Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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Monday 23 May 2005

Project ID # PDP29

This presentation does not contain any proprietary or confidential information

Overview

Timeline

- **Start: January 2005**
- **End: December 2008**
- **Completion: 40%**

Budget

- Funding in FY04

DOE: $200 k, UCB: $50 k

- Funding for FY05

DOE: $200 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

- NREL, ORNL, DaimlerChrysler

Objectives and Approach

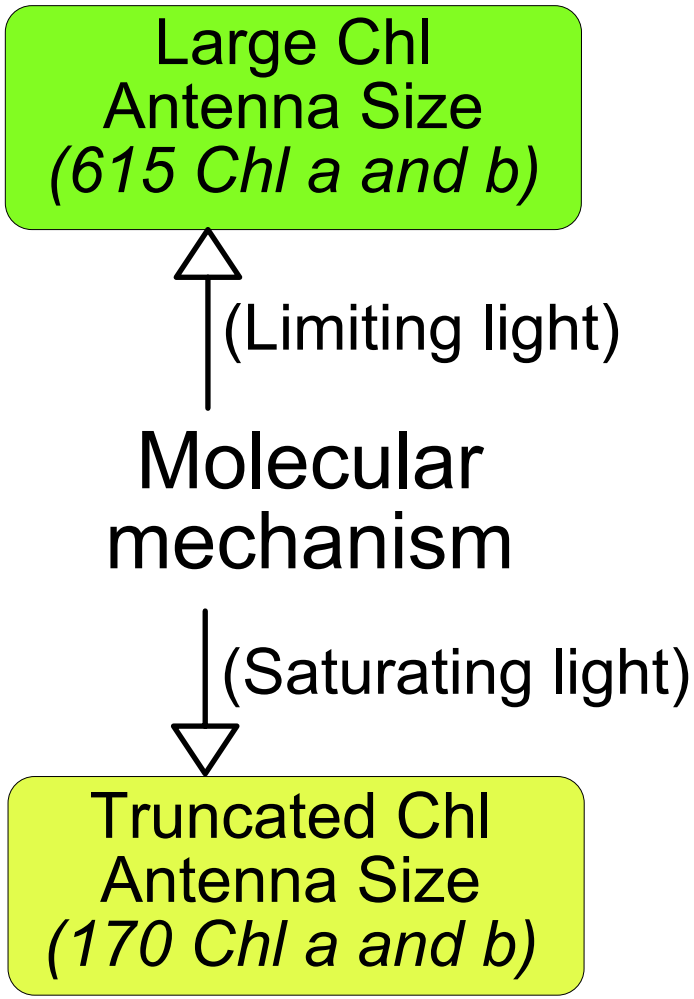
Objective: Minimize the chlorophyll antenna size of photosynthesis to maximize solar conversion efficiency in green algae.

(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.

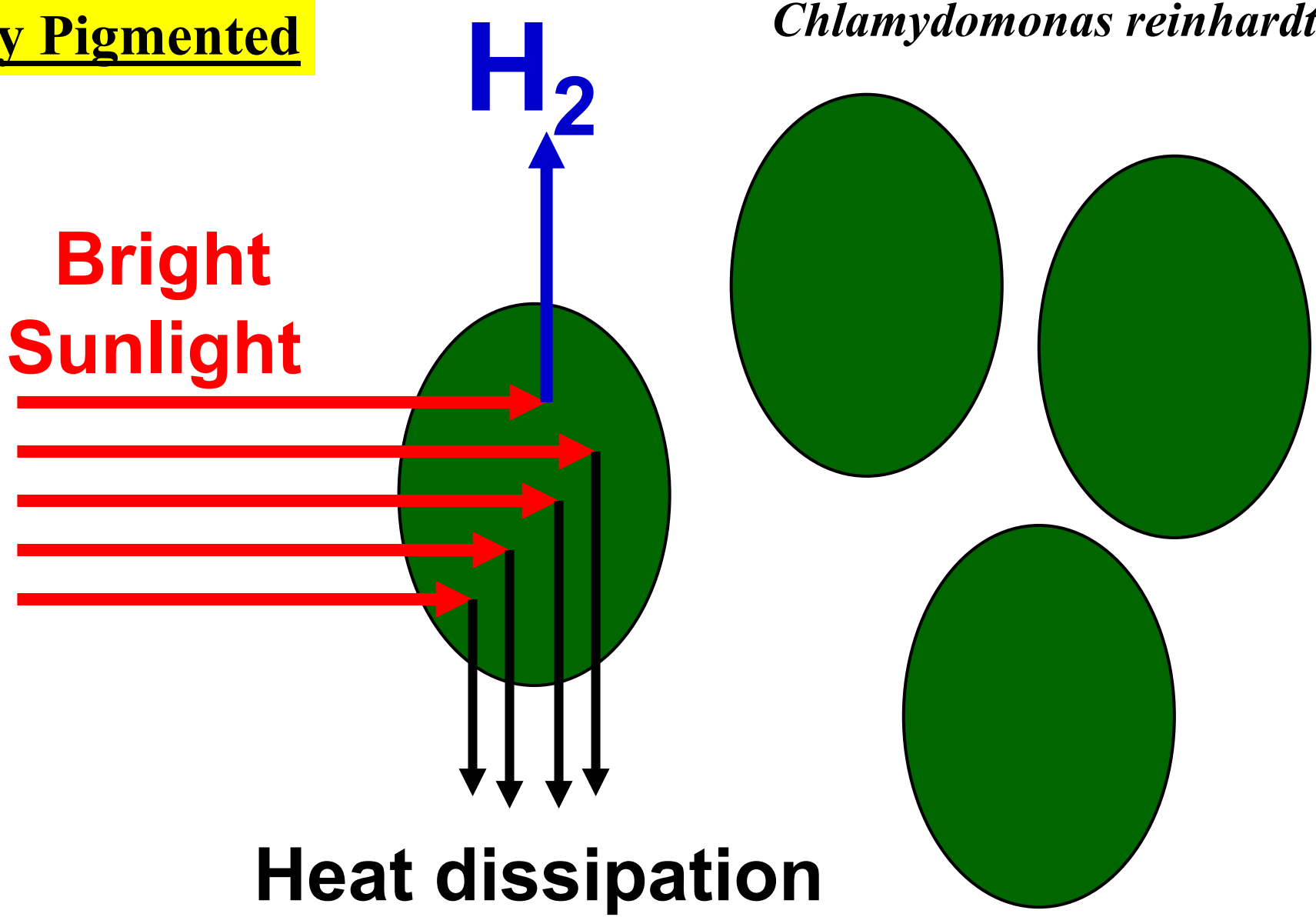
(Employ DNA insertional mutagenesis and high-throughput screening to isolate tagged green algae with a smaller Chl antenna size.)

Regulation of the Chl antenna size

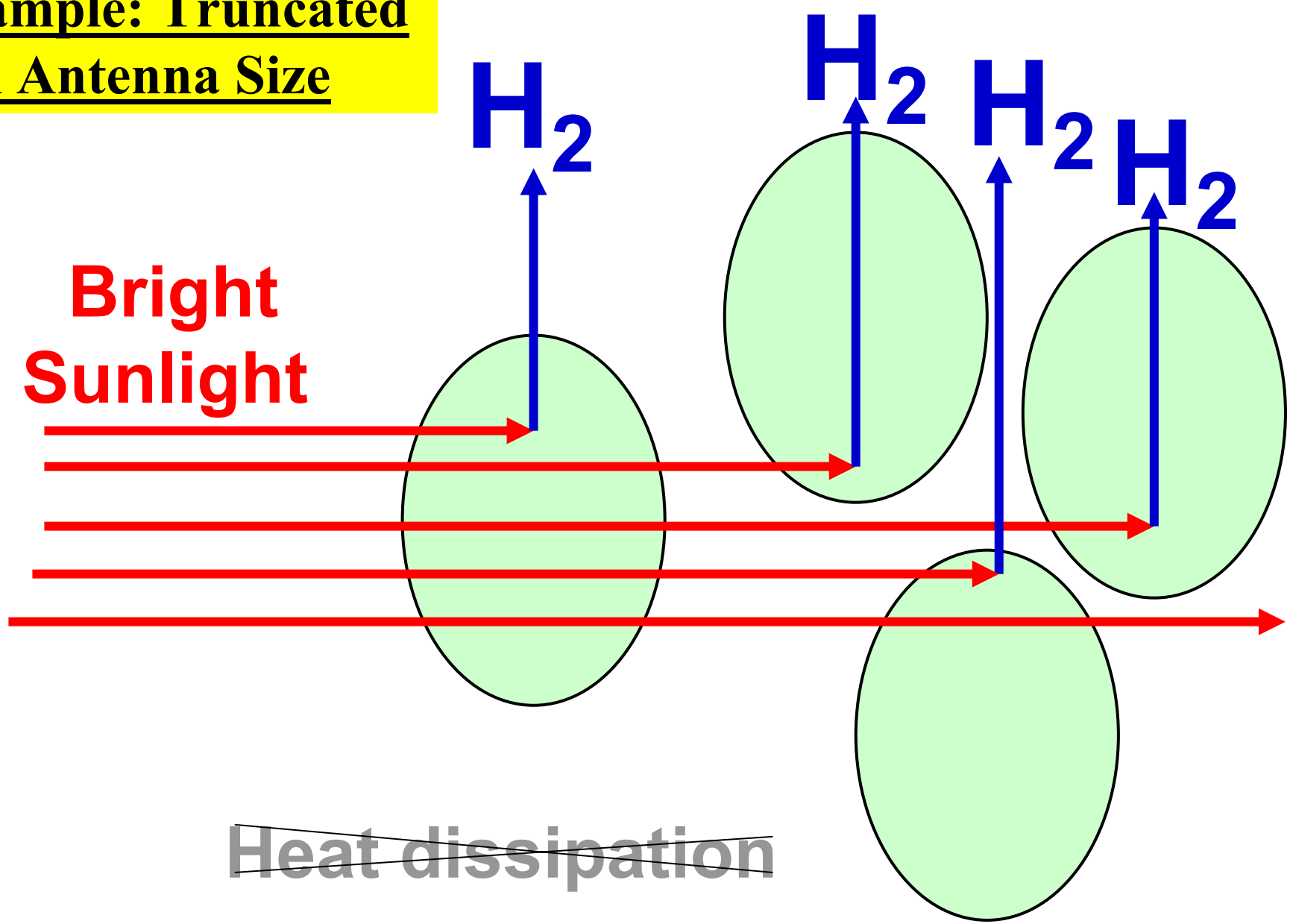


Example:
Fully Pigmented

The green algae
Chlamydomonas reinhardtii

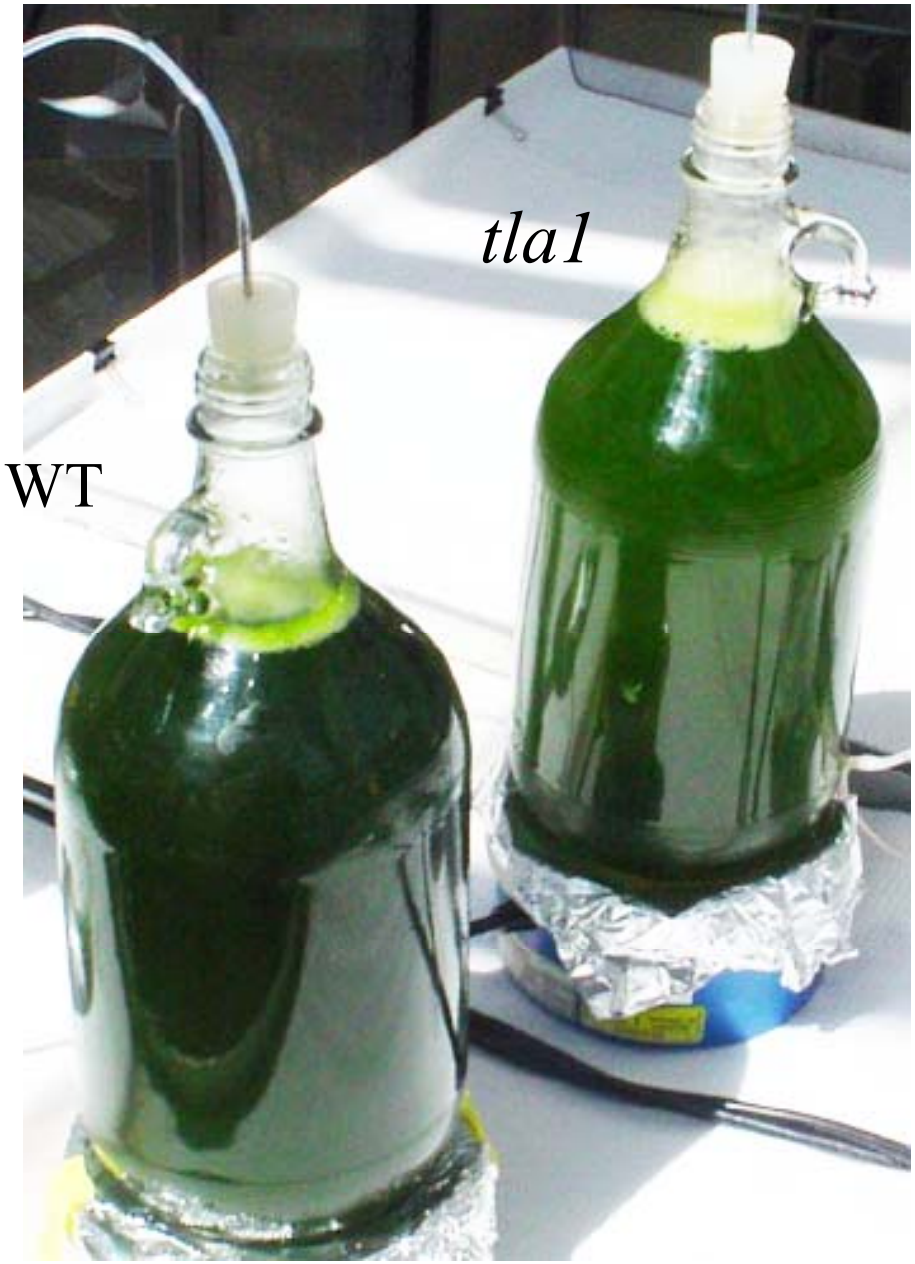


Example: Truncated Chl Antenna Size



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

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 2004: 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%

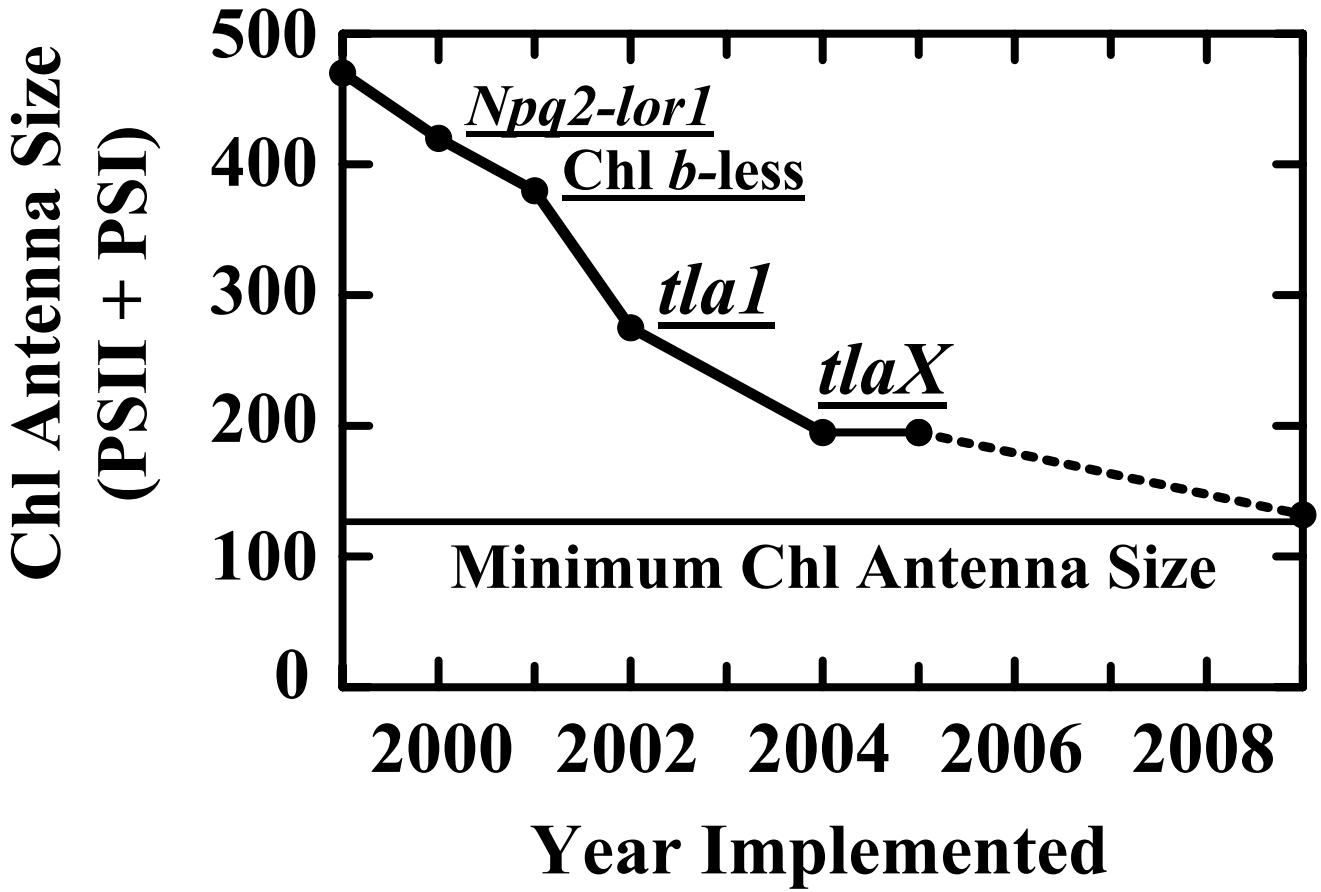
2004 Year Accomplishment

- ***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%

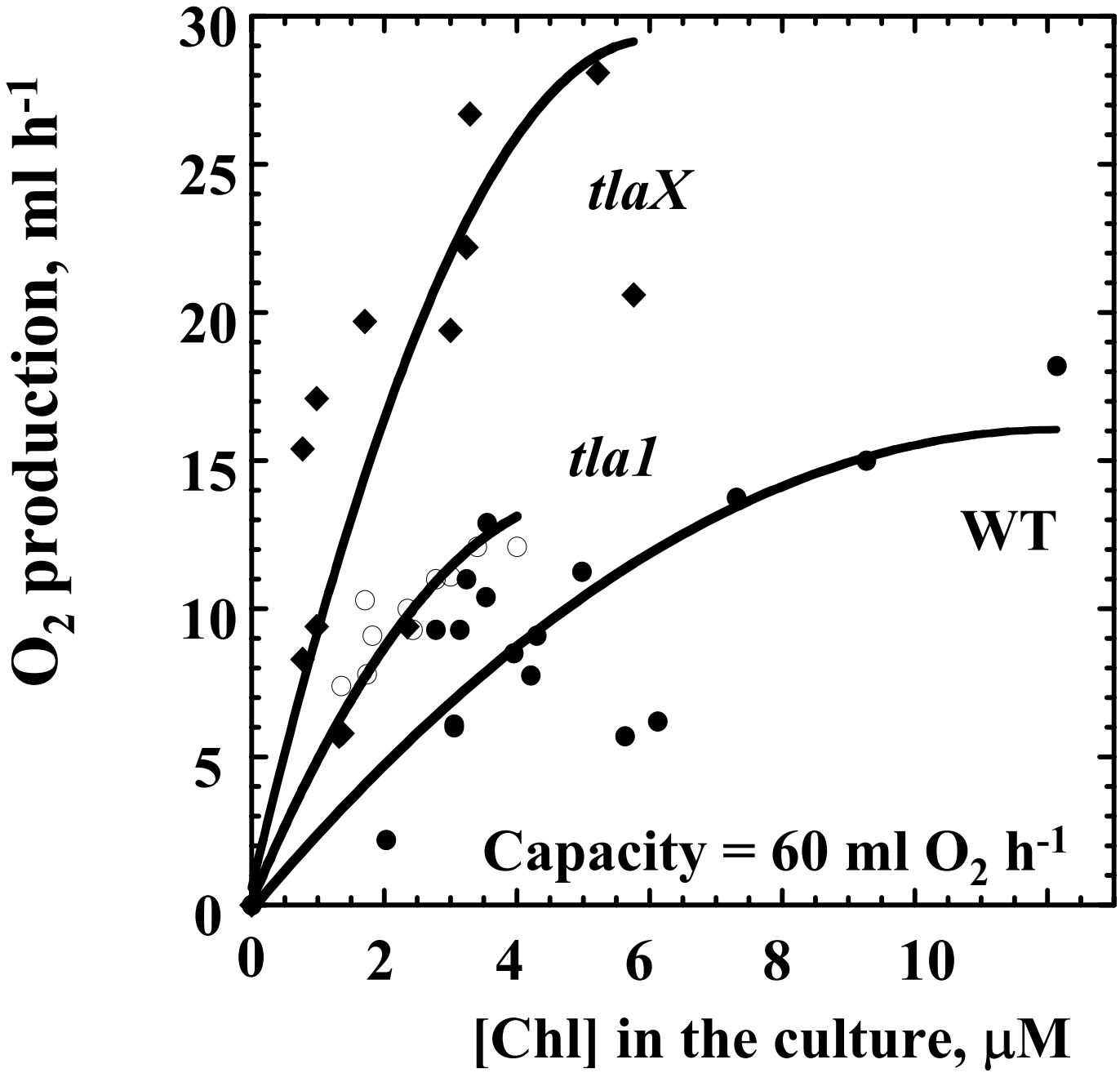
- **Long-term goal: 132 Chl molecules (28% of control)**
  (PSII=37; PSI=95)
  Photon use efficiency of photosynthesis *goal* = ~60%
  Utilization Efficiency of Absorbed Light Energy *goal*: ~30%

Project Timeline

Chlorophyll Antenna Size in Chlamydomonas



Productivity in Scale-up Cultures



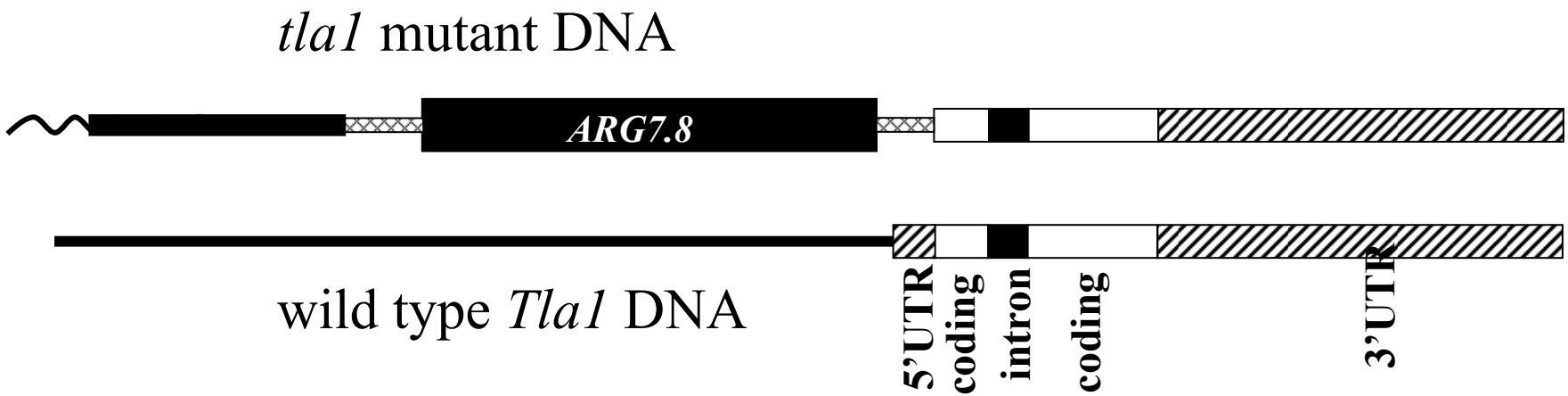
Current Technical Accomplishments

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 in progress.
- **Biochemical analysis of the *tla1* mutation.**
  - Antibodies against the Tla1 protein are being 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.
- **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



5ÕRACE from WT

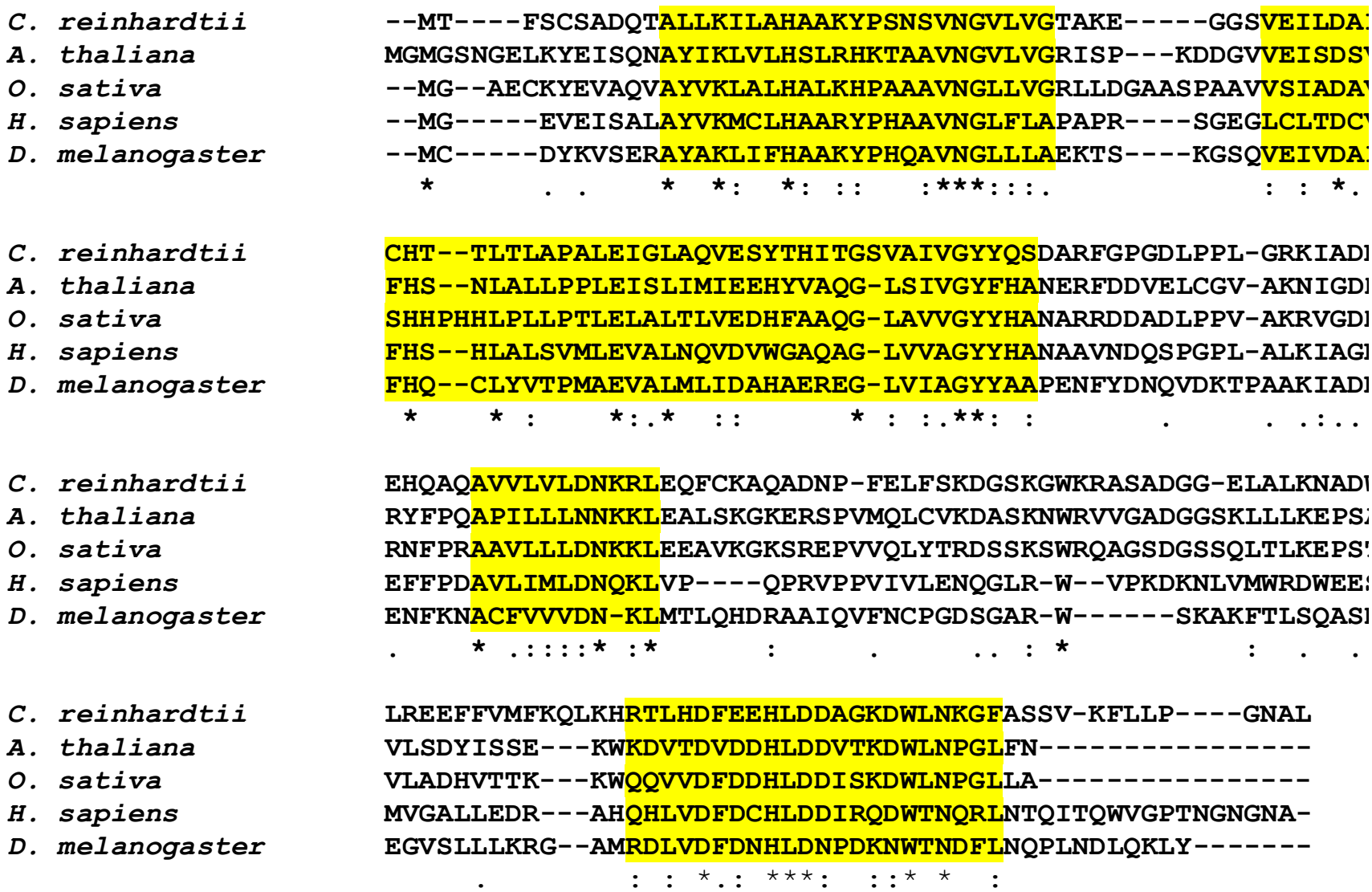
GATGTCGTGTTGACTTTGCGTTACAACCGTGAAGTATATTAGAACTCATTTGCCTGCC
ACAACCTCAGACCAAGAGACGCGCGAAAAACTGACACG**ATG**ACTTTCAGCTGCTCC
GCTGACCAAASCGCGCTCTTAAAGATTCTTGCACACGCGGCTAAGTATCCATCAAAT
AGCGTGAATGGTGTCCTCGTCGGGACAGCGAAGGAGGGCGGCTCTGTCGAAATCCT
GGACGCGATTCCACTGTGTCACACGACGCTGACCCTGGCGCCAGCACTGGAGATAG
GTCTCGCCCAGGTGGAGTCCTACACGCATATCACGGGCAGCGTGGCGATTGTGGGCT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

5ÕRACE from *tla1*

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

Current Technical Accomplishments

Sequence homologies for the Tla1 protein



Responses to Previous Year Reviewers Comments

- ***Systems analysis and engineering should be included***

  This is being done in concert with the other two Photobiological Hydrogen production projects at NREL and ORNL (see http://www.nrel.gov/docs/fy04osti/35593.pdf).

- ***PI should play team leadership role***

  Devised the concept and helped draft the experimental roadmap of the "**Integrated Biological Hydrogen Production**" multiyear plan for the DOE HFCIT program.

- ***Combine the four separate mutations as highest priority***

  This is being planned, requires a full-time geneticist who would perform the crosses and successfully analyze the offspring (a difficult position to fill).

- **Consider second row of mutations and screen on best mutant "*tlaX*"**

  Although contemplated, the high throughput screening of *tlaX* mutants for strains with an even smaller Chl antenna size may be difficult to do. It may be more practical to mutagenize and screen wild type green algae and then, to combine the genetic properties of *tla*-type mutants.

- **Need to more closely relate alga types (mutant vs wild type) in H_2 as well as O_2 production**

  Productivity measurements on the basis of biomass and O_2 are routinely conducted at Berkeley in wild type and each of the *tla* mutants. The sulfur-deprivation method is not suitable for the measurement of H_2-production by the *tla* mutants. Until an alternative steady-state method of green algal H_2-production can become available, only the ORNL "continuous sparging with He" method is suitable for testing the *tla* mutants.

Current Work

Functional and regulation analysis of the *Tla1* gene

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

- Expression levels of the *Tla1* gene increase with the level of irradiance.
  (**leads to smaller Chl antenna size**)
- In the *tla1* mutant, levels of *Tla1* gene transcripts are higher than in the WT.
  (**leads to smaller Chl antenna size**)
- When expression level of the *Tla1* gene is high, expression levels of the *Lhcb* and *Cao* genes are low and Chl-protein content is also low.
  (**leads to smaller Chl antenna size**)

Tentative conclusion

- Enhancing the expression level of the *Tla1* gene minimizes the Chl antenna size of photosynthesis.

Application Hypothesis

- Over-expression of the ubiquitous *Tla1* gene would Maximize Photosynthetic Efficiencies and Hydrogen Production in Microalgal Cultures.

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.**
- **Raise specific polyclonal antibodies against the Tla1 protein; investigate cellular localization of the Tla1 protein; measure levels of Tla1 protein as a function of irradiance in wild type and *tla1* mutant.**
- **Formulate a working hypothesis-model on how enhanced levels of *Tla1* gene expression result in a smaller Chl antenna size.**
- **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' (t*laX* and t*laY*)

- **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.**

Longer Term Future Work - 4

- **Generate and analyze additional *Tla*-type transformants in *Chlamydomonas reinhardtii.***
- **Perform genetic crosses of *Chlamydomonas* strains to combine different *Tla*-like properties. Test for cumulative effects on the Chl antenna size.**
- **Perform solar conversion efficiency and productivity measurements under mass culture conditions in wild type and each of the *Tla*-type mutants generated.**

Publications and Presentations

Posewitz MC, Smolinski SL, Kanakagiri S, Melis A, Seibert M, Ghirardi ML (2004) Hydrogen photo-production is attenuated by disruption of an isoamylase gene in *Chlamydomonas reinhardtii.* Plant Cell 16: 2151-2163

Komine Y and Melis A (2004) *Chlamydomonas reinhardtii* thioredoxin-like protein mRNA, complete cds; nuclear gene. GenBank Accession Number AY762116

Melis A, Seibert M and Happe T (2004) Genomics of green algal hydrogen research. Photosynth. Res. 82: 277-288

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., In Press

White A and Melis A (2004) Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures. Abstracts of the 13th Western Photosynthesis Conference, Asilomar Conference Center, Pacific Grove, CA. January 8-11, p. 33.

Melis A, Polle J and Kanakagiri S (2004) Genes for the regulation of the light-harvesting chlorophyll antenna size in *Chlamydomonas reinhardtii*. Plant Biology 2004, Abstract # 219 in the American Society of Plant Biologists 2004 Annual Meeting Program Book. p. 80.

Melis A, Polle JE and Kanakagiri S (2004) Genes for the regulation of the light-harvesting chlorophyll antenna size in *Chlamydomonas reinhardtii.* Abstract # 259 of the 13th International Congress of Photosynthesis. pp. 112-113

Hydrogen Safety

The most significant hydrogen hazard associated with this project is:

The presence of pressurized cylinders with hydrogen, nitrogen and argon that are employed in the conduct of this work

These are safely anchored in appropriately designed berth spaces.

Hydrogen Safety

Our approach to deal with this hazard is:

Training of personnel in general, and specific aspects of safety for this project in particular, is mandatory for all employees in this department.

The small amounts (ml quantities) of H_2 involved in this work do not entail a significant hazard, nor do they pose an accident scenario.

Safety oversight is maintained by the University's Environmental Health and Safety office (EH&S).