II.G.4 Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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Fiscal Year (FY) 2012 Objectives

- Publish the work on the *TLA2* gene and show its *modus operandi* as to how it confers a truncated Chl antenna size in *Chlamydomonas reinhardtii*.
- Provide physiological and genetic characterization of the *tla3* mutant, including mapping of the plasmid insert site and cloning of the gene affected in the *tla3* mutation.

Technical Barriers

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

(AG) Light Utilization Efficiency

Technical Targets

The Fuel Cell Technologies Program Multi-Year Plan technical target for this project was to reach a truncated Chl antenna size of about 150 Chl molecules in unicellular green algae by 2015. Progress was achieved ahead of schedule enabling us to reach this goal by 2012.

Approach

- Perform *Chlamydomonas reinhardtii* genomic deoxyribonucleic acid (DNA) mapping at the site of plasmid DNA insertions, followed by identification of open reading frames (ORFs = putative genes) that have been affected by the plasmid insertion.
- Perform complementation-type transformations of the *tla* mutant with each of the affected ORFs to rescue the mutation and, thus, identify the gene that confers a TLA property.
- Clone and characterize the gene(s) that affect the "Chl antenna size" property in *Chlamydomonas reinhardtii*.

FY 2012 Accomplishments (TLA1-MOV34/MPN effort)

- Bioinformatic analysis tentatively identified the truncated light-harvesting chlorophyll antenna-1 (TLA1) protein as a variant of the MOV34/MPN containing proteins.

FY 2012 Accomplishments (TLA2-ΔFTSY effort)

- Physiological characterization of the tla2 mutant was completed.
- Genetic analysis and multiple crosses of the tla2 mutant were completed.
- Mapping of the plasmid insert site in the tla2 mutant was completed.
- Of the five genes adversely affected by the plasmid insertional mutagenesis, gene Cre05.g241450 encoding the CpFTSY protein complemented the mutation.
- The unique functional role of the CpFTSY protein in algae was elucidated.
- Patent application on the function of the TLA2-CpFTSY gene filed.

FY 2012 Accomplishments (TLA3 effort)

- Physiological and genetic characterization of the tla3 mutant was completed.
- Mapping of the plasmid insert site in the tla3 mutant was completed.
- The gene affected in the tla3 mutation is known.
- A Western blot analysis remains to be done for project completion.

TABLE 1. *Chlamydomonas reinhardtii* cellular chlorophyll content, photosystem chlorophyll antenna size and energy utilization efficiency in wild type, *tla1, tla2* and *tla3* mutant strains, as determined by spectrophotometric kinetic analysis (n = 5, ±SD).

	wild type	*tla1*	*tla2*	*tla3*	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%

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.

The rationale for this work 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 Fuel Cell Technologies Program. This objective has been successfully 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 the nuclear-encoded and chloroplast-localized FTSY gene (TLA2-CpFTSY) was identified as causing the *tla2* mutation. The TLA2-CpFTSY gene deletion, causing the *tla2* phenotype, was cloned by mapping the insertion site and upon successful complementation with the *C. reinhardtii* TLA2-CpFTSY gene, whose occurrence and function in green microalgae has not hitherto been investigated. Functional analysis showed that the nuclear encoded and chloroplast-localized CrCpFTSY protein specifically operates in the assembly of the peripheral components of the Chl *a-b* light-harvesting antenna. Figure 3 shows TLA2-CpFtsY

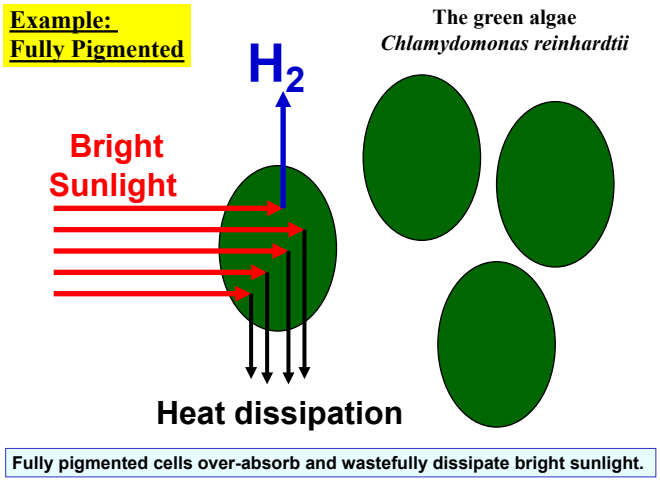

FIGURE 1. Schematic presentation of the fate of absorbed sunlight in fully pigmented (dark green) algae. Individual cells at the surface of the culture over-absorb incoming sunlight (i.e., they absorb more than can be utilized by photosynthesis), and 'heat dissipate' most of it. Note that a high probability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

TABLE 2. Progress achieved vs the DOE targets. Chlorophyll antenna size in wild type and mutants (minimum possible = 130 Chl molecules).

Year	2000	2003	2005	2007	2008	2010	2011	2012	2015
Targets (Chl Antenna Size)	600 (wild type)		300			200			150
TLA strain identified	600 (wild type)	300 *tla1*	195 *tla2*		150 *tla3*				
Gene cloning and functional elucidation				TLA1-Mov34-MPN			TLA2-CpFTSY	TLA3	

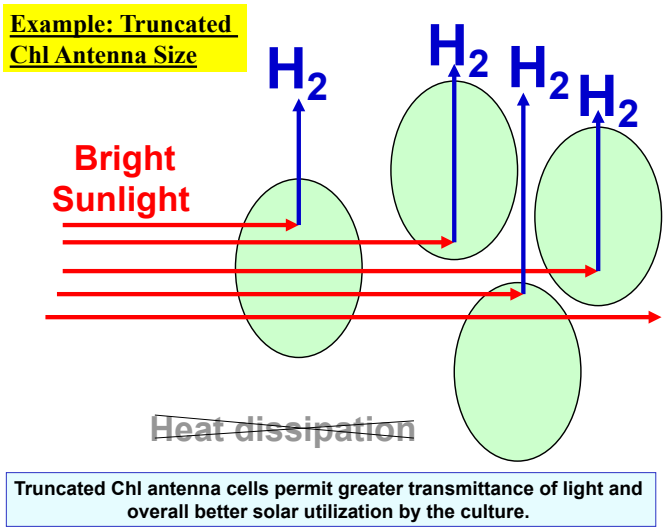

FIGURE 2. Schematic of sunlight penetration through cells with a truncated chlorophyll antenna size. Individual cells have a diminished probability of absorbing sunlight, thereby permitting penetration of irradiance and H2-production by cells deeper in the culture.

protein amino acid sequence and polypeptide structure revealing domains that are critical for its function [6].

In higher plants, a *cpftsy* null mutation inhibits assembly of both the light-harvesting complex and photosystem complexes, thus resulting in a seedling-lethal phenotype. The work shows that *cpftsy* deletion in green algae, but not in higher plants, can be employed to generate tla mutants. The latter exhibit improved solar energy conversion efficiency and photosynthetic productivity under mass culture and bright sunlight conditions. This molecular and genetic analysis has been completed and results have been published.

Work further described the isolation and biochemical and physiological characterization of a new mutant of *Chlamydomonas reinhardtii*, termed *tla3*. Properties of the *tla* "truncated Chl antenna size" strains so far isolated are summarized in Tables 1 and 2, and Figure 3**.** The *tla3* mutant has the smallest yet Chl antenna size known in green algae.

Future efforts will be directed toward the cloning and characterization of gene(s) responsible for the ***tla*** phenotype in the *tla3* mutant, as well as ***tla***-type cyanobacteria.

Conclusions

- Significant progress was achieved in terms of characterization of "truncated Chl antenna size" mutants, cloning of the respective *TLA* genes, and elucidation of the properties of the proteins encoded by these genes.
- Results and analyses on the molecular mechanism for the regulation of the Chl antenna size by the *TLA1* gene [7] and by the *TLA2* gene [6] were published.

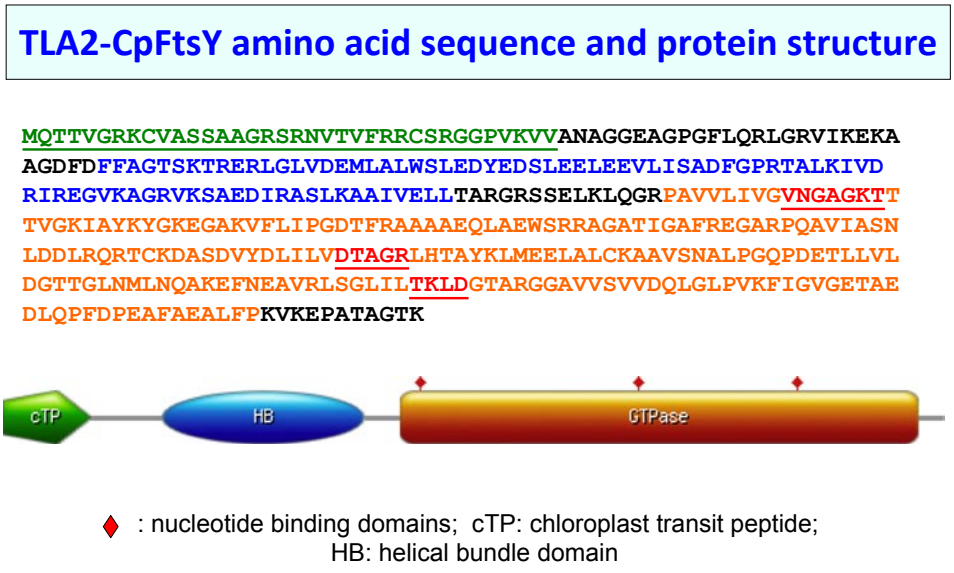

FIGURE 3. Top, Amino acid sequence of the C. reinhardtii chloroplast-localized FTSY protein. Domains of the CrCpFTSY protein are defined as follows: amino acids 1 to 36, transit peptide (green font). Amino acids 66 to 147, helical bundle domain (Pfam), SRP54-type protein (blue font). Amino acids 162 to 370, GTPase domain (Pfam), SRP54-type protein (orange font). Amino acids 164 to 183, P-loop nucleotide binding motif. Amino acids 170 to 176, 258 to 262, and 322 to 325, homologous nucleotide binding (red underlined). Bottom, Domain presentation of the CrCpFTSY protein. CpTP, Chloroplast transit peptide; HB, helical bundle domain; GTPase, GTPase domain. (From [6])

- Completion of the work on the *TLA3* gene is nearly at hand.

Future Directions

- Complete the Western blot analysis for the *tla3* mutant and proceed to peer-reviewed publication of the results.
- Demonstrate feasibility of the TLA concept in cyanobacteria. (Currently in progress.)
- Advance the exploration of the "*extended photosynthetically active radiation*" (ePAR) concept. (Proprietary design not disclosed.)

FY 2012 Publications/Presentations

Peer Reviewed Publications

1. Blankenship RE, Tiede DM, Barber J, Brudvig GW, Fleming G, Ghirardi ML, Gunner MR, Junge W, Kramer DM, Melis A, Moore TA, Moser CC, Nocera DG, Nozik AJ, Ort DR, Parson WW, Prince RC, Sayre RT (2011) Comparing photosynthetic and photovoltaic efficiencies and recognizing the potential for improvement. Science 332:805-809 .

2. Mitra M, Ng S, Melis A (2012) The *TLA1* protein family members contain a variant of the plain MOV34/MPN domain. Amer J Biochem Mol Biol. 2(1): 1-18.

3. Melis A (2012) Photosynthesis-to-Fuels: From sunlight to hydrogen, isoprene, and botryococcene production. Energy Environ. Sci. 5(2): 5531-5539.

4. Kirst H, Garcia-Cerdan JG, Zurbriggen A, Melis A (2012) Assembly of the light-harvesting chlorophyll antenna in the green alga *Chlamydomonas reinhardtii* requires expression of the *TLA2-CpFTSY* gene. Plant Physiol 158: 930–945.

5. Mitra M, Dewez D, García-Cerdán JG, Melis A (2012) Polyclonal antibodies against the TLA1 protein also recognize with high specificity the D2 reaction center protein of PSII in the green alga *Chlamydomonas reinhardtii*. Photosynth Res 112:39-47.

Patent Application Filed

1. Melis A and Kirst H (2012) Suppression of *TLA2-CpFTSY* gene expression for improved solar energy conversion efficiency and photosynthetic productivity in algae.

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. 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, 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, 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.

6. Kirst H, Garcia-Cerdan JG, Zurbriggen A, Melis A (2012) Assembly of the light-harvesting chlorophyll antenna in the green alga *Chlamydomonas reinhardtii* requires expression of the *TLA2-CpFTSY* gene. Plant Physiol 158: 930–9451.

7. Mitra M, Ng S, Melis A (2012) The *TLA1* protein family members contain a variant of the plain MOV34/MPN domain. Amer J Biochem Mol Biol. 2(1): 1-18.