II.F.2 Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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Objectives

- Minimize, or truncate, the chlorophyll (Chl) antenna size in green algae to maximize photobiological solar conversion efficiency and H₂-production.
- Demonstrate that a truncated Chl antenna size would minimize absorption and wasteful dissipation of sunlight by individual cells, resulting in better light utilization efficiency and greater photosynthetic productivity by the green algal mass culture.

Technical Barriers

This project addresses the following technical barriers from the Hydrogen Production section of the Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year Research, Development and Demonstration Plan:

(AG) Light Utilization Efficiency

Technical Targets

The Hydrogen, Fuel Cells and Infrastructure Technologies Multiyear Program Plan technical target for 2005 for this project was to reach a 10% utilization efficiency of absorbed light energy (out of a theoretical maximum of 30% possible) in unicellular green algae. Progress through this project has currently achieved a green alga utilization efficiency of absorbed light energy of about 25% (tentative and unpublished; please see Table 2).

Approach

- Employ deoxyribonucleic acid (DNA) insertional mutagenesis, screening, biochemical and molecular genetic analyses for the isolation of “truncated Chl antenna size” mutants in the green alga *Chlamydomonas reinhardtii*.
- Clone and characterize the gene(s) that affect the “Chl antenna size” property in *Chlamydomonas reinhardtii*.
- Apply such genes to generate a “truncated Chl antenna size” in this and other green algae.

Accomplishments

1. Completed the molecular and genetic analysis of the *tla1* mutant.
2. Completed the functional analysis of the *Tla1* gene.
3. Published manuscript on the utility of the *Tla1* gene in conferring a truncated chlorophyll antenna size and on the mechanism by which it maximizes light utilization efficiency and hydrogen production in microalgal cultures.
4. Disclosed the DNA sequence of *Rdp1*, a gene that overlaps *Tla1* and provided a molecular biological and genetic analysis of the *Rdp1* vis-à-vis the *Tla1* gene.
5. Isolated and characterized new “truncated chlorophyll antenna size” mutants *tlaX* and *tlaR*. Properties of these strains are given in Tables 1 and 2.

![Table 1](image)

**Table 1.** *Chlamydomonas reinhardtii* cellular chlorophyll content, photosystem chlorophyll antenna size and energy utilization efficiency in wild type, *tla1, tlaX* and *tlaR* mutant mutant strains, as determined by spectrophotometric kinetic analysis (n = 5, ±SD).

<table>
<thead>
<tr>
<th></th>
<th>wild type</th>
<th><em>tla1</em></th>
<th><em>tlaX</em></th>
<th><em>tlaR</em></th>
<th>Long-term goal</th>
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<tr>
<td>Chl/cell mol x 10⁷</td>
<td>2.4 ± 0.5</td>
<td>0.9 ± 0.06</td>
<td>0.93 ± 0.1</td>
<td>0.7 ± 0.1</td>
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<tr>
<td>Chl-PSII</td>
<td>222 ± 26</td>
<td>115 ± 36</td>
<td>80 ± 30</td>
<td>50 ± 30</td>
<td>37</td>
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<tr>
<td>Chl-PSI</td>
<td>240 ± 4</td>
<td>160 ± 12</td>
<td>115 ± 10</td>
<td>105 ± 10</td>
<td>95</td>
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<tr>
<td>Light Utilization Efficiency</td>
<td>~3%</td>
<td>~10%</td>
<td>~15%</td>
<td>~25%</td>
<td>~30%</td>
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</table>
Introduction

The goal of the research is to generate green algal strains with enhanced photosynthetic productivity and hydrogen production under mass culture conditions. To achieve this goal, it is necessary to optimize the light absorption and utilization properties of the cells [1,3,5]. A cost-effective way to achieve this goal is to reduce the number of Chl molecules that function in the photosystems of photosynthesis. Thus, efforts are under way to isolate microalga mutants with a truncated chlorophyll antenna size.

The rationale for this research and development is that a truncated light-harvesting Chl antenna size in green algae will prevent individual cells at the surface of the culture from over-absorbing sunlight and wastefully dissipating most of it (Figure 1). A truncated Chl antenna size will permit sunlight to penetrate deeper into the culture, thus enabling many more cells to contribute to useful photosynthesis and hydrogen production (Figure 2). It has been shown that a truncated Chl antenna size will enable about 3-4 times greater solar energy conversion efficiency and photosynthetic productivity than could be achieved with fully pigmented cells [2].

Approach

The immediate objective of the research is to identify genes that control the Chl antenna size of photosynthesis and, further, to elucidate how such genes confer a truncated Chl antenna size in the model green alga Chlamydomonas reinhardtii. Identification of such genes in Chlamydomonas will permit a subsequent transfer of this property, i.e., “truncated Chl antenna size”, to other microalgae of interest to the DOE Hydrogen, Fuel Cells and Infrastructure Technologies Program. This objective is currently being approached through DNA insertional mutagenesis/screening and biochemical/molecular/genetic analyses of Chlamydomonas reinhardtii cells.

Results

The Chlamydomonas reinhardtii mutant tla1 (truncated light-harvesting chlorophyll antenna size) was earlier generated upon DNA insertional mutagenesis and shown to specifically possess a smaller than wild type chlorophyll antenna size in both photosystems. This

<table>
<thead>
<tr>
<th>Year</th>
<th>2000</th>
<th>2003</th>
<th>2005</th>
<th>2008</th>
<th>2013</th>
<th>2018</th>
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<td>Program Targets</td>
<td>3%</td>
<td>10%*</td>
<td>15%</td>
<td>20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actual Progress Achieved</td>
<td>3%</td>
<td>10%</td>
<td>15%</td>
<td>25%</td>
<td></td>
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</tbody>
</table>

* Target adjusted upward to match ahead-of-schedule progress achieved.
strain exhibited a 10% utilization efficiency of incident solar light energy, i.e., substantially greater than the 3% utilization efficiency of the wild type. Molecular and genetic analysis revealed that the exogenous plasmid DNA was inserted at the end of the 5’ untranslated region (UTR) and just prior to the Adenine, Thymine, Guanine start codon of a hitherto unknown gene (termed Tla1), which encodes a protein of 213 amino acids. The Tla1 gene in the mutant is transcribed with a new 5’ UTR sequence, derived from the 3’ end of the transforming plasmid. This replacement of the 5’ UTR resulted in enhanced transcription of the tla1 gene in the mutant but inhibition in the translation of the respective tla1 message ribonucleic acid. These results provided evidence that down-regulation of the Tla1 expression is necessary and sufficient to truncate the chlorophyll antenna size and to improve solar utilization efficiencies in a green algal mass culture. Specific applications of the Tla1 gene in H₂-production were discussed.

Work further described the isolation and biochemical and physiological characterization of a new mutant of Chlamydomonas reinhardtii, termed tlaX, having a truncated light-harvesting chlorophyll antenna size. Properties of the tlaX putative “truncated Chl antenna size” strain are summarized in Table 1. More recent work resulted in the isolation and partial characterization of a new mutant of Chlamydomonas reinhardtii, termed tlaR, also having a truncated light-harvesting chlorophyll antenna size. The tlaR mutant has the smallest yet Chl antenna size known in green algae (Figure 3). These advances were achieved upon generating and screening an additional 4,500 algae (Figure 3). These advances were achieved

![Graph](image-url)

**FIGURE 3.** Project timeline and publications record on the truncated chlorophyll antenna size project. Arrows show publication year of peer-reviewed paper for each of the truncated Chl antenna size mutants. Note that work with the tlaX and tlaR strains has not yet reached the stage of a peer-reviewed paper.

Transformants, following the protocol of Polle et al. [4]. Properties of the tlaR putative “truncated Chl antenna size” strain are also summarized in Table 1.

Future efforts will be directed toward the cloning of the genes responsible for the truncated light-harvesting chlorophyll antenna size phenotype in tlaX and tlaR mutants.

**Conclusions**

- Significant, ahead-of-schedule progress was achieved in terms of acquiring “truncated Chl antenna size” mutants. This demonstrates feasibility of the approach chosen and success of the methods employed.
- A truncated light-harvesting chlorophyll antenna size in the tla-type mutants leads to enhanced solar conversion efficiencies and greater photosynthetic productivity of the algae under bright sunlight conditions.
- Insights on the molecular mechanism for the regulation of the Chl antenna size by the Tla1 gene were obtained (results not shown pending publication of these findings in a peer reviewed journal).

**Future Directions**

- Advance the biochemical and molecular characterization of the tlaX and tlaR strains.
- Clone the genes that confer the tlaX and tlaR phenotypes.
- Establish transformation (sense and antisense) protocols with Tla-type genes to enhance the down-regulation of the Chl antenna size in Chlamydomonas reinhardtii.
- Perform comparative green-alga light utilization efficiency and photosynthetic productivity measurements under mass culture conditions in wild type and tla-type mutants.

**References**


**FY 2008 Publications/Presentations**

Peer reviewed publications (1); Technical DNA disclosures (2,3); Abstracts published (4-11); Invited seminars and lectures (12-17)


