Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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University of California - Berkeley
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Project ID # PDP33

This presentation does not contain any proprietary, confidential, or otherwise restricted information
Overview

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

Budget
- Total Project Funding
  DOE: $330 k, UCB: $225 k
- Funding for FY06
  DOE: $50 k, UCB: $75 k
- Funding for FY07
  DOE: $90 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

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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 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.
The green algae Chlamydomonas reinhardtii

Example: Fully Pigmented

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

Heat dissipation

Truncated Chl antenna cells permit greater transmittance of light and overall better solar utilization by the culture.
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>
</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

[Graph showing the relationship between O₂ production and [Chl] in the culture, with curves for tlaX, tla1, and WT, and a capacity of 60 ml O₂ h⁻¹]
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.
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%
Chlorophyll Antenna Size in Chlamydomonas

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Current Technical Accomplishments
Analysis of the \textit{tla1} and \textit{tlaX} mutants

\begin{itemize}
  \item \textbf{Molecular analysis of the \textit{tla1} mutation.}
      Genomic, cDNA and protein sequences for the \textit{Tla1} gene were published.
      Complementation of the \textit{tla1} mutant with the \textit{Tla1} gene succeeded.
      Analysis of the complemented strains was implemented.
  
  \item \textbf{Biochemical analysis of the \textit{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.

  \item \textbf{Functional analysis of the \textit{Tla1} gene.}
      Regulation of the chlorophyll antenna size by the \textit{Tla1} gene completed.

  \item \textbf{Biophysical and biochemical analyses of the \textit{tlaX} mutant.}
      Chlorophyll antenna size, relative productivity, LHC expression levels.
\end{itemize}
Current Technical Accomplishments

Mapping of the *tla1* mutation and WT *Tla1* gene structure

*tlal* 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>Species</th>
<th>Sequence Homology</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. reinhardtii</em></td>
<td>--MT-----FSCSADQTALLKILAHAAYKPSNSNVSNGVLVVTAK--GGSVEILDAGMGSNGELKYEIQNAYTKLVVLHSLRHKTAANVGVLVGRISP----KDDGVEI</td>
</tr>
<tr>
<td><em>A. thaliana</em></td>
<td>--MG-----AECKYEVAQVAVKLALHALKHPAAVNGLLVGRLLDGAASPAAVVSIADAVGMGSNGELKYEIQNAYTKLVVLHSLRHKTAANVGVLVGRISP----</td>
</tr>
<tr>
<td><em>O. sativa</em></td>
<td>--MG-----EVEISALYVKMCLHAARYPHAAAVNGLLFAPAPR----SGEGLCLTDCVGMGSNGELKYEIQNAYTKLVVLHSLRHKTAANVGVLVGRISP----</td>
</tr>
<tr>
<td><em>H. sapiens</em></td>
<td>--MC-----DYKVSERAYAKLIFHAAKYPHQAVNGLLAEKTS----KGSQVEIVDAHGMGSNGELKYEIQNAYTKLVVLHSLRHKTAANVGVLVGRISP----</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>--MC-----DYKVSERAYAKLIFHAAKYPHQAVNGLLAEKTS----KGSQVEIVDAHGMGSNGELKYEIQNAYTKLVVLHSLRHKTAANVGVLVGRISP----</td>
</tr>
<tr>
<td><em>C. reinhardtii</em></td>
<td>CHT--TLTLAPALEIGLAQVESYTHITGSVAIVGYQSDARFGPGDLPPL--GRKIAD          CHT--TLTLAPALEIGLAQVESYTHITGSVAIVGYQSDARFGPGDLPPL--GRKIAD</td>
</tr>
<tr>
<td><em>A. thaliana</em></td>
<td>FHS--NLALLPLEISLIMIEEHYVAQG--LSIVGYFHAHERFDDEVLCVG--AKNIGD          FHS--NLALLPLEISLIMIEEHYVAQG--LSIVGYFHAHERFDDEVLCVG--AKNIGD</td>
</tr>
<tr>
<td><em>O. sativa</em></td>
<td>SHHPHHLPLLPTLEALTLVEDHFGAQG--LVAVGYYHANARRDDADLPPV--AKRVGDI          SHHPHHLPLLPTLEALTLVEDHFGAQG--LVAVGYYHANARRDDADLPPV--AKRVGDI</td>
</tr>
<tr>
<td><em>H. sapiens</em></td>
<td>FHS--HLALSVMLAEVQNVDGQAG--LVVAGYHANAANVQDQPGPL--ALKIAGI          FHS--HLALSVMLAEVQNVDGQAG--LVVAGYHANAANVQDQPGPL--ALKIAGI</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>FHQ--CLYVTPMAEVALMLIDAHAELEG--LVIYAGYYAQPENFYDNQVDKTPAAKIAD          FHQ--CLYVTPMAEVALMLIDAHAELEG--LVIYAGYYAQPENFYDNQVDKTPAAKIAD</td>
</tr>
<tr>
<td><em>C. reinhardtii</em></td>
<td>EHQAQAVVLVLDNKRLEQFCKAQADNP--FELFSKDGSGBKWSKgcSADGG--ELALKNAD          EHQAQAVVLVLDNKRLEQFCKAQADNP--FELFSKDGSGBKWSKgcSADGG--ELALKNAD</td>
</tr>
<tr>
<td><em>A. thaliana</em></td>
<td>RYFPQAPIPLLNNKKEALSGKERSPVMLCVIKADSKWRVVGADGGSKLLKEPS;          RYFPQAPIPLLNNKKEALSGKERSPVMLCVIKADSKWRVVGADGGSKLLKEPS;</td>
</tr>
<tr>
<td><em>O. sativa</em></td>
<td>RNFPRAAVVLLDNKKLEEAVKGSREPVVQLYTRDSKSWQAAGSGDSSLQTLKEPS;          RNFPRAAVVLLDNKKLEEAVKGSREPVVQLYTRDSKSWQAAGSGDSSLQTLKEPS;</td>
</tr>
<tr>
<td><em>H. sapiens</em></td>
<td>EFPDAVLMIDNQKLVP------QPRVQPVIVLENQGLWR--VPNKDNKLVMWRDWE;          EFPDAVLMIDNQKLVP------QPRVQPVIVLENQGLWR--VPNKDNKLVMWRDWE;</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>ENFKNACFVVVDNLKMTLQHDRAAIQVFNCPSGAR--W----------SKAKFTLSQAS;          ENFKNACFVVVDNLKMTLQHDRAAIQVFNCPSGAR--W----------SKAKFTLSQAS;</td>
</tr>
<tr>
<td><em>C. reinhardtii</em></td>
<td>LREEFFVMFKQLKHKRTLHDFDHEEVIDIAKDWLNGFASSV--KFLLP----------GNAL          LREEFFVMFKQLKHKRTLHDFDHEEVIDIAKDWLNGFASSV--KFLLP----------GNAL</td>
</tr>
<tr>
<td><em>A. thaliana</em></td>
<td>VLSGYSISS-----KWKDVDVDLHDDVDKDLNPFLN--------                      VLSGYSISS-----KWKDVDVDLHDDVDKDLNPFLN--------</td>
</tr>
<tr>
<td><em>O. sativa</em></td>
<td>VLADHVYTK-----KQVQVDFDDHDDLSDKDLNPGALL--------                      VLADHVYTK-----KQVQVDFDDHDDLSDKDLNPGALL--------</td>
</tr>
<tr>
<td><em>H. sapiens</em></td>
<td>MVGQLEDRT------AHQHLVDVFDCRADDIRQDWTNQRNLSTITQWGPPTNGNA-          MVGQLEDRT------AHQHLVDVFDCRADDIRQDWTNQRNLSTITQWGPPTNGNA-</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>EGVSLLLLKRG--AMRDLVDFDNHDLNPDKNWTDNLQPLNDLQKLY         EGVSLLLLKRG--AMRDLVDFDNHDLNPDKNWTDNLQPLNDLQKLY</td>
</tr>
</tbody>
</table>
Summary of Accomplishments
Analysis of the \textit{tla1} and \textit{tlaX} mutants

• Competed the biochemical characterization of the \textit{tla1} mutant and the molecular analysis of the \textit{Tla1} gene.

• Down-regulation of the ubiquitous \textit{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 \textit{tlaX} mutant. Encountered difficulties in the molecular analysis of this mutant.
## Progress achieved vs the DOE targets

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

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<tr>
<td><strong>Program Targets</strong></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>3%</td>
<td>10%</td>
<td></td>
<td>15%</td>
<td>20%</td>
</tr>
<tr>
<td><strong>Progress</strong></td>
<td>3%</td>
<td>10%</td>
<td>15%</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.
Current Work

Perform functional analysis of the *Tla1* gene (How is *Tla1* regulated under different conditions?)

- Investigate levels of expression of the *Tla1* gene as a function of growth irradiance.
- Investigate cellular localization of the Tla1 protein.
- 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*. 

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Future Work

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 substantially 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 enhancement or suppression of TlaX gene expression results 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.
Summary

- Completed first part of work on the *Tla1* gene.
- Filed patent application on the *Tla1* gene.
- Published findings in peer reviewed journal:
  Tetali SD, Mitra M and Melis A (2006)
  Development of the light-harvesting chlorophyll antenna in the green alga *Chlamydomonas reinhardtii* is regulated by the novel *Tla1* gene. Planta 225: 813-829
- **Gave invited presentations on Tla1 work at the:**
  --University of Montreal, Quebec, Canada.
  --Gordon Research Conference on Photosynthesis.
  --University of Minnesota.