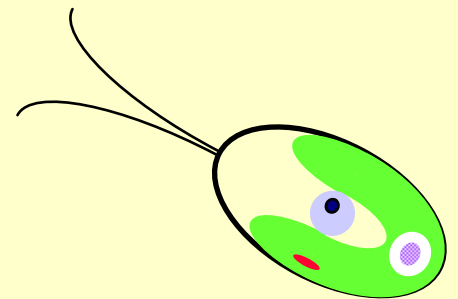
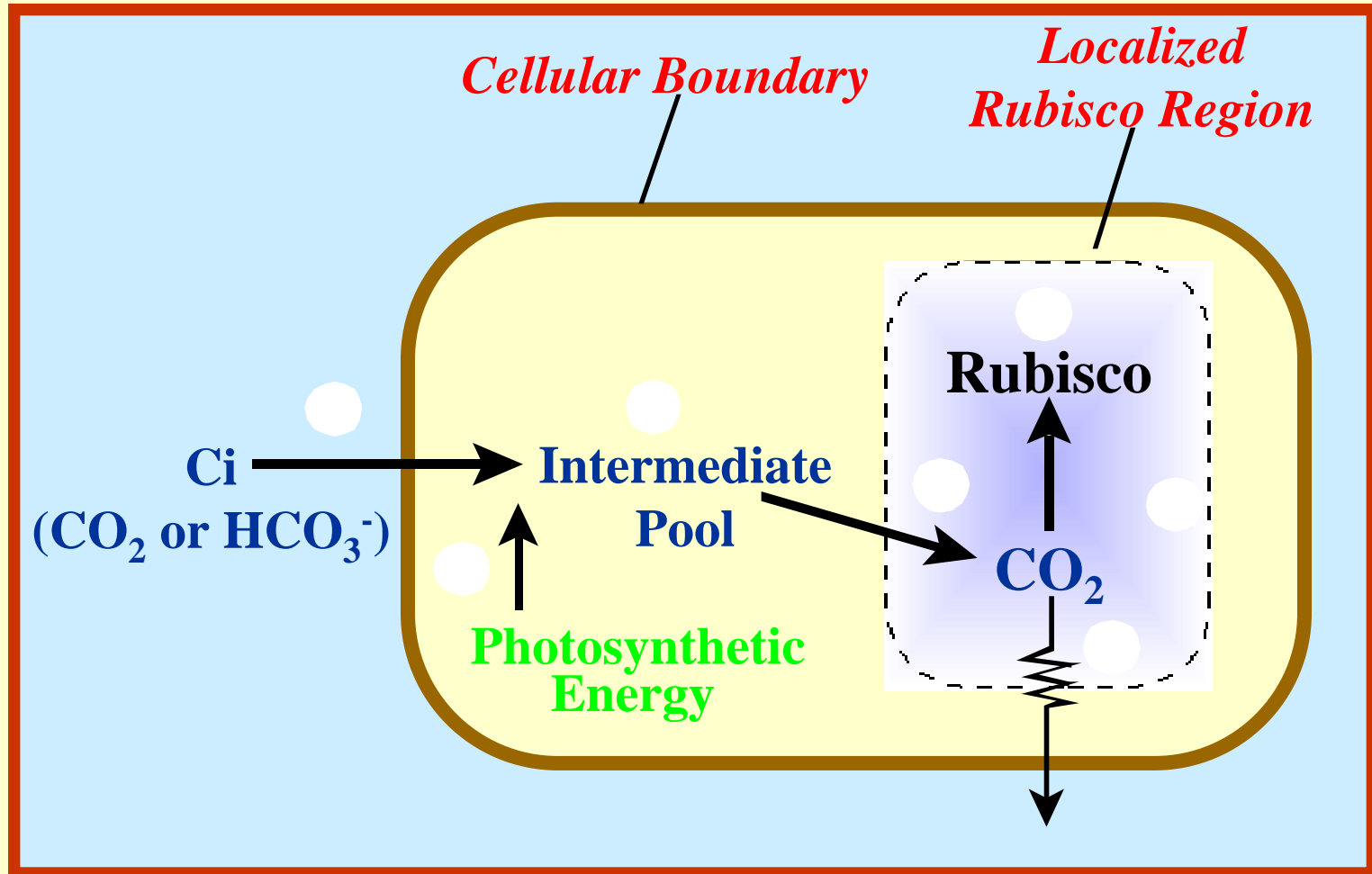

The Role of Inorganic Carbon Transport and Accumulation in the CO₂-Concentrating Mechanism and CO₂ Assimilation in Chlamydomonas

Is there a Role for the CCM in Increasing Biological CO₂ Capture?



Generalized CO₂ Concentrating Mechanism (CCM)



Chlamydomonas reinhardtii

Why *Chlamydomonas*?

- **Fast, convenient growth**
 - Generation time 6h
 - Colonies on plates
 - Small size (~10 μm)
- **Genomics:**
 - Small, sequenced genome (~100 Mb)
 - Easy to transform (all 3 genomes)
- **Classical genetic organism**
 - Large collection of existing mutants
 - Two mating types; tetrad analysis
 - Normally haploid
 - Can grow as diploid
 - Positional cloning possible

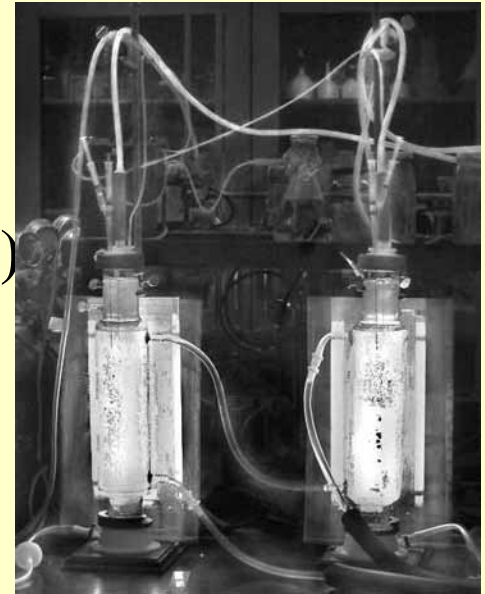


What Do We Know About the Chlamydomonas CCM?

- **Inducible by limiting CO₂ (repressed in high-CO₂); multiple acclimation states**

Multiple Acclimation States

- **CO₂ concentration ranges defined physiologically**
 - High CO₂ (H-CO₂): $\geq 0.5\%$ CO₂
 - Low CO₂ (L-CO₂): 0.03% - 0.4% CO₂
 - Very Low CO₂ (VL-CO₂): $\leq 0.01\%$ CO₂
- **L-CO₂ state**
 - Increased affinity for Ci (inorganic carbon)
 - Induction/up-regulation of many genes
 - CCM activity
 - Low affinity/high capacity Ci transport?
- **VL-CO₂ state**
 - Decreased photosynthetic V_{max}
 - Further increased photosynthetic affinity for Ci
 - High affinity/low capacity Ci transport?

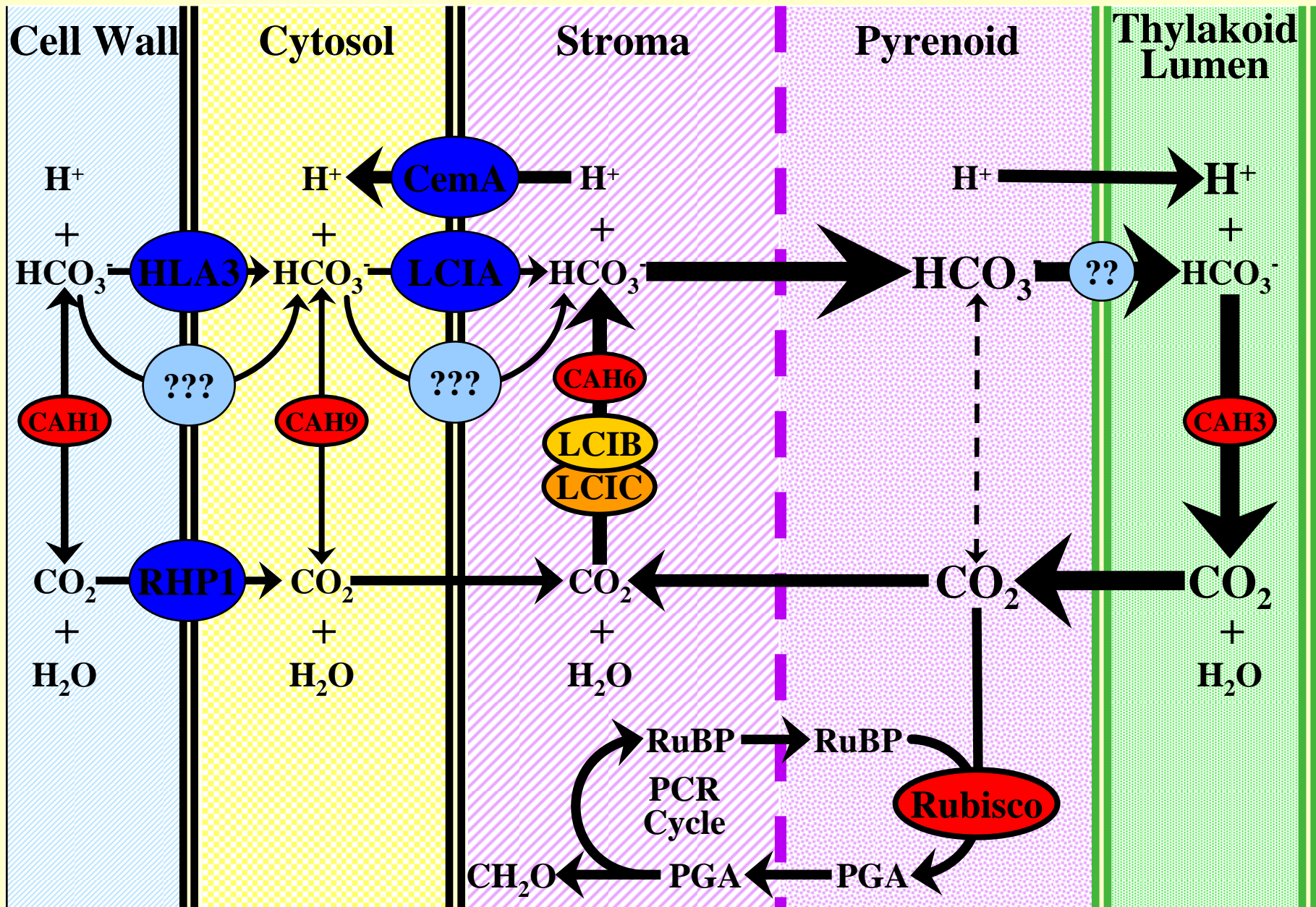


What Do We Know About the Chlamydomonas CCM?

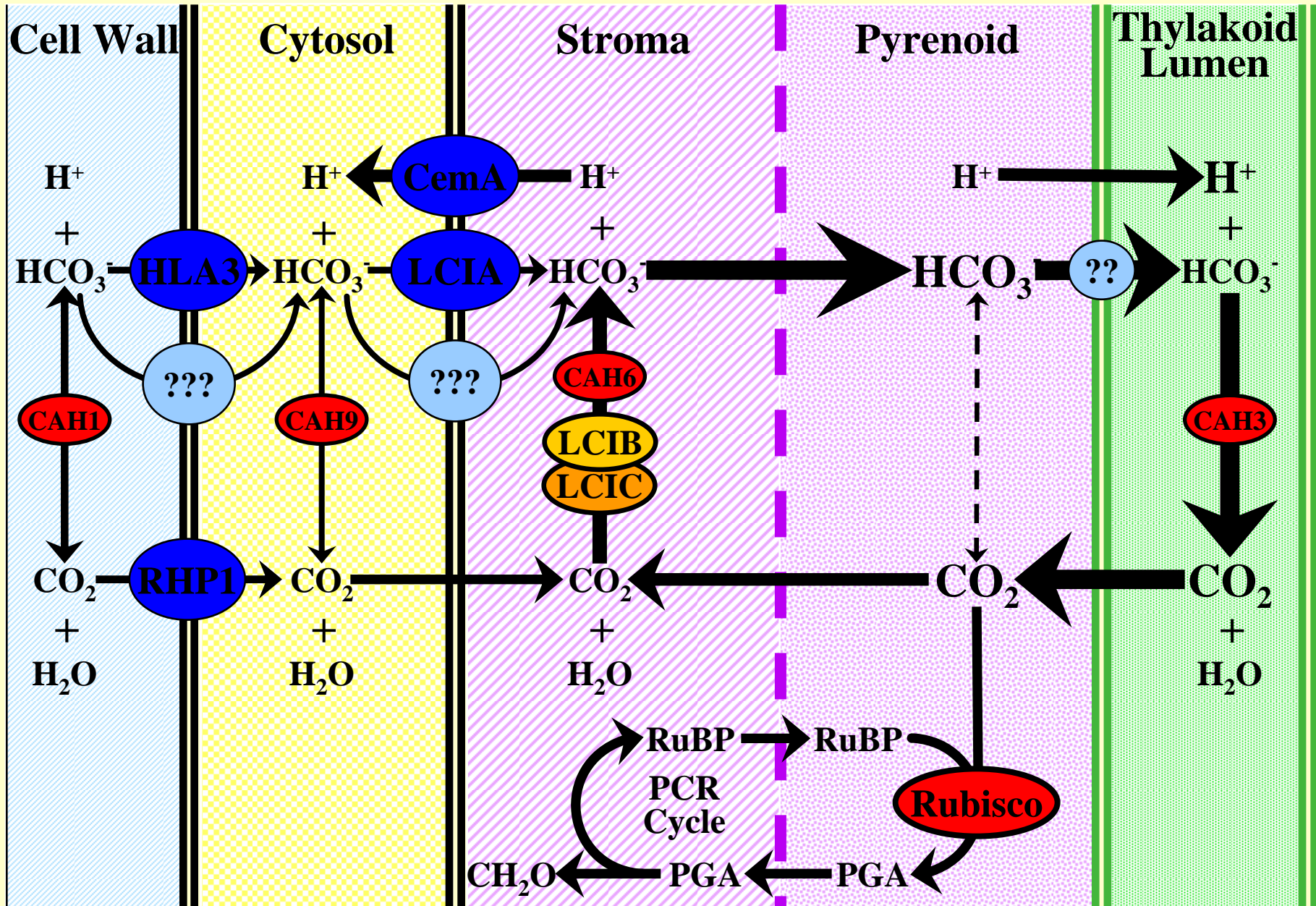
- Inducible by limiting CO₂ (repressed in high-CO₂); multiple acclimation states
- **Requires Ci transporters:**
 - HLA3 (ABC transporter family)
 - LCIA (Formate/Nitrite transporter family)
- **Requires Carbonic anhydrases:**
 - CAH3 (thylakoid lumen CA) required for dehydration of accumulated HCO₃⁻, 8 more CAs with uncertain roles
- **Requires LCIB**
 - soluble chloroplast protein; no recognizable domains
 - required for Ci accumulation in L-CO₂ – “air-dier”
 - “air-dier” mutant phenotype suppressed by CAH3 mutations
 - CO₂ trap, by conversion to HCO₃⁻?

(Wang & Spalding, 2006 *PNAS*; Duanmu et al., 2009 *Plant Physiol*; 2009 *PNAS*)

Schematic Model of Low CO₂ CCM



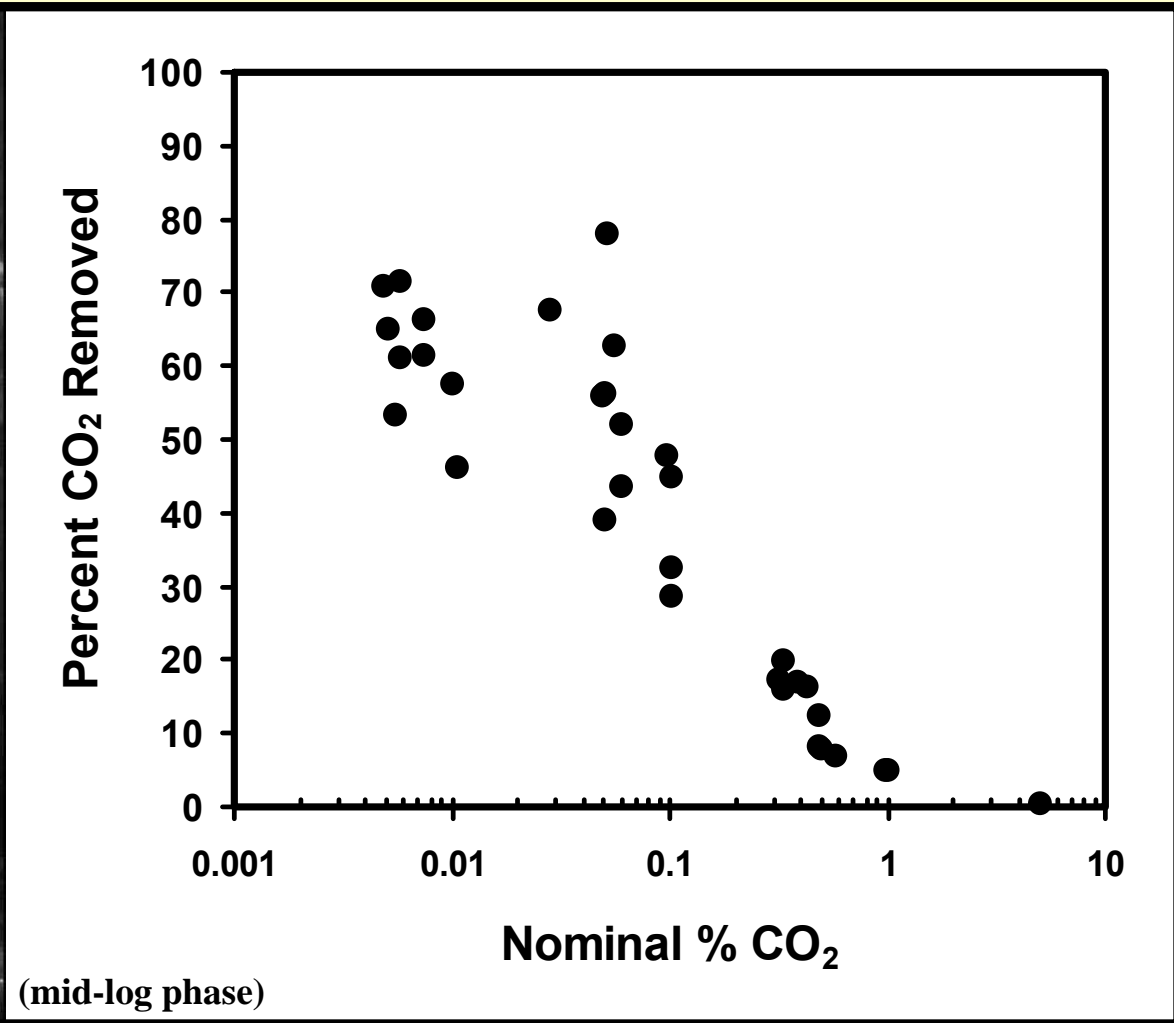
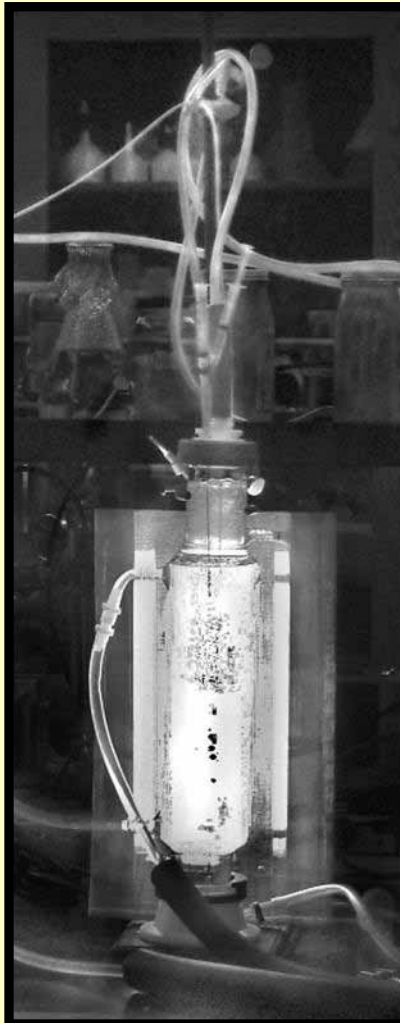
Schematic Model of Low CO₂ CCM



Is there a Role for the CCM in Increasing Biological CO₂ Capture?

- **Is a CCM essential?**
 - Poor growth of CCM mutants, even in H-CO₂ acclimated cells
- **Lessons from CCM studies**
 - Avoid VL-CO₂ range; high affinity but low V_{max}, smaller cell size
 - Could occur in systems without CO₂ enrichment
 - Draw down in CO₂ concentrations, especially at high cell densities

CO₂ Draw-Down

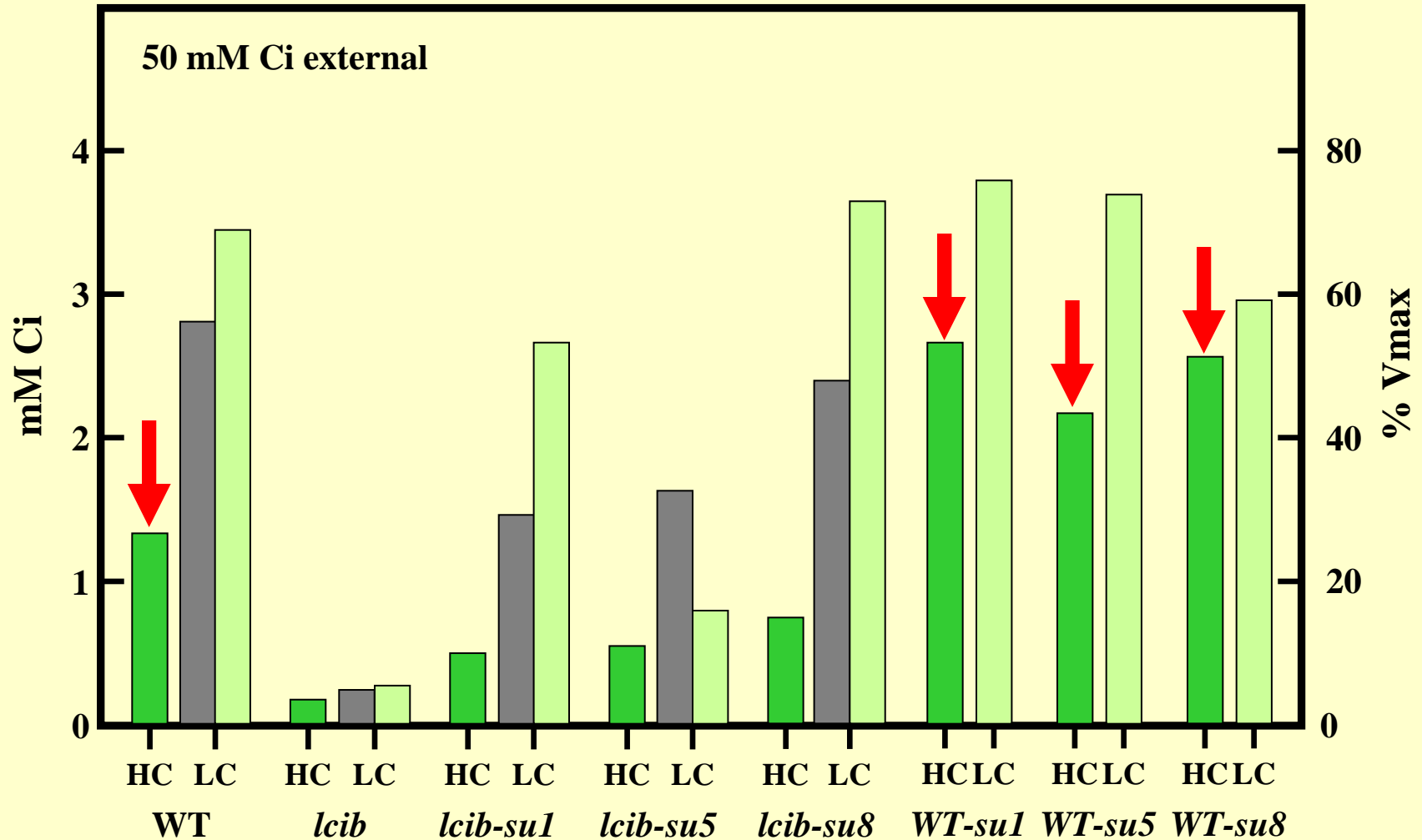


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- **Can the CCM be improved for commercial applications?**
 - **Enhanced H-CO₂ state activity in *lcib* suppressors – could it help at high densities?**

Photosynthesis and Ci Uptake

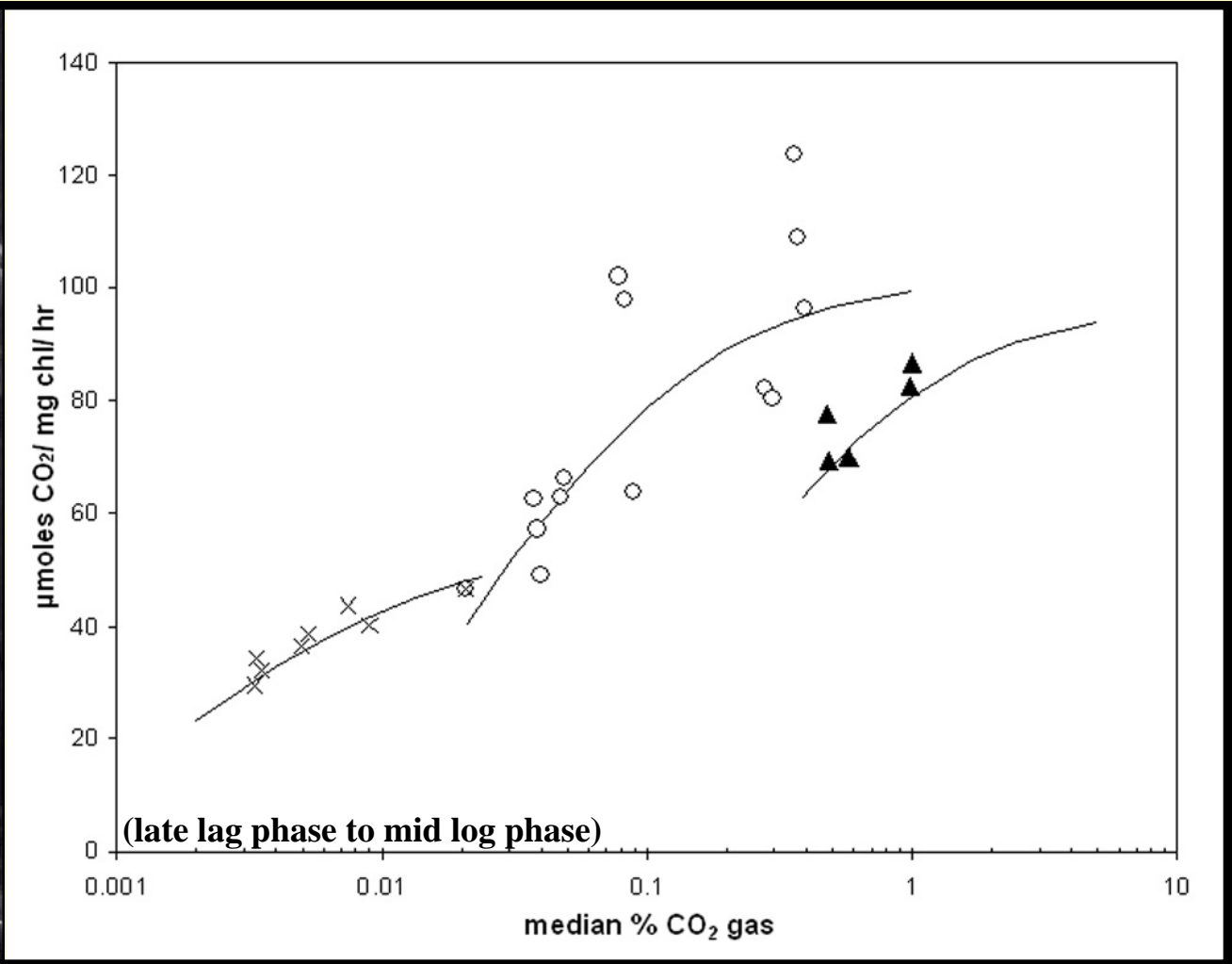
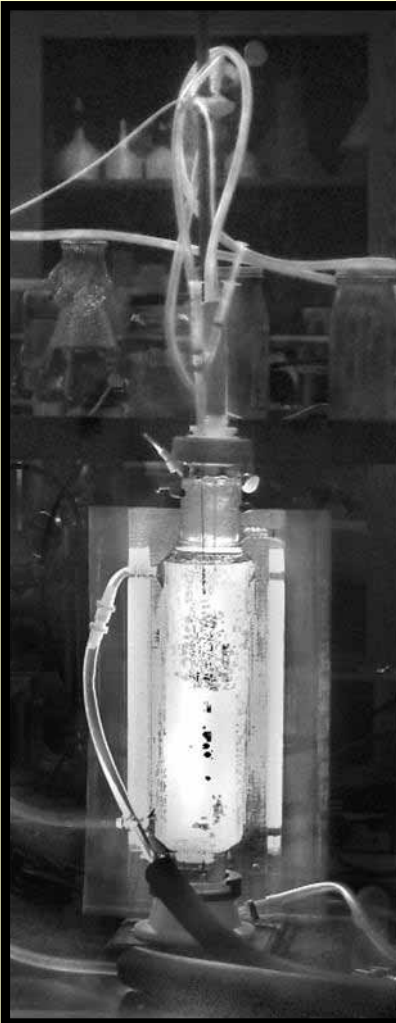
lcib Suppressors, *su1*, *su5* & *su8*



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 - **Could it help at high densities (draw-down in CO₂)?**

Drop-off in Photosynthesis at High CO₂

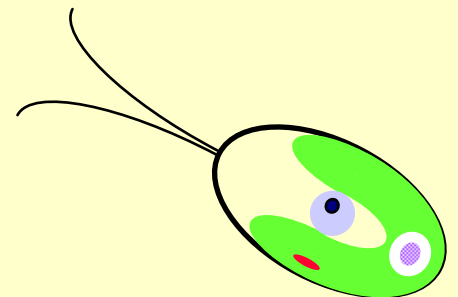


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 - Could it help at high densities (draw-down in CO₂)?
 - **Enhance CCM function in L-CO₂ by over-expression of key genes?**

Acknowledgements

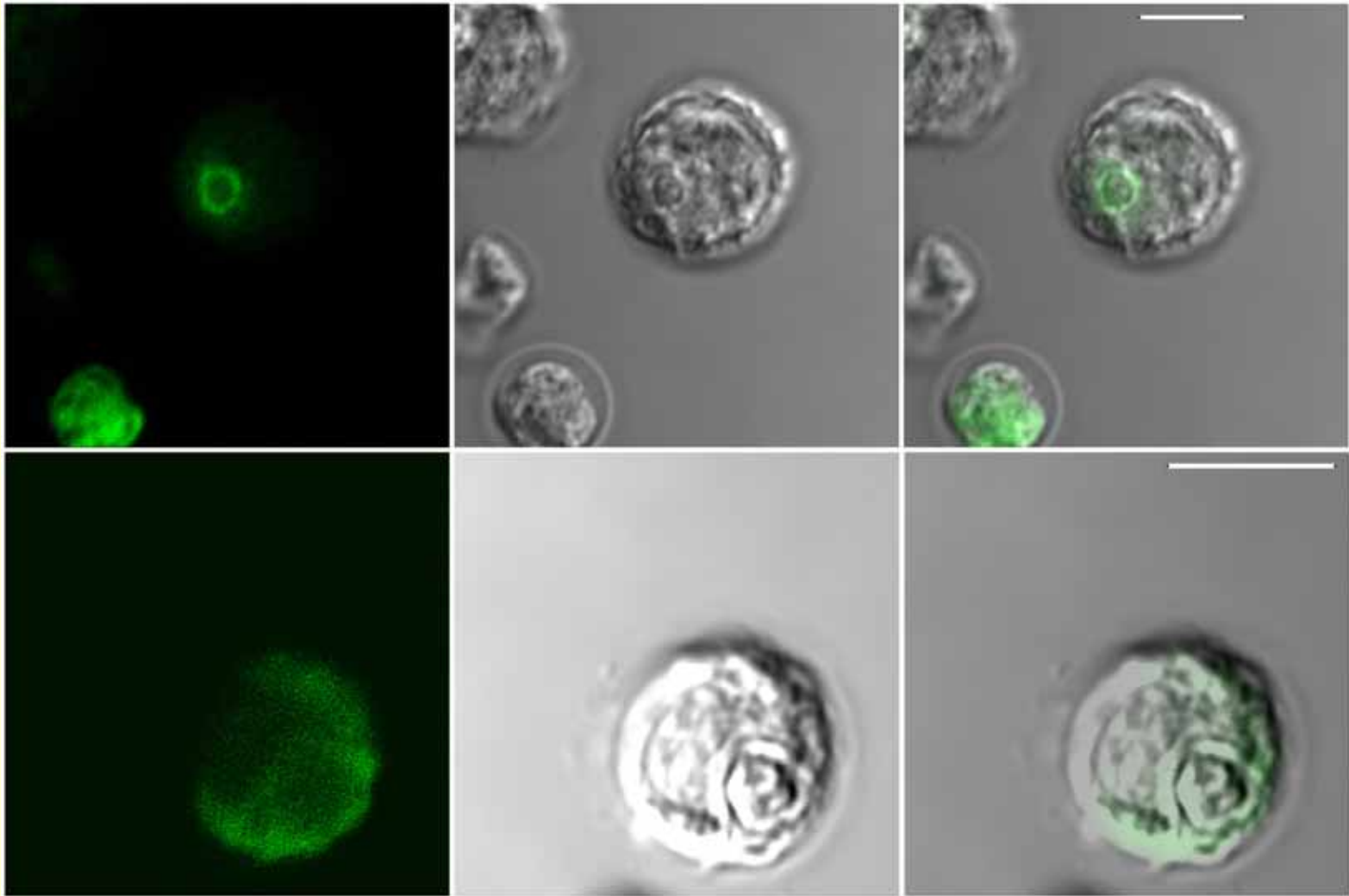
- **Spalding Lab**
 - Deqiang Duanmu
 - Yingjun Wang
 - Wei Fang
 - Han Guo
 - Bo Chen
 - Peter Vance
 - Kyujung Van
- **Don Weeks Lab (Nebraska)**
 - Kemp Horken
 - Amy Miller



LCIB Characteristics

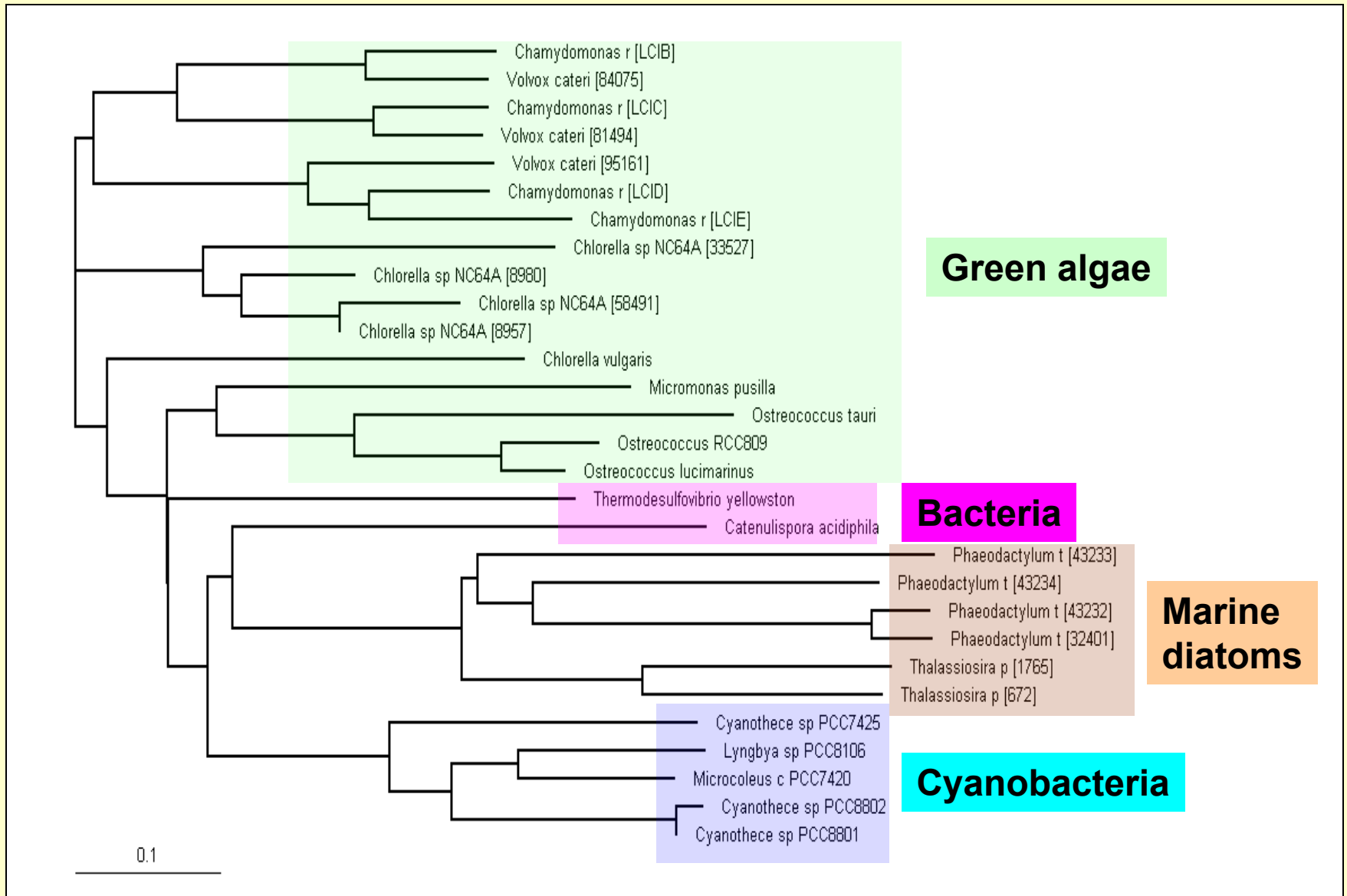
- **LCIB - limiting-CO₂ inducible, soluble chloroplast protein**
 - No recognizable domains
- ***LCIB* mutants: air-dier phenotype**
 - Grow in H-CO₂ (>0.5%) & VL-CO₂ (0.03-0.4%); die in L-CO₂ (<0.01%)
 - Mutants can accumulate Ci internally in VL-CO₂ but not in L-CO₂
 - Ci transport/accumulation defective specifically in L-CO₂
 - Not essential in H-CO₂ & VL-CO₂ but clearly important in both
- **LCIB interactions & localization**
 - Forms complex with LCIC (Yeast 2-hybrid; pull-down assays)
 - Localization in L-CO₂ & VL-CO₂ changes – what function?
- ***LCIB* gene family in *Chlamydomonas***
 - *LCIB*, *LCIC*, *LCID* & *LCIE*, limiting-CO₂ induced
 - Homologs only in green microalgae, diatoms & cyanobacteria
 - High similarity and many conserved regions

LCIB Localization

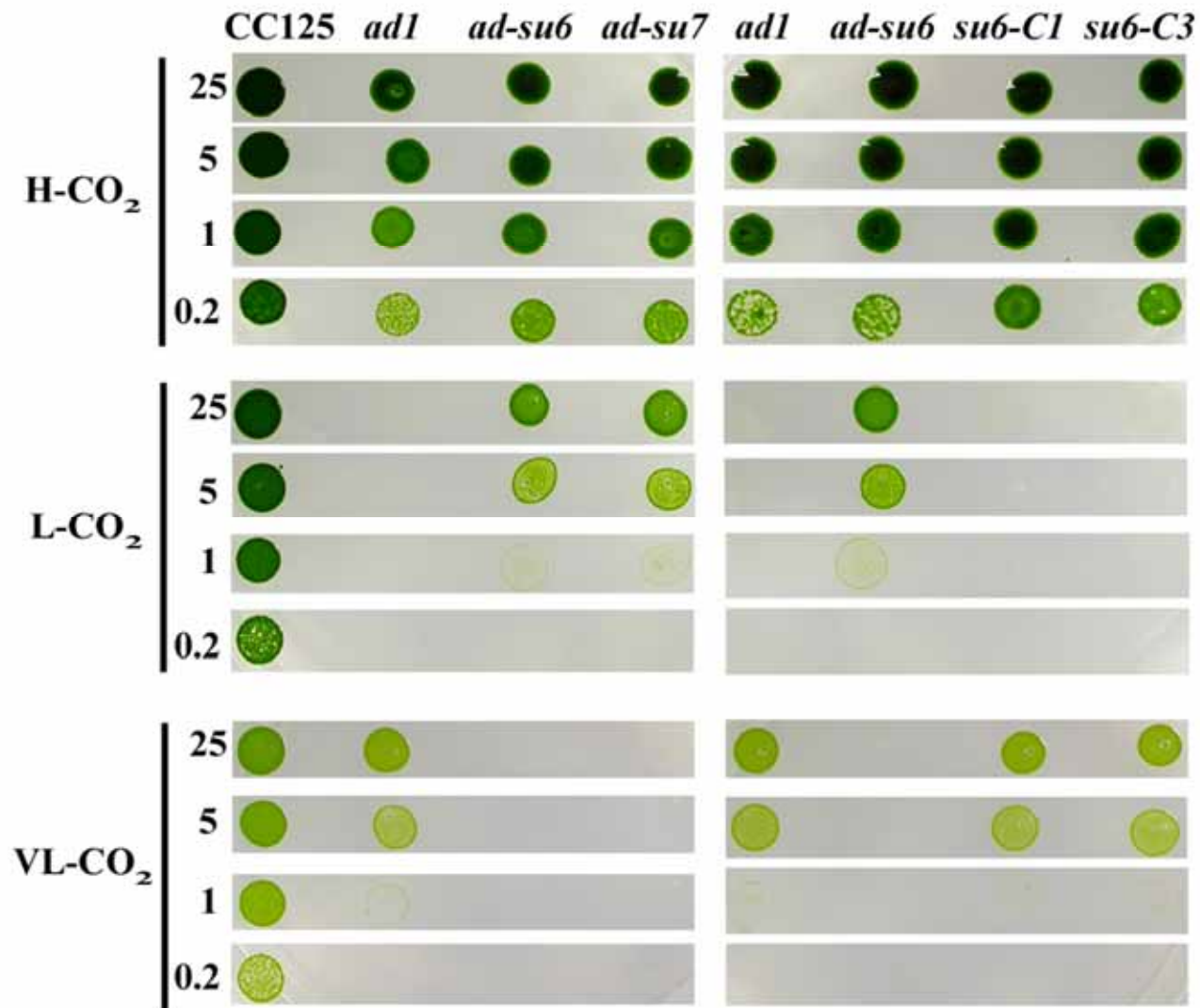


LCIB immunofluorescent localization. Top row: VL-CO₂ cells. Bottom row: L-CO₂ cells. Arrow: pyrenoid. Bars = 5 μm.

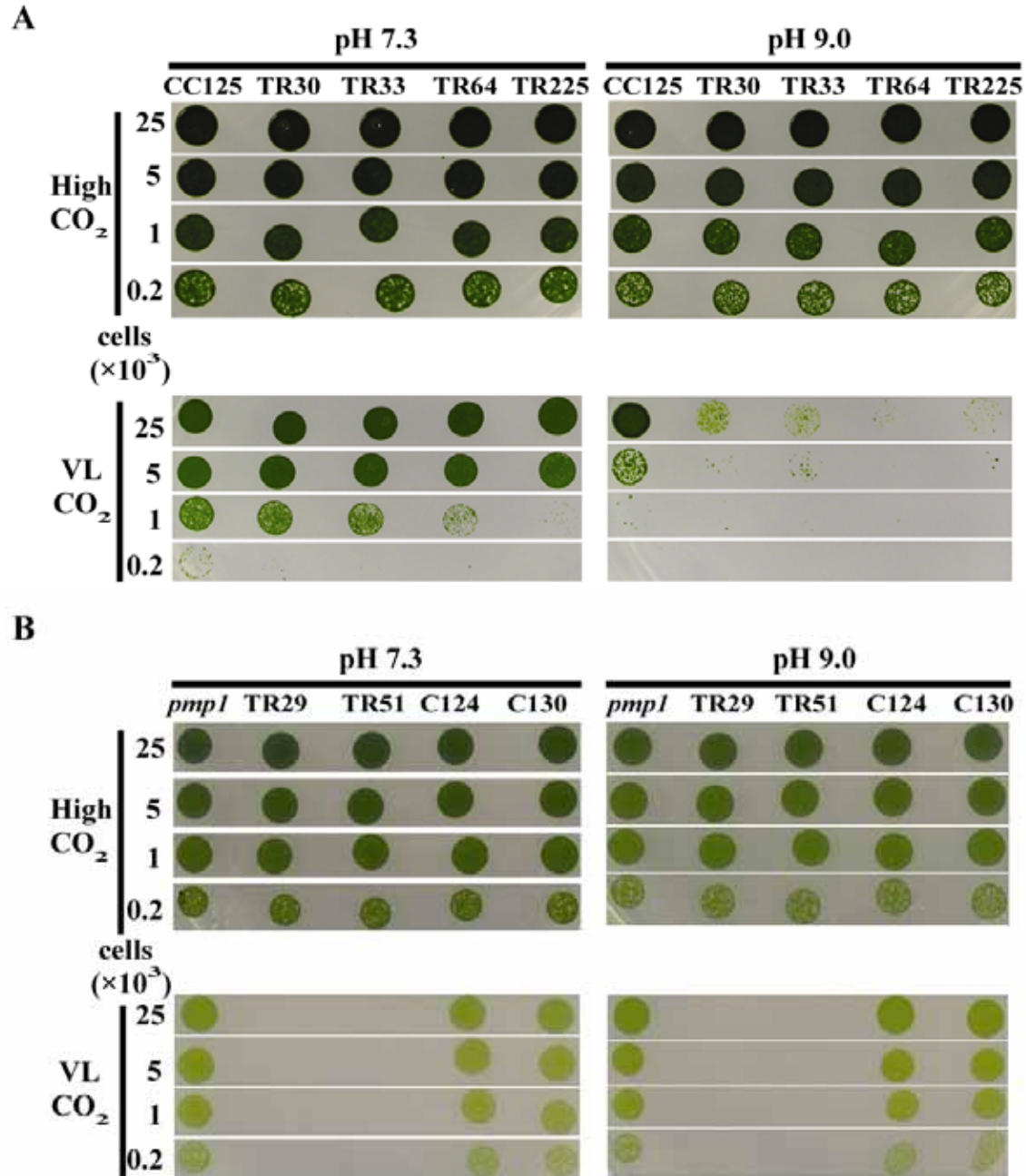
Distribution of LCIB-like Genes



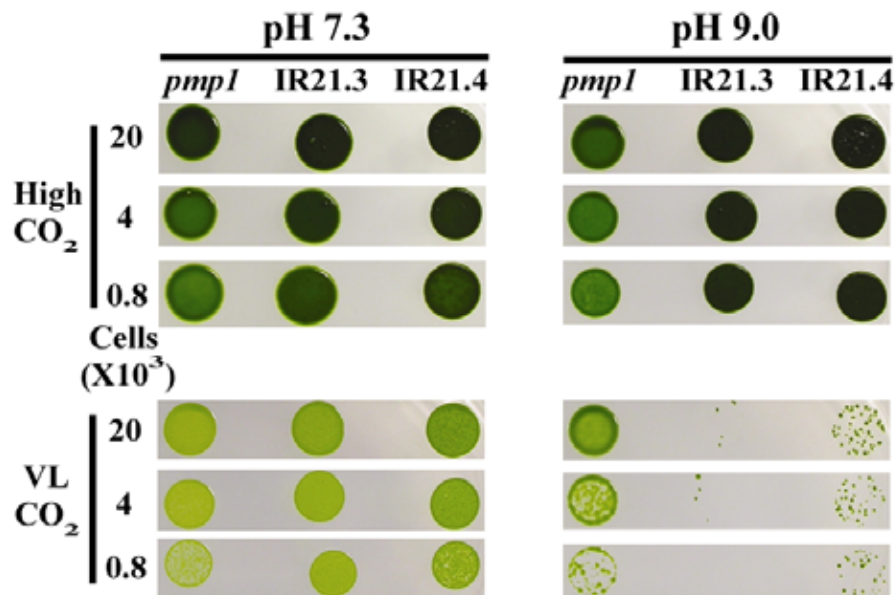
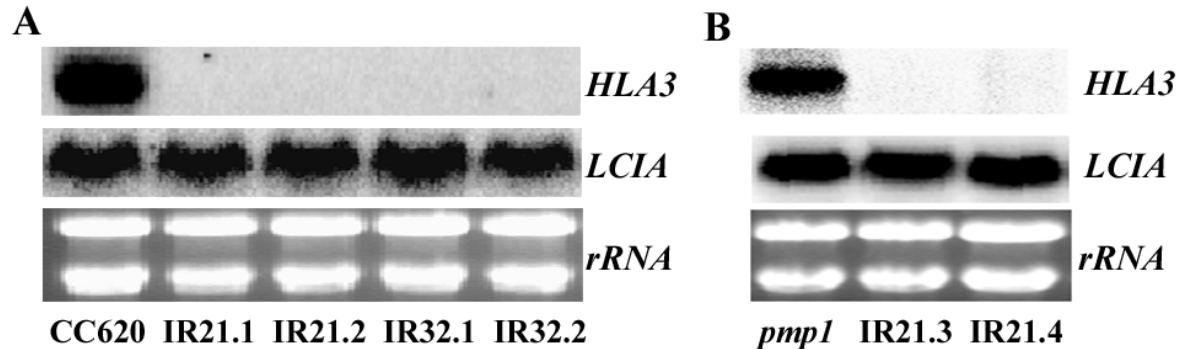
Suppressors of *LCIB* Mutations



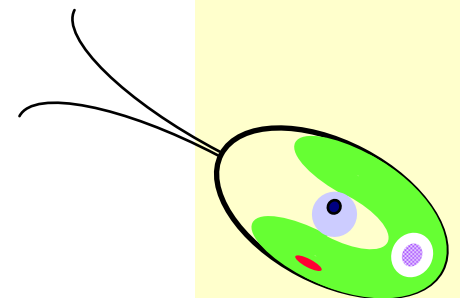
Growth of HLA3 IR-RNAi Knockdowns



HLA3 IR-RNAi Knockdowns Target *HLA3* Specifically

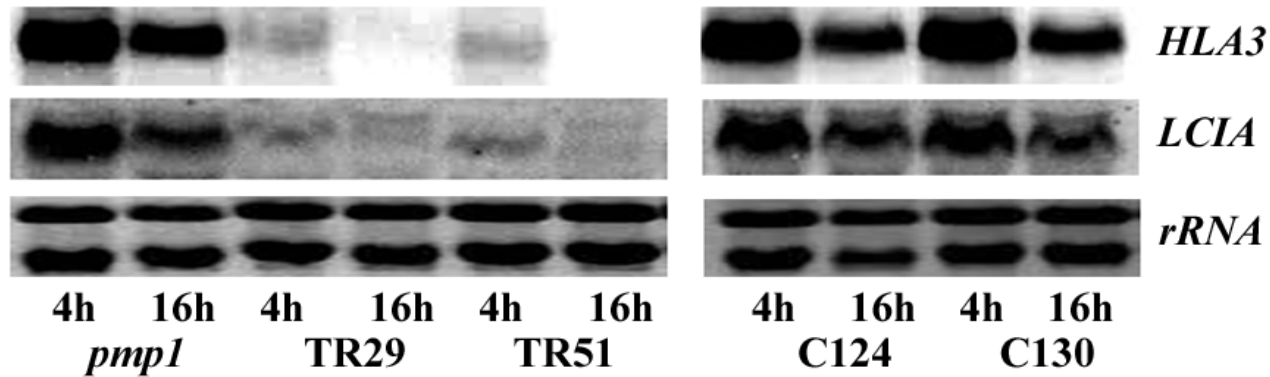


Growth defect only
at high pH

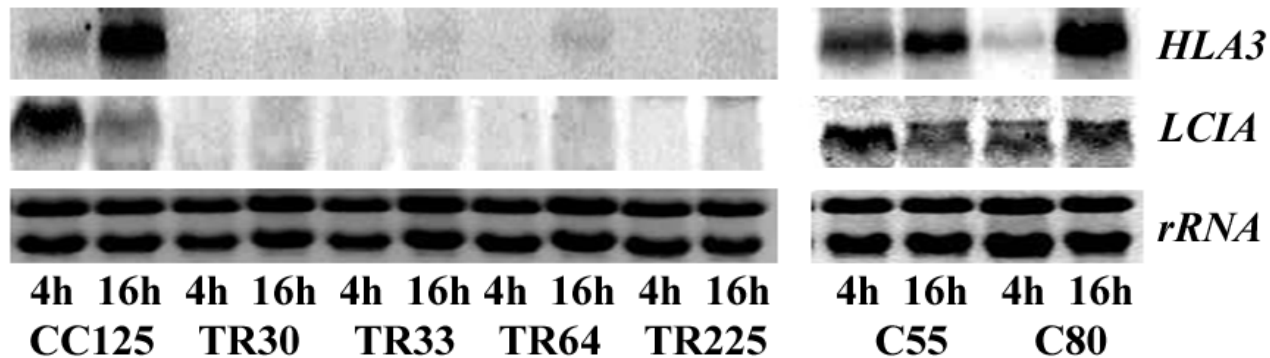


HLA3 TR-RNAi Knockdowns Also Decrease *LCIA* mRNA

A



B

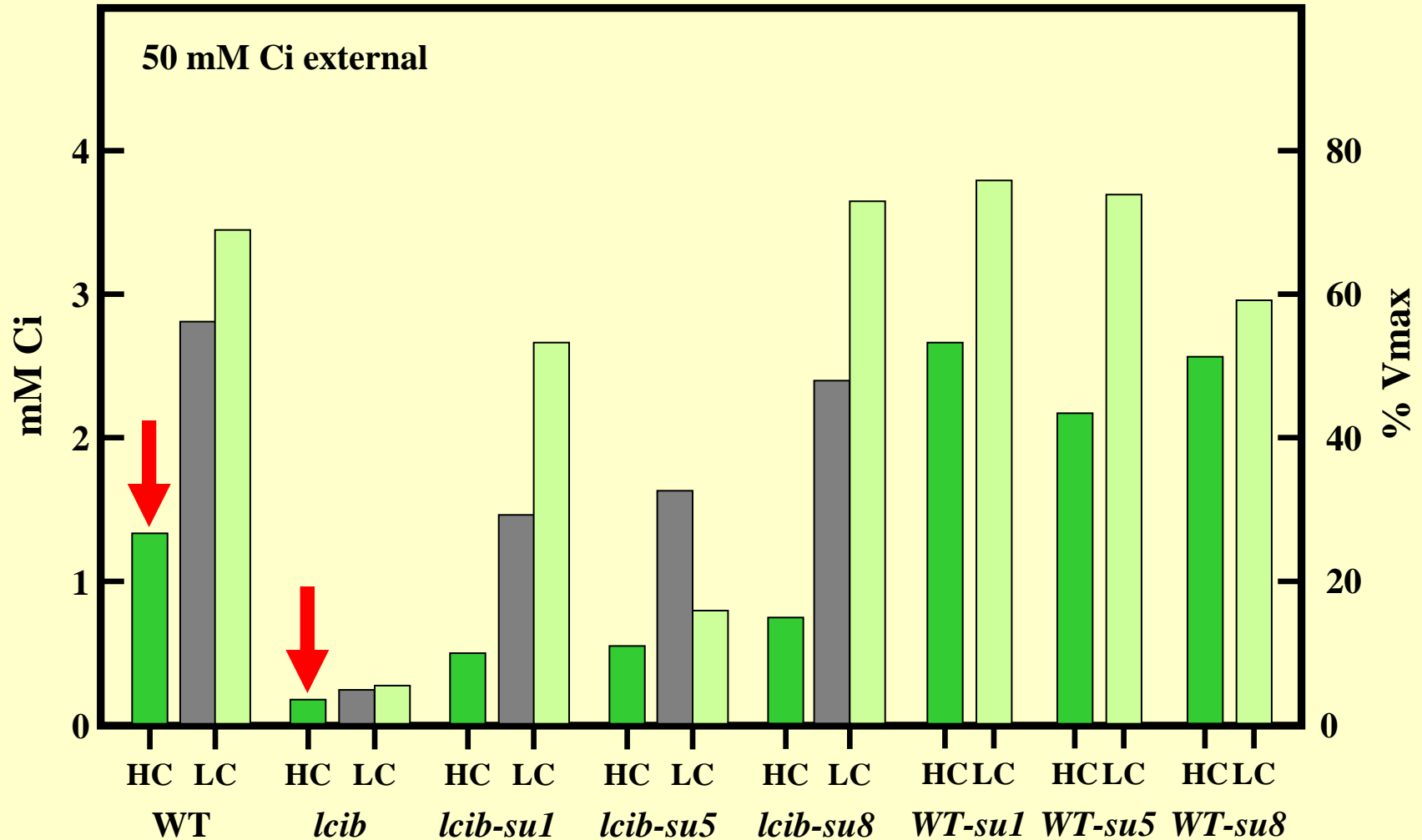


Suppressors of *LCIB* Mutations

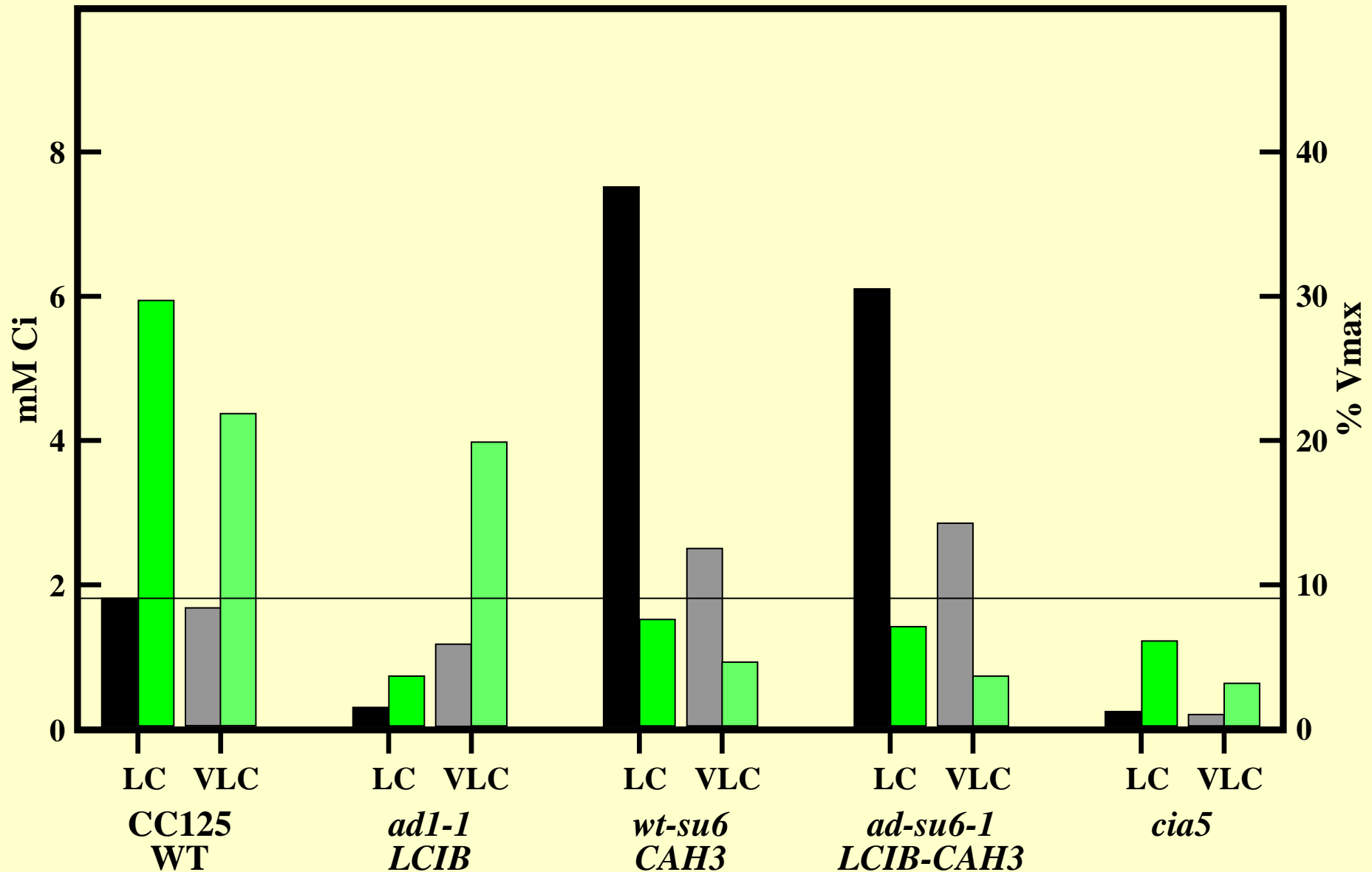
- Six suppressors from insertional mutagenesis
- *su1*: WT-like phenotype; recessive mutation; unlinked
- *su4/su5*: intermediate phenotype; dominant mutation; unlinked
- *su8*: WT-like phenotype; linked to insert; probable disrupted gene gene no homology to known genes
- *su6* & *su7*:
 - both suppress “air-dier” phenotype in LC but die in VLC; *su6* linked; *su7* unlinked
 - both defective in thylakoid lumen carbonic anhydrase, CAH3

Photosynthesis and Ci Uptake

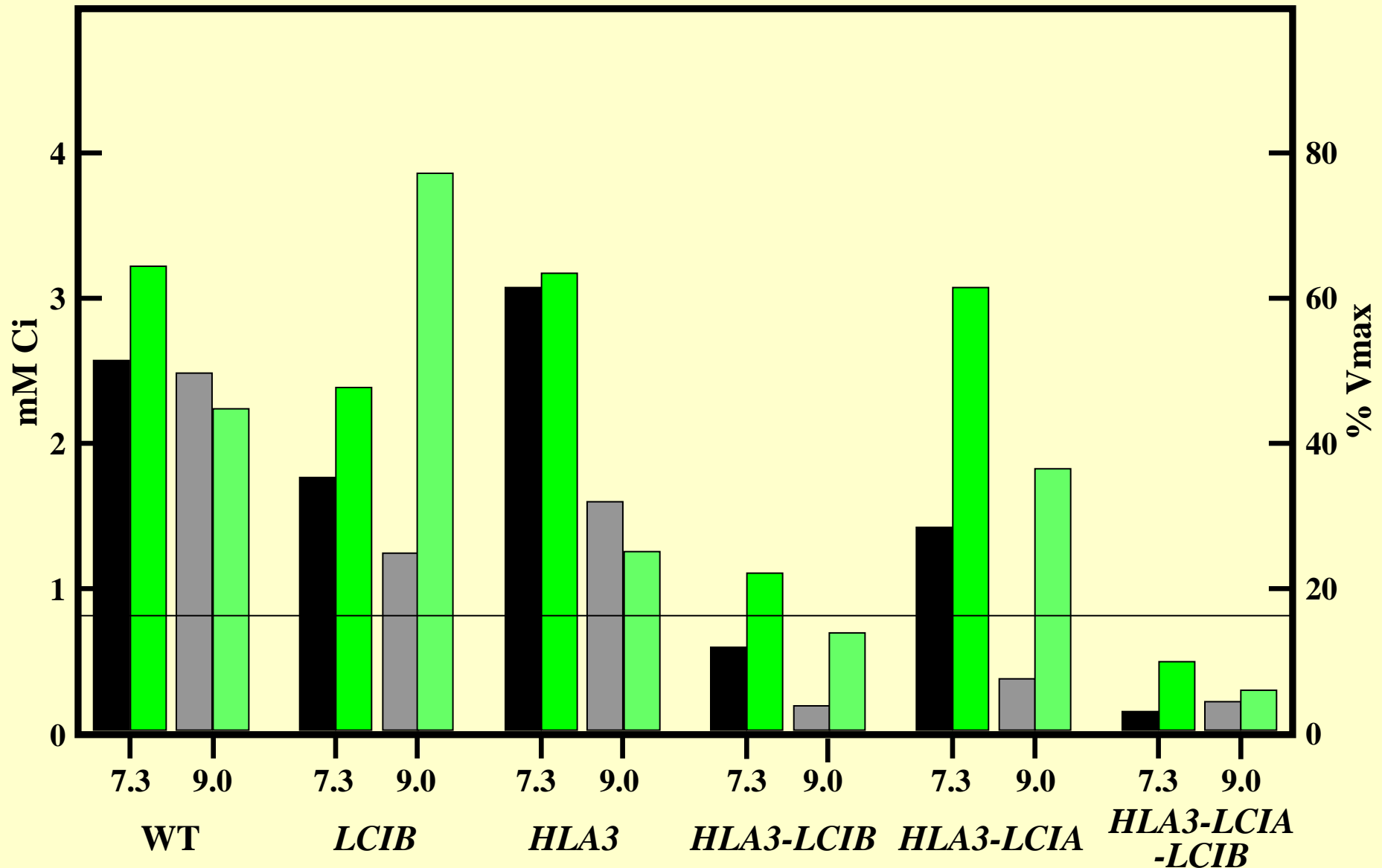
lcib Suppressors, *su1*, *su5* & *su8*



Photosynthesis and Ci Uptake



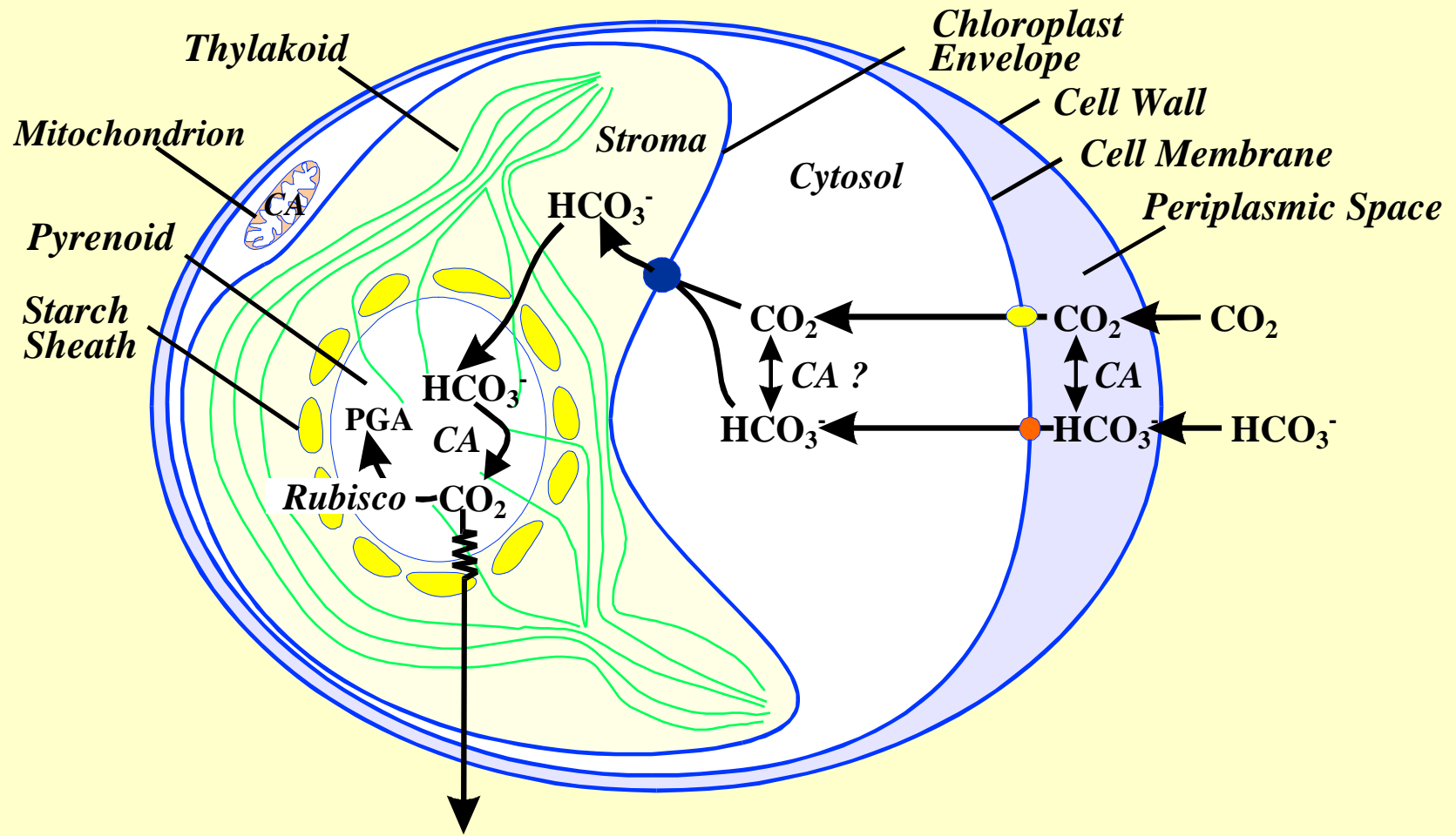
Photosynthesis and Ci Uptake



Inorganic Carbon (Ci) Availability

- **Enormous variation in aquatic systems**
 - **Nearly zero to 5-10% CO₂**
- **Dependent on CO₂/HCO₃⁻ source, pH, mixing, consumption, etc.**
- **Effect of pH: variation in CO₂ vs HCO₃⁻ vs CO₃⁻²**
- **Aquatic organisms typically must acclimate to variable Ci availability**
 - **Ci scavenging via an inducible CCM when limiting**
- **CO₂ diffusion in water 10⁴ fold slower than in air**

The CCM in Chlorophyte Algae



Ci Transporter Candidates

- ***CCP1/CCP2*: chloroplast envelope proteins**
 - Mitochondrial transporter superfamily
 - RNAi knockdown results in minor growth phenotype
- ***LC11*: predicted membrane protein; Ci regulated**
 - No domains & no identified homologs even in other microalgae
- ***LC1A*: predicted chloroplast envelope protein**
 - Closely related to nitrite transporters
 - Reported to confer bicarbonate transport in *Xenopus* oocytes
- ***HLA3/MRP1*: predicted plasma membrane protein**
 - ABC transporter of Mrp family
- ***LC1B*: predicted soluble chloroplast protein; Ci regulated**
 - Homologs found in green microalgae, diatoms & cyanobacteria
 - Mutants grow in HC & VLC but die in LC (air-dier phenotype)
 - Mutants unable to accumulate Ci internally in LC but not VLC

Other Eukaryotic CCMs

- **Is the Chlamydomonas CCM a good model for other eukaryotic CCMs?**
 - **LCIB broadly represented in green algae and diatoms – not in red algae or other eukaryotes**
 - **HLA3 & LCIA, distribution not clear, because the gene families also play other roles**
 - **Basic mechanism may be similar but details probably vary (Cyanobacterial model)**