The Prospect of Using Cyanobacterial Bicarbonate Transporters to Improve Leaf Photosynthesis in C_3 Crop Plants[W]

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The photosynthetic CO_2-fixing enzyme Rubisco arose some 3.5 billion years ago, in an environment when CO_2 was high and oxygen (O_2) was low. Under these conditions, it was CO_2 saturated and presumably performed well (Badger et al., 1998). However, since the advent of oxygenic photosynthesis, the levels of O_2 have risen dramatically and CO_2 has fallen to very low levels. This has gradually created conditions where CO_2 has become limiting for Rubisco and allowed O_2 to act as an alternative inhibitory substrate for the enzyme. To cope with these dramatic environmental changes, two major strategies have evolved to help Rubisco maximize its carboxylation rate at ambient levels of limiting CO_2. First, the enzyme has evolved better kinetic properties, where the $K_m(CO_2)$ has decreased and the ability to distinguish against O_2 has increased at the expense of catalytic rate (Badger et al., 1998). Alternatively, many photosynthetic organisms, ranging from cyanobacteria to algae to land plants, have developed active CO_2-concentrating mechanisms (CCMs) to turbo-charge Rubisco's CO_2 supply at a minor metabolic cost (Badger et al., 1998). Most notably, among plants this has led to the development of C_4 photosynthesis (Sage, 2004).

Most of the important grain crops (rice [*Oryza sativa*], wheat [*Triticum aestivum*], barley [*Hordeum vulgare*], canola [*Brassica napus*], soybean [*Glycine max*]), tuber crops, and vegetable crops are C_3 species and have applied the first strategy and lack any form of CCM at the leaf or chloroplast level. Much of the inherent inefficiency in C_3 photosynthesis revolves around the need to gain CO_2 through passive diffusion through the leaf pores (stomata), across cell walls and cytoplasm, and eventually through to the chloroplasts. Diffusive resistance to CO_2 passage results in a drawdown of the effective CO_2 concentration in the chloroplast, and C_3 plants have adopted strategies to maximize the diffusive conductivity for CO_2 by appressing chloroplasts against the intracellular airspaces and having large chloroplast surface area-to-leaf area ratios (Evans and von Caemmerer, 1996). Low chloroplast CO_2 concentrations exacerbate the CO_2 limitations and increase the wasteful Rubisco oxygenation reaction of ribulose 1,5-bisphosphate (RuBP) to produce phosphoglycolate, which must be recycled back to RuBP through a complex set of reactions known as the photorespiratory cycle. This is worsened by increased temperature, with the affinity for CO_2 dropping and the oxygenase reaction being relatively enhanced (Kubien and Sage, 2008). To achieve acceptable high rates of photosynthetic CO_2 fixation, typical C_3 species invest up to 30% of soluble protein and some 25% of leaf nitrogen into Rubisco protein. Evolution of the CCM in C_4 plants effectively circumvented a number of the inefficiencies, creating the present-day impetus for attempting to introduce C_4 CCMs into important C_3 crops such as rice (Hibberd et al., 2008).

However, while the C_4 CCM is one approach to elevating CO_2 around Rubisco, drawing from our knowledge of single-cell CCMs in cyanobacteria (Price et al., 2008), there are also opportunities to elevate CO_2 around Rubisco at the individual leaf chloroplast level. These prospects are expanded upon below, but in brief we consider two scenarios. The first, and simplest, approach is to consider the transplantation of cyanobacterial bicarbonate transporters to the C_3 chloroplasts to provide marginal but significant improvement in photosynthetic performance. The second, more elaborate, longer term objective would be to engineer a more functional cyanobacterial CCM in the chloroplast.

THE CYANOBACTERIAL CCM

Cyanobacteria have evolved an extremely efficient CCM (Fig. 1; see below), being able to concentrate CO_2 around Rubisco by a factor of up to 1,000-fold. As a result, cyanobacterial CO_2 fixation has been able to retain a Rubisco with a relatively high carboxylation rate, although lower selectivity between CO_2 and O_2, compared with the Rubisco in C_3 plants (Badger et al., 1998). Cyanobacterial cells also have high nitrogen use efficiency, as less nitrogen is devoted to Rubisco than in a C_3 plant (Badger et al., 1998). In addition, Rubisco within a cyanobacterium operates at near CO_2 saturation due to the action of the CCM, such that wasteful photorespiration is largely eliminated.

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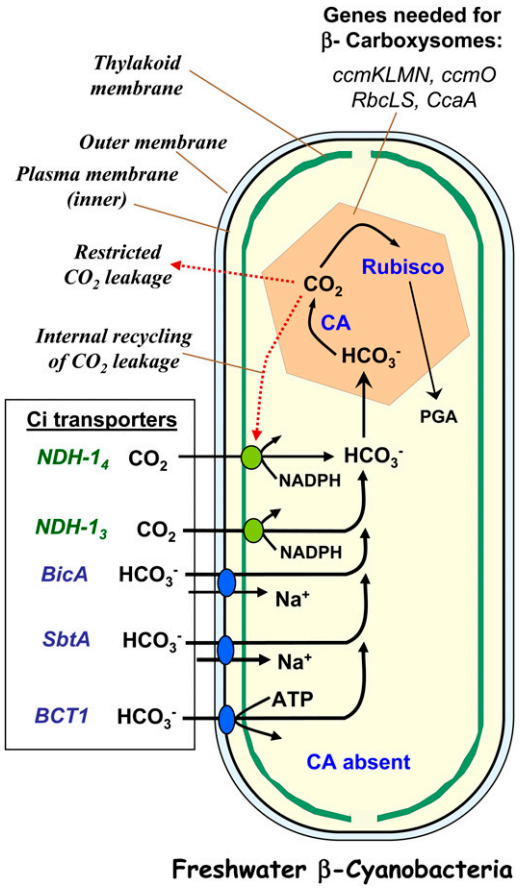

Figure 1. The cyanobacterial CCM utilizes up to five uptake systems for DIC and the polyhedral microcompartments known as carboxysomes, which contain the cell's complement of Rubisco and act as a localized site for the elevation of CO_2 around Rubisco. A key operational feature is that all uptake systems deliver HCO_3^- to the general cytoplasm and that this is kept in a state of dynamic disequilibrium favoring HCO_3^-, owing to the recycling of internally generated CO_2 through the CO_2 pumps and the absence of CA in the general cytoplasm. A specific, low level of CA activity is only present in the carboxysomes. In general, BicA, SbtA, BCT1, and NDH-I_3 uptake systems are only induced under DIC-limiting conditions (e.g. liquid in near equilibrium with air CO_2 levels or less). The carboxysomes are typically 90 to 200 nm in diameter (enlarged in this schematic), and a cell may possess five to 15 carboxysomes. By comparison, typical unicellular cyanobacteria are up to 3 μm in length, and typical C_3 chloroplasts are up to 50 μm in diameter.

Given that an early cyanobacterial progenitor is considered to have become the original endosymbiont for chloroplast evolution in algae and land plants, the question arises as to why present-day land plants lack any apparent chloroplast-based CCM. Cyanobacterial progenitors first appeared some 2.7 billion years ago (Buick, 1992), but it is almost certain that cyanobacteria have been subjected to periods of rapid evolutionary change throughout this period. In particular, the marked drop in CO_2 levels, and the rise in O_2 levels, that occurred around 400 to 350 million years ago (Berner, 1990) represents a likely trigger that forced the evolution of adaptations to cope with photorespiration and low-efficiency CO_2 fixation (Fig. 2). The poor availability of CO_2 in water, where diffusion is 10^4 times slower than in air and where large unstirred layers can exist, probably provided additional evolutionary pressure. In addition, with a pK_a around 6.4, CO_2 is a rarer species at alkaline pH, whereas HCO_3^- is considerably more abundant in many aquatic environments. Evolutionary adaptations to deal with these combined pressures would have included transporters for the active uptake of dissolved inorganic carbon species (DIC; CO_2 and HCO_3^-), the subsequent localized elevation of CO_2 around Rubisco, and the partitioning of Rubisco into microcompartments known as carboxysomes (Badger et al., 2002; Price et al., 2008; see below). This may have also been the stage when microalgae developed CCMs. If, as seems likely, cyanobacteria did not evolve fully functional CCMs until 350 million years ago, then this is well after the first terrestrial plants are thought to have evolved from eukaryotic algae at around 450 million years ago (Kenrick and Crane, 1997) and long after the original endosymbiotic event that gave rise to microalgae at around 1.5 billion years ago (Dyall et al., 2004). This probably explains why present-day crop plants lack any form of chloroplast-based CCM derived from cyanobacterial or microalgal ancestors.

The cyanobacterial CCM functions to actively transport and accumulate DIC into the cell, where the accumulated HCO_3^- pool is utilized to generate elevated CO_2 levels around Rubisco (Badger et al., 2002; Price et al., 2008). Rubisco is encapsulated in unique microcompartments known as carboxysomes that are typically 90 to 200 nm in diameter. The functional importance of these proteinaceous, icosahedral bodies that are composed of 20 equilateral triangular sides is that they act as the site of CO_2 elevation within the cell, with the supply rate of CO_2 from accumulated HCO_3^- being catalyzed by a carboxysome-located carbonic anhydrase (CA). The carboxysome shell in these cyanobacteria is composed of just six to eight proteins (Price et al., 2008), and the average unicellular cyanobacterial cells would normally possess five to 15 carboxysomes per cell. The key to the efficiency of any CCM revolves around the ability to minimize the loss of CO_2 from the elevation zone. In model cyanobacteria, this is accomplished by a combination of (1) the accumulation of the ionic form of DIC, which is less membrane permeable than CO_2, (2) the complete elimination of CA activity from the general cytosol to help reduce CO_2 leakage out of the cell, (3) the special properties of the carboxysome protein shell acting to retard CO_2 leakage, and (4) the action of the CO_2 pumps in recycling CO_2 leakage from the carboxysome back into the HCO_3^- pool (Maeda et al., 2002; Price et al., 2008).

The localization of CA, which catalyzes the reversible hydration and dehydration of CO_2 and HCO_3^-, is a key element of cyanobacterial CCMs. The absence of CA in the cytosol, and the action of the directional CO_2 uptake systems that convert CO_2 to HCO_3^- at the

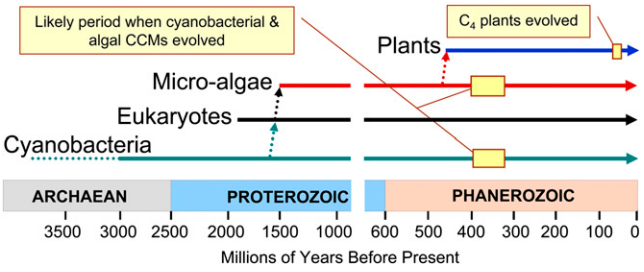

Figure 2. A timeline indicating that CCMs possibly arose in cyanobacteria and microalgae at around 400 to 350 million years ago, well after the evolution of early land plants.

thylakoid membrane, allow the cell to accumulate HCO_3^- and keep it out of rapid chemical equilibrium with CO_2. This is very effective in minimizing the concentration of the diffusible CO_2 molecule owing to the slow dehydration of HCO_3^- in the absence of CA (Walker et al., 1980). The importance of accumulating HCO_3^- in the cytosol, and maintaining an internal HCO_3^- pool out of chemical equilibrium, was shown by an experiment where human CA was expressed in the cytoplasm of a model cyanobacterium, *Synechococcus elongatus* PCC7942. The ectopic expression caused complete dissipation of the accumulated HCO_3^- pool due to the CA-mediated equilibration between CO_2 and HCO_3^-, which in turn led to increased CO_2 diffusion out of the cell (Price and Badger, 1989). This is very different from the situation in C_3 chloroplasts, where CA is highly abundant in the stroma in order to maximize the diffusion of CO_2 across the envelope and throughout the chloroplast (Badger and Price, 1994).

Five distinct transport systems for DIC uptake have been identified in cyanobacteria (Fig. 1; Table I; for more details and related references, see Price et al., 2008). (1) BCT1, which is inducible under DIC limitation and is a high-affinity HCO_3^- transporter (uniporter) belonging to the traffic ATPase family. (2) SbtA, an inducible, high-affinity Na^+-dependent HCO_3^- transporter (Price et al., 2004; Shibata et al., 2002) that apparently acts as a Na^+/HCO_3^- symporter with relatively low flux rate. (3) BicA, a low-affinity, high-flux, Na^+-dependent HCO_3^- transporter belonging to the widespread SulP family and related to the human SLC26 family of anion transporters (Price et al., 2004); BicA is a probable Na^+/HCO_3^- symporter. (4) NDH-I_4, a constitutive CO_2 uptake system based on a specialized NADPH dehydrogenase (NDH-I) complex; this system uses NADPH as an electron donor to drive the conversion of CO_2 to HCO_3^- during the uptake step (Price et al., 2002). Each complex is composed of 10 core subunits that are common to the respiratory NDH-I complex and three specialized subunits required for CO_2 uptake. Interestingly, NDH-I-type CO_2 uptake systems appear to be located on the thylakoid membranes, where they use CO_2 diffusing from outside the cell or arising from leakage from the carboxysomes as a substrate for directional conversion to HCO_3^-. (5) NDH-I_3, a second CO_2 uptake system based on a modified NDH-I complex that is inducible under DIC limitation and is of higher uptake affinity than NDH-I_4, located on the thylakoid membranes in *Synechocystis* PCC6803.

ADDING A HCO_3^- TRANSPORTER TO THE CHLOROPLAST ENVELOPE OF CROP PLANTS

With the objective of attaining a modest elevation of CO_2 levels in the C_3 chloroplast, the simplest approach would be to express a cyanobacterial HCO_3^- transporter on the inner envelope of the C_3 chloroplast (Fig. 3). Single-subunit HCO_3^- transporters such as BicA and SbtA are the most obvious initial candidates. However, within technical restraints, the transfer of multisubunit transporters such as the BCT1 HCO_3^- transporter (four genes) is also possible. Additionally, the use of HCO_3^- transporters from microalgae such as *Chlamydomonas* can also be considered as viable candidates (Duanmu et al., 2009). From a technical viewpoint, the addition of DIC transporters mentioned above would be dependent on host genome transformation techniques using *Agrobacterium tumefaciens*, which are generally available for a range of important crop species. Chloroplast transformation techniques would not be required for this approach, and this is especially important because chloroplast transformation in crop species in not yet available. As can be seen from the associated modeling presented in this report (Fig. 4), the approach of installing BicA and/or SbtA transporters into the chloroplast inner envelope could achieve a 5% to 15% improvement in photosynthetic CO_2 fixation rates at constant substomatal CO_2 levels (see below).

It is clear that a CO_2 diffusion gradient or drawdown exists between the CO_2 level in the substomatal cavity of the leaf (C_i) and the steady-state level of CO_2 in chloroplast (C_{chlo}), with the magnitude of this gradient

Table I. *A summary of the properties of cyanobacterial DIC transporters*

Transport Type	Mechanism	Substrate Affinity	Flux Rate	Photosynthetic Affinity ($k_{0.5}$)
BicA	Na^+-dependent HCO_3^- uptake	Low-medium	High	90–170 μM HCO_3^-
SbtA	Na^+-dependent HCO_3^- uptake	High	Low	<5 μM HCO_3^-
BCT1	Traffic ATPase, HCO_3^- uptake	High	Low	10–15 μM HCO_3^-
NDH-I_4	NADPH-driven CO_2 uptake via conversion to HCO_3^-	Medium	High	10–15 μM CO_2
NDH-I_3	NADPH-driven CO_2 uptake via conversion to HCO_3^-	High	Low	1–2 μM CO_2

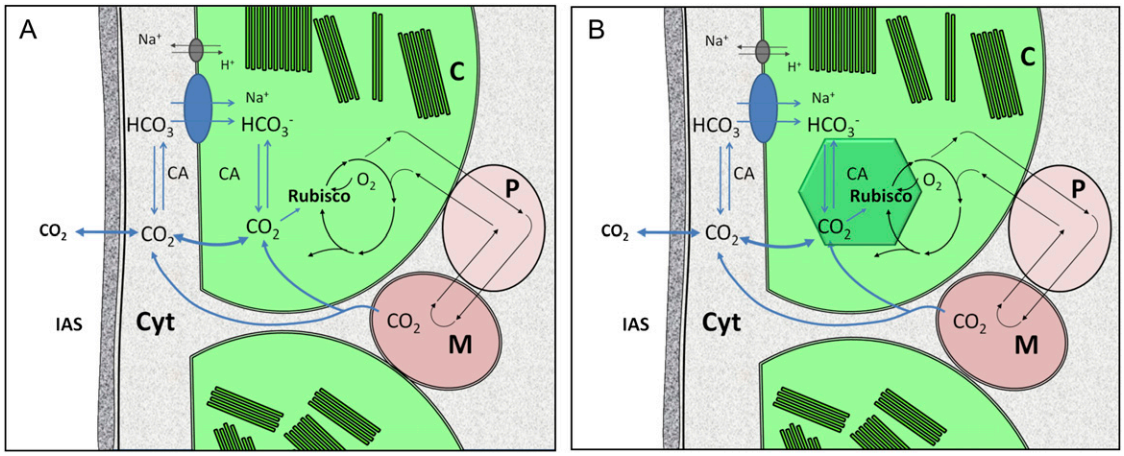

Figure 3. Schematic representations illustrating the concepts of adding a cyanobacterial HCO_3^- transporter to the chloroplast envelope of a notional C_3 leaf chloroplast (A) and the longer term prospect of constructing a more fully functional cyanobacterial or microalgal CCM in the C_3 chloroplast (B). The diagrams show CO_2 moving from the intracellular airspace (IAS; substomatal cavity) of a mesophyll leaf cell through the cell wall to the cytoplasm (Cyt) before entering the chloroplast by CO_2 diffusion or via entry through a HCO_3^- transporter. The hexagonal structure represents the icosahedral carboxysomes that would contain the full complement of Rubisco in the chloroplast, with a specific CA partitioned to this compartment and stromal CA removed. Linkages between the carbon reduction in the chloroplast and photorespiration involving peroxisomes (P) and mitochondria (M) are also shown.

sitting about 40% below C_i at high irradiance (Evans and von Caemmerer, 1996). It is important to recognize that in the first instance, the primary objective of adding a HCO_3^- pump would be to diminish the size of this CO_2 drawdown at the chloroplast and not to elevate it significantly above the external CO_2 concentration. This minimizes the risk of wasteful CO_2 leakage. Such a situation is very similar to the concept of introducing a C_4 cycle into C_3 cells (Matsuoka et al., 2001), which has been modeled for transplantation into a typical C_3 chloroplast (von Caemmerer, 2003) and found to be theoretically capable of raising the steady-state CO_2 level within the chloroplast. More specific modeling data on the theoretical engineering of BicA into a chloroplast is shown in Figure 4 and discussed below. Notably, for the addition of HCO_3^- pumps to work, there is no need to modify CA levels in the chloroplast, since CA is needed to promote rapid equilibrium between accumulated HCO_3^- and CO_2 within the stroma.

In terms of establishing active HCO_3^- uptake across the chloroplast envelope, the question arises as to whether a Na^+-dependent HCO_3^- transporter could function in a chloroplast. Estimates indicate that at least 250 μM HCO_3^- is present in the cytosol of a leaf cell in ambient air (Evans and von Caemmerer, 1996), and this appears to be maintained by cytosolic CA activity. The uptake affinities of SbtA (low flux rate) and BicA (high flux rate) for HCO_3^- in cyanobacteria are 5 to 15 μM and 90 to 170 μM, respectively (Shibata et al., 2002; Price et al., 2004) and would indicate that either transporter would operate well above its intrinsic K_m. An additional concern relates to the question of energization and the involvement of Na^+ gradients across the chloroplast envelope. Both SbtA and BicA require about 1 mM Na^+ for half-maximal activity in the form of a standing inward gradient for Na^+ (Shibata et al., 2002; Price et al., 2004). The leaf cytosol possesses 1 to 3 mM Na^+ (Karley et al., 2000), and proteomic analyses have revealed that the Arabidopsis chloroplast envelope possesses several potential Na^+-coupled transporters and Na^+/H^+ antiporters that are homologous to cyanobacterial forms (Rolland et al., 2003). Thus, there are good prospects that the chloroplast possesses and maintains an inwardly directed Na^+ gradient. As a potential enhancement, the transfer of a cyanobacterial Na^+/H^+ antiporter (or a version from the C_4 chloroplast) could also be considered if this Na^+ gradient needed to be augmented, perhaps at the expense of any existing H^+ gradient (proton motive force) inferred from the existence of H^+-coupled transporters in the envelope (Weber et al., 2005). Any possible perturbation of osmotic and pH homeostasis in the chloroplast by elevating steady-state HCO_3^- levels by up to 15%, or even by as much as 25-fold relative to air-exposed leaves, would be expected to be minimal (Wagner et al., 1990).

Other problems in establishing SbtA or BicA in C_3 chloroplasts would include ensuring the correct targeting to the chloroplast envelope and uncertainty about whether these transporters need to be post-translationally activated. In the case of targeting, we expect that SbtA and BicA can be fused to the cDNAs for known envelope-targeted proteins such that details on targeting are not initially required. We have determined the membrane topology structure of BicA and SbtA as an initial step in identifying the most likely cytoplasmic regulatory domains in these transporters (Shelden et al., 2010).

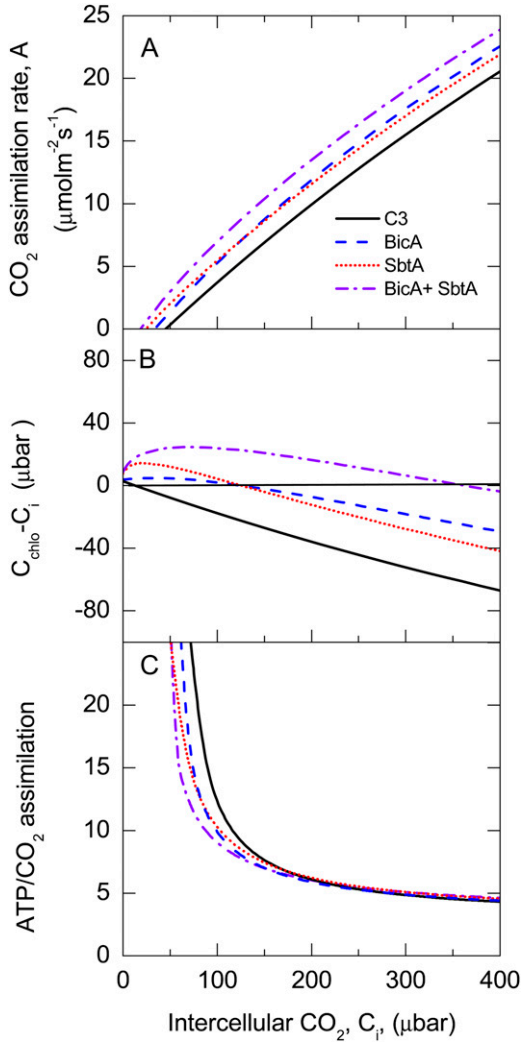

Figure 4. A, Modeled net CO_2 assimilation rate (A) as a function of intercellular CO_2 partial pressure (C_i), with three different options for bicarbonate transport at the chloroplast envelope: (1) a BicA transporter with a maximum activity of 30 μmol m^{-2} s^{-1} and $k_{1/2}$ for HCO_3^- = 90 μM (approximately 140 μbar CO_2 at pH = 7.4); (2) a SbtA transporter with a maximum activity of 15 μmol m^{-2} s^{-1} and $k_{1/2}$ for HCO_3^- = 5 μM (approximately 11 μbar CO_2 at pH = 7.4); (3) with both transporters. Only light-saturated CO_2 assimilation is considered, and maximal Rubisco activity was V_{cmax} = 100 μmol m^{-2} s^{-1}. The diffusive conductance across the cell wall plasmalemma interface, and across the chloroplast envelope, were 1 and 0.5 mol m^{-2} s^{-1} bar^{-1}, respectively. This results in a total conductance of 0.333 mol m^{-2} s^{-1} bar^{-1}. CO_2 assimilation rates are compared with Rubisco-limited C_3 photosynthesis with the same maximal Rubisco activity. Other parameters and model equations used are given in the Supplemental Table S1. (The simulation becomes unrealistic at higher C_i, as it does not consider RuBP regeneration limitation.) B, The difference between chloroplast and intercellular CO_2 ($C_{chlo} - C_i$) as a function of C_i for the examples given in A. C, ATP consumption per net CO_2 assimilation rate for the examples given in A. Considering the proton transport required, we estimate 0.25 ATP per HCO_3^- transported by BicA and 0.5 ATP per HCO_3^- transported by SbtA.

It should be noted that there has been one attempt to place a putative cyanobacterial DIC transporter, IctB, in Arabidopsis and tobacco, resulting in a reported improvement in water use efficiency (WUE; the ratio of CO_2 assimilation rate to transpiration rate) in Arabidopsis plants grown under dry-air conditions; in particular, a drop in the CO_2 compensation point was observed (Lieman-Hurwitz et al., 2003). The basis of this improved WUE is not yet clear, since it is now known that *ictB* does not code for a DIC transporter (Shibata et al., 2002; Price et al., 2008), and its role in cyanobacteria is still unclear.

MODELING THE ADDITION OF BICARBONATE PUMPS TO THE C_3 CHLOROPLAST

Our modeling of the consequences of taking the first step of adding one or two cyanobacterial HCO_3^- transporters to a C_3 chloroplast is based on previous approaches used to consider the theoretical addition of a CO_2 pump of single-cell C_4 type (von Caemmerer, 2003; von Caemmerer and Furbank, 2003); equations and parameters used in the simulations shown in Figure 4 are detailed in the Supplemental Equations S1 and Supplemental Table S1. Much of the discussion of the benefits of introduction of single-cell C_4 photosynthesis into a C_3 leaf applies to the introduction of bicarbonate transporters (von Caemmerer, 2003). The key point issuing from the modeling is that the addition of either HCO_3^- transporter, BicA or SbtA, can lead to an increase in the rate of light-saturated CO_2 assimilation at ambient and low intercellular CO_2 partial pressures (C_i). The magnitude of the increase will be very much dependent on the kinetic properties of the transporters and the conductance to CO_2 diffusion of the chloroplast envelope (von Caemmerer, 2003). The introduction of a transporter elevates chloroplast CO_2 partial pressures (C_{chlo}) above C_i at low C_i values, resulting in a reduced CO_2 compensation point. The addition of the high-affinity SbtA transporter is more effective at reducing the compensation point than the BicA transporter because of its lower K_m, and introduction of both can be more effective again. At higher C_i levels, transporters serve to reduce the drawdown in CO_2 between intercellular CO_2 and the chloroplast (Fig. 4). At a constant C_i level of 250 μbar, a theoretical transgenic plant, with the assumed activities of both HCO_3^- uptake systems, could display an indicative assimilation rate greater than 15% higher than wild-type C_3.

One of the disadvantages of C_4 photosynthesis is the requirement of two ATPs per CO_2 fixed in the C_4 cycle, which makes the introduction of a C_4 cycle into current C_3 leaves energetically inefficient (von Caemmerer, 2003), although there are examples of a number of single-cell C_4 species that have overcome the anatomical limitations inherent in C_3 leaves (Edwards et al., 2004). It is likely that bicarbonate transport is less expensive. Considering the negative electrogenicity of HCO_3^- uptake in cyanobacteria (Ritchie et al., 1996)

and the likely Na^+:proton equivalence, we estimate a required 0.25 ATP per HCO_3^- transported by BicA and 0.5 ATP per HCO_3^- for SbtA. On this basis, the ATP requirement per net CO_2 assimilation rate drops with increasing C_i during C_3 photosynthesis as the cost of photorespiration decreases (Fig. 4) The introduction of bicarbonate transporters reduces the ATP cost at low C_i below that normally experienced during C_3 photosynthesis and increases marginally above the C_3 requirement at higher C_i (Fig. 4).

We have only considered the implications for light-saturated photosynthesis here. For healthy crops, about half the canopy will be operating under these conditions, which should make the introduction of bicarbonate transporters a productive strategy. Enhancing leaf photosynthetic rates also has the potential of increasing leaf WUE, depending on stomatal responses. Bicarbonate transporters provide the largest benefit of a low C_i, and the stomata of a plant with HCO_3^- pump enhancement could afford to be less open while providing the same rate of assimilation, thereby resulting in less loss of water from the leaf stomates. The SbtA transporter could be capable of improving WUE under dry-air conditions more effectively than BicA, and the addition of both transporters is likely to be even better. If SbtA or SbtA + BicA can be successfully introduced into C_3 plants, such species might be able to better survive transient episodes of high water deficit under high light and moderate temperature stress.

ADDING A MORE ELABORATE CYANOBACTERIAL CCM TO THE CHLOROPLAST

A longer term objective, involving the greater technical difficulty of the introduction of multiple genes, could be to establish a more elaborate form of the cyanobacterial CCM in the chloroplast (Fig. 3). This could involve the transfer of one or two functionally active HCO_3^- transporters to the inner envelope membrane combined with the transfer of a CO_2 uptake system to the thylakoid membranes and a Rubisco microcompartment such as the carboxysomes. The C_3 chloroplast would also need to be, like the cyanobacterial cytosol, converted to a HCO_3^--accumulating organelle where the HCO_3^- pool is held in a state of slow chemical interconversion. To do this, it would be necessary to reorganize chloroplastic C_3 Rubisco into effective carboxysome structures and devise an effective means of removing the highly abundant chloroplastic CA so that HCO_3^- accumulation can be optimized. Certainly, it has been possible to remove up to 99% of chloroplastic CA activity in tobacco leaves by antisense RNA approaches (Price et al., 1994). Ideally, a complete removal of CA from the stroma would be more desirable, except for retaining the critically important CA in transferred carboxysomes. One of the most significant uncertainties relates to the conductance of the envelope to CO_2 diffusion, with a range of estimates available (Flexas et al., 2008). Aquaporins seem to play a role in CO_2 conductance; thus, a useful enhancement might be to reduce aquaporin levels in the envelope by RNA interference technology, since lower conductance would aid in reducing CO_2 leakage (Flexas et al., 2006).

Our understanding of carboxysome assembly and function has improved greatly in recent years, to the point where engineering the assembly of a carboxysome in the chloroplast is approaching feasibility. This has been aided by advances in determining the crystal structures of some key components of the shell (Yeates et al., 2008) and our own efforts to identify proteins required as key Rubisco- and shell-organizing proteins (Long et al., 2007, 2010). The remarkable feature of the small shell proteins is an ability to self-assemble (Yeates et al., 2008). This property, shared with some virus coat proteins, could greatly aid the final goal of assembling functional carboxysomes within the chloroplast. The longer term objective of engineering a more potent form of the cyanobacterial CCM into the chloroplast may provide greater photosynthetic enhancements than the introduction of bicarbonate transporters alone.

CONCLUSION

Modeling indicates that the addition of cyanobacterial (or microalgal) HCO_3^- pumps at the chloroplast envelope of a typical C_3 plant could provide a significant boost to the photosynthetic performance of leaf photosynthesis, either as increased assimilation rate or as improved WUE. A next research focus is to target BicA and SbtA transporters to the chloroplast of a model C_3 plant and to extend our understanding of activation and energization processes for cyanobacterial HCO_3^- transporters. However, it should also be clear that parallel transgenic strategies, such as improving the performance of Rubisco or raising capacities for RuBP generation or light interception, would provide complementary improvements to crop performance, as discussed in other articles in this issue.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Equations S1. Modeling equations used in generation of data in Figure 4.

Supplemental Table S1. Photosynthetic parameters used in the model simulation.

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