Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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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

Large Chl Antenna Size (615 Chl a and b)

(Limiting light)

Molecular mechanism

(Saturating light)

Truncated Chl Antenna Size (170 Chl a and b)
Example: Fully Pigmented

Bright Sunlight

Heat dissipation

The green algae Chlamydomonas reinhardtii
Example: Truncated Chl Antenna Size

Bright Sunlight

Heat dissipation
Measurement in Scale-up Cultures

Cultures in the Greenhouse

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT</th>
<th>tla1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell/mL (x10^6)</td>
<td>6.36</td>
<td>10.0</td>
</tr>
<tr>
<td>[Chl] (uM)</td>
<td>25.6</td>
<td>15.4</td>
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</tbody>
</table>
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

Chl Antenna Size (PSII + PSI)

Npq2-lor1
Chl b-less

tla1
tlaX

Minimum Chl Antenna Size

Year Implemented

Productivity in Scale-up Cultures

O$_2$ production, ml h$^{-1}$

[Chl] in the culture, $\mu$M

Capacity = 60 ml O$_2$ h$^{-1}$
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

*tla1* mutant DNA

wt Tla1 DNA

5'UTR  intron  coding  3'UTR

ARG7.8

5' RACE from WT

GATGTGTCGTTCAGTTCAACAACGTGAATATATAGAATCTCATTGGCCTGCC
ACAACCTCAGACCAAAGACGCGCAGAAACCTGACACGAGACTTTCAAGTATTTGACCCT
GCTGACAAASCCGCGCTTTAAAAGATTTCTTGACACCGCGGCTAAGTATCCATCAAAT
AGCGTGAATGTTGGTCCTGGAGACCGAAGGAGGGCCCGCTCTGTCGAAATCCTGGAGAC
ATGTGGAATGGTGTCCTCGGTACAGCGAAGGAGGGCGGCTCTGTCGAAATCCTGGAGAC
GTCTCGGCCTACGTGGATGATCCTACACACGTACACGGGACGCGATTTGTTGCT
ACTACCAATCACAGCGACGTTCACCGCCCGGG

5' RACE from tla1

acgcataggagcggtgcgtggggaatagga
cgatccgcgcaagagcgccggaagtgacctacgcaacaagccagtctgacatcgcaggg
agcttgactgtaagctacagctagctgactgtgacatatctgcaacatttaactgtgataaactaccgc
Current Technical Accomplishments

Sequence homologies for the Tla1 protein

C. reinhardtii: --MT----FSCSADQTALLKILAHAAKYPSNSVNGVLVGTAKE----GGSVEILDA
A. thaliana: MGMTSNGELKYEQSNAYKLYQVHLRLHSAHAAVNGVLVGRIQP----KDDGVVEIDS
O. sativa: --MG--AECKYEVAQVYKLHAIHHPAAAVNGVGLVRLGDAASPAVVIADA
H. sapiens: --MG------EVEISALAYVMCMLAHARYPHAANVGLFALPAPR-----SGEGLCLTD
D. melanogaster: --MC------DYKVSERAAYKLFHAAKYPHQAENGLLLEAEKT-----KSQVEIVDA

C. reinhardtii: CHT--TLTLAPEIGLAQVESYTHITGSAVIGYQSDARFGPGLPPL--GRKIAD
A. thaliana: FHS--NLALLPPLEISLIMIEEHYVAQG-LSIVGYFHANERFDVDELCGV--AKNIGD
O. sativa: SHHPHHPLPLLPTLELALTLIVEDHFAAQG--LAVGYGHANARRDADLPPV--AKRVGI
H. sapiens: FHS--HLALSVMLEAVNQVDVGAQAG-LVVEGYYHANAAVNDQSPGPL--ALKIAGI
D. melanogaster: FHQ--CLYVTPMAELAIMLDAAREG--LVIAGYYAAPENFYDNQVDTPAAKID

C. reinhardtii: EHQAQAVVLVLKNNKLEIQFCAAQADNP--FELFSKDGSKGWKRASADGG--ELALKNAD
A. thaliana: RYFPQAPIILLMNKKLEALSQKESPVMQLCVDASKKWRVVGADGGSKLLKEPS
O. sativa: RNFFPRAAVLLDNLDKLEAVKGSREPVQLVLYTRDSSKSWRQAGSDGGSSLTIKPS
H. sapiens: EFFPADVILMLNDQKLVP------QPRVPFVTVLENQGLR--VPDKRNLVMWMDWEE
D. melanogaster: ENFKNACFVVVDN--KLMTPHQDRAIQFVNGDSGAR--W------SKAKFTLSQAS

C. reinhardtii: LREEFFVFMKQLKHRTLHDFEEHLDAAKGDKWNGPSAV--KFLLP----GNAL
A. thaliana: VLSDYISSE--KWKDVDTVDWHDHDDVTKDWNPGFLN----------------
O. sativa: VLADHTTK--KWQVVFDDHDHLDDHSKIDWNLPGFLA----------------
H. sapiens: MVGALLEDR----AHQLVDVDFCHLDDIRQDWTNQRLNTQITQWVGPNTNGNA
D. melanogaster: EGVSLLLKRG--AMRDLVVDNHDNPKNWTNDFNQPLNDLQKLY----------------
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](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 H2 as well as O2 production**
  Productivity measurements on the basis of biomass and O2 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 H2-production by the tla mutants. Until an alternative steady-state method of green algal H2-production can become available, only the ORNL “continuous sparging with He” method is suitable for testing the tla mutants.
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.
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 H₂-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 TlaX 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 H₂-producing unicellular green algae (Scenedesmus and Chlorella).
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.
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.


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.
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 H₂ 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).