II.F.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 Hydrogen 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 algae. Progress through this project has currently achieved a green alga utilization efficiency of absorbed light energy of about 25% (tentative and unpublished; please see Table 2).

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.

Accomplishments

1. Completed the molecular and genetic analysis of the *tla1* mutant.
2. Completed the functional analysis of the *Tla1* gene.
3. 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.
4. Disclosed the DNA sequence of *Rdp1*, a gene that overlaps *Tla1*. and provided a molecular biological and genetic analysis of the *Rdp1* vis-à-vis the *Tla1* gene.
5. Isolated and characterized new "truncated chlorophyll antenna size" mutants *tlaX* 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, tlaX* and *tlaR* mutant mutant strains, as determined by spectrophotometric kinetic analysis (n = 5, ±SD).

	wild type	*tla1*	*tlaX*	*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	~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	2013	2018
Program Targets	3%	10%*			15%	20%
Actual Progress Achieved	3% WT	10% *tla1*	15% *tlaX*	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 hydrogen production under mass culture conditions. To achieve this goal, it is necessary to optimize the light absorption and utilization properties of the cells [1,3,5]. 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 mutants with a **truncated chlorophyll antenna** size.

The rationale for this research and development 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 about 3-4 times greater solar energy conversion efficiency and photosynthetic productivity than could be achieved with fully pigmented cells [2].

Approach

The immediate 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, 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

The *Chlamydomonas reinhardtii* mutant *tla1* (truncated light-harvesting chlorophyll antenna size) was earlier generated upon DNA insertional mutagenesis and shown to specifically possess a smaller than wild type chlorophyll antenna size in both photosystems. This

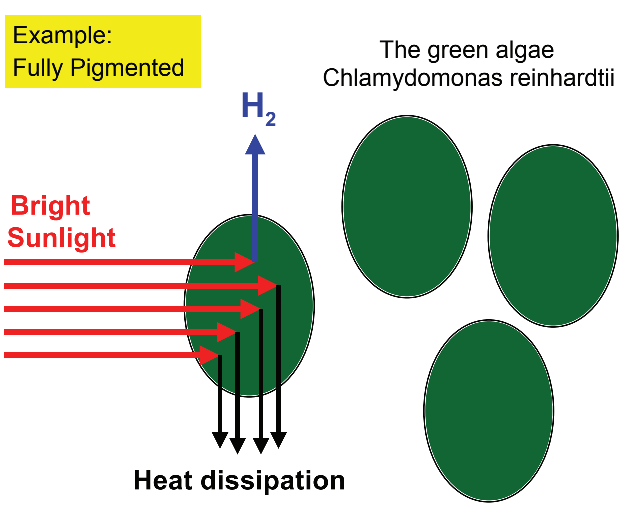

FIGURE 1. Schematic presentation of the fate of absorbed sunlight in fully pigmented (dark green) algae. Individual cells at the surface of the culture over-absorb incoming sunlight (i.e., they absorb more than can be utilized by photosynthesis), and 'heat dissipate' most of it. Note that a high probability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

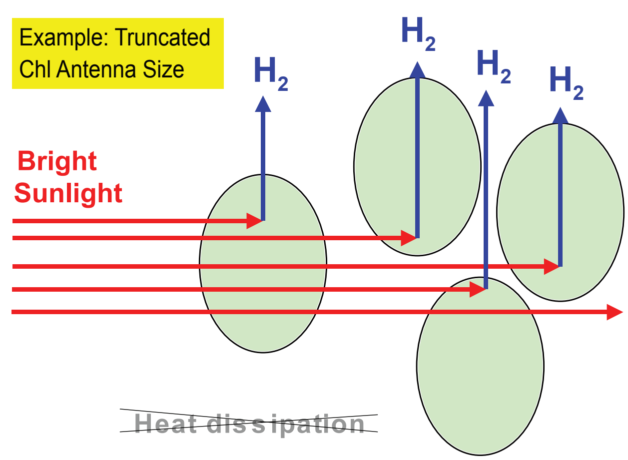

FIGURE 2. Schematic of sunlight penetration through cells with a truncated chlorophyll antenna size. Individual cells have a diminished probability of absorbing sunlight, thereby permitting penetration of irradiance and H_2-production by cells deeper in the culture.

strain exhibited a 10% utilization efficiency of incident solar light energy, i.e., substantially greater than the 3% utilization efficiency of the wild type. Molecular and genetic analysis revealed that the exogenous plasmid DNA was inserted at the end of the 5' untranslated region (UTR) and just prior to the Adenine, Thymine, Guanine start codon of a hitherto unknown gene (termed *Tla1)*, which encodes a protein of 213 amino acids. The *Tla1* gene in the mutant is transcribed with a new 5' UTR sequence, derived from the 3' end of the transforming plasmid. This replacement of the 5' UTR resulted in enhanced transcription of the *tla1* gene in the mutant but inhibition in the translation of the respective *tla1* message ribonucleic acid. These results provided evidence that down-regulation of the Tla1 expression is necessary and sufficient to truncate the chlorophyll antenna size and to improve solar utilization efficiencies in a green algal mass culture. Specific applications of the *Tla1* gene in H_2-production were discussed.

Work further described the isolation and biochemical and physiological characterization of a new mutant of *Chlamydomonas reinhardtii*, termed *tlaX,* having a **t**runcated **l**ight-harvesting chlorophyll **a**ntenna size. Properties of the *tlaX* putative "truncated Chl antenna size" strain are summarized in Table 1. More recent work resulted in the isolation and partial characterization of a new mutant of *Chlamydomonas reinhardtii*, termed *tlaR,* also having a **t**runcated **l**ight-harvesting chlorophyll **a**ntenna size. The *tlaR* mutant has the smallest yet Chl antenna size known in green algae (Figure 3). These advances were achieved upon generating and screening an additional 4,500

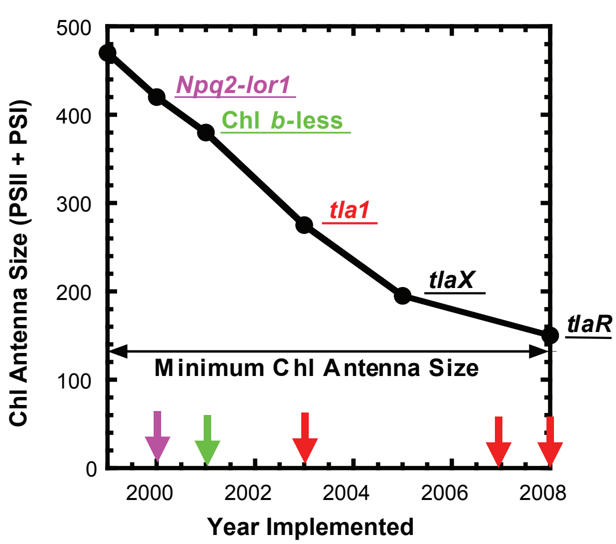

FIGURE 3. Project timeline and publications record on the truncated chlorophyll antenna size project. Arrows show publication year of peer-reviewed paper for each of the truncated Chl antenna size mutants. Note that work with the *tlaX* and *tlaR* strains has not yet reached the stage of a peer-reviewed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

transformants, following the protocol of Polle et al. [4]. Properties of the *tlaR* putative "truncated Chl antenna size" strain are also summarized in Table 1.

Future efforts will be directed toward the cloning of the genes responsible for the truncated light-harvesting chlorophyll antenna size phenotype in *tlaX* 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***.**
- 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.
- 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 *tlaX* and *tlaR* strains.
- Clone the genes that confer the *tlaX* 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.*
- Perform comparative green-alga light utilization efficiency and photosynthetic productivity measurements under mass culture conditions in wild type and *tla*-type mutants.

References

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 D.C., pp 63-75.

2. 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: 515-52.

3. Myers J (1957) Algal culture. In: Kirk RE, Othmer DE (eds), Encyclopedia of chemical technology. Interscience, New York, NY, pp 649-668.

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.

5. Radmer R and Kok B (1977) Photosynthesis: Limited yields, unlimited dreams. Bioscience 29: 599-605.

FY 2008 Publications/Presentations

Peer reviewed publications (1); Technical DNA disclosures (2,3); Abstracts published (4-11); Invited seminars and lectures (12-17)

1. Tetali SD, Mitra M, Melis A (2007) Development of the light-harvesting chlorophyll antenna in the green alga *Chlamydomonas reinhardtii* is regulated by the novel *Tla1* gene. Planta 225: 813-829.

2. Mitra M, Melis A (2008) *Chlamydomonas reinhardtii* RING-like domain protein 1 (RDP1) gene, complete cds. gi|189354188|gb|EU717142.1|[189354188]. GenBank Accession Number EU717142.

3. Mitra M, Melis A (2008) *Chlamydomonas reinhardtii* RING-like domain protein 1 (RDP1) mRNA, complete cds. gi|189354190|gb|EU717143.1|[189354190]..GenBank Accession Number EU717143.

4. Melis A (2007) Photosynthetic hydrogen production: genes, proteins and effects. Photosynth Res 91 (Nos 2-3), p. 136 (PS1.2 - Bioenergy).

5. Mitra M and Melis A (2007) Tla1, a novel protein functions in the regulation of the chlorophyll antenna size in *Chlamydomonas reinhardtii*. Photosynth Res 91 (Nos 2-3), p. 253 (PS15.16 - Regulation of Light-harvesting).

6. Melis A (2007) Genetic engineering for microalgal H_2 production. Hydrogenase and Hydrogen Production 2007: The 8th International Hydrogenase Conference. Abstract L.38. Page 48.

7. Melis A (2007) Optical properties of microalgae for enhanced biofuels production. Frontiers in Optics 2007 – Optical Society of America 91st Annual Meeting. Page 66 SMC4.

8. Melis A (2007) Material issues in photobiological hydrogen production. Book of Abstracts of the International Symposium on Material Issues in a Hydrogen Economy. Richmond, VA. Pp. O-21.

9. Kirst H, Melis A (2008) Characterization of DNA insertional mutagenesis transformants of Chlamydomonas reinhardtii with a truncated light-harvesting chlorophyll antenna size. 17th Western Photosynthesis Conference, Asilomar Conference Grounds, Pacific Grove, CA, Abstracts page 14.

10. Mitra M, Melis A (2008) Optical properties of microalgae for enhanced photosynthetic productivity. Abstract TAM-5-4 of the 34th Meeting of the American Society for Photobiology. Burlingame, CA June 20-25, 2008. pp. 36-37.

11. Kirst H, Melis A (2008) Optical properties of microalgae for enhanced photosynthetic productivity. Abstract TAM-5-5 of the 34th Meeting of the American Society for Photobiology. Burlingame, CA June 20-25, 2008. pp. 37.

12. 14th International Congress on Photosynthesis, Glasgow, Scotland; Symposium on Bioenergy and Photosynthesis. Title of Lecture: Photosynthetic Hydrogen Production: Genes, Proteins and Effects. Monday 23-July-2007.

13. The 8th International Hydrogenase Conference. August 5-10, 2007, Breckenridge, Colorado. Title of Lecture: Genetic engineering for microalgal H_2 production. Thursday 09-Aug-2007.

14. 91st Annual Meeting of the Optical Society of America. September 16-20, 2007. San Jose, California. Title of Lecture: Optical properties of microalgae for enhanced biofuels production. Monday 17-Sep-2007.

15. International Symposium on Material Issues in a Hydrogen Economy. November 12-15, 2007. Richmond, Virginia. Title of Lecture: Material issues in photobiological hydrogen production. Tuesday 13-Nov-2007.

16. University of Nebraska, Lincoln, Redox Biology Center, Biology and Chemistry Seminar Series. Title of Seminar: Photosynthetic Biofuels: Generating Hydrogen and Hydrocarbons. Tuesday 08-Apr-2008.

17. XLVII Congress of the Italian Society for Plant Biology. Pisa, Italy. Title of Lecture: Transgenic microalgae as a source of photosynthetic biofuels. Tuesday 01-Jul-2008.