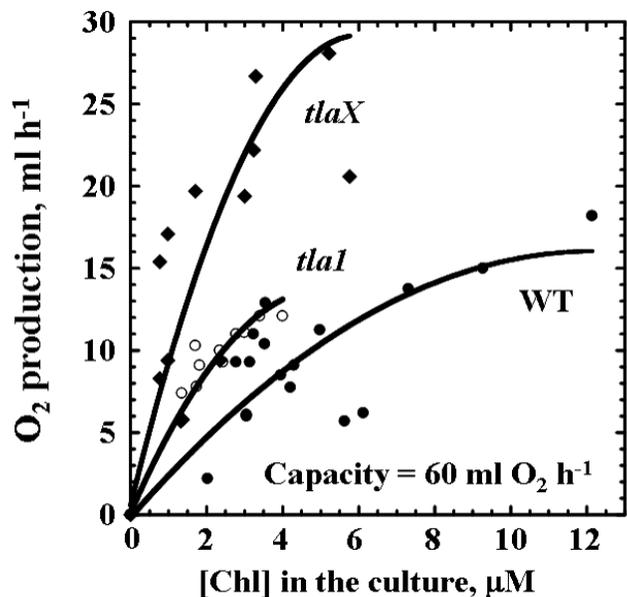


Figure 4. Photosynthetic productivity of wild type and *tla*-type strains. Measurements were conducted with wild type, *tla1* and *tlaX* mutants of *Chlamydomonas reinhardtii* as a function of chlorophyll concentration in a mass culture (Polle et al. 2003). Strains were grown in 2.5-liter bottles at variable cell densities and Chl concentrations. Measurements of photosynthetic productivity were performed based on the volume of oxygen produced. Productivity of the cultures was measured under direct sunlight ($1,800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$).

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FY 2004 Publications

- 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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Special Recognitions & Awards

- Received the DOE HFCIT Program's 2004 Research Achievement Award