Advance Molecular and Cell Biology II

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Oct, 23, 2015

What biological phenomena is attractive to you?

Which organism is suitable to answer your questions ?

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To me, survival from hard enviromental conditions.

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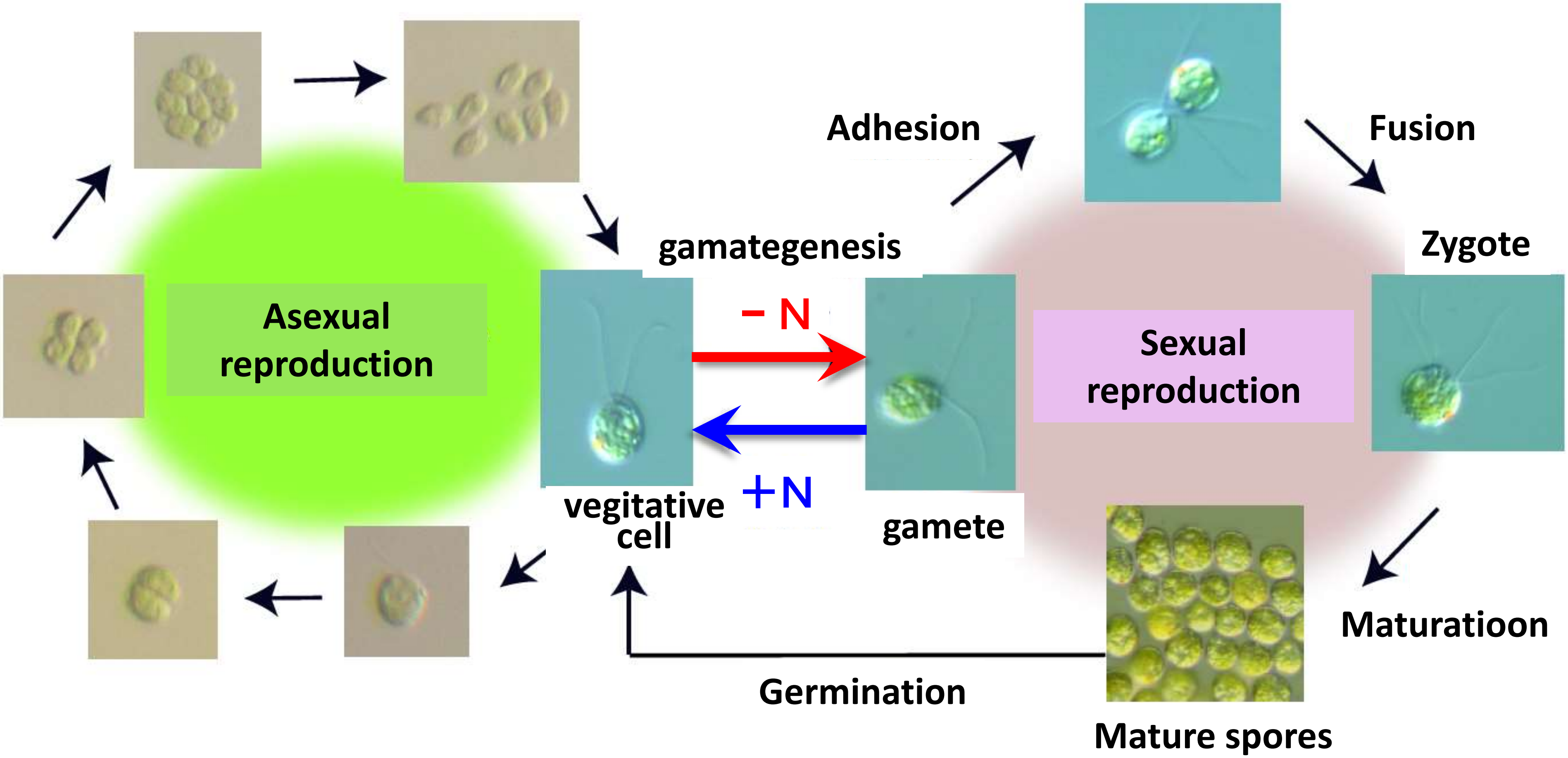
To me, survival from hard enviromental conditions.

Which organism is suitable to answer your questions ?

I think photosynthetic eukaryotes !

After consuming all the nutrients such as nitrogen, how cells can keep their life?

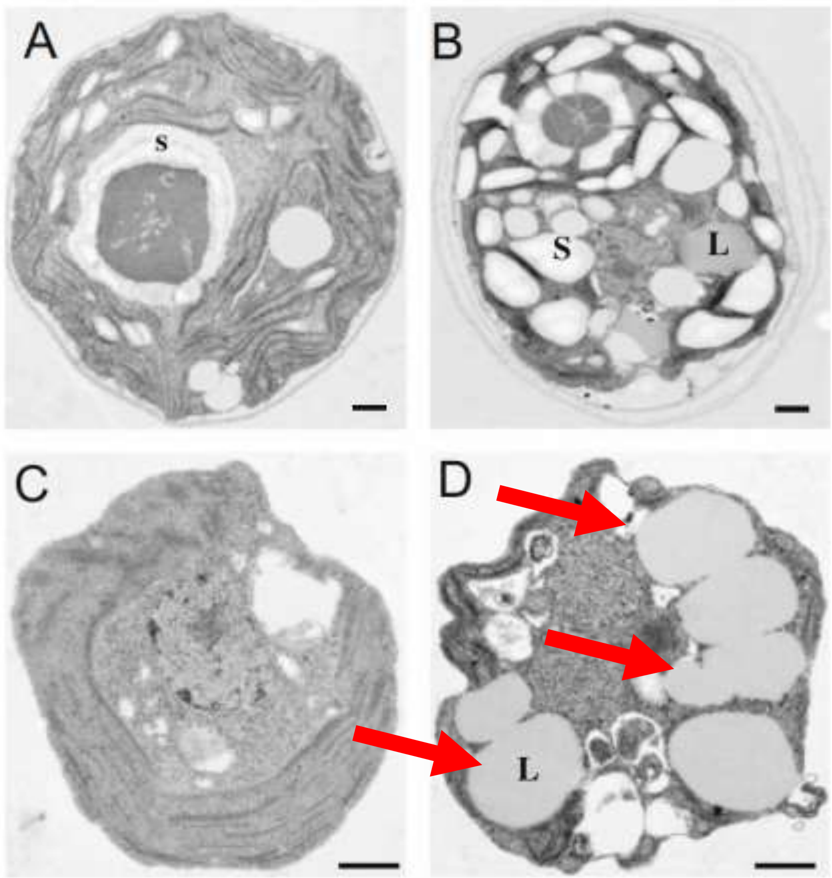
Life cycle of a green alga, *Chlamydomonas reinhardtii*



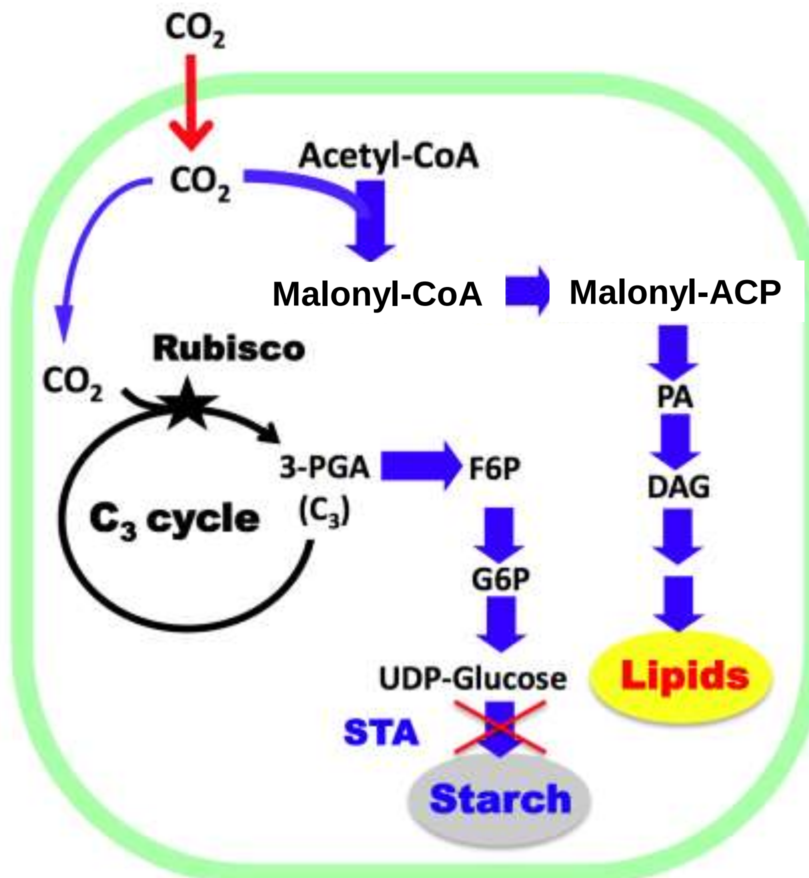
Under nitrogen-deficient conditions, cells accumulate triacylglycerol (TAG) and starch.
Starch less mutant stores much more TAG

Wild type

Mutant *sta6*

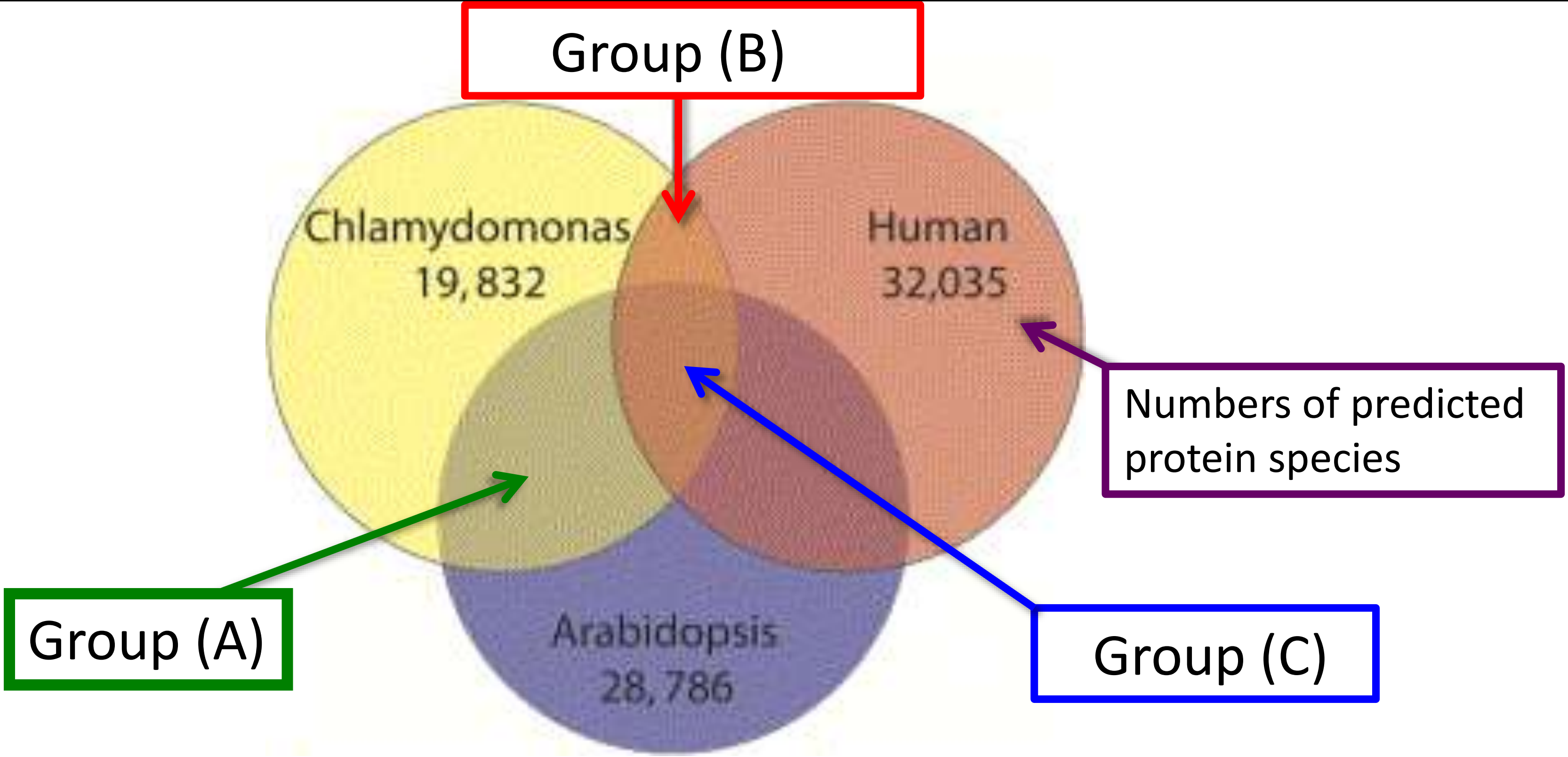


40μE, 2 days   400μE (–N), 2 days



(Li, Y. *et al.* Metabolic Engineering 2010)

The green alga *Chlamydomonas* is classified in both animal and plant kingdims

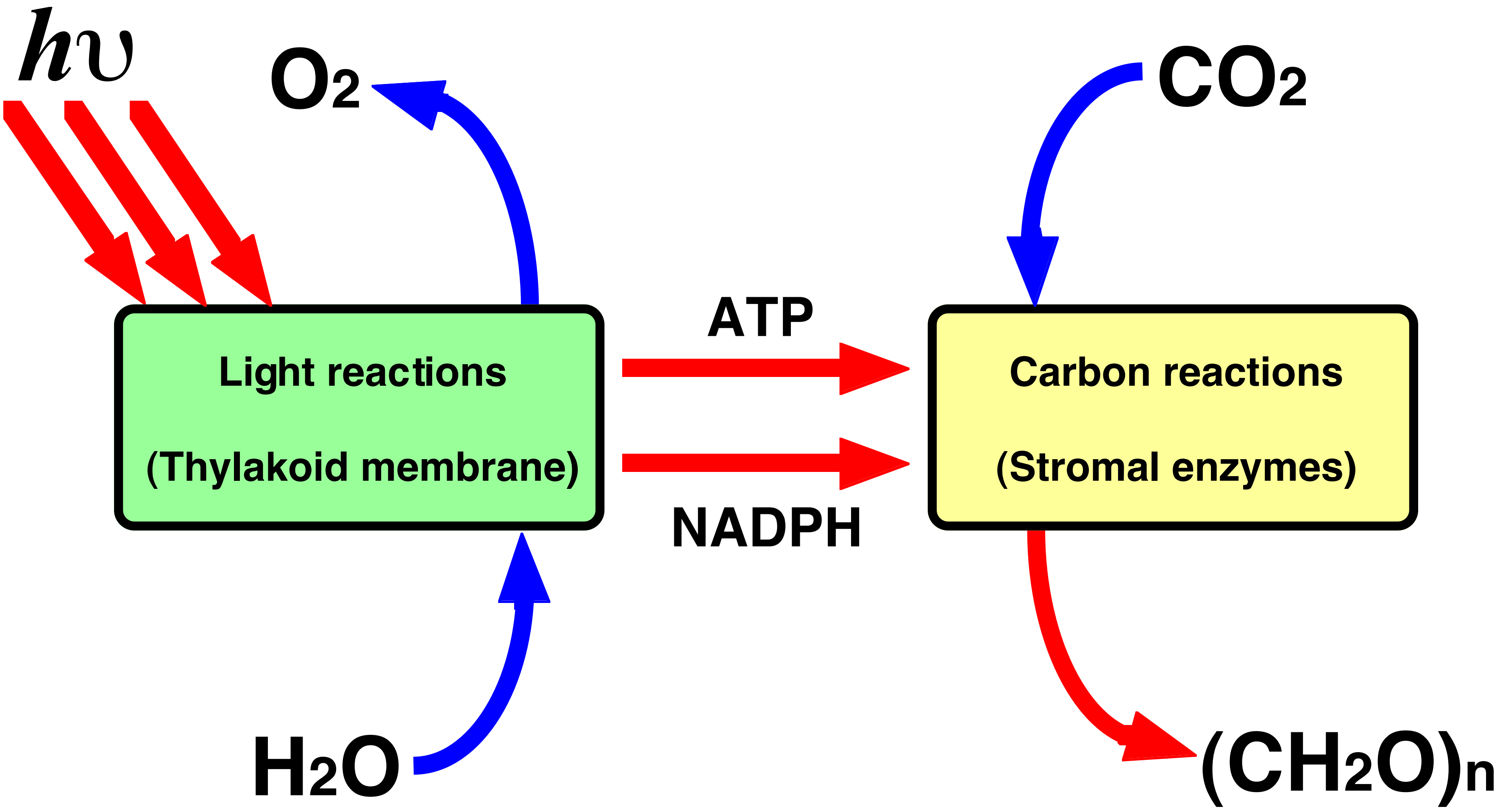


（Q.1） What kinds of proteins are included in the group (A)?

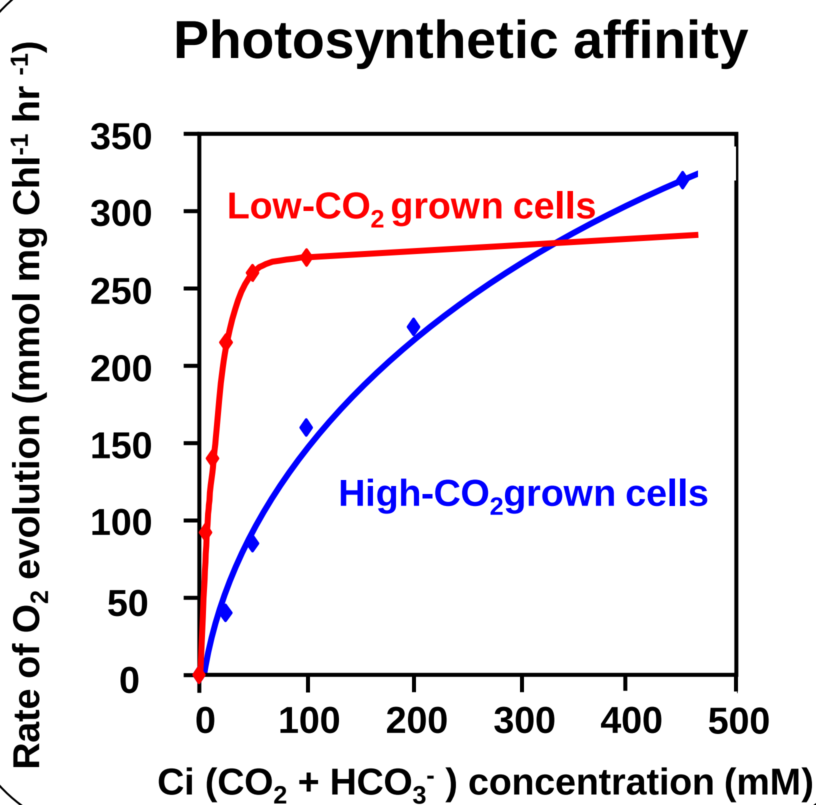
（Q.2） What kinds of proteins are included in the group (B)?

（Q.3） What kinds of proteins are included in the group (C)?

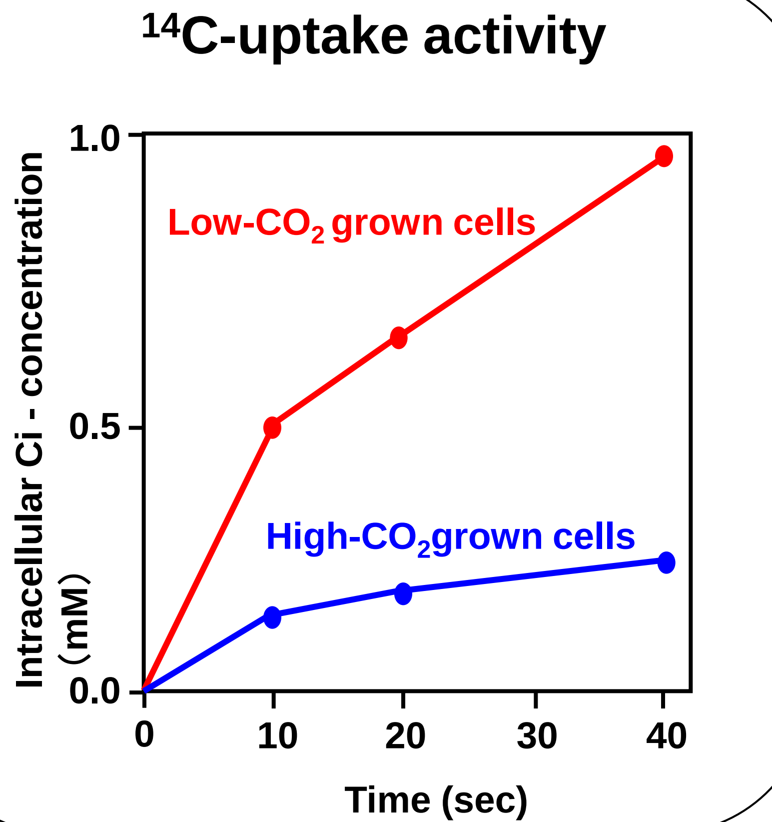
Two teps in photosynthesis



Aquatic photosynthetic organisms can acclimate to CO_2-limiting stress by inducing CO_2-concentrating mechanism



(Badger et al. 1980)

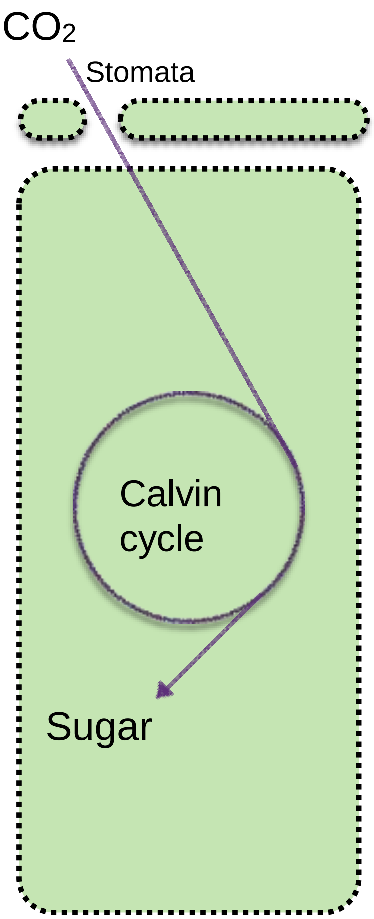


(Fukuzawa et al. 1998)

Low-CO_2: ordinary air containing 0.04% CO_2

High-CO_2: air containing 5%CO_2

Three types of photosynthesis in land plants and three types of CO_2–concentrating mechanism (CCM)

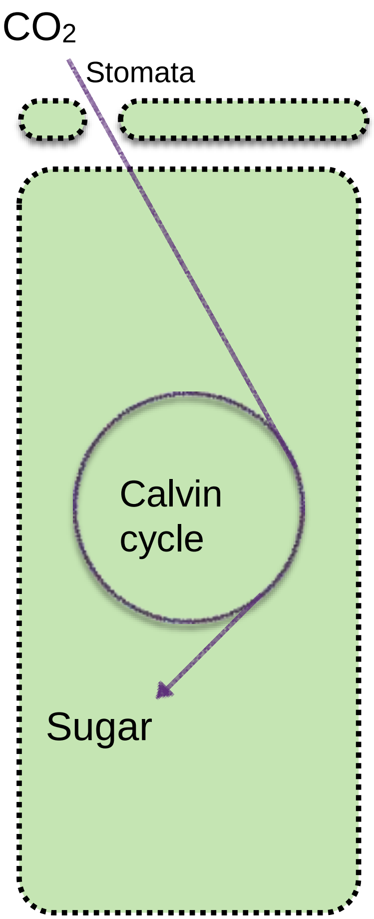


C3 photosynthesis

Nobel Prize 1961:
Melvin Calvin

No CCM

Three types of photosynthesis in land plants and three types of CO_2-concentrating mechanism (CCM)



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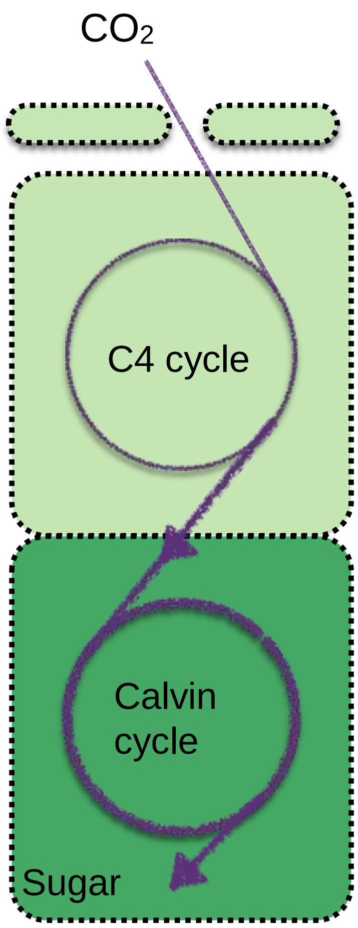
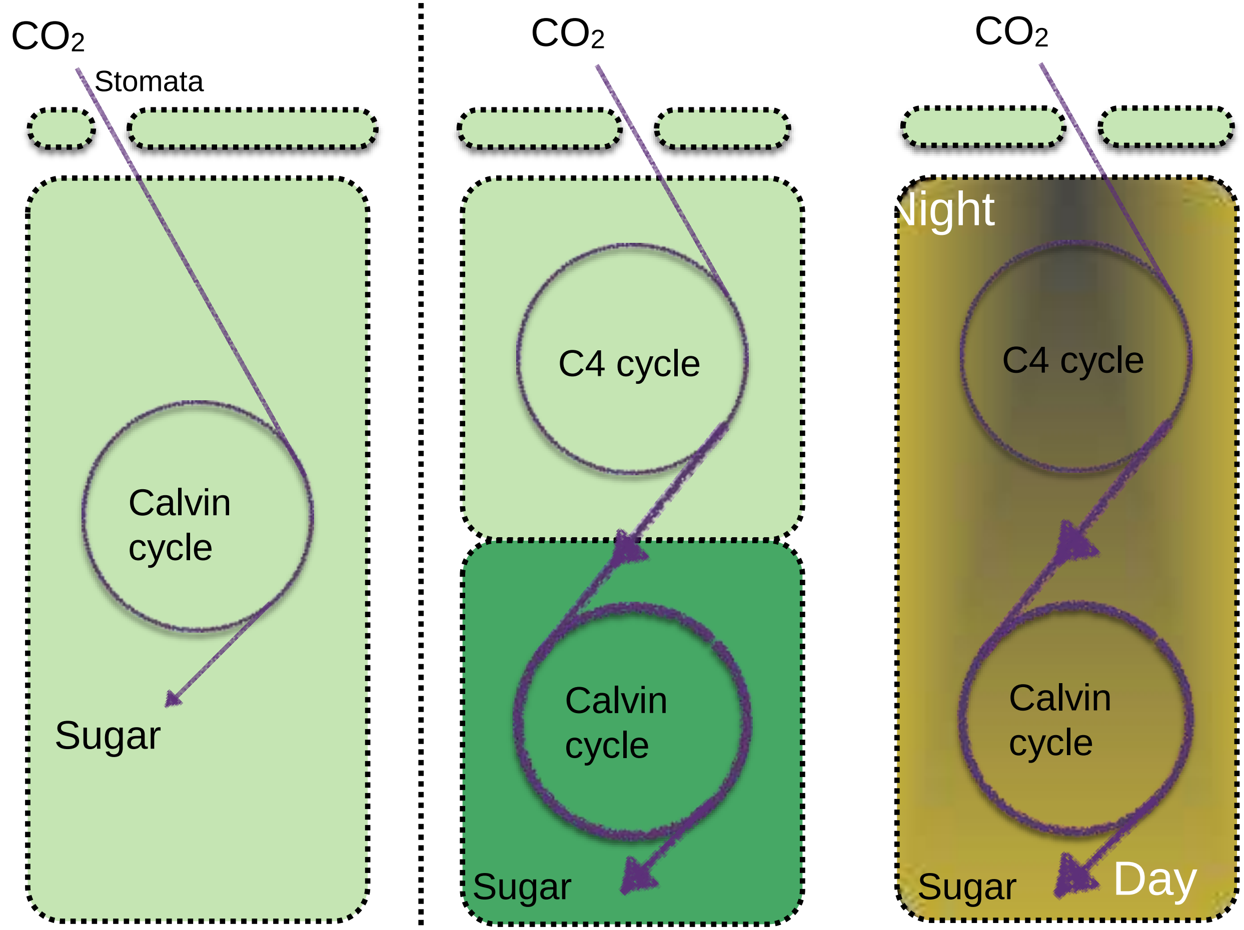


C4 photosynthesis
(Spatial separation)

Slack and Hatch 1967

CCM to overcome the CO_2-limiting stress

Three types of photosynthesis in land plants and three types of CO_2-concentrating mechanism (CCM)



C3 photosynthesis

Nobel Prize 1961:
Melvin Calvin

C4 photosynthesis
(Spatial separation)

Slack and Hatch 1967

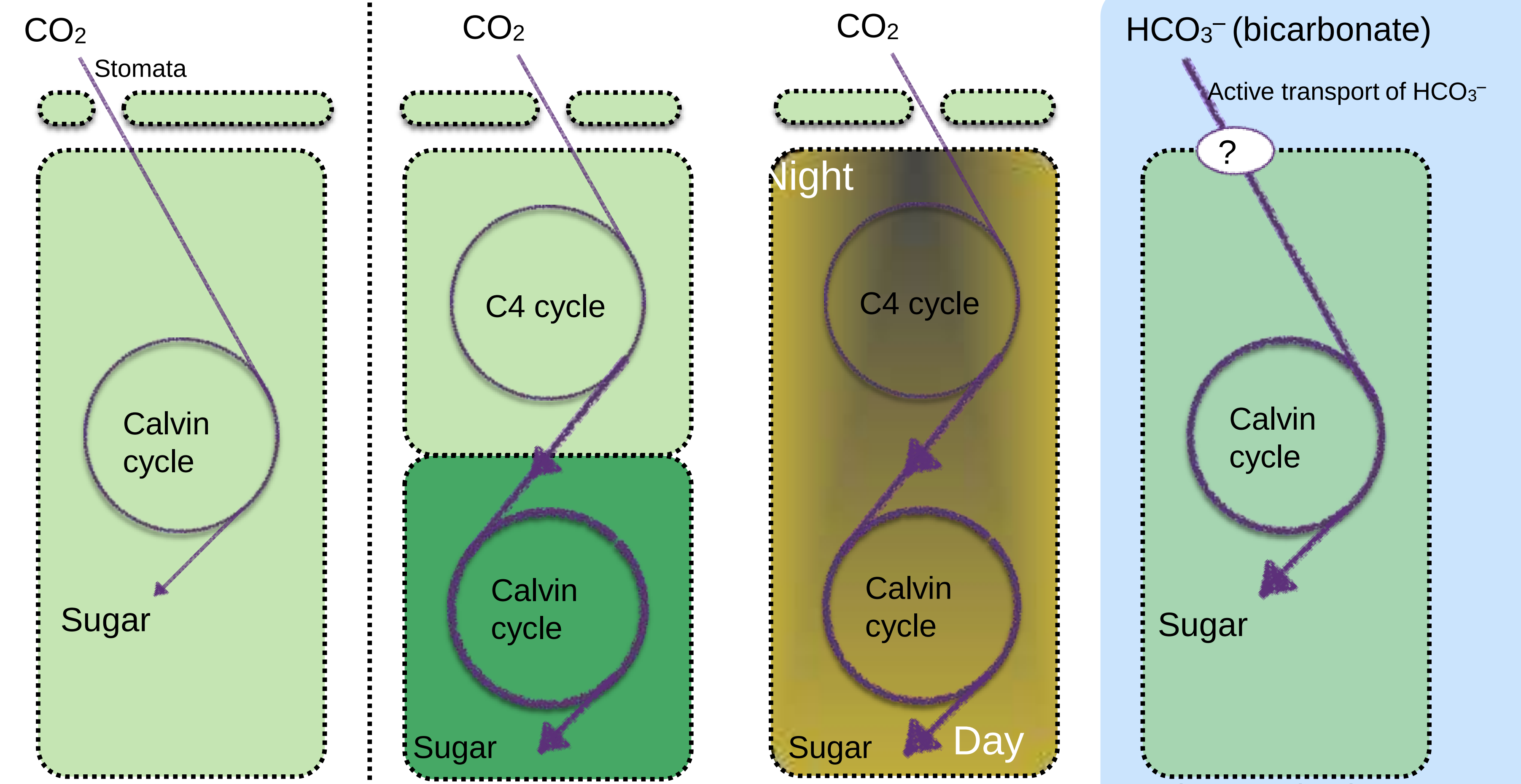
CAM photosynthesis
(Temporal separation)

Ranson and Thomas 1960

No CCM

CCM to overcome the CO_2-limiting stress

Three types of photosynthesis in land plants and three types of CO_2-concentrating mechanism (CCM)



C3 photosynthesis

Nobel Prize 1961:
Melvin Calvin

C4 photosynthesis
(Spatial separation)

Slack and Hatch 1967

CAM photosynthesis
(Temporal separation)

Ranson and Thomas 1960

C3 photosynthesis
(Biophysical uptake of HCO_3^-)

Badger et al. 1980

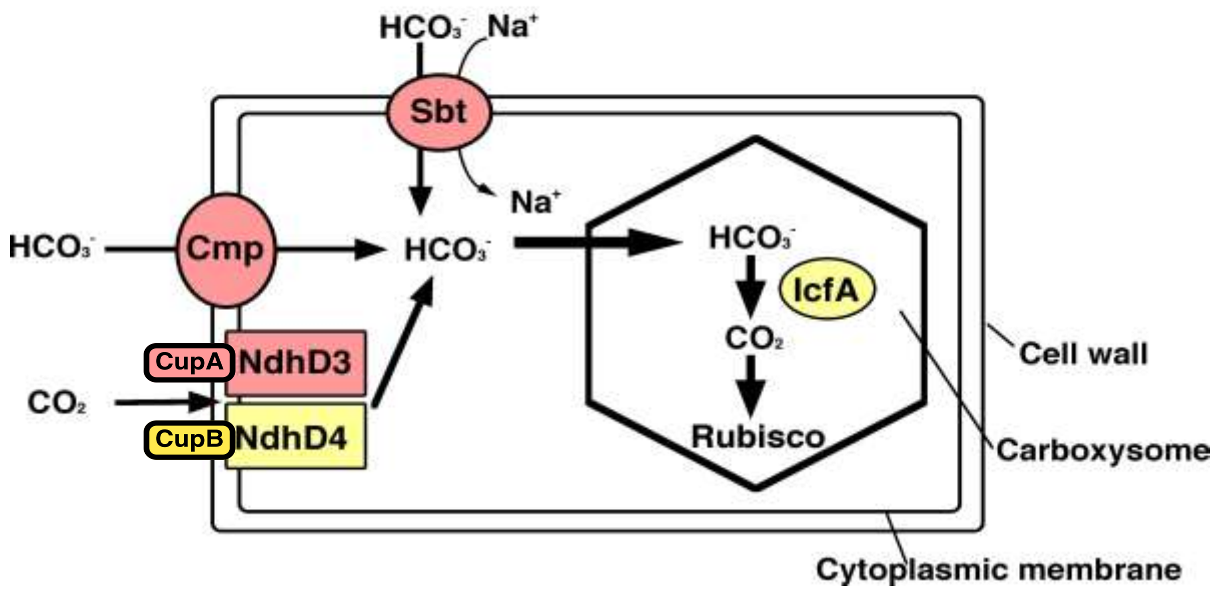
No CCM

CCM to overcome the CO_2-limiting stress

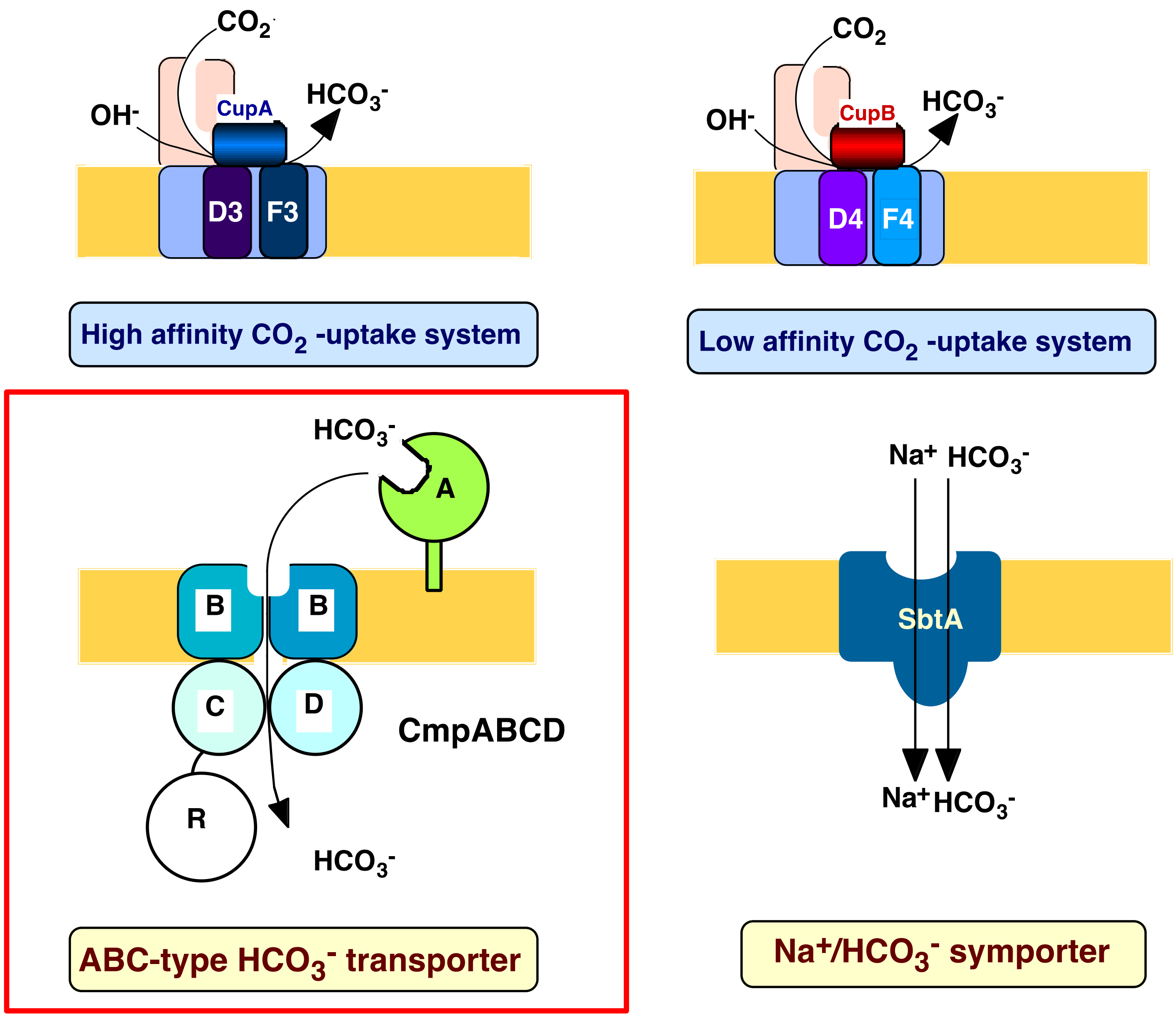
ABC-transporters

in cyanobacteria

Carbon concenbtrating mechanism in a Cyanobacterium, *Synechocystis* PCC6803



Four systems for inorganic carbon acqusition in a cyanobacterium, *Synechocystis* PCC6803

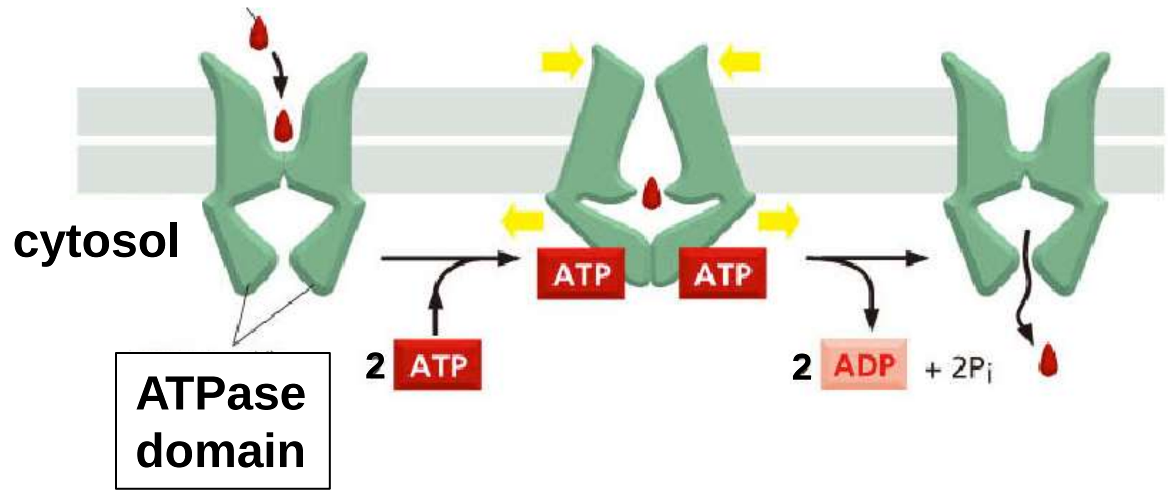


ABC-transporters

in human and eukaryotic algae

ABC-transporters function as an importer as well as an exporter and are evolutionaly conserved among bacteria, animals and plants.

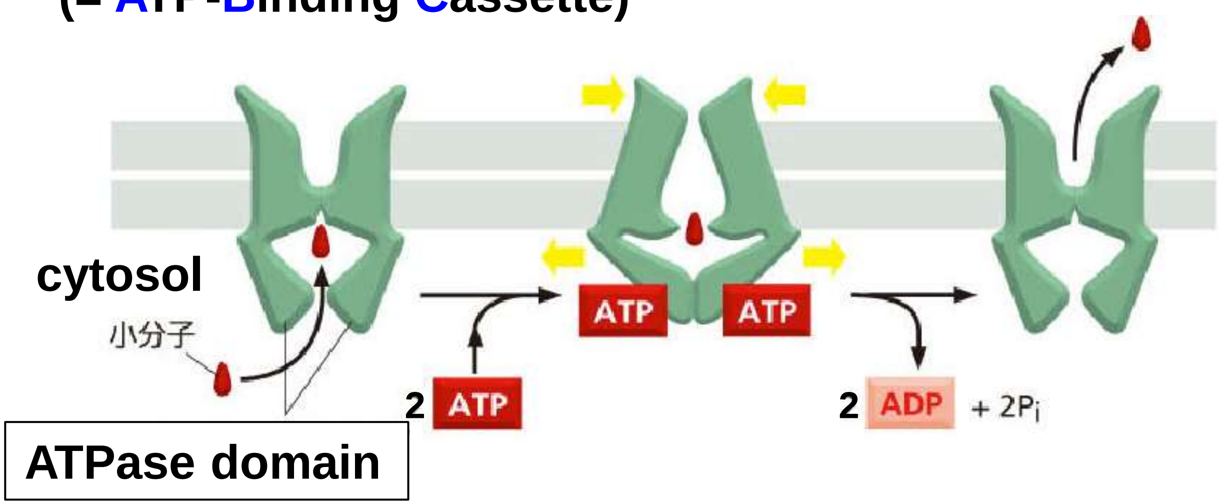
(A)



Import

(= ATP-Binding Cassette)

(B)



Export

Cell, Vol. 47, 381–389, November 7, 1986, Copyright © 1986 by Cell Press

Internal Duplication and Homology with Bacterial Transport Proteins in the *mdr*1 (P-Glycoprotein) Gene from Multidrug-Resistant Human Cells

Chang-jie Chen,* Janice E. Chin,* Kazumitsu Ueda,† Douglas P. Clark,†‡ Ira Pastan,† Michael M. Gottesman,† and Igor B. Roninson*

ous lipophilic compounds from multidrug-resistant cells (Dano, 1973). Other mechanisms for multidrug resistance have been proposed, including decreased drug influx

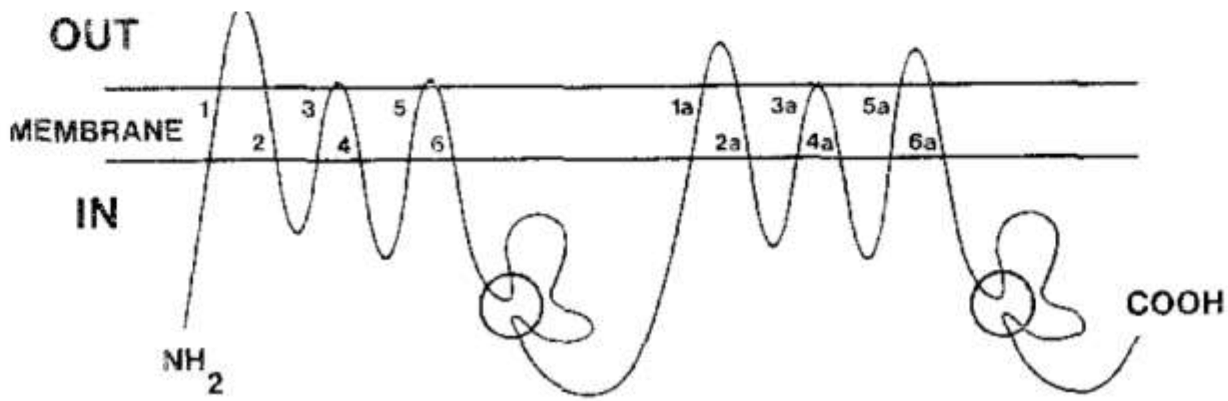
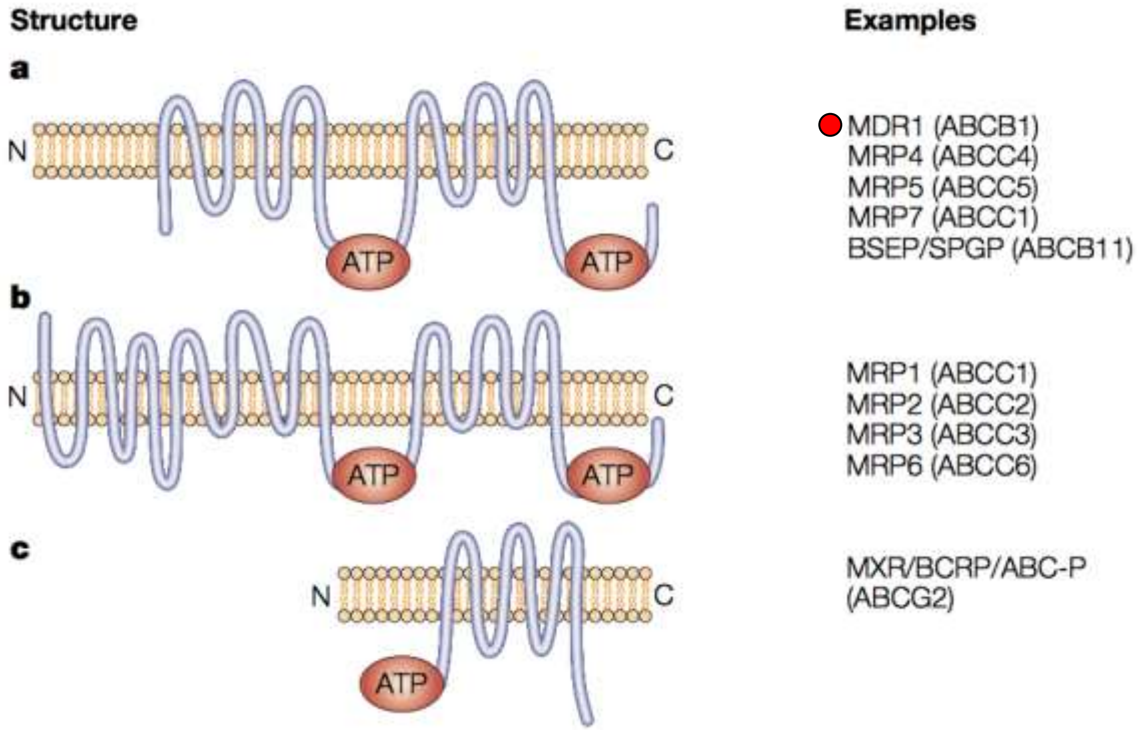


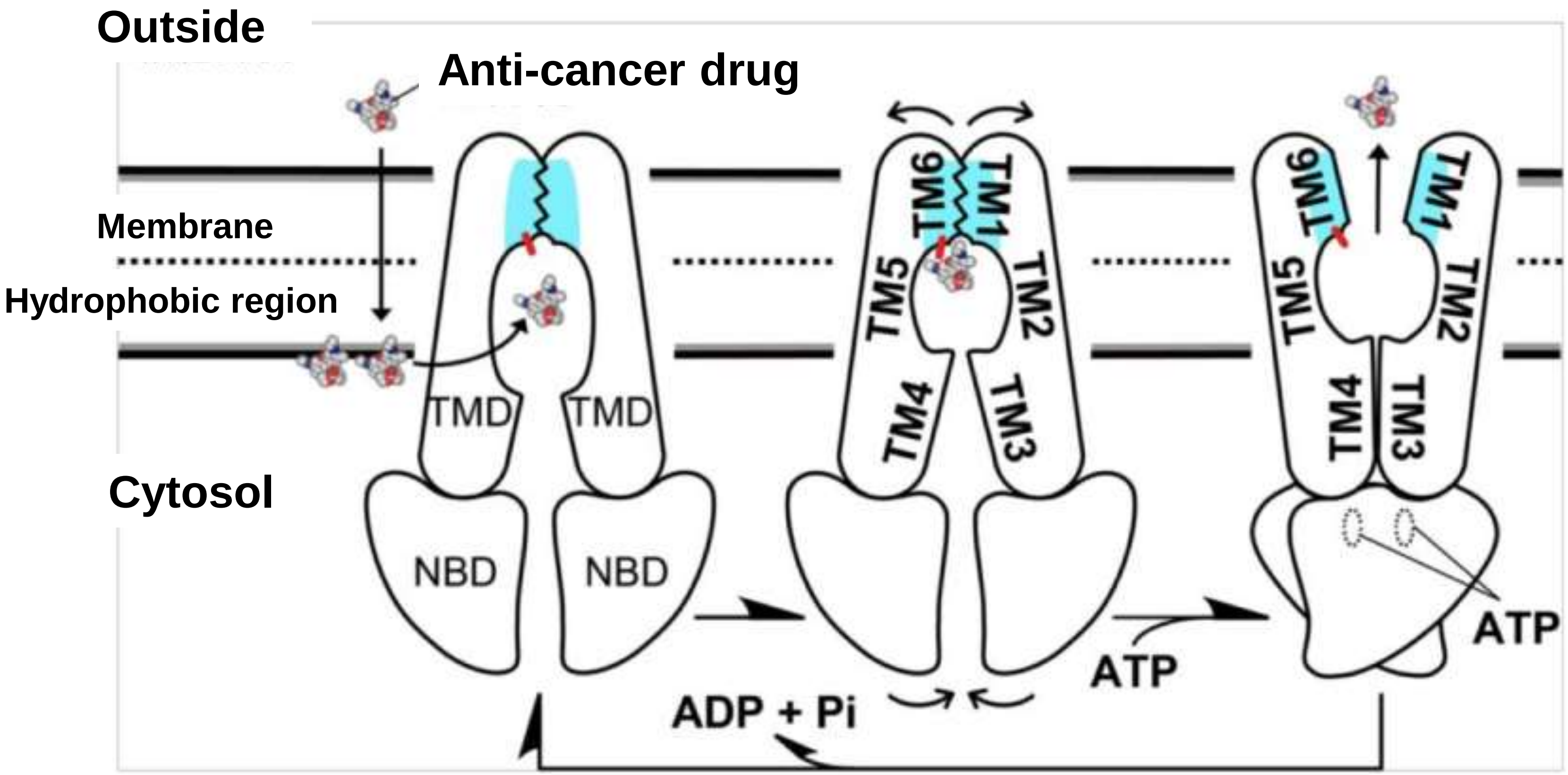
Figure 7. Model for the Transmembrane Orientation of P-Glycoprotein

The transmembrane segments, predicted by the algorithm of Eisenberg et al. (1984), are as follows: 1, residues 52–72; 2, residues 120–140; 3, residues 189–209; 4, residues 216–236; 5, residues 297–317; 6, residues 326–346; 1a, residues 711–731; 2a, residues 757–777; 3a, residues 833–853; 4a, residues 854–874; 5a, residues 937–957; and



Structures of ABC transporters known to confer anti-cancer drug resistance in human.

- Multidrug resistance of cancer cells is a potentially surmountable obstacle to effective chemotherapy of cancer.
- ATP-binding cassette (ABC) transporters, including MDR1 (ABCB1), MRP1 (ABCC1) and ABCG2, can confer multidrug resistance to cancer cells *in vitro*.
- Inhibitors of ABC transporters such as MDR1/P-glycoprotein have been tested in clinical trials.



Outside
Anti-cancer drug
Membrane
Hydrophobic region
Cytosol
TMD
TMD
NBD
NBD
TM6
TM1
TM5
TM2
TM4
TM3
TM6
TM1
TM5
TM2
TM4
TM3
ATP
ATP
ADP + Pi

Crystal structures of a eukaryotic P-glycoprotein homolog, CmABCB1 from a thermophilic red alga, *Cyanidioschyzon merolae*

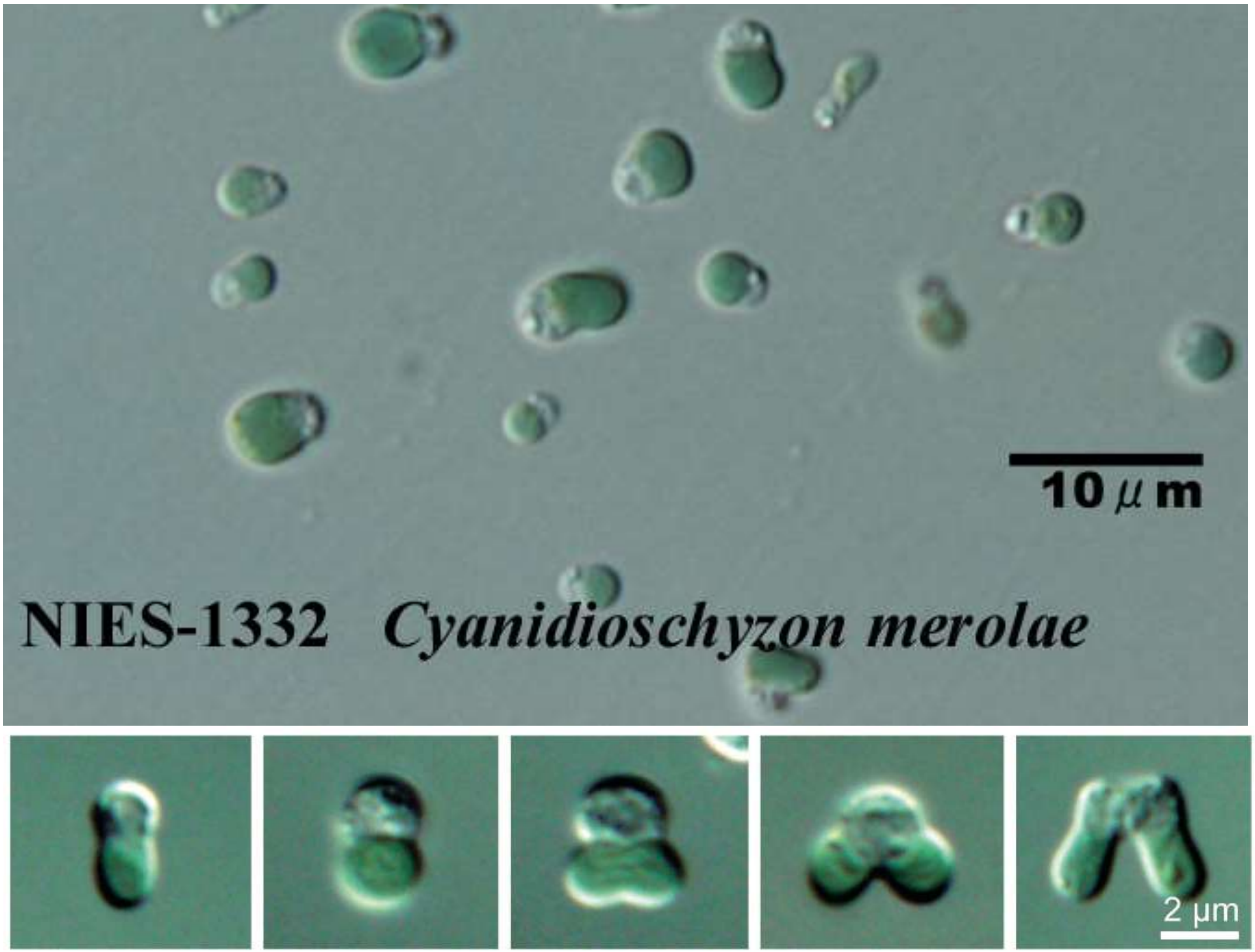


Table 1. LysR-type regulators in *Synechocystis* sp. PCC 6803.

Gene Name	Synechocystis ORF	Function	Co-regulatory Metabolites	Reference
ndhR (*ccmR*)	sll1594	Repressor high affinity C_i uptake (genes for CupA, SbtA, Na^+-NDH-1)	α-KG, $NADP^+$	[31,32,89,97]
cmpR	sll0030	Activator of ABC-type bicarbonate transporter (*cmp* operon and *psbA* genes)	RuBP, 2PG	[32,35,89,97]
ycf30, *rbcR*	sll0998	Activation of CBB genes	NADPH, 3PGA, RuBP	[89,97,98]
ntcB	slr0395	Activation of nitrate assimilation genes	nitrite	[99]

Burnap et al. Life 5, 348-371 (2015)

Appl Microbiol Biotechnol (2015) 99:2451–2462
DOI 10.1007/s00253-015-6397-4

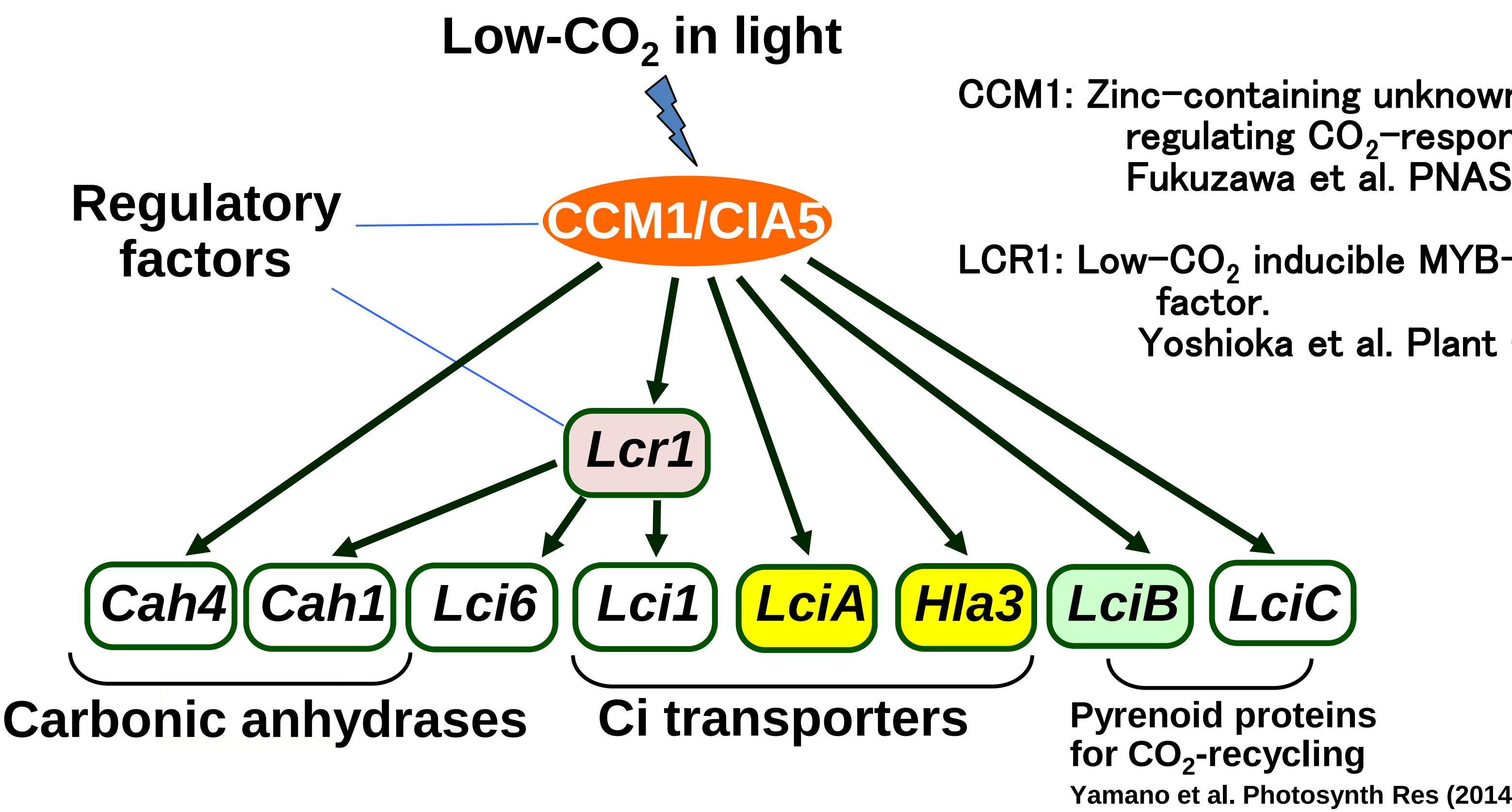
BIOENERGY AND BIOFUELS

Impacts of CO_2 concentration on growth, lipid accumulation, and carbon-concentrating-mechanism-related gene expression in oleaginous *Chlorella*

Jianhua Fan · Hui Xu · Yuanchan Luo · Minxi Wan · Jianke Huang · Weiliang Wang · Yuanguang Li

Chlorella pyrenoidosa grew well under CO_2 concentrations ranging from 1 to 20 %. The highest biomass and lipid productivity were 4.3 g/L and 107 mg/L/day under 5 % CO_2 condition. Switch from high (5 %) to low (0.03 %, air) CO_2 concentration showed significant inhibitory effect on growth and CO_2 fixation rate. The amount of the saturated fatty acids was increased obviously along with the transition. Low CO_2 concentration (0.03 %) was suitable for the accumulation of saturated fatty acids. Reducing the CO_2 concentration could significantly decrease the polyunsaturated degree in fatty acids. Moreover, the carbon-concentrating mechanism-related gene expression revealed that most of them, especially *CAH2*, *LCIB*, and *HLA3*, had remarkable change after 1, 4, and 24 h of the transition, which suggests that *Chlorella* has similar carbon-concentrating mechanism with *Chlamydomonas*

A model for CO_2-resposive regulation of CCM-related genes



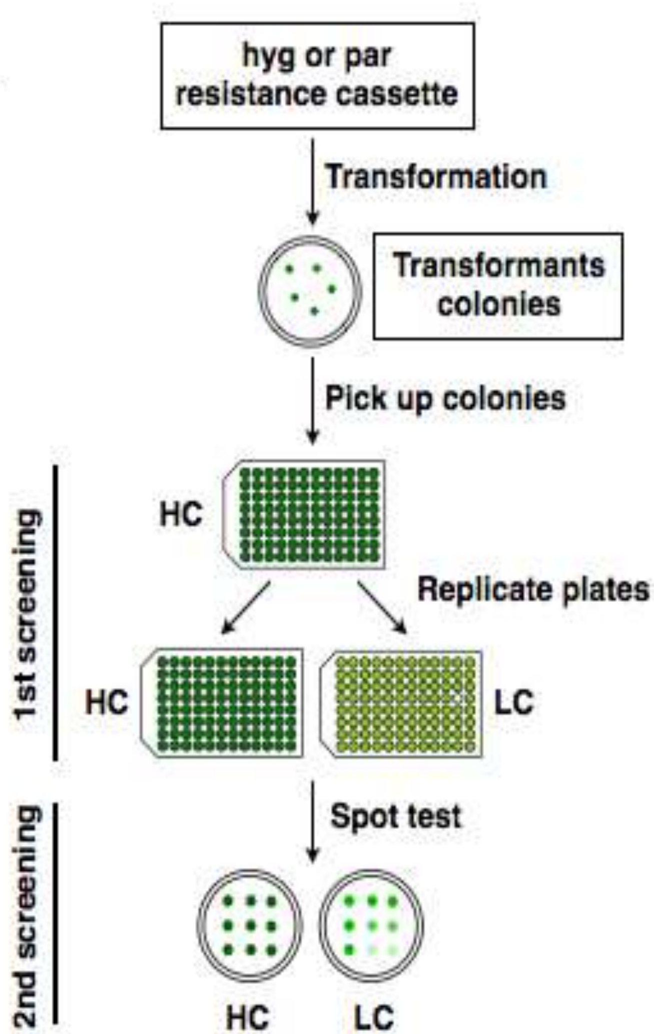
CCM1: Zinc-containing unknown protein regulating CO_2-responsive genes.
Fukuzawa et al. PNAS (2001)

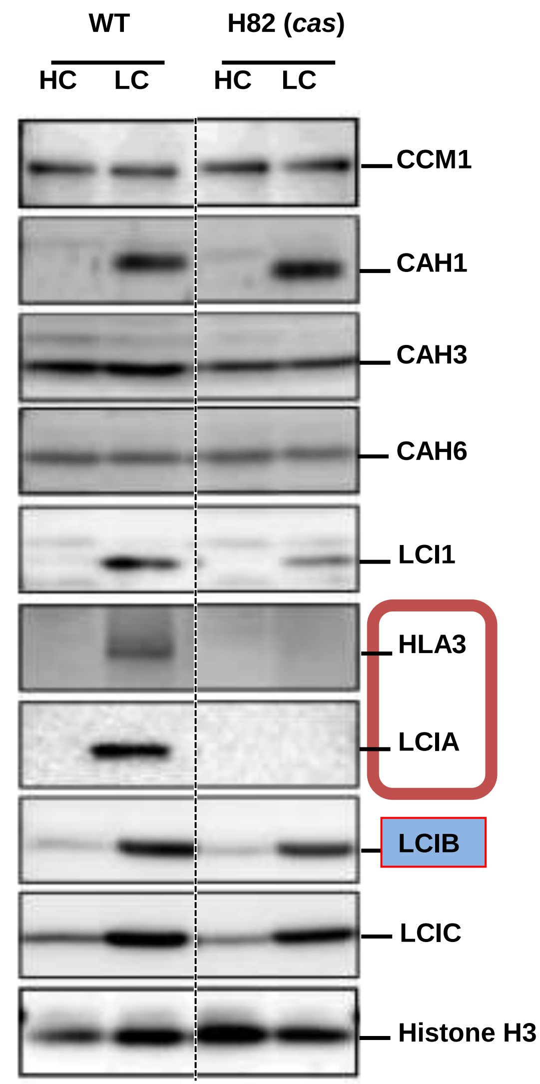
LCR1: Low-CO_2 inducible MYB-transcription factor.
Yoshioka et al. Plant Cell (2004)

LCIA: Chloroplast envelope bicarbonate channel.

HLA3: Plasma membrane bicarbonate transporter.

CO_2-requiring mutant H82 induces low-CO_2 resposive proteins including LCIB except for two membrane proteins, HLA3 and LCIA

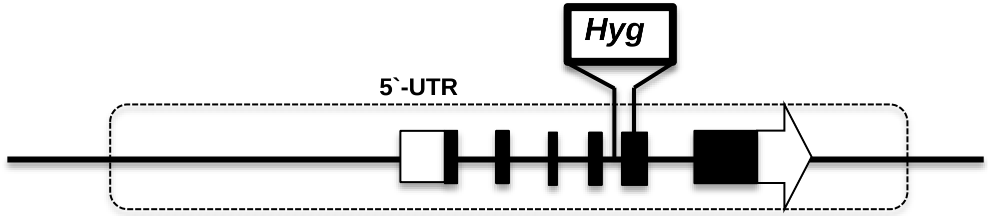




Wang et al. Photosynth Res (2014)

The high-CO_2 (HC)-requiring phenotype of the mutant H82 was complemented by intact CAS gene

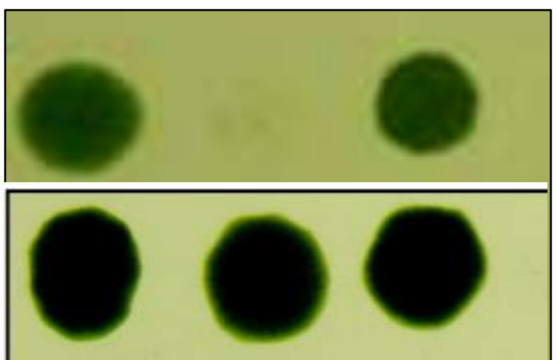
***CrCAS*::*Hyg* in H82**



Wang et al. Photosynth Res (2014)

Genomic fragment containing the intact CAS gene with its own promoter was used for complementation

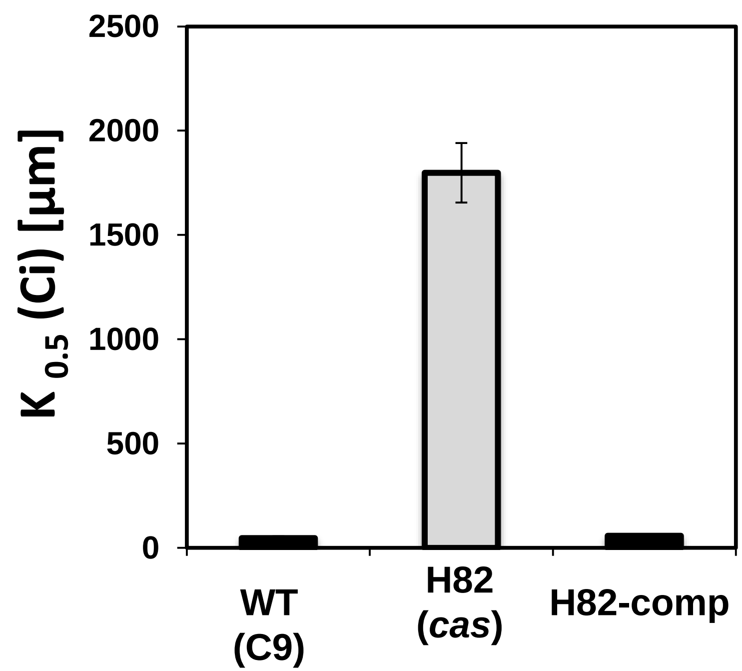
WT (C9) | H82 (*cas*) | H82-comp



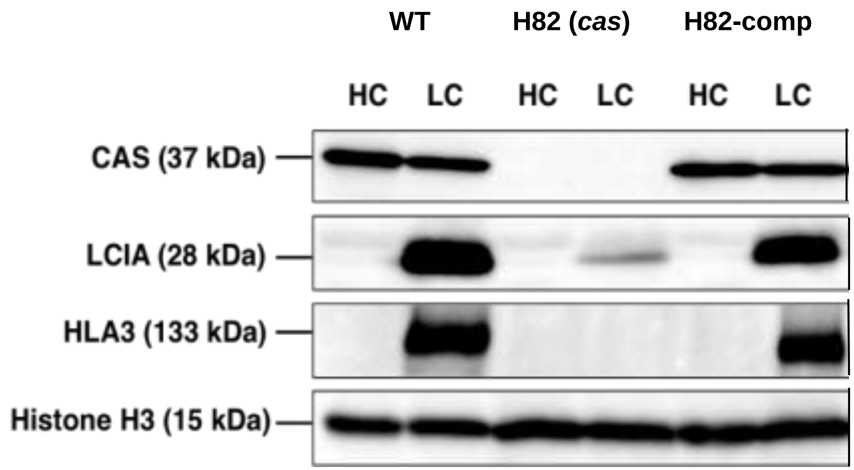
LC: Low-CO_2 (0.04 % CO_2)

HC: High-CO_2 (5 % CO_2)

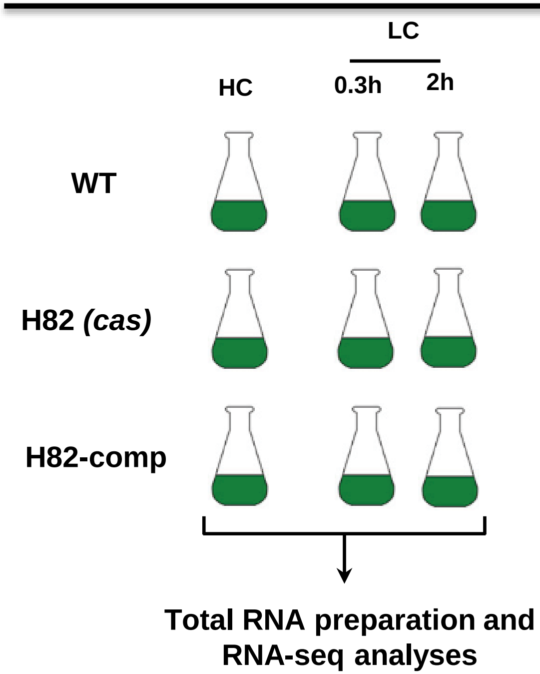
High-CO_2 (HC) requiring in light at 120μE

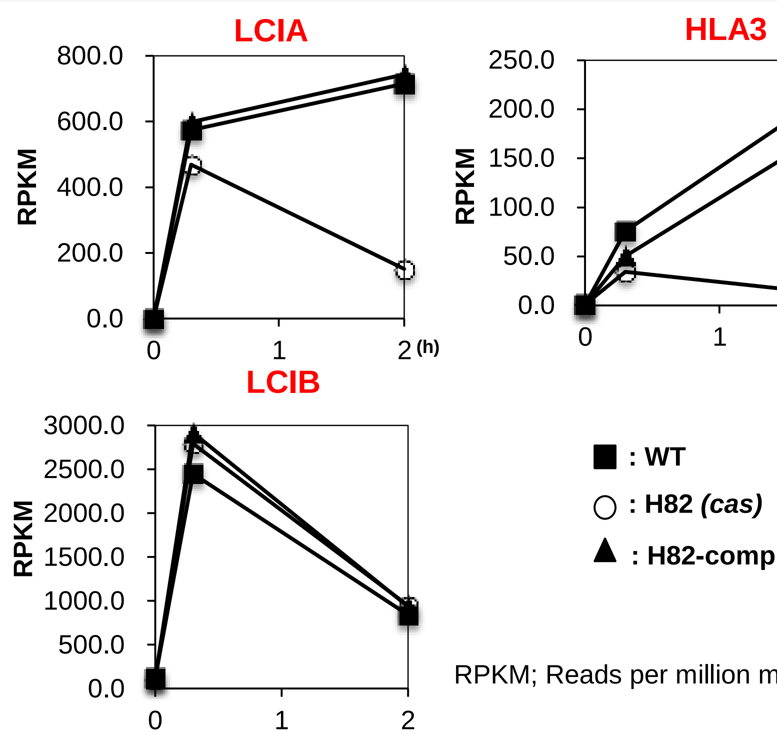


Expression of transporters, LCIA and HLA3, was recovered by introducing *CAS* gene into the *cas* mutant H82



HC: 5 % CO_2
LC: 0.04 % CO_2





RPKM; Reads per million mapped reads.

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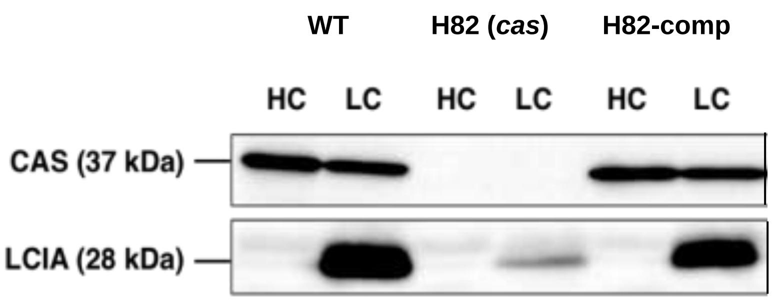
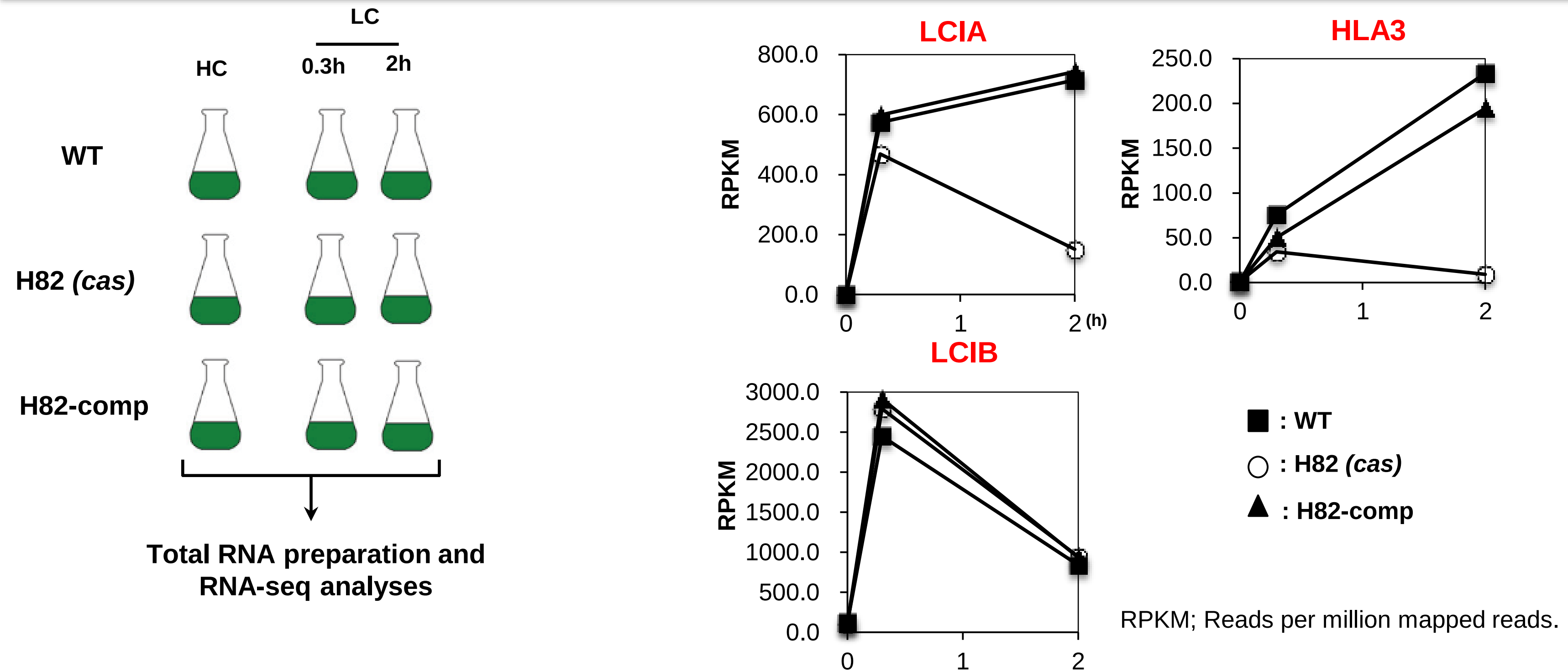


Expression of transporters, LCIA and HLA3 was regulated at the mRNA level by CAS



What is CAS?

In *Arabidopsis,*

・controls <u>stomatal closure</u> as a low-affinity / high-capacity Ca^{2+}-binding protein in guard cell. Han et al. Nature (2003), Nomura et al. Plant J. 53:988 (2008)

・involved in chloroplast-mediated activation of plant <u>immune signalling</u>.

Nomura et al. Nat Commun 26; 926 (2012)

In *Chlamydomonas*,

・Required for <u>photoacclimation by inducing LHCSR3i</u> (Light-harvesting complex stress-related protein), in calcium-dependent manner.

Petroutsos et al. Plant Cell 23: 2950-63 (2011)

・Down-regulation results in <u>inhibition of photosynthetic cyclic electron transfer</u>.

・Forms supercomplex with ANR1 and PGRL1 (PGR5-Like 1).

Terashima et al. PNAS 109: 17717–22 (2012)

Thylakoid-localized CAS protein is reported to function in calcium-dependent regulation of cyclic electron transfer with PGRL1 and ANR1 in photosynthesis

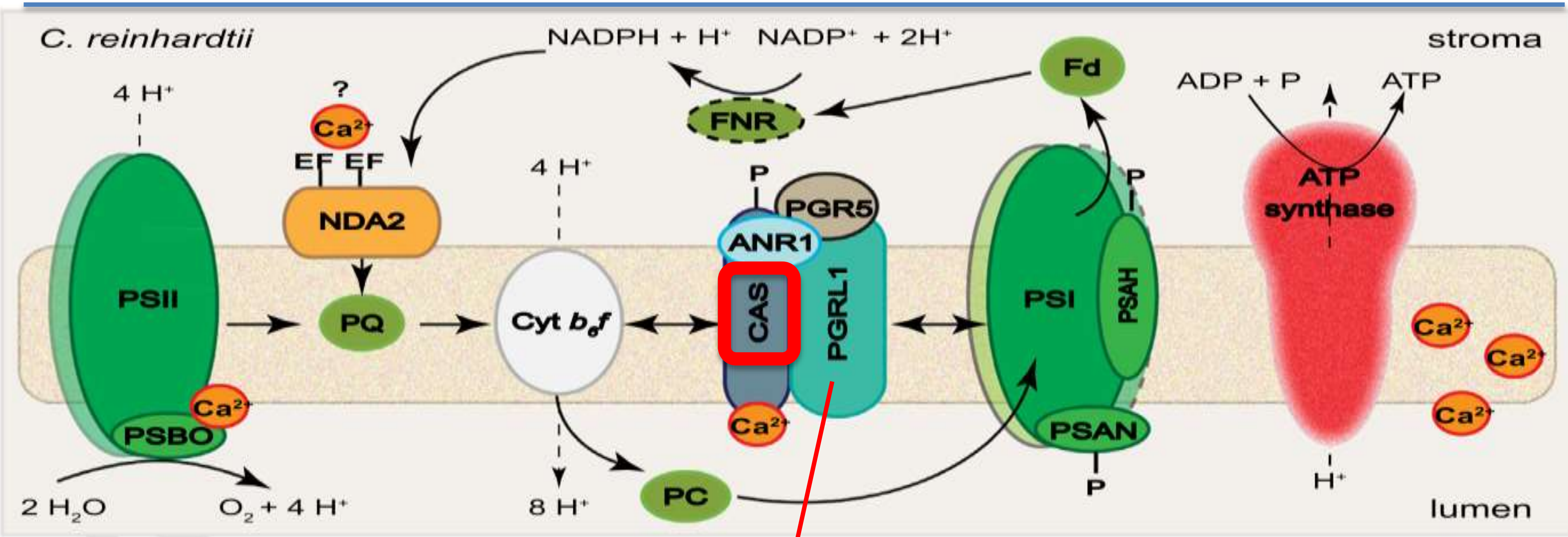
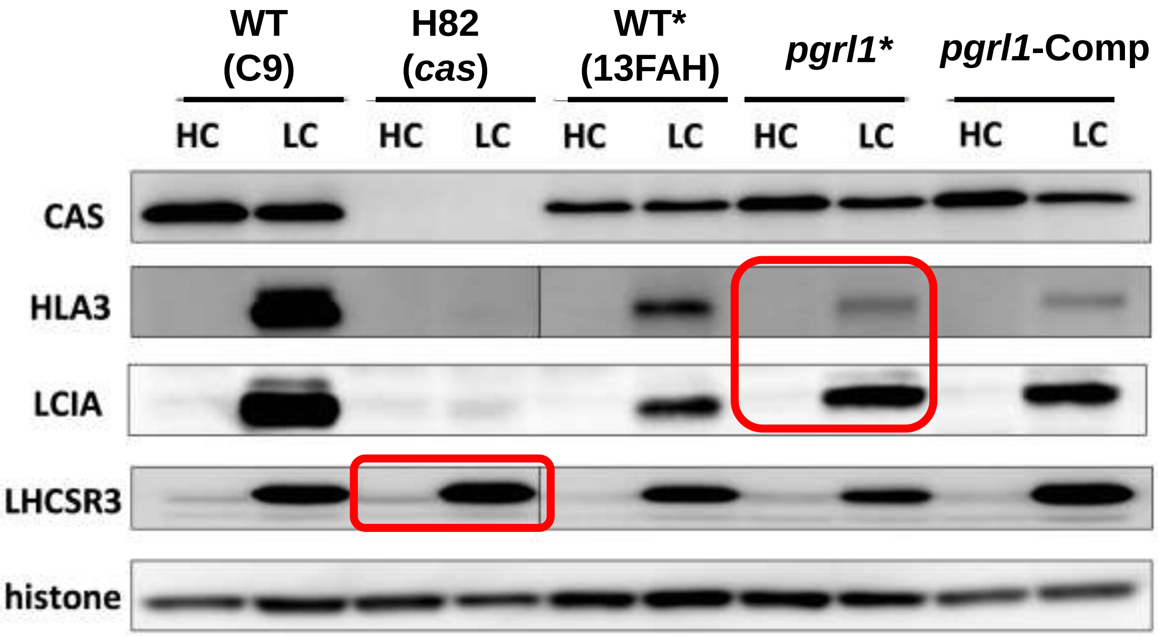


Figure 1D in Hochmal et al. Biochim. Biophys. Acta (2015) Feb. 14

PGRL1 (Proton Gradient Regulation-Like-1) :
ferredoxin-plastoquinone reductase Hetle et al. Mol. Cell 49: 511-523 (2013)

Effects of *cas- and pgrl1-mutations* on expression of LHCSR3 and Ci-transporters



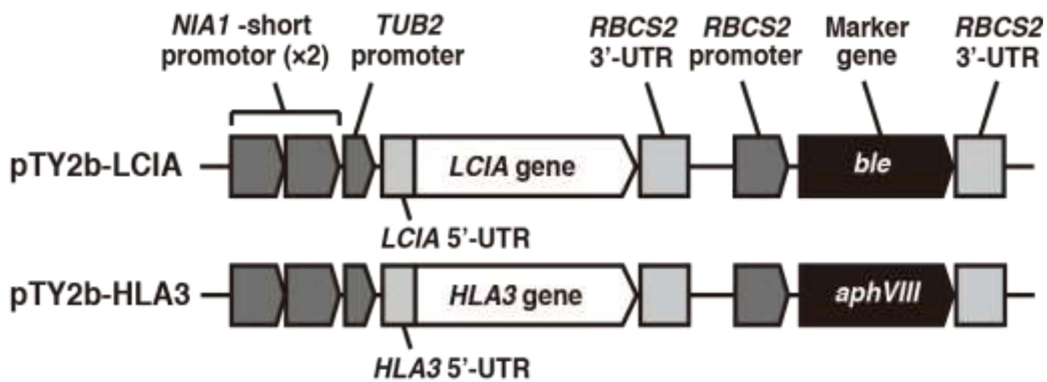
Light intensity: 120 μE m^{-2} s^{-1}
HC: 5 % CO_2
LC: 0.04 % CO_2

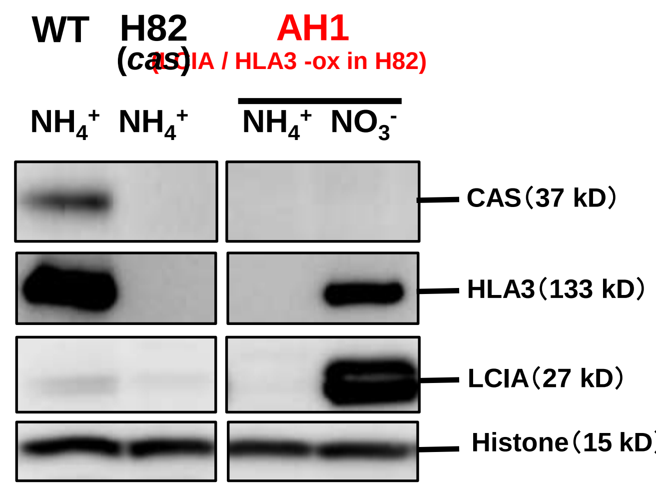
*From Dr. Xenie Johnson

- Although CAS Is reported to be required for photoacclimation, CAS is not essential to induce LHCSR3 (Light-Harvesting Complex Stress-Related protein 3) and non-photochemical quenching (NPQ).

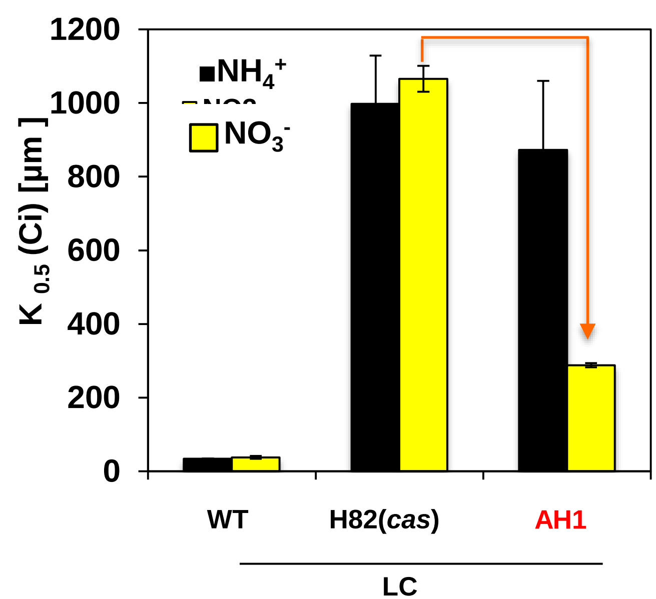
- Ci-transporters, HLA3 and LCIA, are not controled by PRGL1.

Conditionial over-expression of LCIA and HLA3 partially restored the photosynthetic Ci affinity of *cas* mutant





Expression of LCIA and HLA3 was induced by switching the nitrogen source from NH_4^+ to NO_3^-, under the H82 background. Overexpression both of LCIA and HLA3 results in 76% recovery of the photosynthetic Ci affinity. However, The $K_{0.5}$(Ci) value of AH1 was 7.6 times higher than that of WT, suggesting that the defect in accumulation of LCIA and HLA3 was not the only reason for the HC-requiring phenotype of H82.



Summary

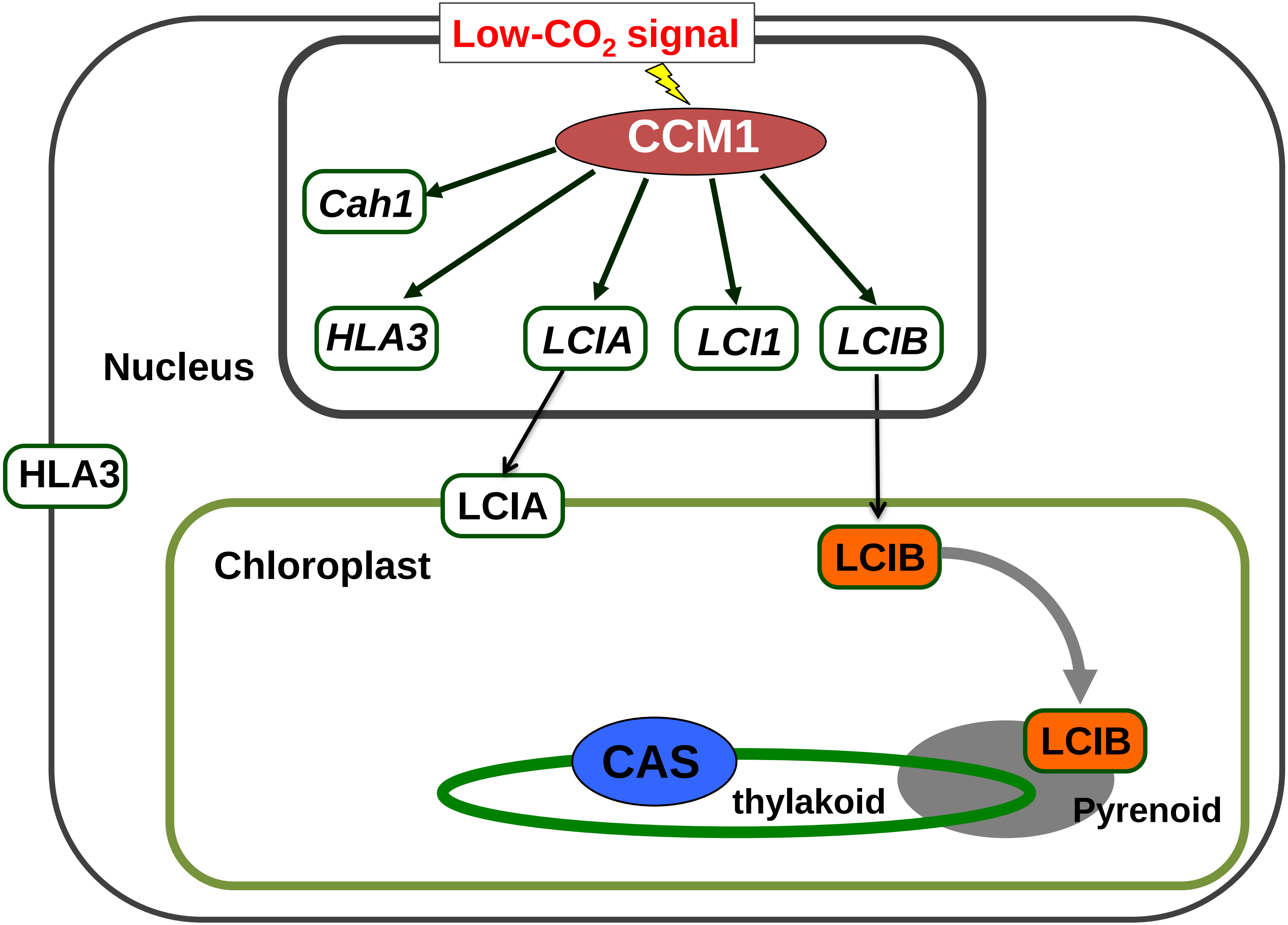
1) High-CO_2 requiring phenotype of H82 was complemented by introducing genomic DNA containing intact *CAS* gene.

2) expression of nuclear-encoded genes, LCIA and HLA3 as well as relocalization of LCIB are regulated by Chloroplast CAS.

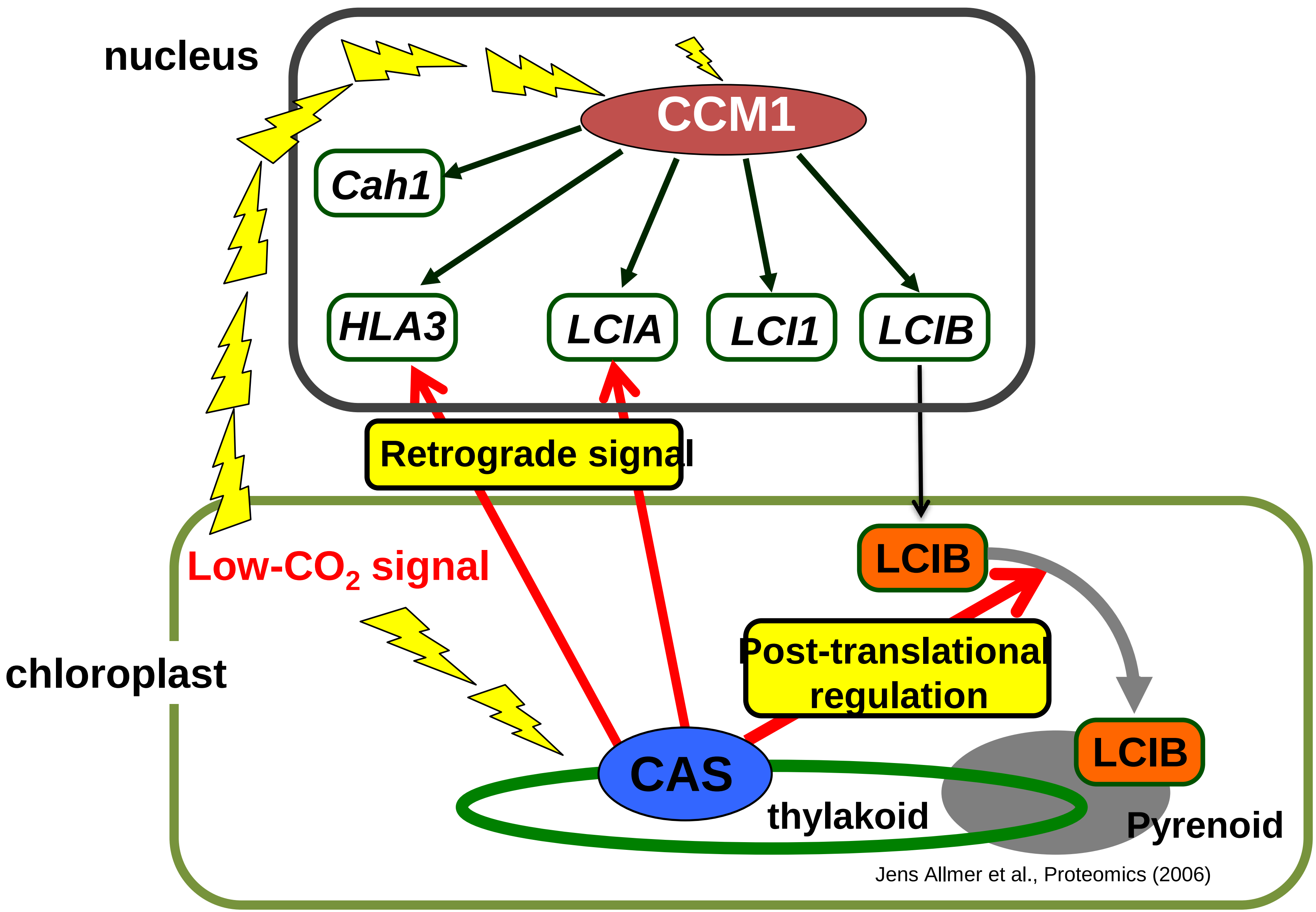
3) Overexpression of LCIA and HLA3 partially restored the photosynthetic Ci affinity of the CAS mutant.

Based on these findings and the following scheme, draw a possible model of the CO_2-sensing regulatory network in the Chlamydomonas cells.

Previous model of CO_2-dependent regulatory pathway



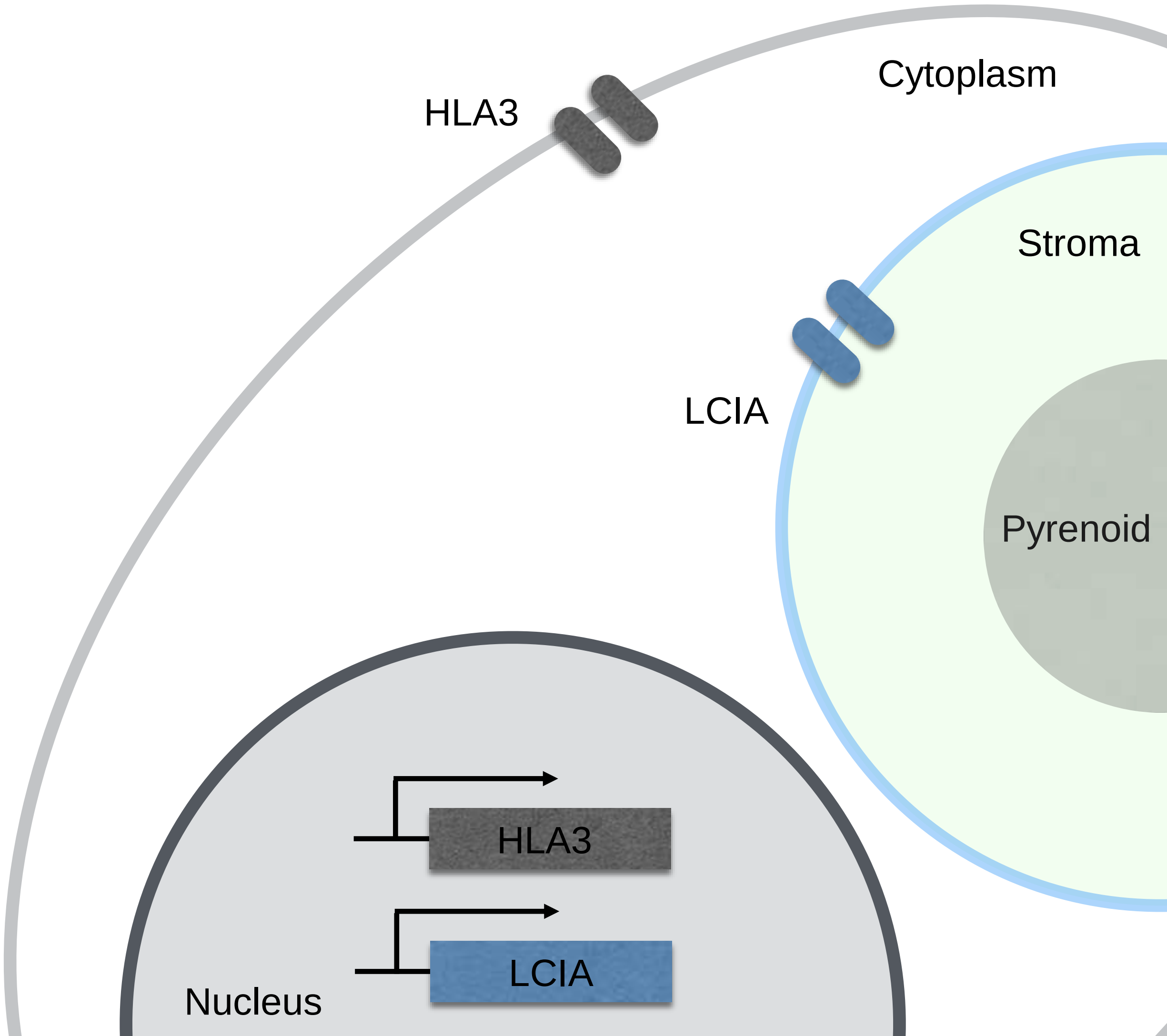
A model of CO_2-dependent regulatory network



Jens Allmer et al., Proteomics (2006)

Conclusion and Discussion

1. *HLA3* and *LCIA* are induced in LC conditions and respective proteins are localised to PM and CE.



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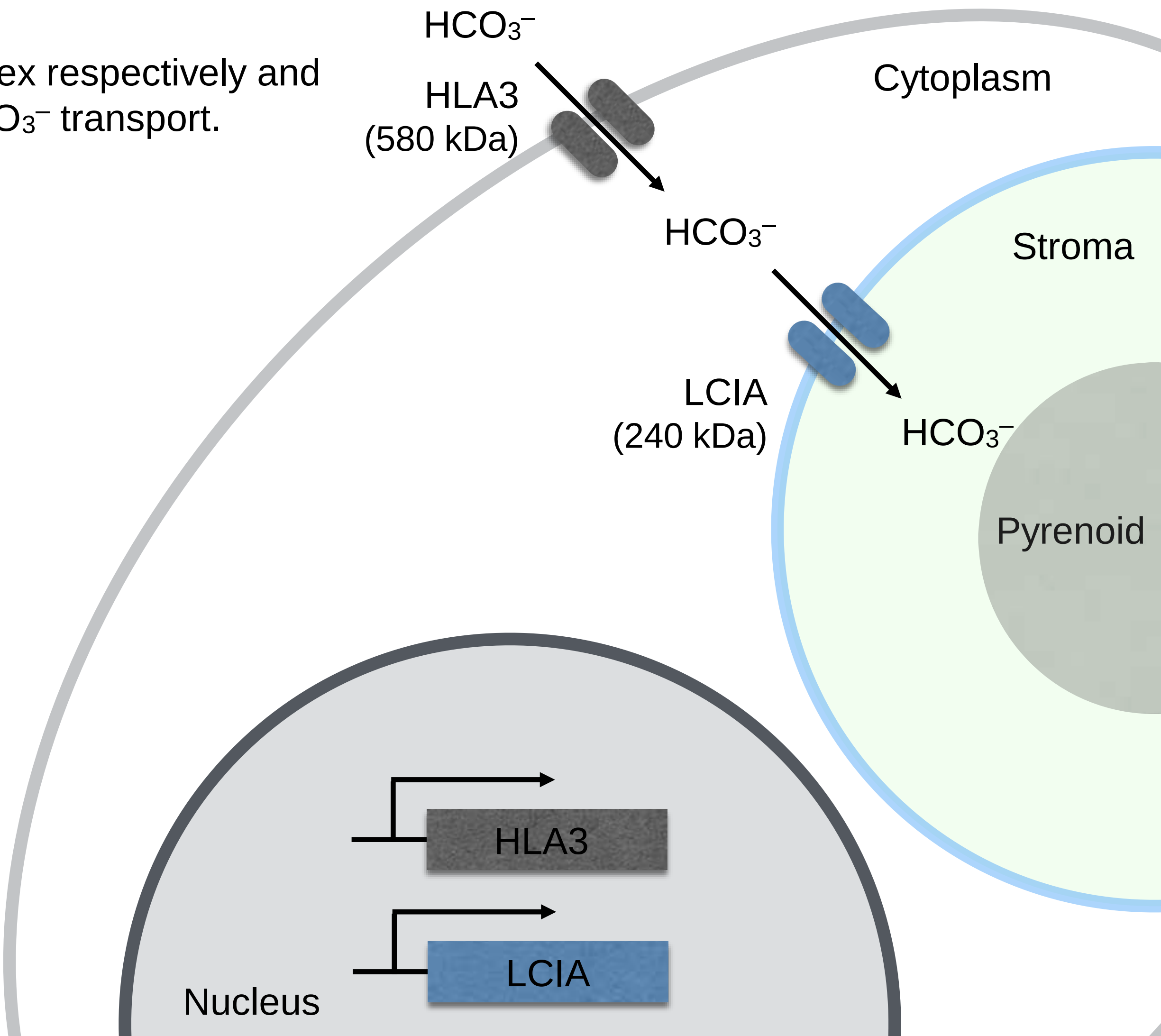
2. HLA3 and LCIA form protein complex respectively and are cooperatively associated with HCO_3^- transport.



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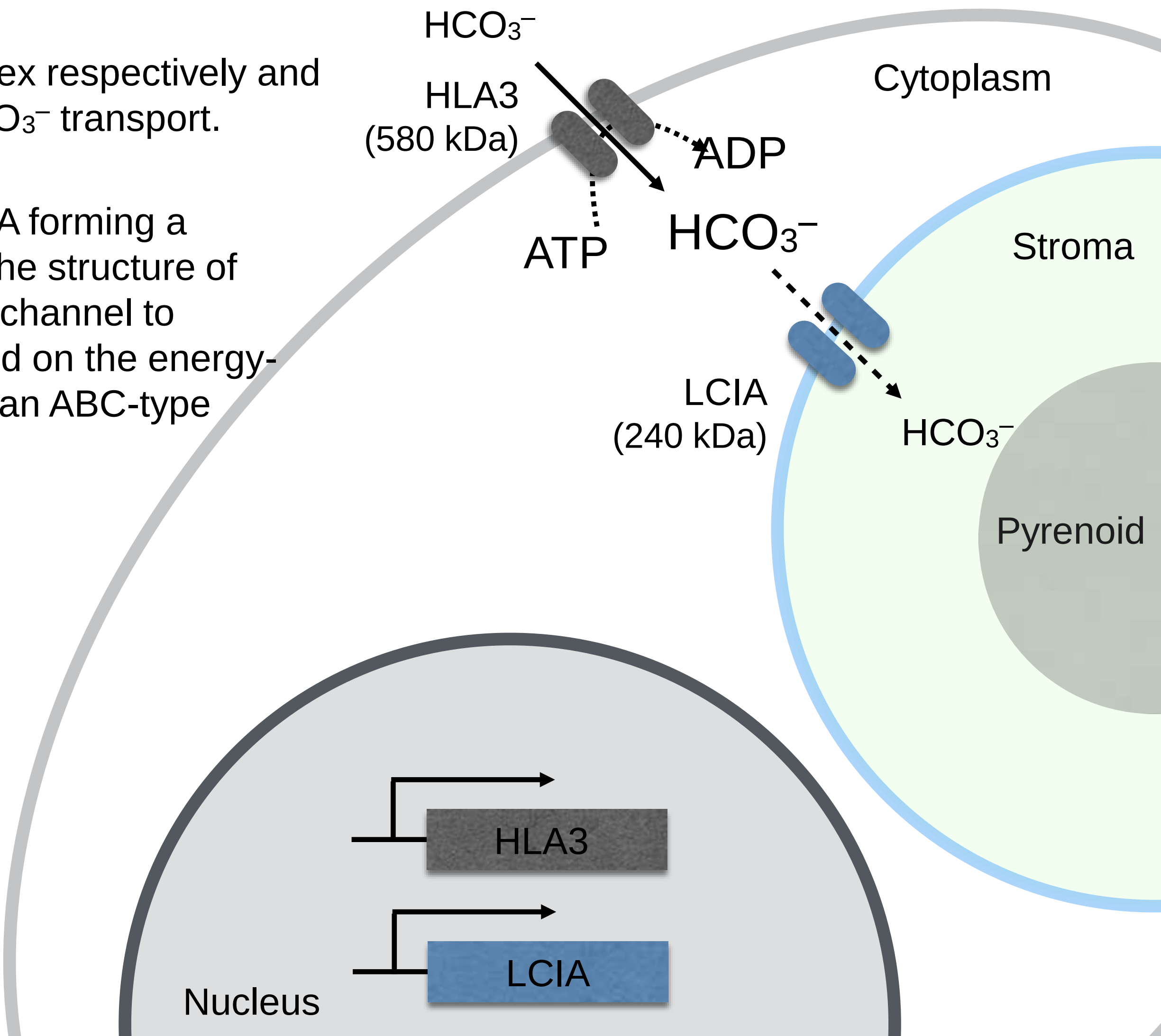
3. Because LCIA is a homolog of FocA forming a symmetric pentamer that resembles the structure of aquaporin, LCIA could functions as a channel to transport HCO_3^- passively and depend on the energy-dependent active transport by HLA3, an ABC-type transporter.



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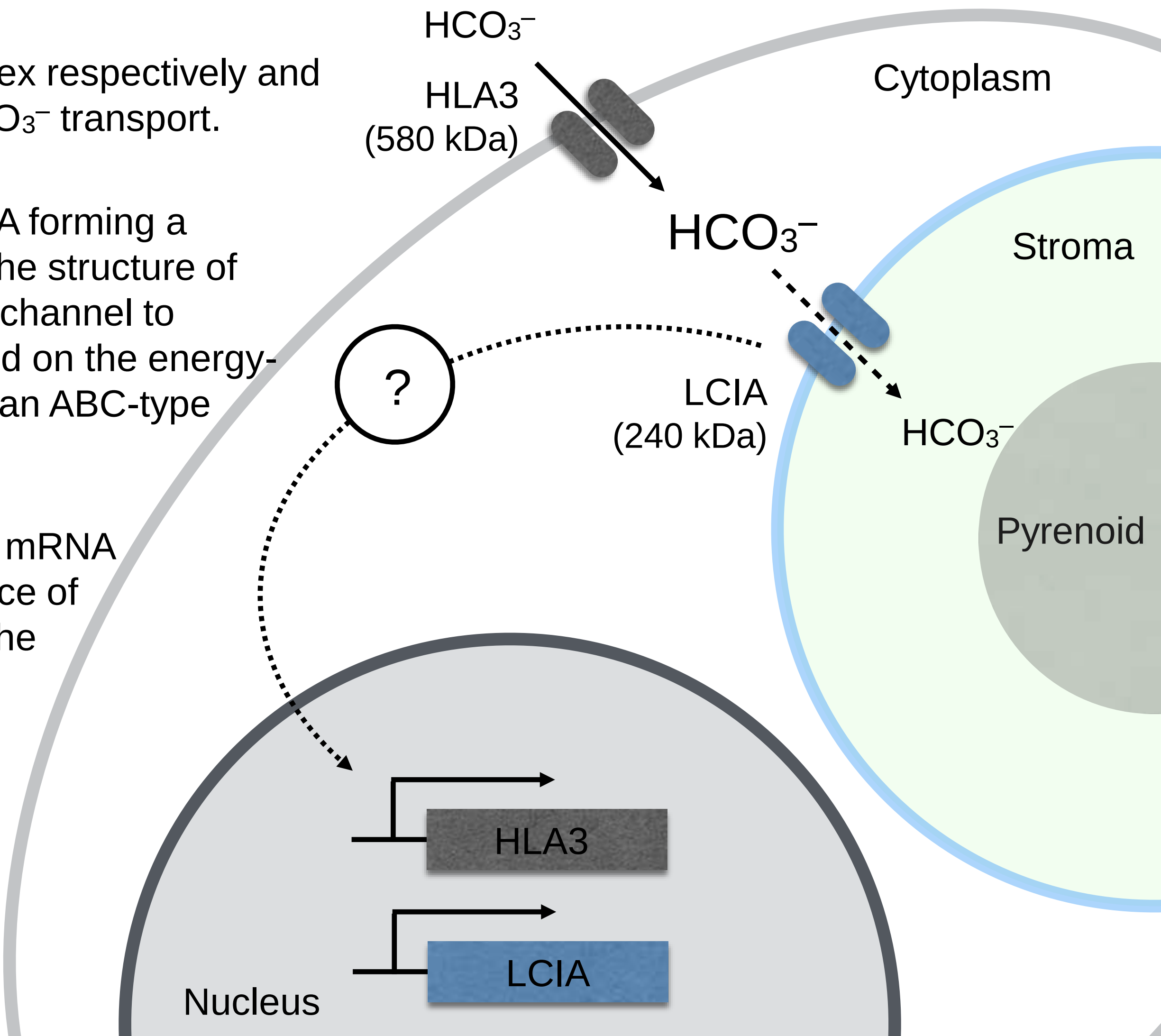
4. Absence of LCIA decreased *HLA3* mRNA accumulation, suggesting the presence of unidentified retrograde signals from the chloroplast to nucleus.



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5. Chloroplast Ca^{2+}-sensor homologue is necessary for induction CCM.