The Role of Inorganic Carbon Transport and Accumulation in the CO$_2$-Concentrating Mechanism and CO$_2$ Assimilation in Chlamydomonas

Is there a Role for the CCM in Increasing Biological CO$_2$ Capture?
Generalized CO₂ Concentrating Mechanism (CCM)

Badger & Spalding 2000 Photosynthesis: Physiology and Metabolism, pp. 369-397
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): ≥ 0.5% CO₂
  - Low CO₂ (L-CO₂): 0.03% - 0.4% CO₂
  - Very Low CO₂ (VL-CO₂): ≤ 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 Vmax
  - 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

Cell Wall

H⁺

HCO₃⁻

HLA3

CAH1

CO₂

H₂O

Cytosol

H⁺

CemA

???

CAH9

CO₂

H₂O

Stroma

H⁺

LCIA

???

CAH6

CH₂O

Rubisco

RHP1

CO₂

H₂O

Pyrenoid

H⁺

HCO₃⁻

???

CAH3

CO₂

H₂O

Thylakoid Lumen

AUCR2
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 Vmax, smaller cell size
    - Could occur in systems without CO₂ enrichment
    - Draw down in CO₂ concentrations, especially at high cell densities
CO₂ Draw-Down

Vance & Spalding 2005 Can J Bot 83:796-809
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- Can the CCM be improved for commercial applications?
  - Enhanced H-CO$_2$ state activity in $lcib$ suppressors – could it help at high densities?
Photosynthesis and Ci Uptake

*lcib* Suppressors, *su1*, *su5* & *su8*
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    - Drop off in photosynthesis at 0.5% CO₂, when CCM repressed
    - Could it help at high densities (draw-down in CO₂)?
Drop-off in Photosynthesis at High CO2

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• Can the CCM be improved for commercial applications?
  • Enhanced H-CO₂ state activity in lcib suppressors
    • Drop off in photosynthesis at 0.5% CO₂, when CCM repressed
    • Could it help at high densities (draw-down in CO₂)?
  • Enhance CCM function in L-CO₂ by over-expression of key genes?
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Induction of Gene Expression

![Graphs showing induction of gene expression](image-url)
LCIB Characteristics

- **LCIB** - limiting-CO$_2$ inducible, soluble chloroplast protein
  - No recognizable domains
- **LCIB** mutants: air-dier phenotype
  - Grow in H-CO$_2$ (>0.5%) & VL-CO$_2$ (0.03-0.4%); die in L-CO$_2$ (<0.01%)
  - Mutants can accumulate Ci internally in VL-CO$_2$ but not in L-CO$_2$
  - Ci transport/accumulation defective specifically in L-CO2
  - Not essential in H-CO$_2$ & VL-CO$_2$ but clearly important in both
- **LCIB** interactions & localization
  - Forms complex with LCIC (Yeast 2-hybrid; pull-down assays)
  - Localization in L-CO$_2$ & VL-CO$_2$ changes – what function?
- **LCIB** gene family in *Chlamydomonas*
  - **LCIB, LCIC, LCID & LCIE**, limiting-CO$_2$ induced
  - Homologs only in green microalgae, diatoms & cyanobacteria
  - High similarity and many conserved regions
LCIB Localization

Distribution of LCIB-like Genes

Green algae

Bacteria

Cyanobacteria

Marine diatoms
Suppressors of \textit{LCIB} Mutations
Growth of HLA3 IR-RNAi Knockdowns
**HLA3 IR-RNAi Knockdowns**

Target *HLA3* Specifically

**A**

- HLA3
- LCIA
- rRNA

CC620 IR21.1 IR21.2 IR32.1 IR32.2

**B**

- HLA3
- LCIA
- rRNA

pmp1 IR21.3 IR21.4

**pH 7.3**

- High CO₂
  - 20
  - 4
  - 0.8

- Cells (X10^3)

**pH 9.0**

- pmp1 IR21.3 IR21.4

Growth defect only at high pH
**HLA3 TR-RNAi Knockdowns Also Decrease LCIA mRNA**

![Image of Western Blot Results]

**A**
- **HLA3**
- **LCIA**
- **rRNA**

**B**
- **HLA3**
- **LCIA**
- **rRNA**
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

- CC125 WT
- ad1-1 LCIB
- wt-su6 CAH3
- ad-su6-1 LCIB-CAH3
- cia5

(mM Ci) vs. (% Vmax)
Photosynthesis and Ci Uptake

[Graph showing the relationship between mM Ci and % Vmax for different genotypes (WT, LCIB, HLA3, HLA3-LCIB, HLA3-LCIA, HLA3-LCIA-LCIB) at different concentrations of 7.3 and 9.0.]
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 CO₃²⁻
- 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

Badger & Spalding 2000 Photosynthesis: Physiology and Metabolism, pp. 369-397
Ci Transporter Candidates

- **CCP1/CCP2**: chloroplast envelope proteins
  - Mitochondrial transporter superfamily
  - RNAi knockdown results in minor growth phenotype
- **LCI1**: predicted membrane protein; Ci regulated
  - No domains & no identified homologs even in other microalgae
- **LCIA**: predicted chloroplast envelope protein
  - Closely related to nitrite transporters
  - Reported to confer bicarbonate transport in *Xenopus* oocytes
- **HLA3/MPR1**: predicted plasma membrane protein
  - ABC transporter of Mrp family
- **LCIB**: 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)