Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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Project ID # PD036

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Overview

Timeline

- Start: 01-Dec-2004
- End: 30-Nov-2013
- Completion: 85%

Barriers

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

Budget

- **Total Project Funding**
- DOE: $1.74M
- UCB: $675K
- Swedish Res Council: $55.2K (**FY11**)
- **Funding received in FY11: $140K**
- **Planned funding for FY12: $150K**

Partners

- NREL
- Swedish Res Council

Relevance

The TLA concept

(TLA = Truncated Light-harvesting Antenna):

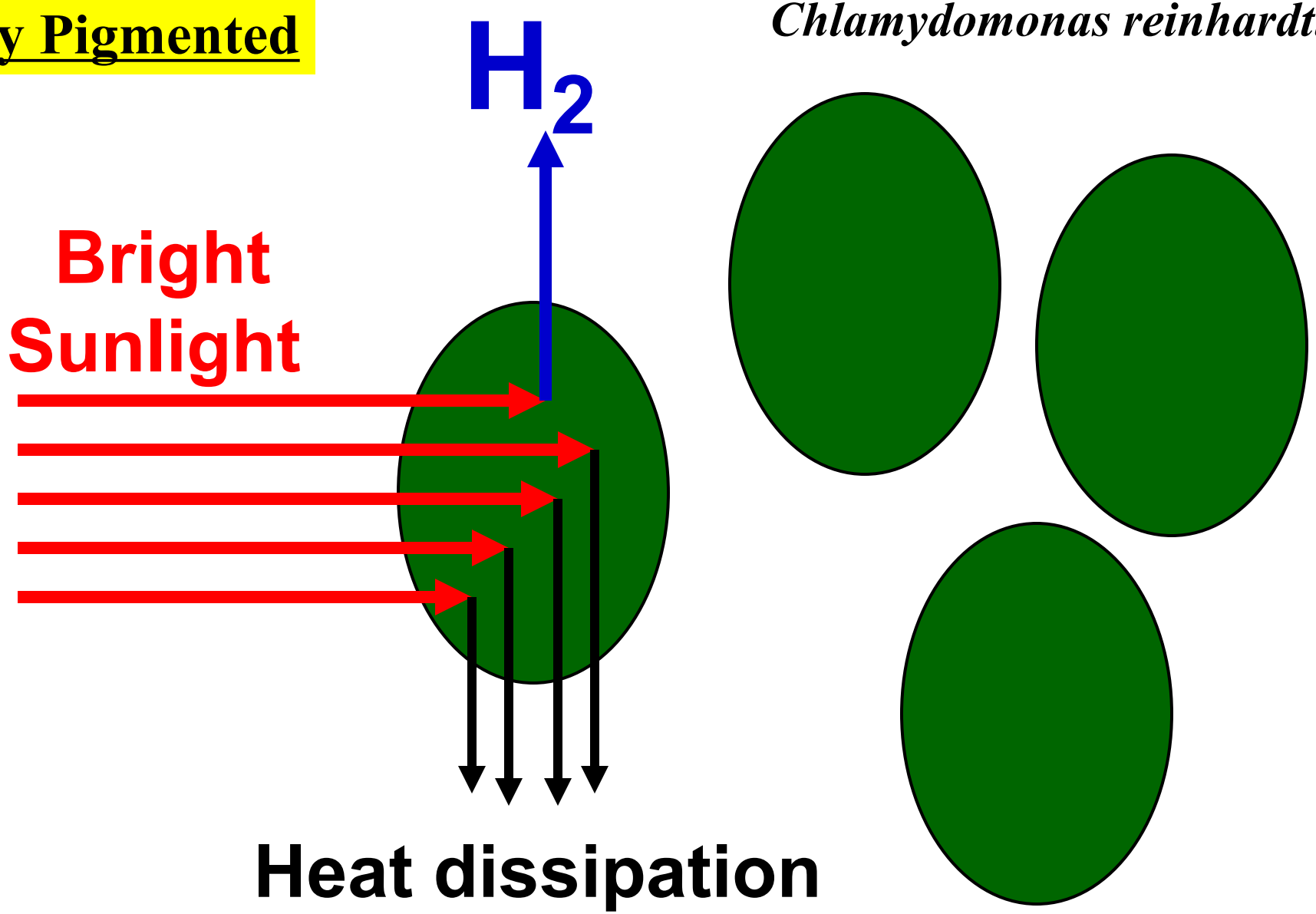
Minimize the light-harvesting antenna size of the photosystems to prevent the early light-saturation of photosynthesis and the associated wasteful dissipation of absorbed sunlight.

Relevance

Improve, by up to 300%, the sunlight-utilization efficiency of photosynthesis, to accordingly improve H_2 or fuels production in microalgae and cyanobacteria.

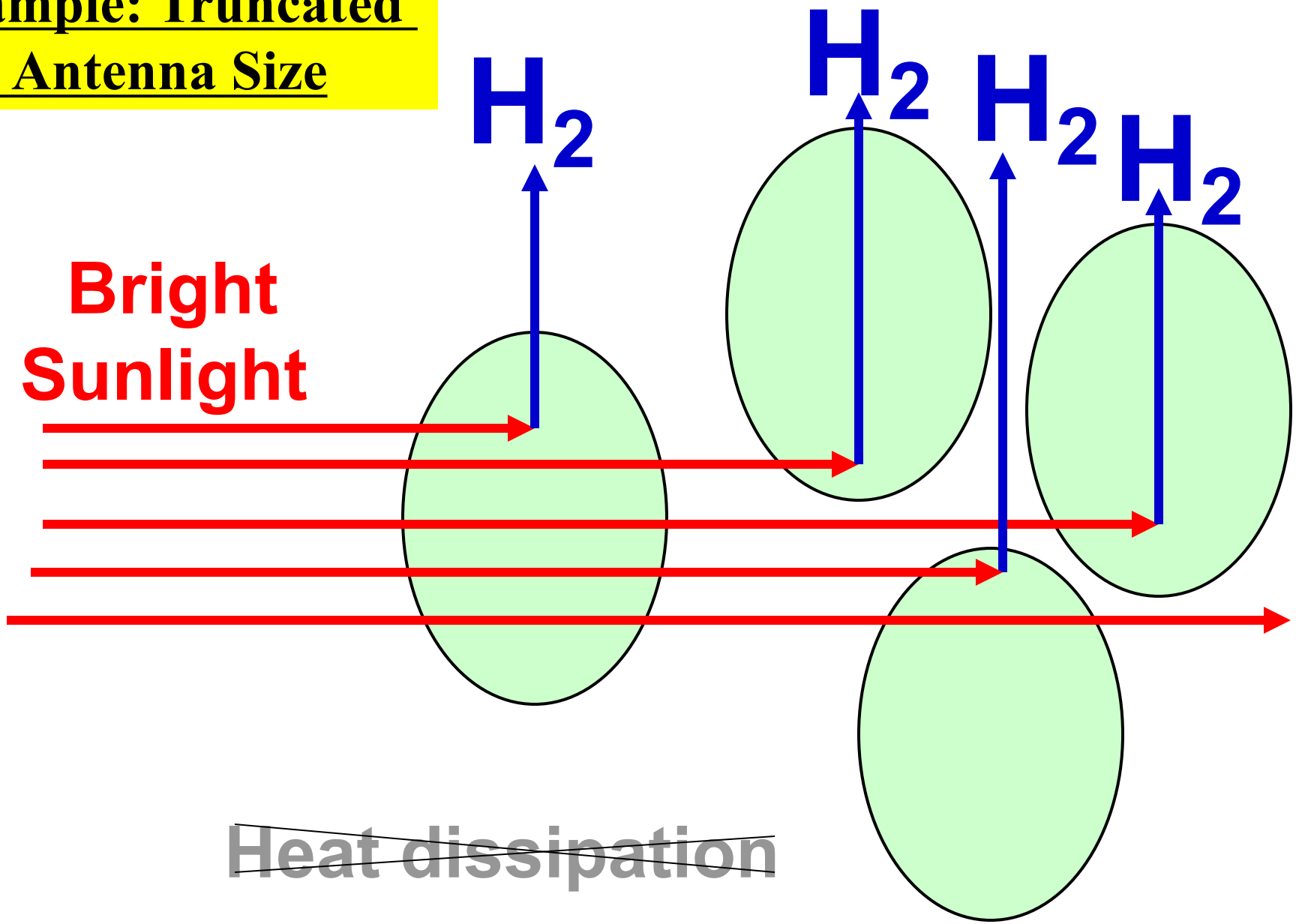
Example:
Fully Pigmented

The green algae
Chlamydomonas reinhardtii



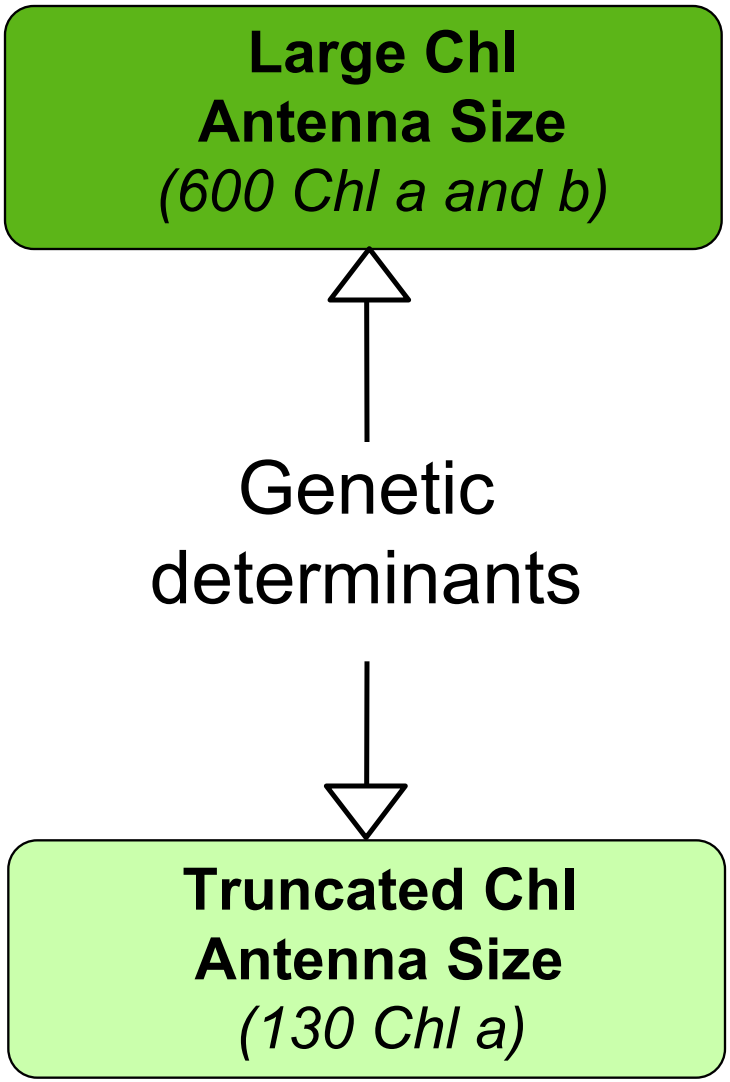
Fully pigmented cells over-absorb and wastefully dissipate bright sunlight.

Example: Truncated Chl Antenna Size



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

Regulation of the Chl antenna size



Identify genes and molecular mechanisms to enable a truncated antenna size in microalgae and cyanobacteria.

Objectives and Approach

Objectives:

Identify genes and associated molecular mechanisms that confer a *T*runcated *L*ight-harvesting *A*ntenna (TLA property) in the ***tla2*** and ***tla3*** strains of *Chlamydomonas reinhardtii.*

Develop protocols for the targeted truncation of the light-harvesting antenna size in cyanobacteria.

Approach:

(a) Cloning of the genes responsible for the the ***tla2*** and ***tla3*** phenotype in *Chlamydomonas reinhardtii*. (b) Identification of genes to be interrupted or deleted in cyanobacteria. (c) Functional analysis of the transformants (Berkeley expertise).

Progress achieved *vs* the DOE targets

Sunlight Utilization Efficiency, % of Incident Solar Energy

(maximum possible = 30%)

	2000	2003	2005	2008	2010	2015
Targets (Light utilization efficiency)	**3%**	**10%**			**15%**	**20%**
Tla strain identified	**3% (WT)**	**10% *tla1***	**15% *tla2***	**25% *tla3***		

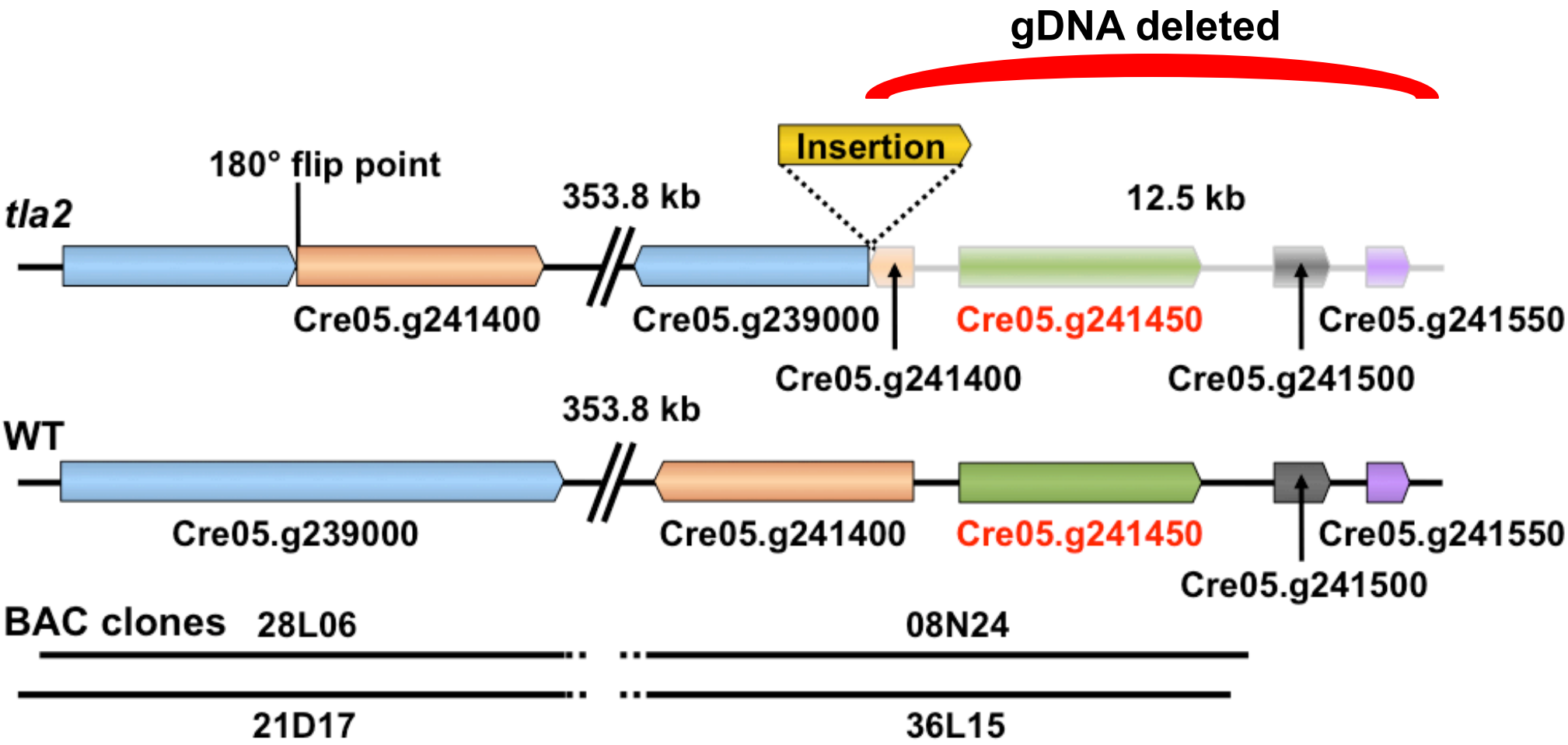
Progress achieved *vs* the DOE targets

Chlorophyll antenna size in wild type and mutants

(minimum possible = 130 Chl molecules)

	2000	2003	2005	2007	2008	2010	2011	2012	2015
Targets (Chl Antenna size	600 (WT)		300			200			150
Tla strain identified	600 (WT)	275 *tla1*	195 *tla2*		150 *tla3*				
Gene cloning and functional elucidation				*TLA1-Mov34 MPN*			*TLA2-FTSY*	*TLA3*	

Progress: *tla2* gDNA Map and Complementation



Progress: *TLA2* gene elucidation

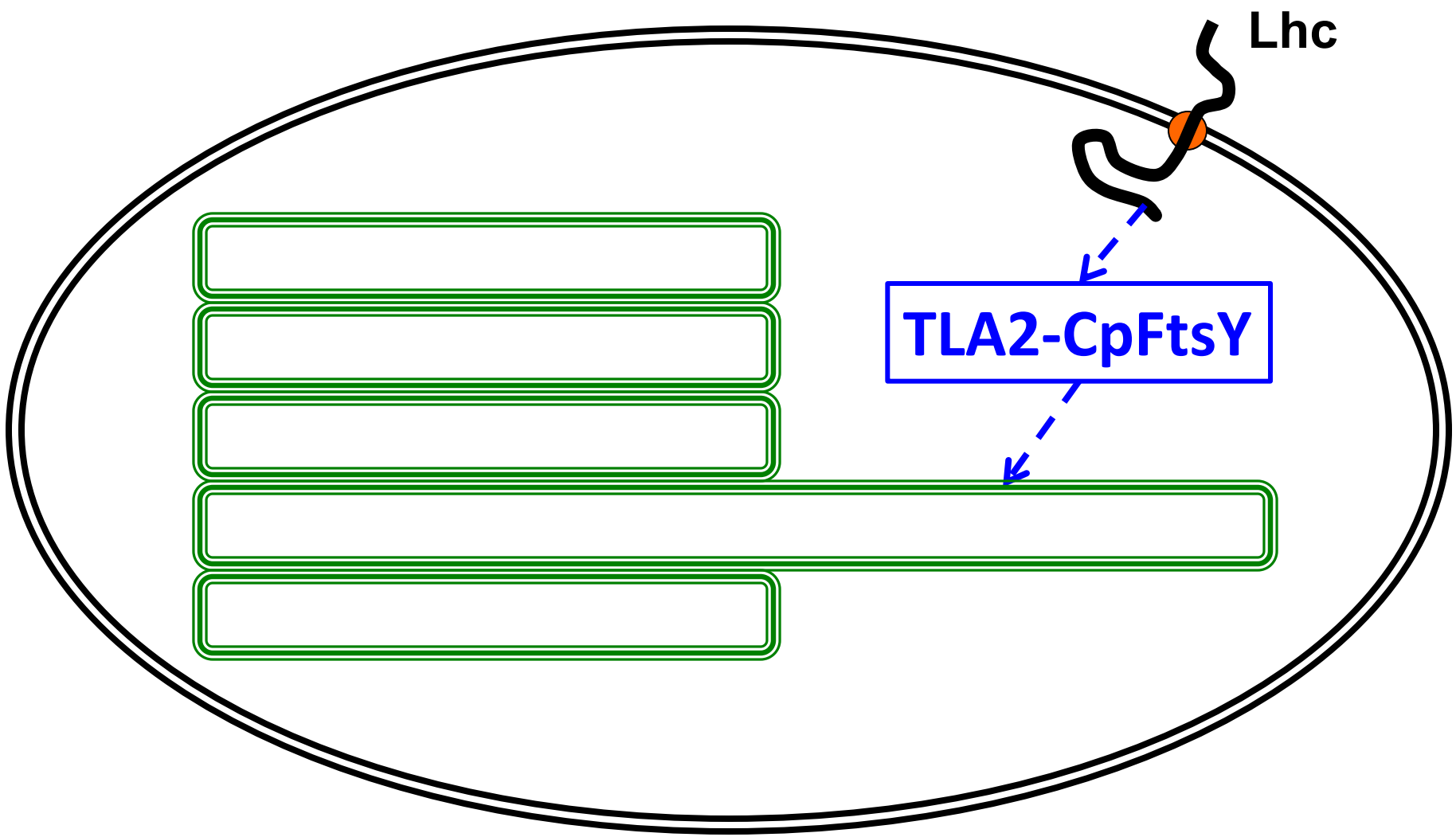
- Cleaning the ***tla2*** mutant genotype through multiple successive genetic crosses with wild type strains; a systematic effort to remove unrelated insertions, DNA fragments, and other anomalies resulting from the mutagenesis approach.
- Mapping the genomic DNA region at the plasmid insertion site, identify genes that were interrupted or deleted in the *tla2* mutant.
- Perform genetic crosses to test for co-segregation of lesion and plasmid.
- Identify genes interrupted or deleted by the DNA insertional mutagenesis process.
- Complementation of the ***tla2*** strain with each of the deleted or interrupted genes to recover the fully pigmented phenotype.
- Elucidation of TLA2 protein structure and function.

Progress: TLA2-CpFtsY protein structure

MQTTVGRKCVASSAAGRSRNVTVFRRCSRGGPVKVVANAGGEAGPGFLQRLGRVIKEKA
AGDFDFFAGTSKTRERLGLVDEMLALWSLEDYEDSLEELEEVLISADFGPRTALKIVD
RIREGVKAGRVKSAEDIRASLKAAIVELLTARGRSSELKLQGRPAVVLIVGVNGAGKTT
TVGKIAYKYGKEGAKVFLIPGDTFRAAAAEQLAEWSRRAGATIGAFREGARPQAVIASN
LDDLRQRTCKDASDVYDLILVDTAGRLHTAYKLMEELALCKAAVSNALPGQPDETLLVL
DGTTGLNMLNQAKEFNEAVRLSGLILTKLDGTARGGAVVSVVDQLGLPVKFIGVGETAE
DLQPFDPEAFAEALFPKVKEPATAGTK

♦ : nucleotide binding domains; cTP: chloroplast transit peptide;
HB: helical bundle domain

Progress: TLA2-CpFtsY protein functions to fold imported Lhc proteins in the developing thylakoid membrane, thereby increasing the chlorophyll antenna size



Summary of Accomplishments FY2012

TLA1-MOV34/MPN effort:

- Bioinformatic analysis tentatively identified the TLA1 protein as a variant of the MOV34/MPN containing proteins

TLA2-ΔFTSY effort:

- Physiological characterization of the *tla2* mutant was completed.
- Genetic analysis and multiple crosses of the *tla2* mutant were completed.
- Mapping of the plasmid insert site in the *tla2* mutant was completed.
- Of the five genes adversely affected by the plasmid insertional mutagenesis, gene Cre05.g241450 encoding the CpFTSY protein complemented the mutation.
- The unique functional role of the CpFTSY protein in algae was elucidated.
- Patent application on the function of the *TLA2-CpFTSY* gene filed.

TLA3-effort:

- Physiological and genetic characterization of the *tla3* mutant was completed.
- Mapping of the plasmid insert site in the *tla3* mutant was completed.
- The gene affected in the *tla3* mutation is known.
- A Western blot analysis remains to be done for project completion.

Evidence of Accomplishments

Peer reviewed publications:

Blankenship RE, Tiede DM, Barber J, Brudvig GW, Fleming G, Ghirardi ML, Gunner MR, Junge W, Kramer DM, Melis A, Moore TA, Moser CC, Nocera DG, Nozik AJ, Ort DR, Parson WW, Prince RC, Sayre RT (2011) Comparing photosynthetic and photovoltaic efficiencies and recognizing the potential for improvement. Science 332:805-809

Mitra M, Ng S, Melis A (2012) The *TLA1* protein family members contain a variant of the plain MOV34/MPN domain. Amer J Biochem Mol Biol. 2(1): 1-18

Melis A (2012) Photosynthesis-to-Fuels: From sunlight to hydrogen, isoprene, and botryococcene production. Energy Environ. Sci. 5(2): 5531-5539

Kirst H, Garcia-Cerdan JG, Zurbriggen A, Melis A (2012) Assembly of the light-harvesting chlorophyll antenna in the green alga *Chlamydomonas reinhardtii* requires expression of the *TLA2-CpFTSY* gene. Plant Physiol 158: 930–945

Mitra M, Dewez D, García-Cerdán JG, Melis A (2012) Polyclonal antibodies against the TLA1 protein also recognize with high specificity the D2 reaction center protein of PSII in the green alga *Chlamydomonas reinhardtii.* Photosynth Res, in press

Patent application filed:

Melis A and Kirst H (2012) Suppression of *TLA2-CpFTSY* gene expression for improved solar energy conversion efficiency and photosynthetic productivity in algae.

Significance of Work

- **Successful cloning of the *TLA2-ΔFTSY and TLA3* genes, and elucidation of their function in defining the antenna size of photosynthesis.**
- **The highly conserved *TLA2* and *TLA3* genes can be applied to improve photosynthetic productivity in all green microalgae.**

Collaborations, Applications and Beneficiaries

→ **The *Tla* concept is commercially applied in microalgae:**

- *Chlamydomonas* for H_2 (NREL) and biomass production (U-Wageningen, The Netherlands); and

- *Nannochloropsis* for commercial production of polyunsaturated fatty acids (PUFAs).

→ **The *tla1 mutant* strain was requested and acquired by universities (x14), industry (x10), government labs (x6), high schools (x2). (Recipients of strains from the US and overseas.)**

Proposed Future Work

- Complete the Western blot analysis for the *tla3* mutant and proceed to peer-reviewed publication of the results.

- Demonstrate feasibility of the TLA concept in cyanobacteria. (Currently in progress.)

- Advance the exploration of the "*extended photosynthetically active radiation*" (ePAR) concept. (Proprietary design not disclosed.)

Technical Backup Slides

Chl Antenna Size vs Light Utilization Efficiency
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%

- ***tla2* antenna size = 195 Chl molecules (42% of control)**
  (PSII=80; PSI=115)
  Photon use efficiency of *tla2* photosynthesis = ~30%
  Utilization Efficiency of Absorbed Light Energy by *tla2*: ~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%