Photosynthesis for Hydrogen and Fuels Production

Tasios Melis,
UC Berkeley
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Feedstock and products

Photosynthesis

H₂O → Photosynthesis → H₂, O₂, Biomass

CO₂ → Photosynthesis → HC

Process offers a renewable fuels supply and mitigation of climate change.
Average US Solar insolation = 5 kWh m\(^{-2}\) d\(^{-1}\)
CA household electricity consumption = 15 kWh d\(^{-1}\)
Can improvement in photosynthesis increase crop yields?

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“Six potential routes of increasing epsilon(c) by improving photosynthetic efficiency were explored, ranging from altered canopy architecture to improved regeneration of the acceptor molecule for CO₂. Collectively, these changes could improve epsilon(c) and, therefore, Y-p by c. 50%.”
Gains upon improving sunlight conversion efficiency: up to 300%
Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

R&D project funded by the DOE-EERE Hydrogen Program

This presentation does not contain any proprietary, confidential, or otherwise restricted information
The unicellular green alga Chlamydomonas reinhardtii

View of a microalga cross-section

Self-repairing and replicating microstructure
The green microalga *Chlamydomonas reinhardtii*
Photosynthetic water oxidation and H₂-production

Potential Energy, mV

-600
-200
0
+200
+600
+1,000 mV

2H₂
4H⁺

~3,000,000 electron transport chains per cell, each capable of transporting 100 electrons per second

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Hypoxic photosynthesis: $O_2$ is consumed by mitochondria

\[
\begin{align*}
2H_2O & \quad \text{Photo phosphorylation} \quad [Fe]-H_2ase \\
& \quad 4H^+ \quad 2H_2 \quad 2H_2O
\end{align*}
\]

\[
\begin{align*}
O_2 & \quad \text{Oxidative phosphorylation} \quad 2NADH \quad [\text{starch}] \\
& \quad 2H_2O
\end{align*}
\]
$\text{H}_2$ bubbles
Microalgal H₂-production facility in the laboratory
Chlamydomonas reinhardtii mass culture

Hydrogen production in a backyard

Chlamydomonas reinhardtii mass culture
Sunlight

Heat dissipation

Fully pigmented cells over-absorb and wastefully dissipate bright sunlight

Example: Cells
Fully Pigmented

The green algae
*Chlamydomonas reinhardtii*
The light-saturation curve of photosynthesis

Oxygen evolution, $\text{mmol } O_2 (\text{mol Chl})^{-1} \text{s}^{-1}$

Light intensity, $\mu\text{mol photons m}^{-2} \text{s}^{-1}$

wild type
Light intensity (PAR) \((\mu \text{mol photons m}^{-2} \text{s}^{-1})\)

**Daily PAR**

\((400 - 700 \text{ nm})\):

50 \text{ mol photons m}^{-2}
The problem of the early light-saturation of photosynthesis

Severe: Green and purple bacteria
Cyanobacteria & red algae
Green & brown algae

Less severe: C3 plants & C4 plants
Photosynthetic Productivities

Theoretical productivity:
~75 g dry weight m\(^{-2}\) d\(^{-1}\)
(8-10\% solar energy conversion efficiency)

(based on the average US solar insolation = 5 kWh m\(^{-2}\) d\(^{-1}\))
Photosynthetic Productivities

Theoretical productivity: 
~75 g dry weight m^{-2} d^{-1}  
(8-10\% solar energy conversion $E$)  

Measured productivities: 
Less than 25 g dry weight m^{-2} d^{-1}  
(3-4\% solar energy conversion $E$)
Example: Truncated Chl Antenna Size

Sunlight

Truncated Chl antenna cells permit greater transmittance of light and overall better solar utilization by the culture
The $Tla$ concept
($Tla =$ Truncated light-harvesting antenna)

Minimize the chlorophyll antenna size of photosynthesis to prevent the early light-saturation effect.
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.
Approach

- Identify genes that confer a truncated antenna in a model organism.

- Apply these genes to other organisms of interest.

- Improve photosynthesis, hydrogen, or fuels production by up to 300%.
DNA insertional mutagenesis and screening

ARG7
Plasmid DNA

TRANSFORMATION
(Random insertion of plasmid DNA in the nuclear genome)

Chlamydomonas reinhardtii

GROW TRANSFORMANTS

SCREEN TRANSFORMANTS

SELECT TRUNCATED ANTENNA STRAINS
DNA insertional mutagenesis and screening

Chlorophyll a fluorescence imaging analysis

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DNA insertional mutagenesis and screening for truncated light-harvesting antenna (tla) mutants

Dot-Spot Colonies of Chlamydomonas reinhardti
Secondary screening for \textit{tla} mutants

Criteria:

- Functional photosystem antenna size \textit{smaller than wild type}
- Number of photosystems per chloroplast should be \textit{the same or greater than wild type}
- High quantum yield of photosynthesis is maintained
- Photosynthesis & productivity per chlorophyll: \textit{inversely proportional to antenna size}

Objective:

- Identify “true positive” \textit{tla} mutants with \textit{improved sunlight utilization efficiency}. 

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Progress achieved vs targets set
Chlorophyll antenna size in wild type and mutants

<table>
<thead>
<tr>
<th></th>
<th>2000 (WT)</th>
<th>2003 (WT)</th>
<th>2005 (WT)</th>
<th>2008 (WT)</th>
<th>2010 (WT)</th>
<th>2015 (WT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targets (Chl Antenna size)</td>
<td>600</td>
<td>300</td>
<td>200</td>
<td>150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progress Achieved</td>
<td>600 (WT) tla1</td>
<td>195 (WT) tla2</td>
<td>150 (WT) tla3</td>
<td></td>
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</tbody>
</table>
Minimum Chl Antenna Size

Chlorophyll Antenna Size in *Chlamydomonas*

**Project Timeline**

- **Npg2-lor1**
- **Chl b-less**
- **tla1**
- **tla2**
- **tla3**

<table>
<thead>
<tr>
<th>Number of Chl molecules per photosynthetic unit</th>
<th>Year Attained</th>
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<tbody>
<tr>
<td>500</td>
<td>2000</td>
</tr>
<tr>
<td>400</td>
<td>2002</td>
</tr>
<tr>
<td>300</td>
<td>2004</td>
</tr>
<tr>
<td>200</td>
<td>2006</td>
</tr>
<tr>
<td>100</td>
<td>2008</td>
</tr>
<tr>
<td>0</td>
<td>2010</td>
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</table>

Minimum Chl Antenna Size
Chlamydomonas tla1 mutant phenotype and its complementation

Complemented tla1 strains with the wild type Tla1 gene (tla1-comp1, tla1-comp2, and tla1-comp3) recovered the green pigmentation phenotype.
Photosynthetic unit chlorophyll antenna size of wild type and *tla1* mutant

**Wild type Chl Antenna Size**
- ~ 600 Chl $a + b$ molecules

**tla1 mutant Chl antenna size**
- 300 Chl $a + b$ molecules
The light-saturation curve of photosynthesis

Oxygen evolution, \( \text{mmol } O_2 \text{ (mol Chl)}^{-1} \text{ s}^{-1} \)

Light intensity, \( \mu \text{mol photons m}^{-2} \text{ s}^{-1} \)

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Productivity in Mass Culture

Cultures in the Greenhouse

<table>
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<tr>
<th>Parameter</th>
<th>WT</th>
<th>tla1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell/mL</td>
<td>6.36 (x10^6)</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.)
Gradient of sunlight penetration through a high density wild type (WT) and tla culture.
Photosynthetic productivity of wild type (WT) and tla1 mutant
The *Tla* concept is commercially applied in green microalgae:
- *Chlamydomonas* for biomass production; and
- *Nannochloropsis* for commercial production of polyunsaturated fatty acids (PUFAs).

The *tla1 mutant* strain was requested and acquired by universities (x5), industry (x5), and government labs (x4).

Successful application of the *Tla1* gene at NREL for enhanced H$_2$-production.
Light intensity-dependent rate of H$_2$-production by immobilized wild type (CC-425) and tla1 antenna mutant (CC-4169)

Kosourova et al. 2010
Increased $Tla$ awareness in the field

Many labs in several countries are now engaged in $tla$ research.
(green microalgae, cyanobacteria, photosynthetic bacteria)
1) Office of Basic Energy Sciences workshop on “What is the Efficiency of Photosynthesis?” May 23-24, 2009

A $Tla$ property in mass culture:

• Prevents the early light-saturation of photosynthesis.
• Facilitates better sunlight penetration.
• Enhances solar energy conversion efficiency and productivity (up to 300% in green microalgae).
Future Tla extensions

- Development of non-genetic approaches to generating Tla strains.

- Application of Tla technologies for biomass, $H_2$ and fuels production in cyanobacteria and photosynthetic bacteria.

- Extension of Tla R&D to include generation of cell-fuel* molecules for application in fuel-cells.

* cell-fuels = small-size high-energy bio-products
Thank You for Listening!