II.D.1 Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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Overall Objectives

- Minimize, or truncate, the chlorophyll antenna size in green algae, and the phycobilisome antenna size in cyanobacteria to maximize photobiological solar energy conversion efficiency and H_2-production.
- Demonstrate that a truncated light-harvesting antenna (TLA) minimizes absorption and wasteful dissipation of bright sunlight by individual cells, resulting in better light utilization efficiency and greater photosynthetic productivity in high-density mass cultures.

Fiscal Year (FY) 2013 Objectives

- Publish the work on the *TLA3* 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.
- Apply the TLA concept in cyanobacteria.

Technical Barriers

The project addresses the following technical barriers from the Photolytic Hydrogen Production from Water (green algae or cyanobacteria) section of the Fuel Cell Technologies Office Multi-Year Research, Development, and Demonstration Plan:

(AN) Light Utilization Efficiency

Technical Targets

The Fuel Cell Technologies Office Multi-Year Research, Development, and Demonstration Plan technical target for this project was to reach a truncated Chl antenna size conferring a 20% light utilization efficiency in unicellular green algae by 2015. Progress was achieved ahead of schedule enabling us to reach this goal by 2012.

FY 2013 Accomplishments (TLA3-ΔCpSRP43 effort)

- Physiological characterization of the *tla3* mutant was completed.
- Genetic analysis and multiple crosses of the *tla3* mutant were completed.
- Mapping of the plasmid insert site in the *tla3* mutant was completed.
- The unique functional role of the CpSRP43 protein in microalgae was elucidated.
- A peer-reviewed paper was published with the following full citation: Kirst H, Garcia-Cerdan JG, Zurbriggen A, Ruehle T, Melis A (2012) Truncated photosystem chlorophyll antenna size in the green microalga *Chlamydomonas reinhardtii* upon deletion of the *TLA3-CpSRP43* gene. Plant Physiol. 160(4):2251-2260.

FY 2013 Accomplishments (TLA effort in cyanobacteria)

- Functional absorption cross section measurements for PSII in wild type and *Δcpc* mutants (10:1 WT/*Δcpc* ratio).
- Functional absorption cross section measurements for PSI in wild type and *Δcpc* mutants (unchanged 1:1 WT/*Δcpc* ratio).
- Completed comparative Light-Saturation Curves of photosynthesis in wild type and *Δcpc* mutants.
- SDS-PAGE and Western blot analysis of protein profiles in wild type and *Δcpc* mutants (verification of the absence of the phycocyanin-binding proteins).
- Growth curves of wild type and *Δcpc* mutants (*Δcpc* growth lags behind the WT at low-light intensities,

whereas growth curves of both are the same at medium-light intensities).

Table 1 summarizes project progress to date.

TABLE 1. Microalgae Milestones and Progress
Sunlight Utilization Efficiency, % of Incident Solar Energy (maximum possible = 30%)

	2000	2003	2005	2007	2008	2010	2011	2012	2015
Targets (Light Utilization efficiency)	3%	10%				15%			20%
Tla strain with the highest efficiency identified	3% (WT)	10% TLA1	15% TLA2		25% TLA3				
Gene cloning from the TLA strains				TLA1: Mov34 MPN			TLA2: FTSY	TLA3: SRP43	

INTRODUCTION

The goal of the research is to generate green algal and cyanobacterial 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 in green microalgae, or phycobilins in cyanobacteria, that function in the apparatus of photosynthesis.

The rationale for this work is that a truncated light-harvesting antenna size in green algae or cyanobacteria will prevent individual cells at the surface of a high-density culture from over-absorbing sunlight and wastefully dissipating most of it (Figure 1). A truncated antenna size will permit sunlight to penetrate deeper into the culture, 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 green microalgal cells [5].

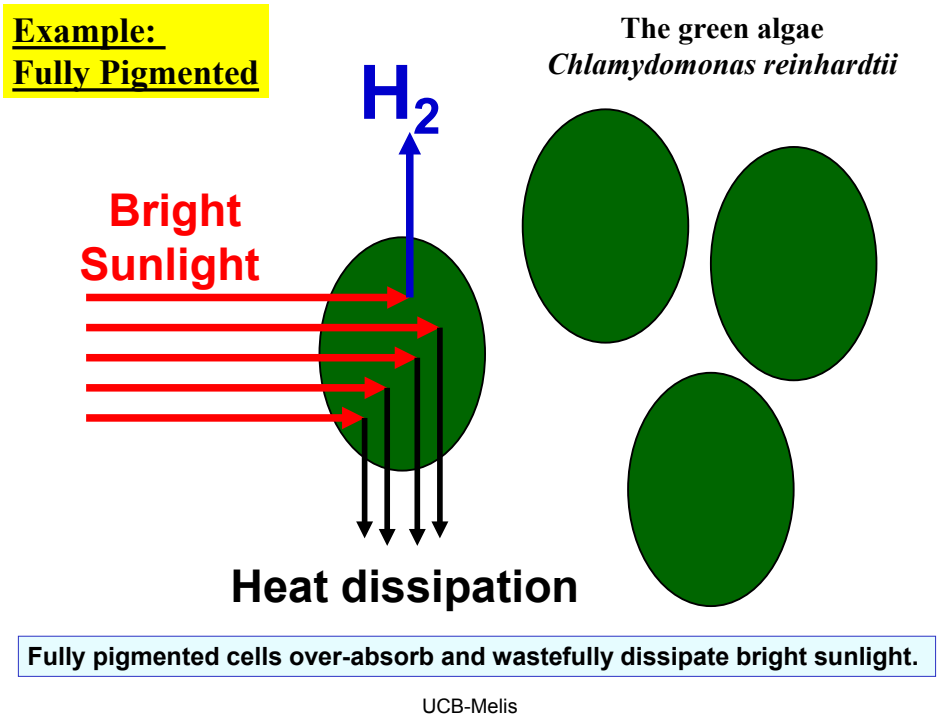

FIGURE 1. Schematic presentation of the fate of absorbed sunlight in fully pigmented (dark green) algae. Individual cells at the surface of the culture over-absorb incoming sunlight (i.e., they absorb more than can be utilized by photosynthesis), and 'heat dissipate' most of it. Note that a high probability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

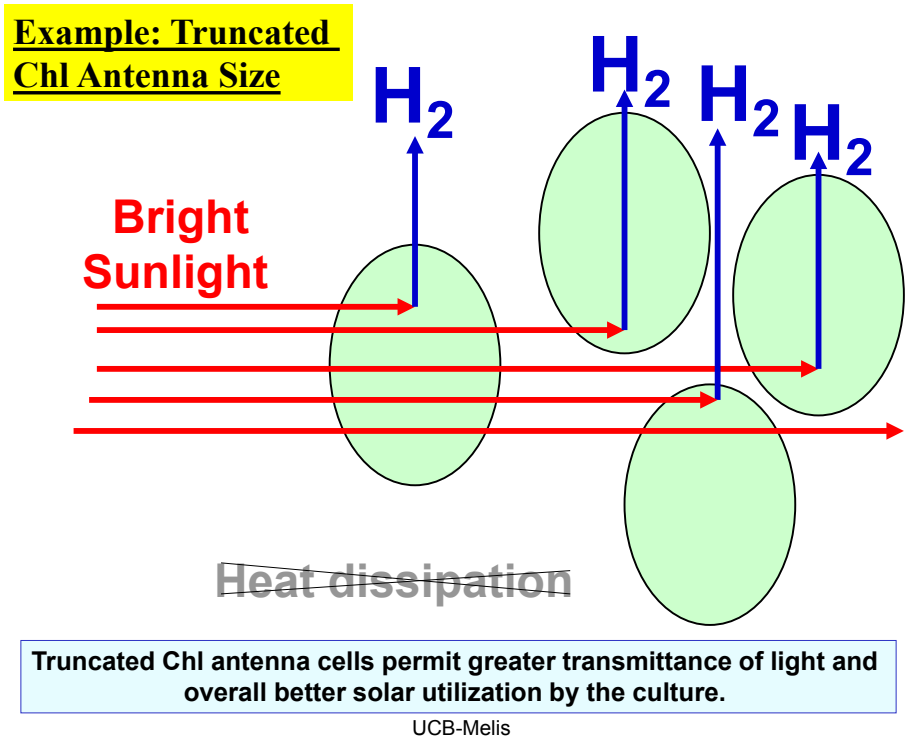

FIGURE 2. Schematic of sunlight penetration through cells with a truncated chlorophyll antenna size. Individual cells have a diminished probability of absorbing sunlight, thereby permitting penetration of irradiance and H_2 production by cells deeper in the culture.

APPROACH

The focal objective of the research is to identify genes that control the antenna size of photosynthesis and, further, to elucidate how such genes confer a truncated antenna size in the model green alga *Chlamydomonas reinhardtii* and model cyanobacterium *Synechocystis* PCC 6803. Identification of such genes will permit application of this TLA property, i.e., "truncated light-harvesting antenna size," to other microalgae and cyanobacteria of interest to the DOE Fuel Cell Technologies Office. This objective has been successfully approached through deoxyribonucleic acid (DNA) insertional mutagenesis/screening (green microalgae) or double homologous recombination and gene deletion (cyanobacteria) and biochemical/molecular/genetic analyses of the resultant TLA *Chlamydomonas reinhardtii* or *Synechocystis* PCC 6803 cells. The following specific approaches were undertaken.

- Performed *Chlamydomonas reinhardtii* genomic 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.
- Performed 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.
- Cloned, characterized, and manipulated gene(s) that affect the "light-harvesting antenna size" property in *Chlamydomonas reinhardtii* and *Synechocystis* PCC6803.

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 in both green microalgae and cyanobacteria.
- Further results and analyses on the manipulation of the *TLA1* gene [6] and deletion of the *TLA3* gene [7] for the regulation of the Chl antenna size in green microalgae were published.
- Application of the TLA concept in cyanobacteria is at a fairly advanced stage, results to be drafted in the form of a manuscript soon.

FUTURE DIRECTIONS

- Complete the work pertaining to the TLA concept in cyanobacteria. (Currently in progress.)

- Advance the exploration of the "*extended photosynthetically active radiation*" (ePAR) concept. (Proprietary design not disclosed.)

FY 2013 PUBLICATIONS/PRESENTATIONS/ PATENT

1. Kirst H, Garcia-Cerdan JG, Zurbriggen A, Ruehle T, Melis A (2012) Truncated photosystem chlorophyll antenna size in the green microalga *Chlamydomonas reinhardtii* upon deletion of the *TLA3-CpSRP43* gene. Plant Physiol. 160(4):2251-2260.

2. Mitra M, Kirst H, Dewez D, Melis A (2012) Modulation of the light-harvesting chlorophyll antenna size in *Chlamydomonas reinhardtii* by *TLA1* gene over-expression and RNA interference. Phil. Trans. R. Soc. B 367:3430-3443.

3. Melis A (2012) Using and abusing photosynthesis to produce fuels and chemicals. European Union Photosynthesis Workshop: from Science to Industry. Marie Curie Program on Training in BioSolar Research. NH Conference Centre, Leeuwenhorst, Noordwijkerhout, The Netherlands. Pp. 26-27.

4. Kirst H, Garcia-Cerdan JG, Zurbriggen A, Melis A (2013) The CrCpSRP pathway proteins CrCpSRP43 and CpFTSY are required for the assembly of the light-harvesting chlorophyll antenna in the green alga *Chlamydomonas reinhardtii*. Western Photosynthesis Conference, Asilomar Conference Grounds, Pacific Grove, CA, pages 23-24.

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7. 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.