Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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Project ID # PDP11

This presentation does not contain any proprietary or confidential information

Overview

Timeline

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

Budget

- Funding in FY05

DOE: $200 k, UCB: $50 k

- Funding for FY06

DOE: $ 50 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

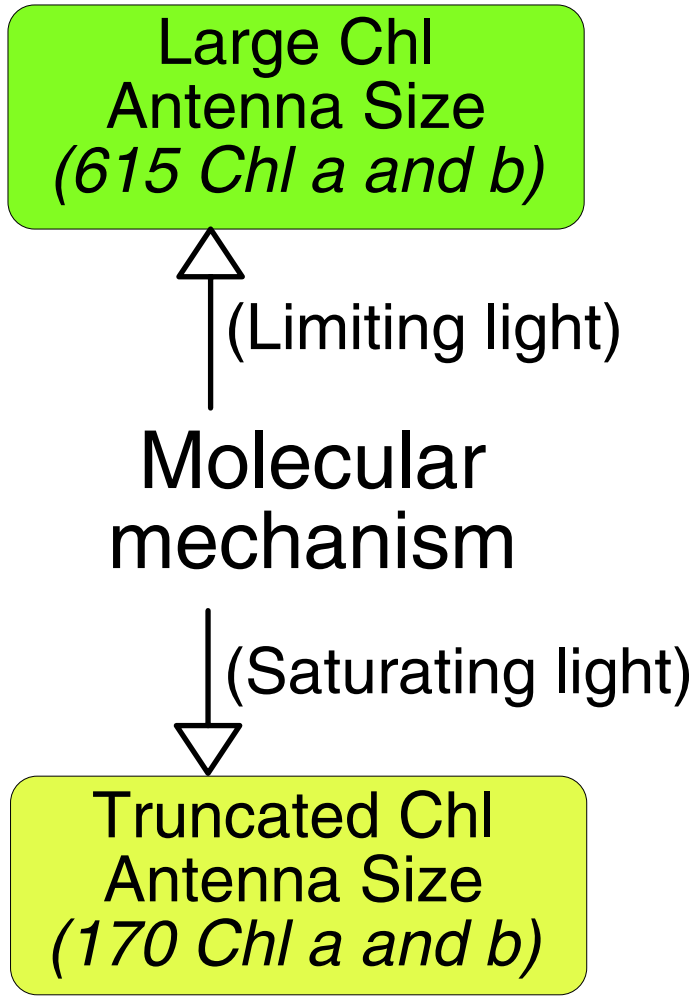
Objective: Minimize the chlorophyll antenna size of photosynthesis to maximize solar conversion efficiency in green algae.

(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.)

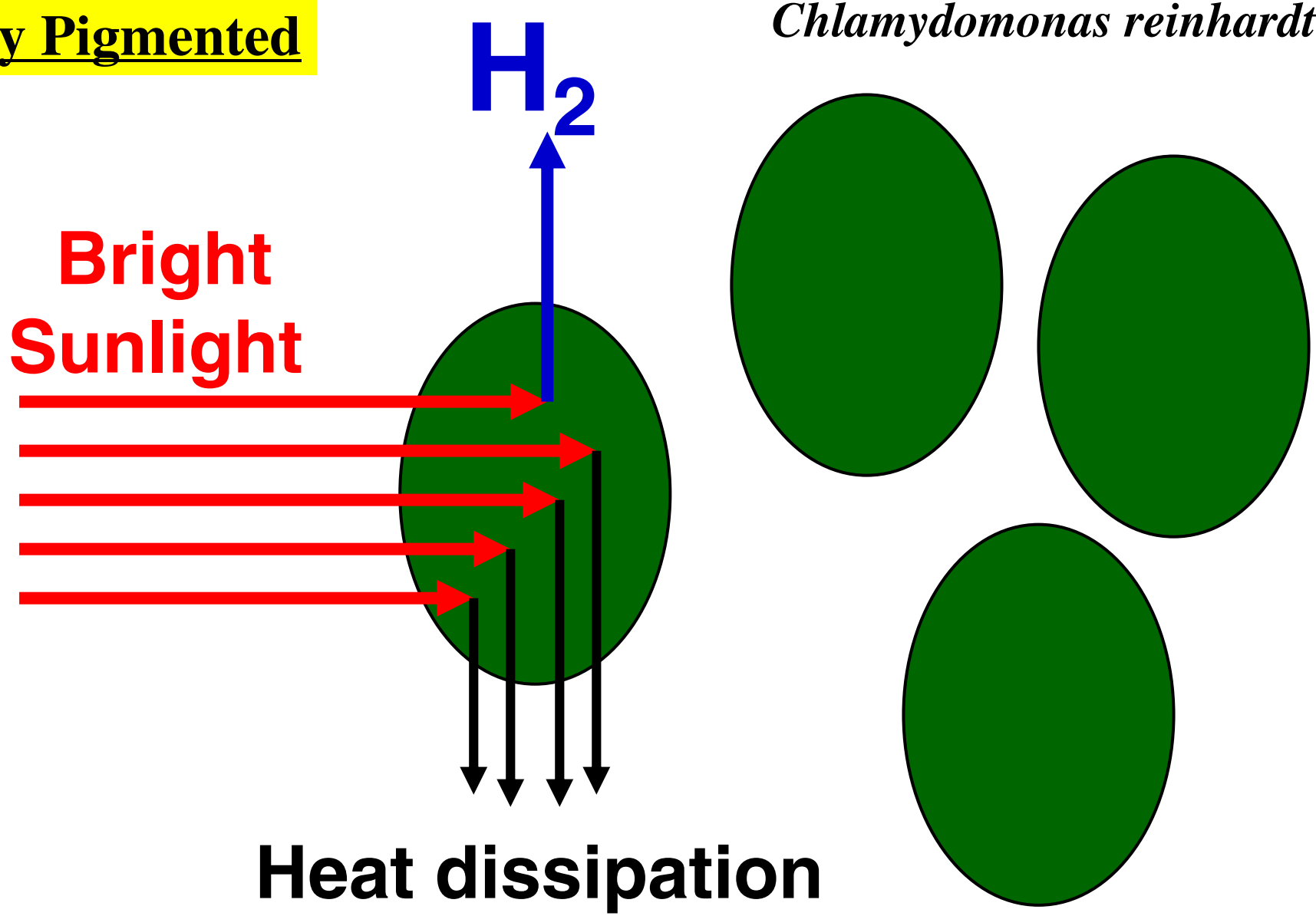
Regulation of the Chl antenna size



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.

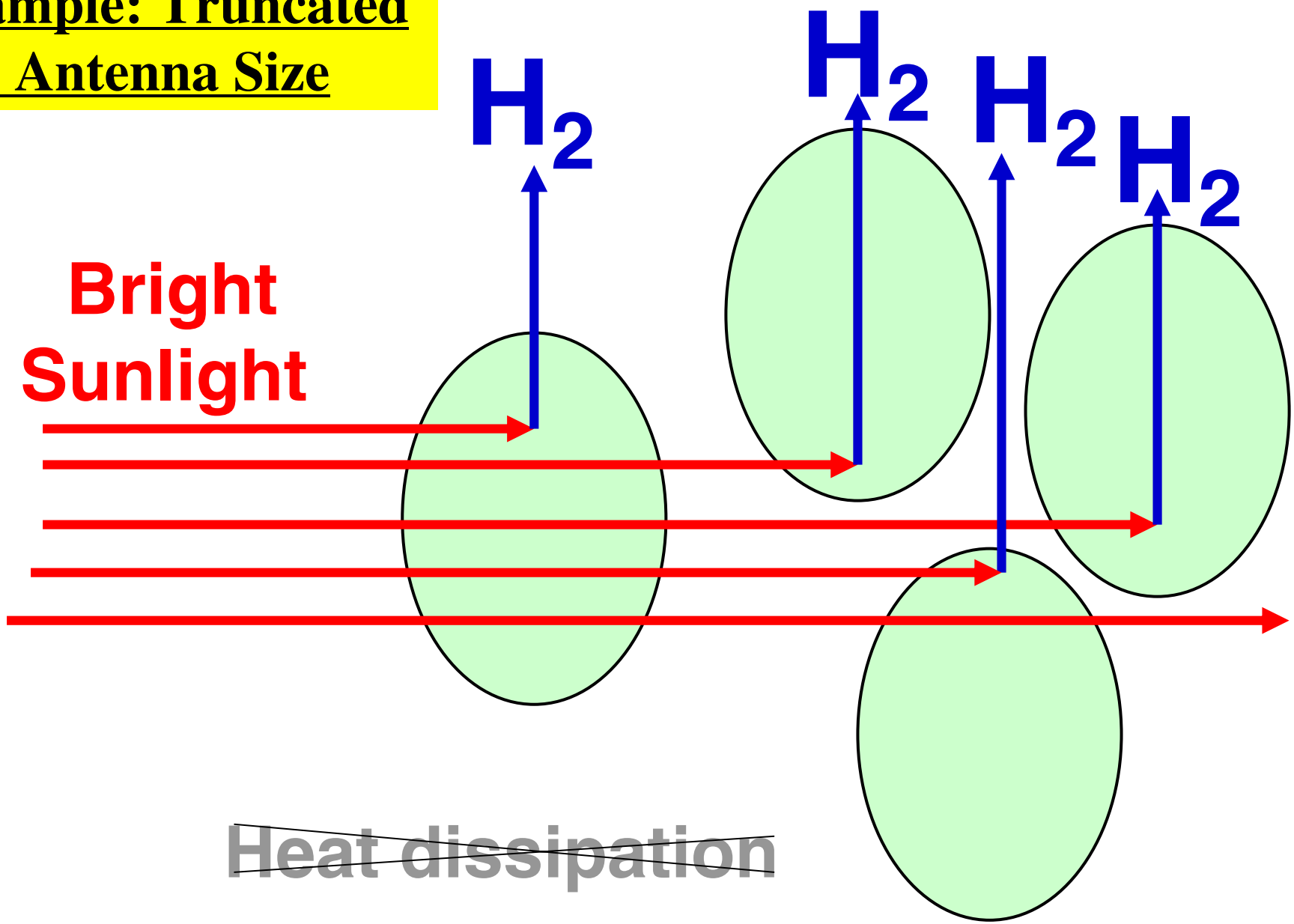
Example:
Fully Pigmented

The green algae
Chlamydomonas reinhardtii



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

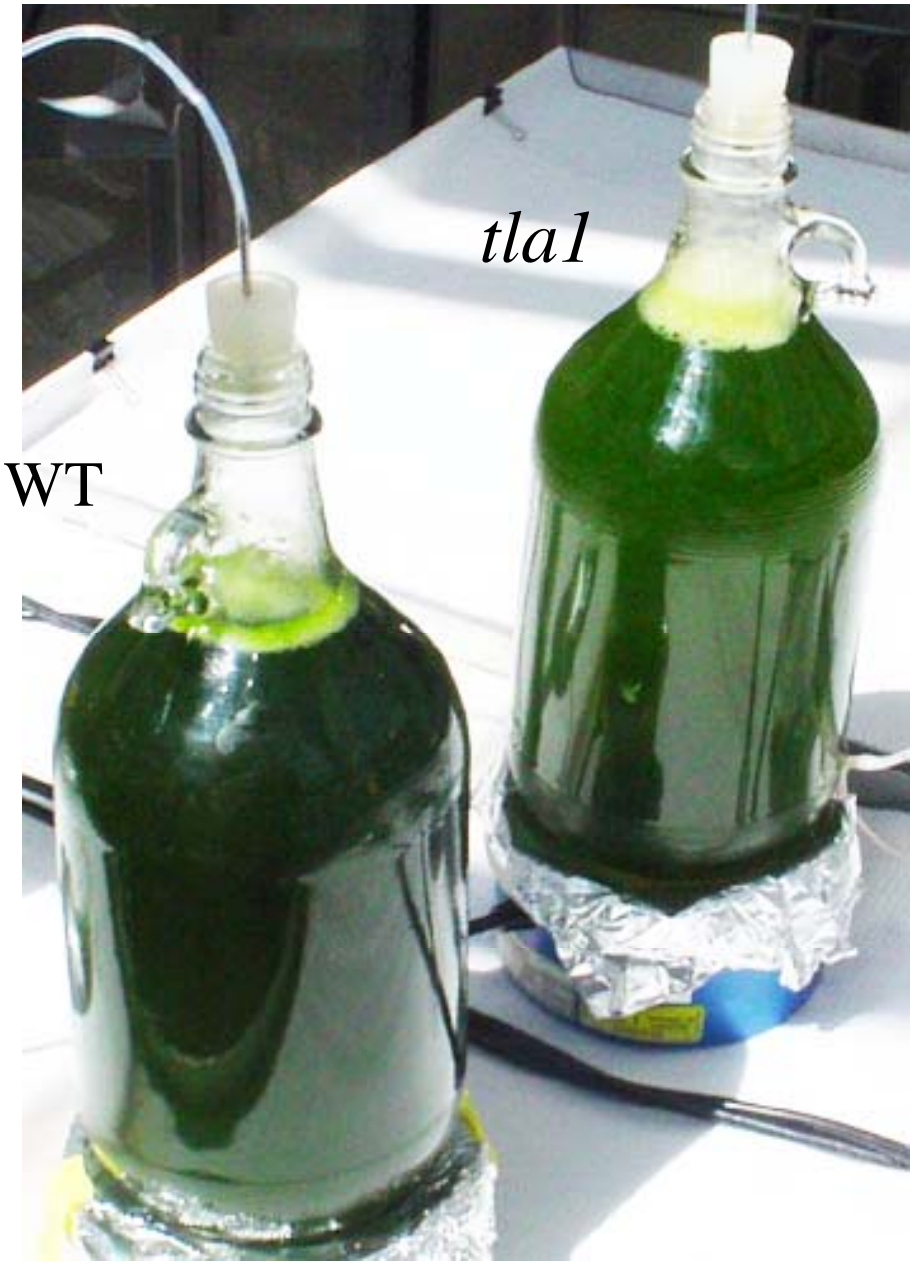
Example: Truncated Chl Antenna Size



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.)

Benefits from this Project

Truncating the Chlorophyll antenna size of microalgae would benefit photobiological:

- **H_2 production,**
- **carbon sequestration,**
- **biomass accumulation,**
- **waste water treatment,**
- **other bio-fuels generation.**

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%

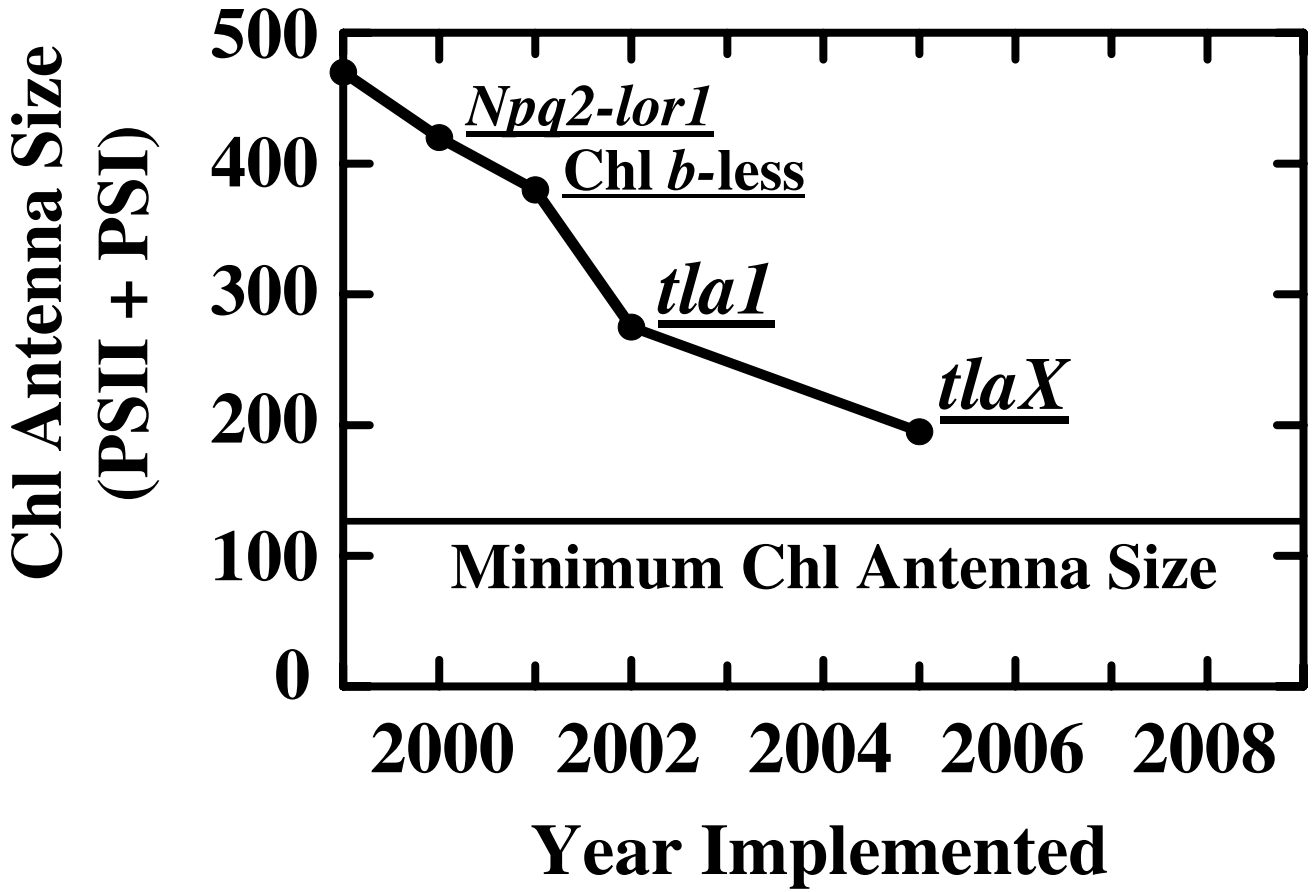
2005 Year Accomplishment

- ***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%

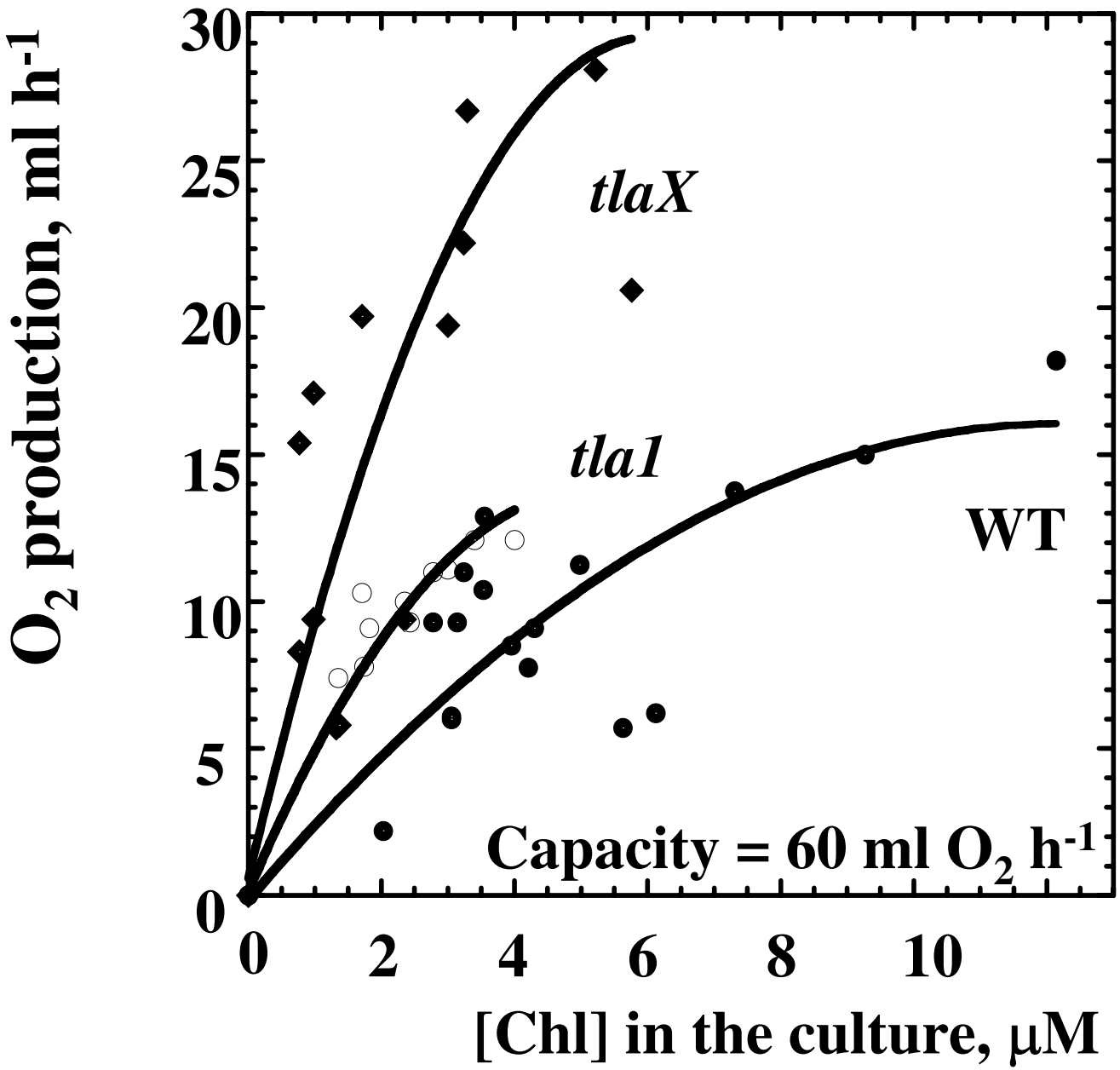
- **Long-term goal: 132 Chl molecules (28% of control)**
  (PSII=37; PSI=95)
  Photon use efficiency of photosynthesis *goal* = ~60%
  Utilization Efficiency of Absorbed Light Energy *goal*: ~30%

Project Timeline

Chlorophyll Antenna Size in Chlamydomonas



Productivity in Scale-up Cultures



Current Technical Accomplishments

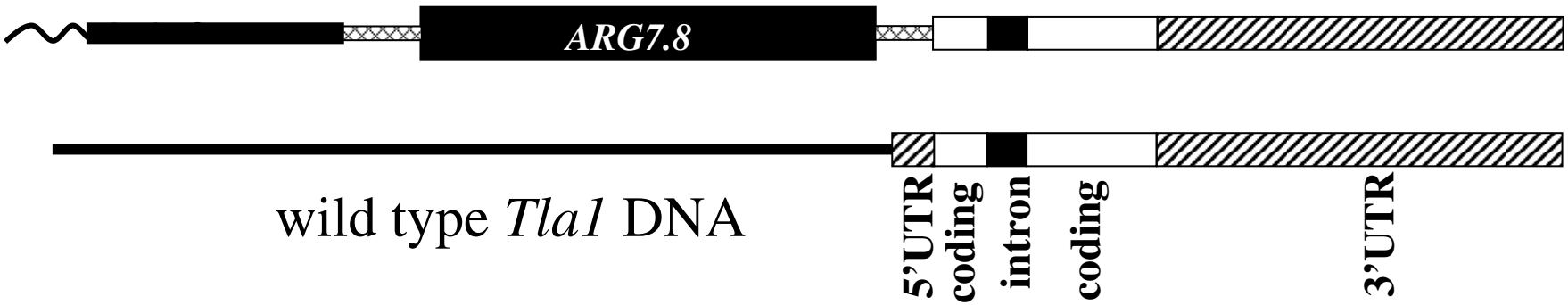
Analysis of the *tla1* and *tlaX* mutants

- **Molecular analysis of the *tla1* mutation.**
  - DNA insertion site in the *tla1* mutant has been mapped.
  - Genomic, cDNA and protein sequences for the *Tla1* gene are at hand.
  - 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 completed.
- **Functional analysis of the *Tla1* gene.**
  - Regulation of the chlorophyll antenna size by the *Tla1* gene initiated.
- **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

tla1 mutant DNA



5' RACE from WT

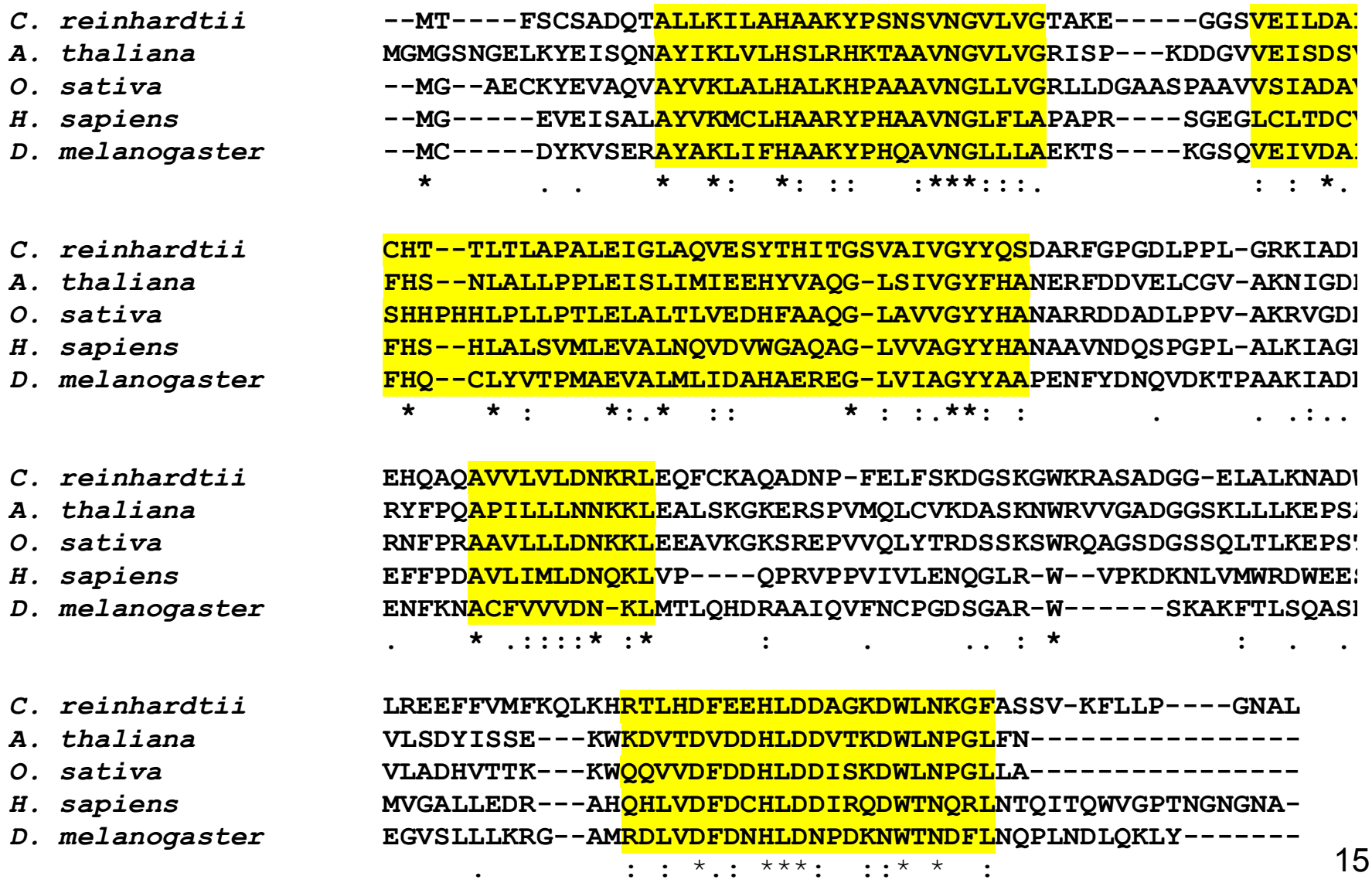
GATGTCGTGTTGACTTTGCGTTACAACCGTGAAGTATATTAGAACTCATTTGCCTGCC
ACAACCTCAGACCAAGAGACGCGCGAAAAACTGACACG**ATG**ACTTTCAGCTGCTCC
GCTGACCAAASCGCGCTCTTAAAGATTCTTGCACACGCGGCTAAGTATCCATCAAAT
AGCGTGAATGGTGTCCTCGTCGGGACAGCGAAGGAGGGCGGCTCTGTCGAAATCCT
GGACGCGATTCCACTGTGTCACACGACGCTGACCCTGGCGCCAGCACTGGAGATAG
GTCTCGCCCAGGTGGAGTCCTACACGCATATCACGGGCAGCGTGGCGATTGTGGGCT
ACTACCAATCAGACGCACGTTTCGGCCCCGGG

5' RACE from *tla1*

acgccatagtgactggcgatgctgtcggaatgga
cgatatcccgcaagaggcccggcagtaccggcataaccaagcctatgcctacagcatccagggtgacggtgccgaggatgacgatgag
cgcattgttagattccatacacggtgcctgactgcgttagcaatttaactgtgataaactaccgc**ATG**ACTTTCAGCTGCTCC
GCTGACCAAACCGCGCTCTTAAAGATTCTTGCACACGCGGCTAAGTATCCATCAAAT
AGTGTGAATGGTGTCCTCGTCGGGACAGCGAAGGAGGGCGGCTCTGTCGAAATCCT
GGACGCGATTCCACTGTGTCACACGACGCTGACCCTGGCGCCAGCACTGGAGATAG
GTCTCGCCCAGGTGGAGTCCTACACGCATATCACGGGCAGCGTGGCGATTGTGGGCT
ACTACCAATCAGACGCACGTTTCGGCCCCGGG

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.**
- **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.**

Responses to Previous Year Reviewers Comments

Specific recommendations and additions/deletions to the work scope, made by the reviewers.

- **Comment: Document progress against the DOE targets as the project proceeds.**
- Response: A Table has been added (slide #18) that documents progress achieved against the DOE targets, as requested.

- **Comment: Develop a working hypothesis for Tla1 function.**
- Response: We are preparing a publication on the Tla1 project. (This manuscript will probably be in the peer-review process by the time of the DOE Hydrogen Program meeting.) The paper reports on the molecular and functional aspects of the *Tla1* gene and how it applies in the regulation of the Chl antenna size. We would prefer not to discuss it until the end of the outside peer review process.

Progress achieved *vs* the DOE targets

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

	2000	2003	2005	2010	2015
Program Targets	3%	10%		15%	20%
Progress	3%	10%	15%		

Current Work

Functional and regulation analysis of the *Tla1* gene

Investigation of the regulation of the chlorophyll antenna size by the *Tla1* gene

- In the wild type, expression levels of the *Tla1* gene are being tested as a function of increasing irradiance.
- In the *tla1* mutant, expression levels of the Tla1 protein are being investigated as a function of increasing irradiance, response compared to that of the WT.
- Expression levels of the *Tla1* gene are being correlated with that of the *Lhcb* and *Cao* genes, known to affect the Chl antenna size. Coordination of gene expression is being assessed.

These and related measurements will help define approaches by which to genetically apply the *Tla1* gene in minimizing the Chl antenna size of microalgae.

Future Work - 1

Perform functional analysis of the *Tla1* gene (how does it work?)

- **Investigate levels of expression of the *Tla1* gene as a function of growth irradiance in wild type and *tla1* mutant.**
- **Investigate cellular localization of the Tla1 protein; measure levels of Tla1 protein as a function of irradiance in wild type and *tla1* mutant.**
- **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.***
- **Probe for the presence of the *Tla1* gene (Southern blot) and of the Tla1 protein (Western blot) in other H_2-producing unicellular green algae (*Scenedesmus* and *Chlorella*).**

Future Work - 2

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 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 enhanced or suppressed levels of *TlaX* gene expression result 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.***
- **Probe for the presence of the *TlaX* gene (Southern blot) and of the TlaX protein (Western blot) in other H_2-producing unicellular green algae (*Scenedesmus* and *Chlorella*).**

Future Work - 3

Reports, Publications and Meetings

- **Included are quarterly and annual reports to the DOE Hydrogen Program, attending meetings of the Photobiological Hydrogen Production Working Group, the Annual DOE Hydrogen Program Peer Review meeting, and publication of the results from this work in peer-reviewed journals.**

Publications and Presentations

1. **Melis A (2005) Bioengineering of green algae to enhance photosynthesis and hydrogen production. Chapter 12 in *Artificial Photosynthesis: From Basic Biology to Industrial Application,* AF Collins and C Critchley (eds.), Wiley-Verlag & Co., pp. 229-240.**
2. **White AL and Melis (2006) Biochemistry of hydrogen metabolism in *Chlamydomonas reinhardtii* wild type and a Rubisco-less mutant. Intl. J. Hydrogen Energy 31: 455-464.**
3. **Melis A, Zhang L, Benemann JR, Forestier M, Ghirardi ML, Seibert M (2006) Hydrogen production using hydrogenase-containing oxygenic photosynthetic organisms. United States Patent 6,989,252 B2 (issued 24-Jan-2006).**

1A. Mitra M, Kanakagiri, SD and Melis A (2005) Chlorophyll antenna size adjustments in Chlamydomonas reinhardtii involve coordinate regulation of Tla1, CAO and Lhcb gene expression. Plant Biology 2005, Abstract # 236 in the American Society of Plant Biologists 2005 Annual Meeting Program Book. p. 142

2A. Melis A (2005) Integrated photobiological H-2 production. Society for Industrial Microbiology and Biotechnology (SIM) Annual Meeting Program and Abstracts. Abstract S43, p. 65

3A. Mitra M, Kanakagiri SD and Melis A (2006) Chlorophyll antenna size adjustments by irradiance in Chlamydomonas reinhardtii involve coordinate regulation of Tla1, CAO and LhcB gene expression. 15th Western Photosynthesis Conference 5-8 January 2006 book of abstracts. p. 22-23

Critical Assumptions and Issues

- **<u>Question</u>: How could others benefit from these advances, when, for example, generation of microalgae with a "truncated Chl antenna size" is needed?**

- **<u>Answer</u>: The end-point of this research is discovery of novel genes and elucidation of how they function to define the Chl antenna size in the model organism *Chlamydomonas reinhardtii*. This know-how could be applied by anyone to *Chlamydomonas reinhardtii,* and/or to other unicellular green algae of interest to the DOE Hydrogen Program, to recreate the "truncated Chl antenna size" mutation. Thus, our elucidation of gene function for the regulation of the Chl antenna size in microalgae will be a lasting contribution to this field.**