



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

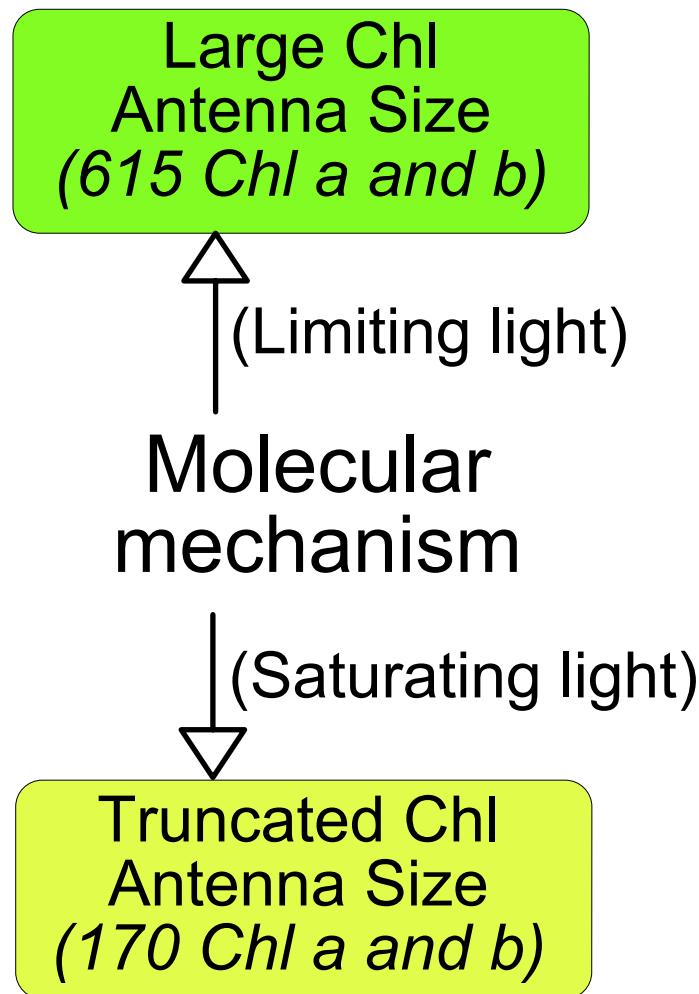
**Objective:** Minimize the chlorophyll antenna size of photosynthesis to maximize solar conversion efficiency in green algae.

(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.)

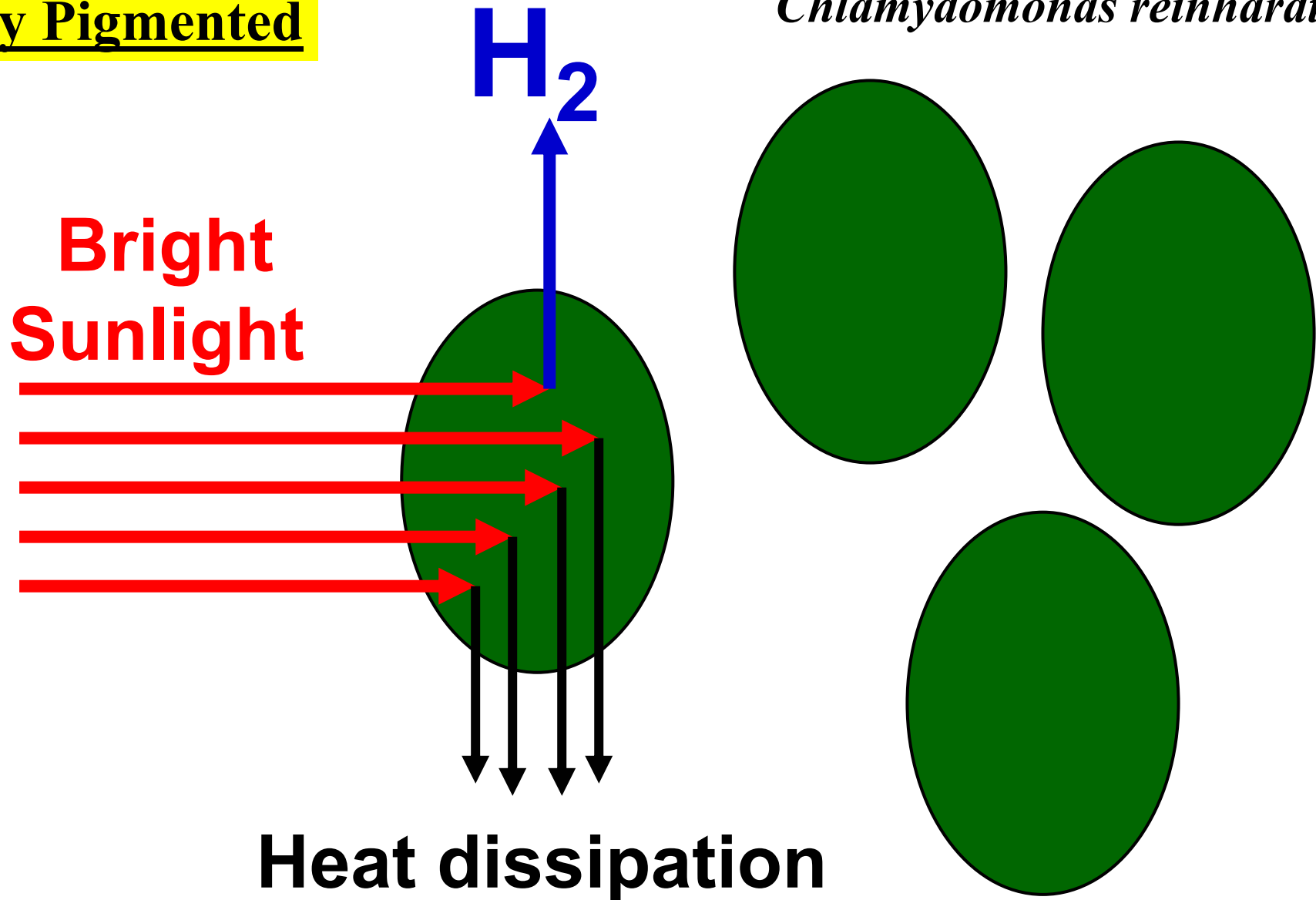
# Regulation of the Chl antenna size



**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.**

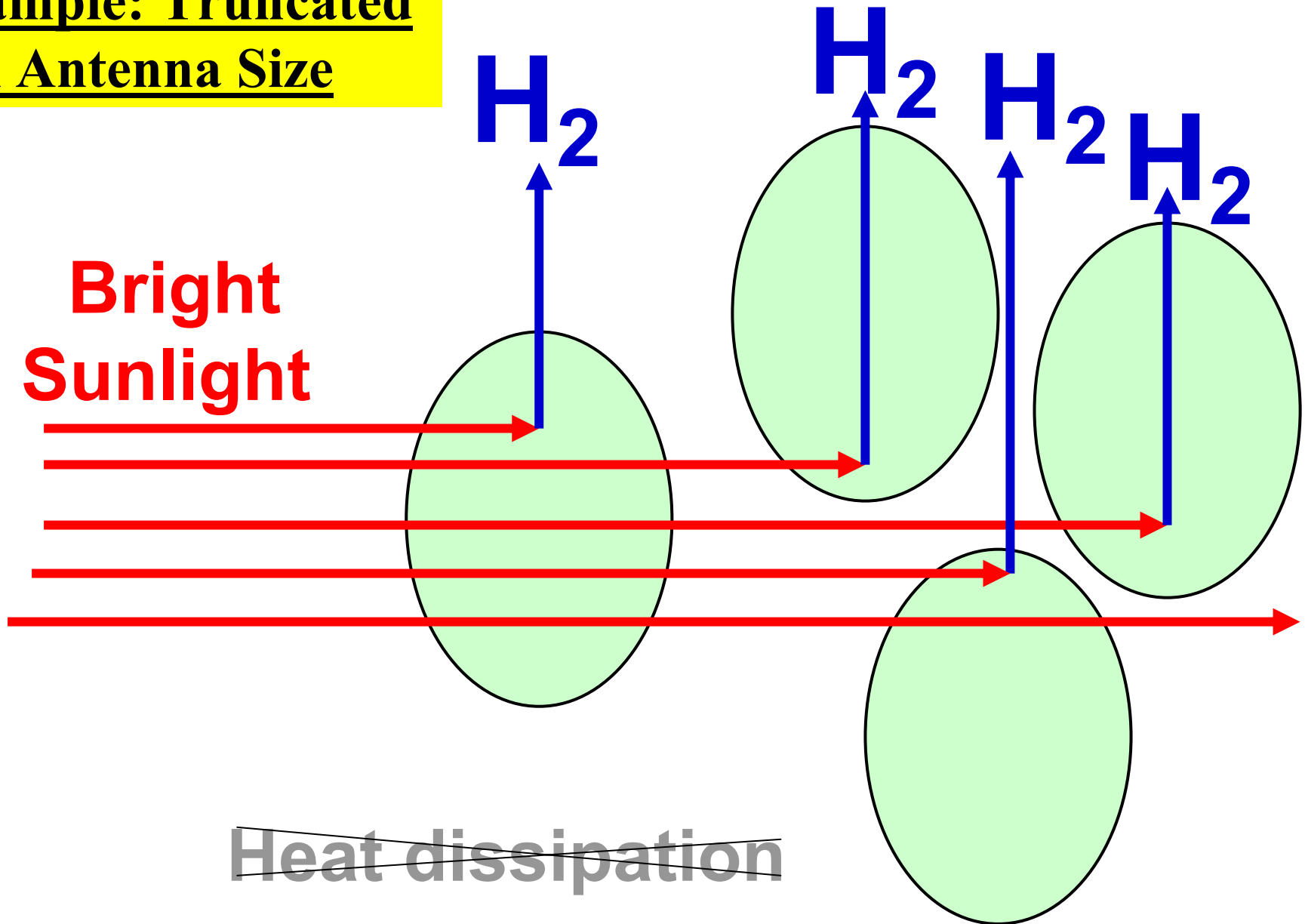
**Example:**  
**Fully Pigmented**

The green algae  
*Chlamydomonas reinhardtii*



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

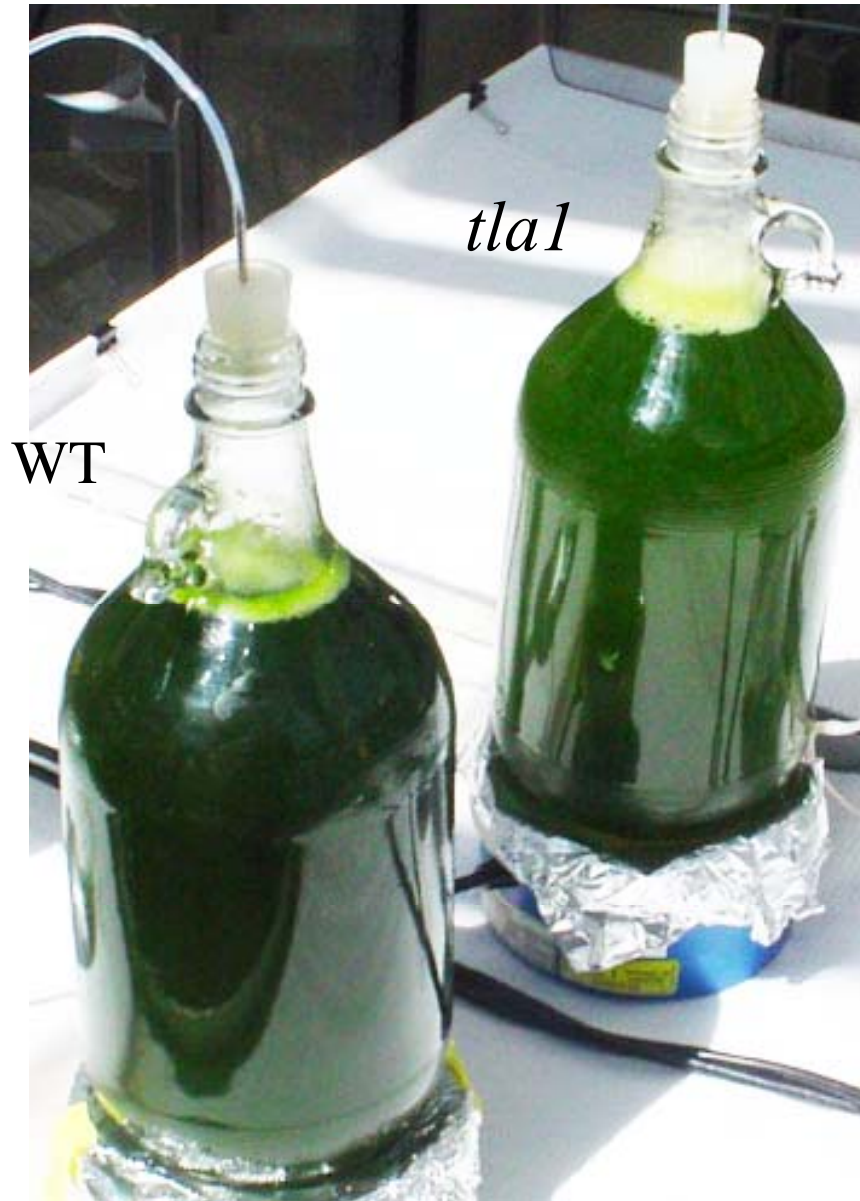
## Example: Truncated Chl Antenna Size



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

# Measurement in Scale-up Cultures

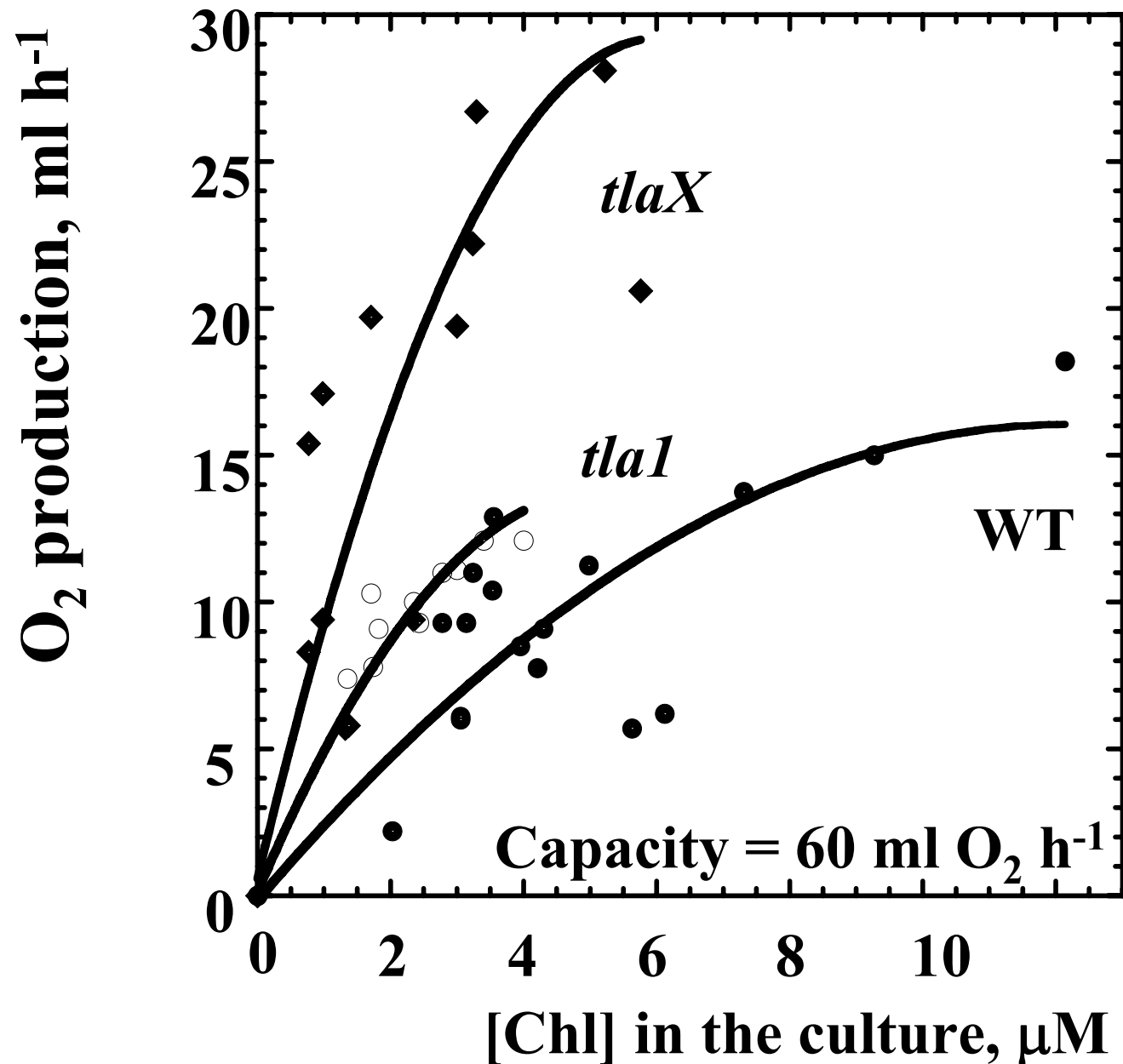
## Cultures in the Greenhouse



<u>Parameter</u>	<u>WT</u>	<u><i>tla1</i></u>
Cell/mL (x10 <sup>6</sup> )	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%

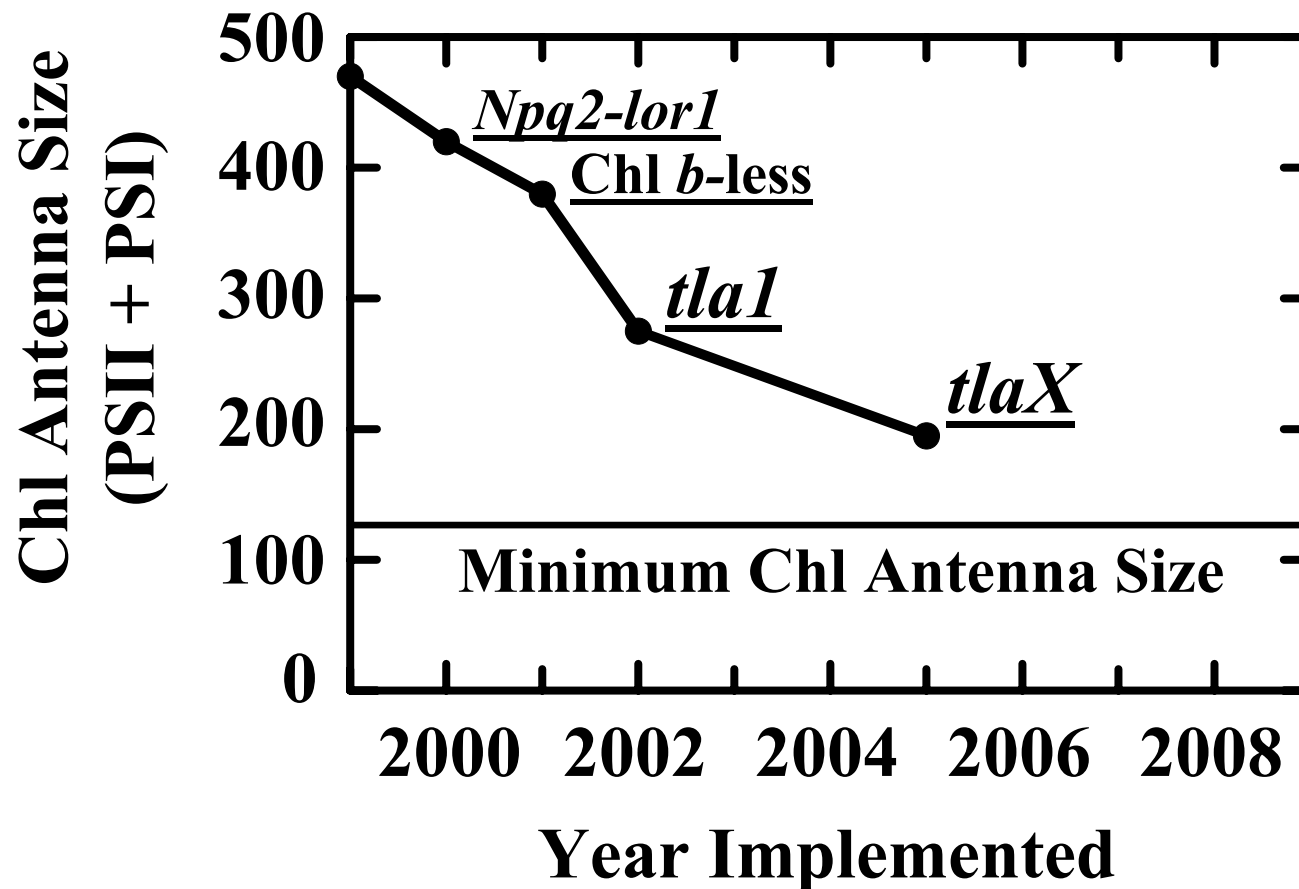
- Wild type antenna size = 470 Chl molecules (100%)  
(PSII=230; PSI=240)  
Photon use efficiency of WT photosynthesis = ~6-10%  
Utilization Efficiency of Absorbed Light Energy by WT: ~3-5%
- *tla1* antenna size = 275 Chl molecules (59% of control)  
(PSII=115; PSI=160)  
Photon use efficiency of *tla1* 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.**

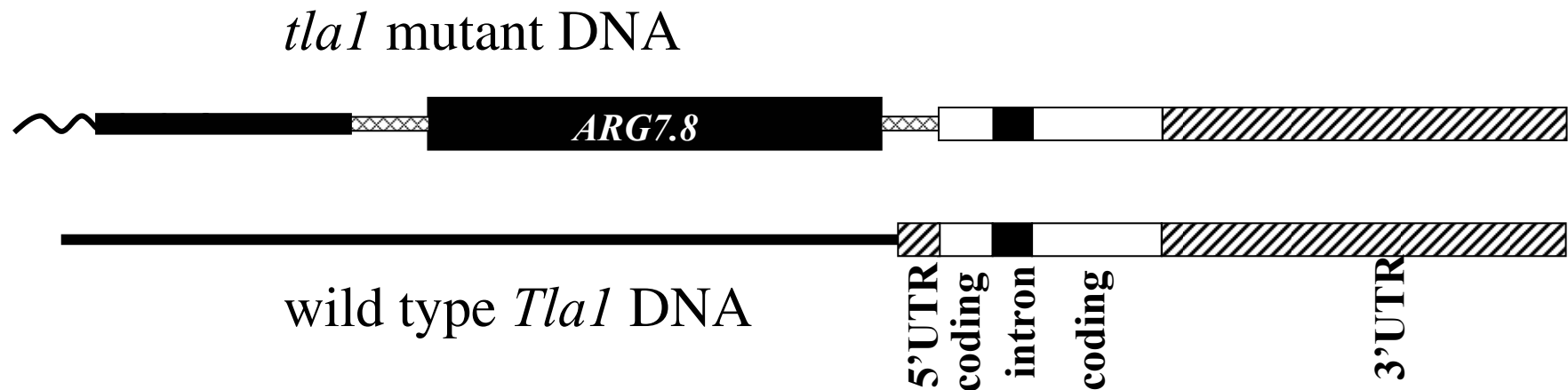
Regulation of the chlorophyll antenna size by the *Tla1* gene completed.

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

Chlorophyll antenna size, relative productivity, LHC expression levels.

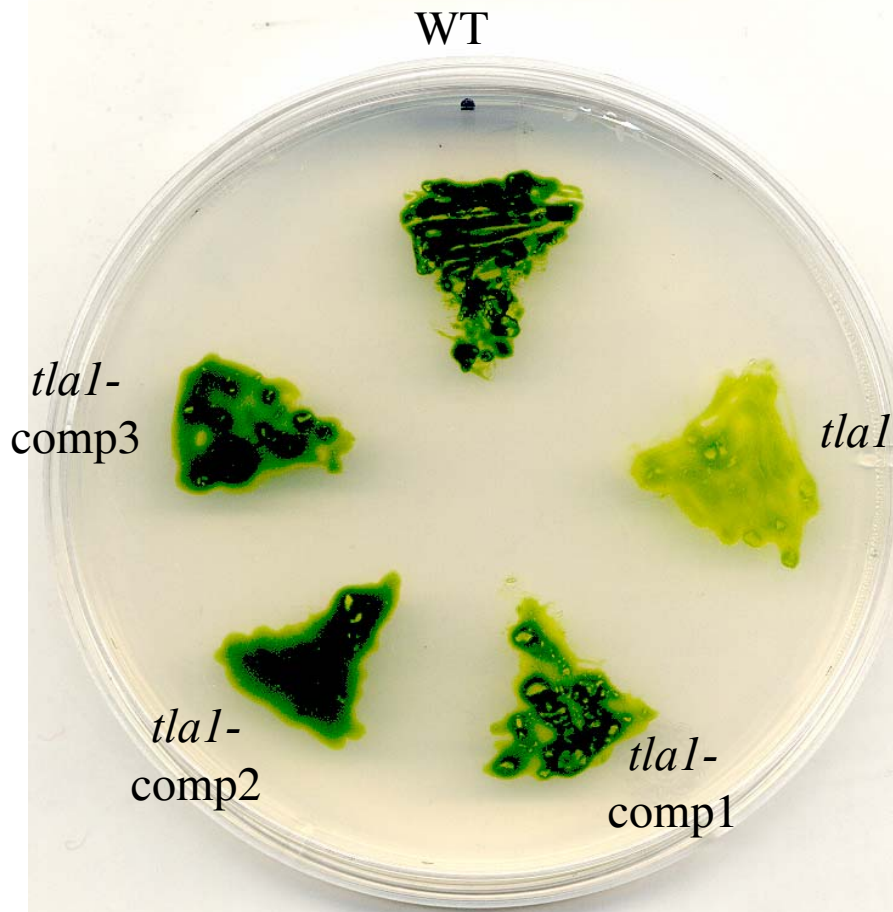
# Current Technical Accomplishments

## 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

<i>C. reinhardtii</i>	--MT----FSCSADQTALLKILAHAAKYPSNSVNGVLVGTAKE-----GGSV
<i>A. thaliana</i>	MGMGSNGELKYEISQNAIYIKLVLSLRHKTAAVNGVLVGRISP---KDDGV
<i>O. sativa</i>	--MG--AECKYEVAQVAVVKLALHALKHPAAAVNGLLVGRLLDGAASPA
<i>H. sapiens</i>	--MG-----EVEISALAYVKMCLHAARYPHA
<i>D. melanogaster</i>	--MC-----DYKVSERAYAKLI
	* . . * *: *: :: :***::: : : *

<i>C. reinhardtii</i>	CHT--TLTLAPALEIGLAQVESYTHITGSVAIVGYYQSDARFGPGDLPPL-GRKIADI
<i>A. thaliana</i>	FHS--NLALLPPLEISLIMIEEHYVAQG-LSIVGYFHANERFDDVELCGV-AKNIGDI
<i>O. sativa</i>	SHHPHHLPLLPTLELALTIVEDHFAAQG-LAVVGYHANAARRDDADLPPV-AKRVGDI
<i>H. sapiens</i>	FHS--HLALSVMLEVALNQVDVWGAQAG-LVVAGYYHANAAVNDQSPGPL-ALKIAGI
<i>D. melanogaster</i>	FHQ--CLYVTPMAEVALMLIDAHAREG-LVIAGYYAAPENFYDNQVDKTPAAKIADI
	* * : *:.* :: * : :.*: : . . . .

<i>C. reinhardtii</i>	EHQAQAVVLVLDNKRLEQFCKAQADNP-FELFSKDGSKGWKRASADGG-ELALKNADI
<i>A. thaliana</i>	RYFPQAPIILLNNKKLEALSKGKERSPVMQLCVKDASKNWRVVGADGGSKLLLKEPSI
<i>O. sativa</i>	RNFPRAAVLLLDNKKLEEAVKGKSREPVVQLYTRDSSKSWRQAGSDGSSQLTLKEPSI
<i>H. sapiens</i>	EFFPDVAVLIMLDNQKLVP----QPRVPPVIVLENQGLR-W--VPKDKNLVMWRDWEEI
<i>D. melanogaster</i>	ENFEKNACFVVVDN-KLMTLQHDRAAIQVFNCPGDSGAR-W-----SKAKFTLSQASI
	. * ::::* :* : . . : * : . .

<i>C. reinhardtii</i>	LREEFFVMFKQLKHRTLHDFEEHLDDAGKDWLNKGFASSV-KFLLP----GNAL
<i>A. thaliana</i>	VLSDYISSE---KWKDVTDVDDHLDVTKDWLNPGLFN-----
<i>O. sativa</i>	VLADHVTTK---KWQQVVDFFDHLDDISKDWLNPGLLA-----
<i>H. sapiens</i>	MVGALLEDL---AHQHLVDFDCHLDDIRQDWTNQRLNTQITQWVGPTNGNGNA-
<i>D. melanogaster</i>	EGVSLLLKRG--AMRDLVDFDNHLDNPDKNWTNDFLNQPLNDLQKLY-----
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# Summary of Accomplishments

## Analysis of the *tla1* and *tlaX* mutants

- **Completed 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,  $E_0 \times E_1$ , %

	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 *TlaX* 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.