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

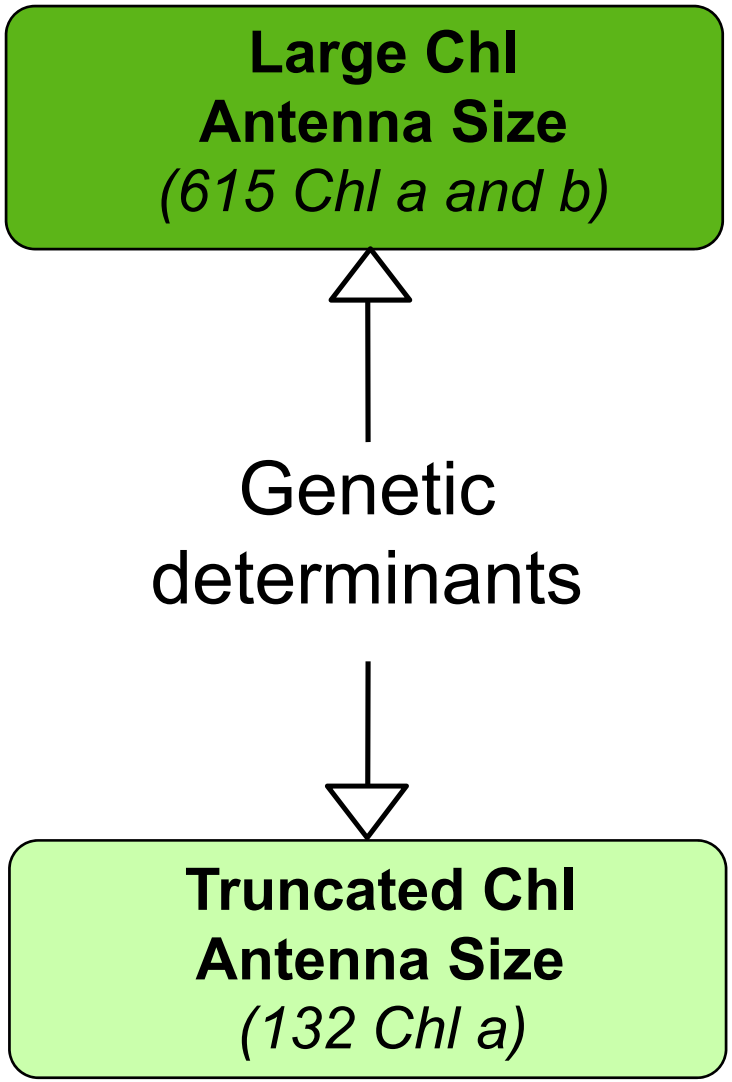
Objective: Minimize the chlorophyll antenna size of photosynthesis to maximize solar conversion efficiency in green algae.

(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.)

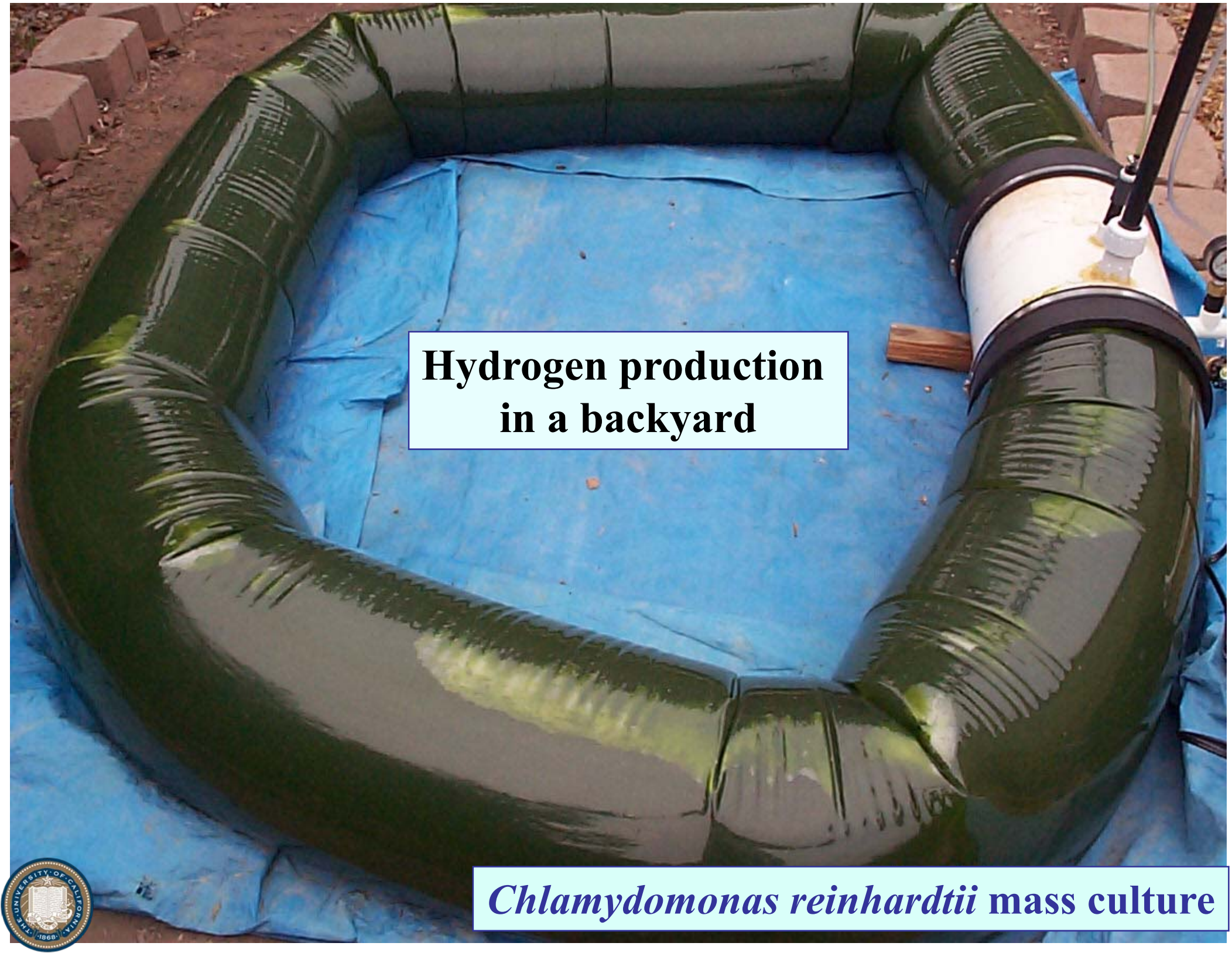
Approach: Interfere with the molecular mechanism for the regulation of the chlorophyll antenna size.

(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

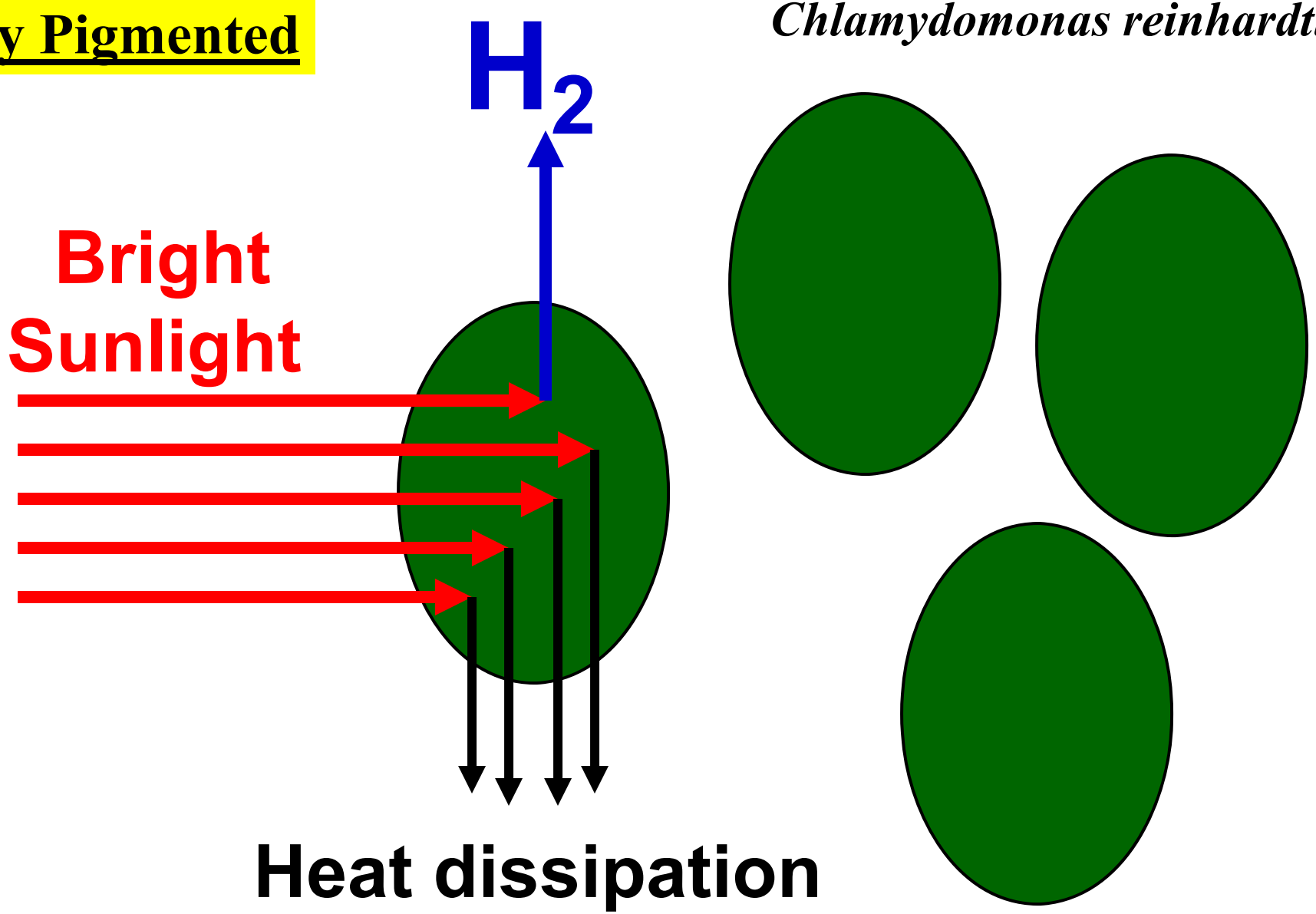


Interference with the genetic mechanism for the regulation of the Chl antenna size, to derive a permanently truncated Chl antenna size, is the goal of this R&D.


Hydrogen production
in a backyard
Chlamydomonas reinhardtii mass culture

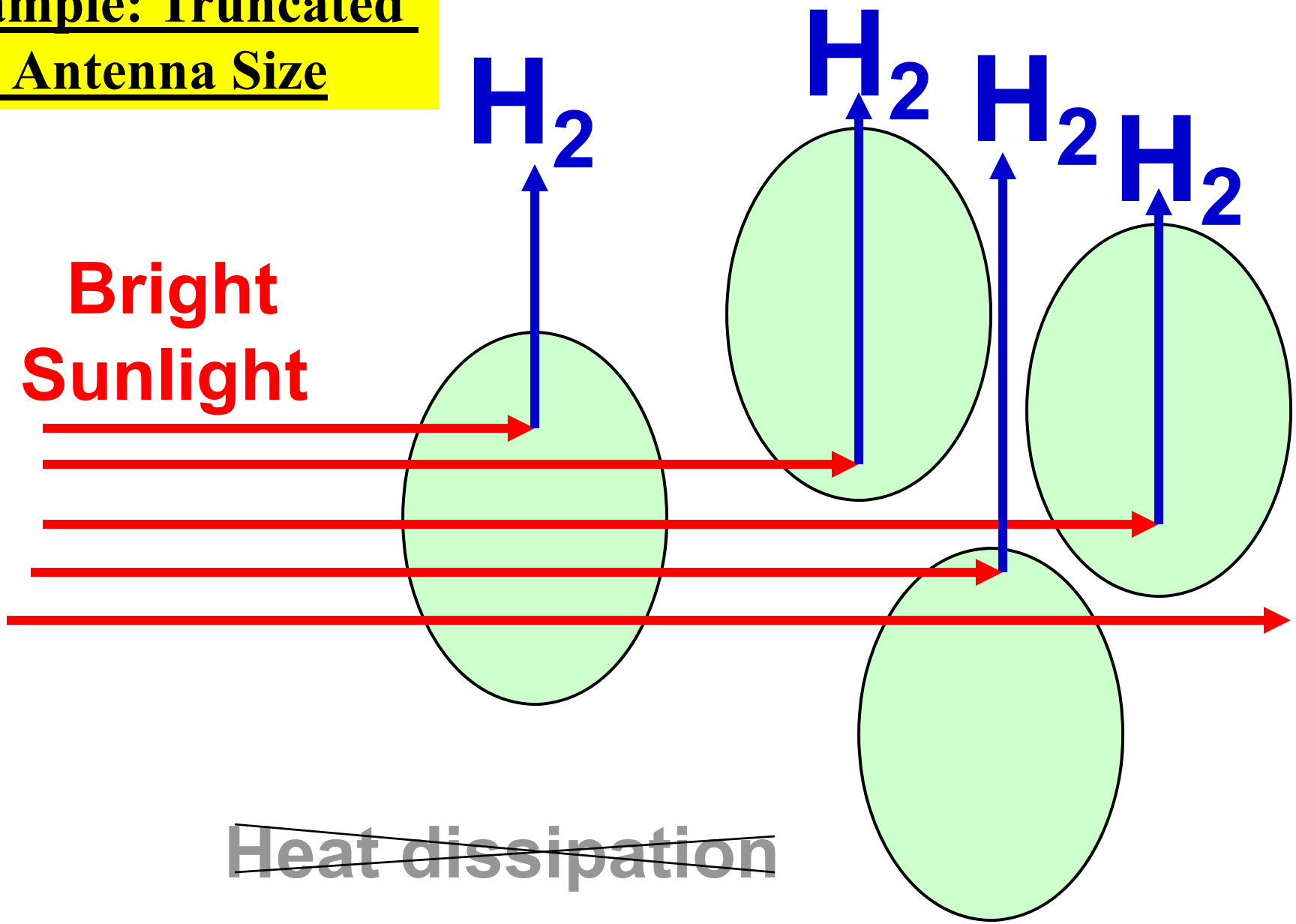
Example:
Fully Pigmented

The green algae
Chlamydomonas reinhardtii



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

Example: Truncated Chl Antenna Size

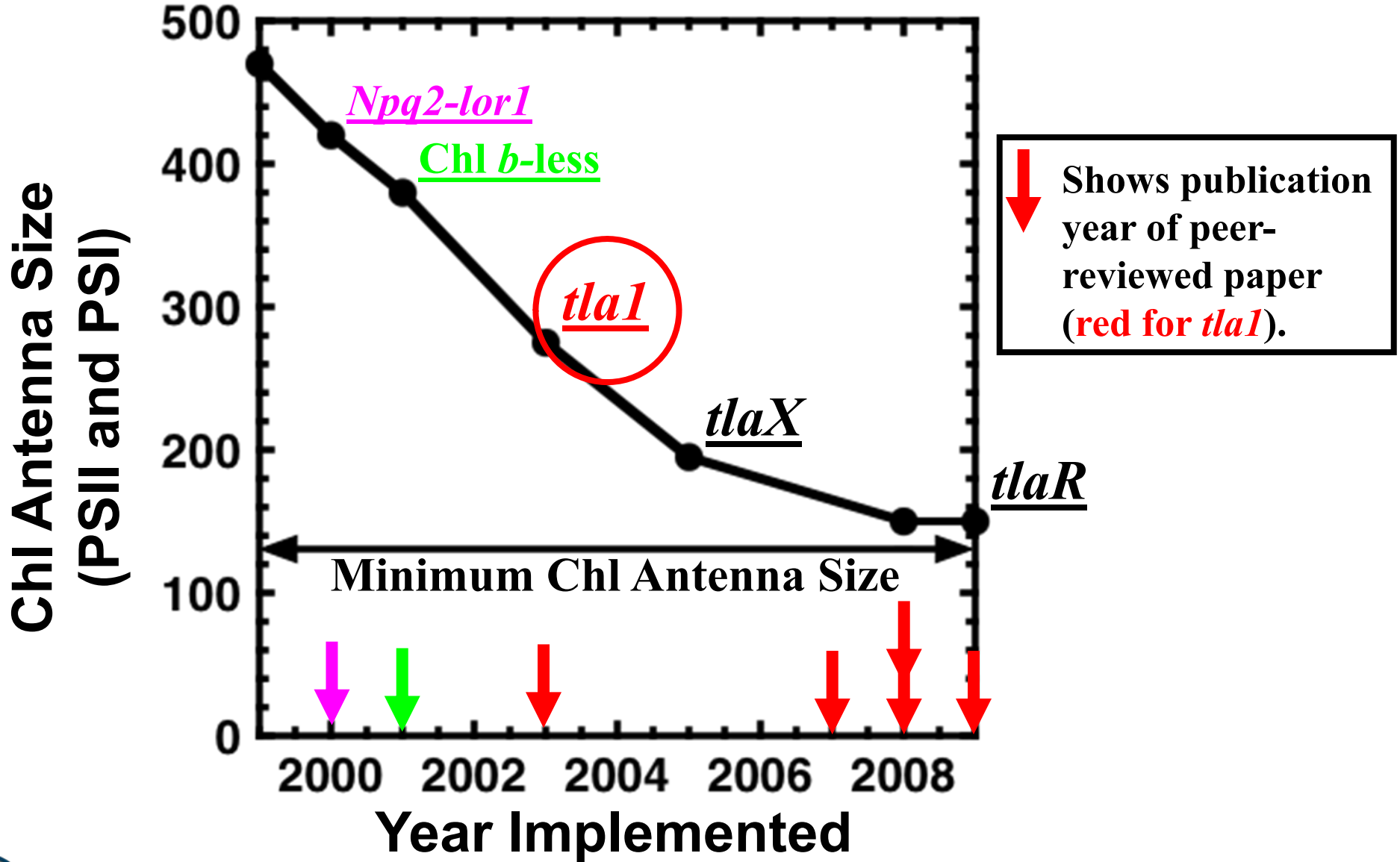


Truncated Chl antenna cells permit greater transmittance of light and overall better solar utilization by the culture.

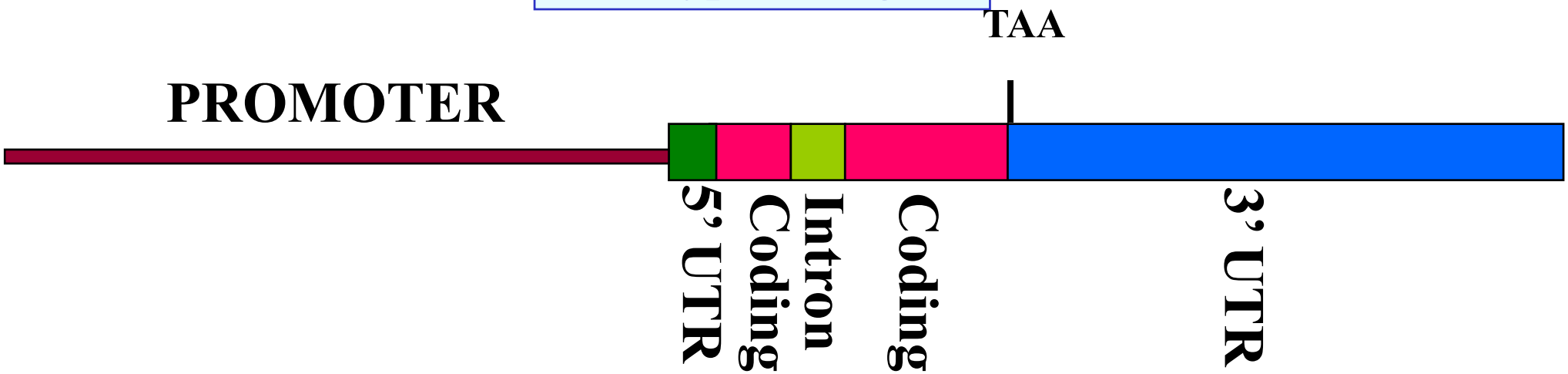
Technical Barriers and Targets

- **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.**

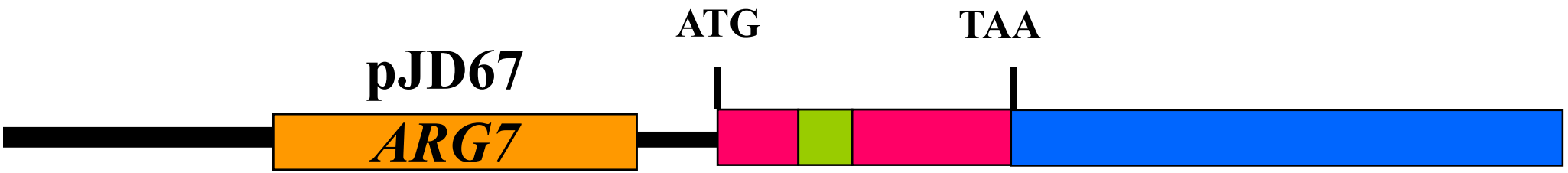
Project Timeline
Chlorophyll Antenna Size in Chlamydomonas



Wild type *Tla1* gene

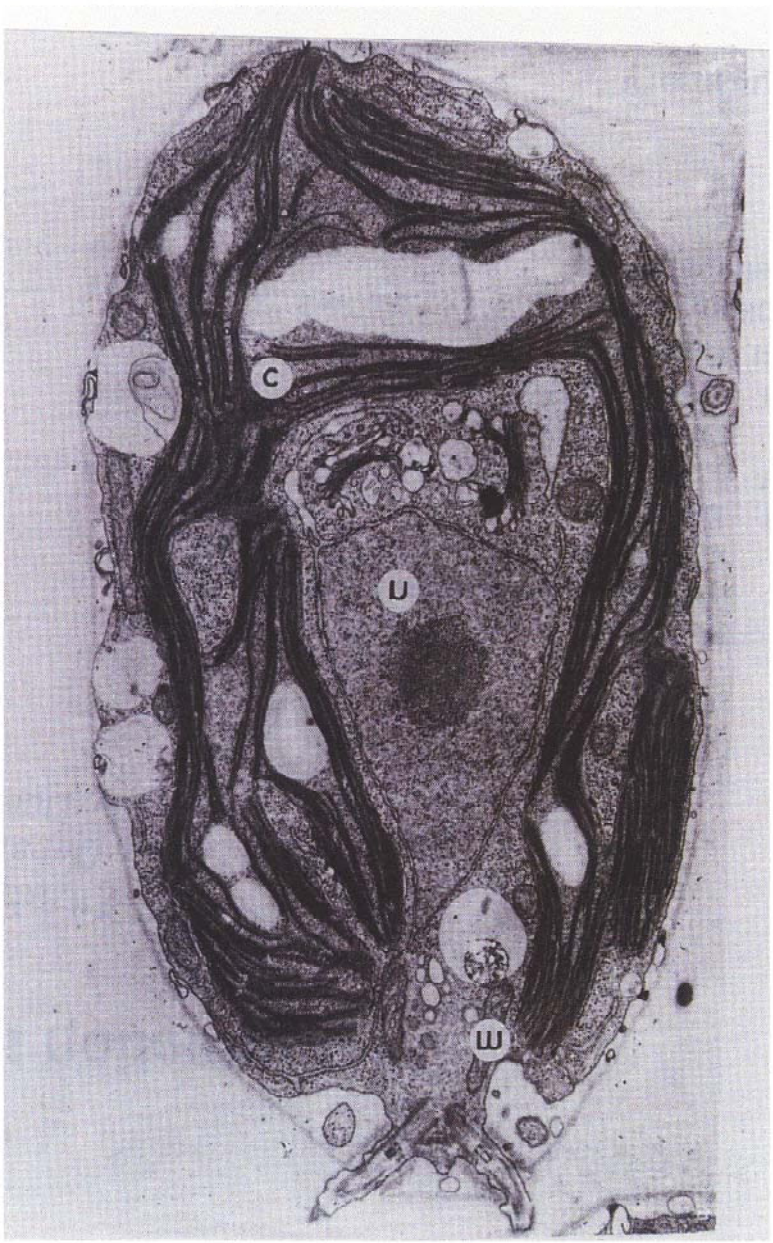


tla1 mutant gene



Localization of the Tla1 protein in *C. reinhardtii*

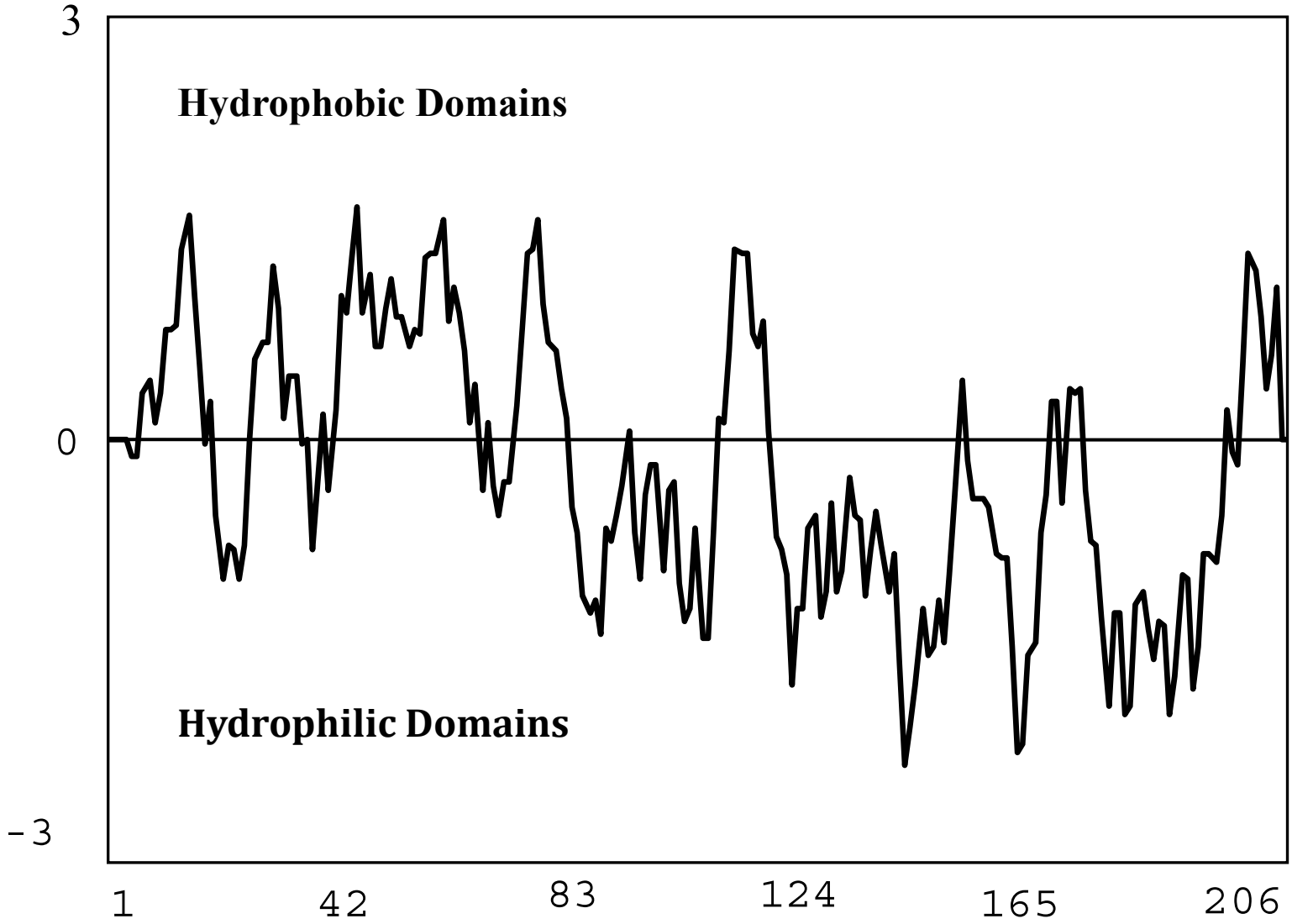
The unicellular green alga *Chlamydomonas reinhardtii*



Environmentally friendly self-repairing and replicating microstructure

Current Technical Accomplishments

Hydropathy plot of the Tla1 protein: Tla1 is hydrophilic



Current Technical Accomplishments

Localization of the Tla1 protein in a cellular compartment

Cell Fractionation and Quantitative Western blot analysis was applied.

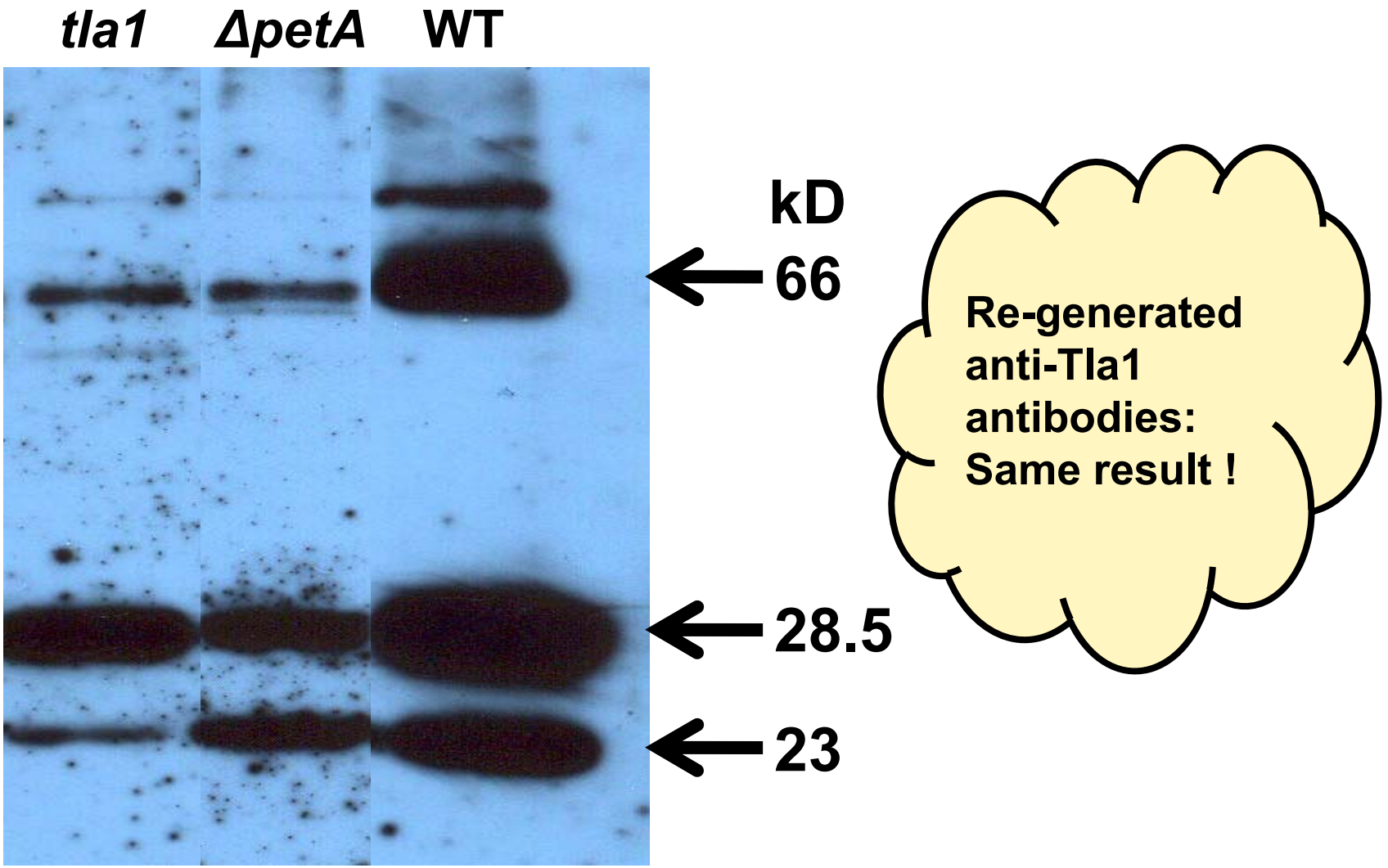
Problem encountered: Tla1 specific polyclonal antibodies recognized the 23 kD Tla1 protein AND a 28.5 kD unknown protein (unexpected complication).

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.**



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 tfrafnptqa
241 eetysmvtan rfwsqifgva fsnkrwlhff mllvpvtglw msaigvvgla lnlraydfvs
301 qeiraaedpe fetfytknil lnegirawma aqdqpherlv fpeevlprgn al
```

Current Technical Accomplishments

A CLUSTAL 2.0.10 multiple sequence alignment of D2 and Tla1 revealed no similarity between the two proteins

```
D2     MTIAIGTYQEKRTWFDDADDWLRQDRFVFVGWSGLLLFPCAYFALGGWLTGTTFVTSWYT 60
Tla1   ------------------------------------MTFSCSADQT-ALLKILAHAAKYPS 24
                                           : *.*:     . *.  :..:.: :

D2     HGLATSYLEGCNFLTAAVSTPANSMAHSLLFVWGPEAQGDFTRWCQLGGLWAFVALHGAF 120
Tla1   NSVNGVLVGTAKEGGSVEILDAIPLCHTTLTLAPALEIG-------LAQVESYTHITGSV 77
       :.:    :  .:   :.    * .:.*: * :  .   *       *. : ::. : *:.

D2     GLIGFMLRQFEIARSVNLRPYNAIAFSAPIAVFVSVFLIYPLGQSGWFFAPSFGVAAIFR 180
Tla1   AIVGYYQSDARFGPGD----------------------LPPLGR---------------- 99
       .::*:   : .:. .                        : ***:

D2     FILFFQGFHNWTLNPFHMMGVAGVLGAALLCAIHGATVENTLFEDGDGANTFRAFNPTQA 240
Tla1   ------------------------KIADKVSEHQAQAVVLVLDN----KRLEQFCKAQA 130
                               *   : * * .   ::::    : :. *  :**

D2     EETYSMVTANRFWSQIFGVAFSNKRWLHFFMLLVPVTGLWMSAIGVVGLALNLRAYDFVS 300
Tla1   DNPFELFSKD-----------GSKGWKR-----------ASADGGELALKNADWKKLRE 167
       ::.:.:.: :            ..* * :            ** *      *    .: .

D2     QEIRAAEDPEFETFYTKNILLNEGIRAWMAAQDQPHERLVFPEEVLPRGNAL 352
Tla1   EFFVMFKQLKHRTLHDFEEHLDDAGKDWLNKGFASSVKFLLPGNAL------ 213
       : :   :: :..*::  :  *::. : *:     .  ::::* :.*
```

Current Technical Accomplishments

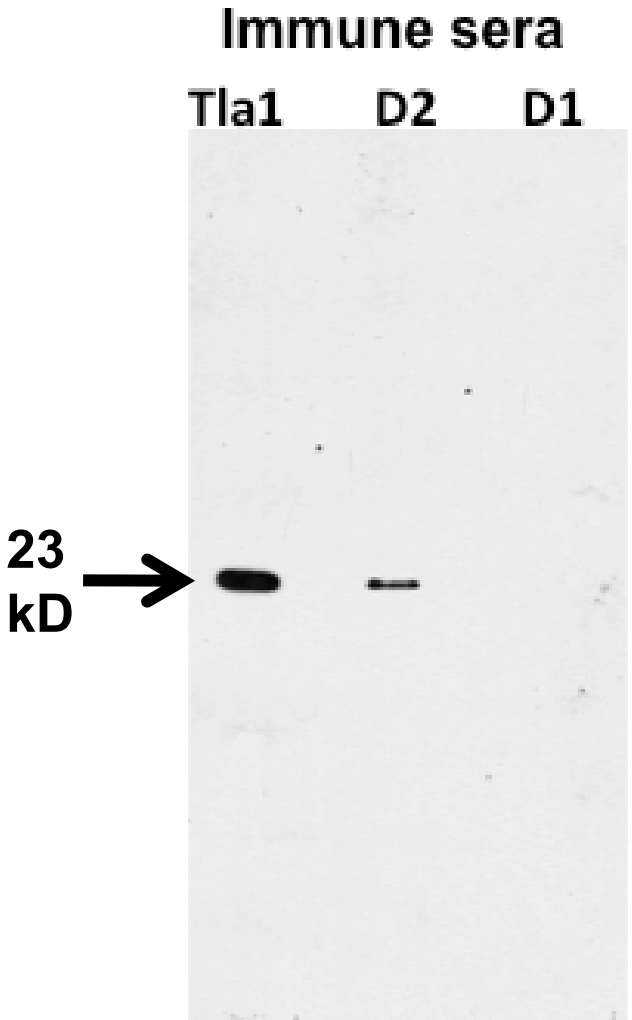
A CLUSTAL 2.0.10 partial sequence alignment of the C-termini of D2 and Tla1 revealed essential identity among 9 consecutive amino acids

```
D2                  V-FPEEVLPRGNAL 13
Tla1                VKF---LLP-GNAL 10
                    * *    :** ****
```

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

Current Technical Accomplishments

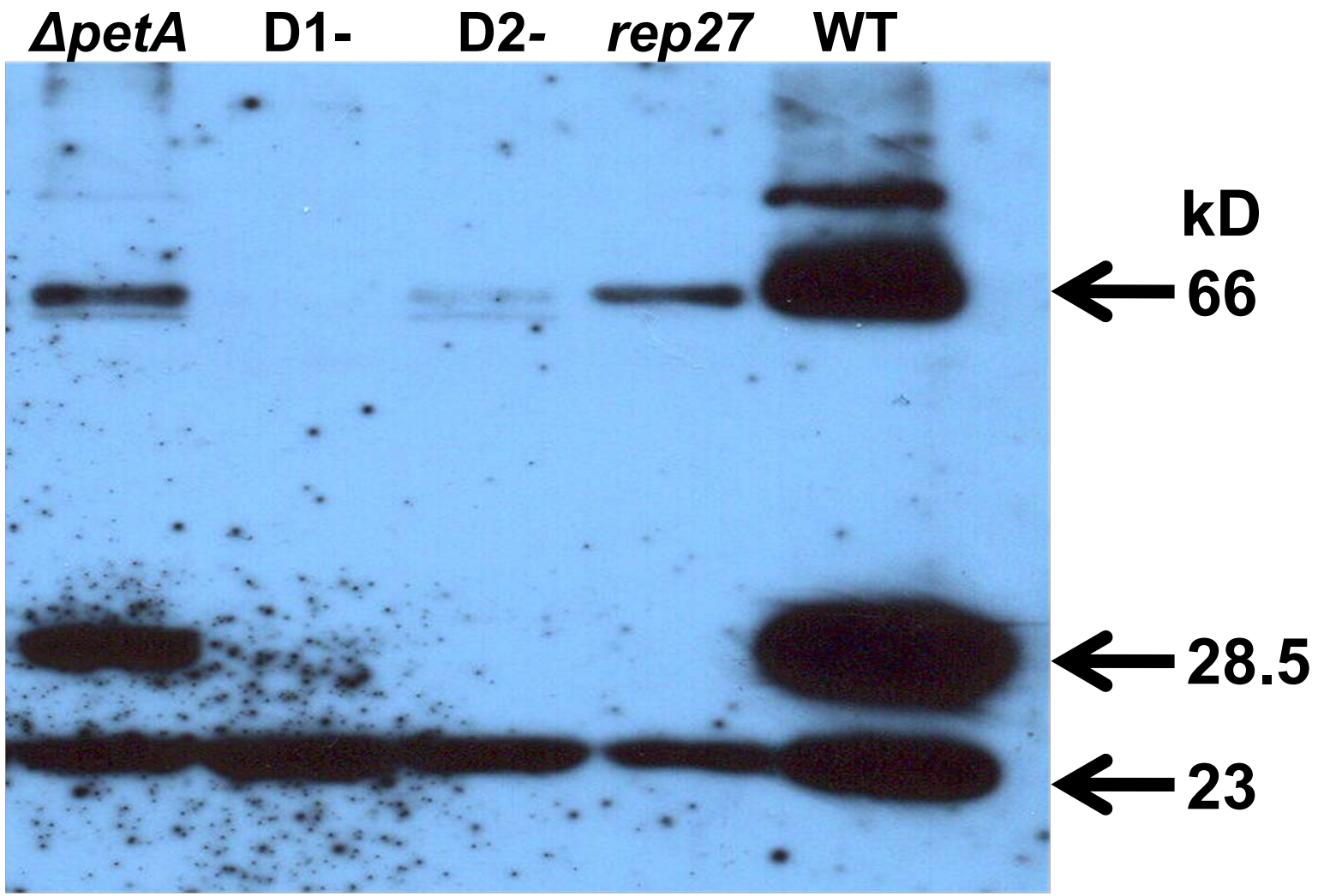
- Immune sera against the Tla1 and D2 proteins both recognize the Tla1 recombinant protein. However, D1 immune sera do not.



0.5 ng of recombinant Tla1 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*.**



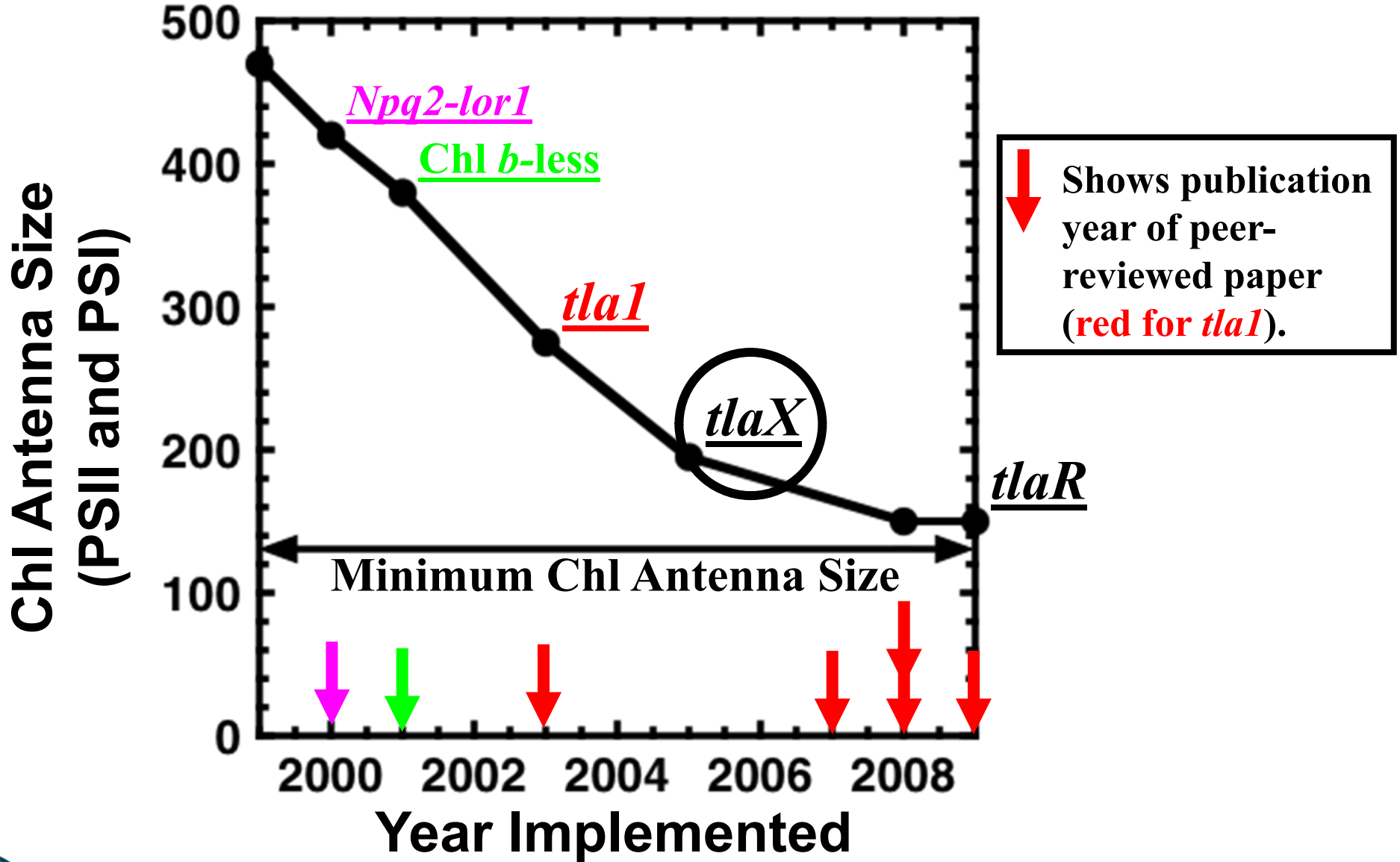
Current Technical Accomplishments

- **Conclusion:**

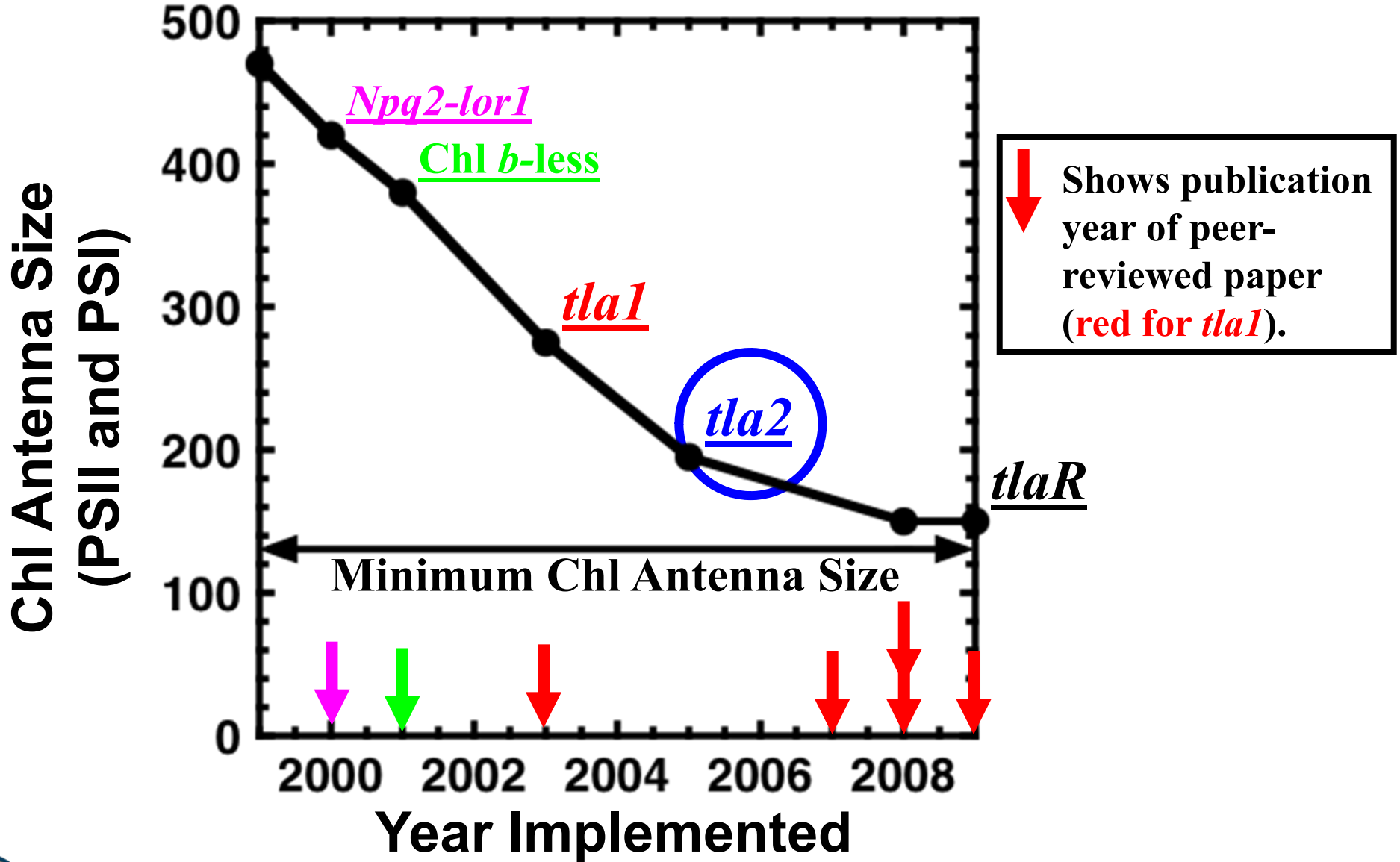
  The Tla1 and D2 proteins have a common 9 amino acid epitope in their C-terminus, that is antigenic enough to generate a strong antibody response against either protein.

  This unexpected property has complicated the analysis of the Tla1 function, but it is now solved.

Project Timeline
Chlorophyll Antenna Size in Chlamydomonas

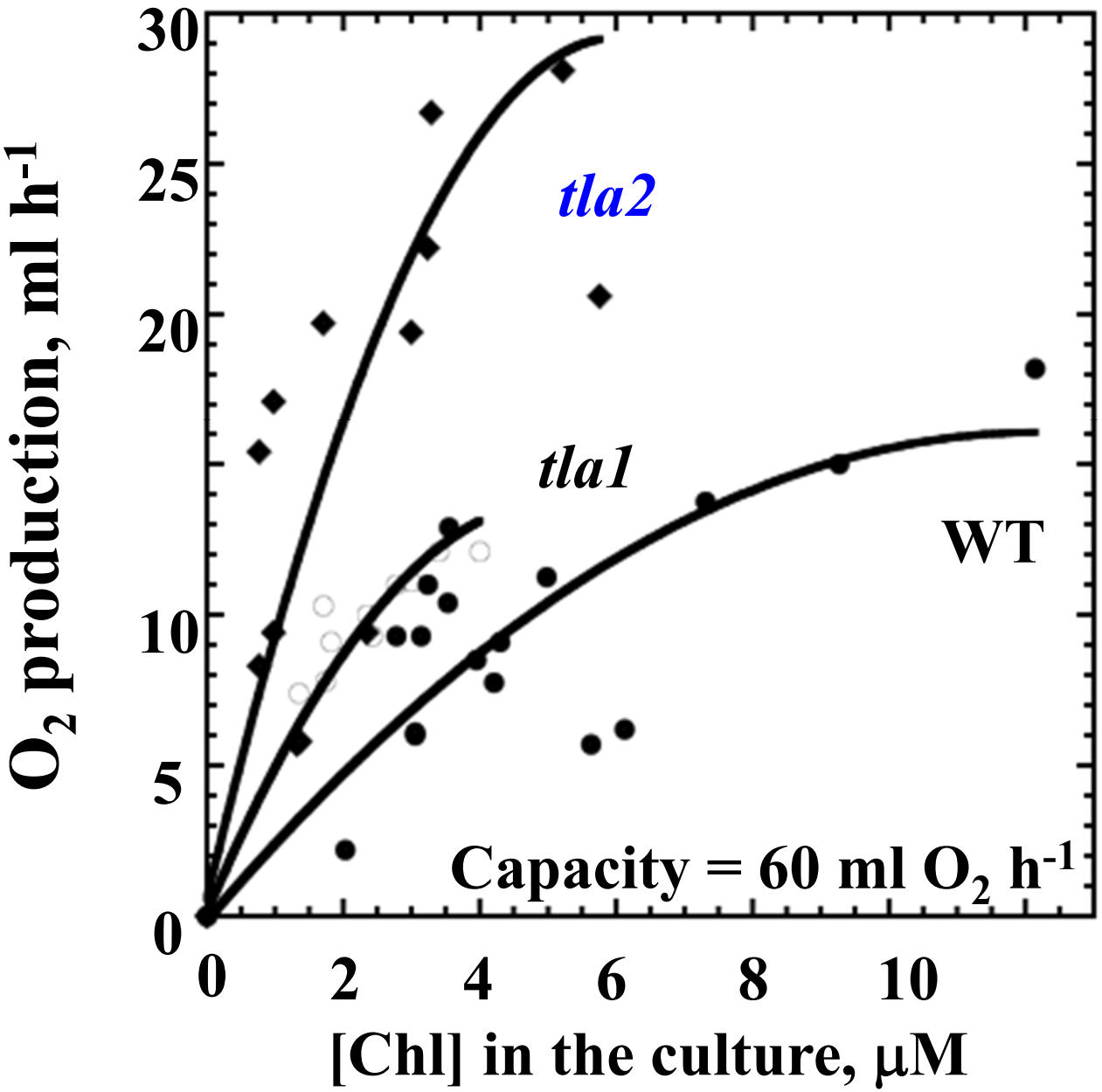


Project Timeline
Chlorophyll Antenna Size in Chlamydomonas

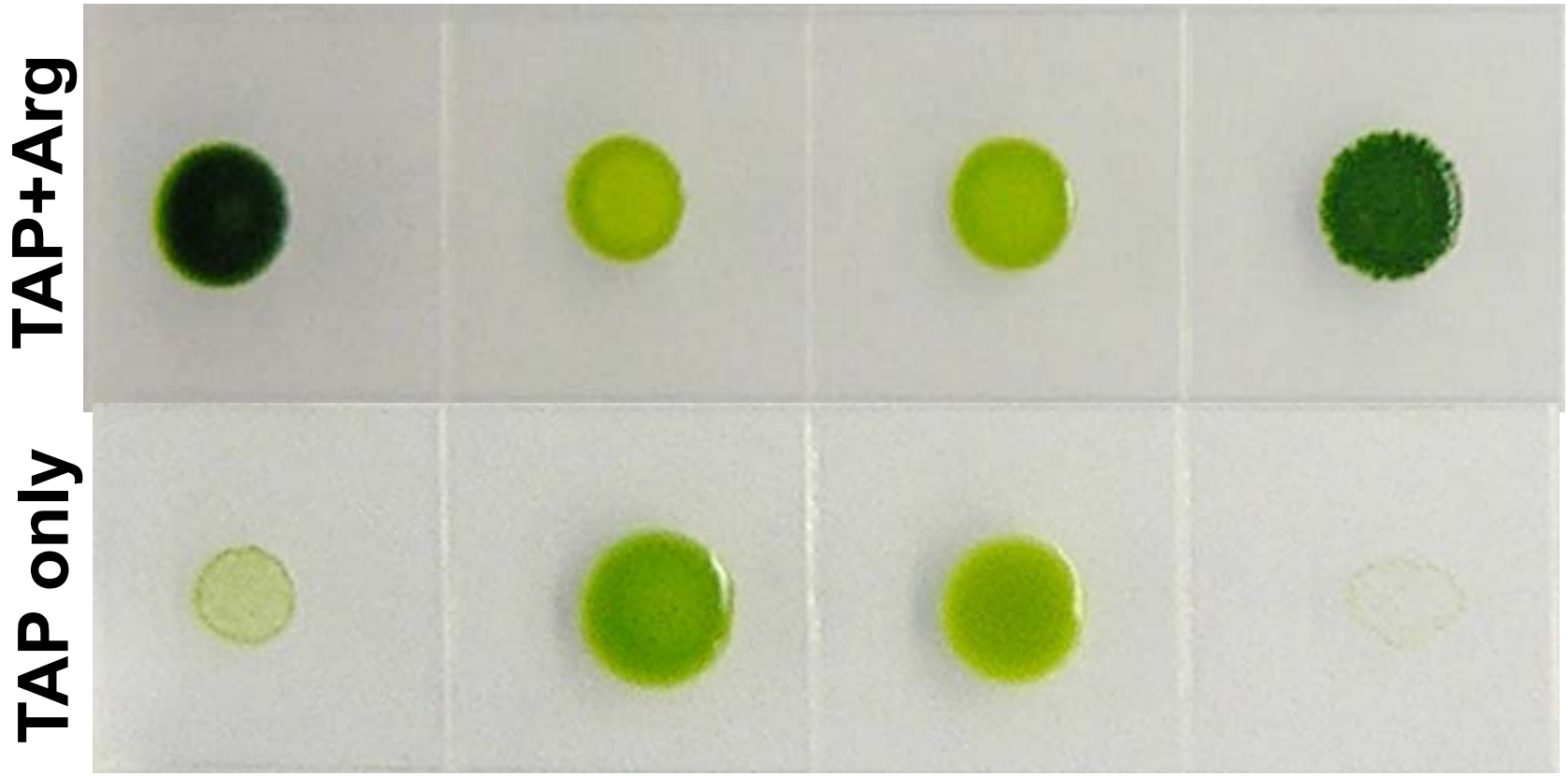


Productivity in Scale-up of Cultures

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



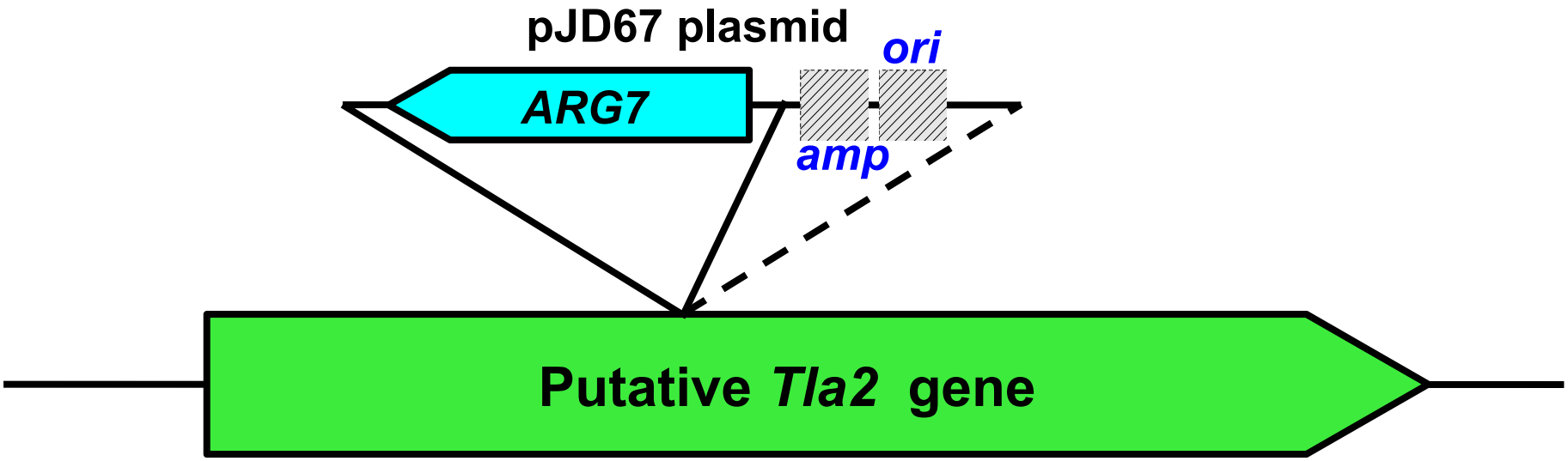
"Tetrad analysis" of progeny from a single *tla2 x Arg*$^{-}$ zygospore



***Arg*(-)**	***Arg*(+)**	***Arg*(+)**	***Arg*(-)**
pJD67(-)	**pJD67(+)**	**pJD67(+)**	**pJD67(-)**
***a/b*=2.5**	***a/b*=8.0**	***a/b*=8.0**	***a/b*=2.6**

Arg^{-} = AGIx3.24 = 4A(mt-)Arg(-)

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



***Chlamydomonas reinhardtii* genomic DNA**

<u>Outcome of Genetic Analysis (so far)</u>:

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-put 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, $E_0 x E_1$, %

	2000	2003	2005	2008	2010	2015
Program Targets	3%	10%			15%	20%
Progress	3%	10% *tla1*	15% *tla2*	25% *tlaR*		

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*$^{+}$. 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 236th 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.

92nd 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%