1. RELEVANCE / OBJECTIVE

Project Title: Maximizing Photosynthetic Efficiencies and Hydrogen Production in Microalgal Cultures

Principal Investigator: Tasios Melis, UC Berkeley

Technical Barrier: Low Light Utilization Efficiency in Photobiological Hydrogen Production due to a Large Photosystem Chlorophyll Antenna Size (Barrier K)

Technical Target: Molecular Genetics of Organisms for Photobiological Hydrogen Production. Use DNA insertional mutagenesis and high-throughput screening methods to select organisms that have a smaller Chl antenna size and increased light conversion efficiency (Target 17)

2. APPROACH

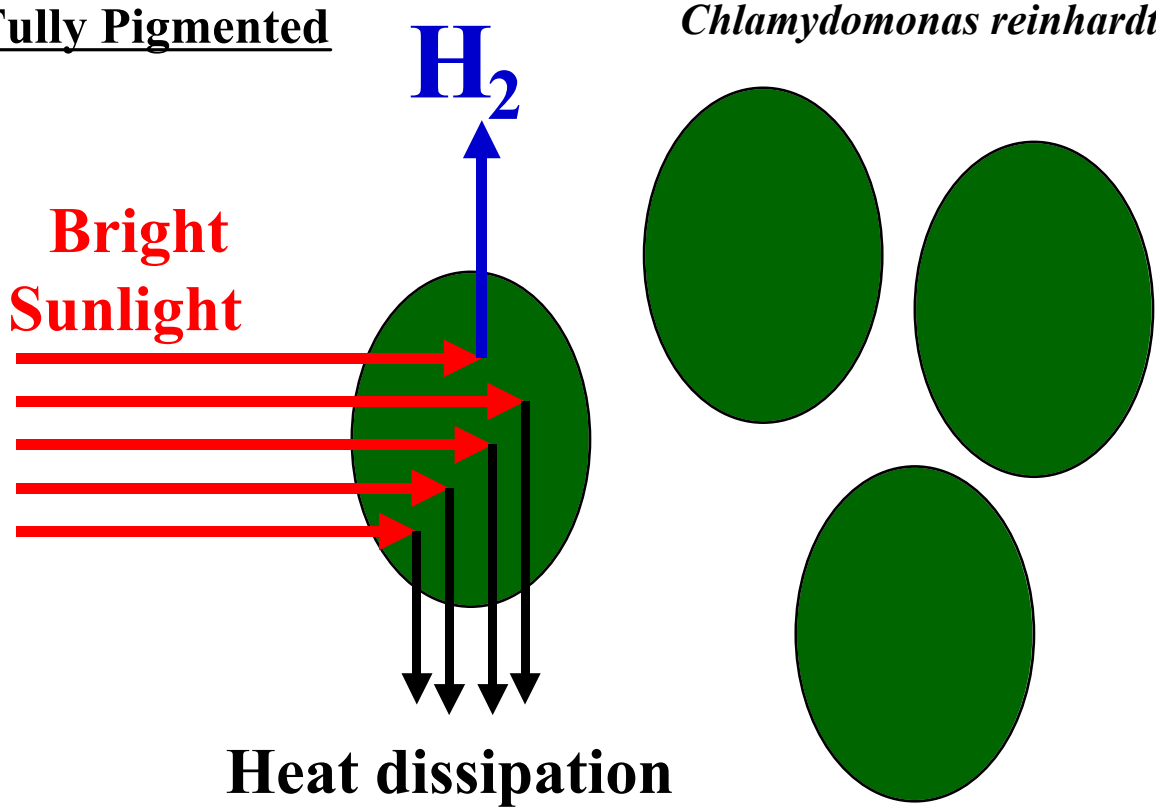
Identify and manipulate genes that attenuate, or truncate, the chlorophyll antenna size in green algae.

DNA insertional mutagenesis, screening, biochemical and molecular genetic analyses of the green alga *Chlamydomonas reinhardtii* are employed.

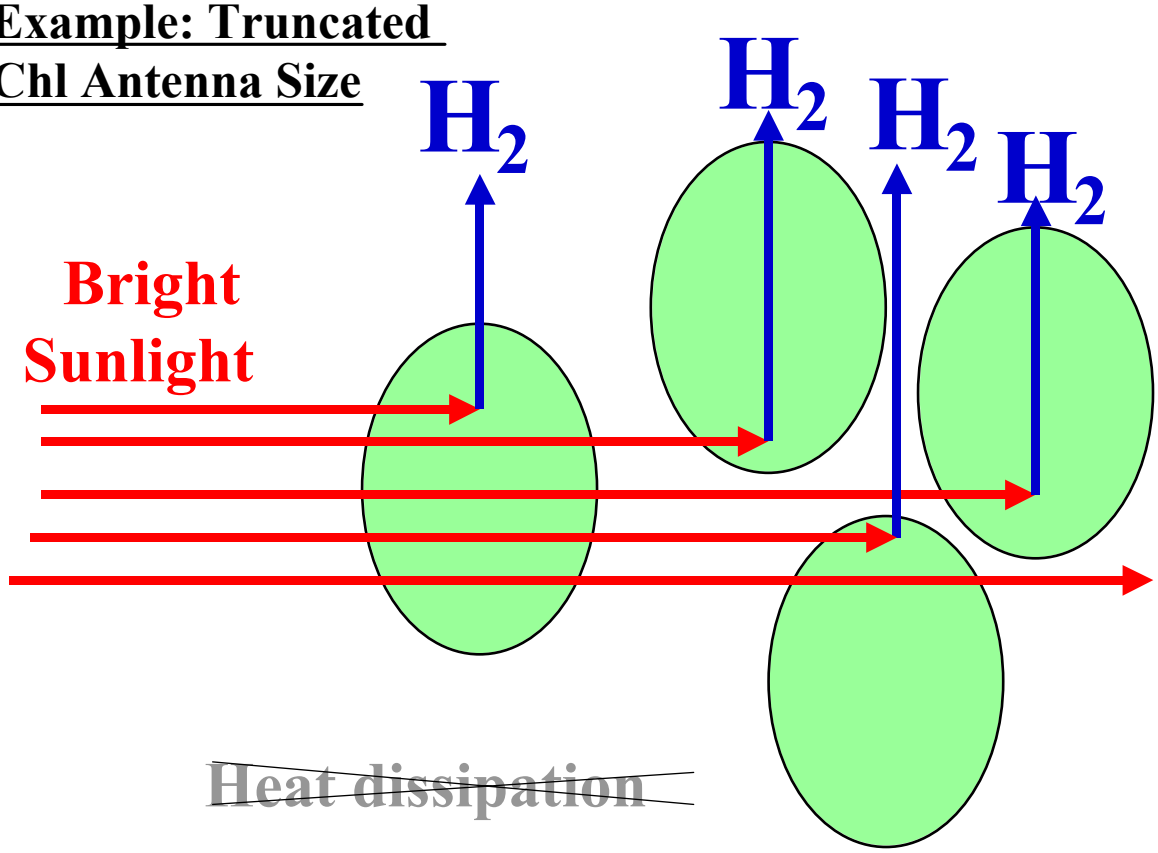
A truncated Chl antenna size would minimize absorption and wasteful dissipation of sunlight by individual cells, resulting in better light utilization efficiency and greater yield of H_2-production by the green alga culture.

Example:
Fully Pigmented

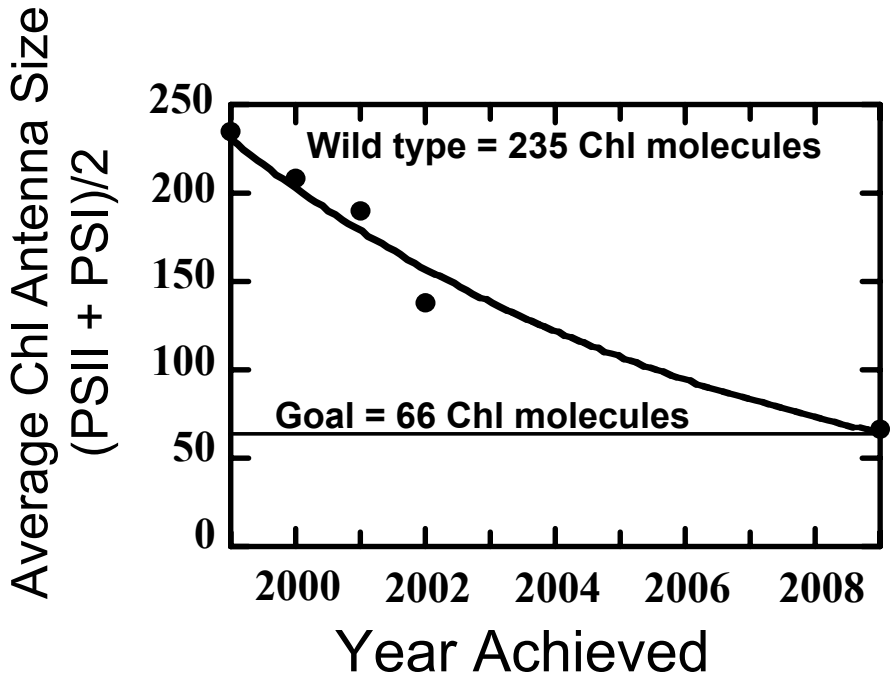
The green algae
Chlamydomonas reinhardtii



Example: Truncated
Chl Antenna Size



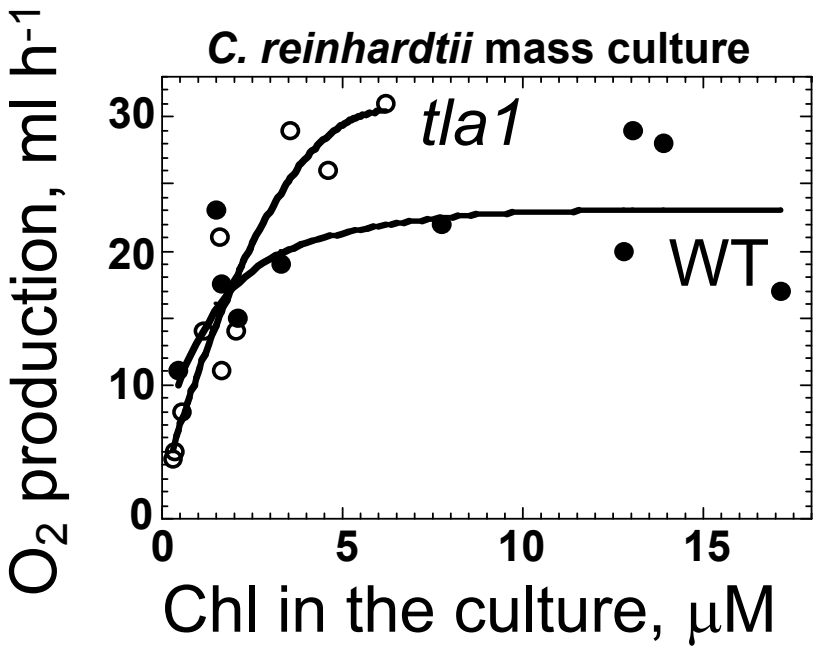
3A. Project Timeline-Graph



3B. Technical Progress - Table

	Wild type	Chl *b*-less	Lutein-less	*Tla1*	LT Goal
Chl-PSII	230	90	125	115	37
Chl-PSI	240	290	290	160	95
Average (PSII+PSI)/2	235	190	208	135	66
Year Achieved	Concept Validation	2000	2001	2002	2009

3C. Productivity in scale up cultures in the greenhouse



3D. Summary of Accomplishments

First-time cloning of *Tla1,* a 'Chl antenna size regulatory gene' (30 year breakthrough).

Genetic interference of the *Tla1* gene, resulted in a truncated PSII Chl antenna size, down to 50%, and a PSI Chl antenna size, down to 67%.

Progress toward the goal: *tla1* mutant showed enhanced productivity under mass culture conditions (see 3C).

The DOE's *Joint Genome Institute* confirmed the sequence of the *Tla1* gene (GenBank Accession No. AF534570 and AF534 571) **and assigned a function on the basis of our work.**

Results from this work apply directly to green alga hydrogen-production, biomass accumulation, and carbon sequestration efforts.

Other branches of the DOE (carbon sequestration) benefited from this work (see 4A, Technology transfer).

4A. Technology Transfer

Mera Pharmaceuticals operates in Hawaii, under the auspices of the DOE, a large-scale, carbon sequestration pilot program with green algae. Mera Pharma is currently negotiating with UC Berkeley to obtain truncated chlorophyll antenna size strains of green algae from the Melis laboratory in order to enhance yields of biomass accumulation and carbon sequestration.

4B. Collaborations

In the US

- **With whom are we working?**

  NREL, ORNL as part of an integrated effort
- **Who else is working in the Chl antenna area?**

  Sole Source

Outside of the US

- **In what international collaborations do you actively participate?**

  Tokyo Institute of Technology, Hokkaido University, University of Bonn
- **Who else is working in the area?**

  Sole Source

5A. Plans, FY 2003 Milestones

A. Solar conversion efficiency (hydrogen) measurements in wild type and *tla1* mutant

B. Complementation studies of the *tla1* mutant

C. Initiate analysis of two additional DNA insertional transformants (*1A-26, 1B-2*)

D. Functional analysis of the *Tla1* gene (how does this gene work?)

E. Preparation of reports/publications in peer-reviewed journals

5B. What needs to be done beyond FY2003

Identify additional *Tla*-type genes.

Functionally characterize these genes (how do they work?)

Perform genetic crosses to combine different *tla*-type properties and phenotypes.

Establish transformation protocols w/ *Tla*-type genes (for overexpression or downregulation) in Chlamydomonas and other key green algae.

6A. Technical Challenges

Key challenge

Diminish the Chl antenna size of the photosystems, without diminishing the number of the photosystems in the green algae.

Approach being used to overcome this challenge

The existence of unique biophysical instrumentation at UCB permits independent direct measurements of photosystem concentration and PS Chl antenna size (sole source).

6B. Responses to 1-3 Questions

Question 7a: Publish the *tla1* results.

Genomic, cDNA, and protein sequences were deposited in the GenBank (Accession Nos. AF534570 and AF534571).

A first manuscript has appeared: 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 DOI 10.1007/s00425-002-0968-1.

A second manuscript dealing with the molecular aspects of the *Tla1* gene is now in preparation.

Question 7b: Do H2 measurements in the greenhouse with the *tla1* strain

With the upcoming sunny season in Berkeley, efforts are under way to measure yields of H2 in wild type and truncated Chl antenna mass cultures.

7. Current FY Publications

Kanakagiri S and Melis A (2002) *Chlamydomonas reinhardtii TLA1* nuclear gene for the regulation of the photosystem chlorophyll antenna size in photosynthesis, complete cds; (bases 1 to 2181). GenBank Accession Number AF534570

Kanakagiri S and Melis A (2002) *Chlamydomonas reinhardtii* chlorophyll antenna size regulatory protein (TLA1) mRNA, complete cds GenBank Accession Number AF534571

Melis A (2002) Green alga hydrogen production: progress, challenges and prospects. Intl. J. Hydrogen Energy 27: 1217-1228

Polle JEW, Kanakagiri, S, Jin ES, Masuda, T and Melis A (2002) Truncated chlorophyll antenna size of the photosystems - a practical method to improve microalgal productivity and hydrogen production in mass culture. Intl. J. Hydrogen Energy 27: 1257-1264

Masuda T, Tanaka A and Melis A (2003) Chlorophyll antenna size adjustments by irradiance in *Dunaliella salina* involve coordinate regulation of chlorophyll *a* oxygenase (*CAO*) and *Lhcb* gene expression. Plant Mol. Biol. 51: 757-771

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 DOI 10.1007/s00425-002-0968-1