



DOE Hydrogen Program

# Maximizing Light Utilization Efficiency and Hydrogen Production in Microalgal Cultures

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**Project ID # PD\_16\_melis**

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



# Overview

## Timeline

- **Start: 01-Dec-2004**
- **End: 30-Nov-2010**
- **Completion: 70%**

## Budget

- Total Project Funding  
**DOE: \$1.2 M, UCB: \$450 k**
- Funding for FY08  
**DOE: \$258 k, UCB: \$75 k**
- Funding for FY09  
**DOE: 0, 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**



# Objectives and Approach

**Objective:** Minimize the chlorophyll antenna size of photosynthesis to maximize solar conversion efficiency in green algae.

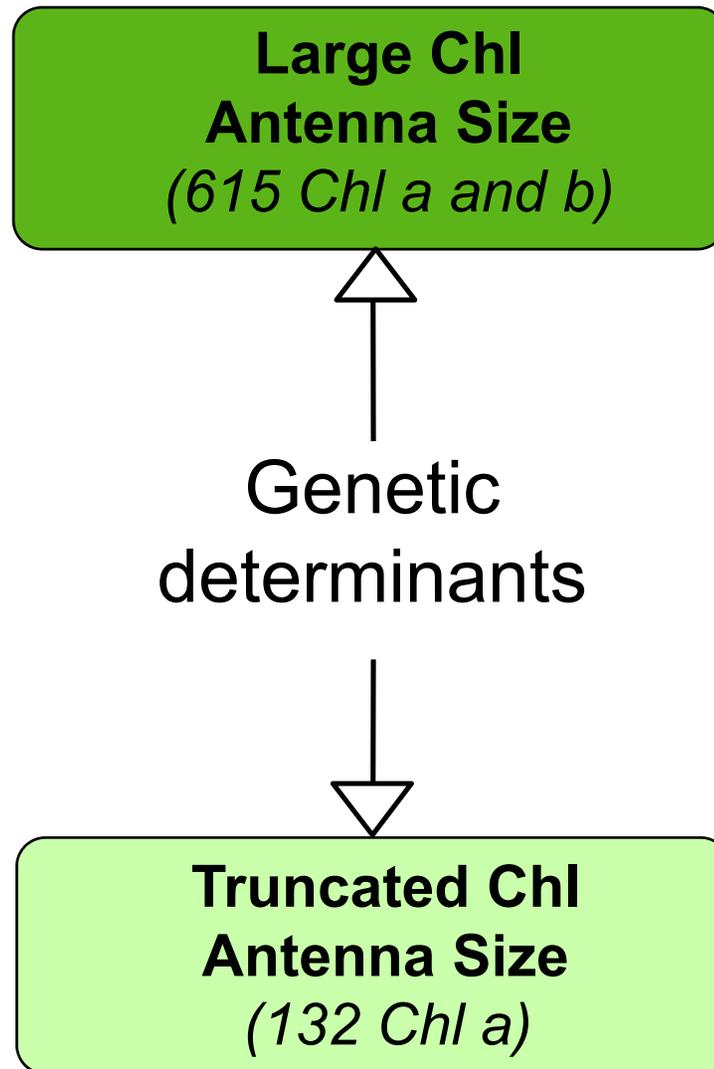
(Identify and characterize genes that regulate the Chl antenna size in the model green alga *Chlamydomonas reinhardtii*. Apply these genes to other green algae, as needed.)

**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.)



# Regulation of the Chl antenna size



**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.**





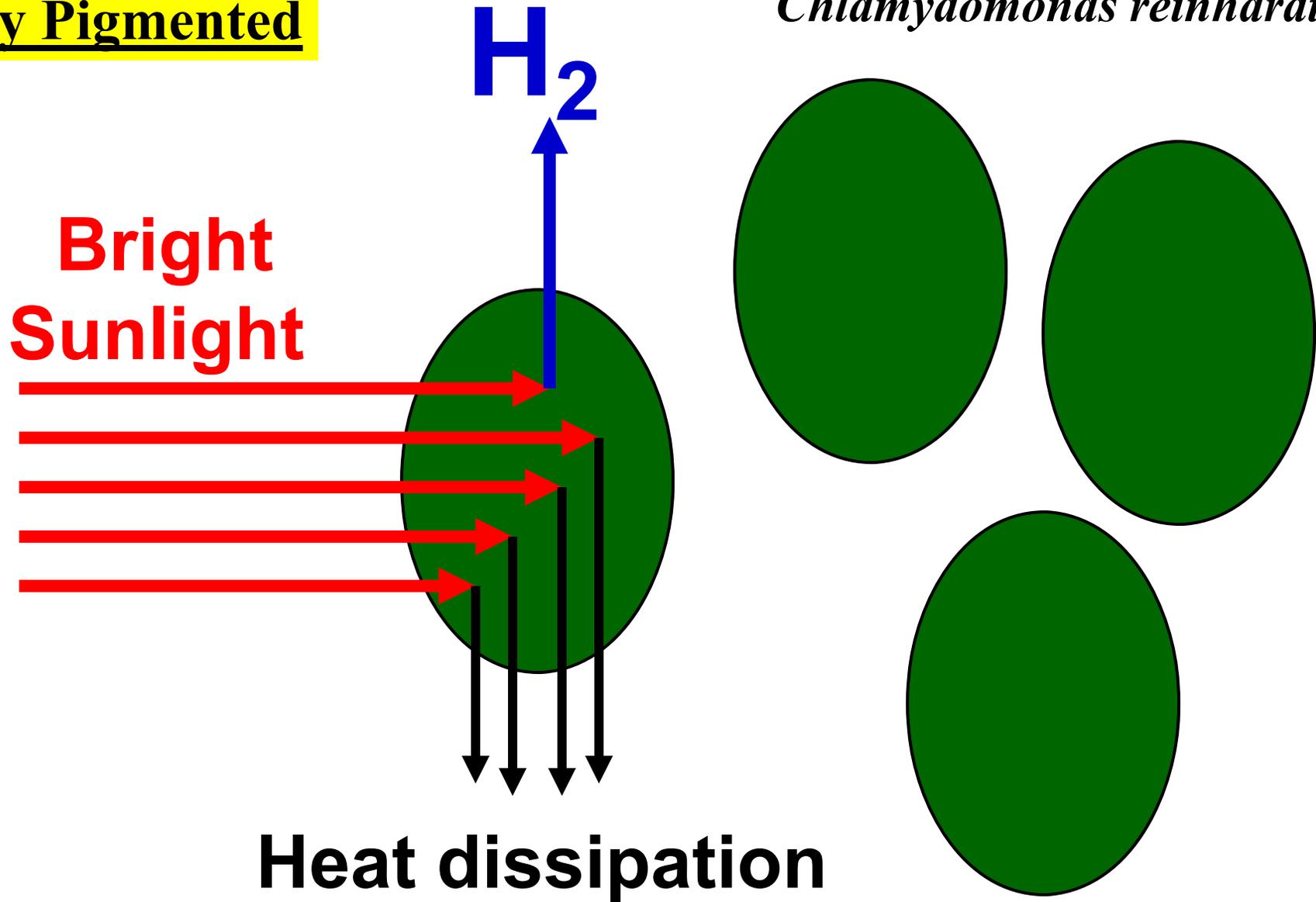
**Hydrogen production  
in a backyard**

*Chlamydomonas reinhardtii* mass culture



**Example:**  
**Fully Pigmented**

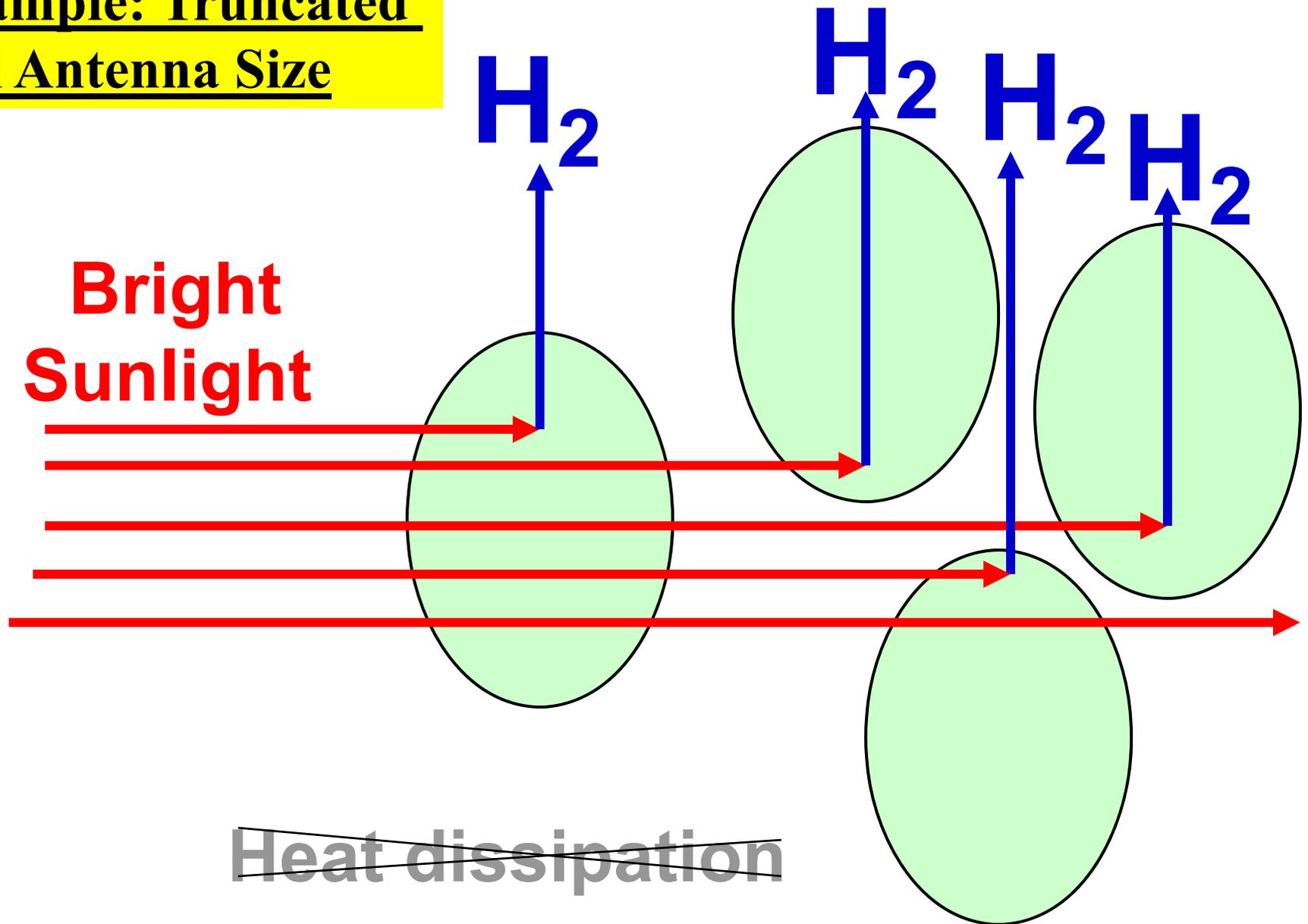
The green algae  
*Chlamydomonas reinhardtii*



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.



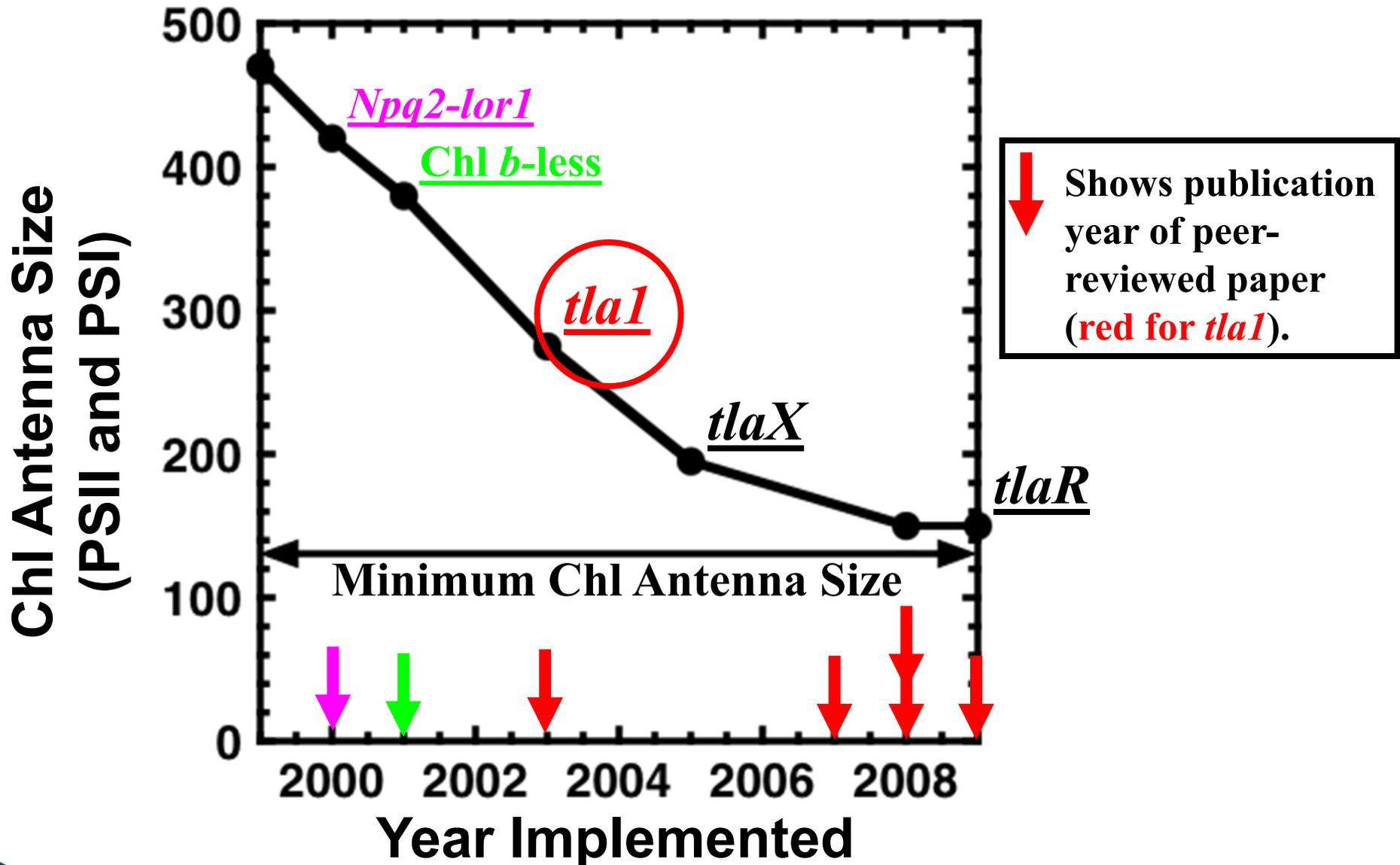
# Technical Barriers and Targets

- **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%**
- **Theoretical maximum efficiency: ~30%**
- **Target for 2010: Reach a 15% Utilization Efficiency of Absorbed Light Energy.**



# Project Timeline

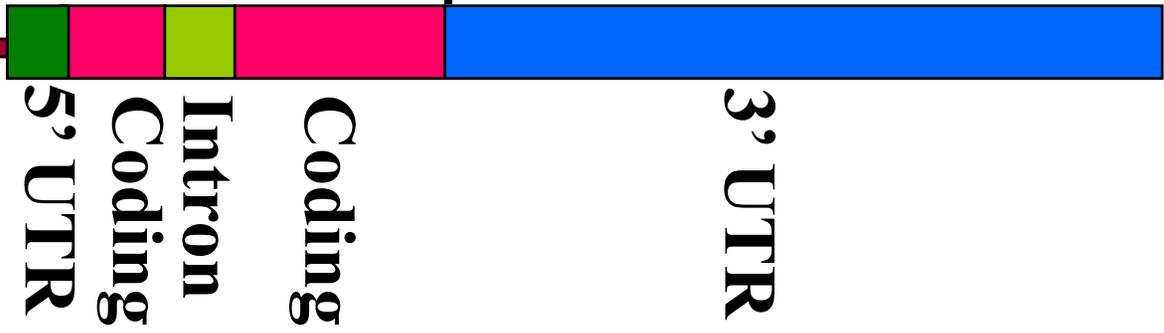
## Chlorophyll Antenna Size in Chlamydomonas



Wild type *Tla1* gene

PROMOTER

TAA



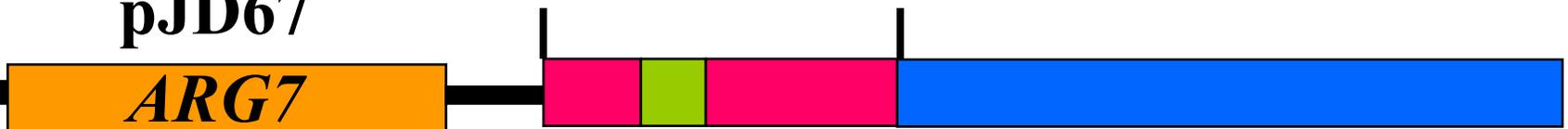
*tla1* mutant gene

pJD67

*ARG7*

ATG

TAA



# Localization of the Tla1 protein in *C. reinhardtii*

## The unicellular green alga *Chlamydomonas reinhardtii*

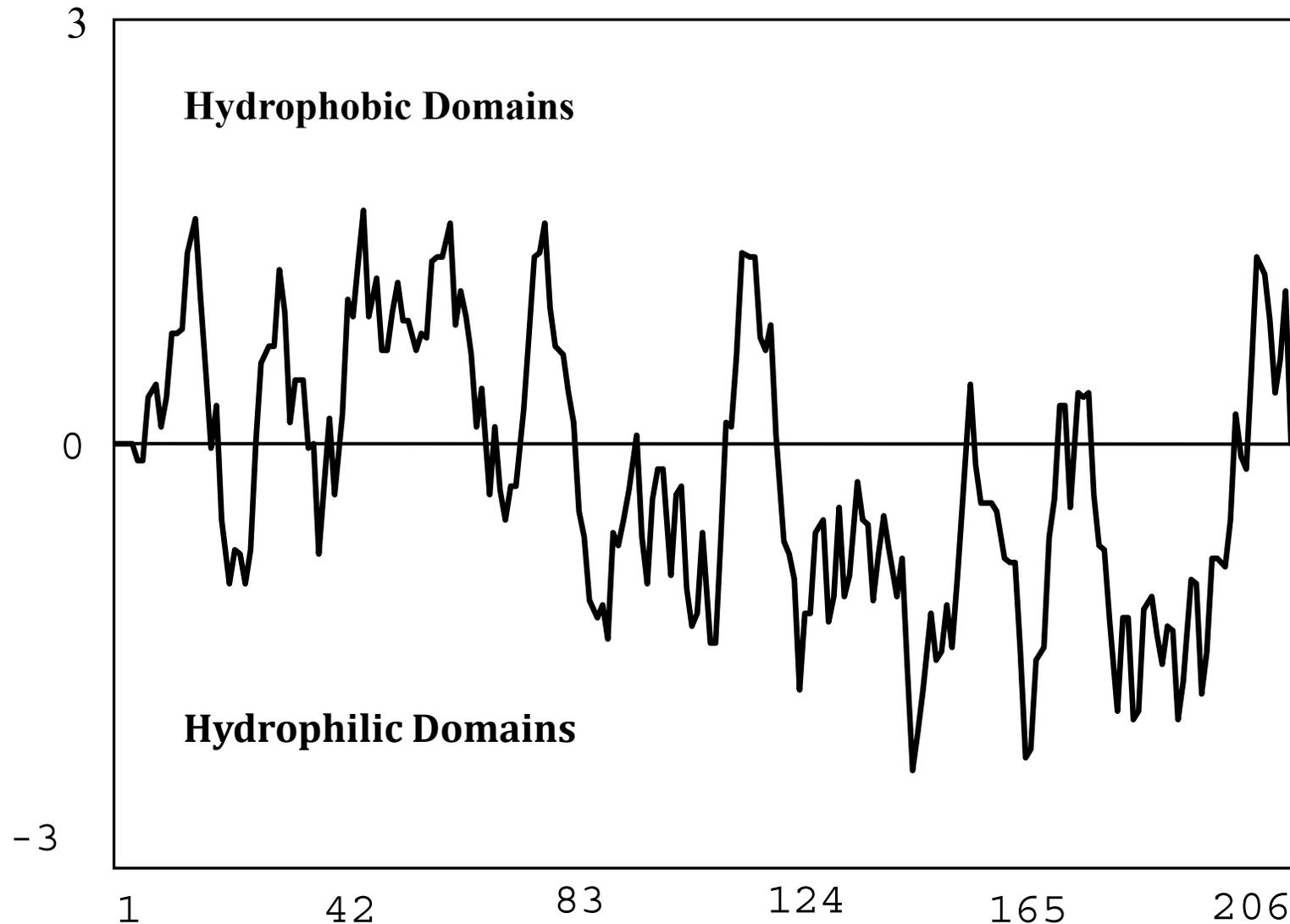


Environmentally friendly  
self-repairing and  
replicating microstructure



# Current Technical Accomplishments

Hydropathy plot of the Tla1 protein: Tla1 is hydrophilic



UCB-Melis



# Current Technical Accomplishments

## Localization of the Tla1 protein in a cellular compartment

Cell Fractionation and Quantitative Western blot analysis was applied.

**Problem encountered:** Tla1 specific polyclonal antibodies recognized the 23 kD Tla1 protein **AND** a 28.5 kD unknown protein (**unexpected complication**).

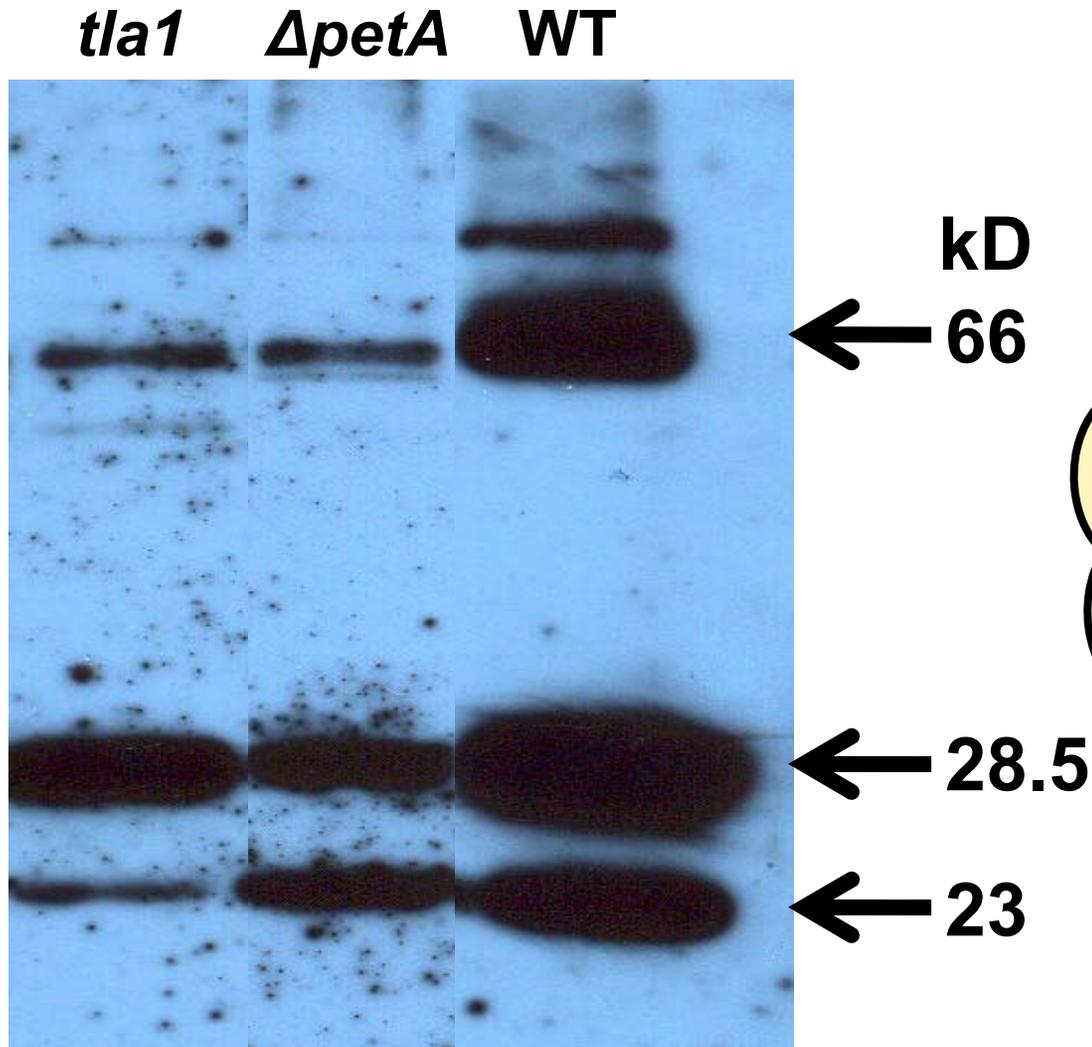
Extensive and time-consuming biochemical analyses revealed that the 28.5 kD protein was the D2 reaction center protein of PSII.

Proteomic analysis revealed a 9 amino acid C-terminus epitope that was nearly identical among the two proteins, explaining how Tla1 and D2 have common antigenic determinants (**complication elucidated**).



# Current Technical Accomplishments

- Tla1 polyclonal antibodies recognizing the 23 kD Tla1 protein, also cross reacted with a 28.5 kD protein.



Re-generated anti-Tla1 antibodies: Same result !



# Current Technical Accomplishments

- **Immuno-precipitation and isolation** of the cross-reacting 28.5 kD protein, followed by mass spec analysis yielded the peptide sequence **R.TWFDDADDWLR.Q**, which is specific for the *psbD/D2* photosystem-II reaction center protein.

```
1  mtiaigtyqe  krtwfddadd  wlrqdrfvfv  gwsglllfpc  ayfalggwlt  gttfvtswyt
61  hglatsyleg  cnfltaavst  pansmahsll  fvwgpeaqgd  ftrwcqlggl  wafvalhgaf
121 gligfmlrqf  eiarsvnlrp  ynaiafsapi  avfvsvfliy  plgqsgwffa  psfgvaaifr
181 filffqgfhn  wtlnpfhmmg  vagvlgaall  caihgatven  tlfedgdgan  tfracfnptqa
241 eetysmvtan  rfwsqifgva  fsnkrwlhff  mllvpvtglw  msaigvvgla  lnlaydfvs
301 qeiraaedpe  fetfytknil  lnegirawma  aqdqpherlv  fpeevlprgn  al
```



# Current Technical Accomplishments

A CLUSTAL 2.0.10 multiple sequence alignment of D2 and T1a1 revealed no similarity between the two proteins

```

D2      MTIAIGTYQEKRTWFDDADDWLRQDRFVFGWSGLLLFPCAYFALGGWLTGTTFFVTSWYT 60
T1a1    -----MTFSCSADQT-ALLKILAHAAKYP      24
                : *.*:      . * . :...: :

D2      HGLATSYLEGCNFLTAAVSTPANSMAHSLLFVWGPEAQGDFTRWCQLGGLWAFVALHGAF 120
T1a1    NSVNGVLVGTAKEGGSVEILDAIPLCHTTLTAPALEIG-----LAQVESYTHITGSV 77
                ::      :  :  :.      *  .:.*: * : .      *      * . : :. : *:.

D2      GLIGFMLRQFEIARSVNLRPYNIAIAFSAPIAVFVSVFLIYPLGQSGWFFAPSFVAAIFR 180
T1a1    AIVGYQSDARFGPGD-----LPPLGR-----          99
                :::*:      :  :. .      : ***:

D2      FILFFQGFHNWTLNPFHMMGVAGVLGAALLCAIHGATVENTLFEDGDGANTFRAFNPQA 240
T1a1    -----KIADKVSEHQQAQAVVLVLDN----KRLEQFCKAQA 130
                *      : * * .      ::::      : :. *      **:

D2      EETYSMVTANRFWSQIFGVAFSNKRWLHFFMLLVPVTGLWMSAIGVVGLALNLRAYDFVS 300
T1a1    DNPFELEFSKD-----GSKGWKR-----ASADGGELALKNADWKKLRE 167
                ::::: :      ..* * :      ** *      *      . : .

D2      QEIRAAEDPEFETFYTKNILLNEGIRAWMAAQDQPHERLVFPEEVLPRGNAL 352
T1a1    EFFVMFKQLKHRTLHDFEEHLDDAGKDWLNKGFASSVKFLLPGNAL----- 213
                : :      : : ...*:: : *::: : * :      .      ::::* :.*
    
```



# Current Technical Accomplishments

A CLUSTAL 2.0.10 partial sequence alignment of the C-termini of D2 and T1a1 revealed essential identity among 9 consecutive amino acids

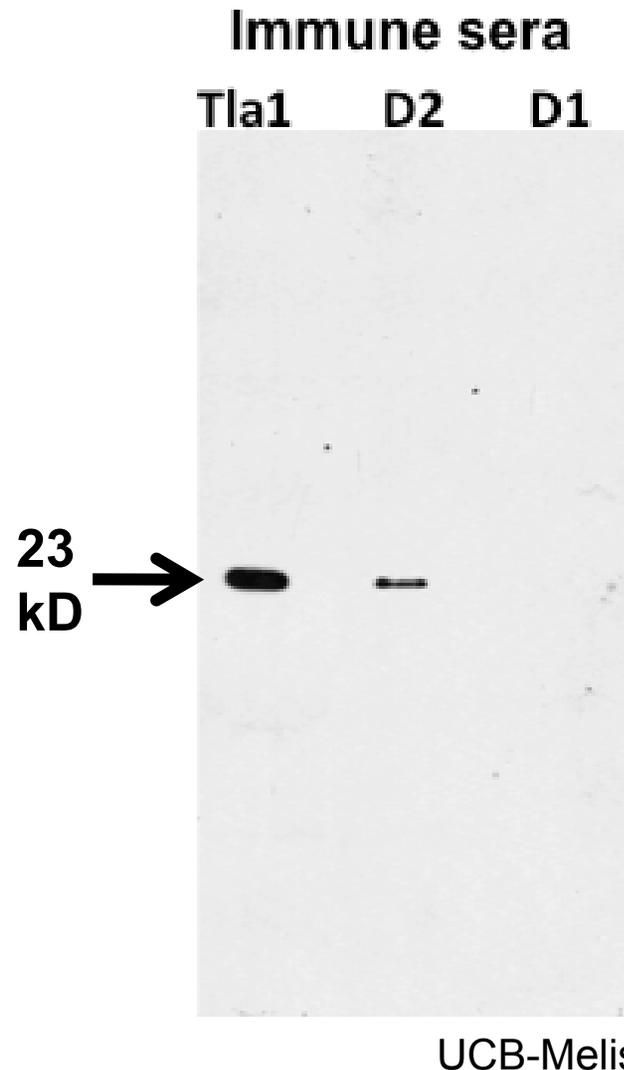
<b>D2</b>	<b>V-FPEEVLPRGNAL</b>	<b>13</b>
<b>T1a1</b>	<b>VKF---LLP-GNAL</b>	<b>10</b>
	<b>* * : ** ****</b>	

These results suggested that 9 amino acids from the C-terminus form a common epitope and serve as common antigenic determinants.



# Current Technical Accomplishments

- Immune sera against the T1a1 and D2 proteins both recognize the T1a1 recombinant protein. However, D1 immune sera do not.

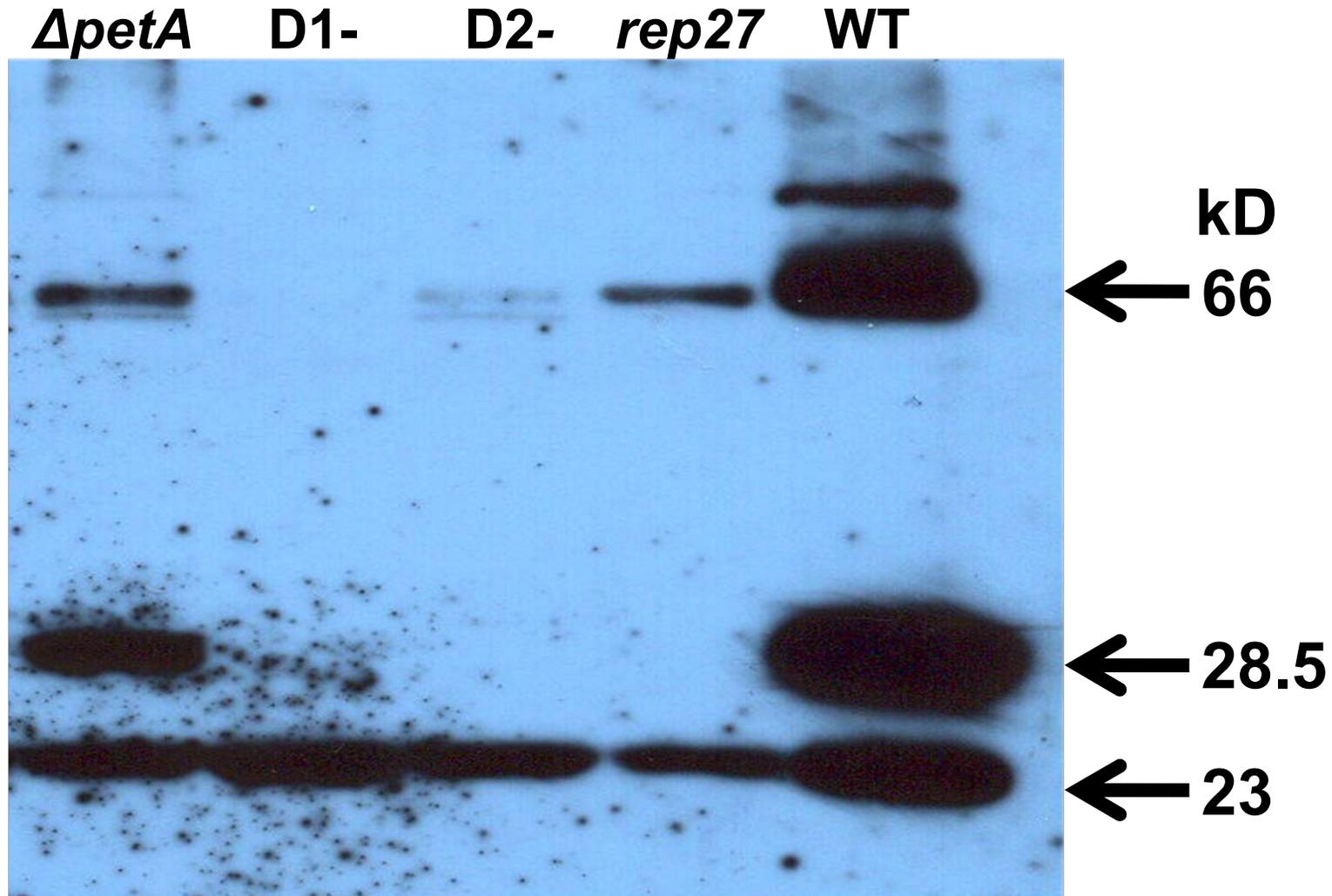


**0.5 ng of recombinant T1a1 protein was loaded in the three lanes**



# Current Technical Accomplishments

- The 28.5 kD protein cross reaction is absent in the D1-less, D2-less, and *rep27* mutants of *Chlamydomonas*.



# Current Technical Accomplishments

- **Conclusion:**

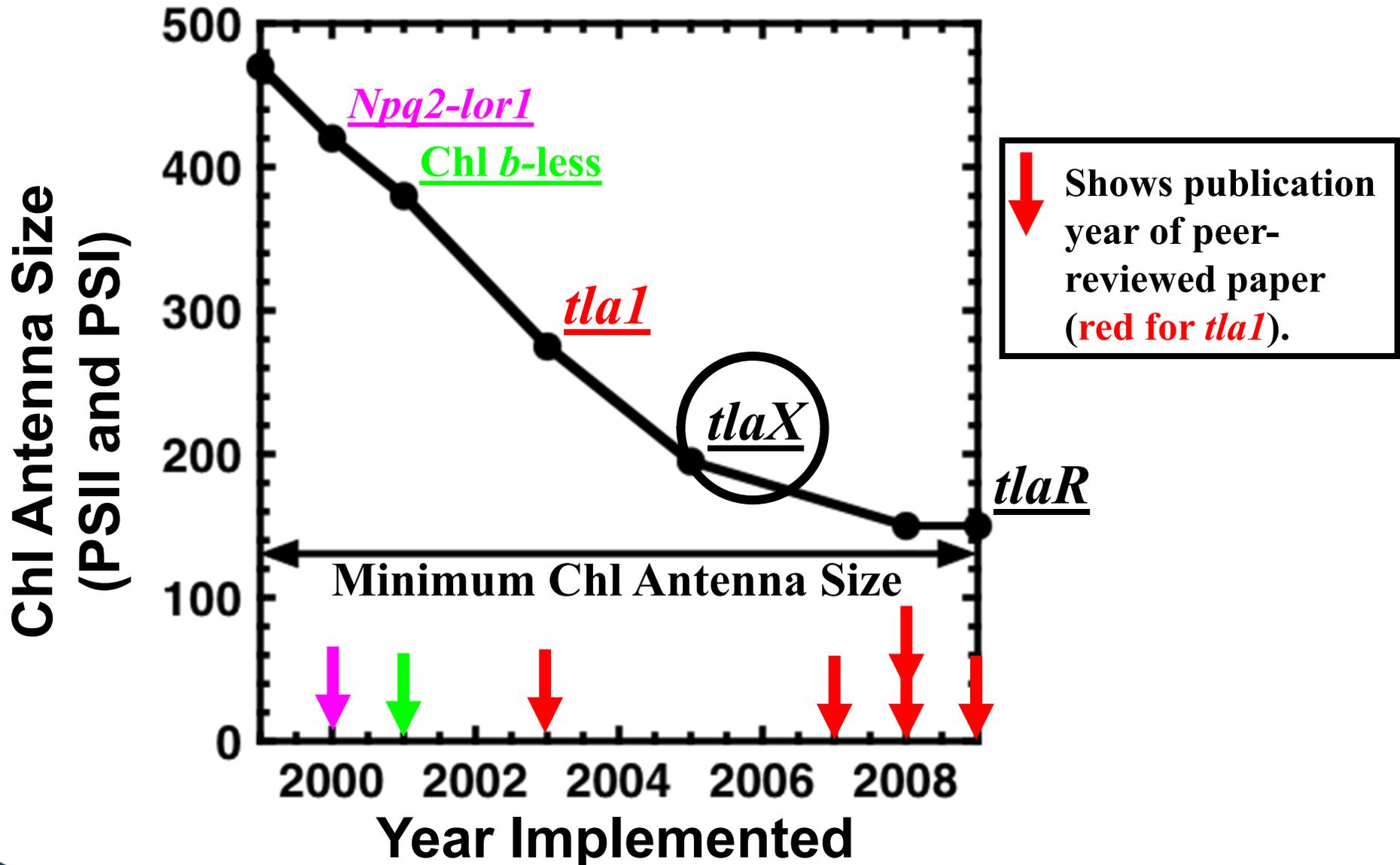
The T1a1 and D2 proteins have a common 9 amino acid epitope in their C-terminus, that is antigenic enough to generate a strong antibody response against either protein.

This unexpected property has complicated the analysis of the T1a1 function, but it is now solved.



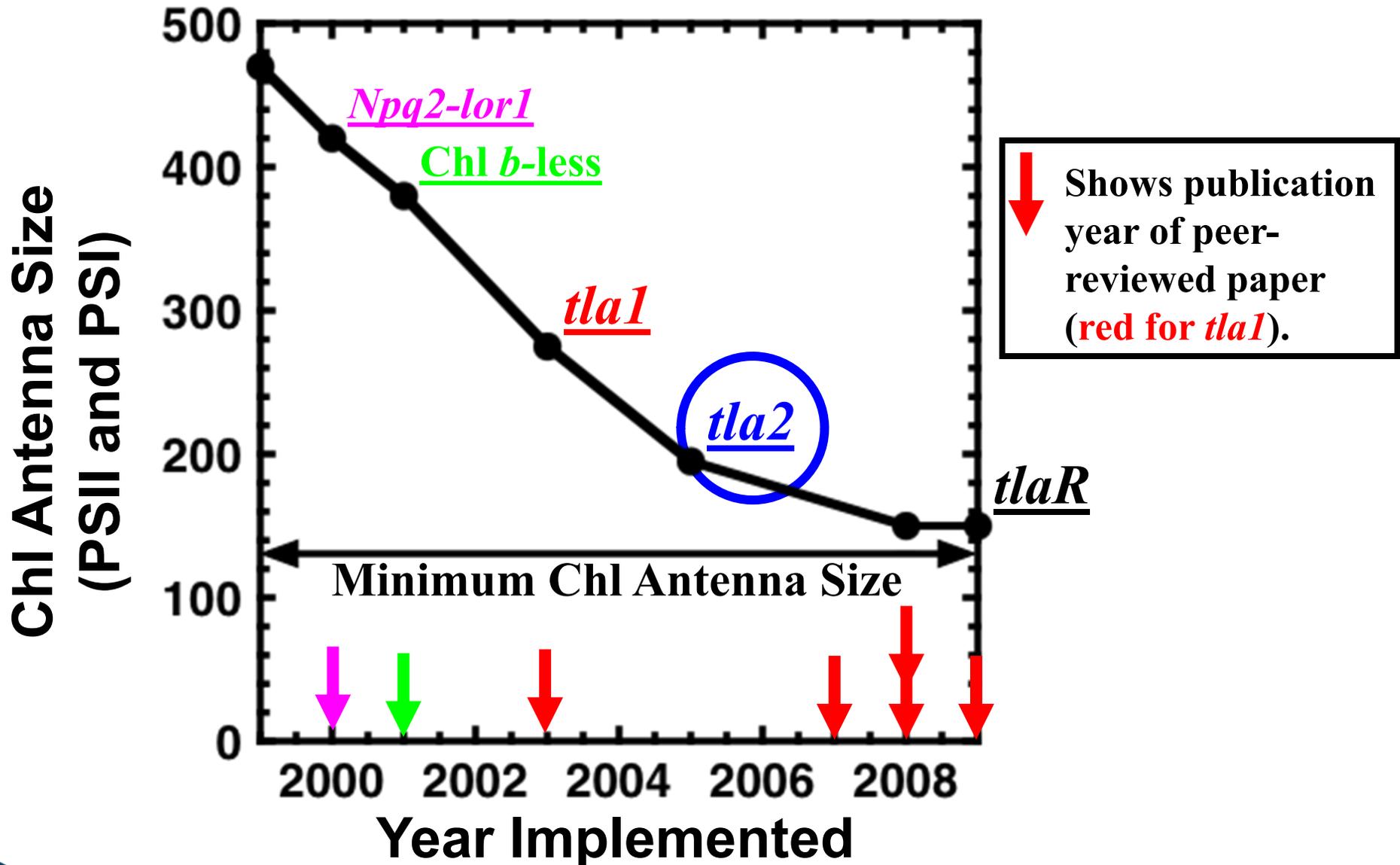
# Project Timeline

## Chlorophyll Antenna Size in Chlamydomonas



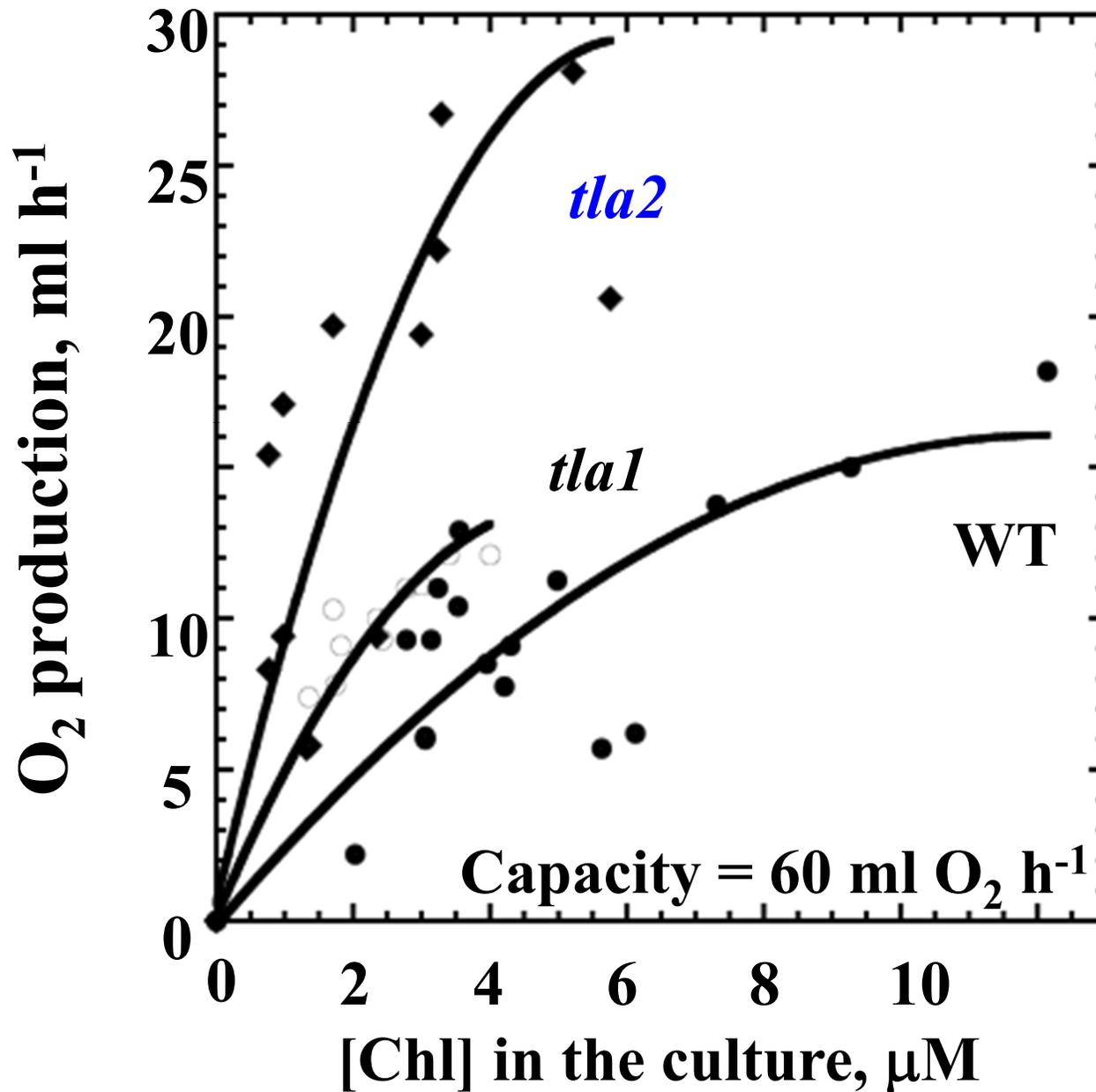
# Project Timeline

## Chlorophyll Antenna Size in Chlamydomonas

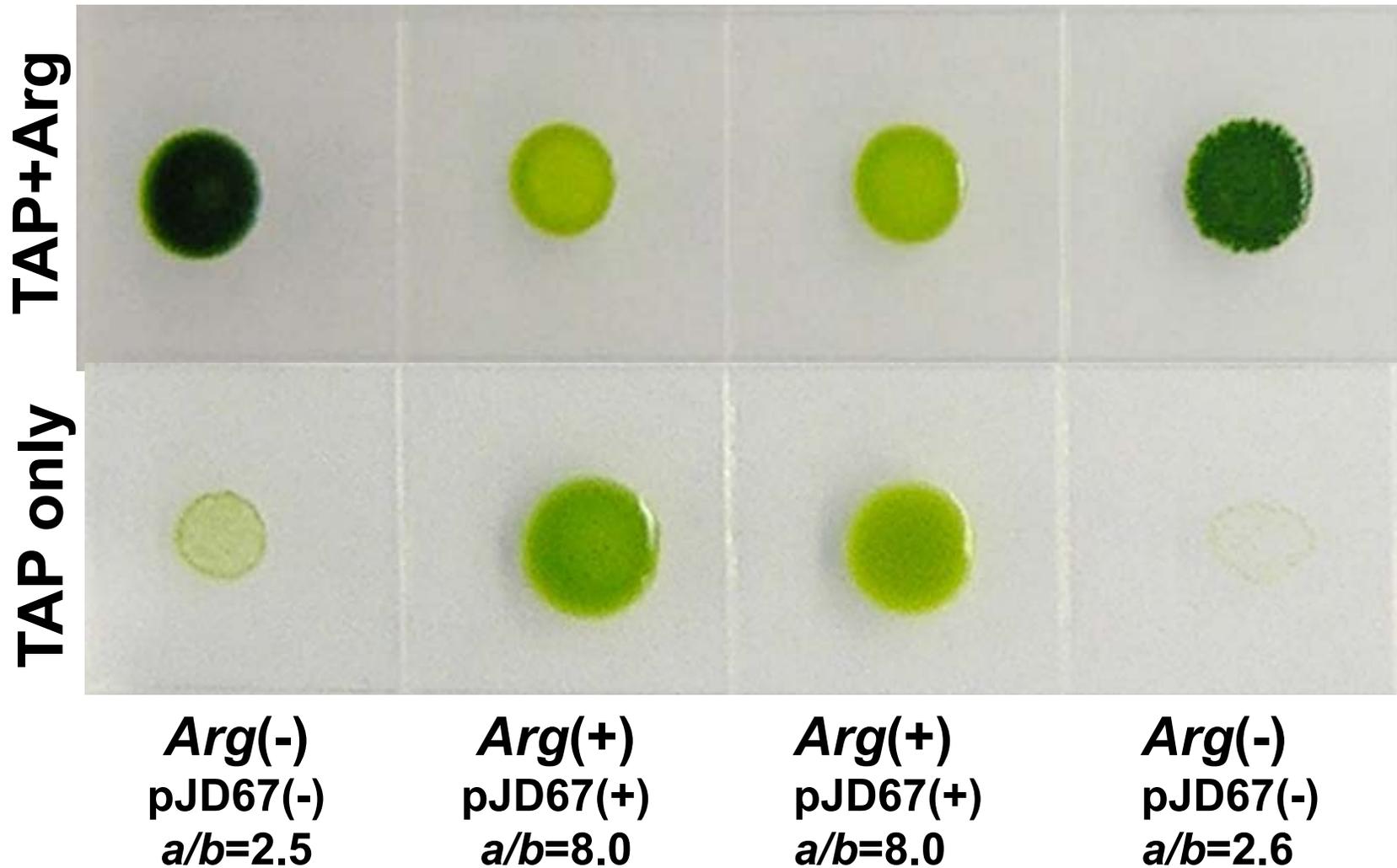


# Productivity in Scale-up of Cultures

(*tla2* outperforms both wild type and *tla1* strains)



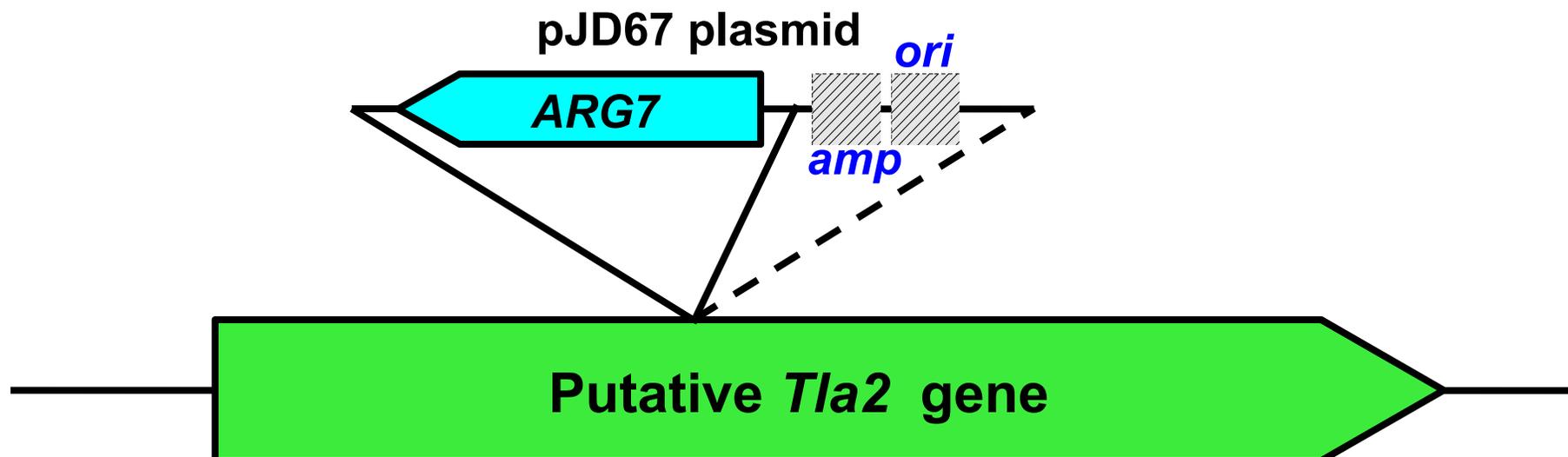
“Tetrad analysis” of progeny from a single *tla2* x *Arg* zygospore



$Arg^- = AGI \times 3.24 = 4A(mt^-)Arg(-)$



## Exogenous *pJD67* plasmid insertion site in the *tla2* mutant



### *Chlamydomonas reinhardtii* genomic DNA

#### Outcome of Genetic Analysis (so far):

*Tla2* knock-out mutant

*pJD67* plasmid *Ori* and *Amp* deleted

Putative *Tla2* gene is ~13.8 kb in size  
(possibly more than 1 genes)

*Tla2* encodes a putative protein of ~138 kD

(unknown function, based on a prediction model)



# Summary of Accomplishments

## Analysis of the Tla1 protein and *tla2* mutant strain

- Sorted-out a nagging but unexpected antigenic complication affecting the analysis of the Tla1 protein.
- Cloned the gene (putative) conferring a truncated Chl antenna size to the *tlaX* mutant (**now called *tla2***).



# Progress achieved vs the DOE targets

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

	2000	2003	2005	2008	2010	2015
Program Targets	3%	10%			15%	20%
Progress	3%	10% <i>tla1</i>	15% <i>tla2</i>	25% <i>tlaR</i>		



# Significance of Work

- **First-time identification and documentation of two different genes (*Tla1* and *Tla2*) that regulate the chlorophyll antenna size in photosynthesis.**
- **Findings could be applied in mass culture to increase solar conversion efficiencies and photobiological hydrogen production.**



# Current Work

**Complete the cellular localization of the Tla1 protein.**

**Elucidate Tla1 function upon application of sense, antisense & RNAi technologies with the *Tla1* gene in *Chlamydomonas reinhardtii*.**

**Advance the characterization of the *Tla2* gene.**



# Future Work

**Continue work with the cloning of gene(s) conferring the “truncated Chl antenna” phenotype in the *tlaR* strain.**

**(Entails molecular, genetic, biochemical, physiological and scale-up studies.)**



# Overall Summary & Publications

**Completed first part of work on the *Tla1* gene.**

**Sorted-out an unexpected immunoblot problem interfering with the localization analysis of the Tla1 protein.**

**Cloned the gene causing the *tlaX* mutant phenotype; termed the newly discovered gene as *Tla2*.**

## **Published findings in peer reviewed journal:**

Berberoglu H, Pilon L, Melis A (2008) Radiation characteristics of *Chlamydomonas reinhardtii* CC125 and its truncated chlorophyll antenna transformants *tla1*, *tlaX*, and *tla1-CW<sup>+</sup>*. Intl J Hydrogen Energy 33: 6467-6483

Mitra M, Melis A (2008) Optical properties of microalgae for enhanced biofuels production. Optics Express 16: 21807-21820



# Invited Presentations on the Tla1 Work

- XLVII Congress of the Italian Society for Plant Biology. Pisa, Italy.** Title of Plenary Lecture: Transgenic microalgae for enhanced photosynthesis. Tuesday 01-Jul-2008.
- J. Craig Venter Institute, Rockville, Maryland.** Title of Seminar: Maximizing light utilization efficiency and hydrogen production in microalgal cultures. Thursday 07-Aug-2008.
- American Chemical Society 236<sup>th</sup> National Meeting, Philadelphia, PA.** Title of “Emerging Technologies: Fuel Biotechnology” Symposium Lecture: Photosynthetic Biofuels: Improvement of *in situ* generation of hydrogen and hydrocarbons. Thursday 21-Aug-2008.
- 92<sup>nd</sup> Annual Meeting of the Optical Society of America.** October 19-24, 2008. Rochester, NY. Title of “Optics for Energy” Symposium Lecture: Optical properties of microalgae for enhanced biofuels production. Thursday 23-Oct-2008.
- “Global Energy” International Congress on Biofuels.** October 30-31, 2008. University of Alicante, Spain. Title of Keynote Lecture: Photosynthetic biofuels from microalgae. Thursday 30-Oct-2008.



# 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%
- *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%

