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The *Chlamydomonas* CO₂-concentrating mechanism and its potential for engineering photosynthesis in plants

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Summary

To meet the food demands of a rising global population, innovative strategies are required to increase crop yields. Improvements in plant photosynthesis by genetic engineering show considerable potential towards this goal. One prospective approach is to introduce a CO₂-concentrating mechanism into crop plants to increase carbon fixation by supplying the central carbon-fixing enzyme, Rubisco, with a higher concentration of its substrate, CO₂. A promising donor organism for the molecular machinery of this mechanism is the eukaryotic alga *Chlamydomonas reinhardtii*. This review summarizes the recent advances in our understanding of carbon concentration in *Chlamydomonas*, outlines the most pressing gaps in our knowledge and discusses strategies to transfer a CO₂-concentrating mechanism into higher plants to increase photosynthetic performance.

I. Introduction

Crop food production will have to increase by ~85% by 2050 to feed a rising global population (Ray *et al.*, 2013). These improved yields are unlikely to be achieved by traditional breeding or agricultural land expansion (Long *et al.*, 2015). An approach that is already proving to be very promising is photosynthetic enhancement by genetic engineering (Simkin *et al.*, 2015; Kromdijk *et al.*, 2016). In nature, photosynthesis is rarely what limits plant growth, which is generally dependent on the availability of fixed nitrogen,

water, light and phosphorous. However, in modern agriculture, these are mostly kept abundant, and CO₂ fixation by photosynthesis can restrict growth (Ainsworth & Long, 2005). This limitation is largely due to the inefficiency of the principal carbon-fixing enzyme, Rubisco. In the current atmosphere, O₂ competes with CO₂ for the active site of Rubisco, resulting in the loss of fixed carbon and nitrogen through a process known as photorespiration. To reduce photorespiration, some photosynthetic organisms operate CO₂-concentrating mechanisms (CCMs) to increase the CO₂:O₂ ratio at the active site of Rubisco.

Modeling has shown that successful introduction of a CCM into major food crops, such as rice, wheat and soya bean, could result in up to a 60% increase in carbon fixation whilst improving water and nitrogen use efficiencies (Long *et al.*, 2015). Several CCM and photosynthesis engineering strategies are currently being pursued (Table 1). One promising approach is the transfer of the CCM from the eukaryotic alga *Chlamydomonas reinhardtii* to crop plants (Atkinson *et al.*, 2016; Meyer *et al.*, 2016; Rae *et al.*, 2017). *Chlamydomonas* operates an efficient CCM, has a well-established genetic toolbox, and is a proven model organism for photosynthesis and CCM research (Merchant *et al.*, 2007; Wang *et al.*, 2015). In addition, it is a member of the green lineage, having similar cellular structure, chloroplasts and photosynthetic apparatus as higher plants, supporting its role as a compatible donor of genetic components (Atkinson *et al.*, 2016).

This review will focus on the engineering of the *Chlamydomonas* CCM into higher plants. It will outline the recent significant advances in our understanding of the *Chlamydomonas* CCM, and highlight the most pressing gaps in our knowledge and novel approaches that could rapidly fill these gaps. It will then explore approaches that could be implemented to identify, test and assemble the minimal components of a functional CCM in a higher plant.

II. Recent advances in our understanding of the *Chlamydomonas* CCM

Chlamydomonas operates a biophysical CCM, which is thought to principally operate by the concentration of bicarbonate (HCO_3^-) inside the cell and its subsequent conversion to CO_2 in the proximity of Rubisco. To further enhance the rate of CO_2 fixation, Rubisco is tightly packaged in a microcompartment called the pyrenoid within the chloroplast (Wang *et al.*, 2015). Biophysical CCMs have three broad yet overlapping features: inorganic carbon (Ci) flux, spatial and structural organization, and regulation. The last 2–3 yr have seen substantial advances and refinements in our understanding of all three features (Fig. 1).

Inorganic carbon (Ci) flux

Although multiple components have been associated with Ci flux from the surrounding environment to Rubisco in the pyrenoid (reviewed in detail by Wang *et al.*, 2015), current data only unambiguously support the necessity of five proteins: HLA3, LCII, LCIA, CAH3 and LCIB (Box 1). Understanding the precise function and cooperation of these proteins has proved to be challenging, due to the lack of functionally characterized homologs in other organisms and the presence of distinct *Chlamydomonas* acclimation states to two different concentrations of limiting CO_2 : 'very low CO_2 ' (<0.03% CO_2) and 'low CO_2 ' (c. 0.03–0.5% CO_2). The precise control of CO_2 growth conditions and the use of double mutants have been invaluable in understanding the molecular mechanisms at play.

At very low CO_2 , the cell appears to predominantly concentrate Ci in the form of HCO_3^- , with HLA3 and LCIA driving HCO_3^- uptake across the plasma membrane and chloroplast envelope,

respectively (Gao *et al.*, 2015; Yamano *et al.*, 2015; Atkinson *et al.*, 2016). The role of LCII at very low and low CO_2 is currently unclear (Ohnishi *et al.*, 2010). HCO_3^- uptake by HLA3 and LCIA appears to be highly cooperative, with uptake only enhanced if both components are over-expressed (Yamano *et al.*, 2015), and the expression of HLA3 is dependent on the presence of LCIA (Yamano *et al.*, 2015). Once in the stroma, HCO_3^- is thought to travel into the thylakoid lumen by a currently unidentified transporter/channel, where CAH3, potentially enriched in the trans-pyrenoid thylakoid tubules (Blanco-Rivero *et al.*, 2012; Mitchell *et al.*, 2014), drives the formation of CO_2 for fixation by Rubisco in the pyrenoid.

Under low CO_2 , the cell switches to an LCIB-dependent CO_2 -based uptake system. Mutants in *LCIB* fail to grow at air concentrations of CO_2 (c. 0.04%) but are partially recovered at very low CO_2 (Wang & Spalding, 2006). By monitoring the photosynthetic performance of *lciA* and *lcib* single and double mutants, whilst varying CO_2 : HCO_3^- ratios, Wang & Spalding (2014) show that: (1) LCIB functions in CO_2 , not HCO_3^- , uptake at low CO_2 , (2) HCO_3^- uptake by LCIA is inhibited by CO_2 at and above approximately air concentrations of CO_2 , and (3) LCIB also has a role at very low CO_2 (Fig. 1). At low CO_2 , LCIB disperses throughout the stroma, where it may drive Ci accumulation by converting CO_2 to HCO_3^- and thus maintaining a CO_2 gradient across the cell. At very low CO_2 , LCIB localizes to the pyrenoid periphery, where it may function in CO_2 recapture from CAH3-driven HCO_3^- to CO_2 conversion in the pyrenoid (Duanmu *et al.*, 2009; Wang & Spalding, 2014).

Spatial and structural organization of the CCM

The spatial separation of biochemical reactions is essential for biological processes to function correctly. In this way, the enrichment of Rubisco in the pyrenoid is necessary for a functional CCM, with cells that fail to fully assemble a pyrenoid having severe growth defects at low CO_2 concentrations (Genkov *et al.*, 2010; Meyer *et al.*, 2012; Mackinder *et al.*, 2016).

Considerable advances have been made in our understanding of pyrenoid formation. We recently identified a pyrenoid protein that appears to function as a Rubisco linker. A mutant lacking Essential Pyrenoid Component 1 (EPYC1) showed a disrupted CCM and severe reductions in Rubisco assembly into the pyrenoid. The EPYC1 protein is composed of four nearly identical repeats, and forms a complex with Rubisco (Mackinder *et al.*, 2016). Potential EPYC1 interaction sites on Rubisco are the two surface-exposed α -helices of the Rubisco small subunit, which are critical for pyrenoid assembly (Meyer *et al.*, 2012). A molecular understanding of the EPYC1–Rubisco interaction will be crucial for future pyrenoid assembly in plant chloroplasts. Additionally, mutants in the putative methyl transferase CIA6 also exhibit disrupted pyrenoids, although the functional role of CIA6 is still unclear (Ma *et al.*, 2011).

Regulation

The *Chlamydomonas* CCM is not only regulated by CO_2 : CCM gene expression, protein abundance and protein localization all

Table 1 Outline of approaches to engineer photosynthesis in higher plants

Engineering approach*	Details	Current progress (highlights)	Major pros and cons	Reference(s)
<i>Chlamydomonas</i> CCM	Engineering of a pyrenoid and HCO ₃ ⁻ transporters to increase CO ₂ concentration at Rubisco's active site. As part of the Combining Algal and Plant Photosynthesis (CAPP) project	Expression of multiple <i>Chlamydomonas</i> CCM components in <i>Arabidopsis</i> and tobacco. Replacement of <i>Arabidopsis</i> native Rubisco small subunit (<i>rbcS</i>) with <i>Chlamydomonas rbcS</i> . No photosynthetic improvements to date	<i>Pros.</i> Evolutionary 'close' donor organism. Recent rapid progress in our understanding of the <i>Chlamydomonas</i> CCM. Correct localization of <i>Chlamydomonas</i> proteins in higher plants. <i>Cons.</i> Gaps in our knowledge of the <i>Chlamydomonas</i> CCM. Complex synthetic biology problem	Reviewed by Rae <i>et al.</i> (2017) and Meyer <i>et al.</i> (2016)
Cyanobacterial CCM	Engineering a carboxysome and associated cyanobacterial inorganic carbon transporters to increase CO ₂ concentration at Rubisco's active site. As part of the Realizing Increased Photosynthetic Efficiency (RIPE) project	Expression of SbtA and BicA HCO ₃ ⁻ transporters and several carboxysome shell components in higher plants. Partial assembly of β-carboxysomes. No photosynthetic improvements to date	<i>Pros.</i> Good understanding of the components and function of the CCM. Modeling supports up to 60% gains in CO ₂ fixation. <i>Cons.</i> Incorrect targeting of cyanobacterial proteins in higher plants. Unknown regulation of HCO ₃ ⁻ transporters. Complex synthetic biology problem	Reviewed by Rae <i>et al.</i> (2017)
C ₃ to C ₄ engineering	Engineering of C ₄ photosynthesis in a C ₃ plant. Part of the C ₄ Rice Project	Large advances in our understanding of the regulation, components and evolution of C ₄ photosynthesis. Overexpression of single or multiple C ₄ cycle enzymes in C ₃ plants. No significant photosynthetic improvements to date	<i>Pros.</i> C ₄ photosynthesis evolved over 66 times, supporting feasibility. <i>Cons.</i> Will require significant anatomical changes to achieve leaf Kranz anatomy. Highly complex synthetic biology problem	Reviewed by Schuler <i>et al.</i> (2016)
Rubisco engineering	Improvement of Rubisco kinetics by protein engineering or Rubisco replacement	Catalytic screening of Rubisco from diverse photosynthetic lineages has identified superior forms. Successful replacement of <i>rbcl</i> in Tobacco. Expression of non-native <i>rbcS</i> in rice and <i>Arabidopsis</i> . No significant photosynthetic improvements to date	<i>Pros.</i> Single target protein engineering approach. <i>Cons.</i> Incompatibility of foreign Rubisco with host chaperones. May require engineering of both the plastid (<i>rbcl</i>) and nuclear (<i>rbcS</i>) genomes. Theoretical improvements (~15%) are less compared to other engineering approaches	Reviewed by Sharwood (2017)
Photorespiratory bypass	Engineering photorespiratory bypasses to improve 2-phosphoglycolate recycling to minimize CO ₂ and ATP losses of photorespiration. As part of the RIPE project	Three photorespiratory bypasses have been successfully engineered in plants. Increases in biomass have been seen in lab-grown plants under specific light regimes	<i>Pros.</i> Relatively simple approach. Promising results from preliminary experiments. <i>Cons.</i> Potential negative side effects of reducing/modifying photorespiration. Theoretical improvements (~15%) are less compared to other engineering approaches, but engineered traits could be stacked	Reviewed by Betti <i>et al.</i> (2016)
Improving nonphotochemical quenching (NPQ)	Reducing energy loss by improving NPQ. As part of the RIPE project	Acceleration of NPQ relaxation in tobacco resulted in a 15% increase in crop productivity in field trials	<i>Pros.</i> Simple genetic engineering approach. Proven to improve CO ₂ fixation in field trials. <i>Cons.</i> Theoretical improvements (~20%) are less compared to other engineering approaches, but engineered traits could be stacked	Kromdijk <i>et al.</i> (2016)
Ribulose-1, 5-bisphosphate (RuBP) regeneration	Optimization of RuBP regeneration in the Calvin cycle	Overexpression of Calvin cycle enzymes resulted in an increase in biomass of glasshouse-grown tobacco plants	<i>Pros.</i> Relatively simple approach. Promising data from glasshouse growth experiments. <i>Cons.</i> Expression levels of multiple genes may need to be changed to realize large photosynthetic improvements	Simkin <i>et al.</i> (2015)

*This is nonexhaustive. Additional approaches include Crassulacean acid metabolism (CAM) engineering, extending the usable light spectrum for photosynthesis and crop canopy rearrangement.

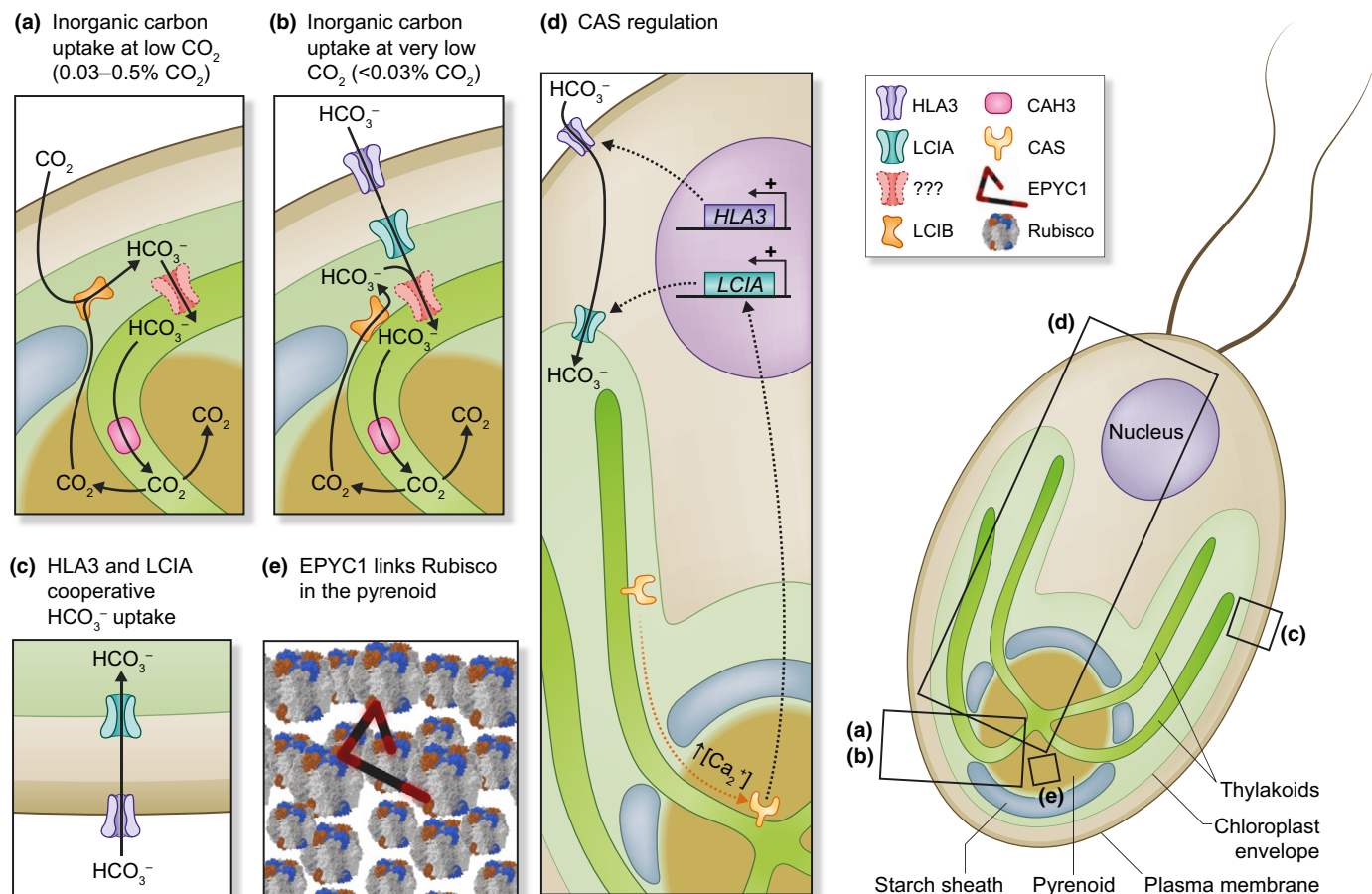


Fig. 1 Recent advances in our understanding of the *Chlamydomonas* CO₂-concentrating mechanism (CCM). *Chlamydomonas* has two known CO₂-limiting acclimation states: (a) a low CO₂ LCIB-dependent CO₂ uptake system and (b, c) a very low CO₂, cooperative HLA3- and LCIA-dependent HCO₃⁻ uptake system. (d) HLA3 and LCIA levels are partially controlled by a Ca²⁺-dependent, retrograde signaling event that involves the movement of the thylakoid membrane-tethered CAS protein into the pyrenoid and an increase in pyrenoid Ca²⁺ concentration. Under both acclimation states, HCO₃⁻ is probably delivered to the thylakoid lumen by a currently unidentified transporter/channel, where it is dehydrated to CO₂ by CAH3 in the pyrenoid tubules (a, b). (e) CO₂ is then fixed by Rubisco, which is linked together to form the pyrenoid by EPYC1.

Box 1 Proteins unambiguously involved in inorganic carbon (Ci) flux

Current data unambiguously support the necessity of five proteins in Ci flux from the external environment to Rubisco's active site, including three transmembrane Ci transporters: HLA3 (high light activated 3), an ATP binding cassette (ABC) family member localized to the plasma membrane (Yamano *et al.*, 2015); LCI1 (low CO₂ inducible gene 1), a protein with no detectable homologs in other species also found in the plasma membrane (Ohnishi *et al.*, 2010); and LCIA, a member of the formate/nitrite transporter family, localized to the chloroplast envelope (Yamano *et al.*, 2015). There are strong data supporting HCO₃⁻ transport for HLA3 and LCIA, whilst the Ci species transported by LCI1 is currently unclear (Ohnishi *et al.*, 2010). The other two Ci flux-associated proteins are CAH3, a thylakoid lumen-localized carbonic anhydrase; and LCIB found in the chloroplast stroma. Although the precise function of LCIB is unclear, LCIB orthologs in other algae function as carbonic anhydrases that are perhaps involved in CO₂ recapture by directional hydration of CO₂ to HCO₃⁻ (Jin *et al.*, 2016; Kikutani *et al.*, 2016).

change in response to both light and circadian signals. In the dark, CCM genes are downregulated and cellular Ci affinity is reduced. However, shortly before the light-phase CCM genes such as *HLA3*, *LCIA* and *LCIB* are upregulated, CAH3 and Rubisco relocate to the pyrenoid and Ci affinity increases (Mitchell *et al.*, 2014).

Two detailed transcriptional studies have revealed significant RNA-level regulation associated with CCM induction (Brueggeman *et al.*, 2012; Fang *et al.*, 2012), with a large fraction of CO₂-regulated genes controlled by the transcriptional regulator CIA5/CCM1 (Fang *et al.*, 2012). Some of the most upregulated transcripts during CO₂ limitation correspond to key CCM genes, including *HLA3*, *LCI1*, *LCIA* and *LCIB*. This suggests that other currently uncharacterized genes that have the same expression profile may also be core CCM components. Interestingly, there are no apparent significant expression changes between the low CO₂ and very low CO₂ acclimation states, indicating that this shift is probably regulated post-transcriptionally. Indeed, phosphorylation of CAH3 and EPYC1 has been demonstrated in response to

limiting CO₂ (Turkina *et al.*, 2006; Blanco-Rivero *et al.*, 2012; Wang *et al.*, 2014).

Very little is known about how *Chlamydomonas* senses and responds to CO₂. Recently, Wang *et al.* (2016) showed that expression of a set of core CCM genes is controlled by CO₂-regulated, Ca²⁺-dependent retrograde signaling from the pyrenoid to the nucleus. Additionally, under CO₂-limiting conditions, [Ca²⁺] is elevated in the pyrenoid, and the Ca²⁺-binding protein (CAS) relocates from the stroma to the pyrenoid tubules. A mutant lacking CAS has a defective CCM and fails to upregulate the HCO₃⁻ transporters, *HLA3* and *LCIA* (Wang *et al.*, 2016), suggesting that Ca²⁺ plays a key role in CO₂ sensing in *Chlamydomonas*.

III. Current gaps in our understanding of the *Chlamydomonas* CCM

There are still several critical gaps in our knowledge of the *Chlamydomonas* CCM. A key priority is to complete the picture of Ci flux from the surrounding environment to Rubisco in the pyrenoid. The current model of the CCM is dependent on the presence of an unidentified transporter or channel in the thylakoid membrane to maintain a flux of HCO₃⁻ from the stroma to CAH3 in the lumen. The CCM is an energy-dependent process, but how the different transport steps are powered is still unresolved. More detailed biochemical and structural characterization, including the kinetic properties of transport by *HLA3*, *LCI1* and *LCIA*, is urgently needed. As discussed above, *LCIB* plays a key role in the CCM, but if and how it functions as a directional carbonic anhydrase has yet to be shown biochemically; this needs to be a priority for the field.

Further, the role of mitochondria in the CCM is unknown. Several of the most upregulated genes during CCM induction (*CCP1*, *CCP2*, *CAH4*, *CAH5*) encode mitochondrial localized proteins (Fang *et al.*, 2012). Mitochondria play a key role in photorespiration and may be critical during the early stages of cellular adaptation to limiting CO₂, before the CCM is fully induced. Alternatively or additionally, they may play a role in CO₂ recapture and recycling once the CCM is operating.

Changes in pH between compartments can be used to drive Ci flux, because different CO₂ : HCO₃⁻ ratios occur at different pH. Understanding how CCM induction affects pH in the pyrenoid, stroma and thylakoid lumen would help inform our understanding of Ci fluxes (Mangan *et al.*, 2016).

Correct pyrenoid assembly appears to be critical for a fully functional CCM. Whether components additional to EPYC1 and CIA6 are necessary for assembly is still uncertain. Furthermore, what nucleates the pyrenoid, how it maintains its central anterior position in the chloroplast and how it divides are all open questions. Detailed cryo-tomography has shown large structural changes to thylakoids as they enter the pyrenoid (Engel *et al.*, 2015); it will be exciting to see what proteins are responsible for these changes in membrane curvature. Other pressing areas are the role of the pyrenoid starch sheath in the CCM (Villarejo *et al.*, 1996) and how Rubisco activase maintains access to tightly packed Rubisco in the pyrenoid.

IV. Approaches to rapidly advance our understanding of the *Chlamydomonas* CCM

To successfully engineer the *Chlamydomonas* CCM into a higher plant, a complete parts list is needed. To achieve this, significant step changes in our understanding of the CCM are essential (Fig. 2). To identify candidate genes, large-scale, genome saturating, forward genetic mutant screens and targeted reverse genetic screens will be key – both of which will be made significantly easier with the recent release of the *Chlamydomonas* mutant library (Li *et al.*, 2016).

To understand the spatial organization of the CCM and to discover new pyrenoid structural components, localization studies of known CCMs and low CO₂-upregulated genes could be implemented. These could be incorporated with affinity-purification MS studies to identify additional components and provide a CCM protein–protein interaction network. Such approaches have proven extremely powerful to understand specific cellular processes in model systems, including mammalian cells and *Caenorhabditis elegans* (Christianson *et al.*, 2012; Sarov *et al.*, 2012).

Additionally, membrane inlet MS-based studies could provide insight into alterations in Ci flux in mutants and between acclimation states to different limiting CO₂ concentrations. An additional approach could be through *in vivo* monitoring of the distribution of Ci by the development of a genetically encoded HCO₃⁻ or CO₂ sensor. The engineering of sensors to a broad range of target molecules, including oxygen, supports the feasibility of a Ci sensor (Hochreiter *et al.*, 2015).

Finally, systems biology approaches to integrate newly derived datasets (i.e. genome-wide mutant screens, proteomic studies) with existing datasets (i.e. transcriptomes) will allow detailed modeling of the CCM, and provide information on the function of uncharacterized components. These components can then be experimentally validated and prioritized for future plant engineering.

V. Engineering a CCM into higher plants

Transferring the *Chlamydomonas* CCM into a higher plant will involve the introduction of components into multiple compartments and membranes as well as the potential removal of native carbonic anhydrase and aquaporins to prevent the CCM from short-circuiting. Early work has been promising, with core *Chlamydomonas* CCM proteins – including *HLA3*, *LCIA* and *LCIB* – localizing correctly in higher plants (Atkinson *et al.*, 2016), and the stable functional expression of *Chlamydomonas* Rubisco in *Arabidopsis* (Atkinson *et al.*, 2017). Modeling has indicated that a partial CCM effect may be achieved by the addition of HCO₃⁻ transporters in the chloroplast envelope (McGrath & Long, 2014). However, for a fully functional CCM the aggregation of Rubisco to form a pyrenoid-like structure will probably be necessary. The number of components needed for pyrenoid assembly is currently unknown. However, the structural diversity (McKay & Gibbs, 1991) and multiple origins of pyrenoids (Villarreal & Renner, 2012) imply that there are minimal constraints for a functional pyrenoid. Also, fundamentally, a pyrenoid appears to be an aggregation of Rubisco around a thylakoid network (McKay &

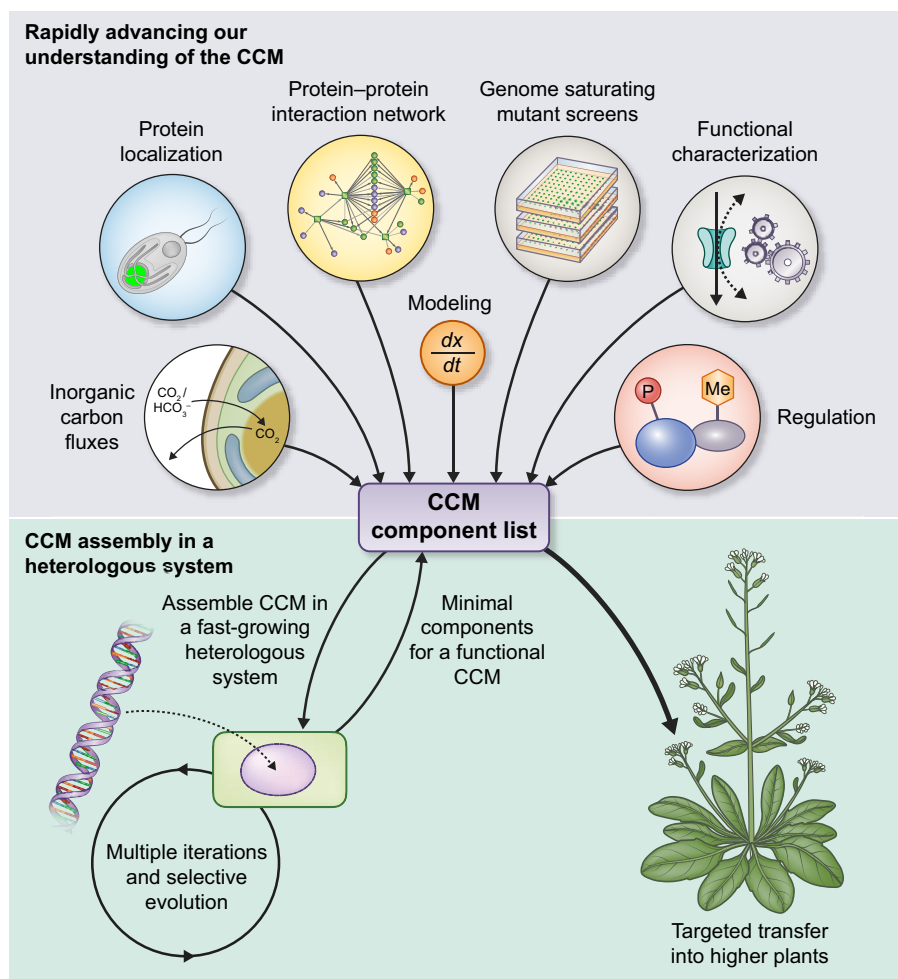


Fig. 2 Approaches to rapidly advance our understanding of the CO₂-concentrating mechanism (CCM) to guide transfer into higher plants. A broad range of high-throughput approaches combined with detailed biochemical characterization of proteins and modeling are needed to gain an exhaustive CCM component list. This component list can then be refined into a minimal set of components needed for a functional CCM using a fast-growing heterologous system. Together, these data will guide the targeted transfer of CCM components into a higher plant model system such as *Arabidopsis* or tobacco.

Gibbs, 1991). Together, these imply that relatively few components may be needed for a functional pyrenoid. To guide future plant engineering, modeling studies are urgently needed to determine what pyrenoid structural aspects are necessary, which of the two limiting CO₂ acclimation states should be inserted and the order that CCM components should be introduced into a C₃ leaf.

Identification of core CCM components that would be needed in a higher plant could be quickly identified by introducing a CCM into a heterologous, fast-growing, photosynthetic system without a CCM (Fig. 2). This could allow simultaneous testing of hundreds of components, and would mirror approaches used to successfully engineer pathways in *Escherichia coli* (Raman *et al.*, 2014). Potential candidates for such a system are the moss *Physcomitrella patens*, the liverwort *Marchantia polymorpha*, plant cell cultures or non-CCM-containing green algae. Alternatively, CCM efficiency could be tested in highly tractable nonphotosynthetic organisms. The recent functional assembly of the Calvin–Benson–Bassham cycle in *E. coli* (Antonovsky *et al.*, 2016) could function as a chassis to build and test CCM components. A minimal core set of components needed for a functional CCM could then be used to guide plant engineering.

Future CCM engineering could involve the development of hybrid systems. CCM components could be picked from a diverse

range of sources, including green algae, cyanobacteria, diatoms, haptophytes and hornworts. Furthermore, synthetic proteins could be designed to perform specific functions, including Rubisco linkers compatible with higher plant Rubisco or chimeric proteins, where multiple components are fused together to ensure correct assembly and stoichiometry of complexes (Gonzalez-Esquer *et al.*, 2015).

VI. Conclusion and outlook

The *Chlamydomonas* CCM elevates CO₂ around Rubisco, thereby enhancing photosynthesis. The evolutionary similarity between *Chlamydomonas* and higher plant chloroplasts means that no or minimal protein modifications may be necessary to ensure the correct localization and function of *Chlamydomonas* CCM components in C₃ plants. Recent, significant advances have been made in our understanding of the *Chlamydomonas* CCM; however, there are still several large gaps in our knowledge. It is anticipated that the pace of advancement will accelerate with both the availability of the *Chlamydomonas* mutant library and the maturation of large-scale systems biology approaches. Modeling studies are urgently needed to guide the step-wise transfer of components from *Chlamydomonas* to higher plants. In addition, approaches adapted from synthetic biology and pathway-engineering fields could facilitate the

assembly of a CCM in a fast growing, 'stepping stone' organism, which could aid our understanding of the minimal components needed for a functional CCM. Engineering a *Chlamydomonas* or hybrid CCM into a C₃ plant is a grand challenge and with the correct resources could become a reality.

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