Global Regulators of Photosynthetic Acclimation to Irradiance

Shaun Bailey
Director, Photosynthesis Research

2013 Algal Biomass Summit
Orlando
10/01/2013
The Open Pond Light Environment

5 cm photic zone

Darkness

Patents Pending
Self Shading and Antenna Cross Section

Both increasing cell density and increasing chlorophyll per cell contribute to the high extinction coefficient in dense culture.

RC
LHC
Saturation of Photosynthesis

- Light absorption continues beyond saturation (Ek)
- Light absorbed in excess of the capacity for photosynthetic electron flow is actively dissipated as heat through Non-Photochemical Quenching processes (NPQ)
Saturation of Photosynthesis

Decreasing the chlorophyll antenna improves light penetration and increases the saturating irradiance level for photosynthesis, ensuring that the culture dissipates less absorbed light as heat.
**Locked In High Light Acclimated (LIHLA) Concept**

High Light acclimated algae typically have:

- Significant decrease in chlorophyll, typically associated with auxiliary antenna
- Significant increase in the maximum rate of photosynthesis (Pmax/Chl) with no decrease in photosynthetic electron transport (PET/cell) or Pmax/cell

- A functional genomics approach was undertaken to isolate mutants with a Locked In High Light Acclimated phenotype and to isolate the global regulators of photo-acclimation and mutants with a naturally balanced decreased antenna.
The LIHLA Forward Genetic Screen

- Mutagenesis and screening for LIHLA
- Molecular characterization of LIHLA and ‘omics
- Utilization of LIHLA

WT

Patents Pending
Many of the LIHLA mutants mapped to genetic lesions associated with just 2 genes, confirmed through whole genome sequencing, in house.
Confirmation that the Lar Lesions Confer LIHLA

Through a combination of complementation, directed gene knockout and RNAi we have definitively associated the Lar genes with the LIHLA phenotype

<table>
<thead>
<tr>
<th>Nanno species 1</th>
<th>Nanno species 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Complementation</strong></td>
<td><strong>Recapitulation</strong></td>
</tr>
<tr>
<td>Native Gene</td>
<td>Epitope Tagged Gene</td>
</tr>
<tr>
<td>LAR1</td>
<td>✓</td>
</tr>
<tr>
<td>LAR2</td>
<td>✓</td>
</tr>
</tbody>
</table>
The LIHLA Phenotype

When grown under conditions that lead to low light acclimation in WT, the Lar mutants retain high light acclimated features. These data show a targeted knockout of Lar1 in *Nannochloropsis* sp. The phenotype is very similar for all Lar mutants and RNAi lines. Complementation restores the ability to low light acclimate.
When grown under conditions that lead to low light acclimation in WT, the Lar mutants retain high light acclimated features including: over 50% less chlorophyll per cell, equivalent Pmax/cell therefore double the Pmax/chl.
The LIHLA Phenotype continued

When grown under conditions that lead to low light acclimation in WT the Lar mutants retain high light acclimated features including: higher saturating PSII electron transport rates (ETR), higher saturating irradiance for photosynthesis (Ek), lower excitation pressure (higher qP at all irradiance levels)

![Graph showing PSII ETR vs Irradiance](attachment:PSII_ETR.png)

![Graph showing Photochemical quenching vs Irradiance](attachment:Photoquenching.png)
The predicted increase in light penetration, coupled with a higher light saturation point for photosynthesis in the Lar mutants, measured at the same biomass density (400 mg L\(^{-1}\)) in open ponds, provides more available light for photochemistry (data shown for a WT and Lar1 mutant).
Transcriptomics: High to Low Light Shift

The Lar mutants remain high light acclimated during a light shift from high to low light, evidenced by the photophysiological response.
Transcriptomics: High to Low Light Shift

The Lar mutants remain high light acclimated during a light shift from high to low light, evidenced by the global deregulation of the photosynthetic transcriptional response.
Transcriptomics: Lar1, Lar2 comparative

A comparison of the transcriptomes of the Lar 1 and Lar2 mutants with each other and relative to WT, during low light growth, reveals the remarkable similarity in the de-regulated response of the mutants to low light acclimation.
Transcriptomics: Network Inference Analysis

Using over 100 whole genome transcriptional profiles, generated in house in response to a wide range of abiotic stress conditions in WT *Nannochloropsis*, sophisticated network inference analysis (NIA) is possible. An example of co-expression analysis for the Lar1 and Lar2 genes under a wide range of abiotic conditions reveals their co-regulation.
Conclusions

- We have isolated light acclimation deficient mutants with a number of favorable traits for growth in mass culture.

- We have identified global regulators of acclimation to irradiance, lacking both a transcriptional and physiological response to low irradiance.
Acknowledgements

**Synthetic Genomics Inc.**

Jonathan Meuser  
Jay McCarren  
Daniel Yee  
Anna Ritchie  
Soyan Lieberman  
Aimee Lim  
Emily Effner  
Leah Soriaga  
Ariel Schwarz  
Rob Brown  
Toby Richardson  
Stan Bower  
James Flatt

**Exxon Mobil**

Joseph Weissman  
Roger Prince  
Robert Nielson  
Kelsey McNeely  
Jennifer Feeley