DOE Hydrogen Program

Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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Project ID # PDP33

Overview

Timeline

- **Start: January 2005**
- **End: December 2008**
- **Completion: 50%**

Budget

- Total Project Funding

DOE: $330 k, UCB: $225 k

- Funding for FY06

DOE: $ 50 k, UCB: $75 k

- Funding for FY07

DOE: $ 90 k, UCB: $75 k

Barriers addressed

- **Low Light Utilization Efficiency in Photobiological Hydrogen Production due to a Large Photosystem Chlorophyll Antenna Size (Barrier X).**

Partners

- None: Sole Source Effort

Objectives and Approach

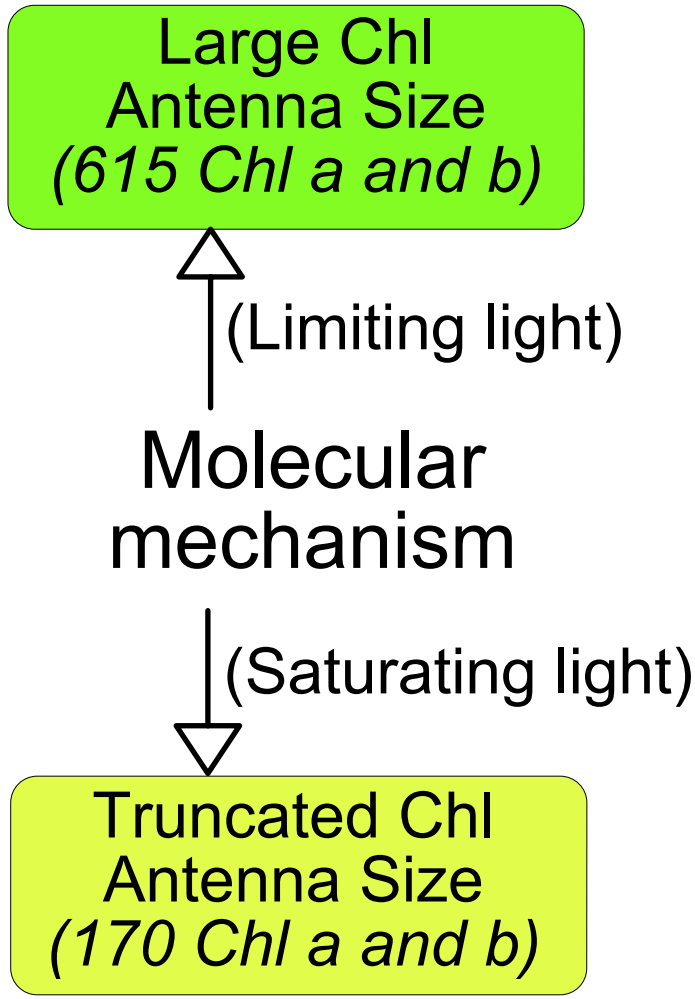
Objective: Minimize the chlorophyll antenna size of photosynthesis to maximize solar conversion efficiency in green algae.

(Identify and characterize genes that regulate the Chl antenna size in the model green alga *Chlamydomonas reinhardtii.* Apply these genes to other green algae, as needed.)

Approach: Interfere with the molecular mechanism for the regulation of the chlorophyll antenna size.

(Employ DNA insertional mutagenesis and high-throughput screening to isolate tagged green algae with a smaller Chl antenna size.)

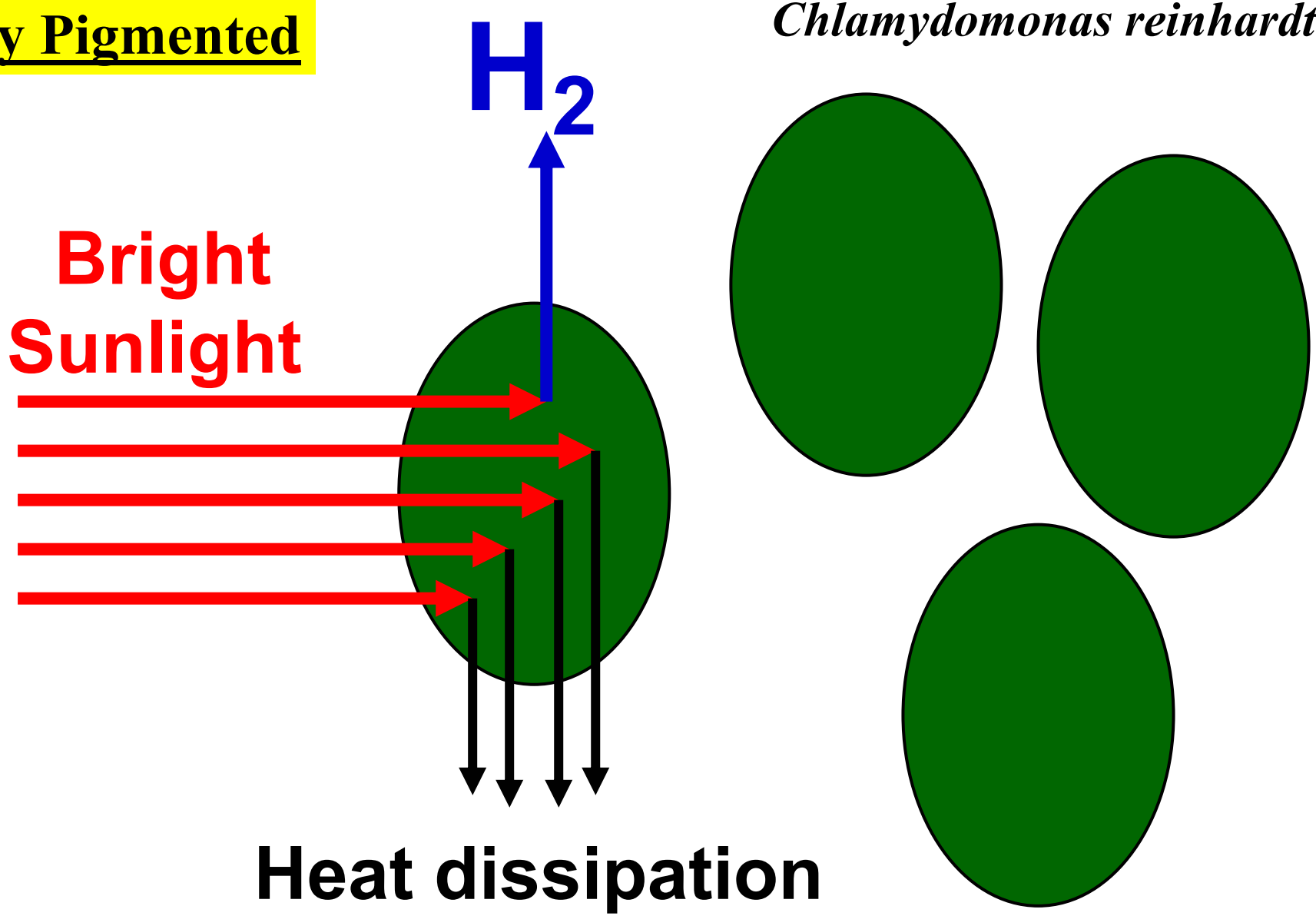
Regulation of the Chl antenna size



Interference with the molecular mechanism for the regulation of the Chl antenna size, to derive a permanently truncated Chl antenna size, is the goal of this R&D.

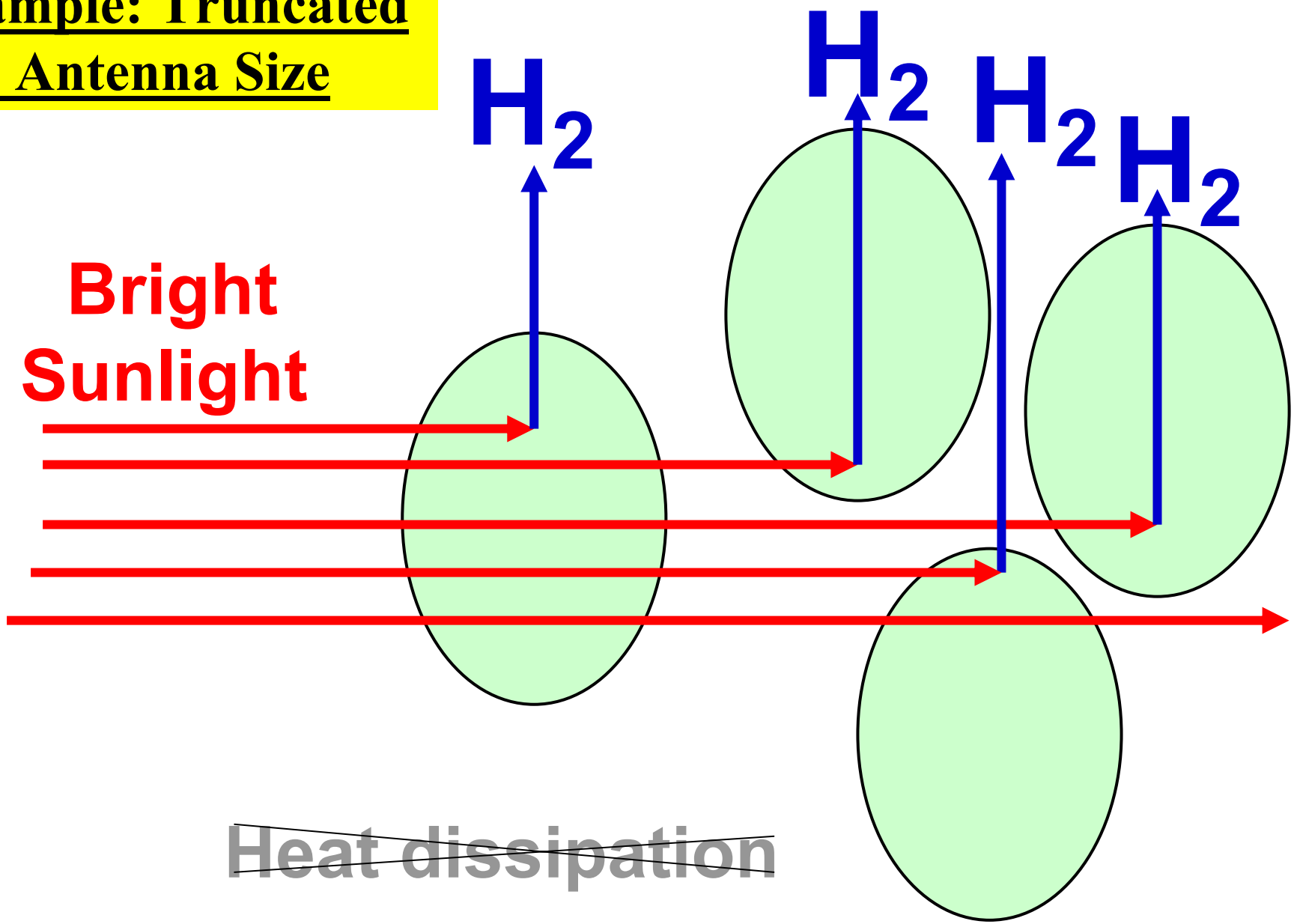
Example:
Fully Pigmented

The green algae
Chlamydomonas reinhardtii



Fully pigmented cells over-absorb and wastefully dissipate bright sunlight.

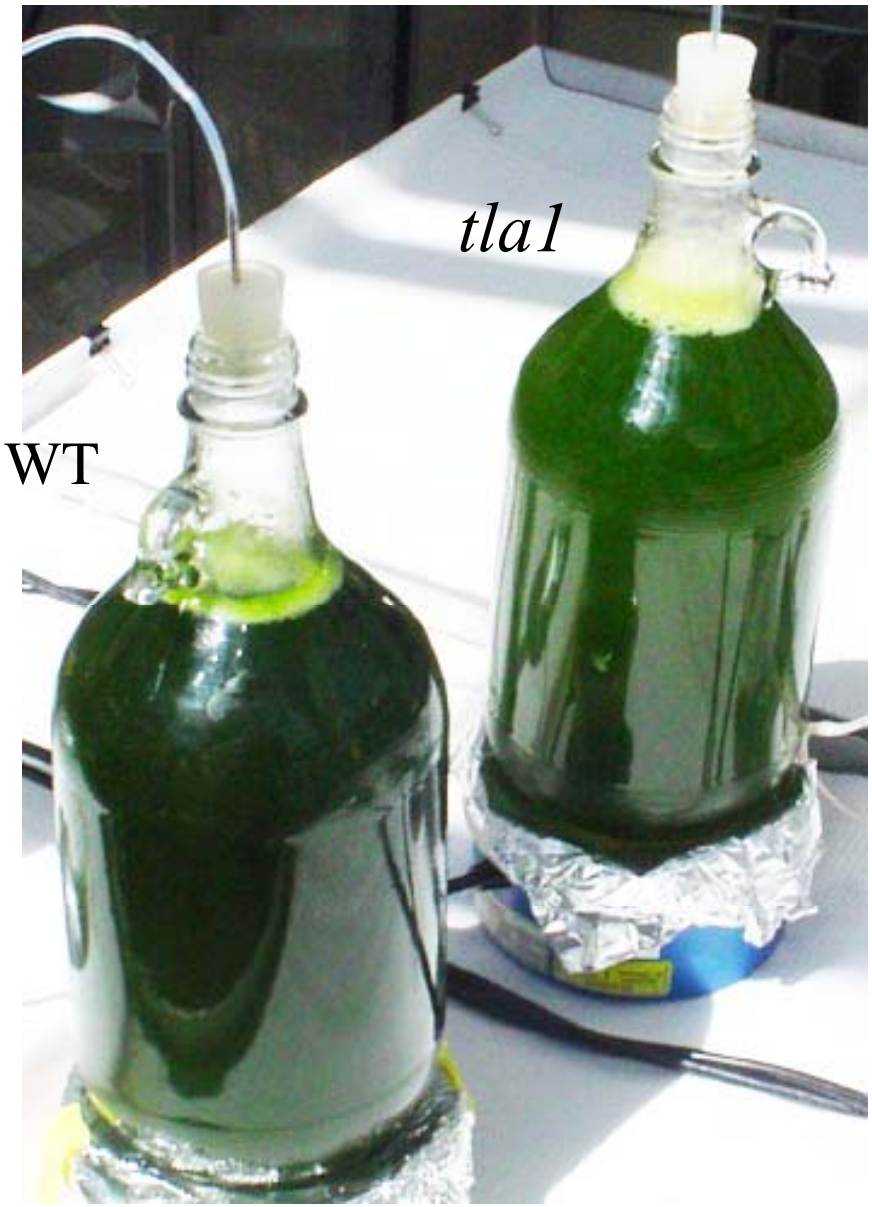
Example: Truncated Chl Antenna Size



Truncated Chl antenna cells permit greater transmittance of light and overall better solar utilization by the culture.

Measurement in Scale-up Cultures

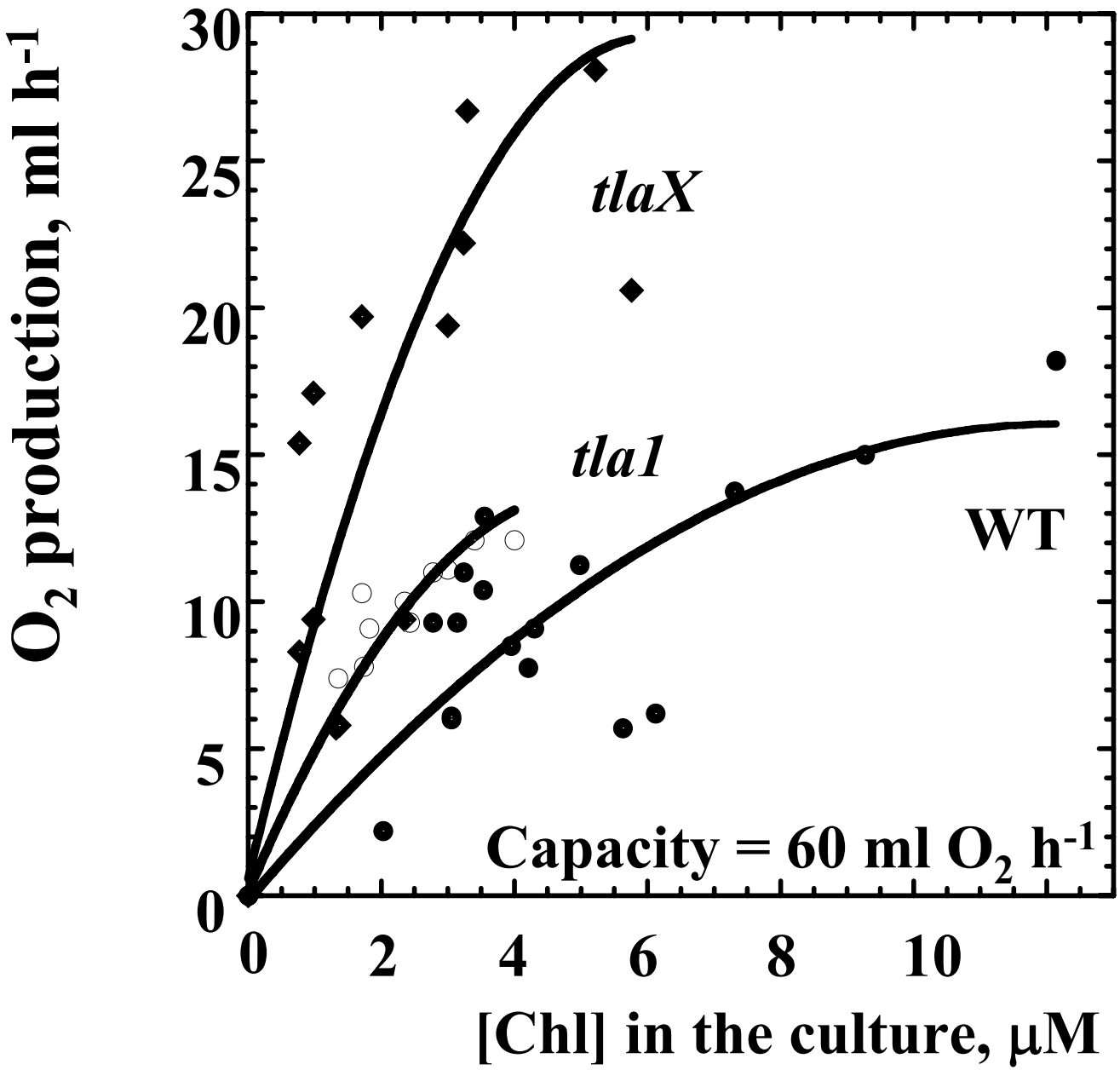
Cultures in the Greenhouse



Parameter	WT	*tla1*
Cell/mL ($x10^6$)	6.36	10.0
[Chl] (uM)	25.6	15.4

The *tla1* strain shows greater productivity than the wild type cells under bright sunlight conditions. (Note relative amounts of gas bubbles produced by the two samples.)

Productivity in Scale-up Cultures



Technical Barriers and Targets

- **Barrier X: Low Light Utilization Efficiency in Photobiological Hydrogen Production due to a Large Photosystem Chlorophyll Antenna Size.**
- **Light Utilization Efficiency of WT green algae: 3-5%**
- **Theoretical maximum efficiency: ~30%**
- **Target for 2010: Reach a 15% Utilization Efficiency of Absorbed Light Energy.**

Chl Antenna Size vs Light Utilization Efficiency

Utilization Efficiency of Absorbed Light Energy

Achievement in 2005: 15%

- **Wild type antenna size = 470 Chl molecules (100%)**
  (PSII=230; PSI=240)
  Photon use efficiency of WT photosynthesis = ~6-10%
  Utilization Efficiency of Absorbed Light Energy by WT: ~3-5%

- ***tla1* antenna size = 275 Chl molecules (59% of control)**
  (PSII=115; PSI=160)
  Photon use efficiency of *tla1* photosynthesis = ~20%
  Utilization Efficiency of Absorbed Light Energy by *tla1*: ~10%

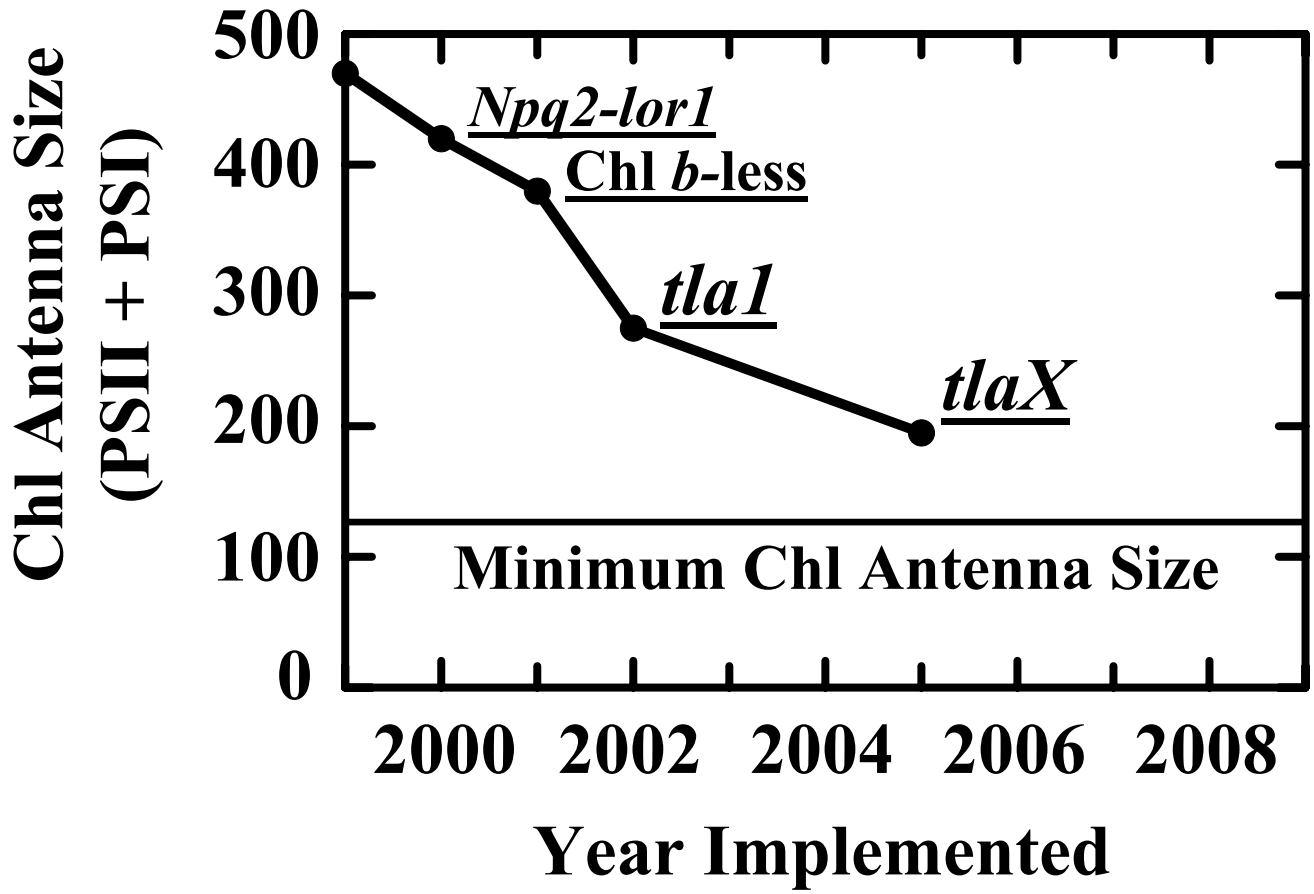
2005 Year Accomplishment

- ***tlaX* antenna size = 195 Chl molecules (42% of control)**
  (PSII=80; PSI=115)
  Photon use efficiency of *tlaX* photosynthesis = ~30%
  Utilization Efficiency of Absorbed Light Energy by *tlaX*: ~15%

- **Long-term goal: 132 Chl molecules (28% of control)**
  (PSII=37; PSI=95)
  Photon use efficiency of photosynthesis *goal* = ~60%
  Utilization Efficiency of Absorbed Light Energy *goal*: ~30%

Project Timeline

Chlorophyll Antenna Size in Chlamydomonas



Current Technical Accomplishments

Analysis of the *tla1* and *tlaX* mutants

- **Molecular analysis of the *tla1* mutation.**

  Genomic, cDNA and protein sequences for the *Tla1* gene were published.

  Complementation of the *tla1* mutant with the *Tla1* gene succeeded.

  Analysis of the complemented strains was implemented.

- **Biochemical analysis of the *tla1* mutation.**

  Antibodies against the Tla1 protein were raised.

  Hydropathy plot of the Tla1 protein measured.

  Sequence homologies for the Tla1 protein and phylogenetics completed.

- **Functional analysis of the *Tla1* gene.**

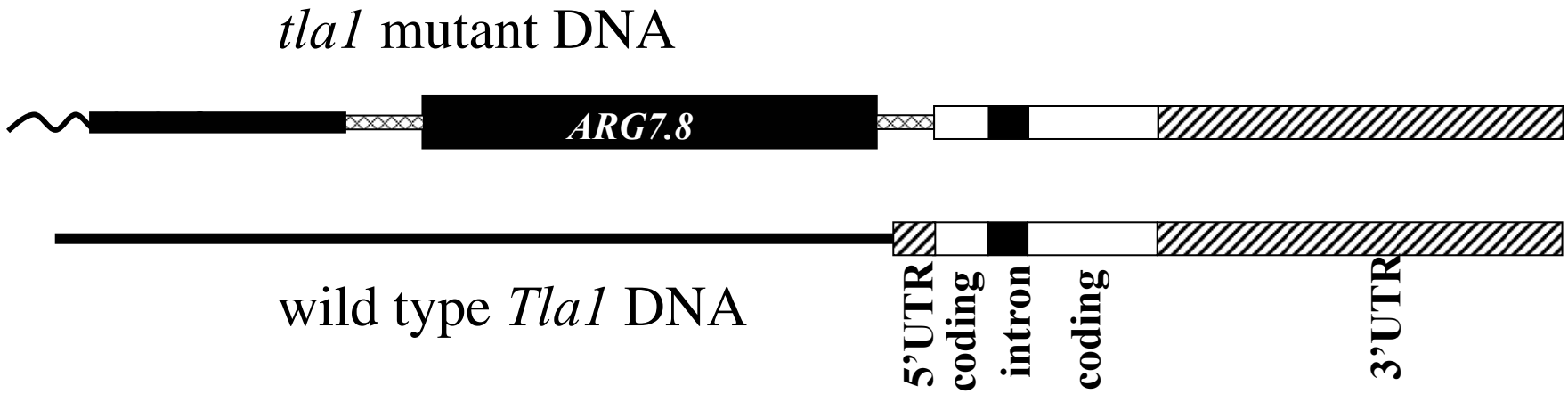
  Regulation of the chlorophyll antenna size by the *Tla1* gene completed.

- **Biophysical and biochemical analyses of the *tlaX* mutant.**

  Chlorophyll antenna size, relative productivity, LHC expression levels.

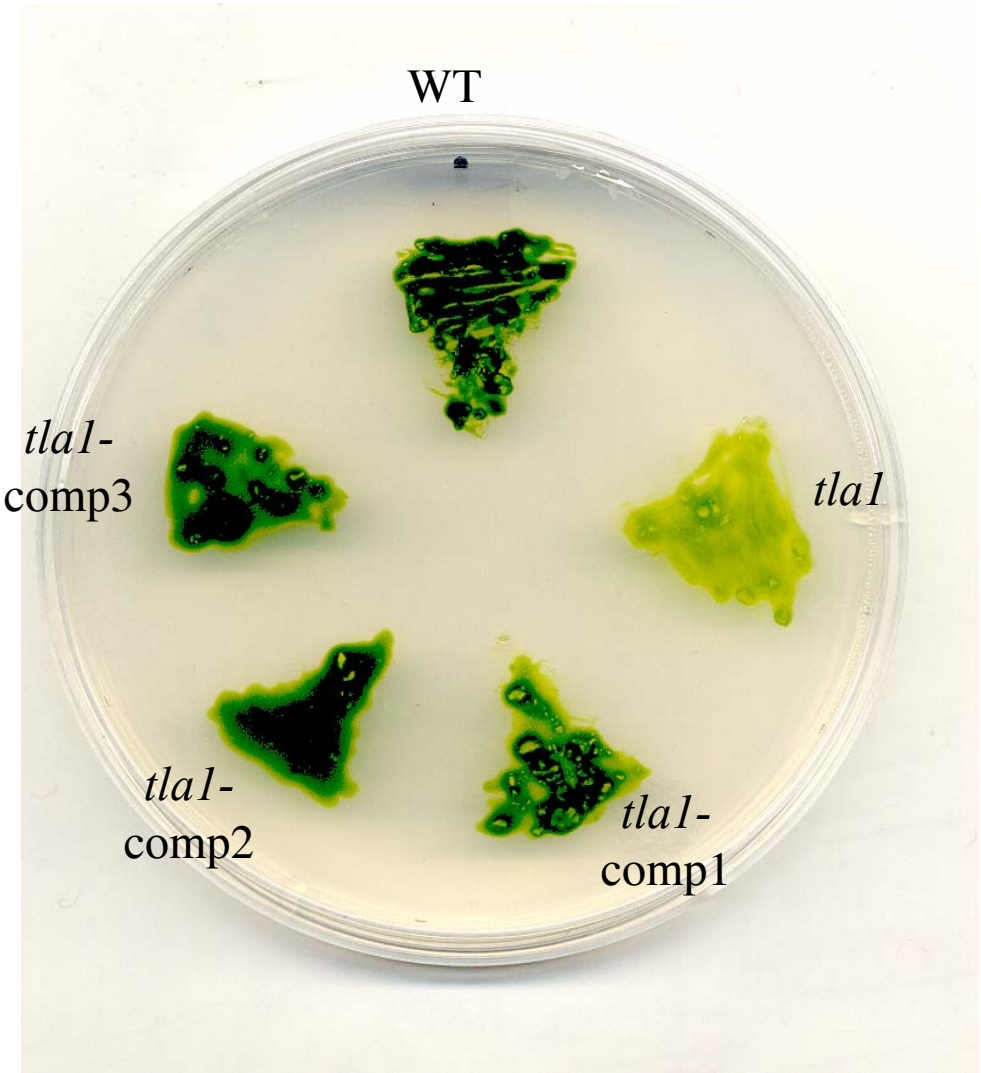
Current Technical Accomplishments

Mapping of the *tla1* mutation and WT *Tla1* gene structure



Current Technical Accomplishments

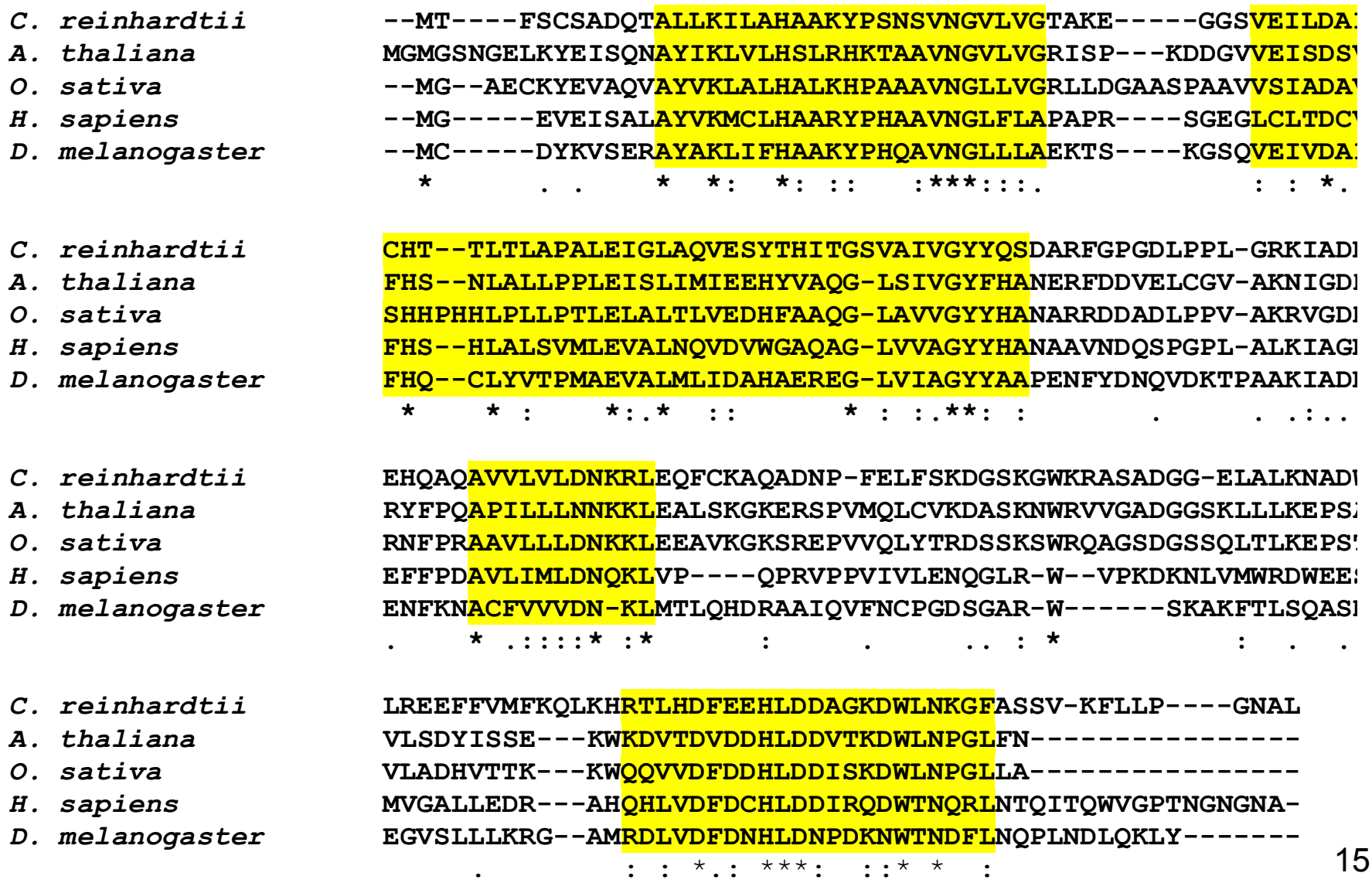
tla1 mutant complementation



Complementation of the pale-green *tla1* mutant with the wild type *Tla1* gene resulted in *tla1*-comp1, *tla1*-comp2, and *tla1*-comp3 strains with restored dense green pigmentation properties.

Current Technical Accomplishments

Sequence homologies for the Tla1 protein



Summary of Accomplishments

Analysis of the *tla1* and *tlaX* mutants

- **Competed the biochemical characterization of the *tla1* mutant and the molecular analysis of the *Tla1* gene.**
- **Down-regulation of the ubiquitous *Tla1* gene could be applied in the regulation of the chlorophyll antenna size in microalgae.**
- **Demonstrated higher yields of photosynthesis in microalgae with a truncated chlorophyll antenna size.**
- **Advanced the biophysical and biochemical analyses of the *tlaX* mutant. Encountered difficulties in the molecular analysis of this mutant.**

Progress achieved *vs* the DOE targets

Utilization Efficiency of Incident Solar Light Energy, E_0xE_1, %

	2000	2003	2006	2010	2015
Program Targets	3%	10%		15%	20%
Progress	3%	10%	15%		

Significance of Work

- **First-time identification and documentation of a gene (*Tla1*) that regulates the development of the chlorophyll antenna size in photosynthesis.**
- **Findings could be applied in the modification of the Chl antenna size in microalgae and higher plants, helping to increase solar conversion efficiencies and photobiological hydrogen production.**

Current Work

Perform functional analysis of the *Tla1* gene

(How is *Tla1* regulated under different conditions?)

- **Investigate levels of expression of the *Tla1* gene as a function of growth irradiance.**
- **Investigate cellular localization of the Tla1 protein.**
- **Establish transformation (sense and antisense) protocols with the *Tla1* gene to enhance the down-regulation of the Chl antenna size in wild type *Chlamydomonas reinhardtii*.**

Future Work

Implement analysis of two additional DNA insertional transformants with a putative 'truncated Chl antenna size' (*tlaX* and *tlaY*)

- **Clone the putative *TlaX* gene, responsible for the substantially truncated Chl antenna phenotype in the *tlaX* strain.**
- **Raise specific polyclonal antibodies against the TlaX protein; investigate cellular localization of the TlaX protein; measure levels of TlaX protein expression as a function of irradiance in wild type and *tlaX* mutant.**
- **Investigate whether enhancement or suppression of *TlaX* gene expression results in a truncated Chl antenna size.**
- **Establish transformation (sense and antisense) protocols with the *Tla*X gene to enhance the down-regulation of the Chl antenna size in *Chlamydomonas reinhardtii.***

Summary

- **Completed first part of work on the *Tla1* gene.**
- **Filed patent application on the *Tla1* gene.**
- **Published findings in peer reviewed journal:**

Tetali SD, Mitra M and Melis A (2006)
Development of the light-harvesting chlorophyll antenna in the green alga *Chlamydomonas reinhardtii* is regulated by the novel *Tla1* gene. Planta 225: 813-829

- **Gave invited presentations on Tla1 work at the:**

--University of Montreal, Quebec, Canada.

--Gordon Research Conference on Photosynthesis.

--International Symposium on Materials Issues in Hydrogen Production and Storage.

--University of Minnesota.