



DOE Hydrogen Program

# **Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures**

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**Project ID # PD33**

# Overview

## Timeline

- **Start: 01-Dec-2004**
- **End: 30-Nov-2010**
- **Completion: 60%**

## Budget

- Total Project Funding  
**DOE: \$1.2 M, UCB: \$450 k**
- Funding for FY08  
**DOE: \$258 k, UCB: \$75 k**  
Funding for FY07  
**DOE: \$660 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

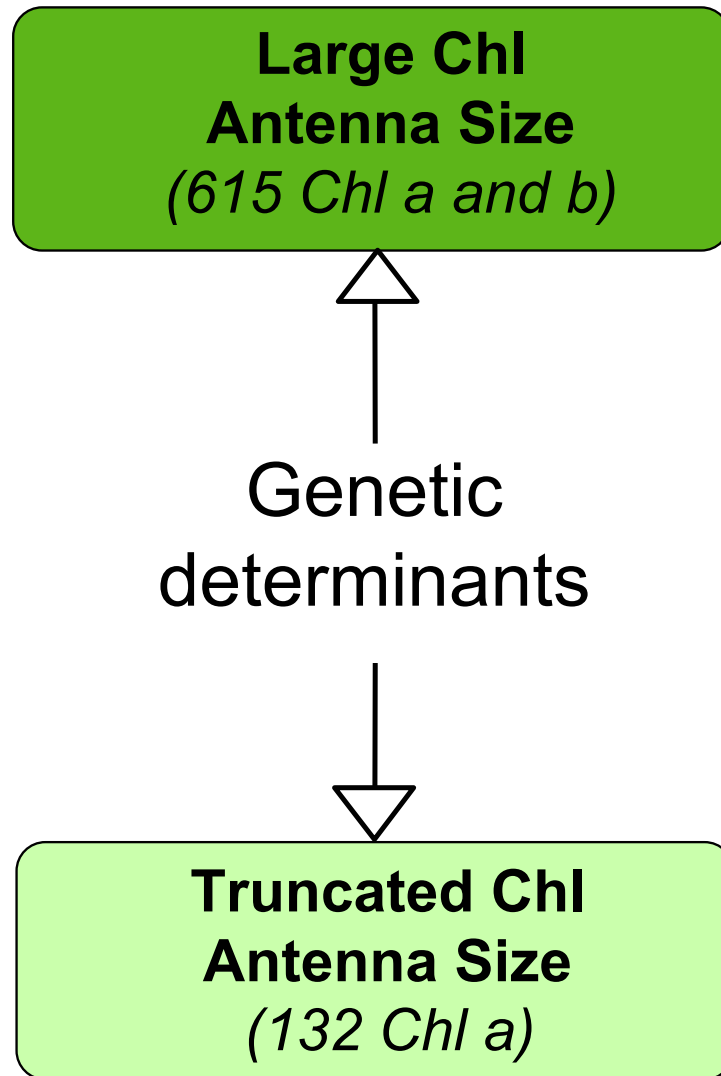
**Objective:** Minimize the chlorophyll antenna size of photosynthesis to maximize solar conversion efficiency in green algae.

(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 genetic mechanism for the regulation of the Chl antenna size, to derive a permanently truncated Chl antenna size, is the goal of this R&D.**

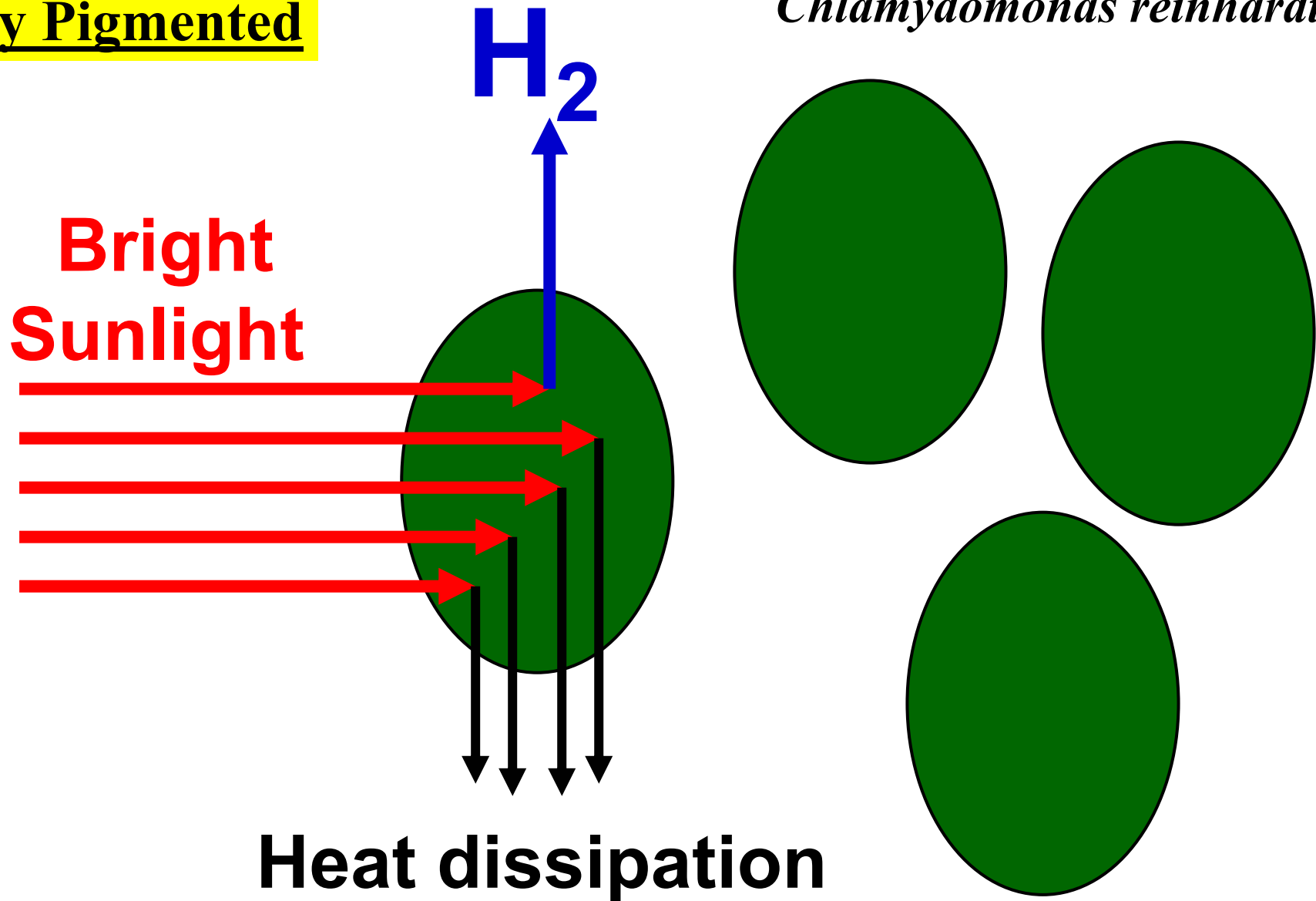


**Hydrogen production  
in a backyard**

*Chlamydomonas reinhardtii* mass culture

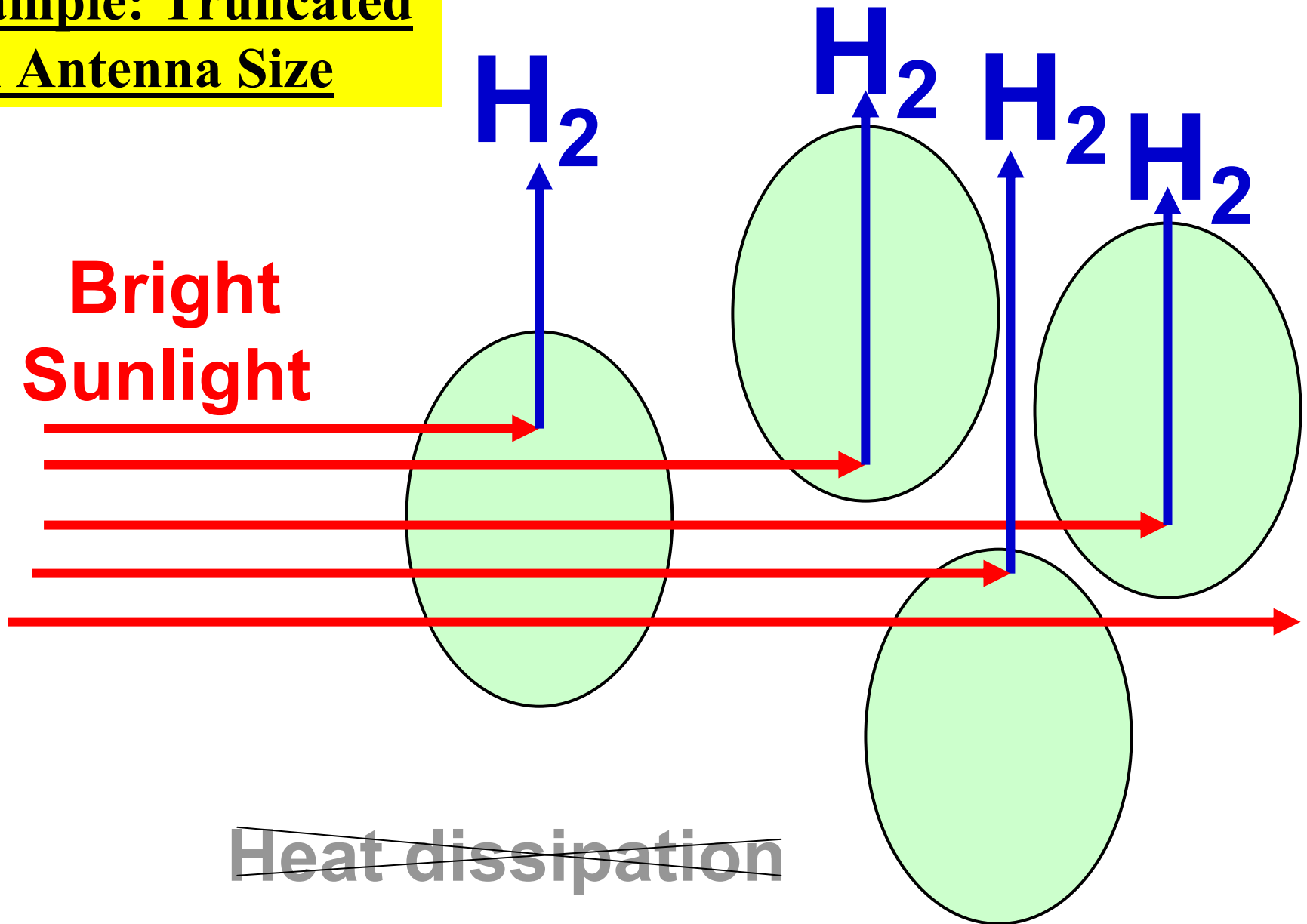
**Example:**  
**Fully Pigmented**

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.**

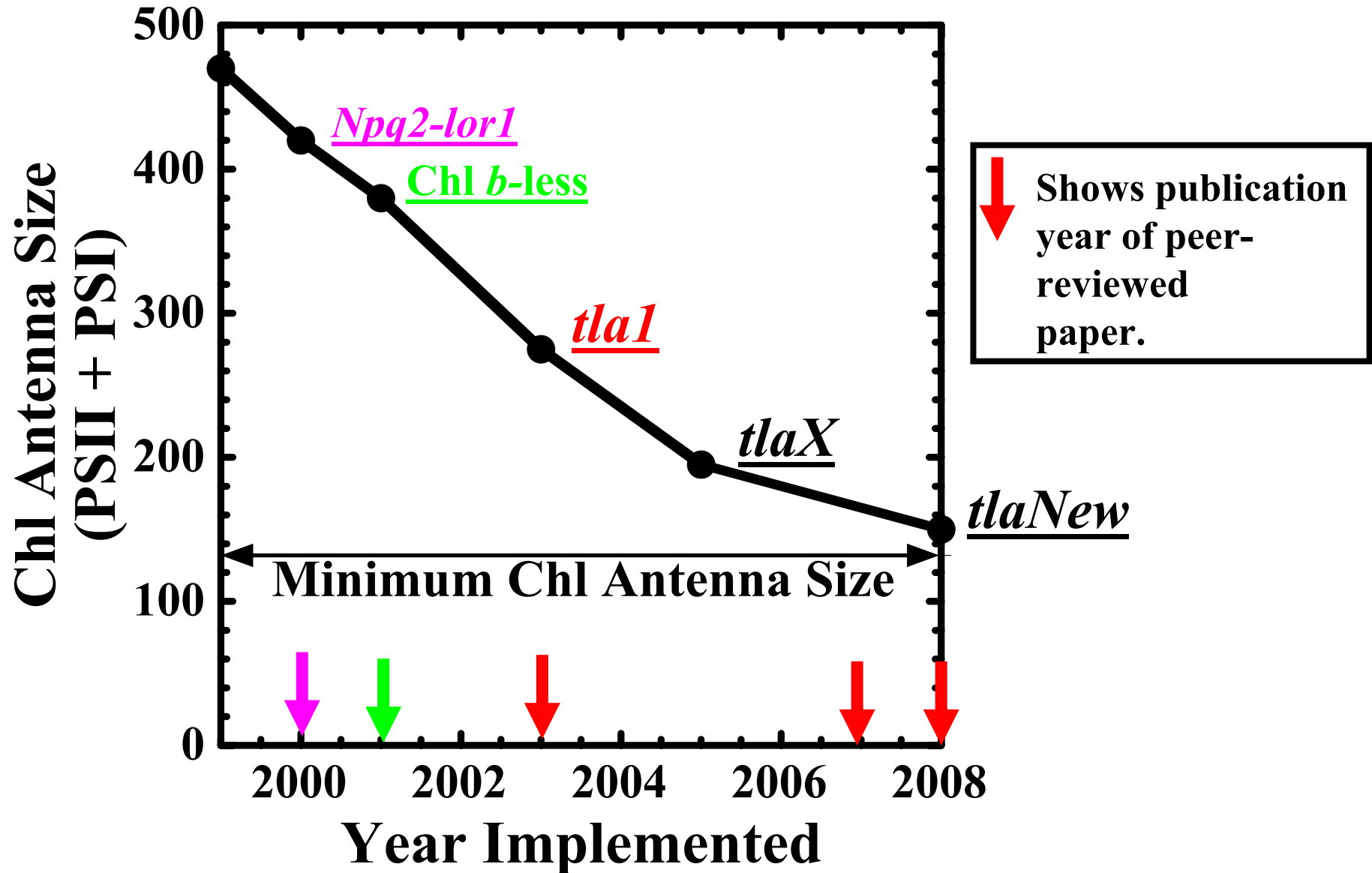
# Technical Barriers and Targets

- **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%**
- **Theoretical maximum efficiency: ~30%**
- **Target for 2010: Reach a 15% Utilization Efficiency of Absorbed Light Energy.**



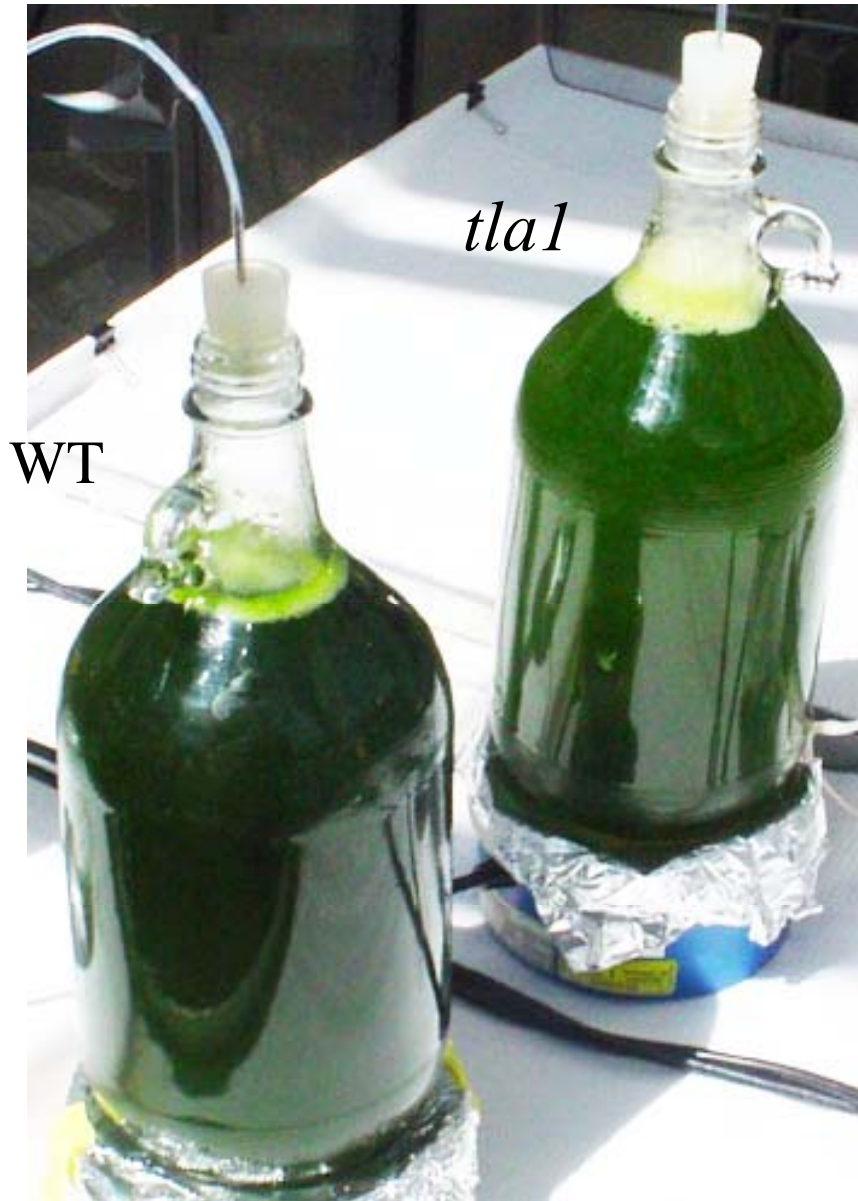
# Project Timeline

## Chlorophyll Antenna Size in Chlamydomonas



# Measurement in Scale-up Cultures

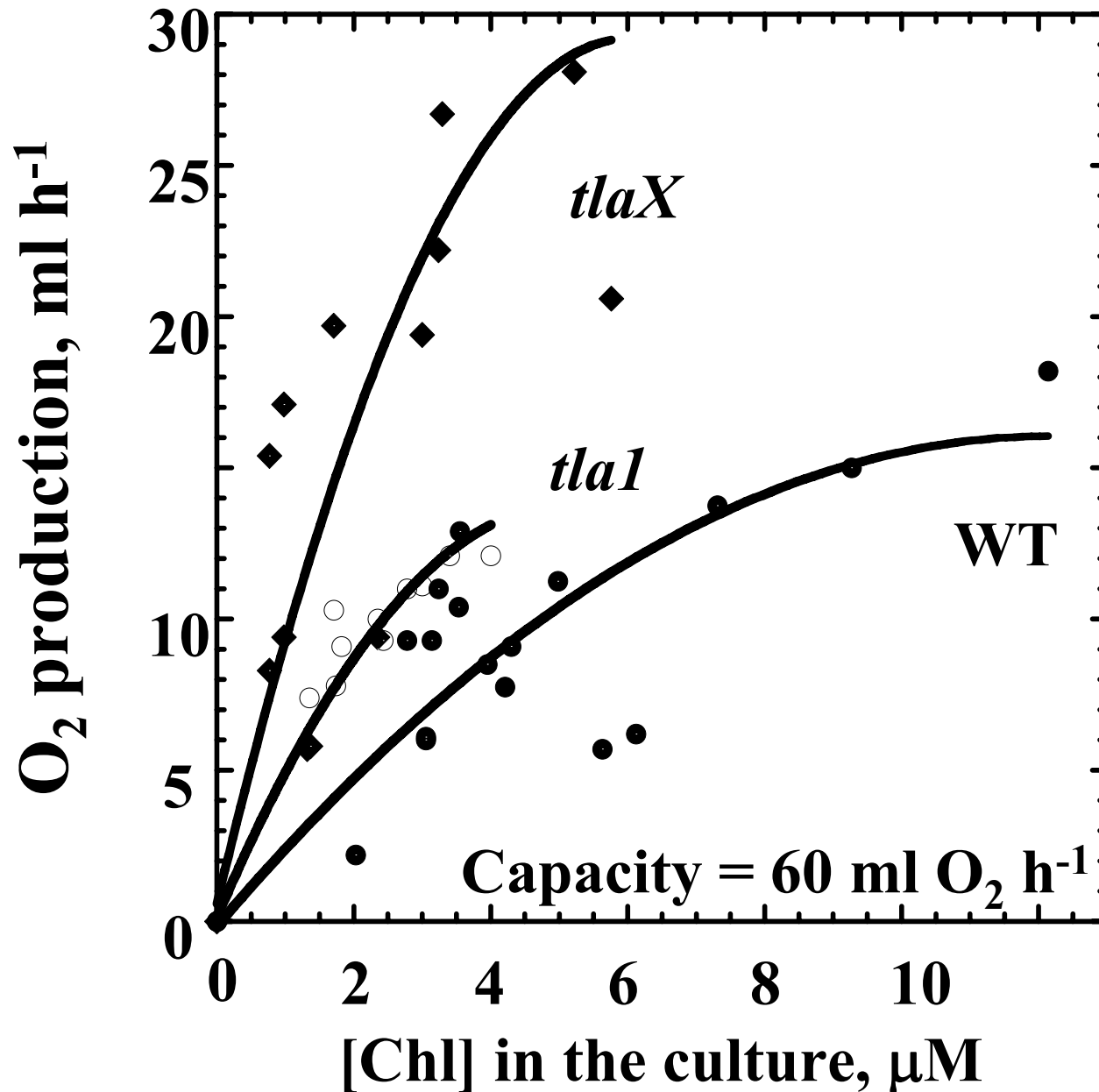
## Cultures in the Greenhouse



<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



# Current Technical Accomplishments

## Analysis of the *tla1*, *tlaX* and *tlaNew* 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.**

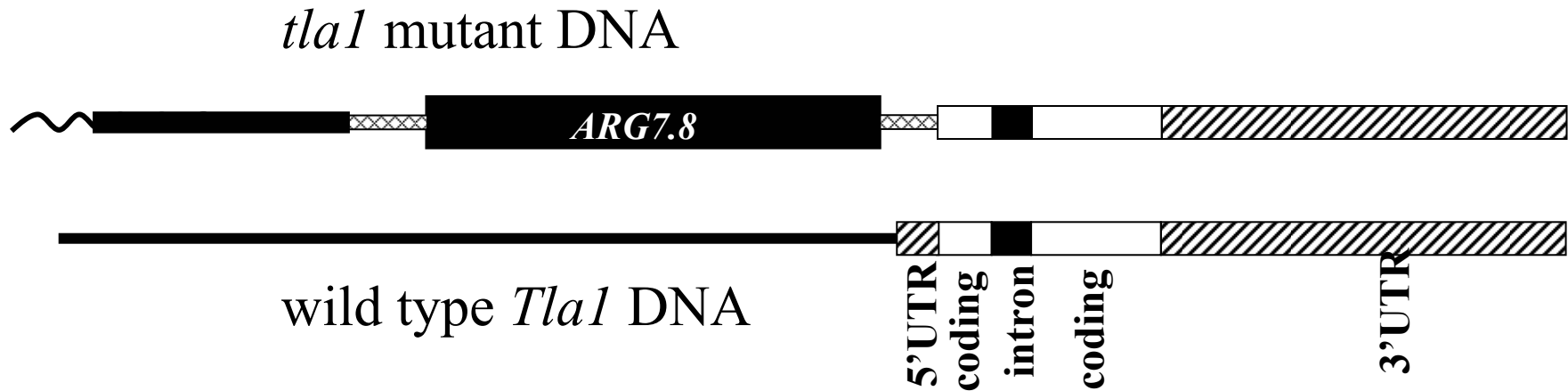
Regulation of the chlorophyll antenna size by the *Tla1* gene completed.

- **Biophysical and biochemical analyses of the *tlaX* and *tlaNew* mutants.**

Chlorophyll antenna size, relative productivity, LHC expression levels.

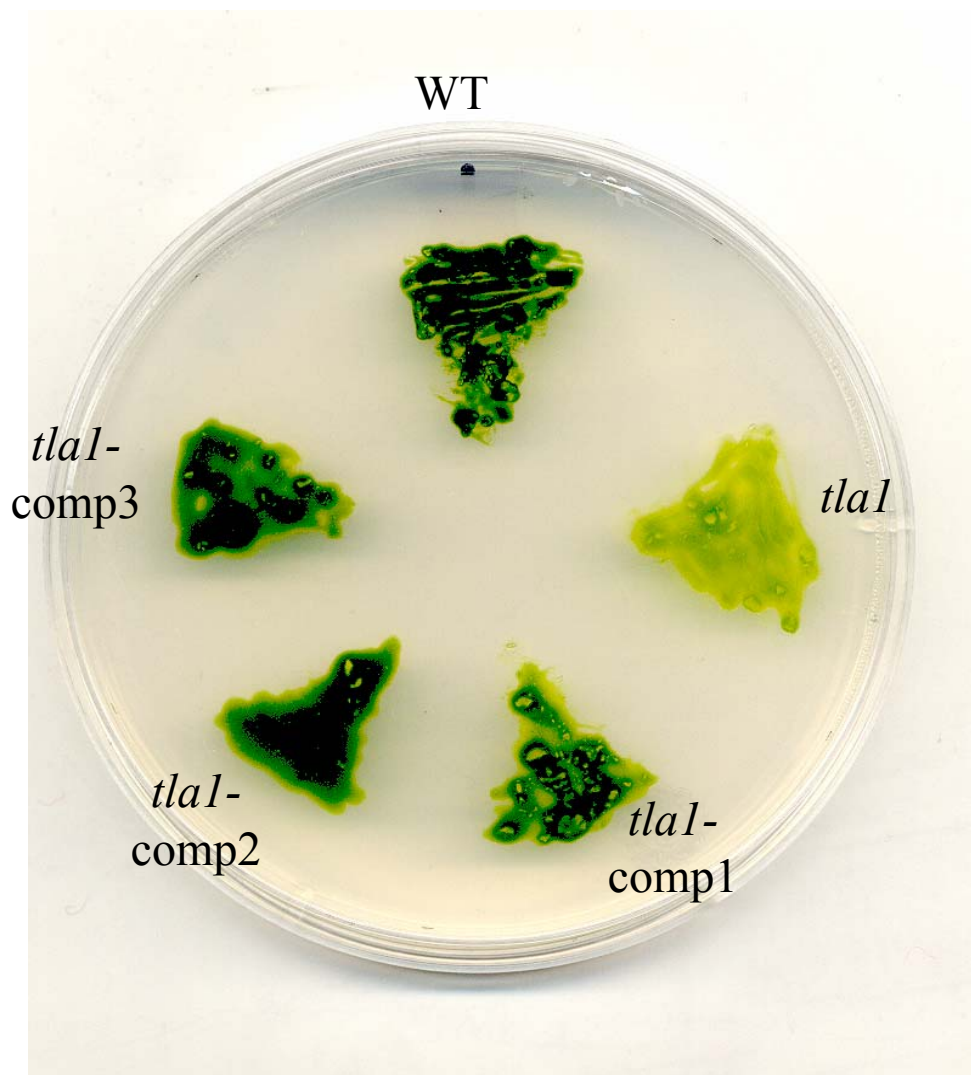
# Current Technical Accomplishments

## 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

*C. reinhardtii* --MT----FSCSADQTALLKILHAHAAKYPSNSVNGVLVGTAKE-----GGSV EILDA  
*A. thaliana* MGMG SNGELKYEISQ NAYIKLV LHS LRHK TAAVNGVLVGRISP---KDDGVVEISDS  
*O. sativa* --MG--AECKYEVAQVAYVKLALHALKHPAAAVNGLLVGRLLDGAASPAAVVSIADAN  
*H. sapiens* --MG-----EVEISALAYVKMCLHAARYPHAAVNGLFLAPAPR---SGEGLCLTDC  
*D. melanogaster* --MC-----DYKVSERAYAKLI FHA AKY PHQA VNGLL LAE KTS---KGSQVEIVDA  
 \* . . \* \*: \*: :: :\*\*\*::: . : : \*

*C. reinhardtii* CHT--TLTLAPALEIGLAQVESYTHITGSVAIVGYYQSDARFGPGDLPPL-GRKIADI  
*A. thaliana* FHS--NLALLPPLEISLIMIEEHYVAQG-LSIVGYFHANERFDDVELCGV-AKNIGDI  
*O. sativa* SHHPHHLPLLPTLELALTIVEDHFAAQG-LAVVGYHANAARRDDADLPPV-AKRVGDI  
*H. sapiens* FHS--HLALSVMLEVALNQVDVWGAQAG-LVVAGYYHANAAVNDQSPGPL-ALKIAGI  
*D. melanogaster* FHQ--CLYVTPMAEVALMLIDAHAEREG-LVIAGYYAAPENFYDNQVDKTPAAKIADI  
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*C. reinhardtii* EHQAQAVVLVLDNKRLEQFCKAQADNP-FELFSKDGSKGWKRASADGG-ELALKNADI  
*A. thaliana* RYFPQAPI LLLNKKLEALS K GKERS PVMQLCVKDASKNWRVVGADGGSKLLLKEPS  
*O. sativa* RNF PRAAV LLLDNKKLEEAVK GKSREP VVQLYTRDSSKSWRQAGSDGSSQLTLKEPS  
*H. sapiens* EFPDAVLI MLDNQKLV P---QPRVPPVIVLENQGLR-W--VPKDKNLVMWRDWEE  
*D. melanogaster* ENFKNACFVVVDN-KLMTLQHDRAAIQVFNC PGDSGAR-W-----SKAKFTLSQASI  
 . \* .::: \* : \* : . . . : \* : . .

*C. reinhardtii* LREEFFVMFKQLKHRTLHDFEEHLDDAGKDWLNKGFASSV-KFLLP----GNAL  
*A. thaliana* VLSDYISSE---KWKDVTVDVDDHLDVTKDWLNPGLFN-----  
*O. sativa* VLADHVTTK---KWQQVVDFDDHLDLISKDWLNPGLLA-----  
*H. sapiens* MVGALLEDR---AHQHLVDFDCHLDDIRQDWTNQRNLNTQITQWVGPTNGNGNA-  
*D. melanogaster* EGVSLLLKRG--AMRDLVDFDNHLDNPNKNTNDFLNQPLNDLQKLY-----  
 . : : \* . : \*\*\* : : \* \* :

# ***Tla1* Hypotheses Investigated**

- **The *Tla1* gene has been recruited by different organisms to perform different functions.**
- **The *Tla1* gene regulates the relationship between nucleus and organelles.**



# Summary of Accomplishments

## Analysis of the *tla1*, *tlaX* and *tlaNew* 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* and *tlaNew* mutant. Encountered difficulties in the molecular analysis of this mutant.**

# Progress achieved vs the DOE targets

Utilization Efficiency of Incident Solar Light Energy,  $E_0 \times E_1$ , %

	<b>2000</b>	<b>2003</b>	<b>2005</b>	<b>2008</b>	<b>2010</b>	<b>2015</b>
<b>Program Targets</b>	<b>3%</b>	<b>10%</b>			<b>15%</b>	<b>20%</b>
<b>Progress</b>	<b>3%</b>	<b>10%</b> <i>tla1</i>	<b>15%</b> <i>tlaX</i>	<b>25%</b> <i>tlaNew</i>		

# 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

**Complete the characterization of the function of the *Tla1* gene and address how can this be applied to other organisms in truncating the Chl antenna size.**

**Employ transformation protocols, such as sense, antisense & RNAi) with the *Tla1* gene to enhance the down-regulation of the Chl antenna size in wild type *Chlamydomonas reinhardtii*.**

# Future Work

**Continue work with the cloning of genes conferring the “truncated Chl antenna” phenotype in strains *tlaX* and *tlaNew*.**

**(Entails molecular, genetic, biochemical, physiological and scale-up studies with these strains.)**

# Summary

- **Completed first part of work on the *Tla1* gene.**
- **Filed patent application on the *Tla1* gene.**
- **UC Berkeley issued non-exclusive license to *Tla1*.**
- **Published findings in peer reviewed journal:**  
Tetali SD, Mitra M and Melis A (2007) Development of the light-harvesting chlorophyll antenna in the green alga *Chlamydomonas reinhardtii* is regulated by the novel *Tla1* gene. *Planta* 225: 813-829
- **Invited presentations on *Tla1* work at the:**
  - **14<sup>th</sup> International Congress on Photosynthesis, Glasgow, Scotland; Symposium on Bioenergy and Photosynthesis.**
  - **91<sup>st</sup> Annual Meeting of the Optical Society of America.**
  - **International Symposium on Material Issues in a Hydrogen Economy.**
  - **University of Nebraska, Lincoln.**