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-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 FY10

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

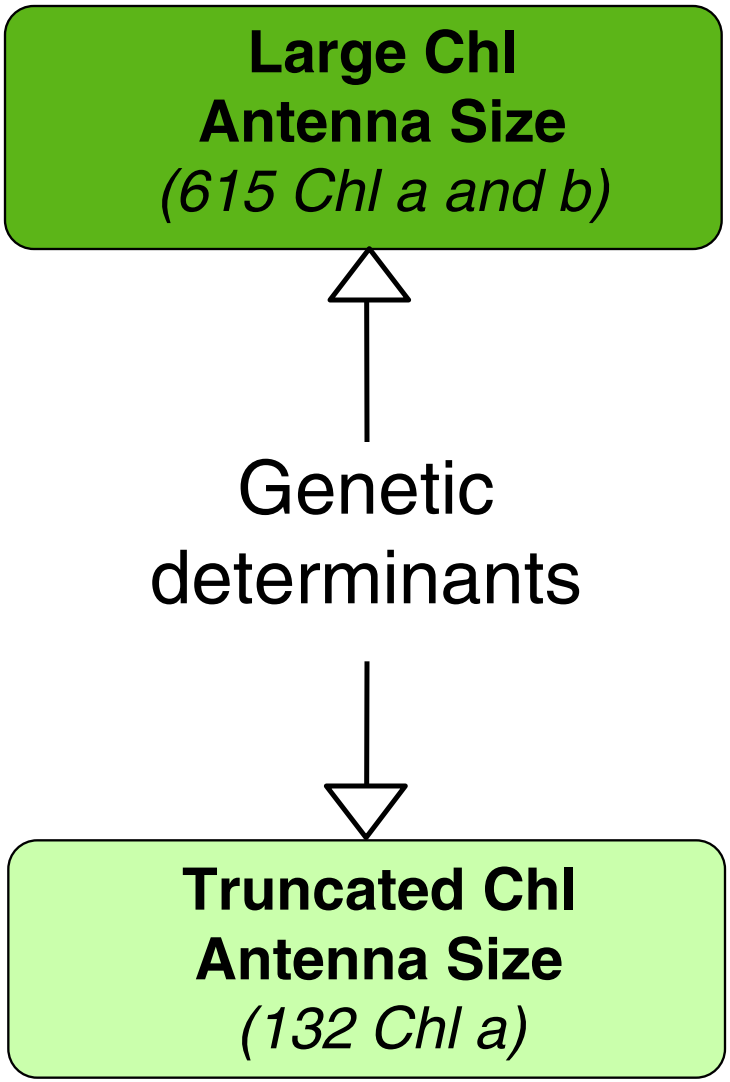
Objective: Minimize the chlorophyll antenna size of photosynthesis to maximize solar conversion efficiency in green algae.

(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

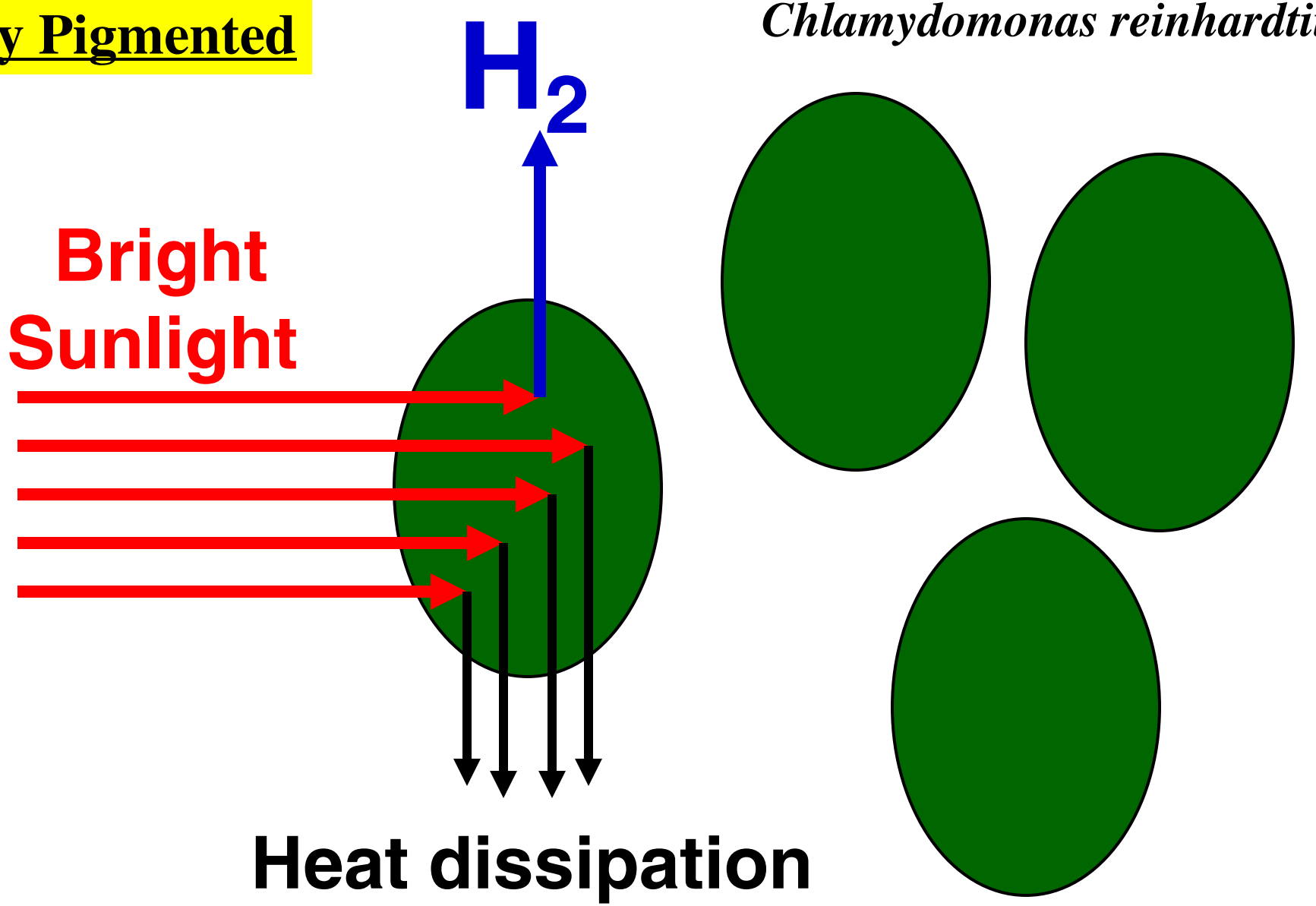


Interference with the genetic mechanism for the regulation of the Chl antenna size, to derive a permanently truncated Chl antenna size, is the goal of this R&D.


Hydrogen production
in a backyard
Chlamydomonas reinhardtii mass culture

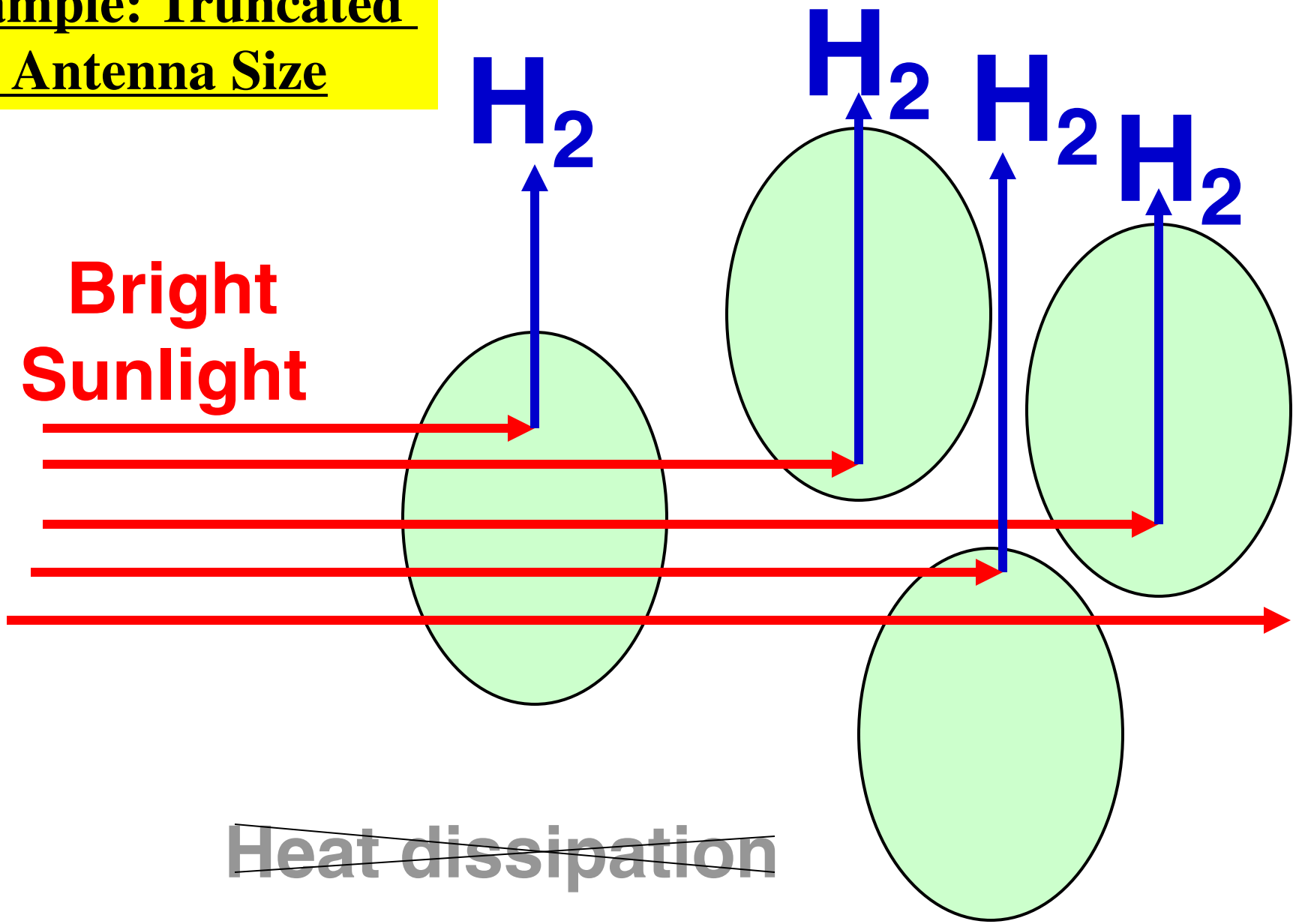
Example:
Fully Pigmented

The green algae
Chlamydomonas reinhardtii



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

Example: Truncated Chl Antenna Size

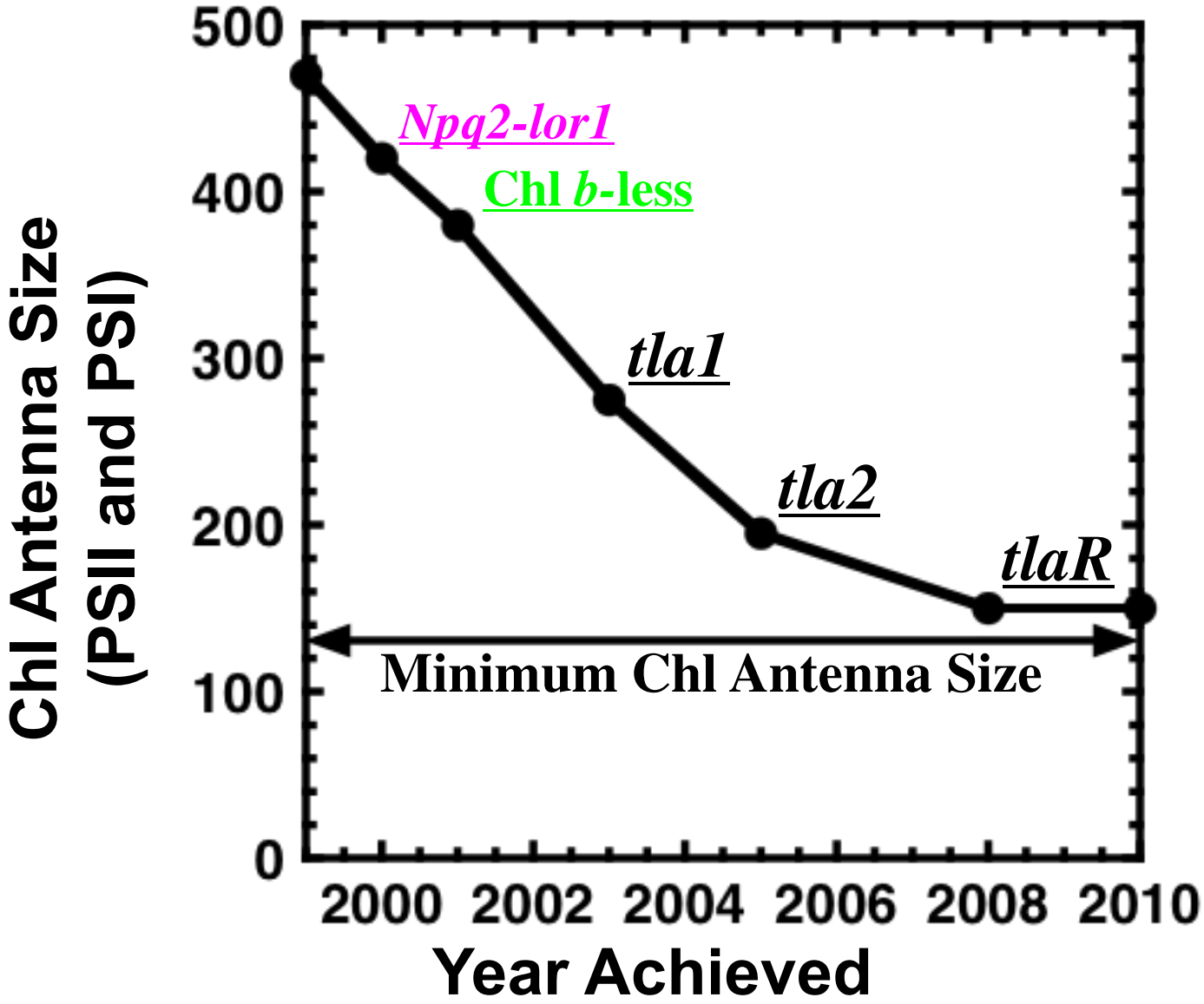


Truncated Chl antenna cells permit greater transmittance of light and overall better solar utilization by the culture.

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 wild type green microalgae (solar-to-chemical): ~3%**
- **Theoretical maximum solar-to-chemical efficiency: ~30%**
- **Target for 2010: Reach a 15% Utilization Efficiency of Absorbed Light Energy.**
- **Ancillary Objective: Identify and characterize genes that confer the "*tla*" property to microalgae.**

Project Timeline
Chlorophyll Antenna Size in Chlamydomonas

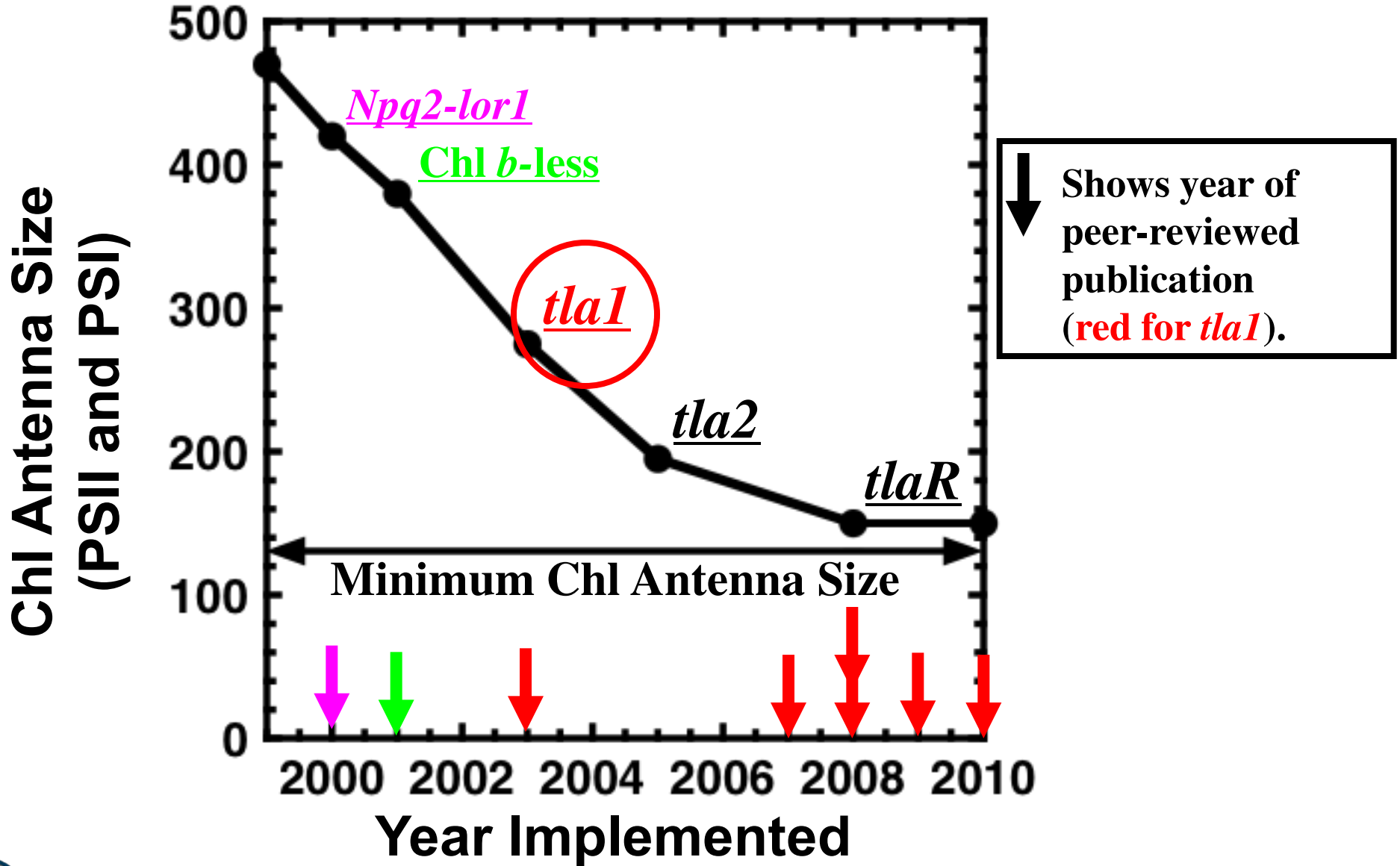


Progress achieved *vs* the DOE targets

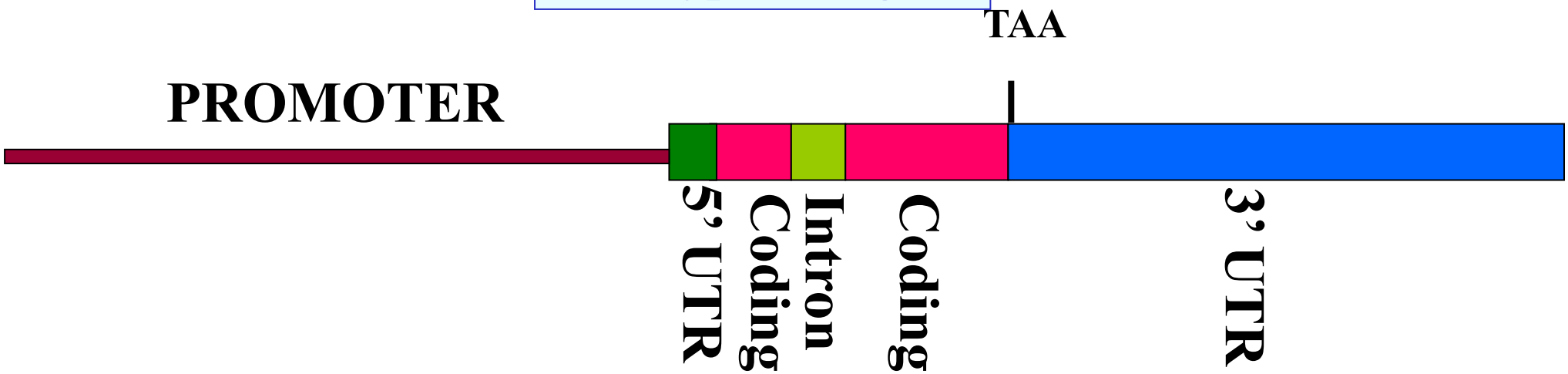
Utilization Efficiency of Incident Solar Light Energy, $E_0 x E_1$, %

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

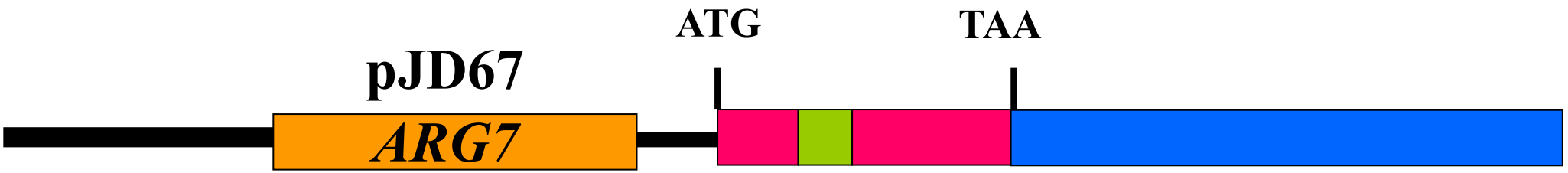
Project Timeline
Chlorophyll Antenna Size in Chlamydomonas



Wild type *Tla1* gene



tla1 mutant gene



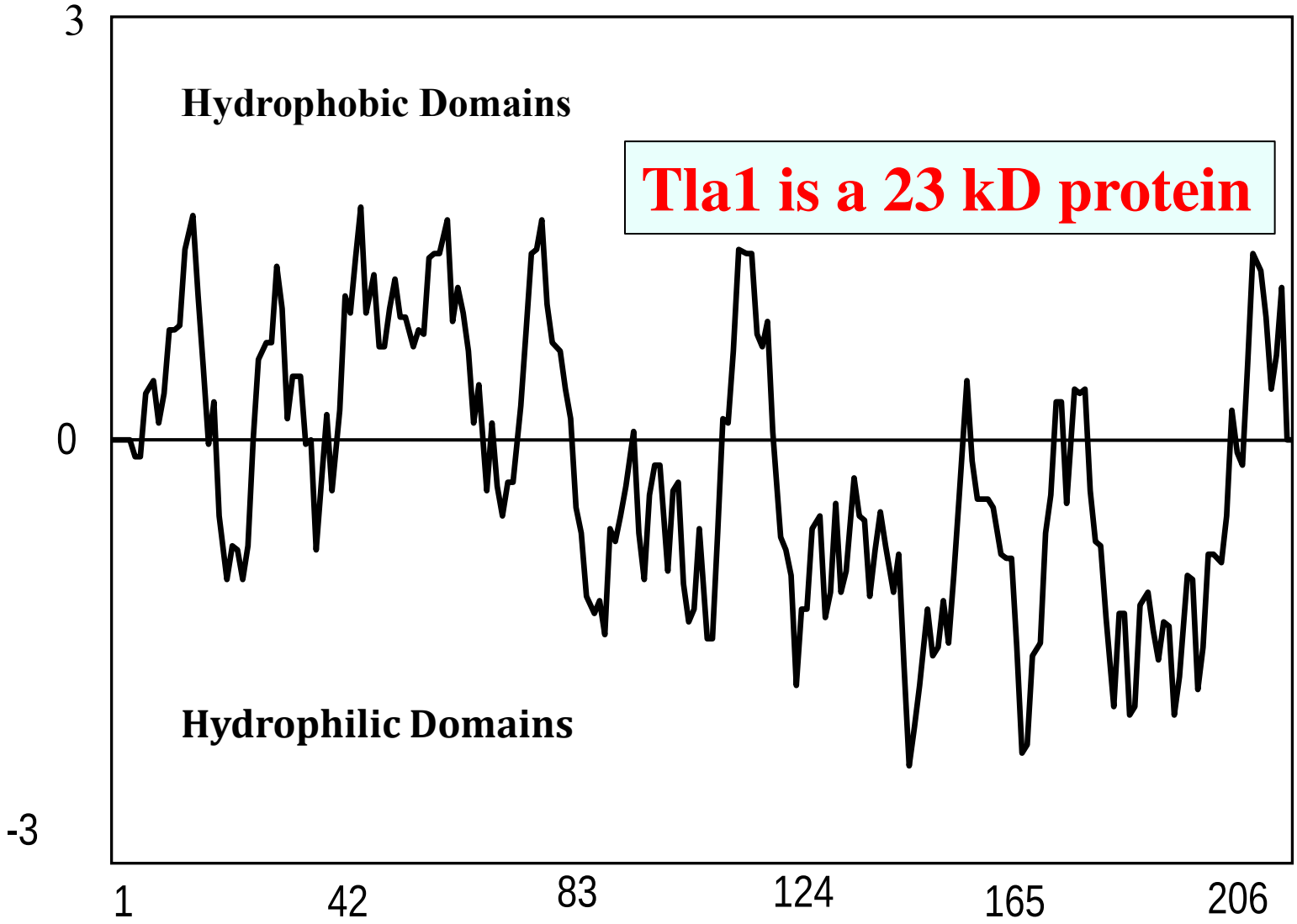
Localization of the Tla1 protein in *C. reinhardtii*

The unicellular green alga *Chlamydomonas reinhardtii*

Environmentally friendly self-repairing and replicating microstructure

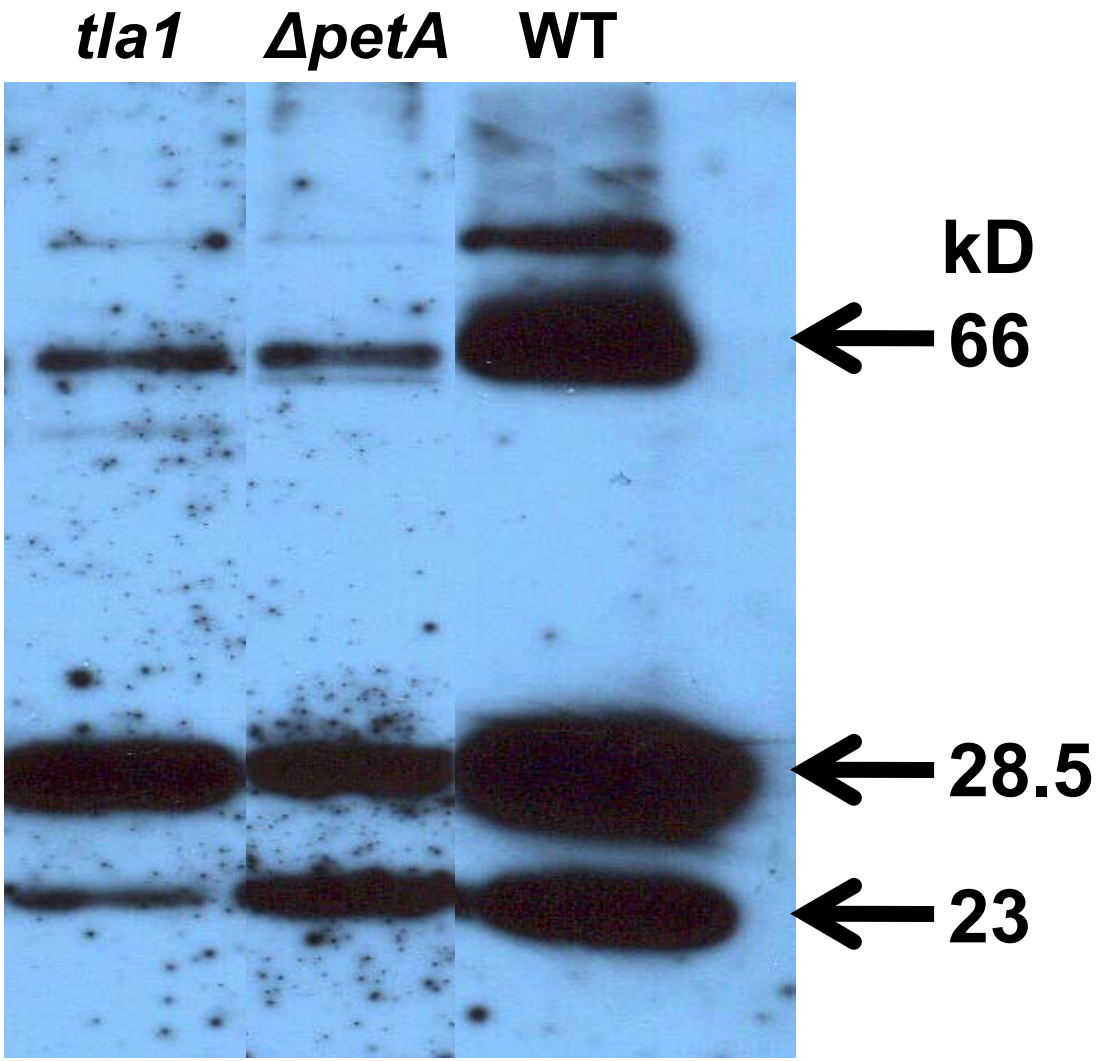
Current Technical Accomplishments

Hydropathy plot of the Tla1 protein



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

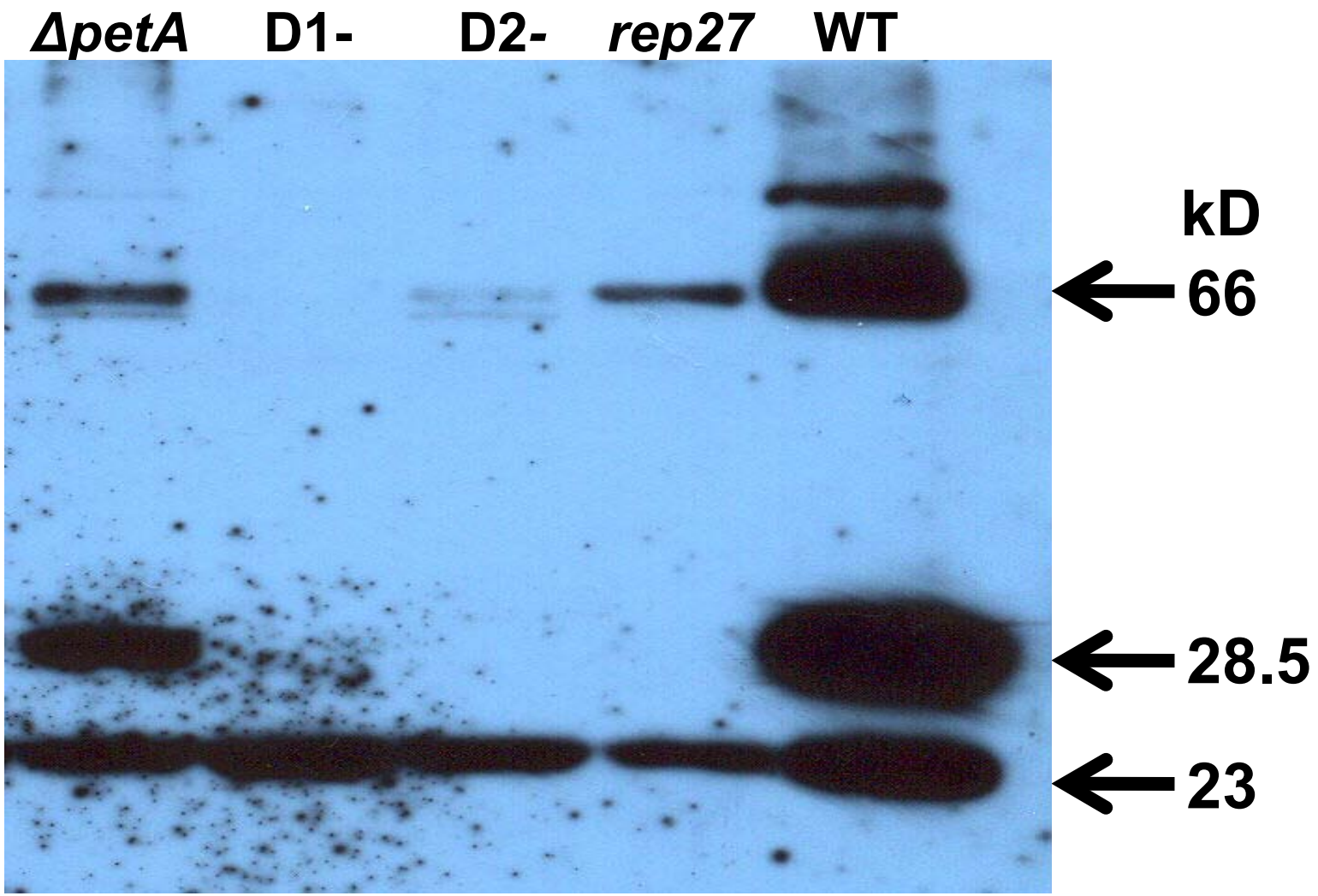
A CLUSTAL 2.0.10 partial sequence alignment of the C-termini of D2 and Tla1 revealed essential identity among 9 consecutive amino acids

```
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

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



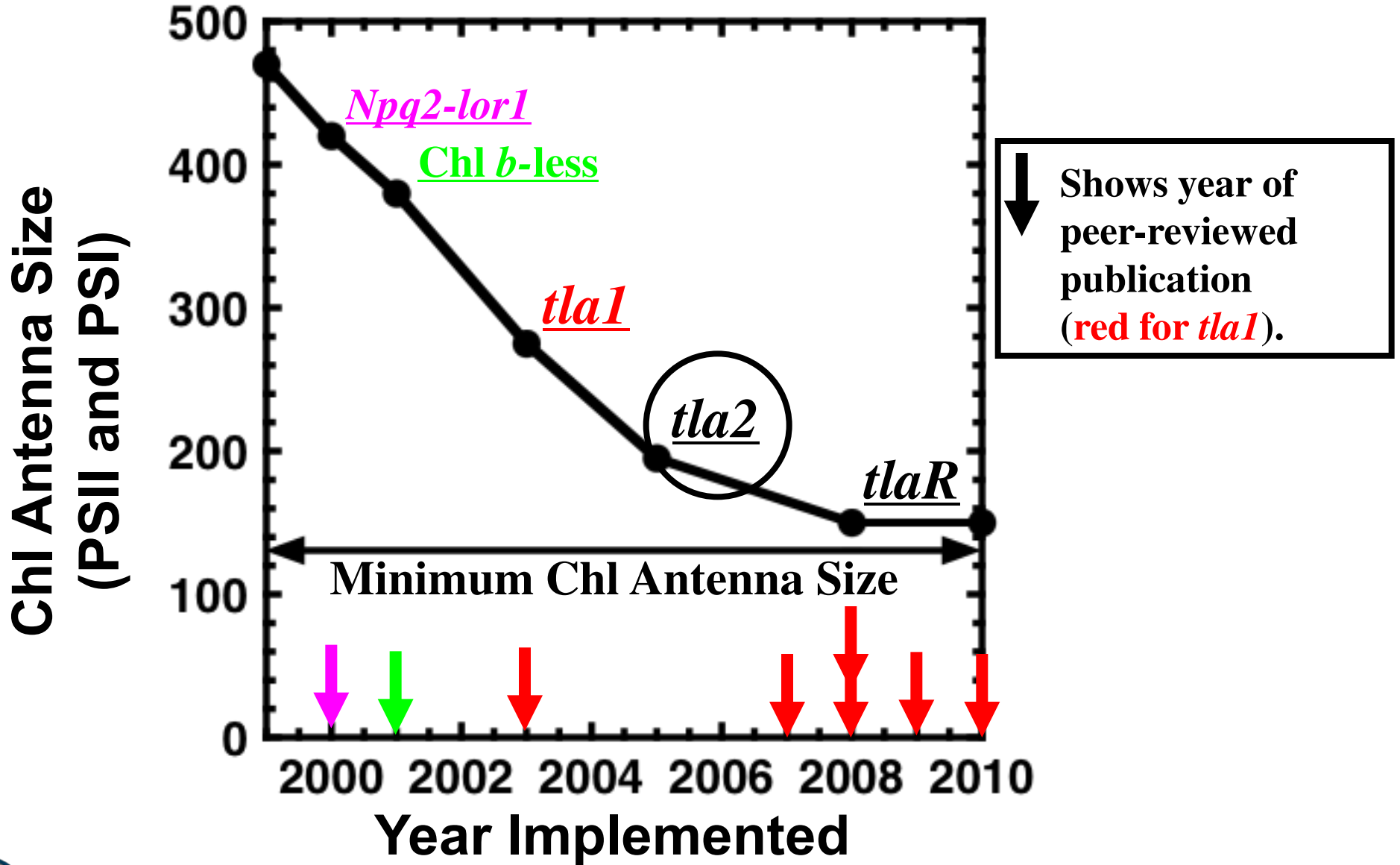
Current Technical Accomplishments

- **Resolution:**

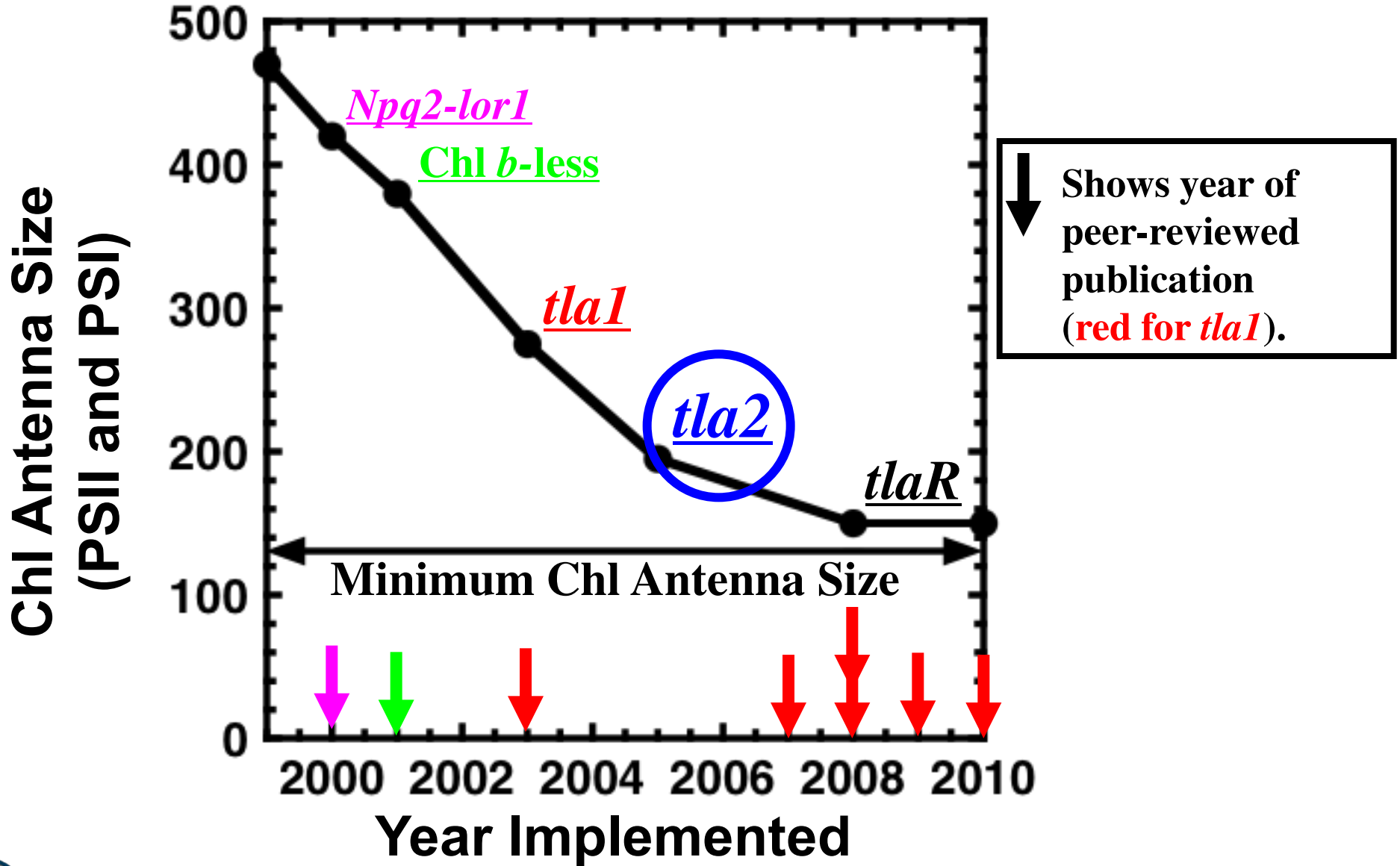
  The Tla1 and D2 proteins have a common 9 amino acid epitope in their C-terminus, that is antigenic enough to generate a strong antibody response against either protein.

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

Project Timeline
Chlorophyll Antenna Size in Chlamydomonas

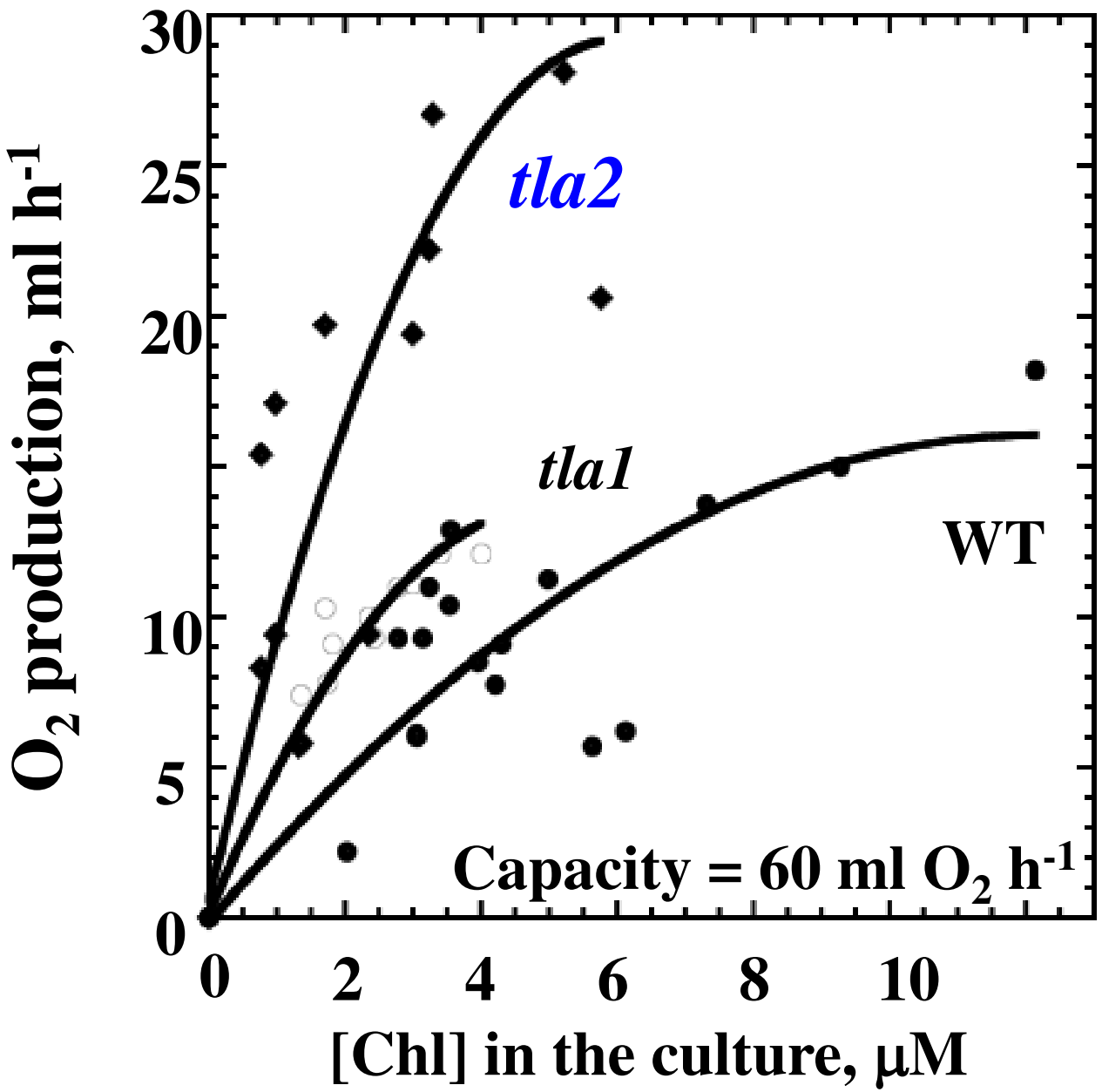


Project Timeline
Chlorophyll Antenna Size in Chlamydomonas

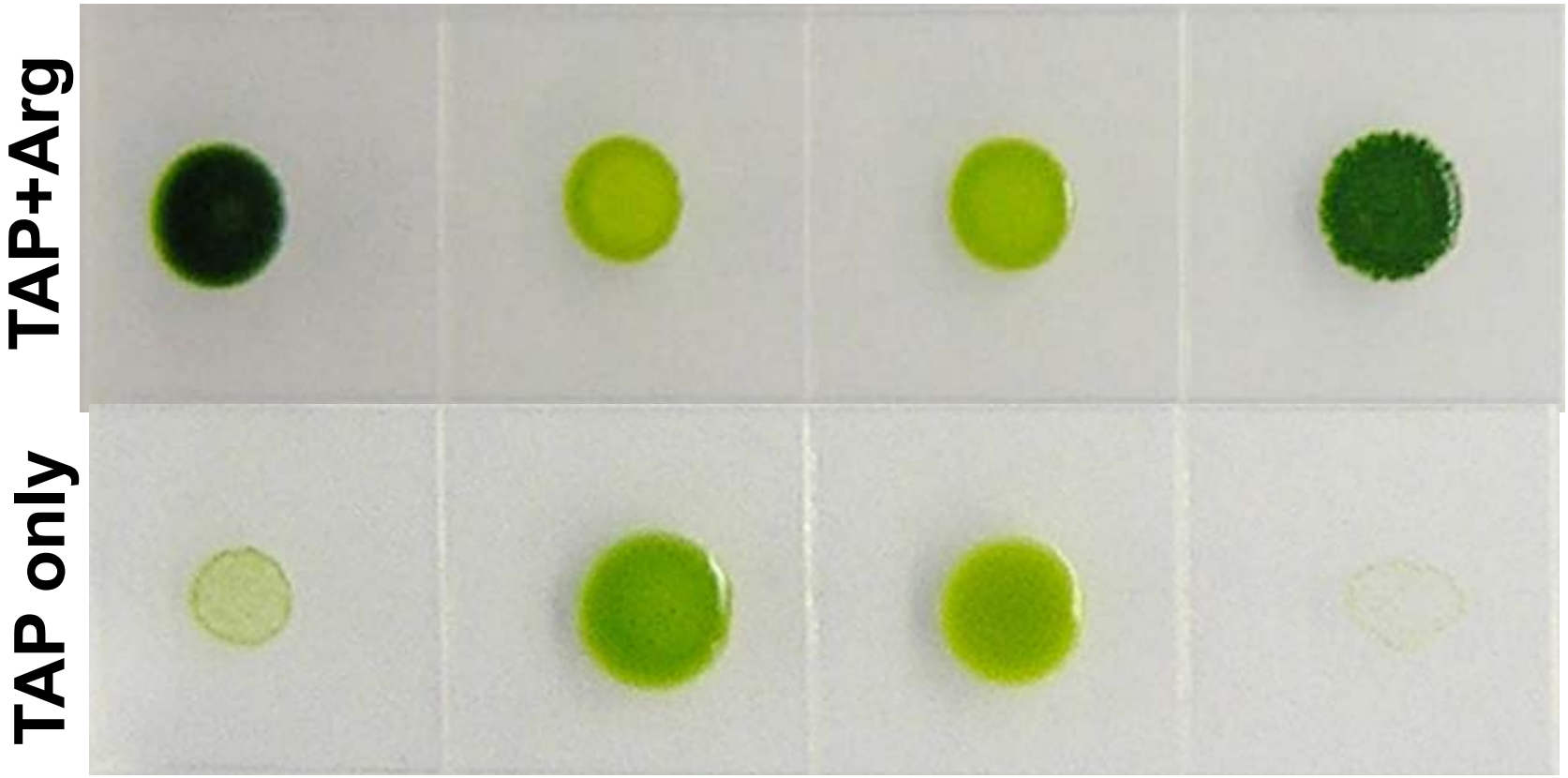


Productivity in Scale-up of Cultures

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



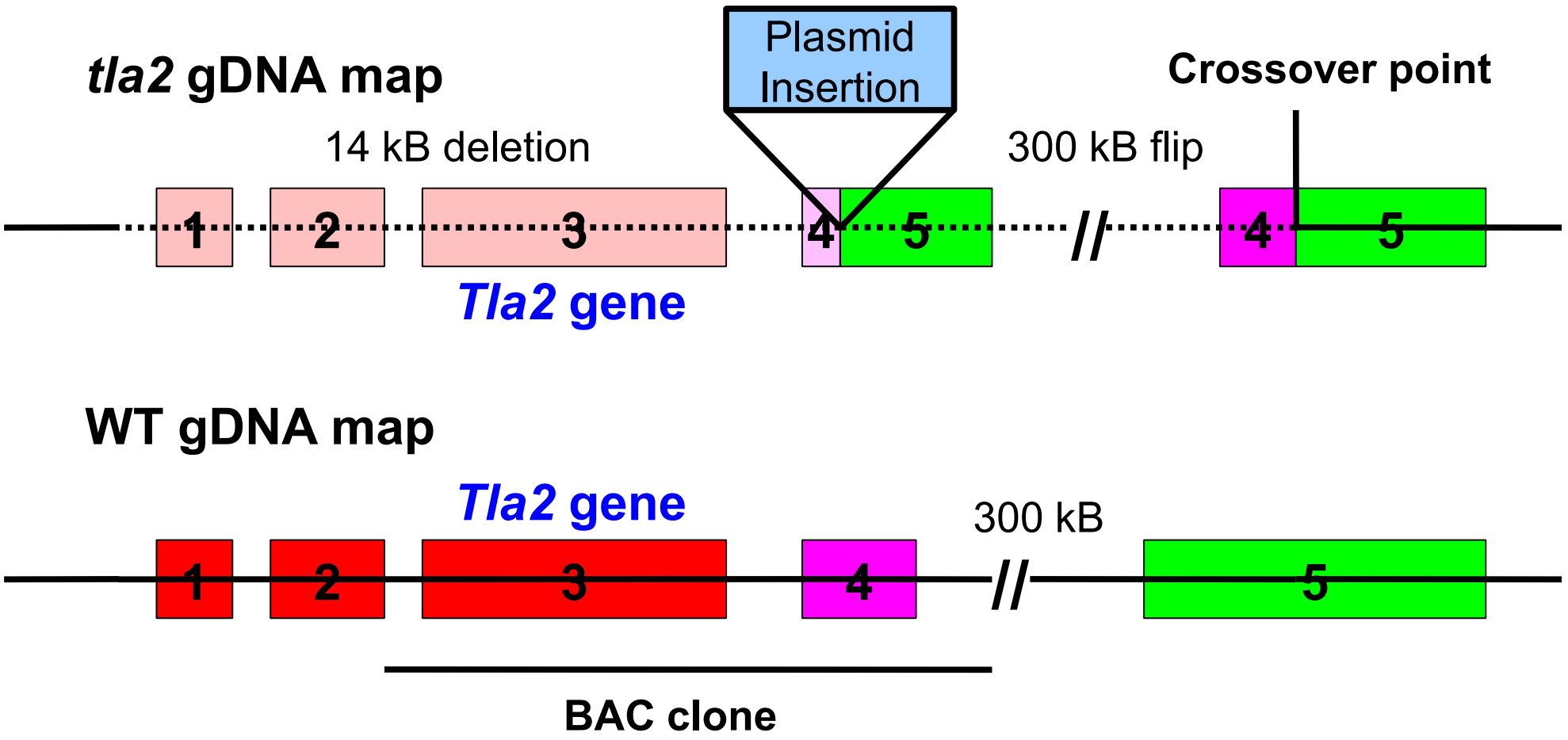
"Tetrad Analysis" of Progeny from a Single *tla2xArg(-)* Zygospore



***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(-)

tla2 gDNA Map and Complementation



Summary of *tla2 Mutant* Properties

- ***Tla2 gene* knock-out.**
- ***pJD67* plasmid *Ori* and *Amp regions* deleted.**
- **Putative *Tla2 gene* is ~6 kb in size.**
- ***Tla2* encodes a putative protein of ~36 kD.**
- ***Tla2* function is currently under investigation.**

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 conferring a truncated Chl antenna size to the *tla2* mutant.**

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.

Complete the genetic and molecular analysis of the *Tla2* gene; publish results.

Elucidate biochemical function 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.)

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%