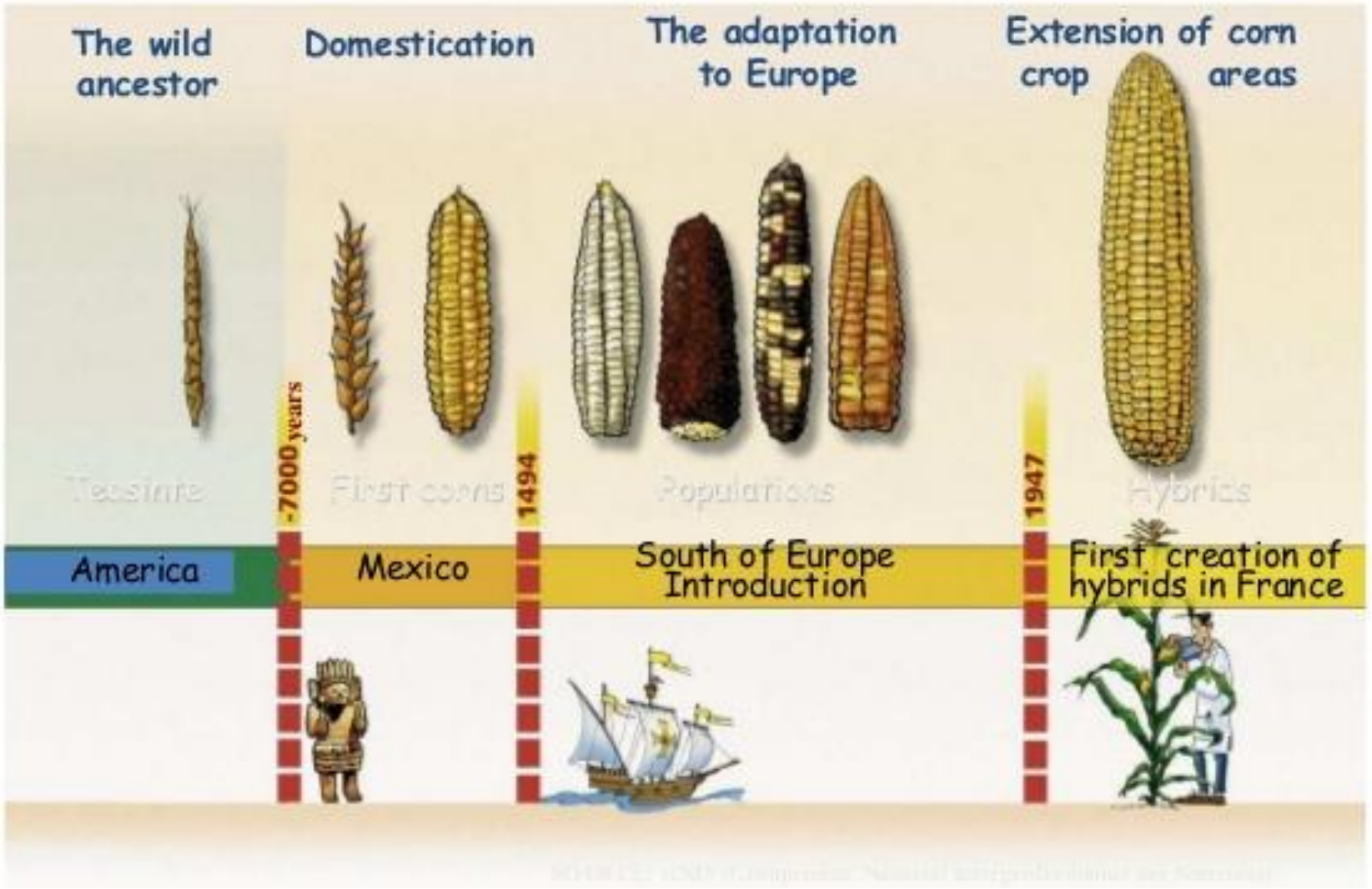
Is it possible to improve primary metabolism efficiency?

Is there still room for the improvement of crop yield?



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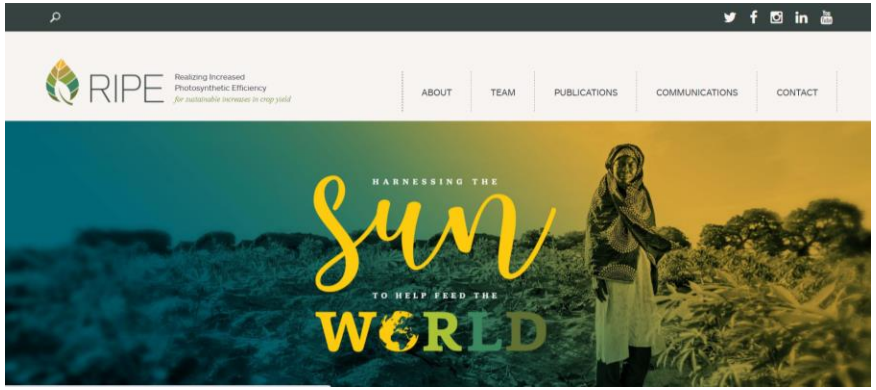

Objectives

Our strategy is to probe a mathematical representation of the whole photosynthetic process in silico to identify which of billions of possible changes might be the most rewarding in practice to boost the yields of key food crops. Top candidates are then tested in a single cultivate of tobacco; why tobacco? Tobacco was selected for our proof-of-concept experiments, not only for its ease of genetic transformation, but also because it is an ideal model crop that is robust in the field, forms a fully closed canopy, and produces large quantities of seed, circumventing the need for numerous seed amplification generations, further accelerating the timeline to field testing. When statistical evidence of productivity increase is achieved in tobacco, then the successful manipulations are transferred to the food crops of interest: cowpea, rice, cassava, and soybean.

Identify

We simulate photosynthesis in silico from gene to canopy to discover opportunities that could improve photosynthesis and boost yield.

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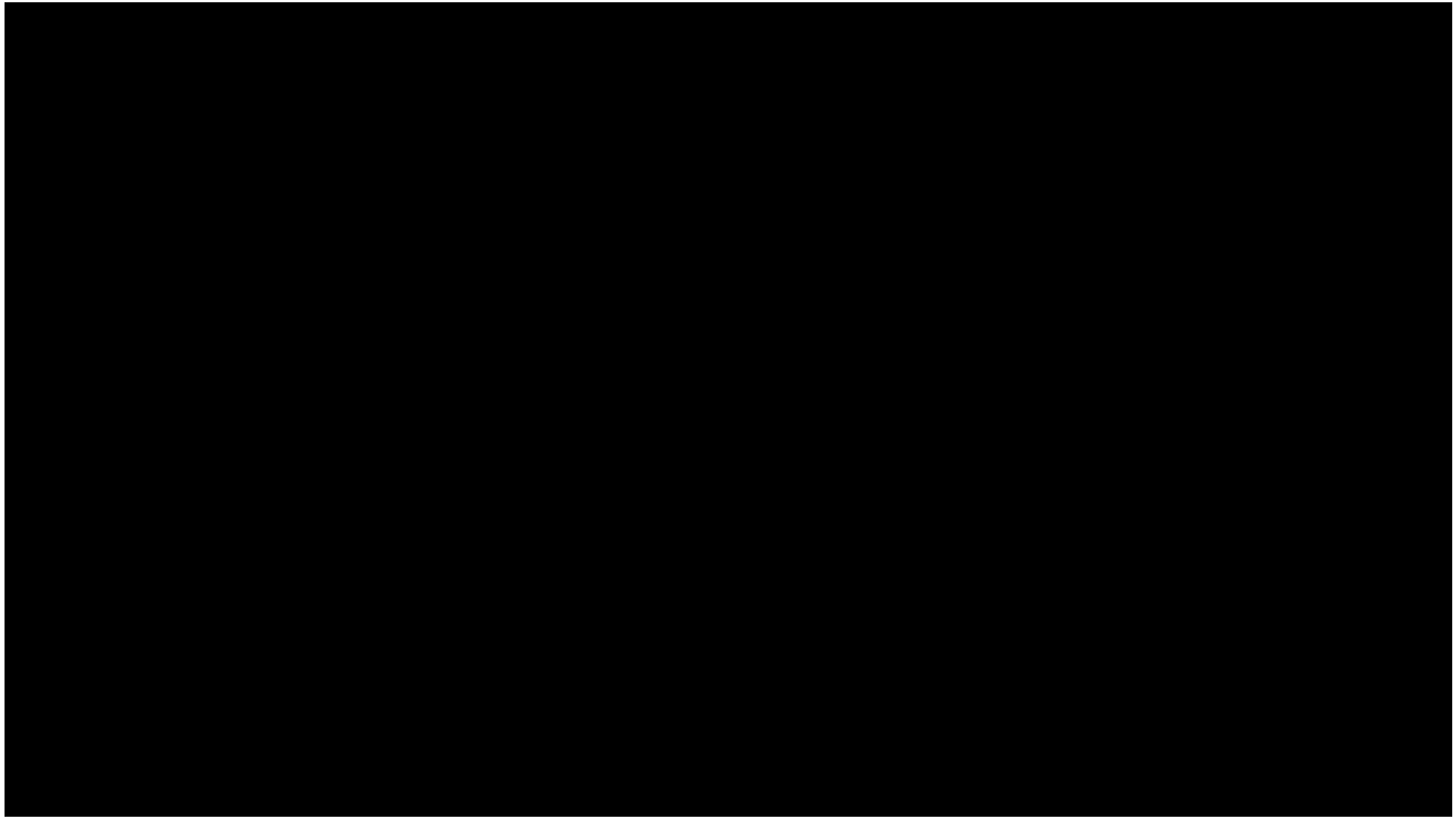
Improve

Next we explore each opportunity to increase photosynthetic efficiency by engineering a model crop, which enables us to make modifications with precision and speed.

- Relaxing Photoprotection
- Photorespiratory Bypass
- RuBP Regeneration
- Improving Rubiscos
- Optimizing Canopies
- Algal Mechanisms
- Mesophyll Conductance

Translate

Finally, we transform our target food crops with the successful modifications to boost the yield of cowpea, cassava, rice, and soybeans.



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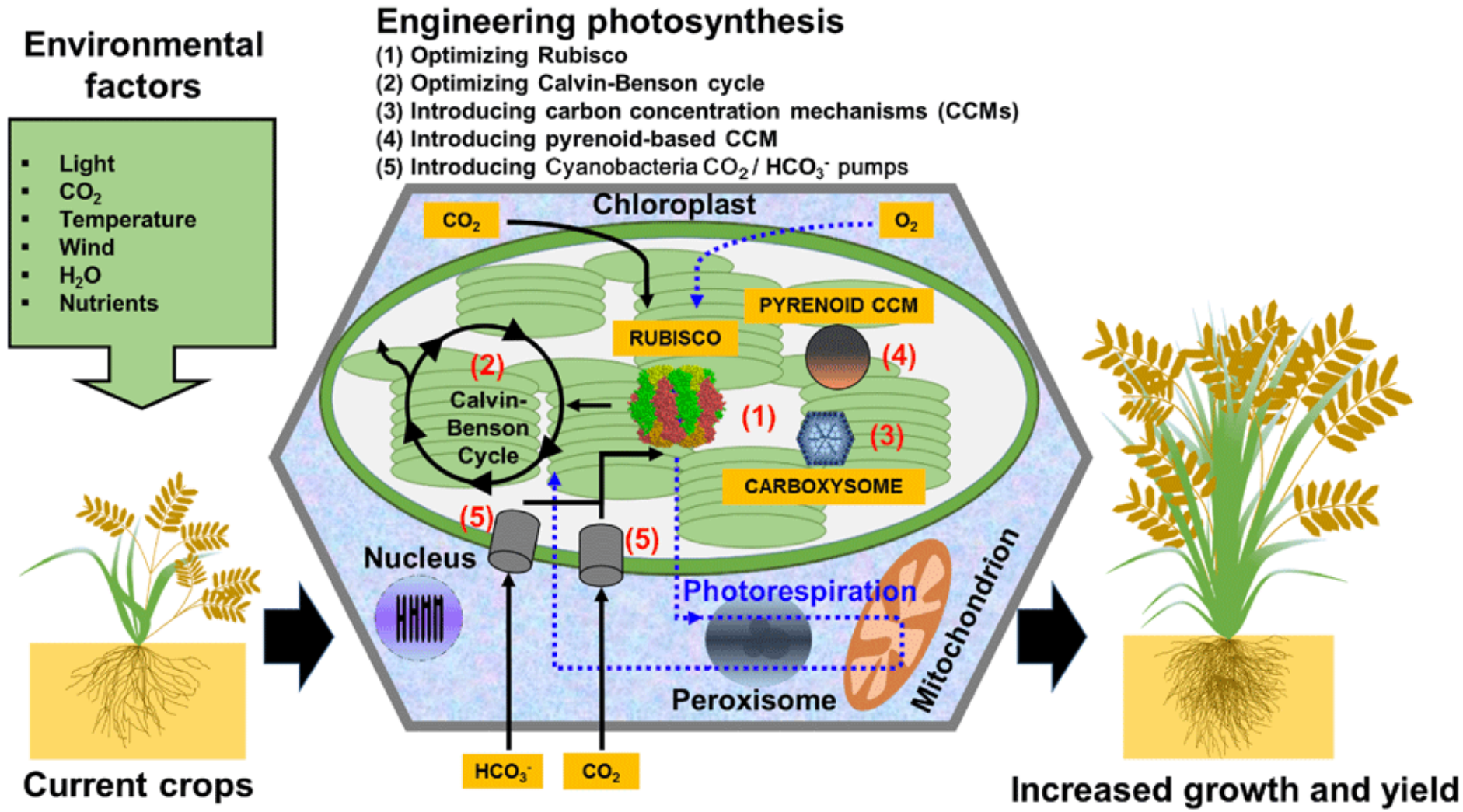
PHOTOSYNTHESIS, THE BASIS OF PRIMARY PRODUCTIVITY ON THE PLANET



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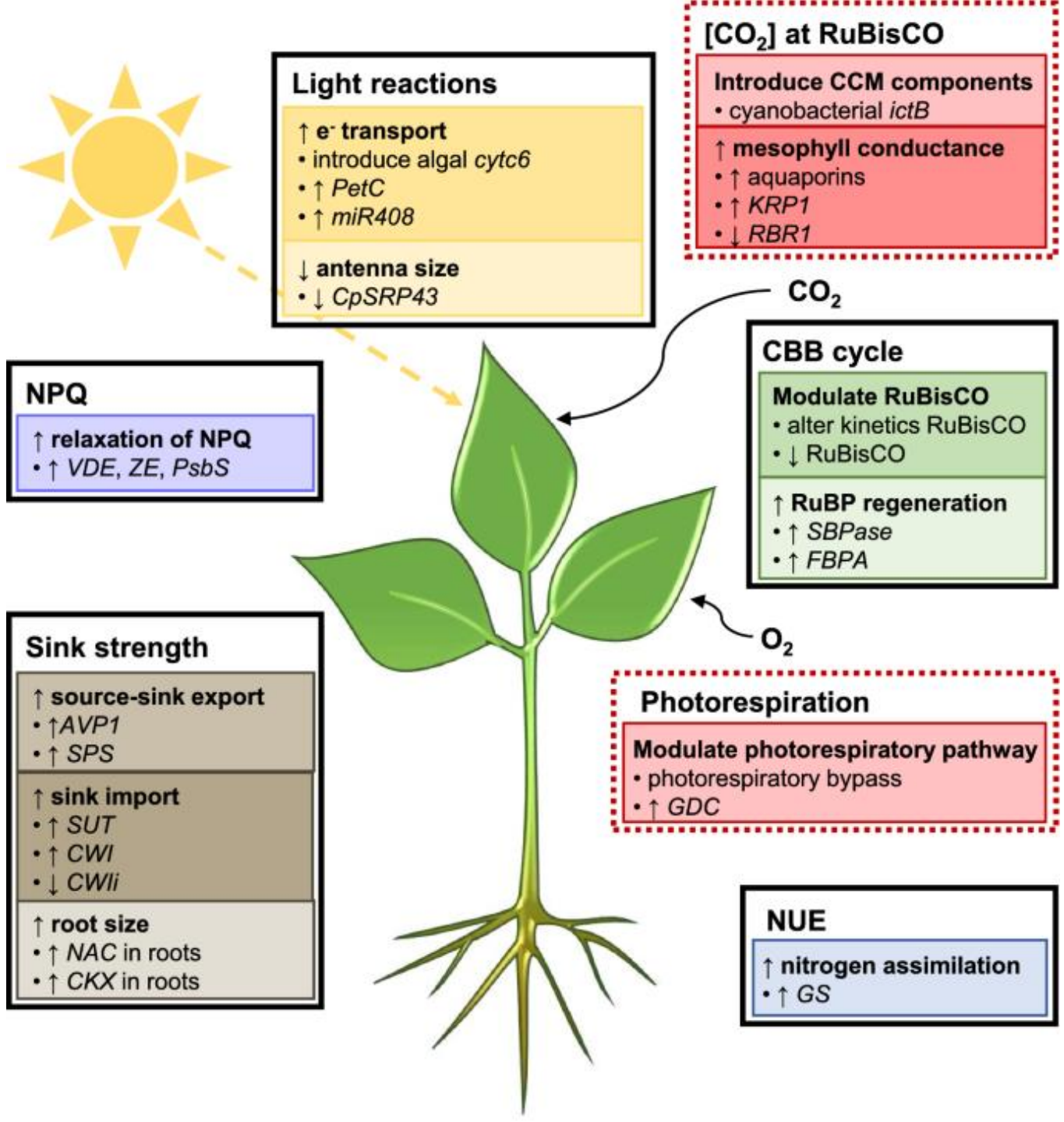
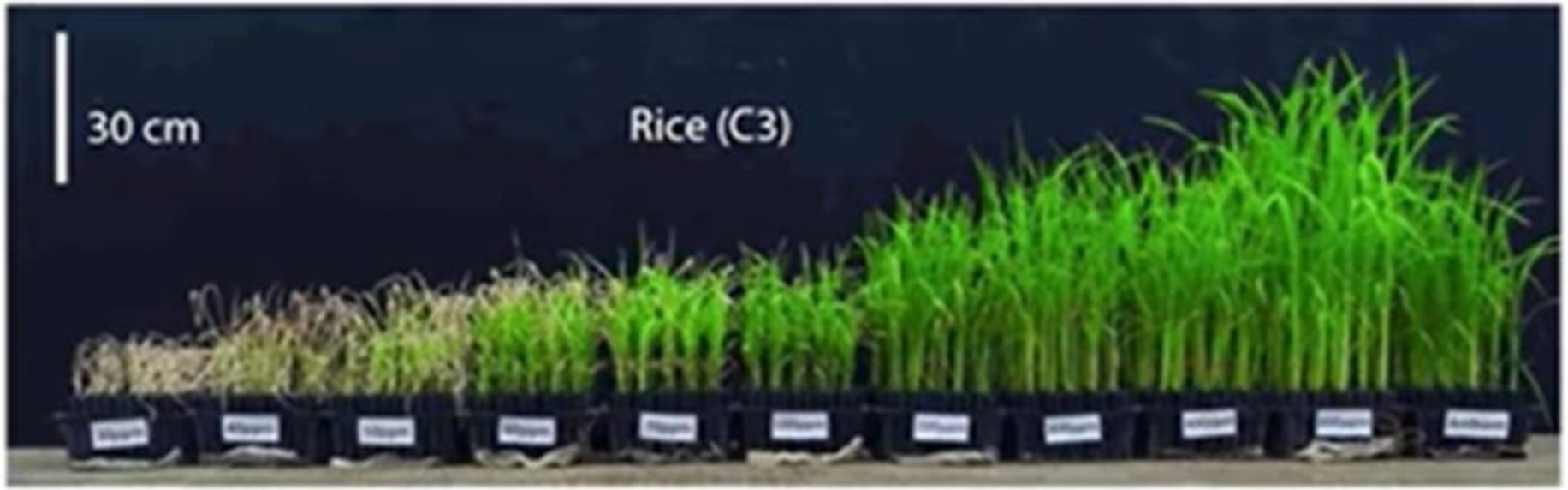
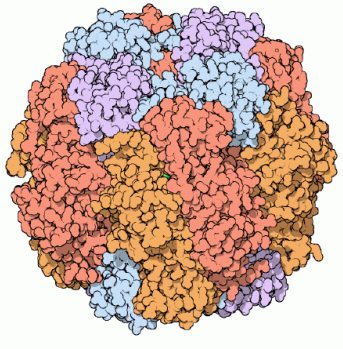

Light reactions
↑ e- transport
• introduce algal cytc6
• ↑ PetC
• ↑ miR408
↓ antenna size
• ↓ CpSRP43
[CO2] at RuBisCO
Introduce CCM components
• cyanobacterial ictB
↑ mesophyll conductance
• ↑ aquaporins
• ↑ KRP1
• ↓ RBR1
CO2
CBB cycle
Modulate RuBisCO
• alter kinetics RuBisCO
• ↓ RuBisCO
↑ RuBP regeneration
• ↑ SBPase
• ↑ FBPA
NPQ
↑ relaxation of NPQ
• ↑ VDE, ZE, PsbS
O2
Photorespiration
Modulate photorespiratory pathway
• photorespiratory bypass
• ↑ GDC
Sink strength
↑ source-sink export
• ↑AVP1
• ↑ SPS
↑ sink import
• ↑ SUT
• ↑ CWI
• ↓ CWIi
↑ root size
• ↑ NAC in roots
• ↑ CKX in roots
NUE
↑ nitrogen assimilation
• ↑ GS

CO_2 Effect on Photosynthesis

Clear risk of low CO_2 and benefit for high CO_2

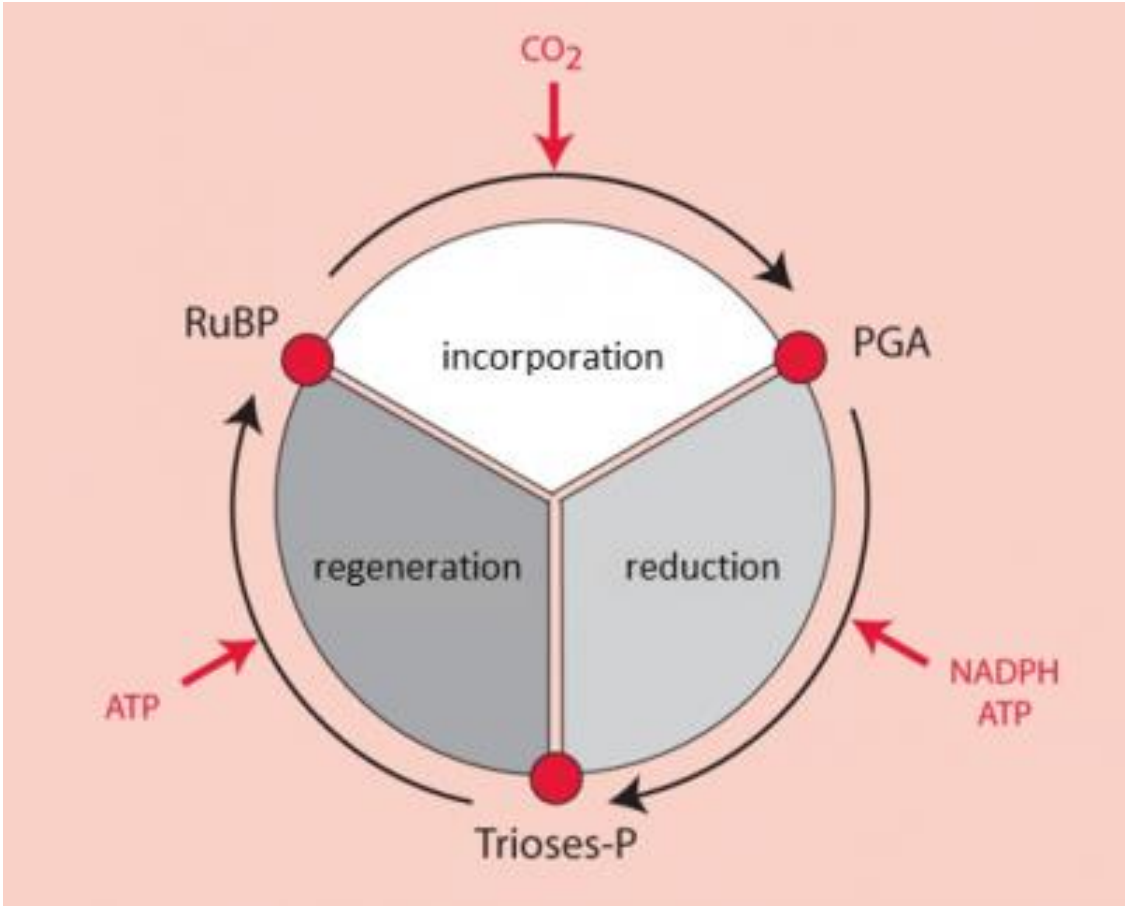


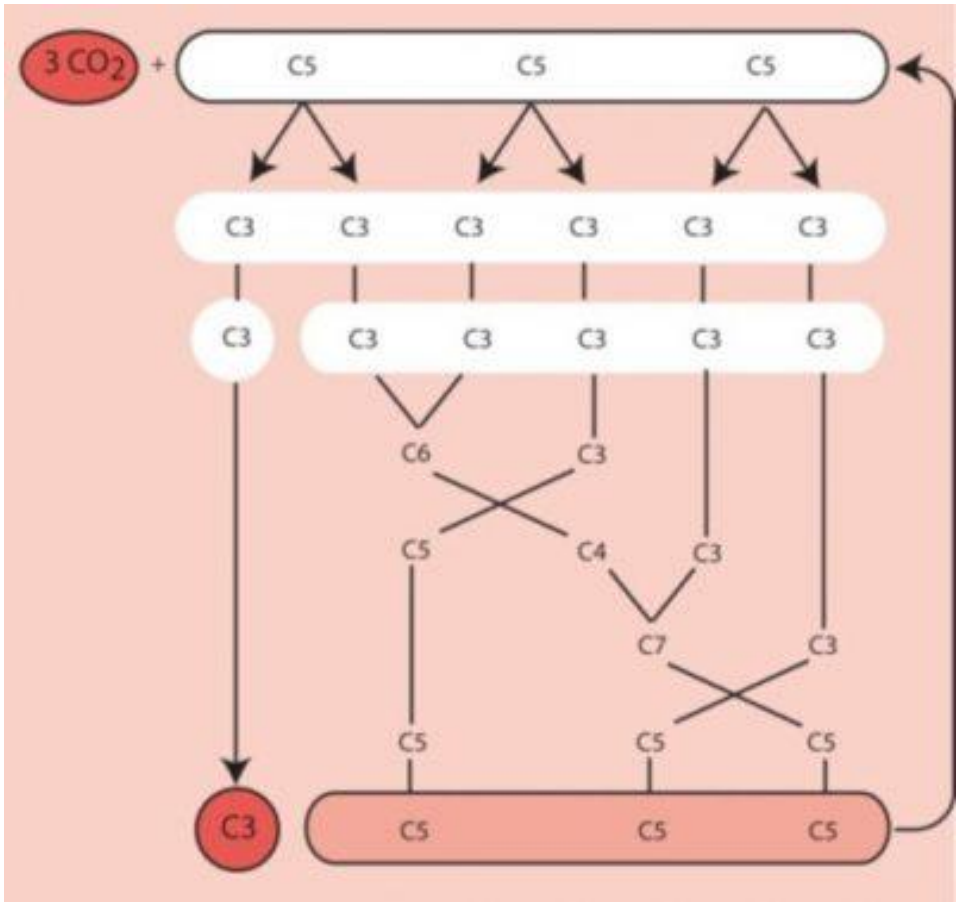
Various plants have shown a significant increase in growth rate when exposed to an elevated atmospheric CO2 concentration due to increased carbon fixation rate



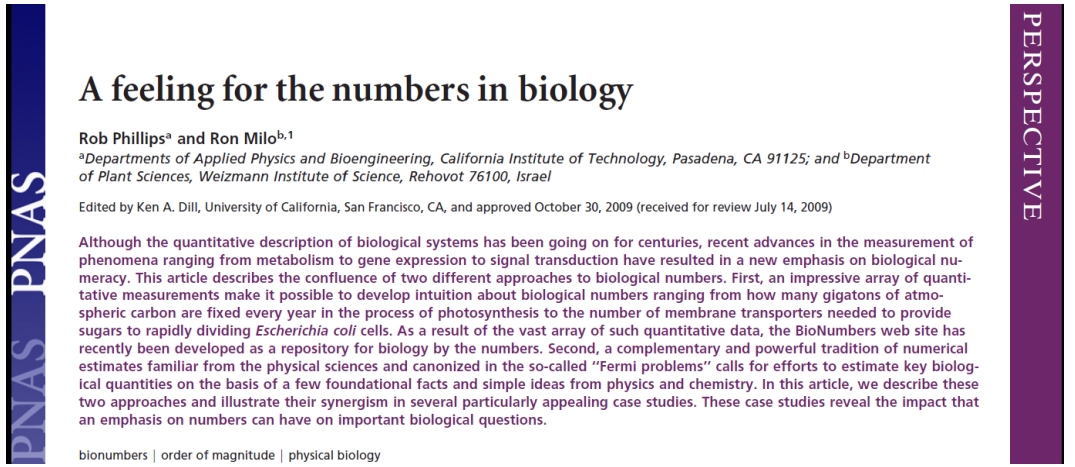
RUBISCO

Most life on Earth depends on photosynthesis, the process whereby inorganic carbon is fixed and converted into organic carbon metabolites





CO_2-fixing enzyme Rubisco
(ribulose 1,5 bisphosphate carboxylase/oxygenase)



PNAS PNAS

A feeling for the numbers in biology

Rob Phillips[a] and Ron Milo[b,1]

[a]*Departments of Applied Physics and Bioengineering, California Institute of Technology, Pasadena, CA 91125; and* [b]*Department of Plant Sciences, Weizmann Institute of Science, Rehovot 76100, Israel*

Edited by Ken A. Dill, University of California, San Francisco, CA, and approved October 30, 2009 (received for review July 14, 2009)

Although the quantitative description of biological systems has been going on for centuries, recent advances in the measurement of phenomena ranging from metabolism to gene expression to signal transduction have resulted in a new emphasis on biological numeracy. This article describes the confluence of two different approaches to biological numbers. First, an impressive array of quantitative measurements make it possible to develop intuition about biological numbers ranging from how many gigatons of atmospheric carbon are fixed every year in the process of photosynthesis to the number of membrane transporters needed to provide sugars to rapidly dividing *Escherichia coli* cells. As a result of the vast array of such quantitative data, the BioNumbers web site has recently been developed as a repository for biology by the numbers. Second, a complementary and powerful tradition of numerical estimates familiar from the physical sciences and canonized in the so-called ''Fermi problems'' calls for efforts to estimate key biological quantities on the basis of a few foundational facts and simple ideas from physics and chemistry. In this article, we describe these two approaches and illustrate their synergism in several particularly appealing case studies. These case studies reveal the impact that an emphasis on numbers can have on important biological questions.

bionumbers | order of magnitude | physical biology

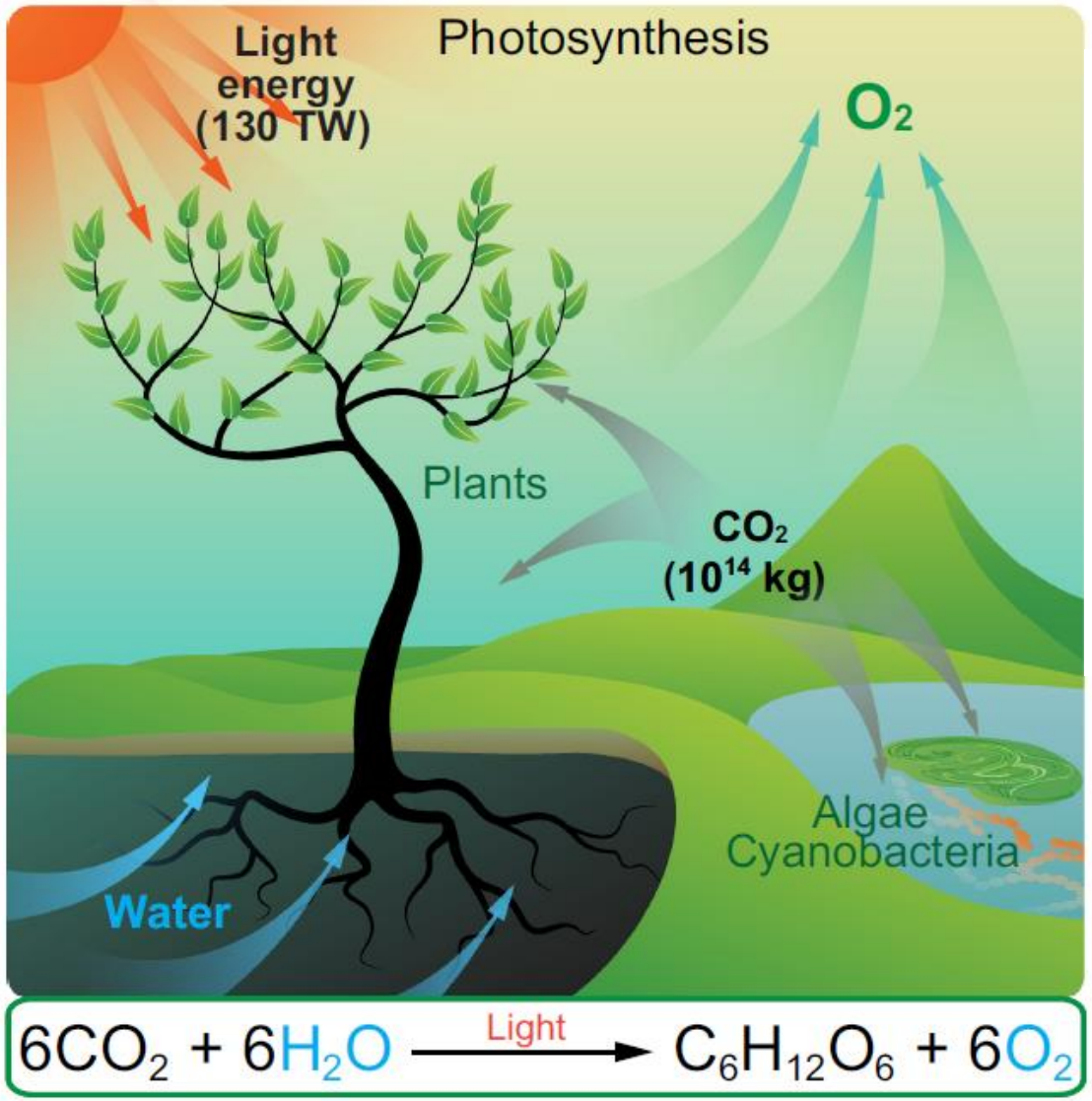
PERSPECTIVE

- More than 90% of the inorganic carbon that is converted into biomass is fixed by the enzyme RubisCO.
- RubisCO catalyzes the carboxylation and cleavage of ribulose-1,5-bisphosphate (RuBP) into two molecules of 3-phospho- glycerate (3PG).
- RubisCO is found in all three domains of life: bacteria, archaea and eukaryotes.
- The enzyme makes up 30–50% of the soluble protein in plant leaf
- For every person on earth there is 5 kg of RubisCO. Altogether, this makes RubisCO one of the most abundant enzymes in the global carbon cycle that literally feeds life on earth.

Review

Chaperone Machineries of Rubisco – The Most Abundant Enzyme

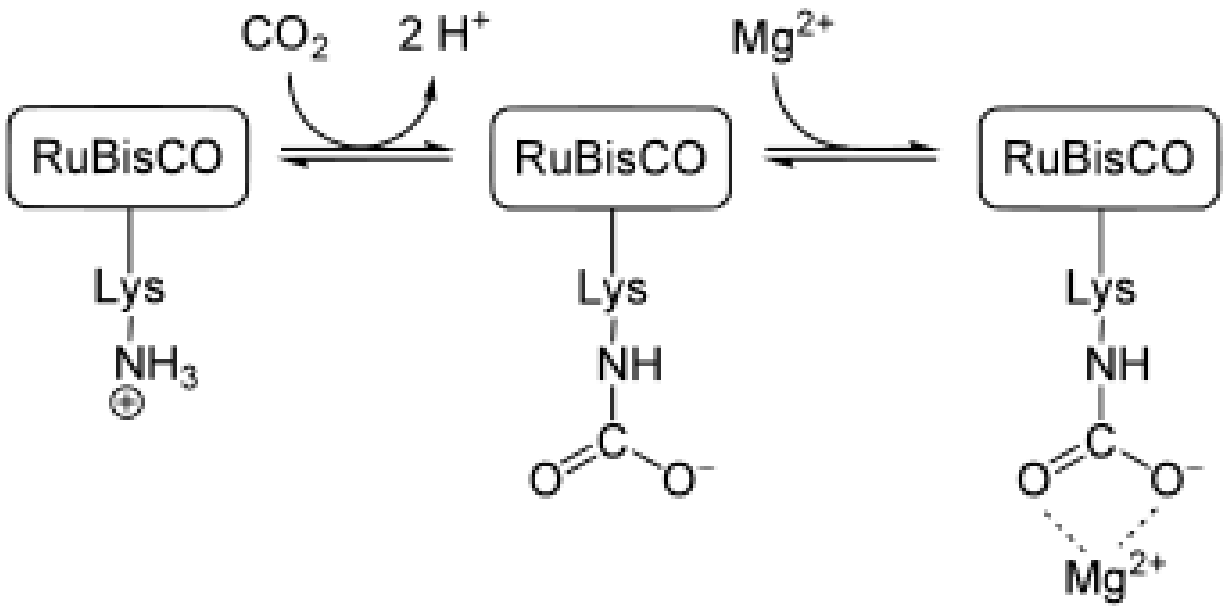
Manajit Hayer-Hartl [1,*] and F. Ulrich Hartl [1,*]

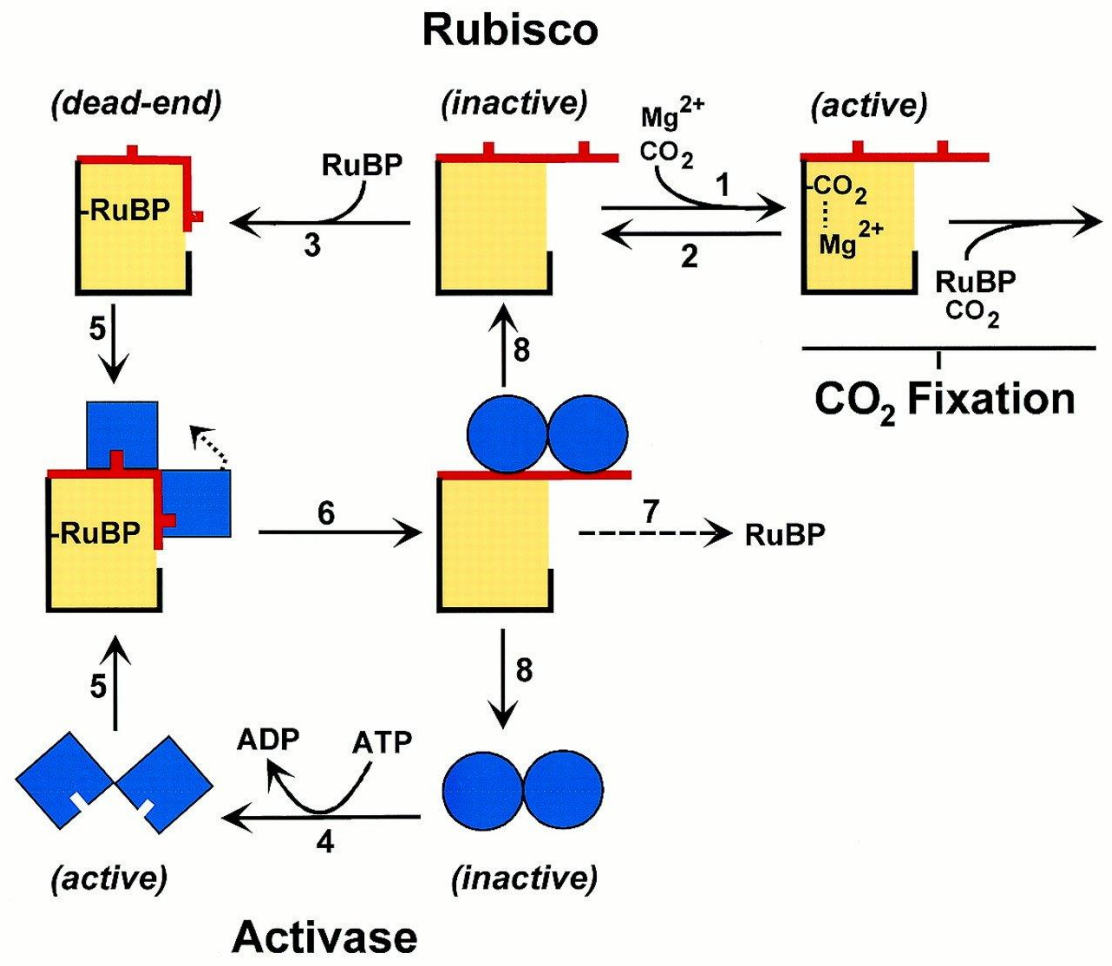


all food sources for life on Earth and maintains the oxygen level of the atmosphere. The human population currently stands at ~7.8 billion, and is expected to grow to ~10 billion by 2050 [1]. However, not only is the current overall **agricultural productivity** (see Glossary) not keeping pace with population growth, it is actually expected to decline in the course of this century due to climate change, especially in the southern regions of the planet [2]. For example, by 2030 Southern Africa could lose more than 30% of maize production, its main crop, and South Asia could suffer losses of more than 10% for many regional staples, such as rice, millet, and maize [3]. Climate change affects agriculture in various ways, including through changes in average and local temperatures, regional climate extremes, and droughts. In the face of these challenges, it seems unlikely that future **food security** can be ensured without increasing photosynthetic carbon fixation by genetically engineering crop plants [4–9]. Making photosynthesis more efficient would not only increase productivity but also enhance agricultural sustainability by optimizing the use of resources and minimizing the impact on the environment [10].

RubisCO peculiarities

1. A **conserved lysine** residue in the active site needs to be carbamylated in order to complex an Mg2+ ion that is in turn required for activity.

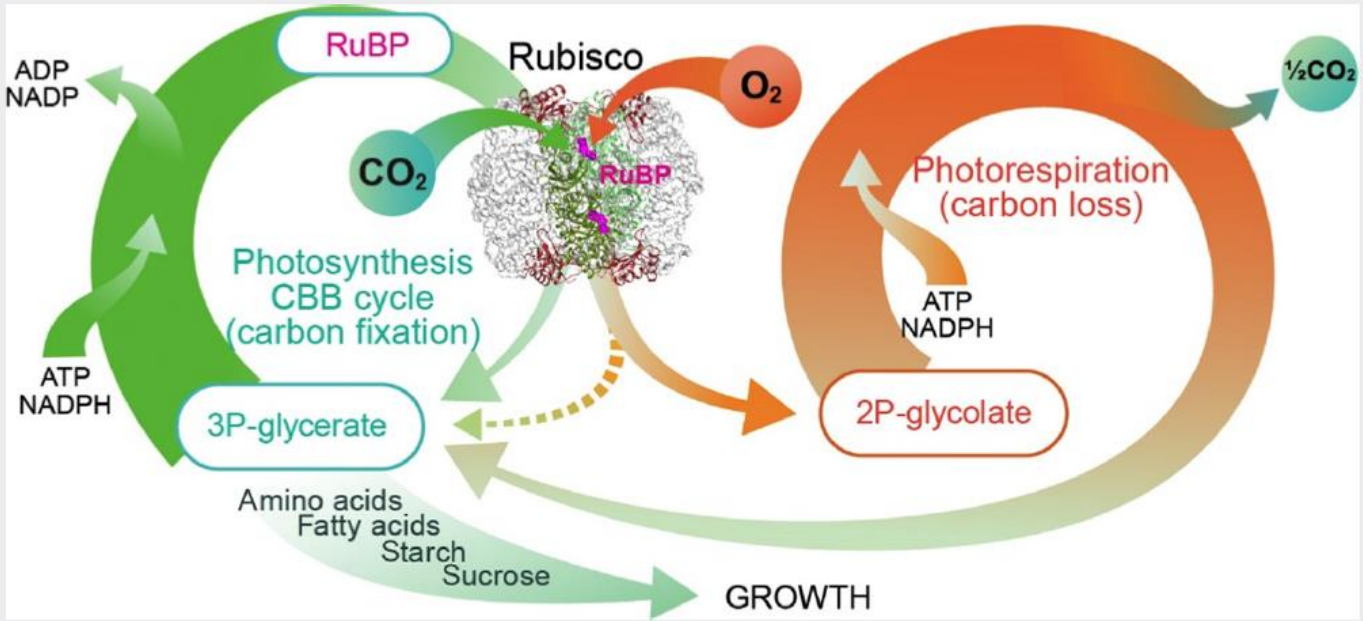




https://www.pnas.org/doi/10.1073/pnas.97.24.12937

RubisCO peculiarities

1. A conserved lysine residue in the active site needs to be carbamylated in order to complex an Mg2+ ion that is in turn required for activity.
2. the enzyme is a rather slow catalyst. The turnover frequency of an average RubisCO is only between 1 and 10 s^{-1} (http:// brenda-enzymes.org), which can make it a limiting factor in photosynthetic CO2-fixation under optimal conditions.
3. RubisCO makes mistakes. Besides the carboxylation reaction RubisCO catalyzes a non-productive oxygenation side-reaction that leads to the formation of 2- phosphoglycolate (2PG).



(B)

Amount of CO_2 fixed globally per annum:	~10^{14} kg
Total mass of Rubisco:	~7 x 10^{11} kg (~96 % terrestrial, ~4 % marine)
Time-averaged catalytic rate:	~0.03 s^{-1} terrestrial Rubisco ~0.6 s^{-1} marine Rubisco
CO_2 molecules fixed per second:	~2 x 10^{32} terrestrial Rubisco ~1.5 x 10^{32} marine Rubisco

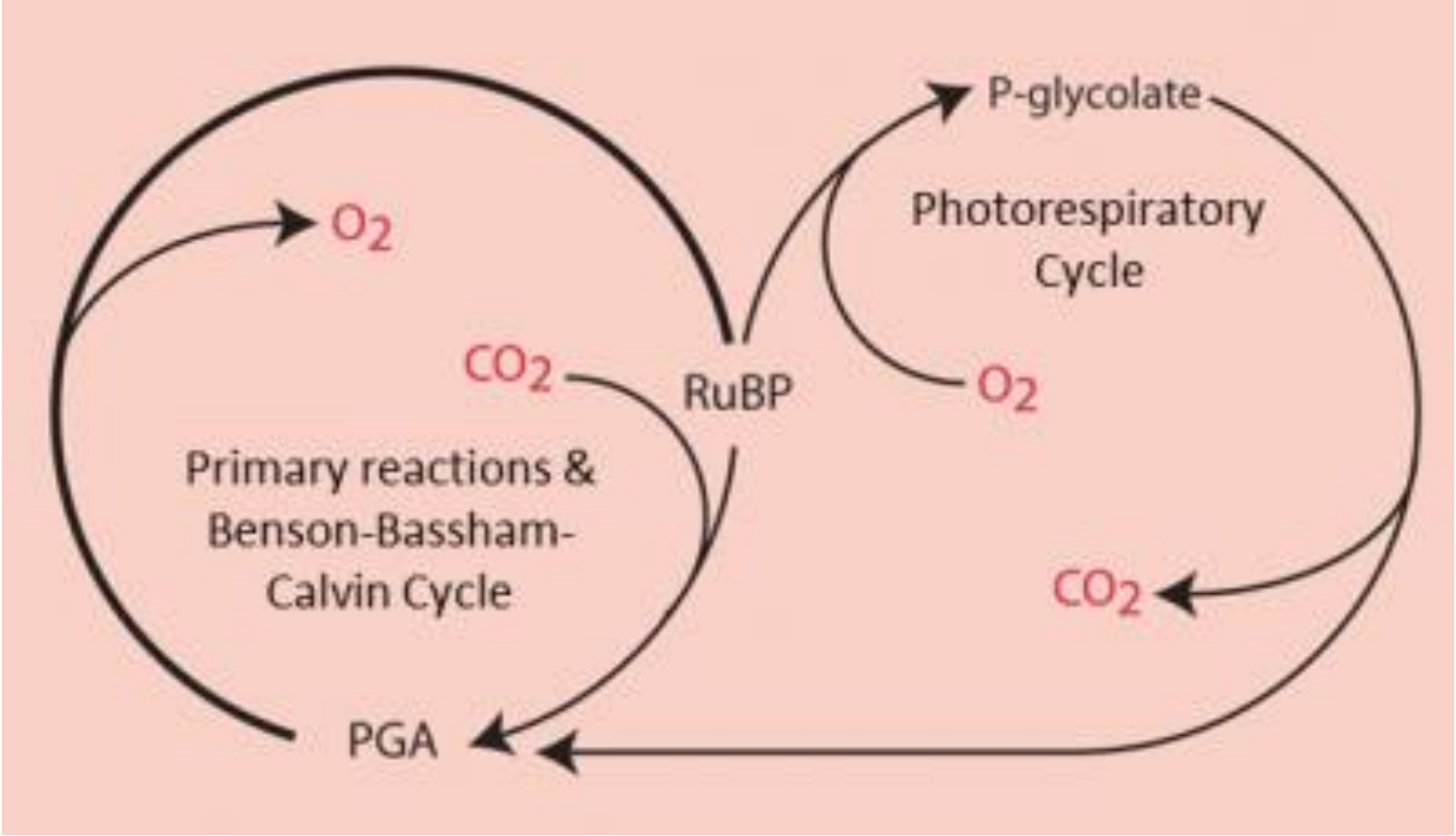
Trends in Biochemical Sciences

An important limitation in photosynthesis is the poor performance of Rubisco due to its dual carboxylase and oxygenase activity.

At ambient CO_2 levels, the carboxylase and oxygenase activities compete with each other, leading to a significant decrease in carbon fixation through photorespiration.

Photorespiration creates a product (2-Pglycolate) that cannot be used to make sugars and hence reduces the efficiency of the Calvin cycle

Photorespiration reduces levels of photosynthesis by up to ~25% in C3 plants, reducing energy yield in these plants



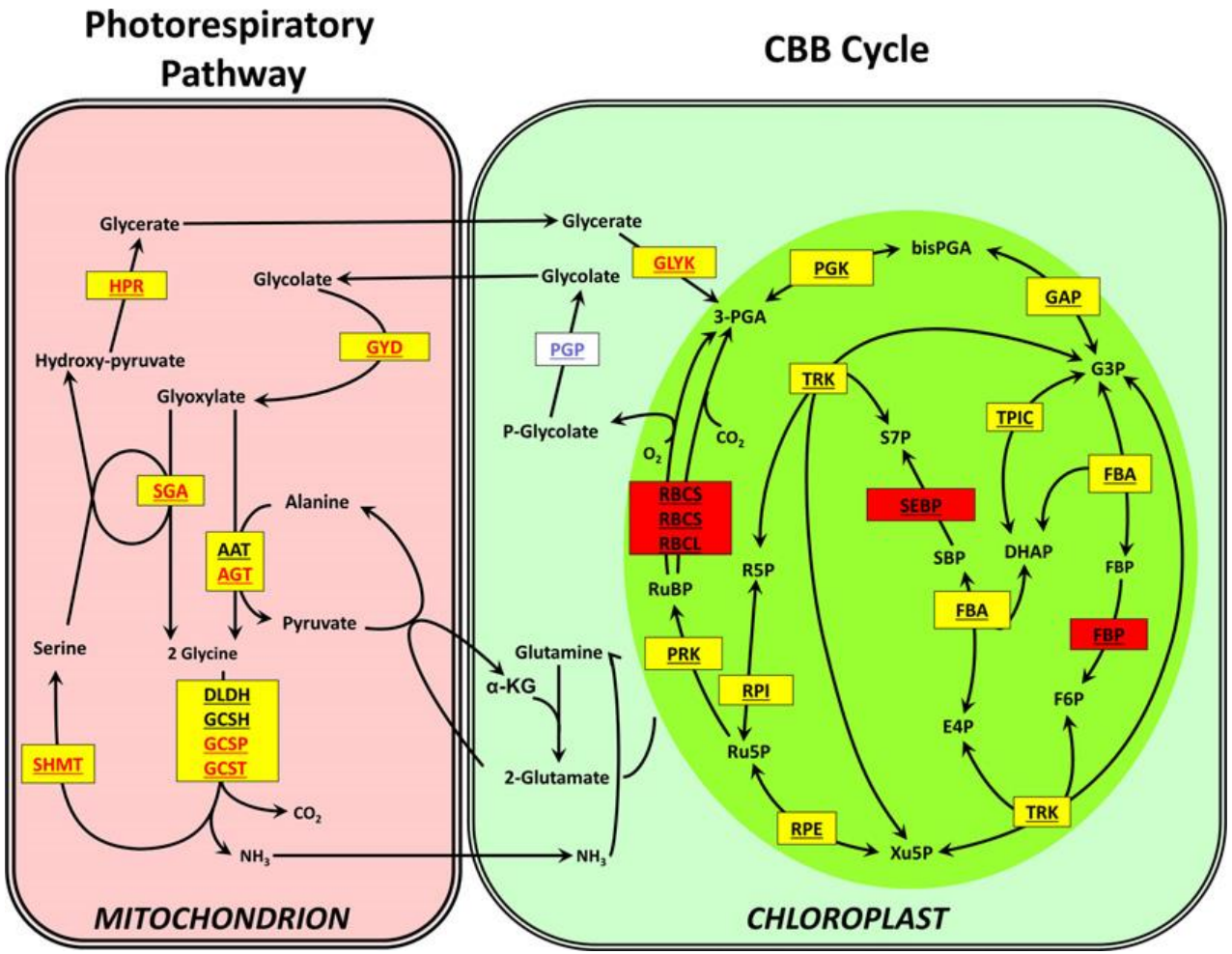
https://www.encyclopedie-environnement.org/en/life/path-carbon-photosynthesis/

The Plant Journal (2015) 82, 429–448 doi: 10.1111/tpj.12829

SI CHLAMYDOMONAS

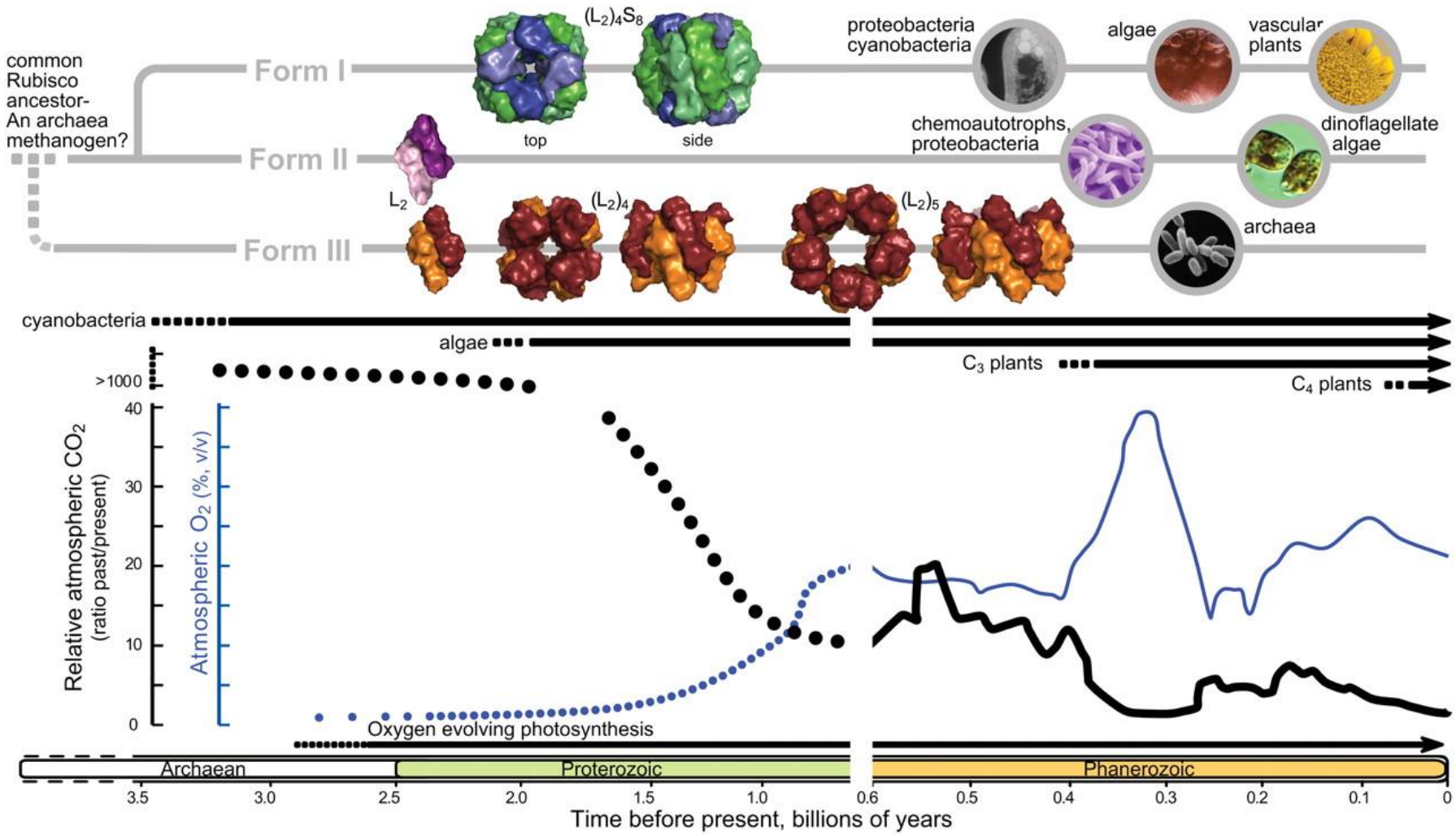
The CO_2 concentrating mechanism and photosynthetic carbon assimilation in limiting CO_2: how Chlamydomonas works against the gradient

Yingjun Wang, Dan J. Stessman and Martin H. Spalding*
Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, Iowa, USA



Chlamydomonas oxidizes glycolate to glyoxylate utilizing a glycolate dehydrogenase located in the mitochondria, donating electrons to the ubiquinone pool of the respiratory electron transport chain.

Over the past **three billion years**, the carbon-fixing enzyme **Rubisco drew down atmospheric concentrations of CO_2** to trace levels, in effect **starving itself** of its substrate.



Why is RubisCO so inefficient?

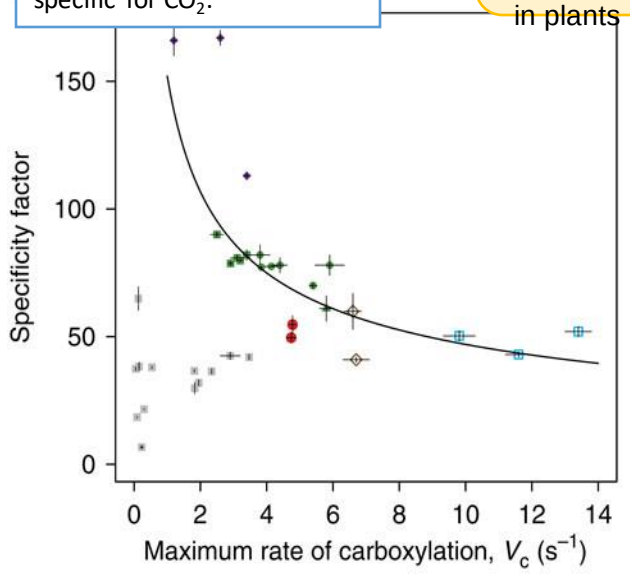
- Evidence accumulated that enzyme activity and specificity are reciprocally linked with each other in RubisCO.
- A faster RubisCO has a higher error rate and a more specific RubisCO has a lower catalytic rate.
- RubisCO evolved before the first great oxygenation event in an atmosphere without oxygen (O2), so that its mechanism was not constrained by O2.
- However, with the rise of atmospheric O2 concentrations to modern-day levels, as a result of the second great oxygenation event, RubisCO had to learn to discriminate between CO2 and O2. Because discrimination usually comes at the cost of reduced catalytic rate, a more specific enzymes almost inevitably becomes a slower catalyst

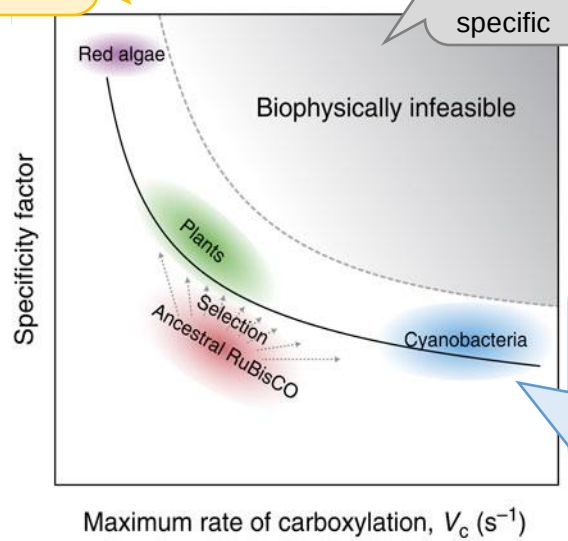
Different forms of Rubisco in nature

Specificity factor indicates the specificity of the enzyme for CO_2 relative to O_2. Higher means more specific for CO_2.

Rubisco in red algae is more specific but slower than that in plants

In many organisms, the enzyme cannot be both fast and specific

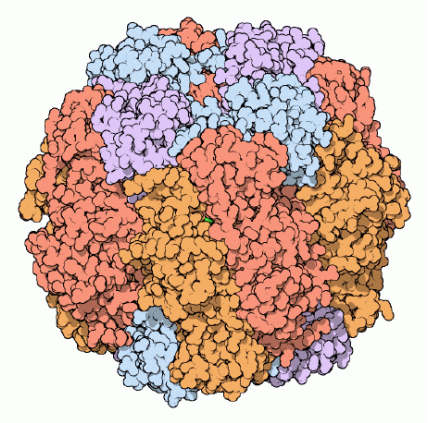




Rubisco in cyanobacteria algae is faster but less specific than that in plants

Shih P.M., Occhialini A., Cameron J.C., Andralojc P.J., Parry M.A.J.and Kerfeld C.A. (2016). Biochemical characterization of predicted Precambrian RuBisCO. Nat Commun 7: 10382.
See also Whitney, S.M., Houtz, R.L. and Alonso, H. (2011). Advancing our understanding and capacity to engineer Nature's CO_2-sequestering enzyme, Rubisco. Plant Physiol. 155: 27-35.

How algae and cyanobacteria solved this problem?



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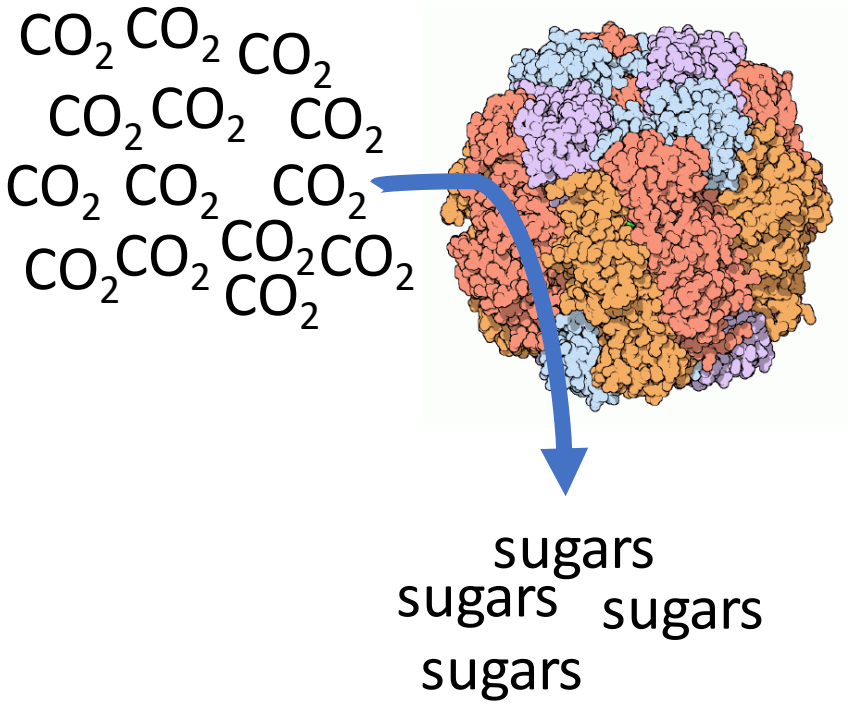
Trends in Biochemical Sciences

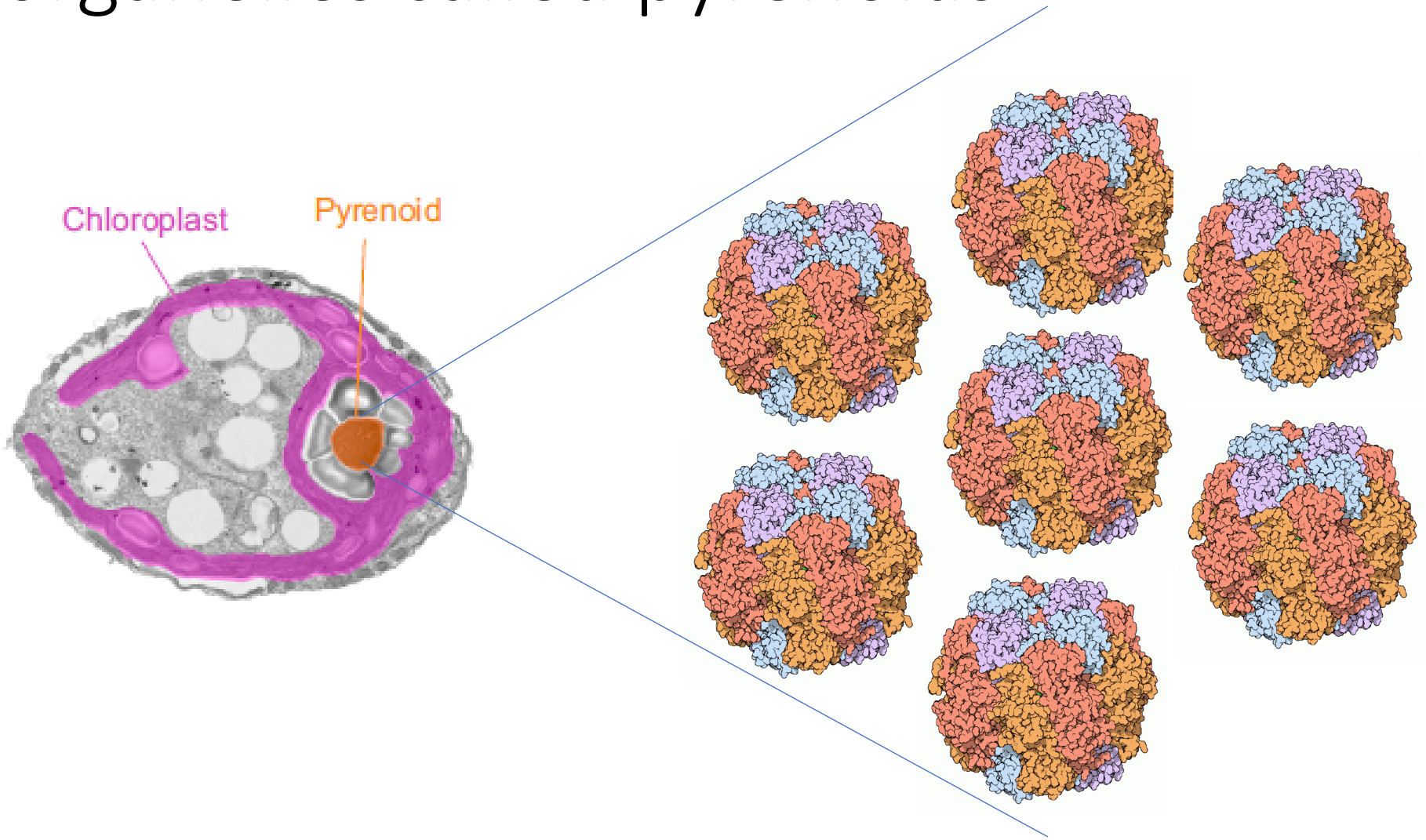
How algae and cyanobacteria solved this problem?

by increasing the level of CO_2 in the vicinity of Rubisco through CO_2-concentrating mechanisms (CCM)



Microalgae and pyrenoids

By packing RUBISCO in specialized organelles called pyrenoids



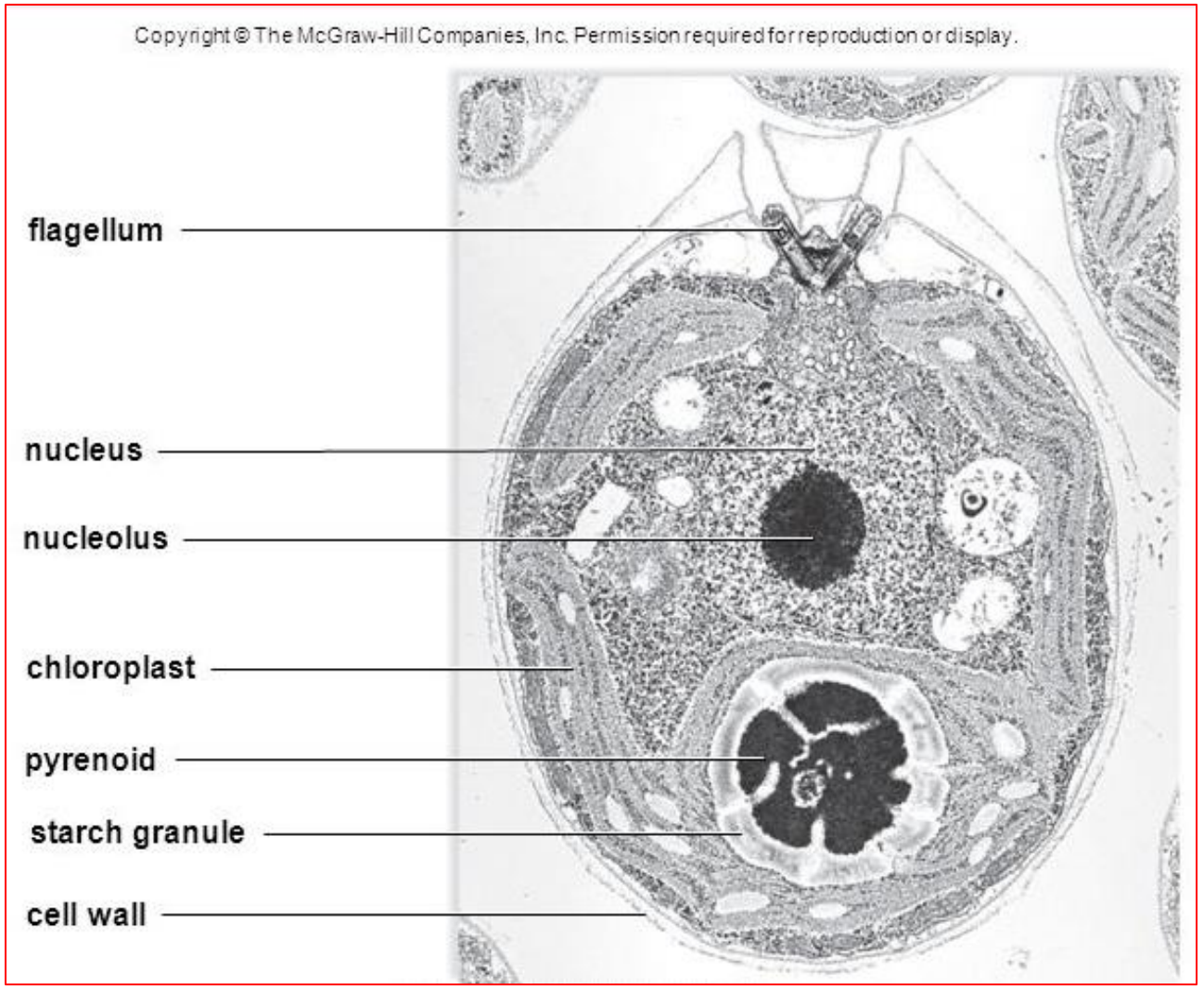
Proposed synthetic biology approach:

Introducing non-plant Carbon Concentrating Mechanisms (CCMs) into C3 plant

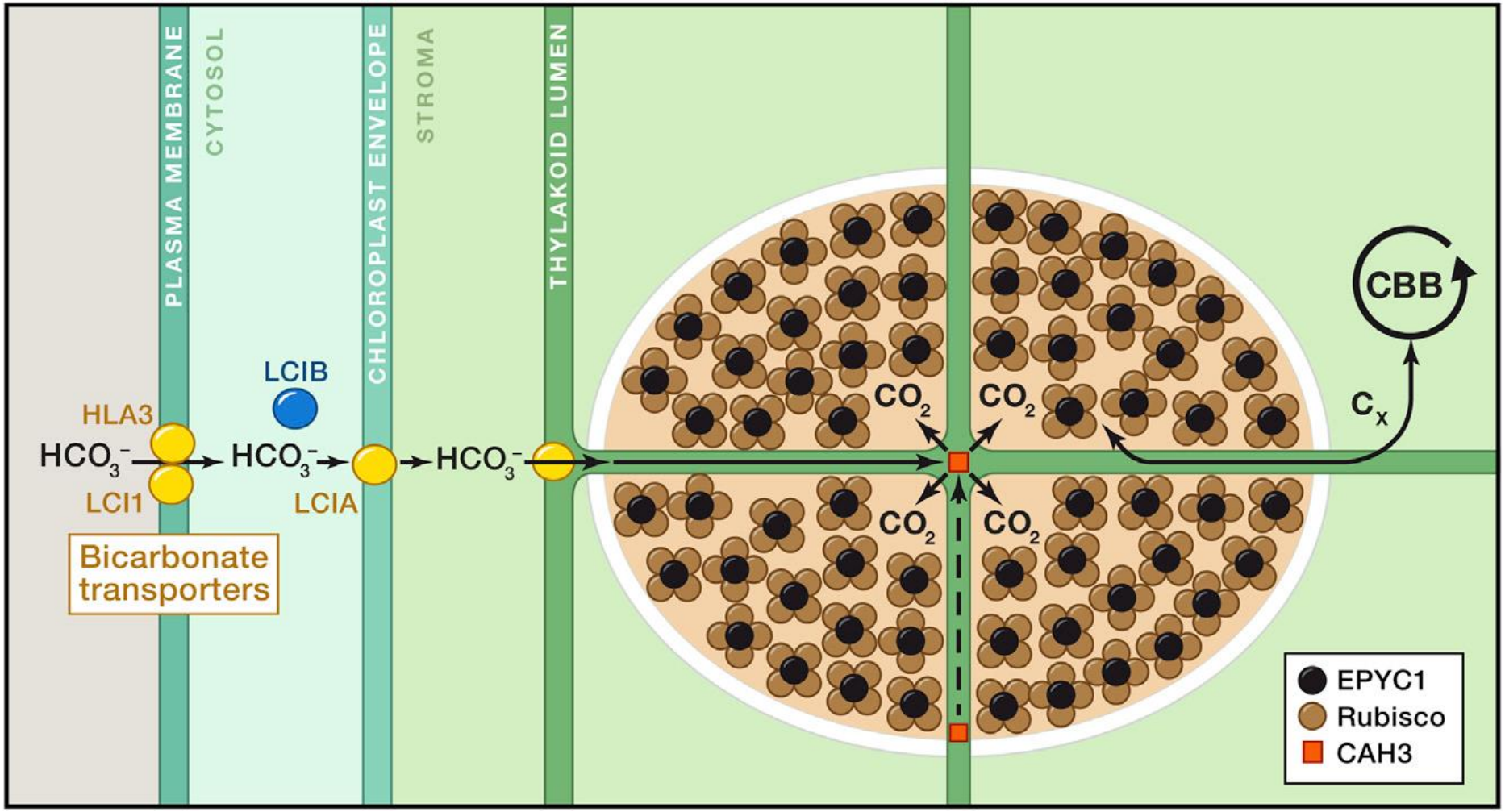
One approach to improving plant photosynthesis is using engineering to implement CCMs from other photosynthetic organisms such as cyanobacteria or algae.

Pyrenoid, a membraneless organelle within chloroplasts, where approximately **one-third of global CO_2 fixation is estimated to occur**

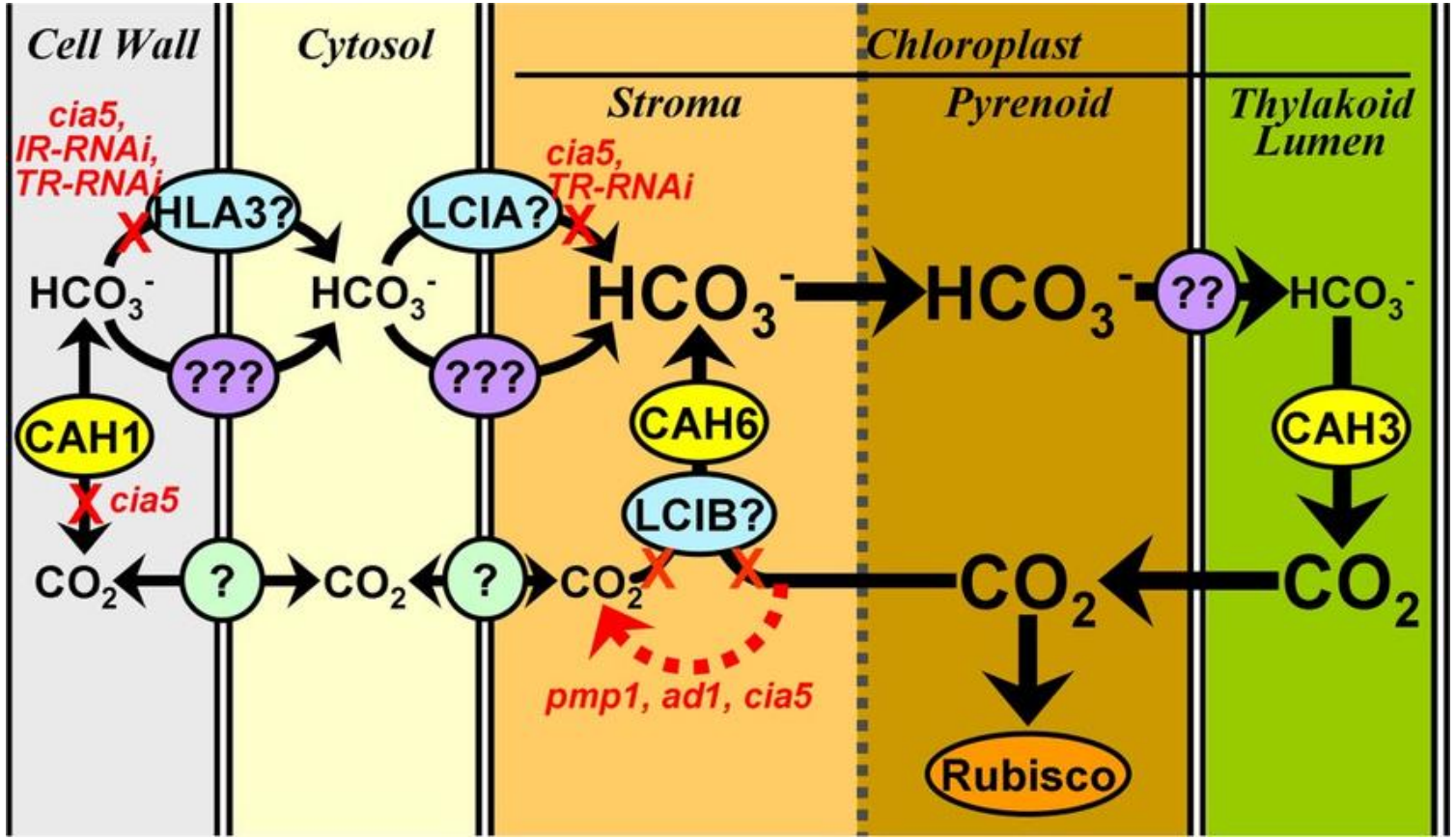
The pyrenoid contains a starch sheath and a matrix in which CO_2 is concentrated together with Rubisco. The matrix is traversed by membrane tubules that are continuous with the thylakoid membranes.



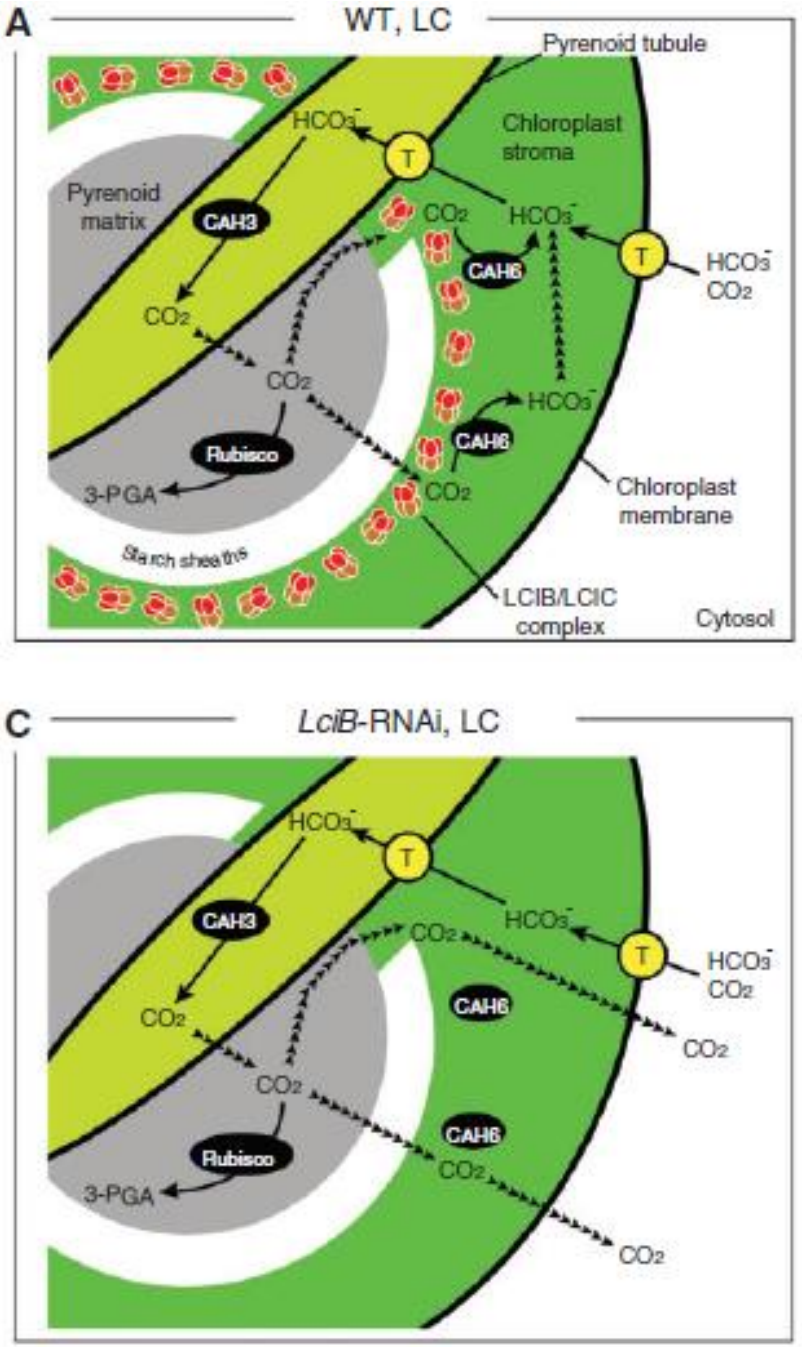
In algae, CCM is mediated by several plasma and chloroplast-membrane inorganic carbon transporters, a set of carbonic anhydrases in strategic locations



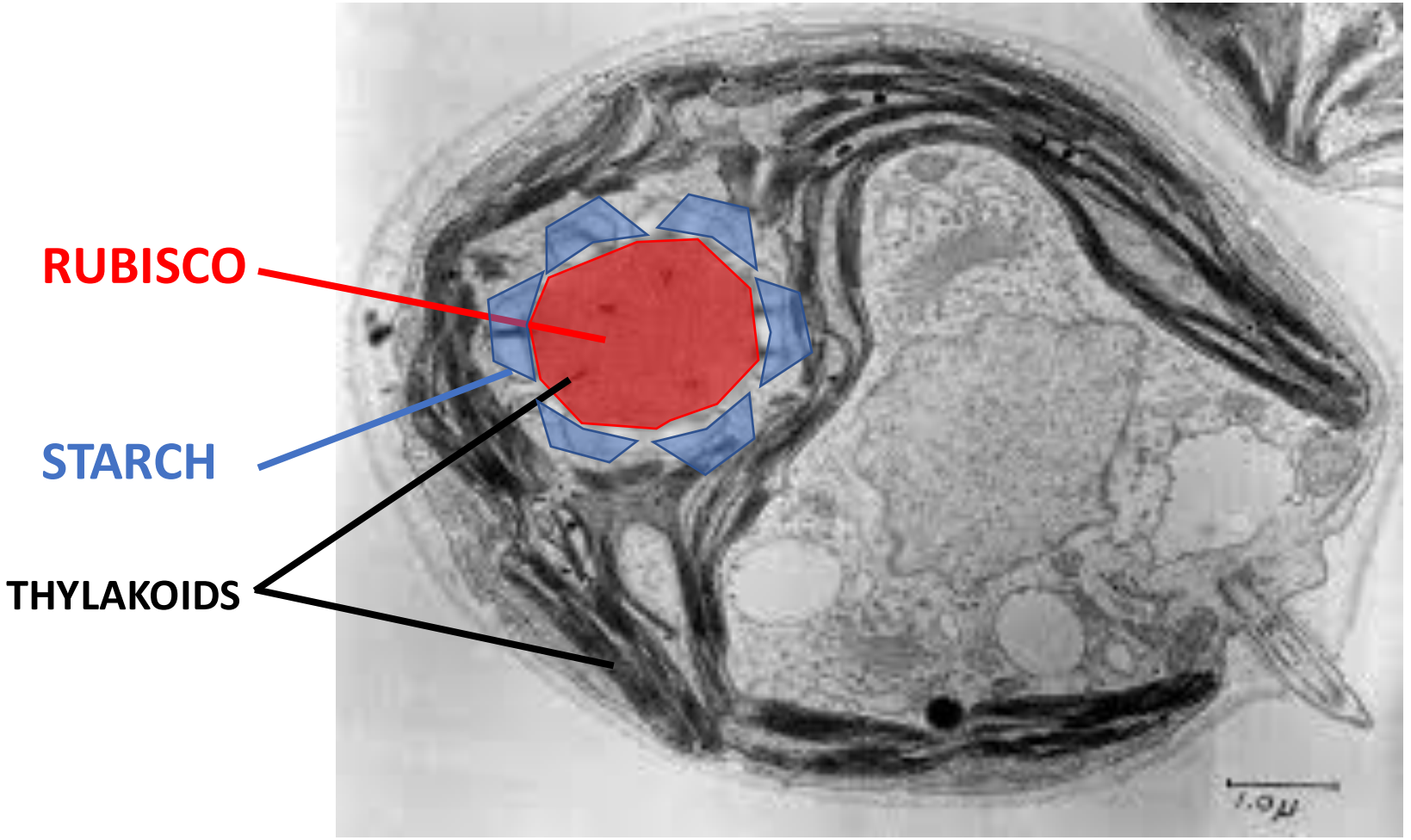
Ci, including the charged species, bicarbonate (HCO_3^-), must cross both the plasma membrane and the chloroplast envelope to reach Rubisco, yet the roles, if any, of proposed and confirmed Ci transporters, including the plasma membrane proteins HLA3 and LimitingCO_2 Inducible1 (LCI1) and the chloroplast envelope proteins LCIA (NAR1.2), CCP1, and CCP2



Many mutants affected in CCM have been identified based on their ability to grow photoautotrophically under high-CO_2 but not under low-CO_2 conditions.



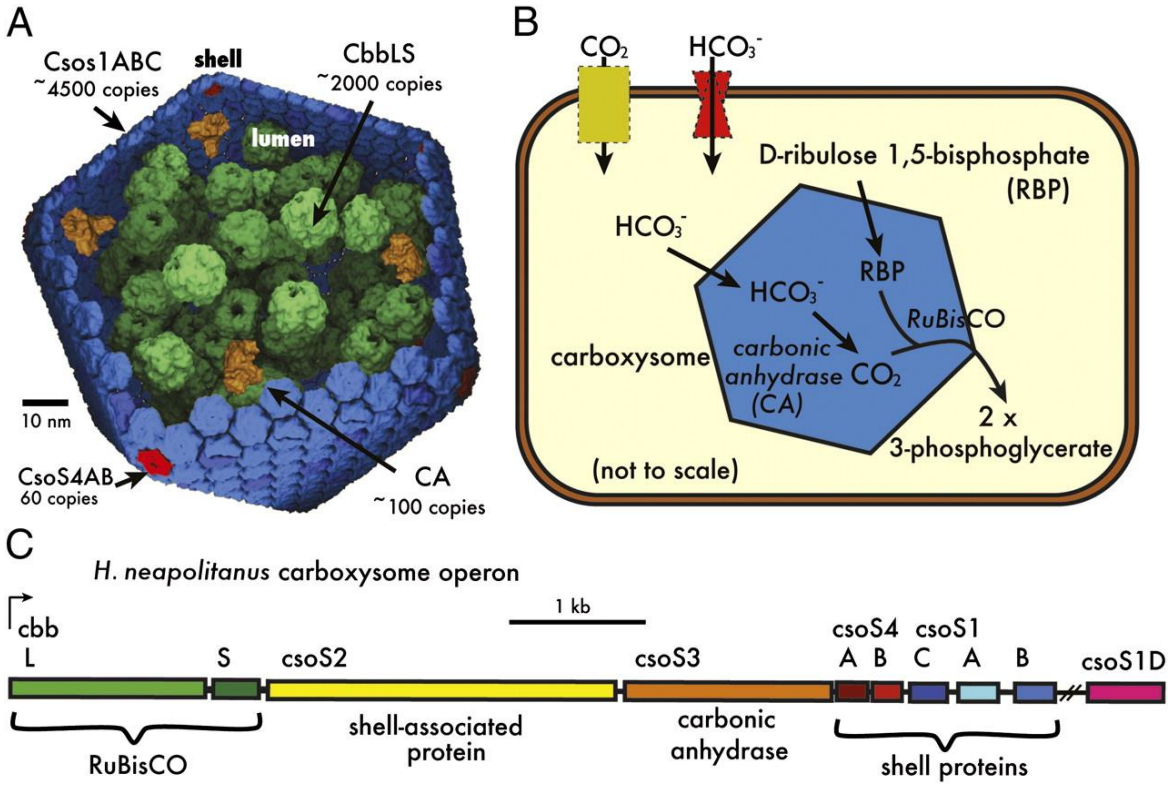
Many mysteries surround pyrenoids, especially about its protein composition, structure, and assembly



1. What proteins form pyrenoids?
2. What keep rubisco inside pyrenoid?
3. How does it divide?

Meanwhile in bacteria…

The components of the **cyanobacterial CCMs have largely been identified**, facilitated in part by the organization of the genes encoding them into **operons**. Knowledge of these components has enabled the detailed characterization of the structure and assembly pathway of the **beta carboxysome**



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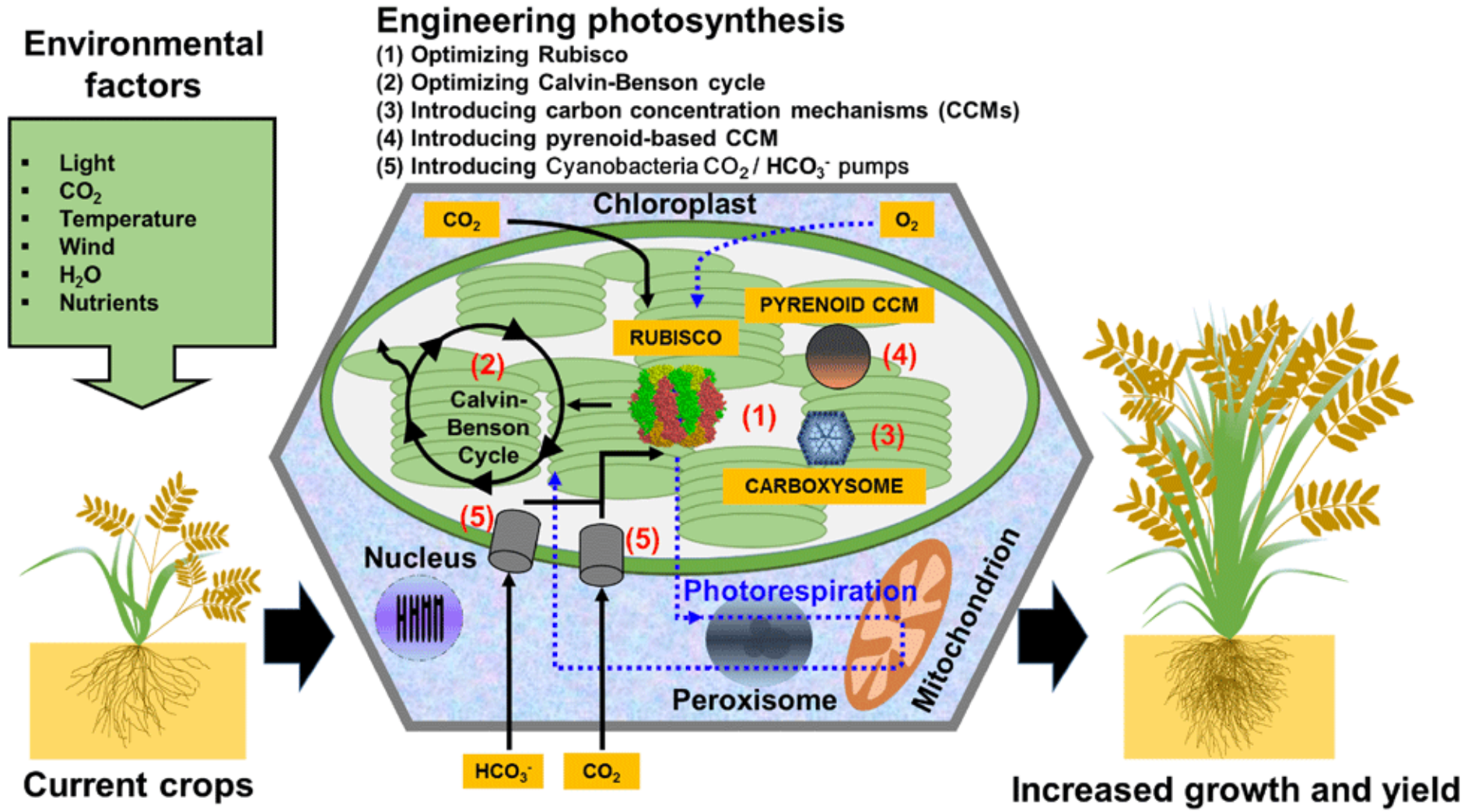
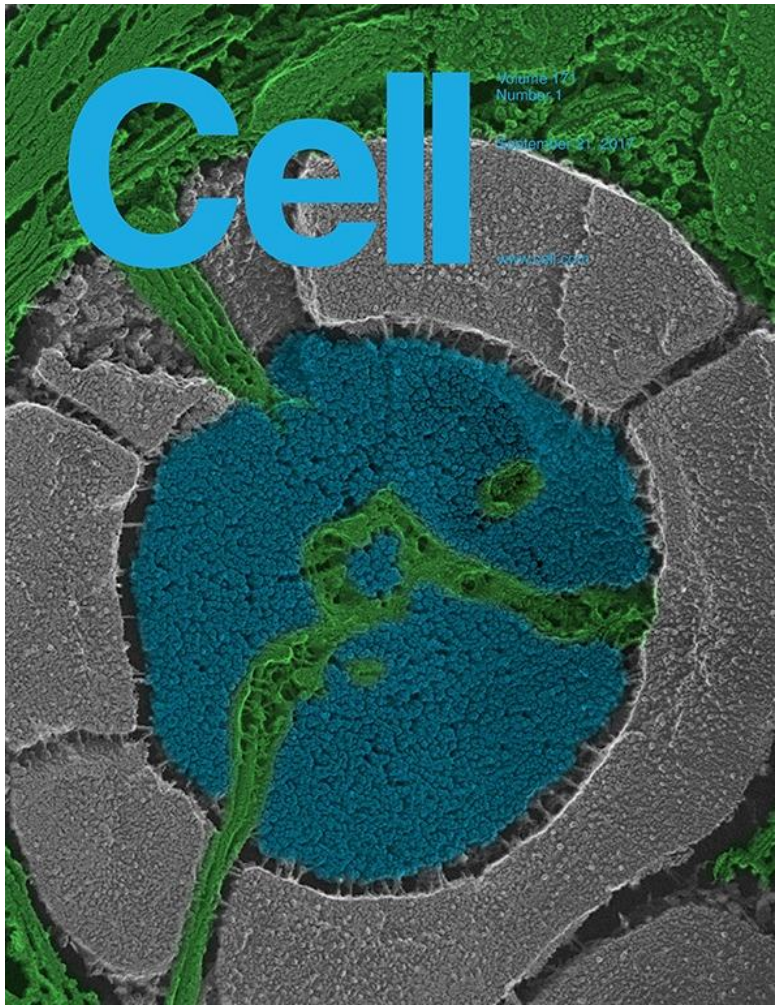


Orr DJ, Pereira AM, da Fonseca Pereira P et al. Engineering photosynthesis: progress and perspectives [version 1]. F1000Research 2017, 6:1891 (doi: 10.12688/f1000research.12181.1)

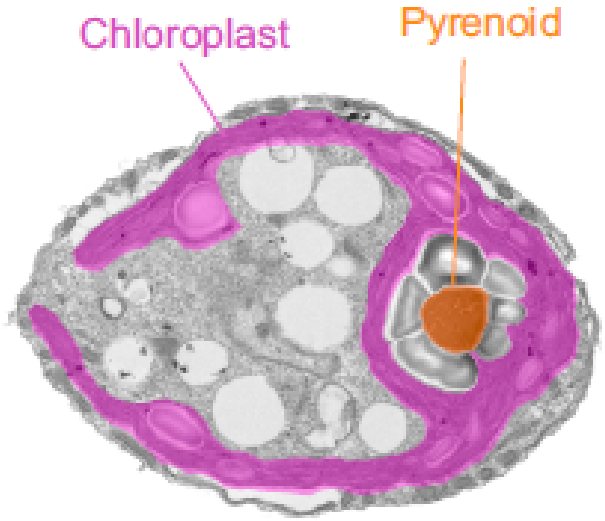
A Spatial Interactome Reveals the Protein Organization of the Algal CO_2-Concentrating Mechanism

Luke C.M. Mackinder,[1,6] Chris Chen,[1,2] Ryan D. Leib,[3] Weronika Patena,[1,4] Sean R. Blum,[1,7] Matthew Rodman,[2] Silvia Ramundo,[5] Christopher M. Adams,[3] and Martin C. Jonikas[1,2,4,8,*]

http://jonikaslab.princeton.edu/



High-Throughput Pipeline for Systematic Localization of Proteins in *Chlamydomonas reinhardtii*

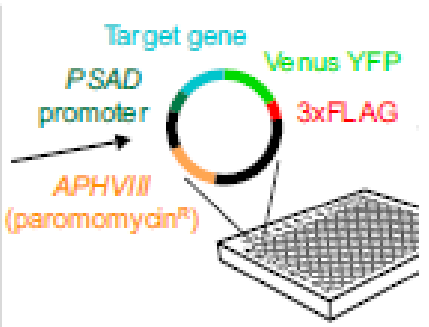


Target genes

Known/putative CCM genes (25)

Candidate CCM genes:
CO_2 regulated (236)
Pyrenoid proteomics (46)
Other (321)

Organelle markers (14)



96 well PCR and cloning

Open reading frames (ORFs) were amplified by PCR from genomic DNA and cloned in frame with a C-terminal Venus **YFP** and a **3xFLAG** epitope, driven by the strong *PsaD* promoter. These constructs were transformed into wild-type *Chlamydomonas,* where they inserted into random locations in the genome.
Paromomycin selection

Potential drawbacks:

Potential drawbacks:

- loss of the endogenous transcriptional regulation of the protein, including information encoded in the promoter, terminator, and genomic locus.
- Additionally, the C-terminal protein tag could obscure subcellular targeting signals or disrupt functional domains.
- Notorious difficulties with expressing tagged genes in Chlamydomonas

the plant journal

SEB SOCIETY FOR EXPERIMENTAL BIOLOGY

The Plant Journal (2009) 57, 1140–1150 doi: 10.1111/j.1365-313X.2008.03746.x

but…

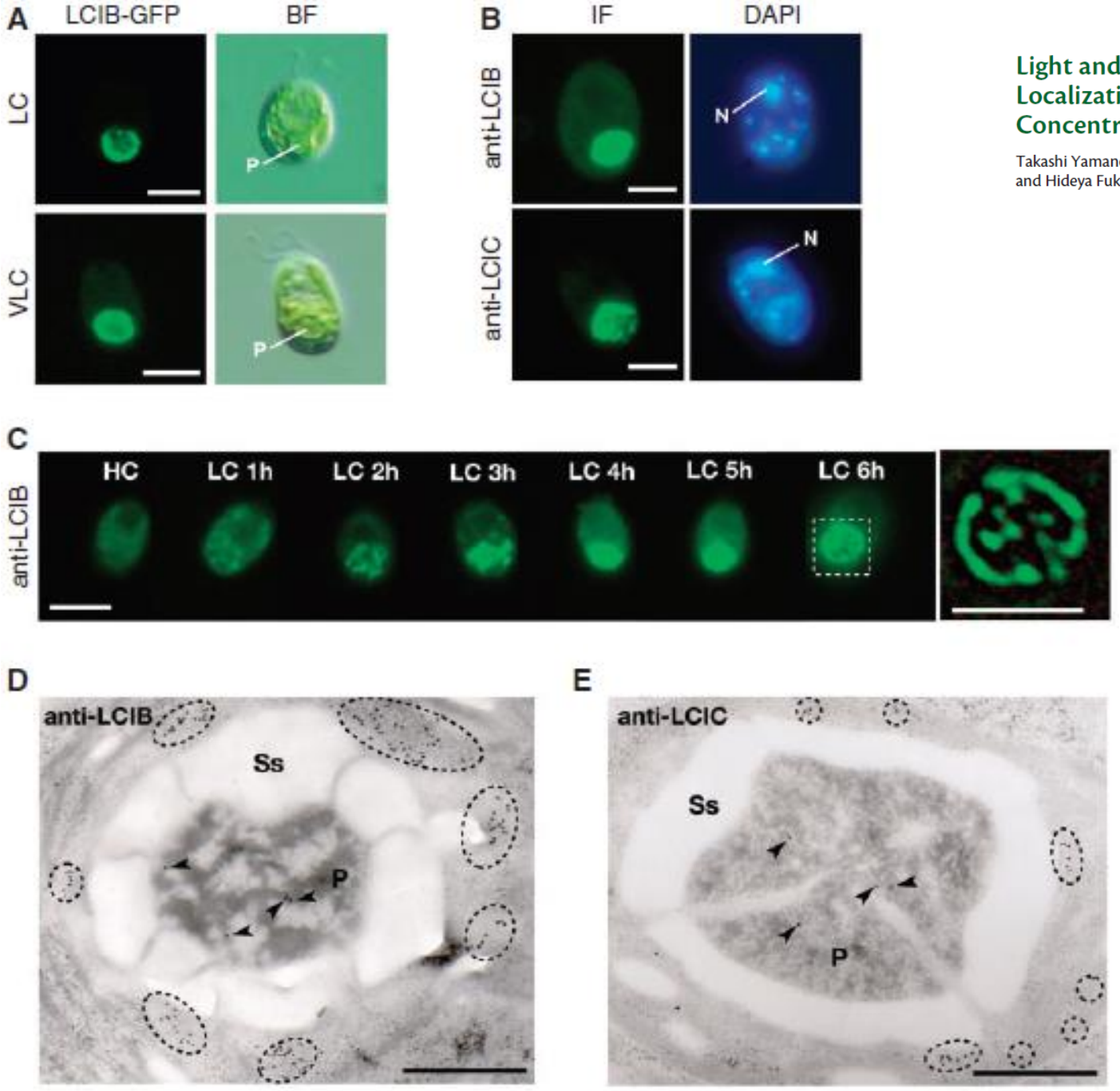
TECHNICAL ADVANCE

Generation of *Chlamydomonas* strains that efficiently express nuclear transgenes

PCP
PLANT & CELL PHYSIOLOGY

Light and Low-CO_2-Dependent LCIB–LCIC Complex Localization in the Chloroplast Supports the Carbon-Concentrating Mechanism in *Chlamydomonas reinhardtii*

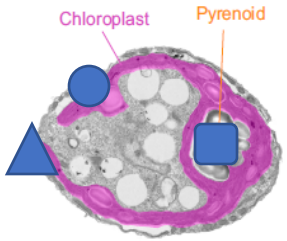
Takashi Yamano[1,4], Tomoki Tsujikawa[1], Kyoko Hatano[2], Shin-ichiro Ozawa[3,5], Yuichiro Takahashi[3] and Hideya Fukuzawa[1,*]



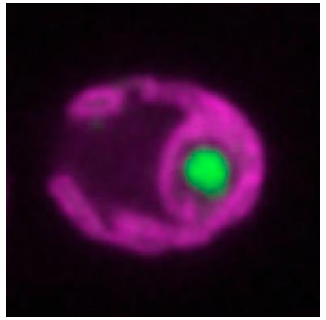
LCIB–LCIC complex could be related to the developing pyrenoid structure and/or formation of starch sheaths.

Some roles to trap CO_2 leaking from the pyrenoid matrix and to transfer the captured CO_2 to stromal carbonic anhydrase CAH6

626 candidate CCM targets



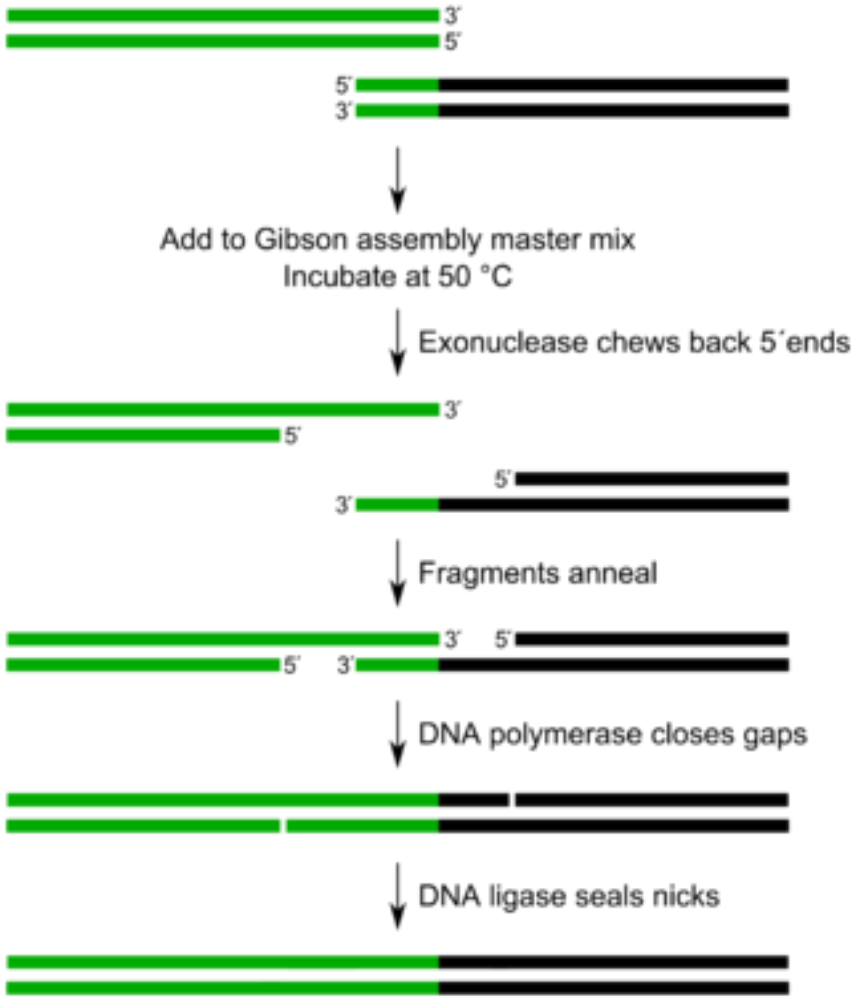
303 transformed and expressed in Chlamydomonas **(48%)**

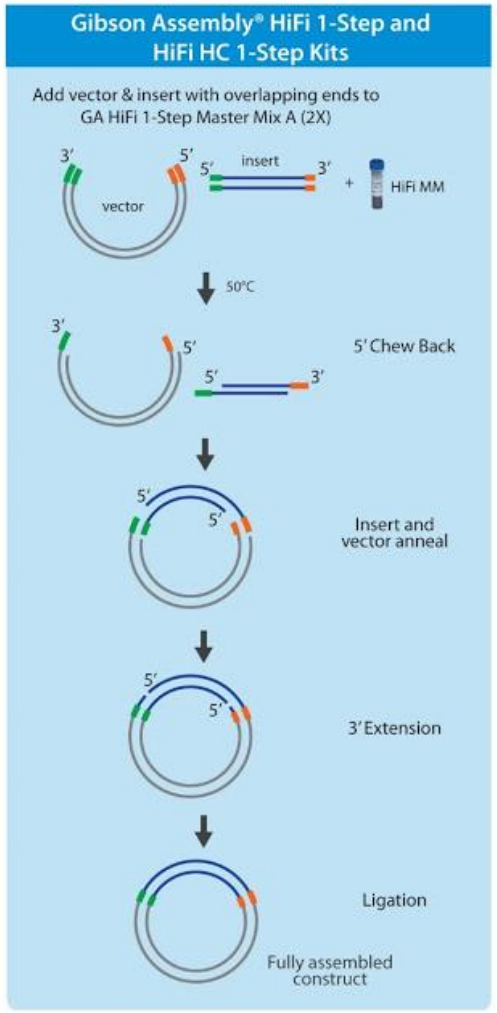


146 were imaged and localized at subcellular level by confocal laser scanner microscopy **(23%)**

Gibson technology to clone high number of cloning sequences

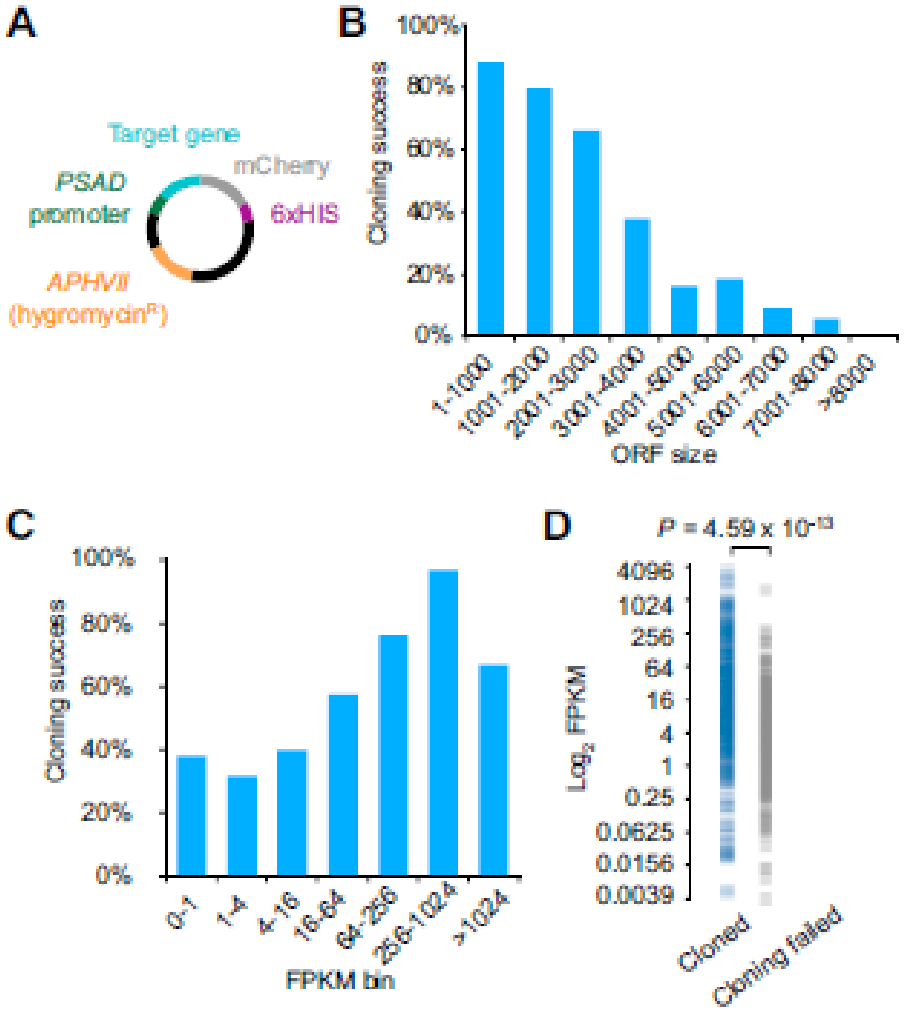
Gibson technology to clone high number of cloning sequences





Uncovering factors that may contribute to cloning and tagging success in Chlamydomonas.

They successfully cloned 303 of the 626 target genes (48%). Cloning success rate decreased with **gene size**.



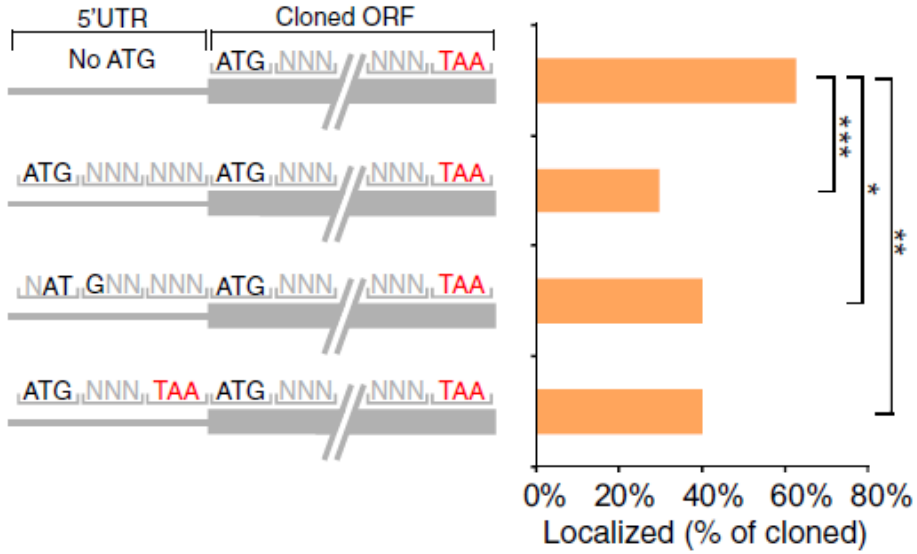
Intriguingly, cloning success was higher for genes with high expression levels ($p = 4 \times 10^{-13}$, Mann-Whitney U test), suggesting that **intrinsic properties of a gene** that influence endogenous expression may also affect PCR efficiency.

Successfully transformed and acquired protein localization data for 146 of the 303 cloned genes (49%).

The two main factors correlated with the ability to obtain localization data were:

(1) high endogenous gene expression level

(2) absence of upstream in-frame ATGs



Successfully transformed and acquired protein localization data for 146 of the 303 cloned genes (49%).

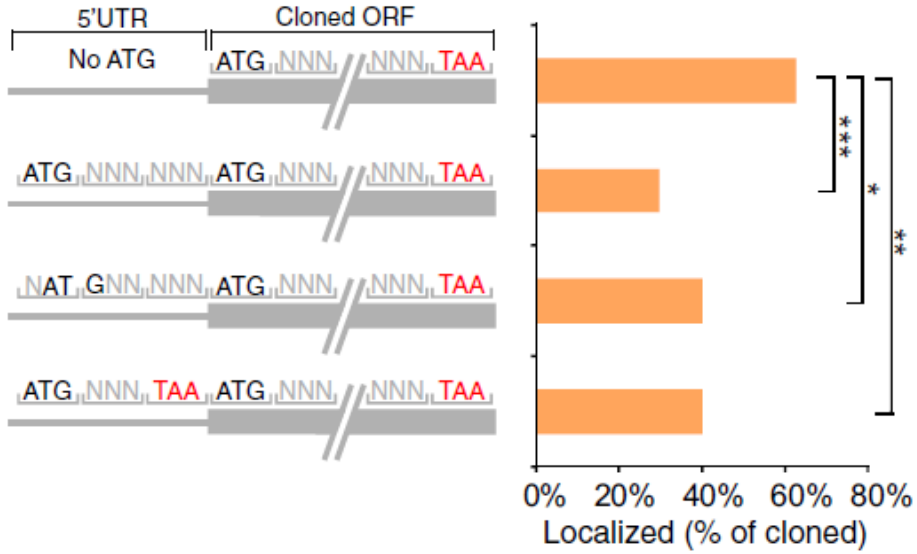
The two main factors correlated with the ability to obtain localization data were:

(1) high endogenous gene expression level

(2) absence of upstream in-frame ATGs

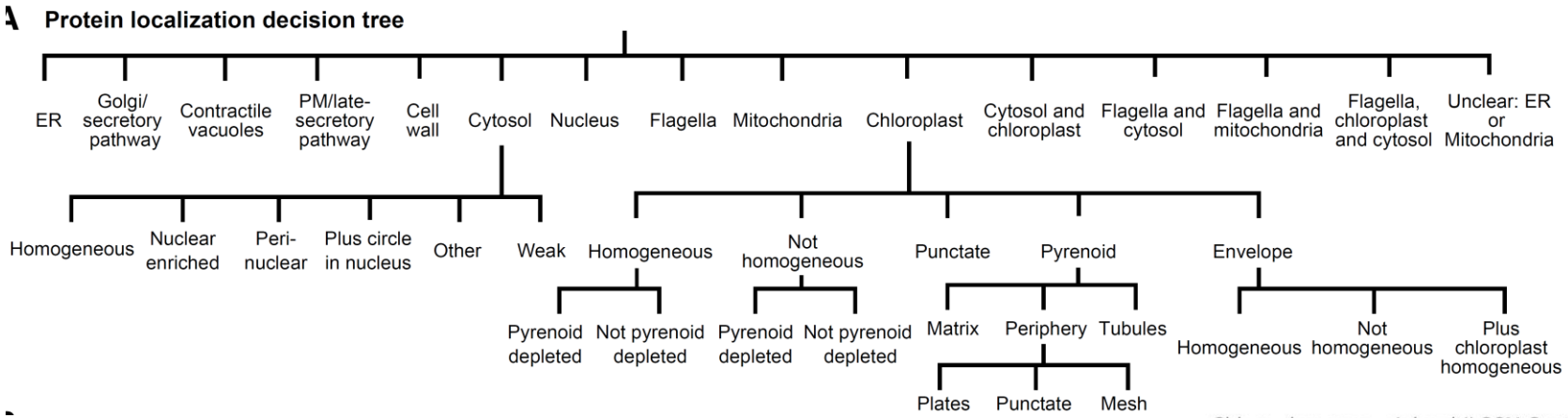
The failure to obtain localization data for genes with in-frame uATGs is likely due to the absence of the correct translational start site in the cloned construct.

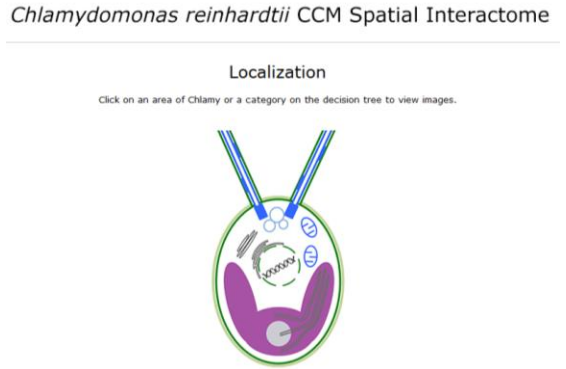
Transcript abundance is predictive for localization success and that future protein expression studies will benefit substantially from improved annotation of Chlamydomonas translation start sites.



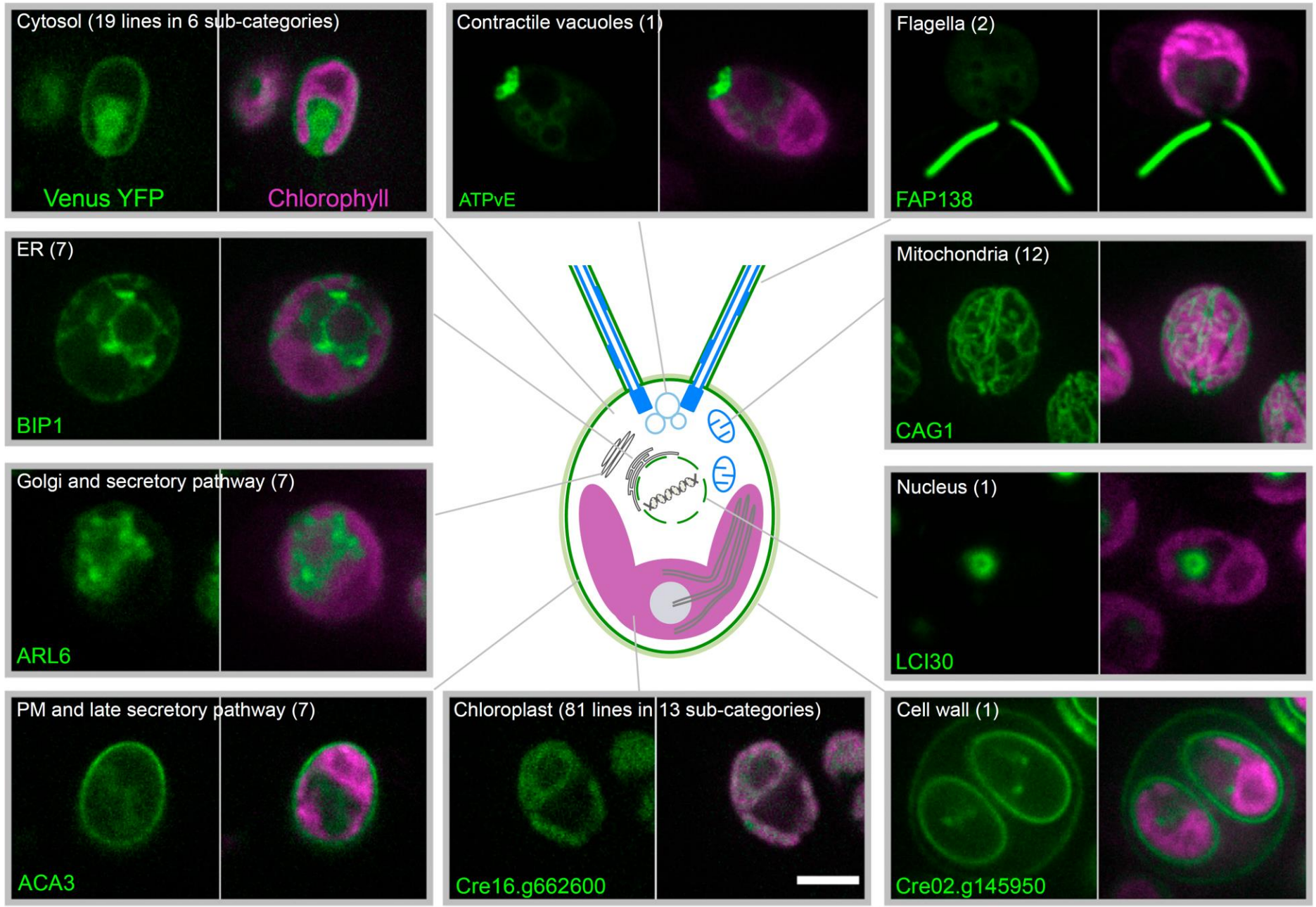
To aid in the classification of unknown proteins to subcellular regions, we tagged a series of conserved, well-characterized organelle and cellular structure proteins.

They employed a **decision tree to classify visually the localization of 135 additional proteins into 29 distinct subcellular regions**, representing nearly all of the known organelles and cellular structures of Chlamydomonas.



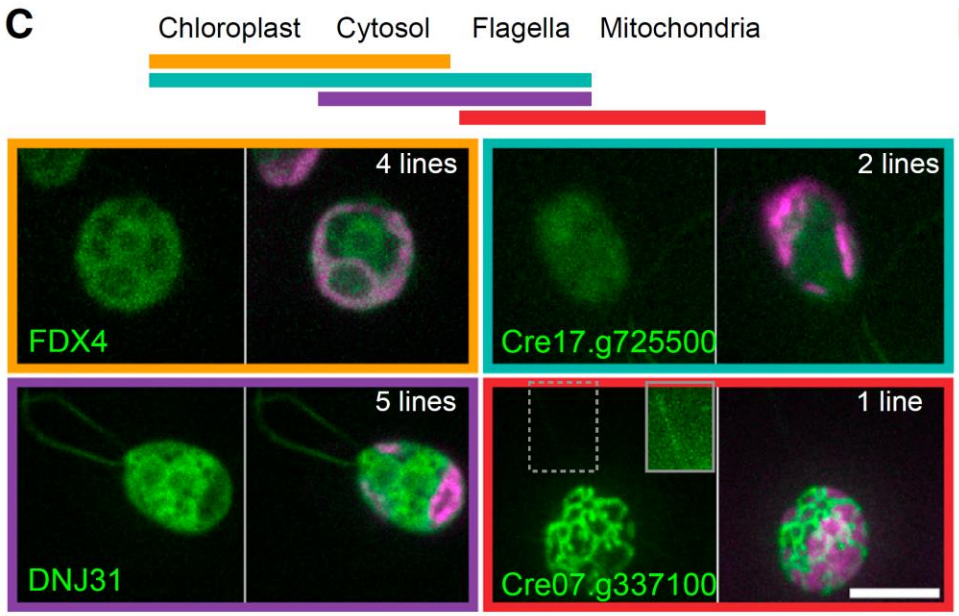


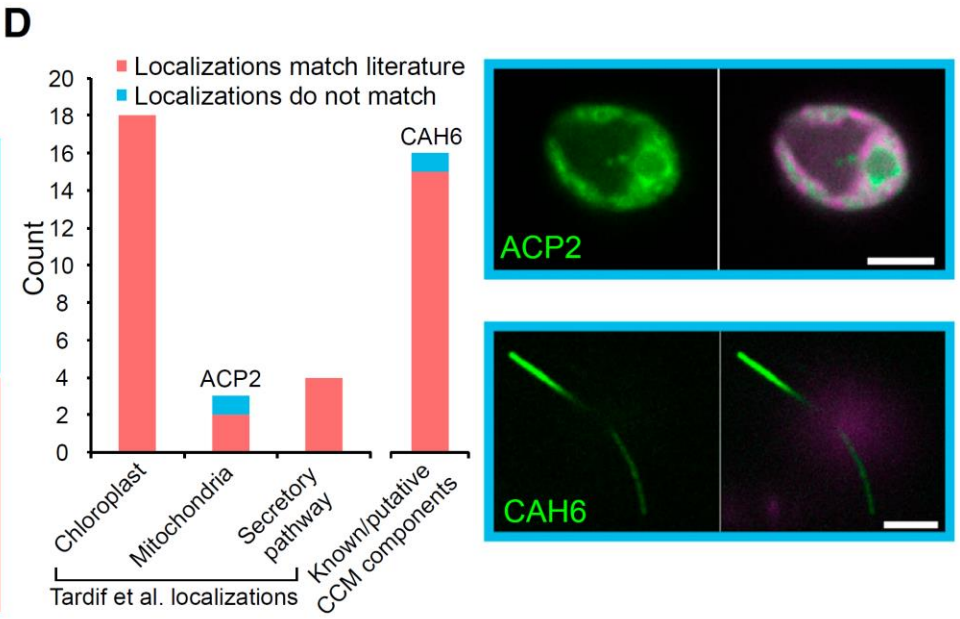
https://sites.google.com/site/chlamyspatialinteractome/


Cytosol (19 lines in 6 sub-categories)
Venus YFP
Chlorophyll
Contractile vacuoles (1)
ATPvE
Flagella (2)
FAP138
ER (7)
BIP1
Mitochondria (12)
CAG1
Golgi and secretory pathway (7)
ARL6
Nucleus (1)
LCI30
PM and late secretory pathway (7)
ACA3
Chloroplast (81 lines in 13 sub-categories)
Cre16.g662600
Cell wall (1)
Cre02.g145950

12 proteins were not confined to one organelle but were seen in multiple compartments

Validation for 39/41 proteins;
2 unexpected resuts





E

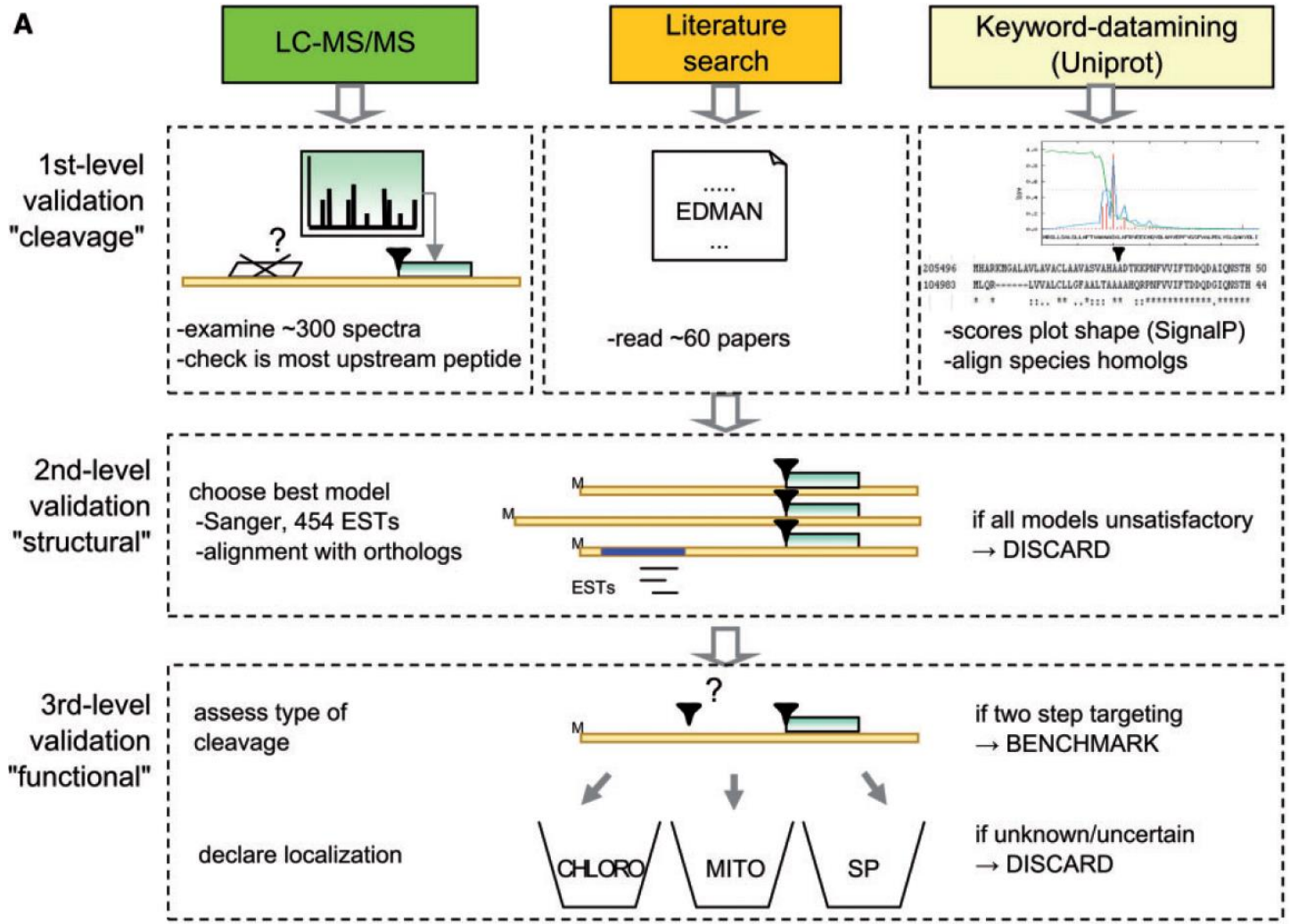
This study		Predalgo prediction				Predalgo analysis		TargetP prediction				TargetP analysis	
		C	M	SP	O	Match	False negative rate	C	M	SP	O	Match	False negative rate
Chloroplast	67	60	3	2	2	90%	10%	21	41	0	5	31%	69%
Mitochondria	13	3	4	0	6	31%	69%	4	2	0	7	15%	85%
Secretory pathway	21	2	0	8	11	38%	62%	0	3	5	13	24%	76%
Other	41	15	0	2	24	59%	41%	7	6	1	27	66%	34%

Prediction tools validation

C-term tagging does not alter protein localization

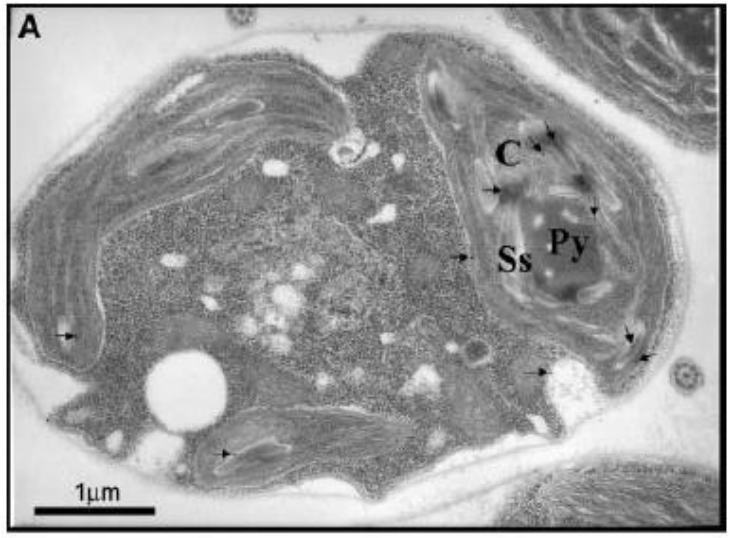
PredAlgo: A New Subcellular Localization Prediction Tool Dedicated to Green Algae

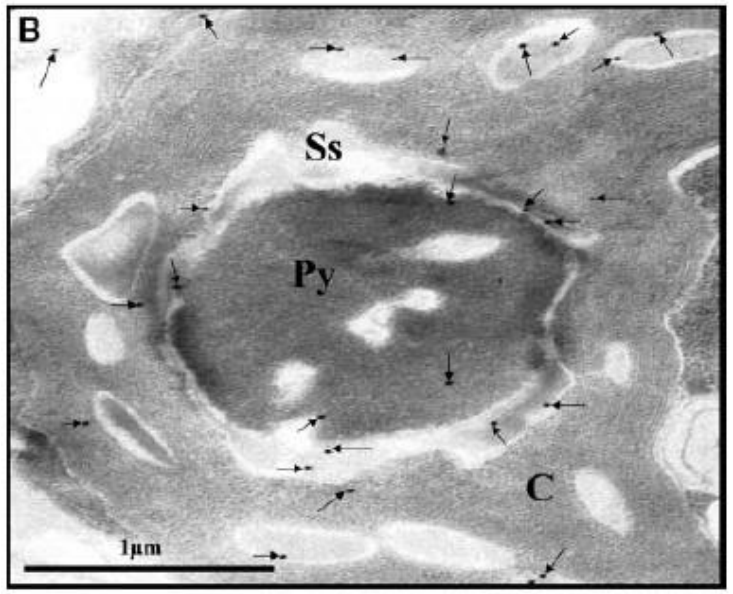
Marianne Tardif,[1,2,3] Ariane Atteia,[†,1,3,4,5] Michael Specht,[6] Guillaume Cogne,[7,8] Norbert Rolland,[1,3,4,5] Sabine Brugière,[1,2,3] Michael Hippler,[6] Myriam Ferro,[1,2,3] Christophe Bruley,[1,2,3] Gilles Peltier,[9,10,11] Olivier Vallon,[12,13] and Laurent Cournac*[,‡,9,10,11]

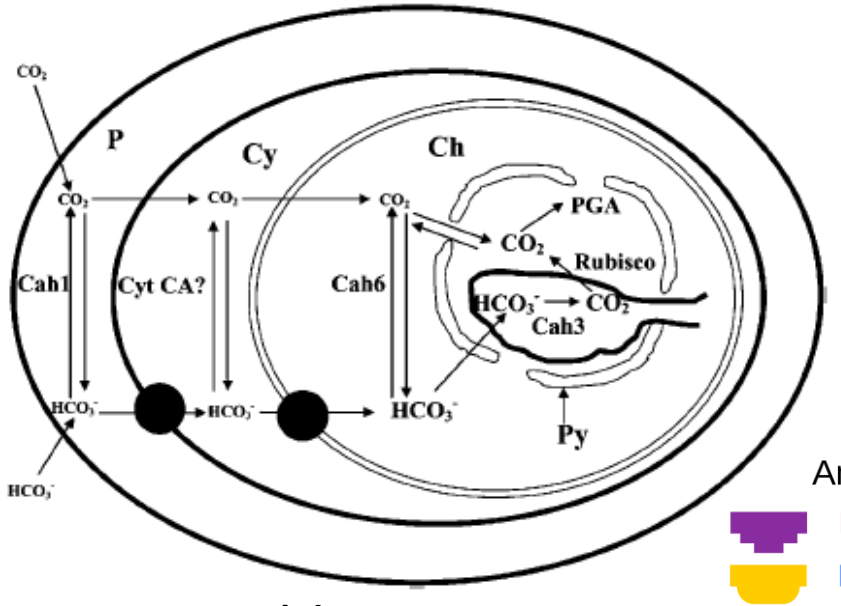


Identification of a New Chloroplast Carbonic Anhydrase in *Chlamydomonas reinhardtii*[1]

Mautusi Mitra, Scott M. Lato, Ruby A. Ynalvez, Ying Xiao, and James V. Moroney*
Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803

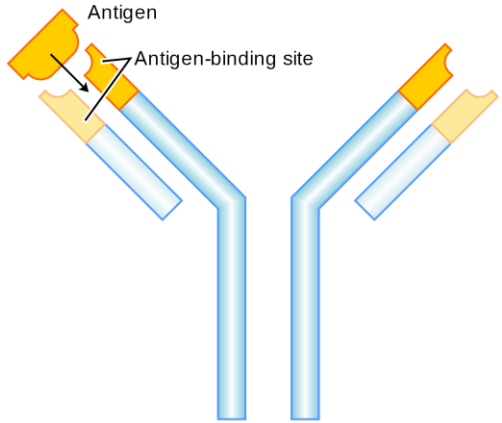






Immunogold gave CAH6 in the stroma, but proteomics in the flagella

Why?



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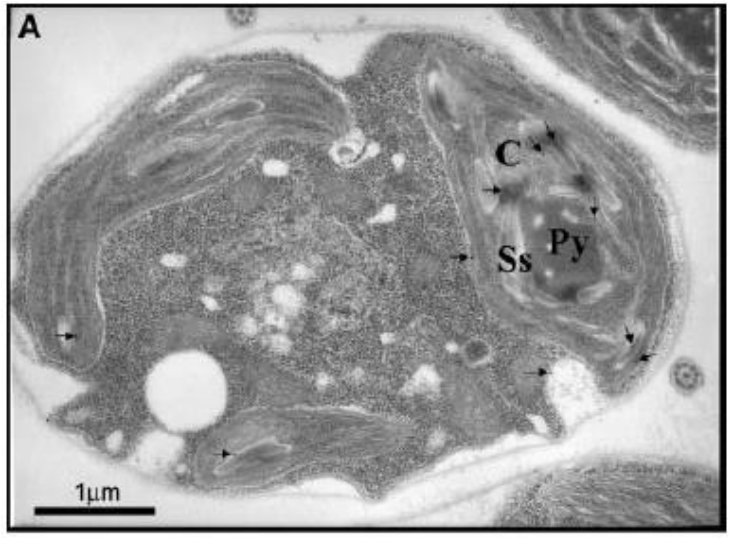
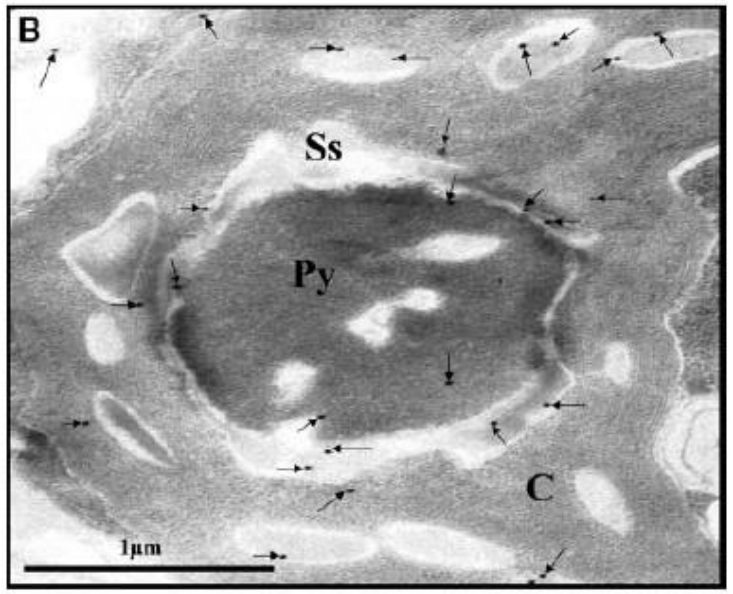
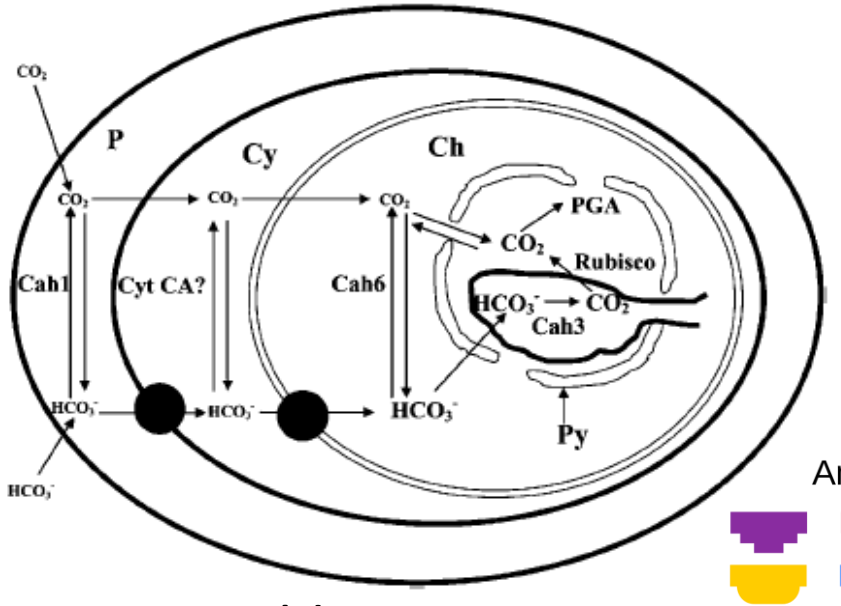
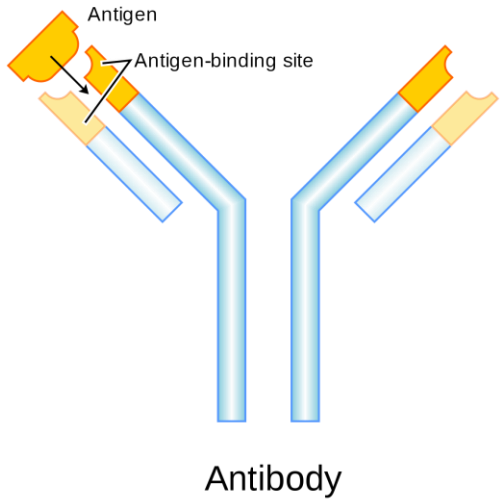






Immunogold gave CAH6 in the stroma, but proteomics in the flagella

Watch out of the antibody you use!



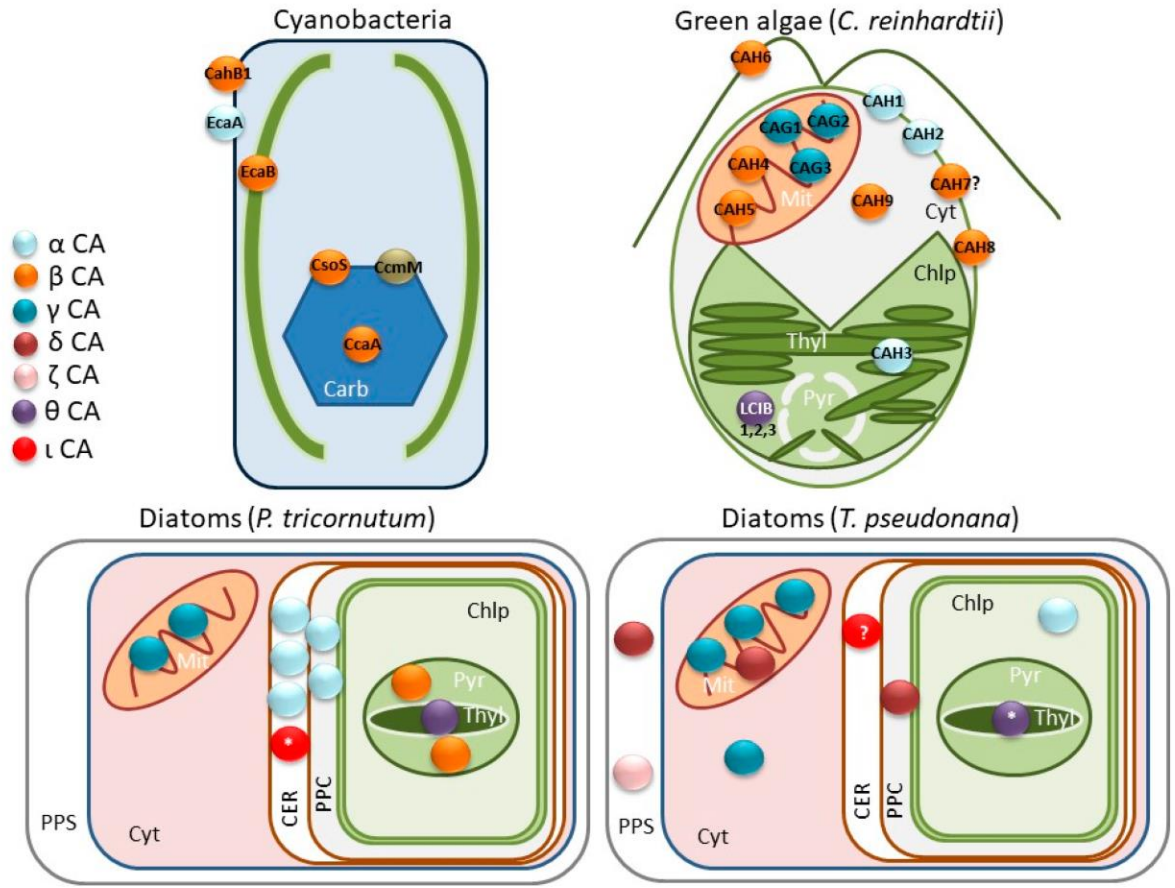
International Journal of
Molecular Sciences

MDPI

Review

Insights on the Functions and Ecophysiological Relevance of the Diverse Carbonic Anhydrases in Microalgae

Erik L. Jensen [1,†], Stephen C. Maberly [2] and Brigitte Gontero [1,*]

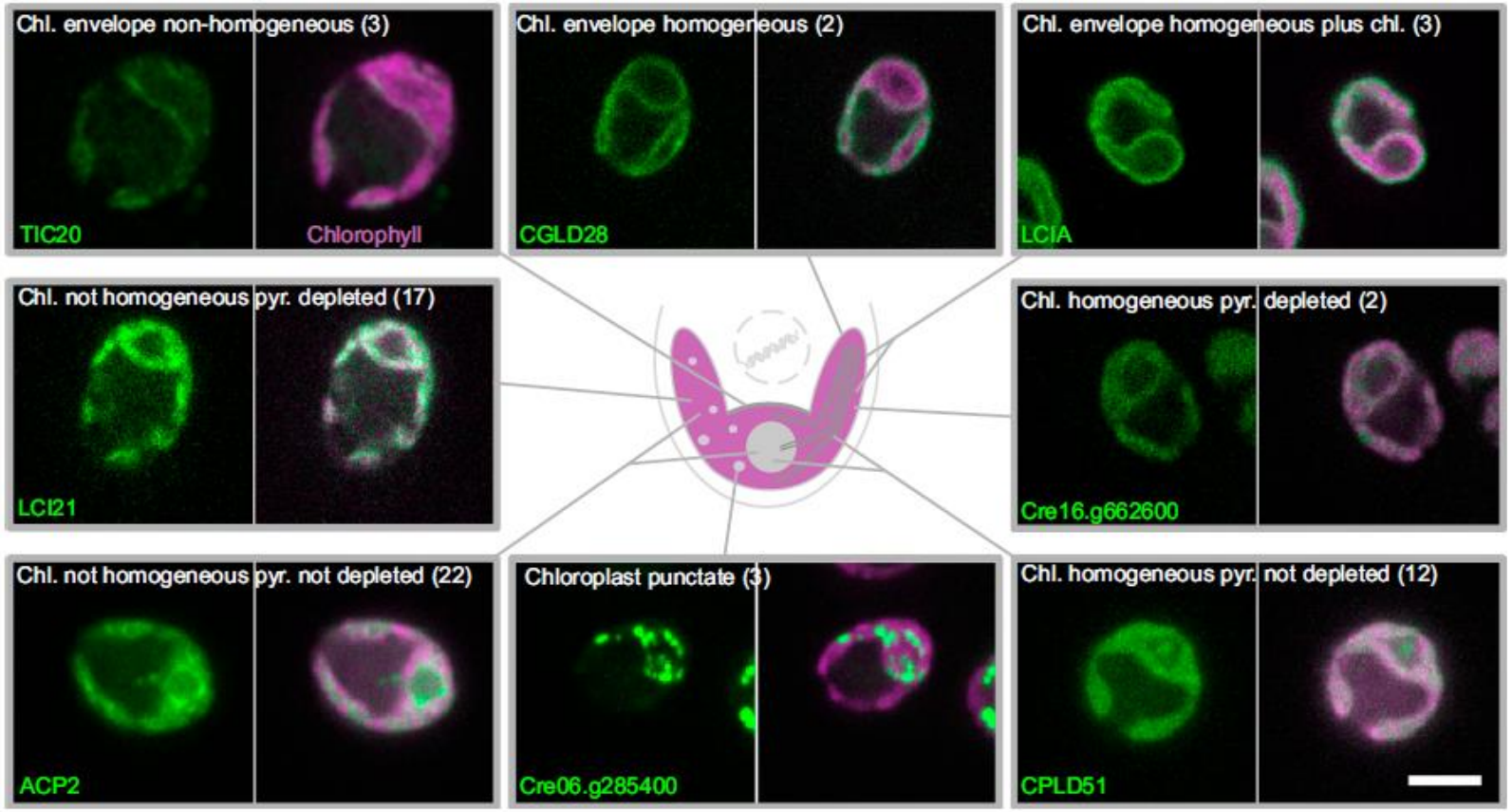


What is CAH6 doing in flagella?

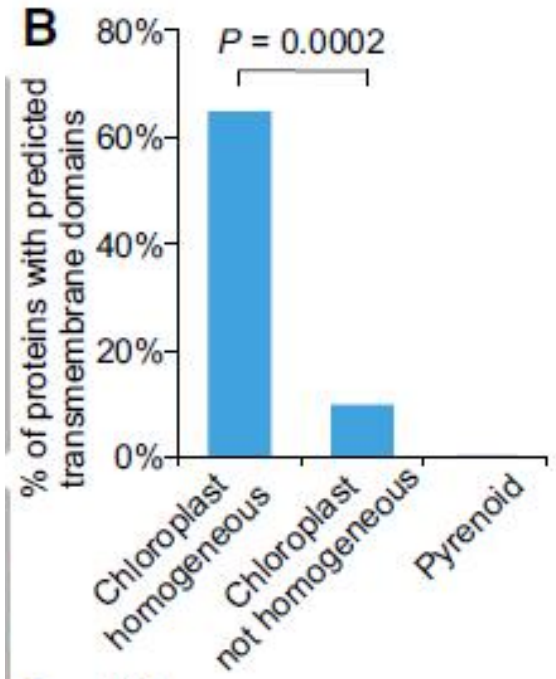
Instead of directly participating in the CCM, **CAH6 could be involved in inorganic carbon sensing**. Chlamydomonas was recently shown to **chemotax toward HCO3-** (Choi et al., 2016), and carbonic anhydrases have been previously implicated in inorganic carbon sensing (Hu et al., 2010).

Localization of sensing machinery to the flagella, which are found at the leading edge of swimming cells, could facilitate chemotaxis.

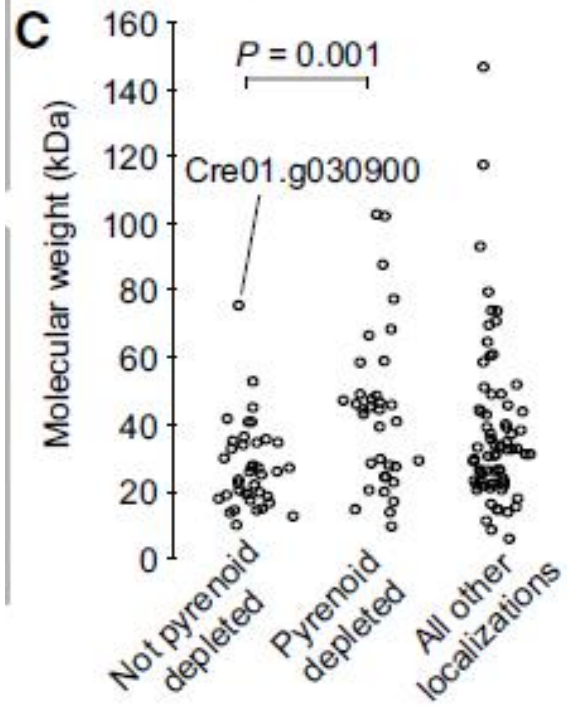
Focus on the chloroplast



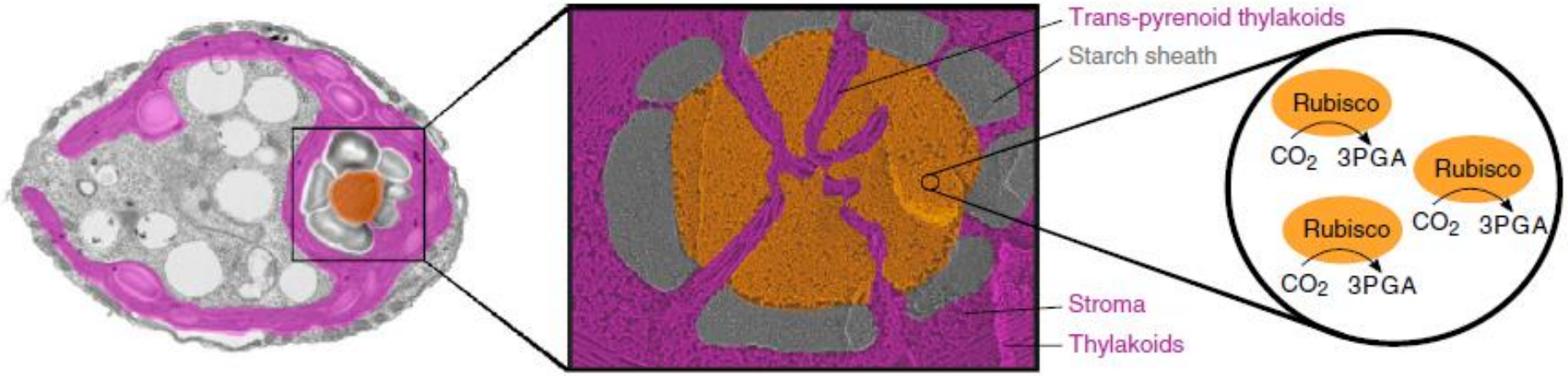
Approximately 56% (82/146) of the proteins localized to the chloroplast. They assigned these 82 proteins to 13 sub-chloroplast locations. Chloroplast envelope proteins showed three subcategories of localization: (1) envelope homogeneous, (2) envelope non-homogeneous, and (3) envelope plus chloroplast homogeneous



homogeneous chloroplast localization is a trait enriched in proteins containing transmembrane domains.
This observation suggests that **proteins with homogeneous localization are most likely thylakoid membrane-associated**.

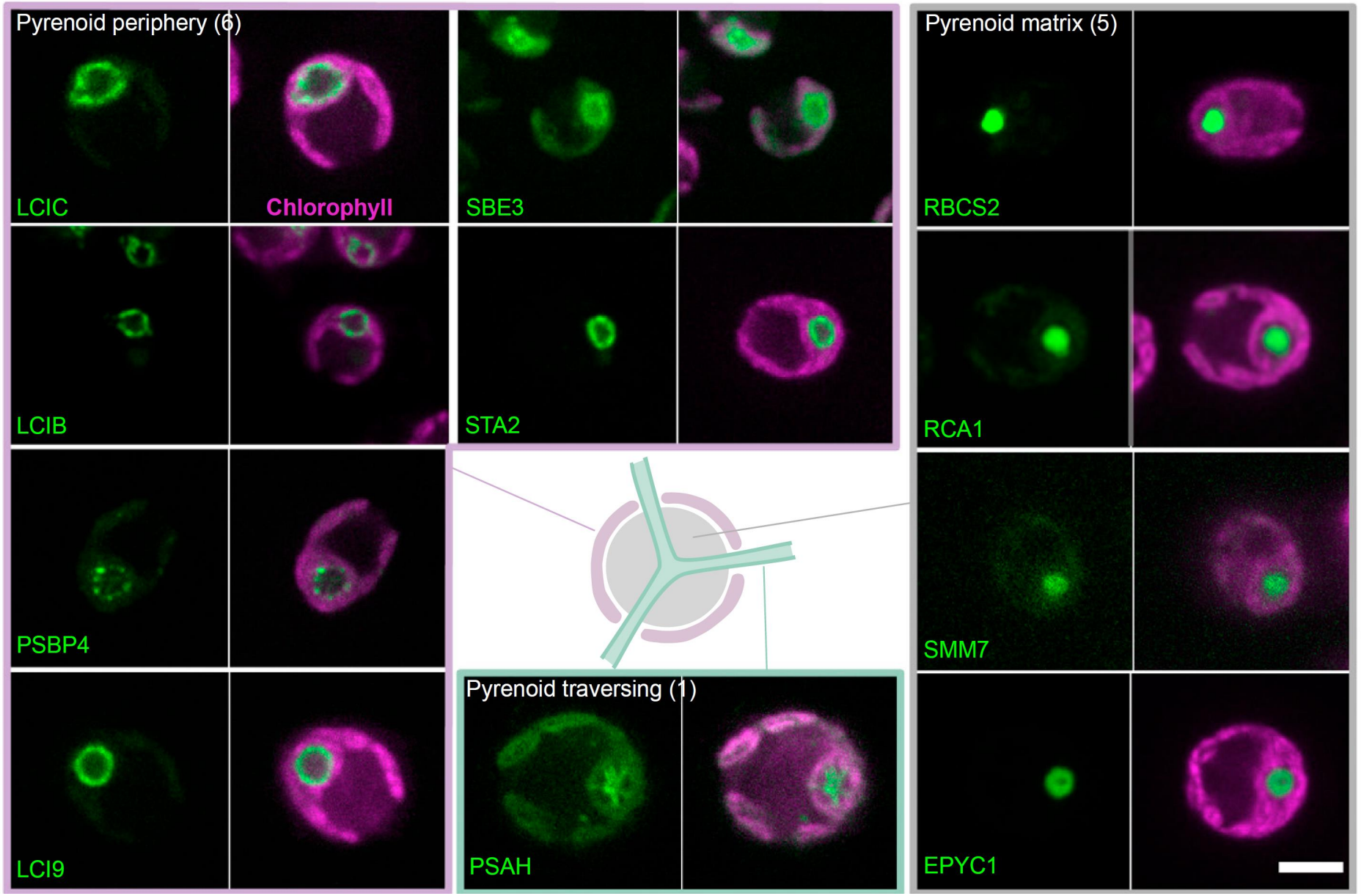


The 39 proteins that are not pyrenoid-depleted are almost all smaller than ~50 kDa (the value of ~ 50 kDa excludes the Venus YFP region, therefore the effective molecular weight is ~ 78 kDa), suggesting that the **pyrenoid may exclude larger proteins**.

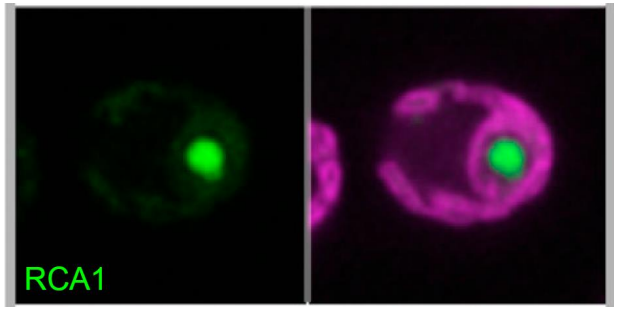


Electron microscopy-based techniques have shown that the Chlamydomonas pyrenoid contains a dense matrix of Rubisco surrounded by a starch sheath and traversed by membrane tubules formed from merged thylakoids

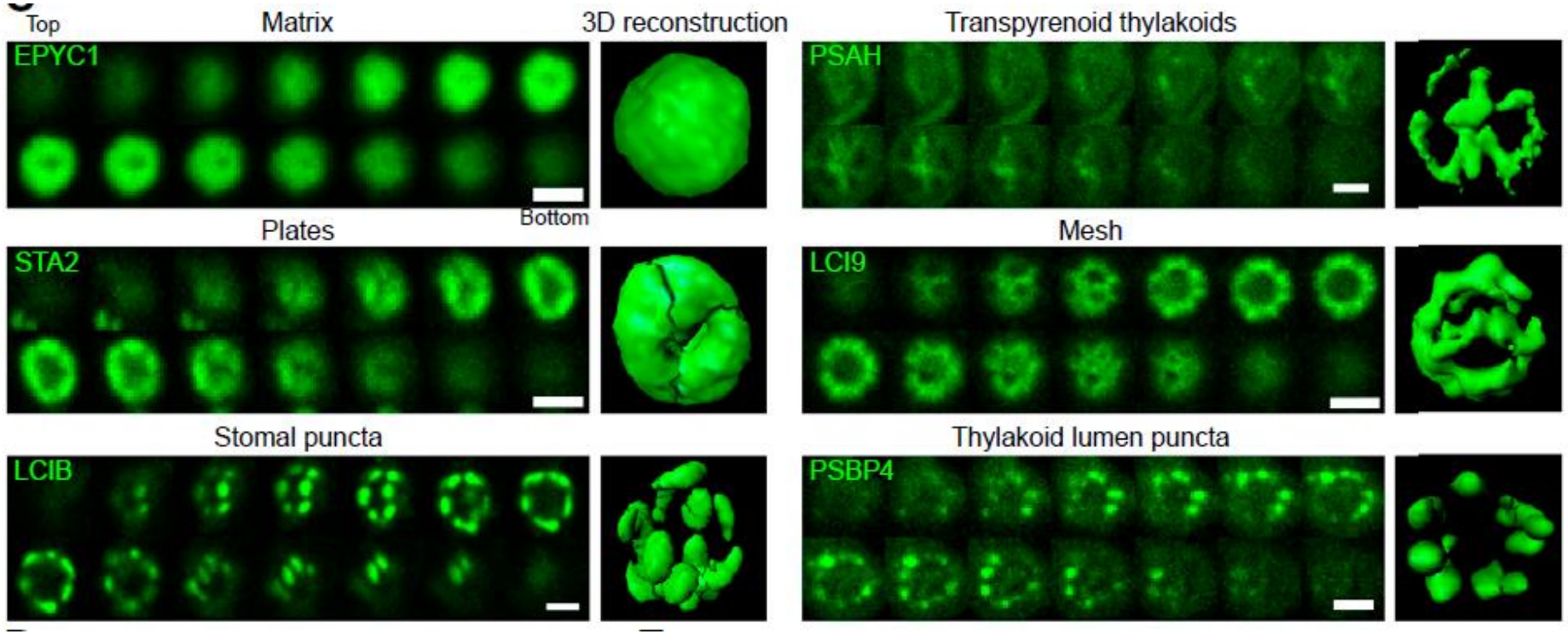
Specific localization inside pyrenoids



Specific localization inside pyrenoids



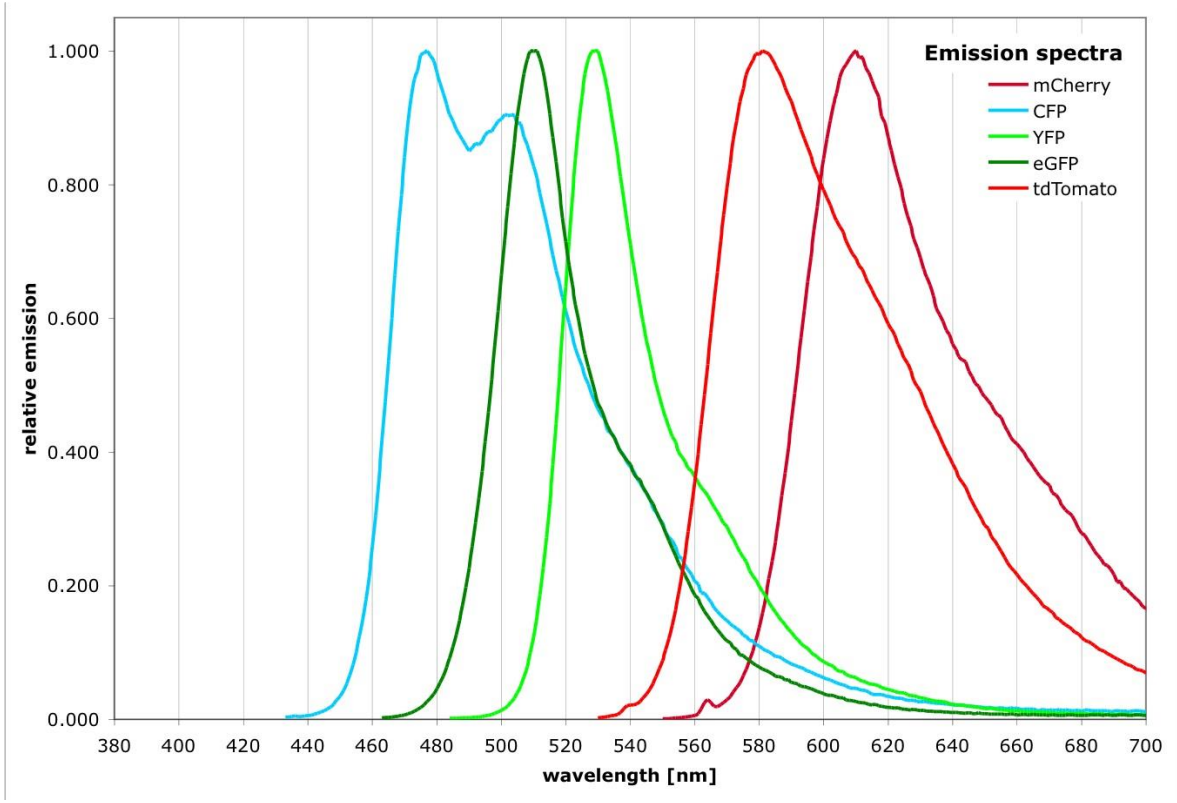
chaperone Rubisco activase (RCA1) is expected in the matrix of pyrenoids



Randon insertion of one set cloned in frame with **YFP** and associated with paramomycin-resistance

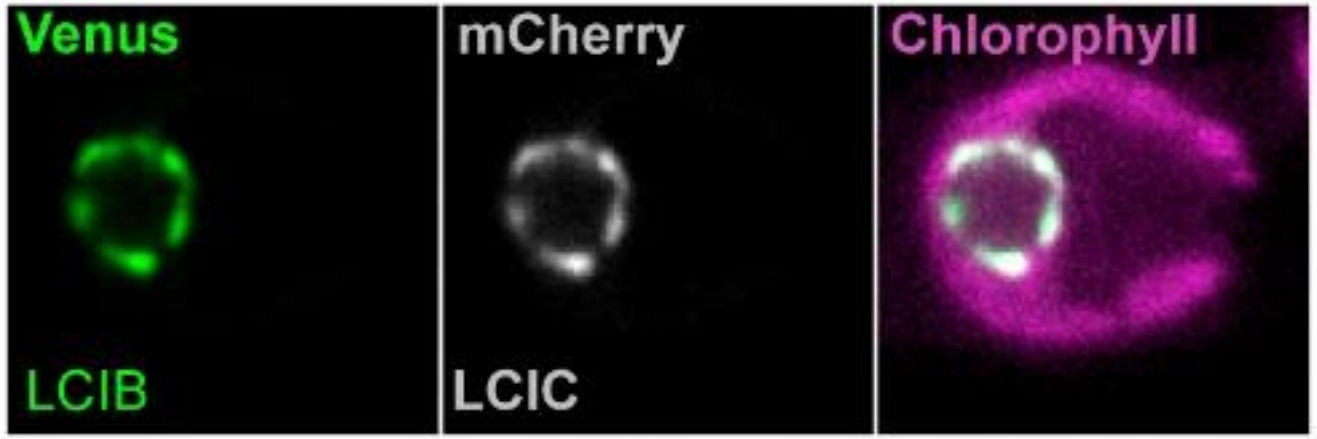
Randon insertion of one set cloned in frame with **mCherry** and associated to hygromicin-resistance

Emission spectra:

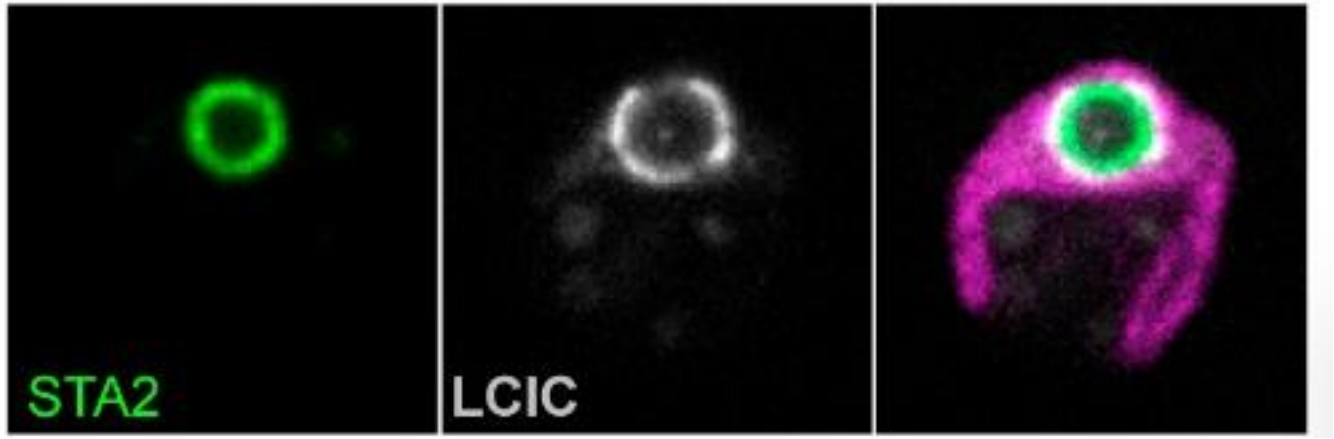


colocalization

The same

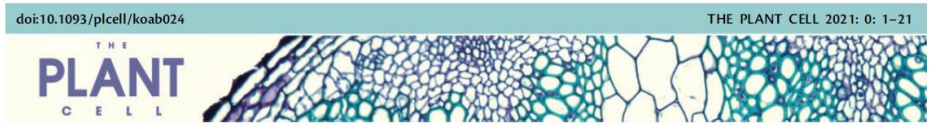


Different



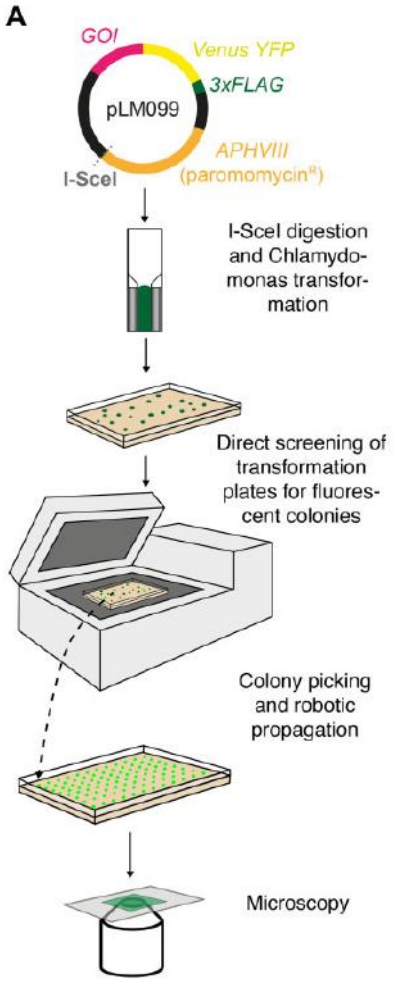
The Pyrenoid Has at Least Four Distinct Outer Protein Layers

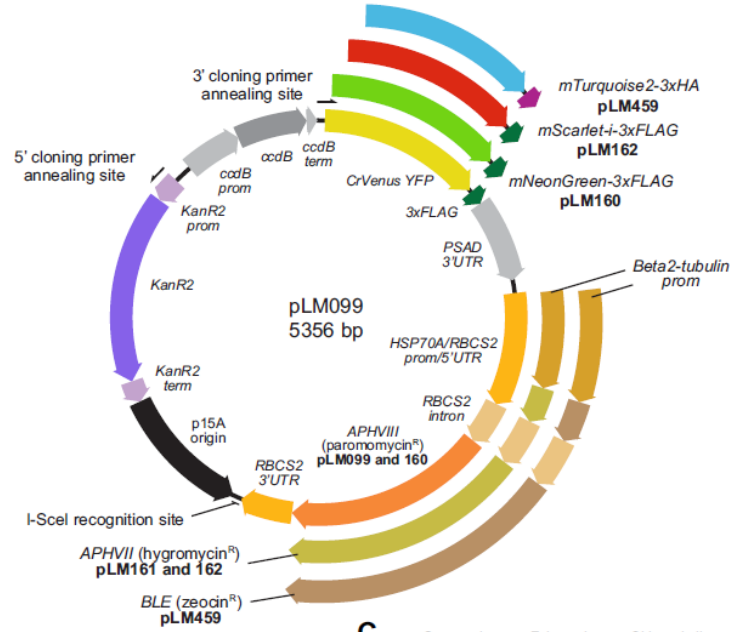
doi:10.1093/plcell/koab024 THE PLANT CELL 2021: 0: 1–21

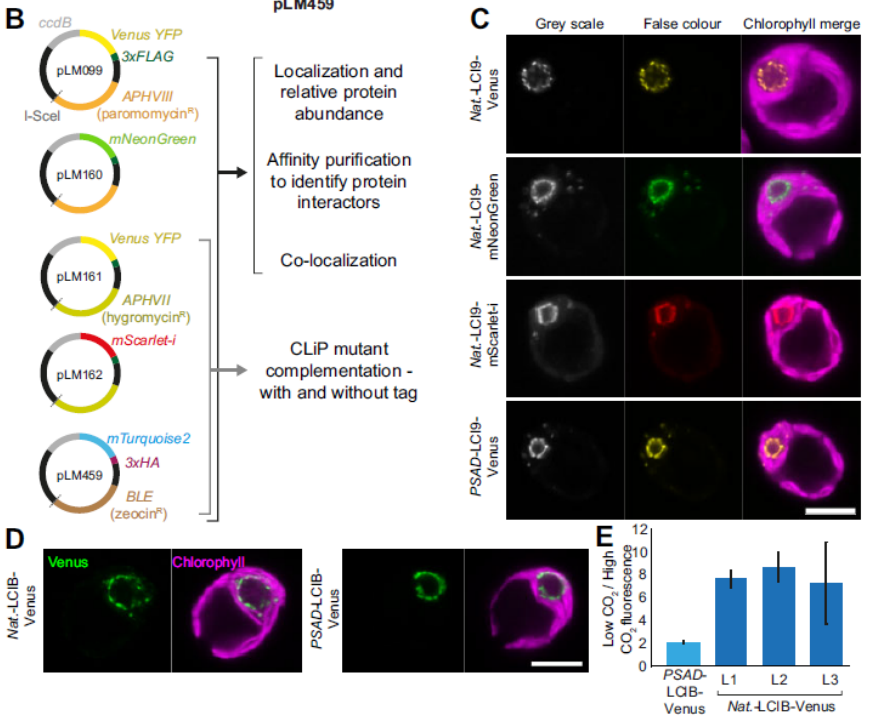


A recombineering pipeline to clone large and complex genes in Chlamydomonas

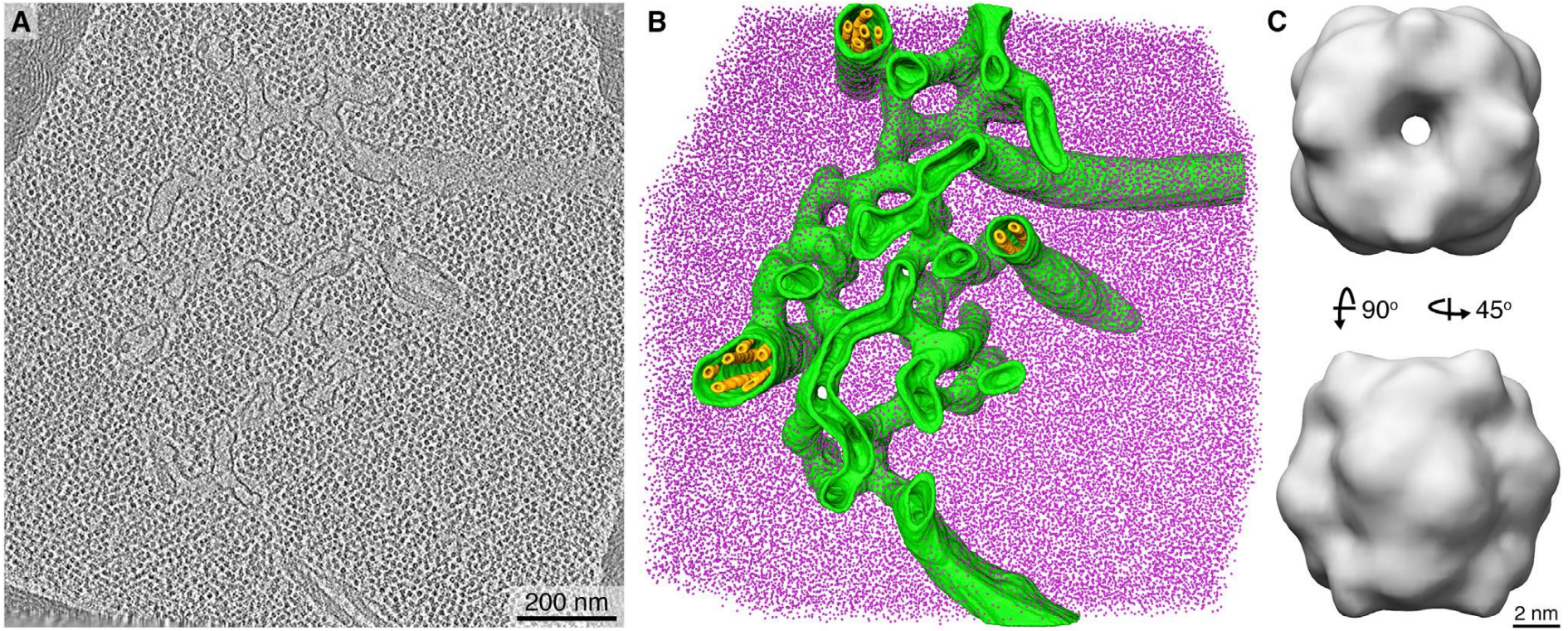
- Endogenous promoters



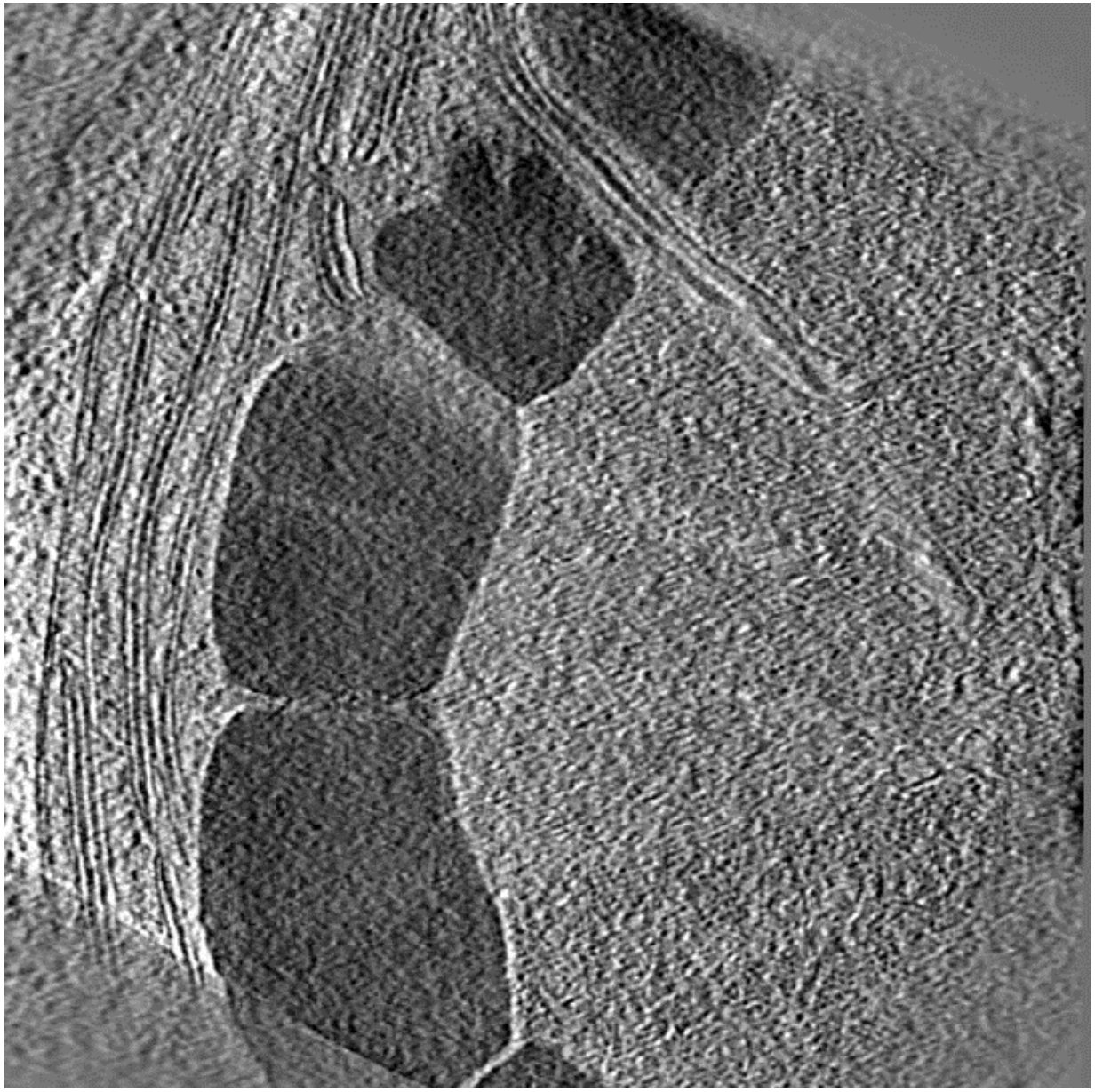




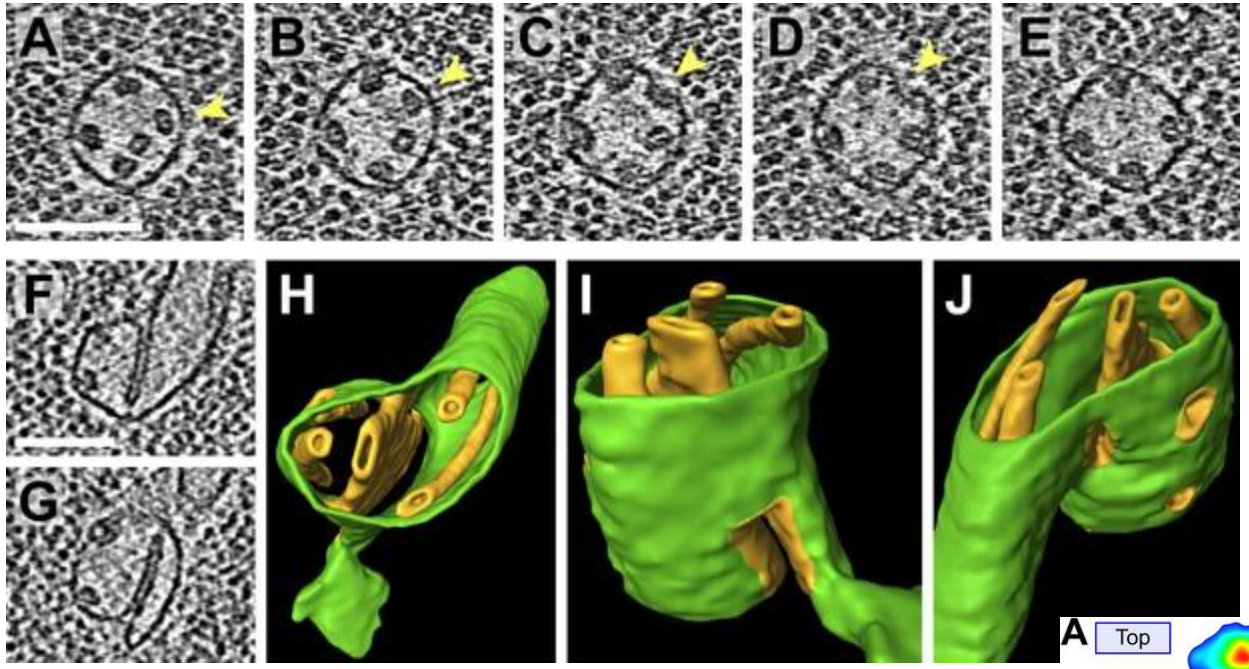
Analysis of the position of individual Rubisco holoenzymes within the Chlamydomonas pyrenoid matrix by *in situ* cryo-electro tomography

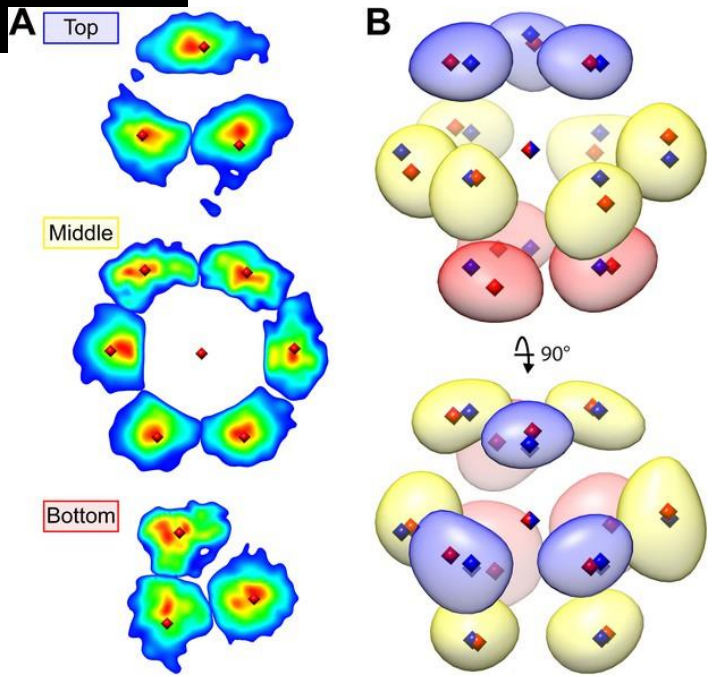


(A) Slice through a tomographic volume of the native Chlamydomonas pyrenoid. (B) Segmentation of the tomogram shown in (A) with localized positions of 46,567 Rubisco holoenzymes (magenta) mapped into the volume. **Green and yellow: pyrenoid tubule membranes**. (C) In situ subtomogram average of Rubisco (16.5 A ° resolution) generated from 30,000 particles extracted from the tomogram shown in (A).

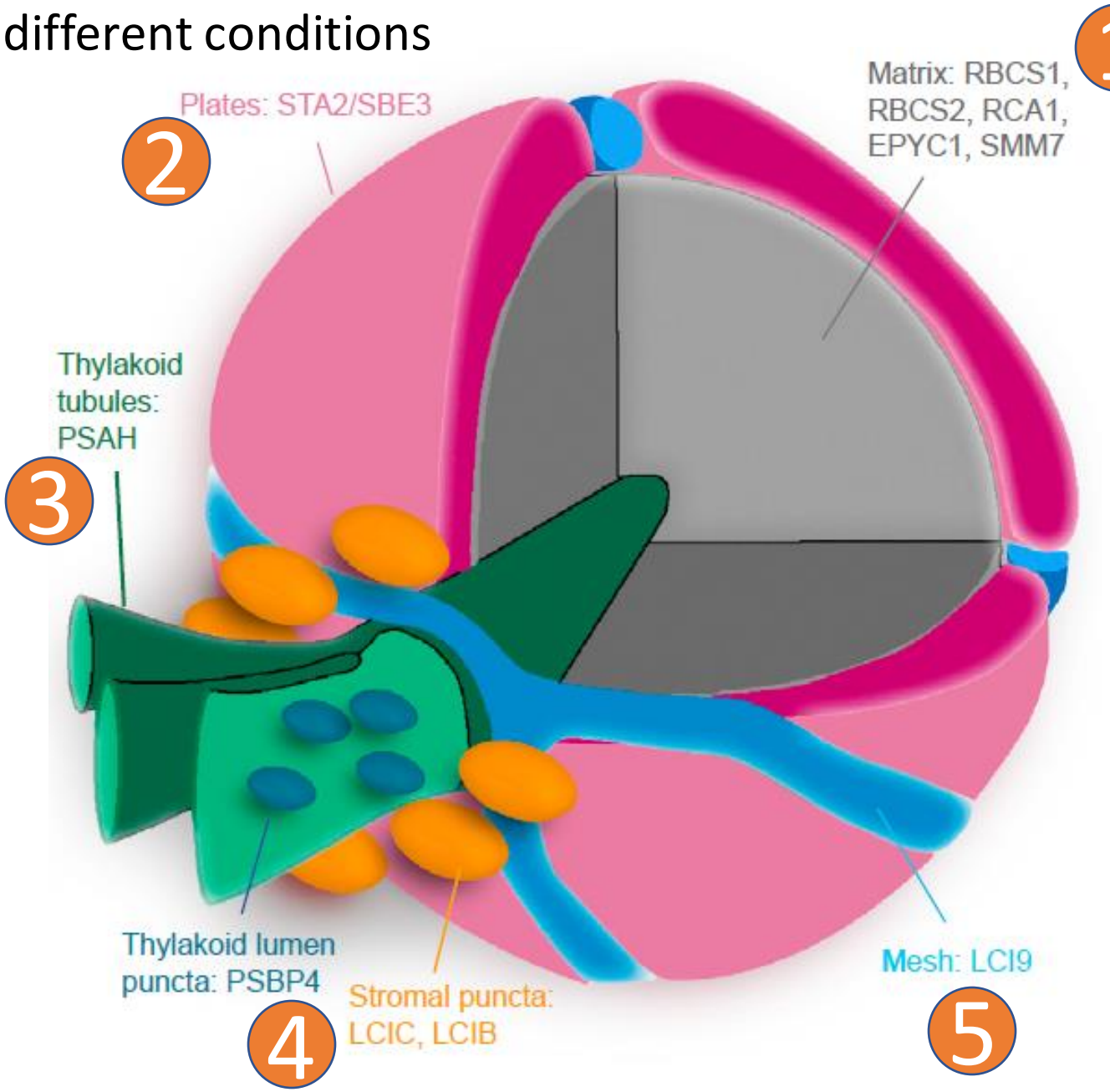


Cite this articleas: eLife 2015;4:e04889 DOI: 10.7554/ELIFE.04889


A
B
C
D
E
F
G
H
I
J

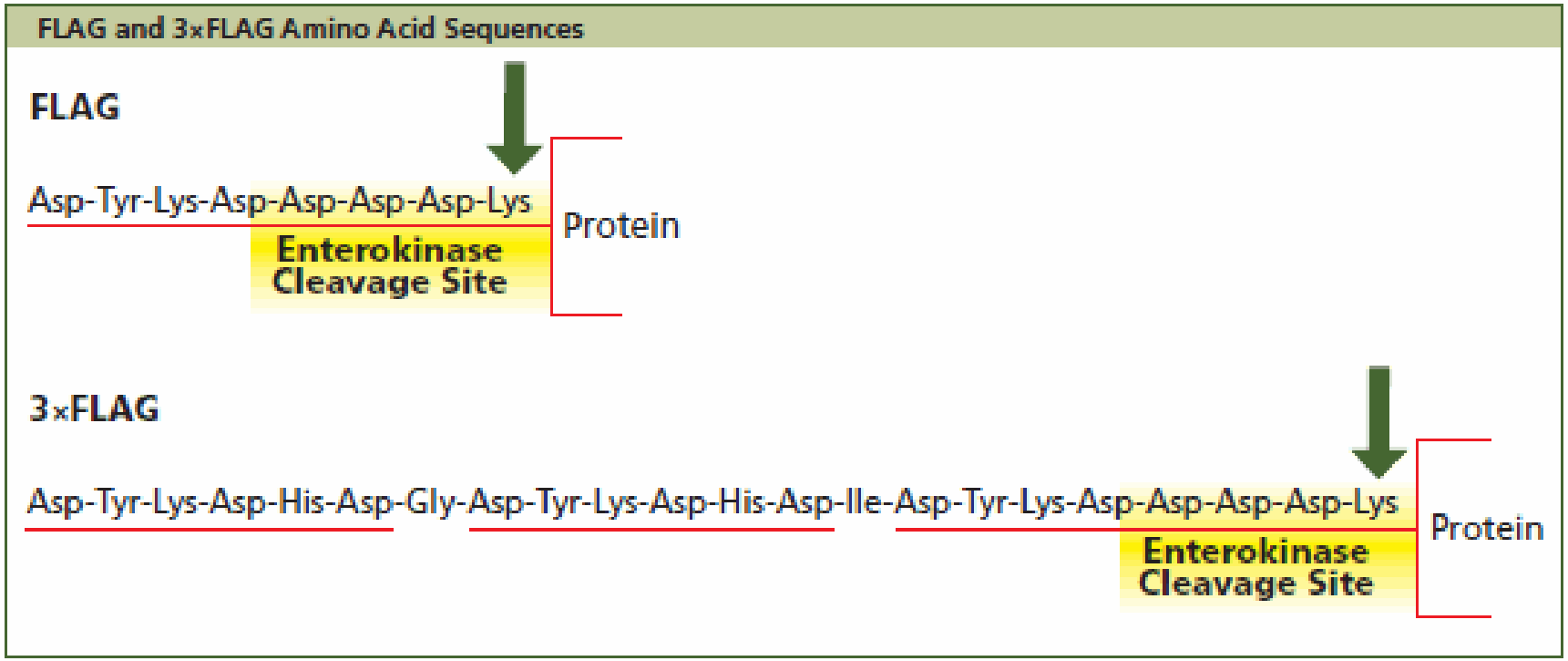

A
Top
Middle
Bottom
B
90°

New model for pyrenoid structure, biogenesis and regulation in different conditions



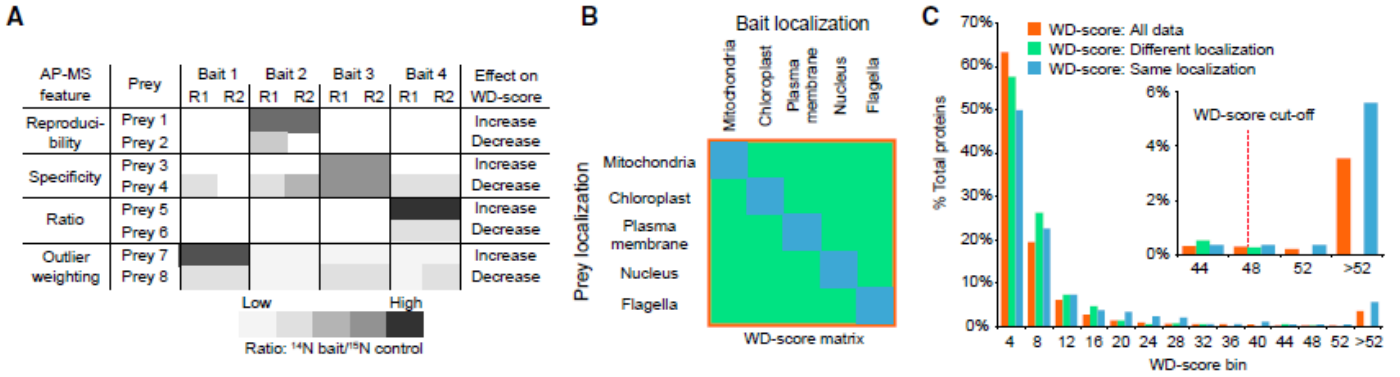
A Putative Methyltransferase Localizes to the Pyrenoid Matrix, role in biogenesis

FLAG


FLAG and 3×FLAG Amino Acid Sequences
FLAG
Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys
Enterokinase Cleavage Site
Protein
3×FLAG
Asp-Tyr-Lys-Asp-His-Asp-Gly-Asp-Tyr-Lys-Asp-His-Asp-Ile-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys
Enterokinase Cleavage Site
Protein

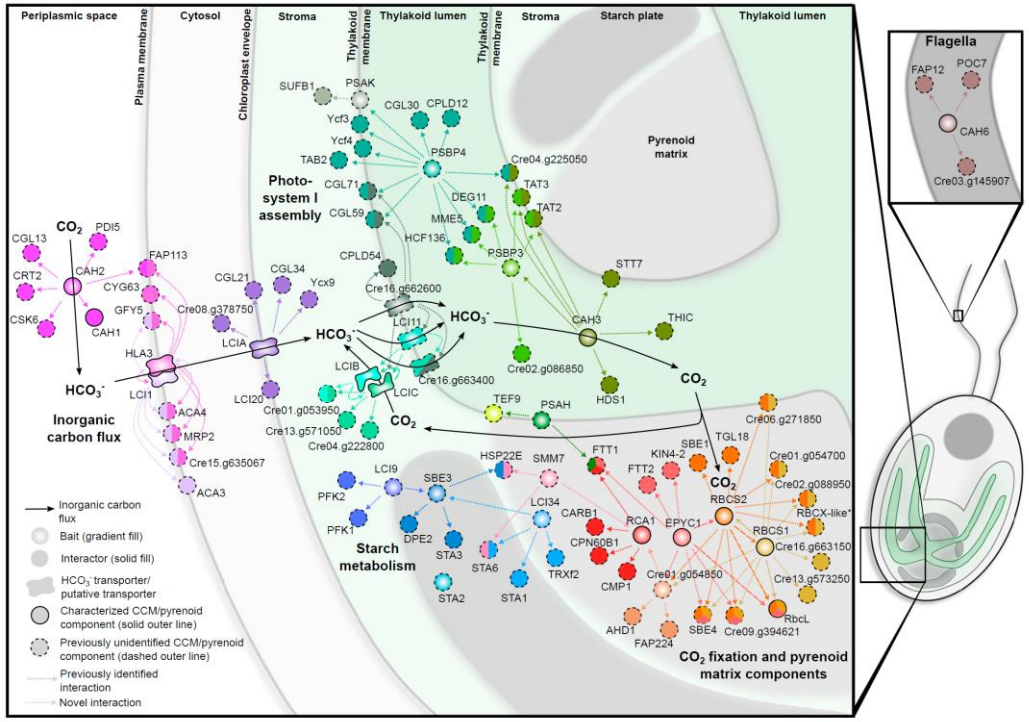
Large-scale affinity purification mass spectrometry (AP-MS) approach

- 38 candidates
- 2 different labeling protocols
- Co-localization criteria



Interesting localization of proteins of photosystem I, photosystem II, CAS and STT7 that are proteins involved in the regulation of photosynthesis activity

Large-scale affinity purification mass spectrometry (AP-MS) approach



Pyrenoid core
*Cre16.g655050|RBCX-like, Cre06.g273050|CGLD10, Cre09.g386200|OPR36, + 5 more HCIPs
Cre10.g429100, Cre03.g213313
Cre10.g452250
Cre09.g416850
Cre03.g156750|CCT5, Cre03.g168450|CCT8, Cre05.g232750|CNK4, + 16 more HCIPs

Thylakoids
Cre11.g467778, Cre16.g692901
Cre06.g266900, Cre02.g142146
Cre16.g684750
Cre01.g014000
Cre12.g524500|RMT2, Cre07.g328200|PSBP6, Cre11.g475850|ZNJ1, + 6 more HCIPs
Cre17.g729800|ALB3.2, Cre01.g056696, Cre03.g176833
Cre07.g339750|FeC, Cre19.g750547|NDA2, Cre02.g111450|TEF4, + 8 more HCIPs

Chloroplast envelope
Cre05.g230850, 28269781|Ycf78, Cre13.g587100|PCYA1, + 34 more HCIPs

Plasma membrane
Cre08.g360600|ERM4, Cre14.g613950|ABC2, Cre16.g657350, Cre17.g700750|GFY3, + 25 more HCIPs

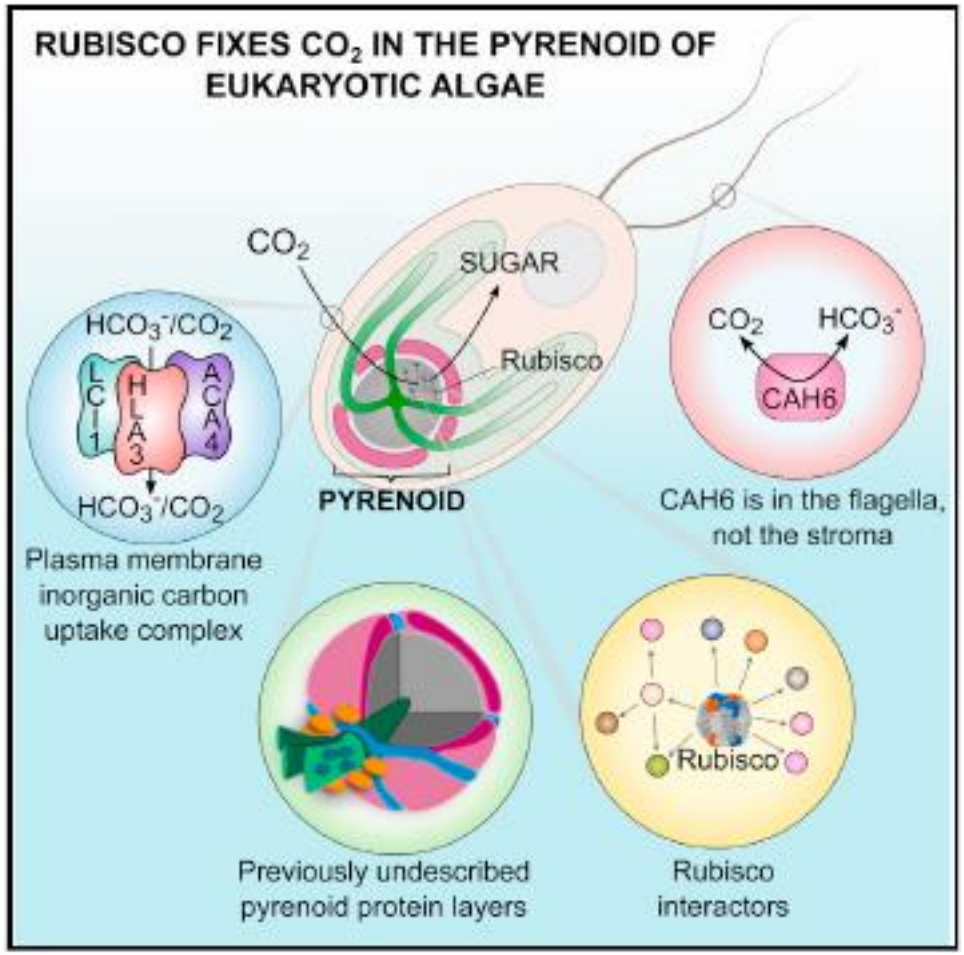
Extracellular
Cre03.g144564|MMP13, Cre02.g093750|NRX2, Cre09.g393700|MMP3, + 21 more HCIPs

Important to look closer in this interactome.

For example a missing step was the transfer of inorganic carbon from the external environment to the pyrenoid.

They identified 3 proteins that might be involved in bicarbonate transport.

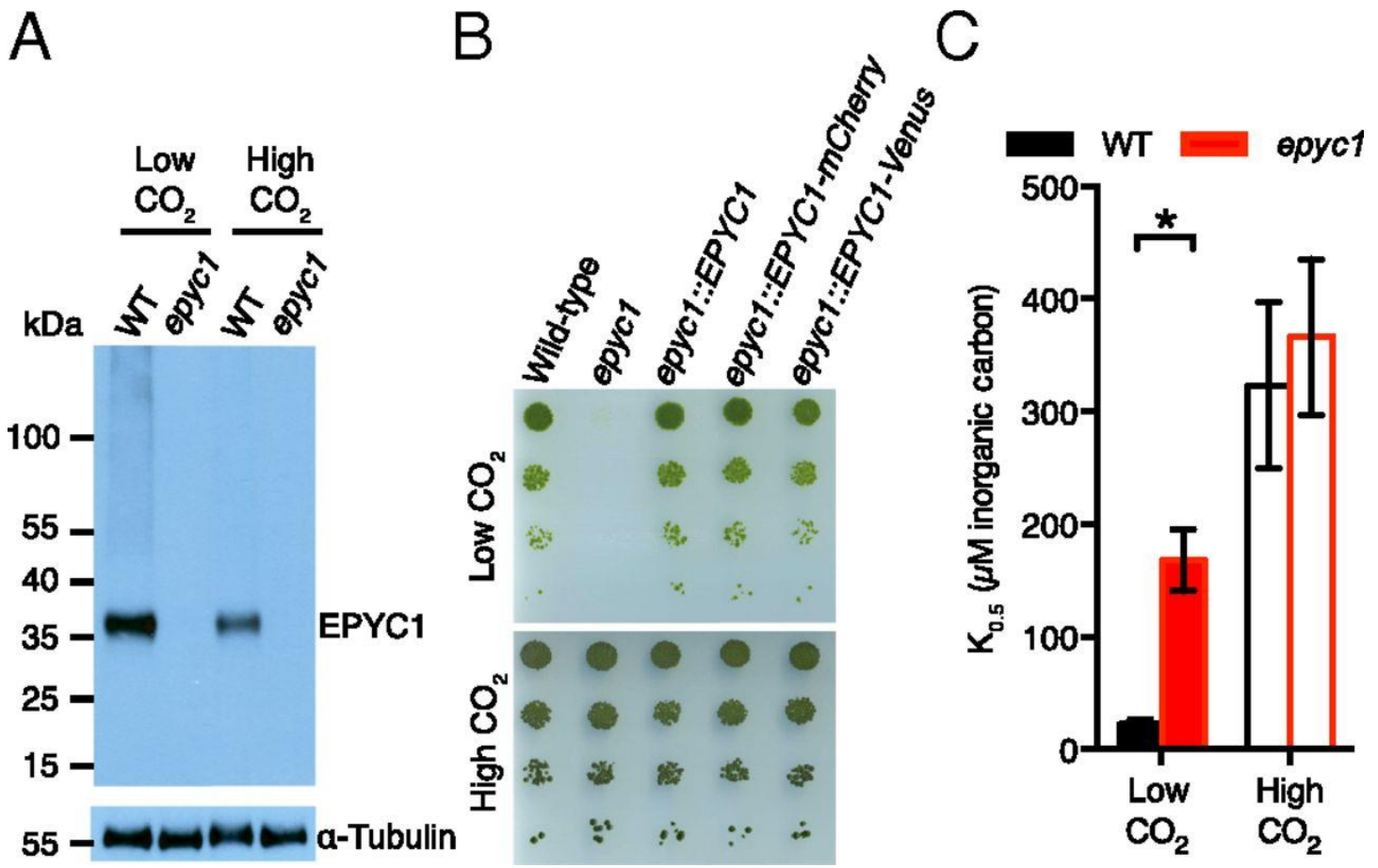
The cellular organelle in algae responsible for one-third of global CO2 fixation



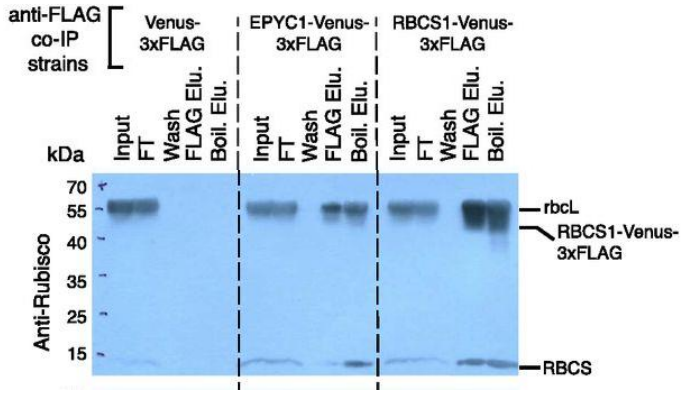
- Localizations and physical interactions of candidate CO2-concentrating mechanism (CCM) proteins were determined
- The data reveal three previously undescribed pyrenoid layers and 89 pyrenoid proteins
- Plasma membrane inorganic carbon transporters LCI1 and HLA3 form a complex
- Carbonic anhydrase 6 localizes to the flagella, changing the model of the CCM

One potential approach for improving yields is the transfer of a CCM into higher plants to increase CO2-fixation rates

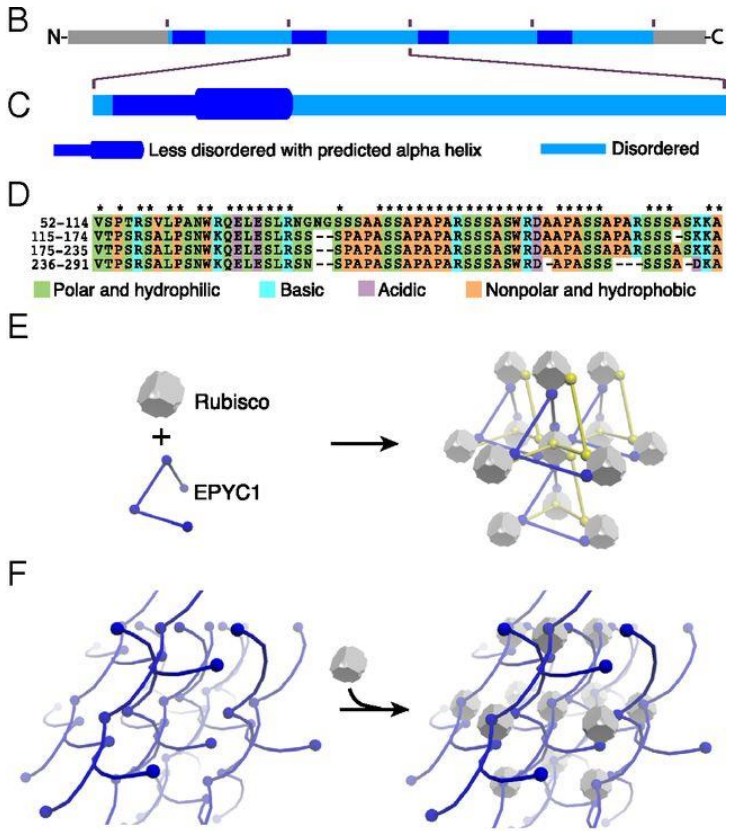
EPYC1, a novel protein correlated with RUBISCO activity

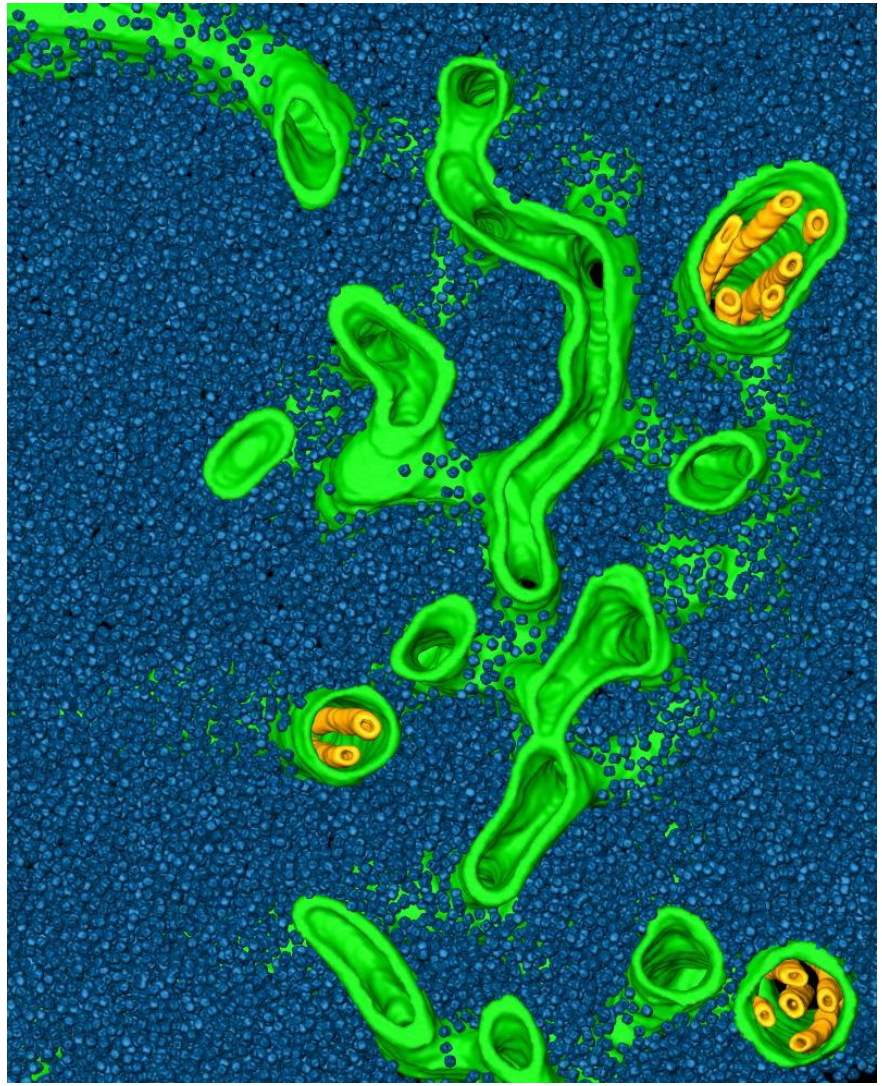


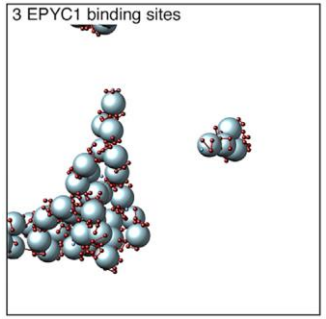
Co-immunopurification

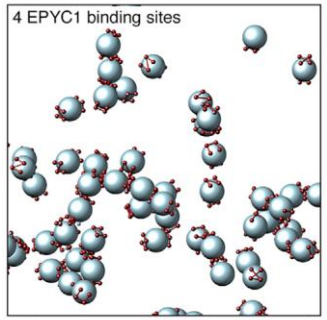


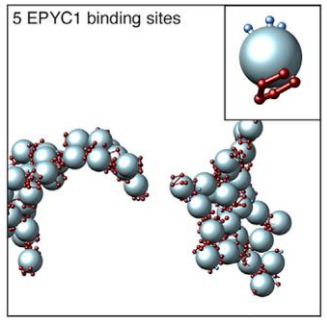
Mathematical models






3 EPYC1 binding sites


4 EPYC1 binding sites


5 EPYC1 binding sites

Cell

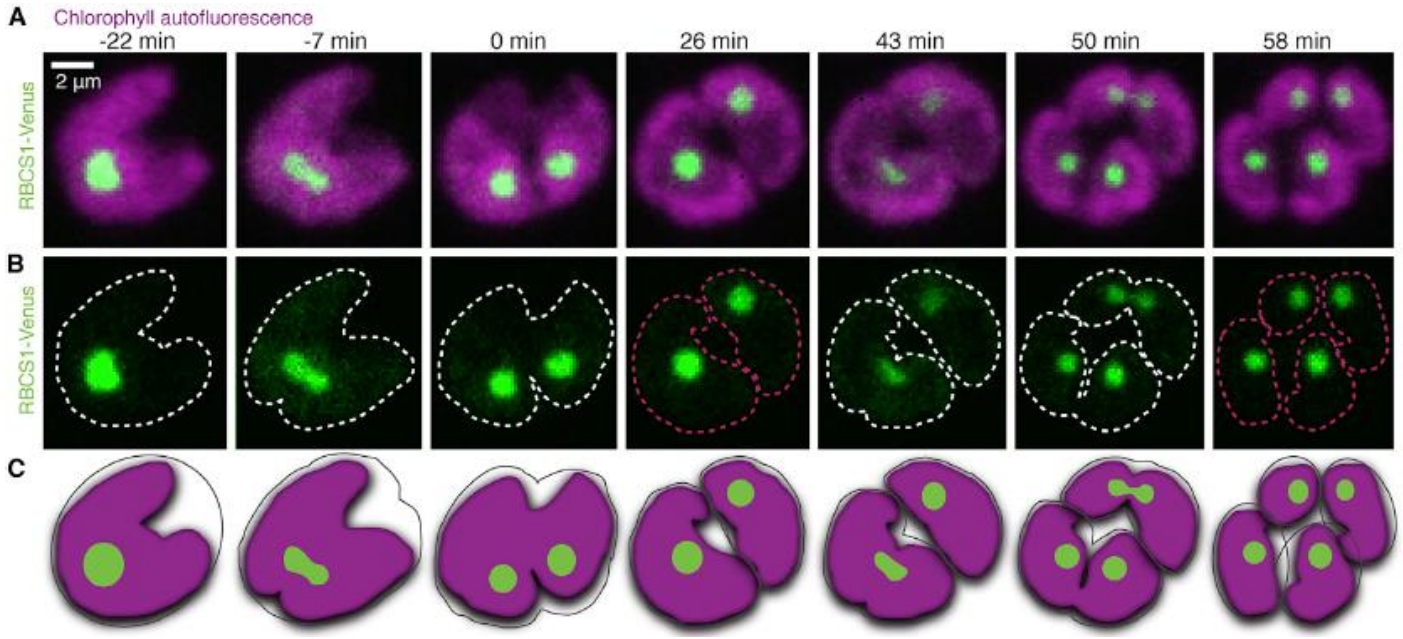
Article

The Eukaryotic CO_2-Concentrating Organelle Is Liquid-like and Exhibits Dynamic Reorganization

Elizabeth S. Freeman Rosenzweig,[1,2] Bin Xu,[3,11] Luis Kuhn Cuellar,[4,11] Antonio Martinez-Sanchez,[4] Miroslava Schaffer,[4] Mike Strauss,[5] Heather N. Cartwright,[2] Pierre Ronceray,[6] Jürgen M. Plitzko,[4] Friedrich Förster,[4,9] Ned S. Wingreen,[7,8,*] Benjamin D. Engel,[4,*] Luke C.M. Mackinder,[2,10,12] and Martin C. Jonikas[1,2,8,11,12,13,*]

The Pyrenoid Exhibits Both Fission and *De Novo* Assembly

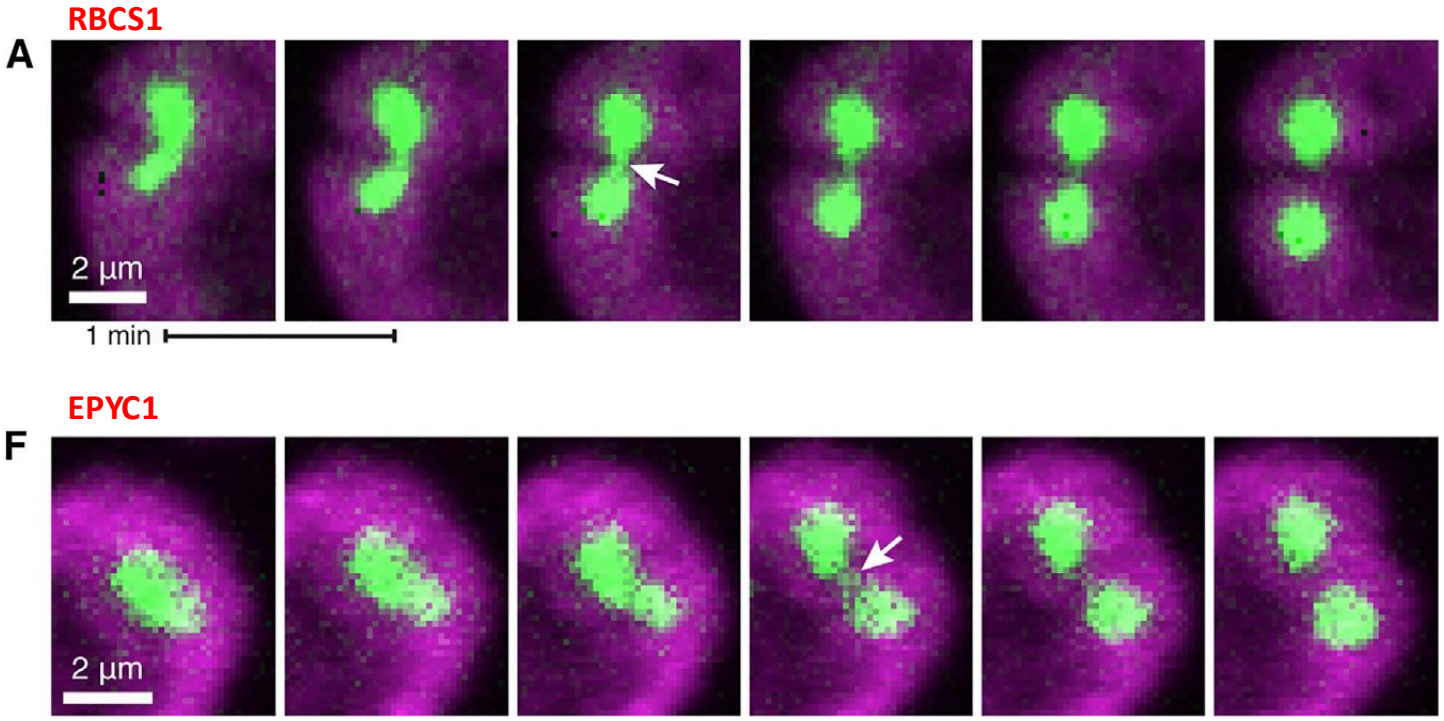
To enable the first observations of pyrenoid matrix dynamics in living cells, we expressed pyrenoid **matrix proteins tagged with the fluorescent protein Venus** and **imaged them in 3D with fluorescence time-lapse microscopy** during photoautotrophic growth.



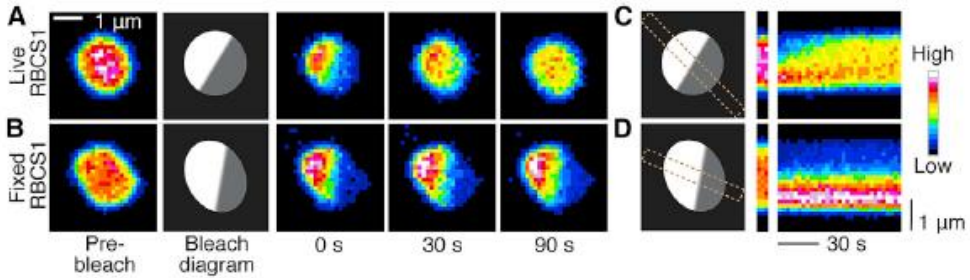
tracked inheritance of the pyrenoid matrix by monitoring Venus-tagged Rubisco small subunit 1 (**RBCS1**) or Venus-tagged **EPYC1** and recorded chlorophyll autofluorescence to follow cellular orientation and chloroplast division

Elongation and then fission

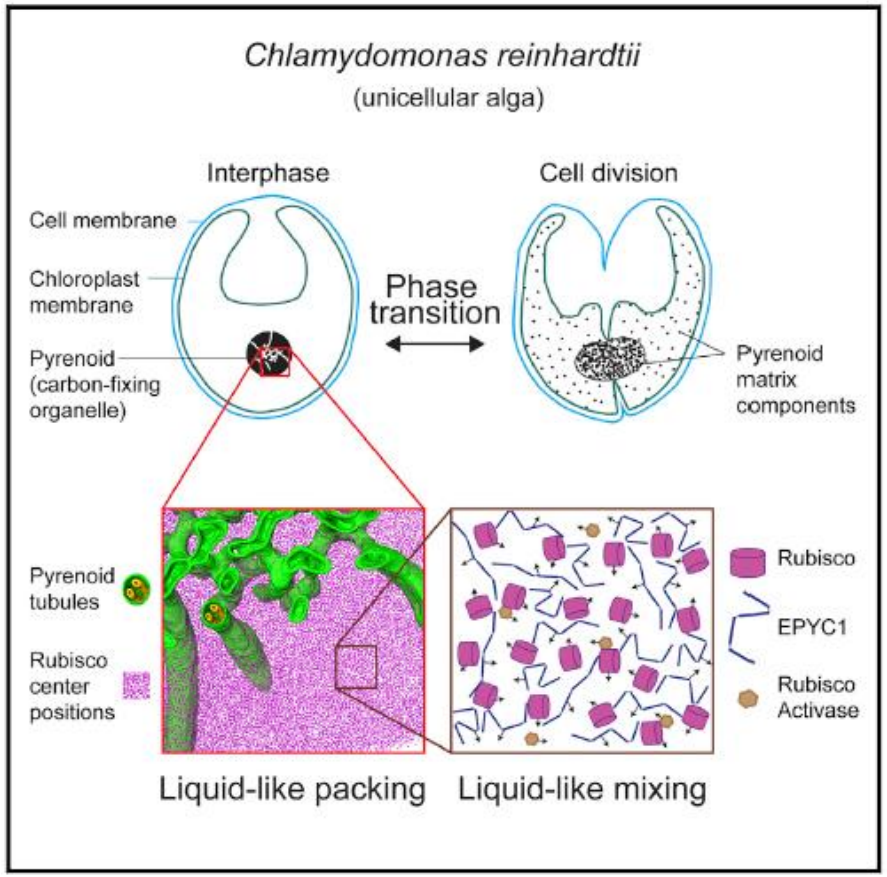
Pyrenoid recall liquid materials with a bridge between two daughter pyrenoids



Need for better esolution…

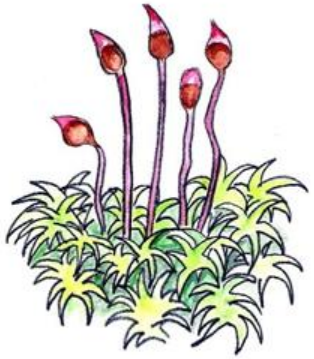


The pyrenoid, a Rubisco-containing organelle that enhances carbon fixation, mixes internally and undergoes phase transitions.

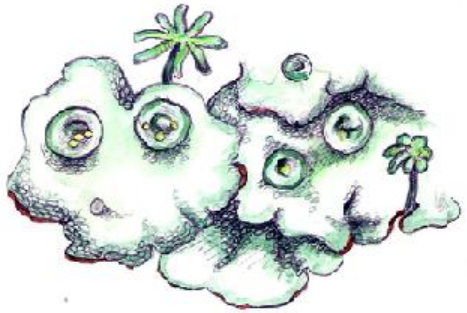


- The pyrenoid undergoes a reversible phase transition during cell division
- Modeling reveals a ‘‘magic number’’ effect that governs phase transitions

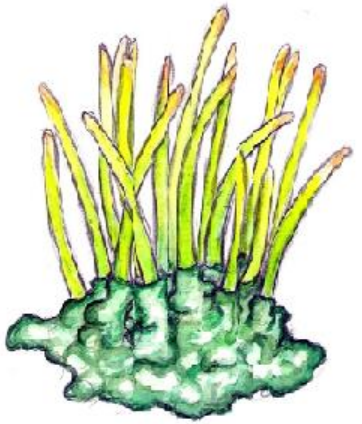
Bryophytes



Moss



Liverwort



Hornwort

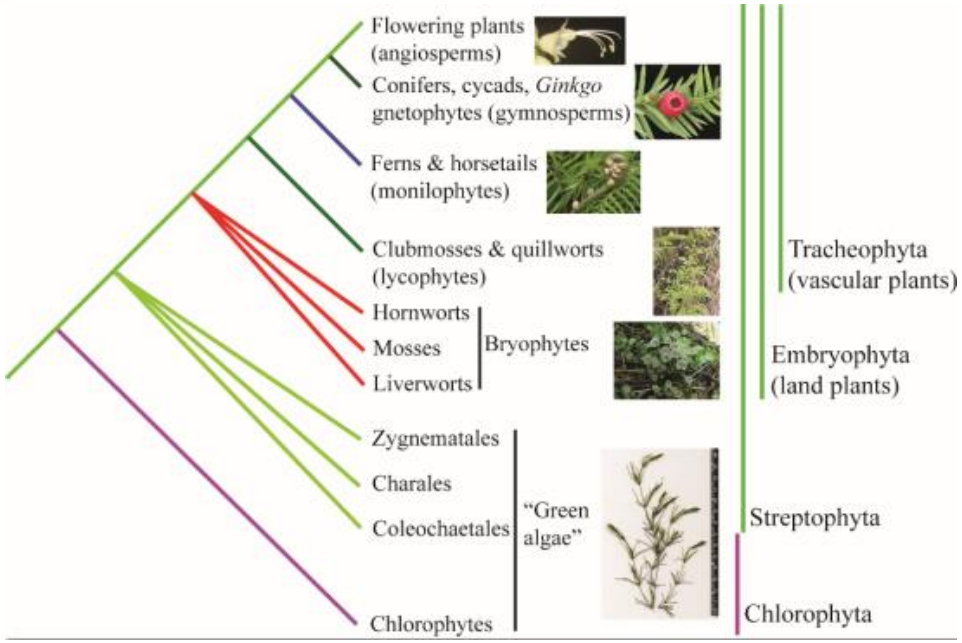
- About 100 species
- Similar to liverwort
- Hornlike sporophyte

PNAS

Hornwort pyrenoids, carbon-concentrating structures, evolved and were lost at least five times during the last 100 million years

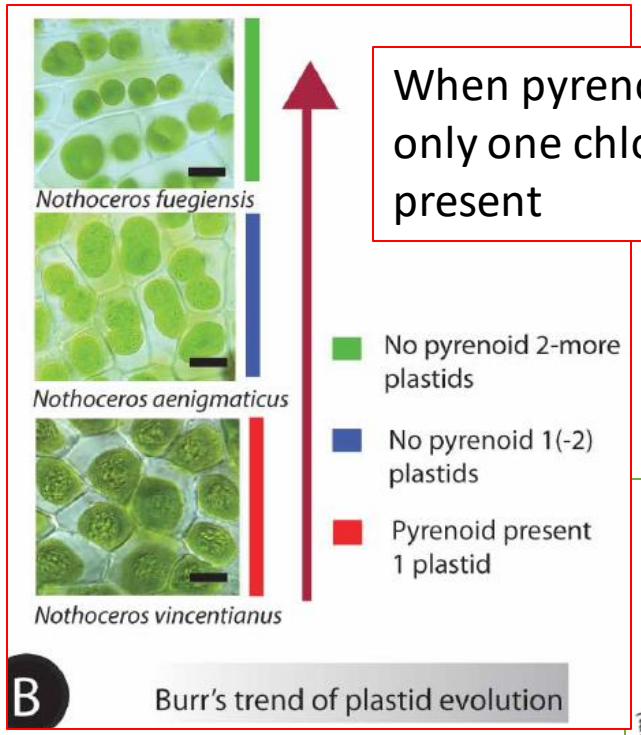
Juan Carlos Villarreal[1] and Susanne S. Renner

Pyrenoids are generally found in algae but they are also present in a group of plants called **hornworts**, suggesting they had an important **role during water-to-land transition in the green lineage**



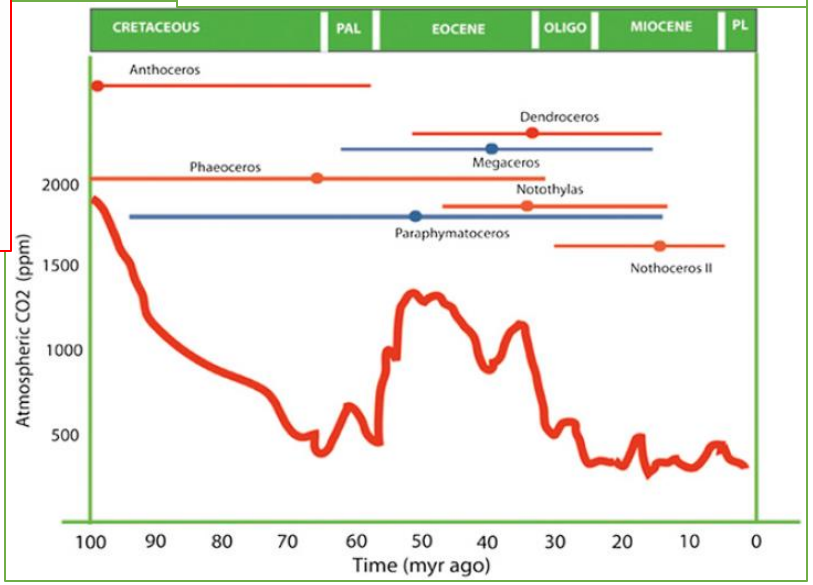
hornworts

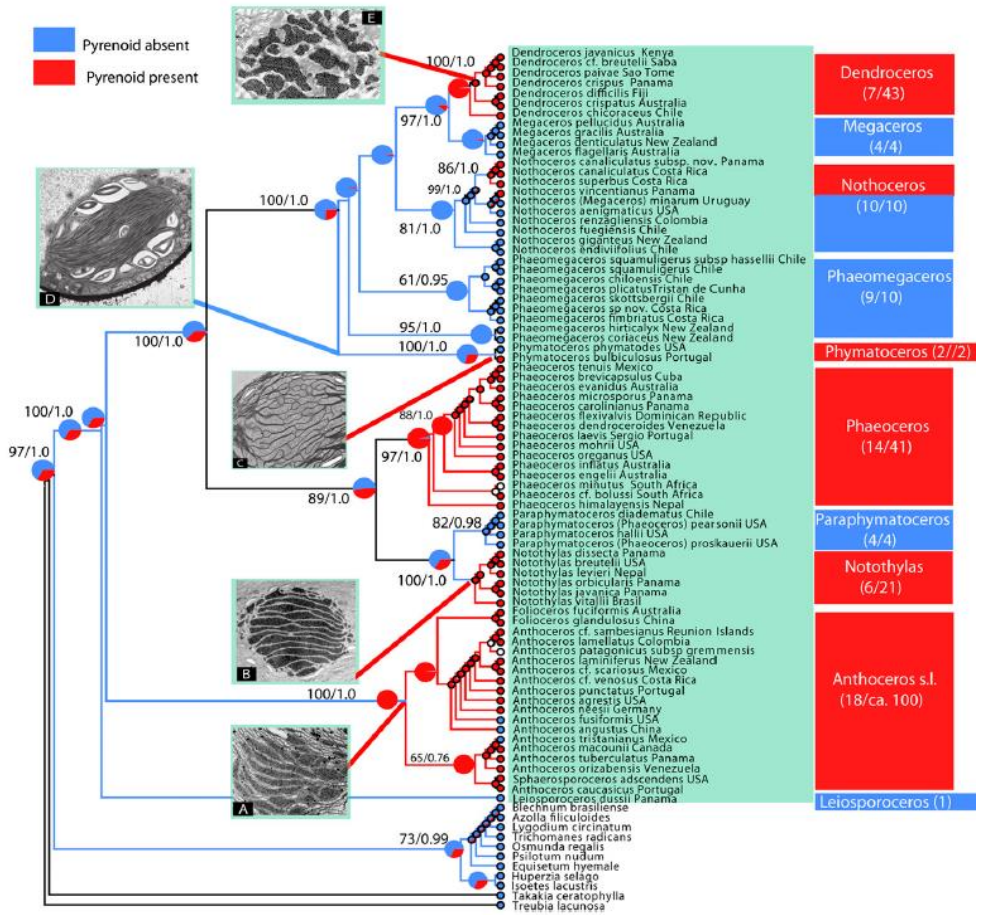
Tree of life, the soltis lab



When pyrenoid is present, only one chloroplast is present

Pyrenoid evolution might be associated to water submergence more then $[CO_2]$ in the atmosphere





In hornwort, the pyrenoid is composed of subunits of various shapes

Gains and losses of the pyrenoid (and putative CCM) in hornworts. Pyrenoids evolved from five to six times.

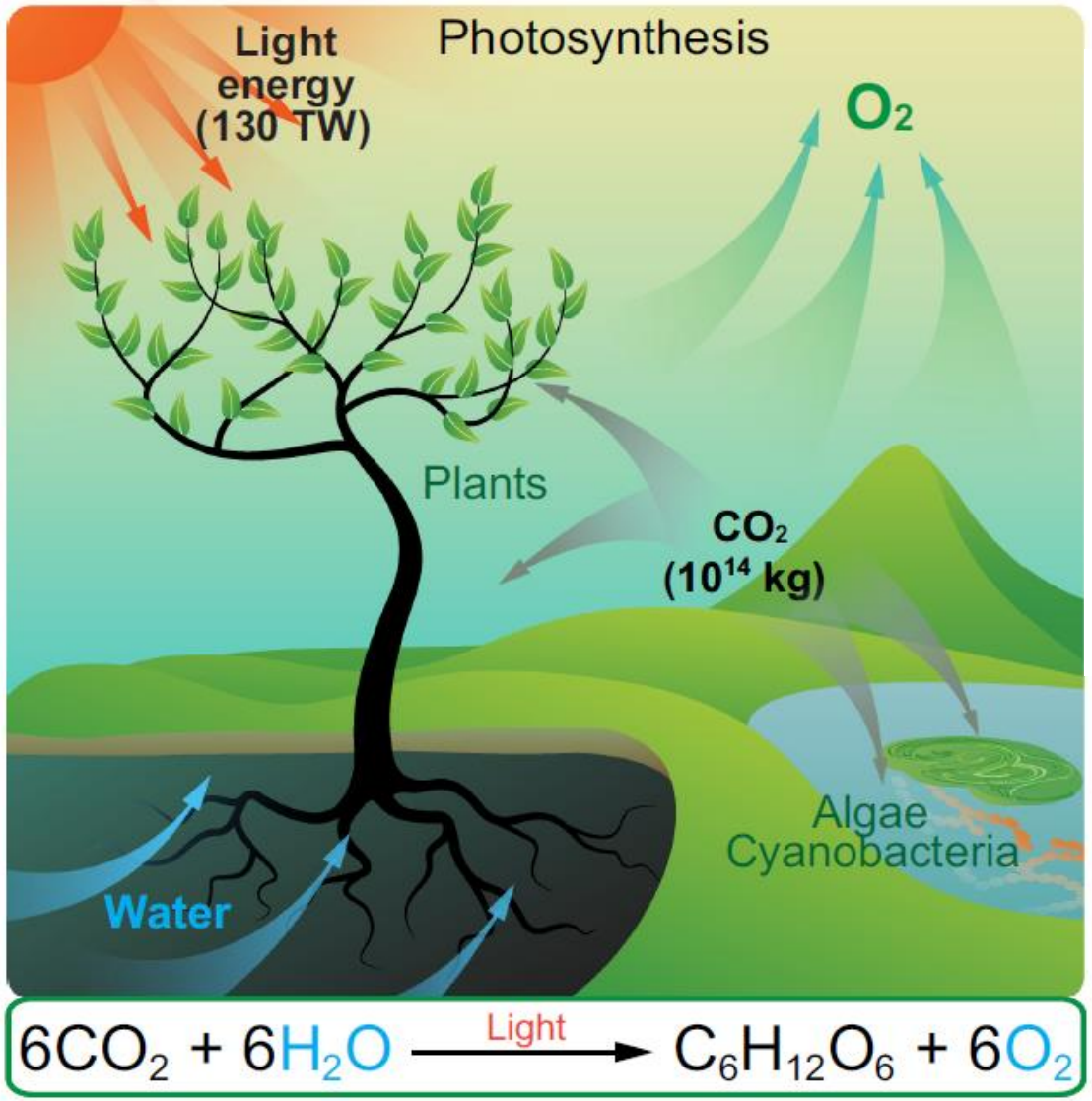
wrap-up

- Pyrenoids convert 30% of world CO_2 into organic carbon
- Pyrenoid-like structures could increase RUBISCO's performances in C3 plants
- High-throughput approaches allowed to identify proteins involved in pyrenoid formation and transmission to new generations
- The pyrenoid undergoes a reversible phase transition during cell division
- Hornworts as possible organisms to engineer plant pyrenoids

Review

Chaperone Machineries of Rubisco – The Most Abundant Enzyme

Manajit Hayer-Hartl [1,*] and F. Ulrich Hartl [1,*]



all food sources for life on Earth and maintains the oxygen level of the atmosphere. The human population currently stands at ~7.8 billion, and is expected to grow to ~10 billion by 2050 [1]. However, not only is the current overall **agricultural productivity** (see Glossary) not keeping pace with population growth, it is actually expected to decline in the course of this century due to climate change, especially in the southern regions of the planet [2]. For example, by 2030 Southern Africa could lose more than 30% of maize production, its main crop, and South Asia could suffer losses of more than 10% for many regional staples, such as rice, millet, and maize [3]. Climate change affects agriculture in various ways, including through changes in average and local temperatures, regional climate extremes, and droughts. In the face of these challenges, it seems unlikely that future **food security** can be ensured without increasing photosynthetic carbon fixation by genetically engineering crop plants [4–9]. Making photosynthesis more efficient would not only increase productivity but also enhance agricultural sustainability by optimizing the use of resources and minimizing the impact on the environment [10].