Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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University of California - Berkeley
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Project ID # PD33
Overview

Timeline

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

Barriers addressed

• Low Light Utilization Efficiency in Photobiological Hydrogen Production due to a Large Photosystem Chlorophyll Antenna Size (Barrier X).

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

Partners

• None: Sole Source Effort

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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.)
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.
Chlamydomonas reinhardtii mass culture

Hydrogen production in a backyard
Example: Fully Pigmented

The green algae *Chlamydomonas reinhardtii*

**Bright Sunlight**

**Heat dissipation**

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

Chl Antenna Size (PSII + PSI)

Year Implemented

Minimum Chl Antenna Size

Npq2-lor1
Chl b-less
tla1
tlaX
tlaNew

Shows publication year of peer-reviewed paper.

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Measurement in Scale-up Cultures

Cultures in the Greenhouse

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

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

O₂ production, ml h⁻¹

[Chl] in the culture, μM

Capacity = 60 ml O₂ h⁻¹

WT

tlaX

tla1
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

*tla1* mutant DNA

wild type *Tla1* DNA

5’UTR coding intron coding 3’UTR
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

<table>
<thead>
<tr>
<th></th>
<th>C. reinhardtii</th>
<th>A. thaliana</th>
<th>O. sativa</th>
<th>H. sapiens</th>
<th>D. melanogaster</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C. reinhardtii</strong></td>
<td>--MT----FSCSADQTALLKILAHAACKYPNSSVNGVLVGTAKIGGSVEILDA</td>
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<tr>
<td><strong>A. thaliana</strong></td>
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<tr>
<td><strong>O. sativa</strong></td>
<td>--MG--AECKYEVQVAYVKLALHALKHPPAAAVNLGVGRLDLGASPAVAVSIAADV</td>
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<tr>
<td><strong>H. sapiens</strong></td>
<td>--MG-----EVEISALYVKMCLHAARYPHAHAVNGFLAPAPRSEGELCLTD</td>
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<tr>
<td><strong>D. melanogaster</strong></td>
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<tr>
<td><strong>C. reinhardtii</strong></td>
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<td><strong>A. thaliana</strong></td>
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<tr>
<td><strong>O. sativa</strong></td>
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<tr>
<td><strong>H. sapiens</strong></td>
<td>FHS--HLALSVMLEVELNQVDWAGQQLVAGYHYHANAVNDQSGPPL--ALKIAG</td>
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<tr>
<td><strong>D. melanogaster</strong></td>
<td>FHQ--CLYVTMPAEVMLIDAHAREG--LVIAGYAAKENFYDNPVDPKAIA</td>
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<tr>
<td><strong>C. reinhardtii</strong></td>
<td>EHQAQAVVVLVDNKRLEROFCKAQADNP--FELFSKDGSKGKWKRASADGG--ELALKNAD</td>
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<td><strong>O. sativa</strong></td>
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<tr>
<td><strong>H. sapiens</strong></td>
<td>EFPDAVLIMLDNQLVP--QRPRPPVIVLENOQGLR--VPKDKNLVMWRDWE</td>
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<tr>
<td><strong>D. melanogaster</strong></td>
<td>ENFKNAACFVVVDN--KLMTLQHDRAAIQVFNCOLSDGAR--W--SRAKAKTLSAS</td>
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<tr>
<td><strong>C. reinhardtii</strong></td>
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<td><strong>D. melanogaster</strong></td>
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**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

• Competed 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.

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<tr>
<td></td>
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<td>tla1</td>
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<td>tlaNew</td>
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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.
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*.
- Invited presentations on *Tla1* work at the:
  -- 14th International Congress on Photosynthesis, Glasgow, Scotland; Symposium on Bioenergy and Photosynthesis.
  -- 91st Annual Meeting of the Optical Society of America.
  -- International Symposium on Material Issues in a Hydrogen Economy.
  -- University of Nebraska, Lincoln.