Advances in understanding the cyanobacterial CO$_2$-concentrating-mechanism (CCM): functional components, Ci transporters, diversity, genetic regulation and prospects for engineering into plants

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Abstract

Cyanobacteria have evolved a significant environmental adaptation, known as a CO$_2$-concentrating-mechanism (CCM), that vastly improves photosynthetic performance and survival under limiting CO$_2$ concentrations. The CCM functions to transport and accumulate inorganic carbon actively (Ci; HCO$_3^-$, and CO$_2$) within the cell where the Ci pool is utilized to provide elevated CO$_2$ concentrations around the primary CO$_2$-fixing enzyme, ribulose bisphosphate carboxylase-oxygenase (Rubisco). In cyanobacteria, Rubisco is encapsulated in unique micro-compartments known as carboxysomes. Cyanobacteria can possess up to five distinct transport systems for Ci uptake. Through database analysis of some 33 complete genomic DNA sequences for cyanobacteria it is evident that considerable diversity exists in the composition of transporters employed, although in many species this diversity is yet to be confirmed by comparative phenomics. In addition, two types of carboxysomes are known within the cyanobacteria that have apparently arisen by parallel evolution, and considerable progress has been made towards understanding the proteins responsible for carboxysome assembly and function. Progress has also been made towards identifying the primary signal for the induction of the subset of CCM genes known as CO$_2$-responsive genes, and transcriptional regulators CcmR and CmpR have been shown to regulate these genes. Finally, some prospects for introducing cyanobacterial CCM components into higher plants are considered, with the objective of engineering plants that make more efficient use of water and nitrogen.

Key words: Carboxysomes, CO$_2$-concentrating-mechanism, CO$_2$-responsive genes, CO$_2$-uptake systems, cyanobacteria, HCO$_3^-$ transporters, genetic regulation, photosynthesis.

Introduction

Present-day cyanobacteria (blue-green algae) occupy an enormous range of ecological habitats including freshwater, polar, hot springs, alkaline, estuarine, and open ocean, saline and symbiotic environments (Badger et al., 2006). Cyanobacterial progenitors first appeared some 2.7 billion years ago (Buick, 1992) and it is highly probable 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 350 million years ago (Berner, 1990, 2006) may have triggered adaptations to cope with photorespiration and low efficiency carbon gain, such as transport mechanisms for the active uptake of inorganic carbon (Ci) and the subsequent localized elevation of CO$_2$ around the primary carboxylating enzyme, ribulose bisphosphate carboxylase-oxygenase (Rubisco) and the partitioning of Rubisco into micro-compartments known as carboxysomes (Badger et al., 2002; Badger and Price, 2003; Raven, 2003; Giordano et al., 2005). Collectively, these adaptations are known as a CO$_2$-concentrating-mechanism (CCM) since the express operational outcome is to raise the concentration of CO$_2$ around Rubisco and...
thereby improve the efficiency of CO₂ fixation. The widespread occurrence of CCMs in present day cyanobacteria may have been aided by rapid evolution as a result of lateral transfer of large gene sets (Badger et al., 2002). Another important secondary adaptation that appears to have accompanied the development of the CCM is the co-evolution of the Rubisco enzyme that is adapted for optimal performance under the elevated CO₂ conditions produced by the CCM (Badger et al., 1998). Thus cyanobacterial Rubiscos have much lower affinities for both CO₂ and O₂ than other algal or higher plant counterparts, but have much higher turnover rates per unit of protein. These enzymatic properties improve the nitrogen use efficiency of photosynthesis since the CCM allows Rubisco to operate near $V_{max}$ thereby requiring a much smaller nitrogen investment in Rubisco to achieve a particular rate of photosynthesis.

The net acquisition of Ci (as CO₂ and HCO₃⁻), in support of photosynthetic CO₂ fixation, represents the largest nutrient flux that cyanobacteria encounter, but ultimately only CO₂ can serve as the substrate for Rubisco. However, in aquatic environments, CO₂ availability and supply rate are often severely limiting. Key reasons for this include the fact that CO₂ diffusion in water is $10^4$ times slower than in air, the chemical equilibrium between HCO₃⁻ (the main source of CO₂ supply) and CO₂ is relatively slow (especially in the pH range 7–8.5), and the total Ci concentration itself is sensitive to the prevailing pH, being less abundant at neutral to acidic pH. Present-day cyanobacterial CCMs function to maximize the efficiency of Ci uptake and CO₂ fixation under conditions of limited CO₂ availability by actively accumulating Ci and then generating an elevated CO₂ level within the carboxysomes. The cyanobacterial CCM can be highly effective, with cells able to achieve a saturated rate of CO₂ fixation at less than 10–15 μM exogenous CO₂ despite the fact that cyanobacterial Rubisco enzymes possess a low affinity for CO₂, with $K_m$ for CO₂ greater than 150 μM (Badger and Andrews, 1987). The higher nitrogen use efficiency with regard to Rubisco nitrogen investment and the near-absence of photorespiration allows cyanobacteria considerable photosynthetic and ecological competitiveness. It is generally assumed that the extra metabolic costs associated with active accumulation of Ci are significantly outweighed by the performance advantages conferred by a CCM (Raven and Lucas, 1985).

In the relatively few cyanobacterial species so far studied, it is clear that a basal form of the CCM is an obligate requirement for growth in most natural aquatic environments (i.e. typical [CO₂] of <15 μM), including seawater environments where the Ci level sits at a fairly constant 2 mM. Particularly in freshwater species (β-cyanobacteria), a heightened level of engagement or effectiveness of the CCM can be up-regulated from a constitutive level by growth under conditions of Ci limitation. This is achieved through transcriptional up-regulation of transport activities and carboxysome components and also kinetic modification of existing transporters.

From the viewpoint of Ci acquisition and CO₂ fixation, cyanobacteria can be divided in two major groups based on Rubisco and carboxysome lineages. This evolutionary divergence relates to the phylogenetic relationships for Form 1 (L₉S₈) Rubisco large subunit as either the Form 1A or 1B types. On this basis the two groupings have been referred to as α-cyanobacteria (chiefly oceanic cyanobacteria) for those containing Form 1A Rubisco and β-cyanobacteria (chiefly freshwater strains) with Form 1B Rubisco (Tabita, 1999). In what is an interesting example of parallel evolution, it is now clear that α-cyanobacteria possess carboxysomes that are proteomically different from the carboxysomes found in β-cyanobacteria, but each type is ultrastructurally similar. These two forms have become known as α-carboxysomes, for those found in Form 1A Rubisco-containing photosynthetic bacteria, including α-cyanobacteria and proteobacteria such as Thiobacillus (Halothiobacillus) species, or β-carboxysomes for those associated with Form 1B Rubisco in the β-cyanobacteria (Badger et al., 2002).

Although the α-cyanobacteria are relatively slow growers (e.g. as low as one cell division per day), often inhabiting low light–low nutrient environments, they are nevertheless both highly abundant and highly productive organisms in open ocean environments. It is estimated that some 50% of global primary productivity occurs in the oceans, with oceanic cyanobacteria contributing a significant fraction of this productivity (Field et al., 1998; Liu et al., 1999). For example, in oligotrophic oceans located between 40° N and 40° S, photosynthetic CO₂ fixation is dominated by marine cyanobacteria of the Synechococcus and Prochlorococcus genera, and together these species have been estimated to contribute 30–80% of primary production (Liu et al., 1997).

The purpose of this review is to cover some of the recent findings about the operation of the cyanobacterial CCM, and aspects of transcriptional regulation and genetic diversity. Finally, some views on prospects for the engineering of CCM components into crop plants are considered with emphasis on improving water-use and nitrogen-use efficiencies. A short review such as this is not intended to be exhaustively complete. Further details can be found in number of previous reviews dealing with cyanobacterial and micro-algal CCMs (Badger and Price, 1994, 2003; Price et al., 1998; Kaplan and Reinhold, 1999; Badger et al., 2002, 2006; Cannon et al., 2002).

**Basic operational features of the cyanobacterial CCM**

The two most important components of the cyanobacteria CCM are the presence of active uptake systems for both
CO₂ and HCO₃⁻, and the existence of the carboxysome compartment where CO₂ can be generated in close proximity to Rubisco. However, as well as these two essential features a number of other operational features need to come together to achieve an effective cyanobacterial CCM; these are depicted in Fig. 1 and include the following.

**Active Ci uptake**
Cyanobacteria need to accumulate Ci actively in order to achieve a satisfactory rate of CO₂ fixation. So far, a total of five different uptake systems have been identified (see below), including three HCO₃⁻ transporters and two CO₂ uptake systems. These uptake systems vary in flux rate and net affinity characteristics, with some transport activities being constitutive, while others are genetically inducible by growth under Ci limitation. Most cyanobacteria possess active uptake systems for both CO₂ and HCO₃⁻ species, however, based on genomic data the *Prochlorococcus* species (oceanic) seem to be an exception since they only have identifiable genes for HCO₃⁻ uptake (Badger et al., 2002, 2006; Badger and Price, 2003). β-cyanobacteria (freshwater and some estuarine strains) tend to face the greatest extremes in Ci availability and accordingly possess the largest number of discrete transporters (Badger et al., 2006).

**Energization of Ci uptake**
Active uptake and accumulation of Ci species requires the input of metabolic energy. Several different mechanisms of energization have been putatively identified and each type of energization requires co-ordination with photosynthetic demand, either directly (energy availability), or indirectly, as an allosteric activation of the transporter during a dark to light transition. Energy sources for Ci uptake may include ATP (BCT1 HCO₃⁻ transporter), NADPH or reduced ferredoxin (CO₂ uptake) or coupling to an electrochemical Na⁺ gradient (SbtA or BicA HCO₃⁻ transport) (Badger et al., 2002; Badger and Price, 2003).

**Accumulation of HCO₃⁻**
Irrespective of the type of uptake substrate, CO₂ or HCO₃⁻, there is a ensuing accumulation of HCO₃⁻ within the cell to ratios as high as 1000-fold with regard to total exogenous Ci levels. Peak internal Ci pools of between 20 mM and 40 mM are regularly measured in cyanobacteria (Price et al., 1998; Sültemeyer et al., 1995; Kaplan and Reinhold, 1999; Woodger et al., 2005b). Furthermore, HCO₃⁻ is apparently maintained at chemical non-equilibrium against CO₂ through the general absence of carbonic anhydrase activity in the cytosol (Volokita et al., 1984; Price and Badger, 1989a). Being an ionic form of Ci, HCO₃⁻ is about 1000-fold less permeable to lipid membranes than the uncharged CO₂ molecule, making it the preferred form of Ci for accumulation, especially where the cytoplasmic pH is 7.8–8.2. The best support for the view that HCO₃⁻ is the accumulated species is that ectopic expression of human carbonic anhydrase within the cytoplasm of *Synechococcus PCC7942* cells leads to a high-flux leakage of CO₂ from the cells due to rapid equilibration between HCO₃⁻ and CO₂ (Price and Badger, 1989a). This shows that HCO₃⁻ is normally held in a steady-state non-equilibrium favouring HCO₃⁻. In addition, this experiment indicated that carboxysomes are the necessary site of CO₂ elevation by logical elimination of the cytosolic compartment.

**Internal pH homeostasis**
The large fluxes of Ci required to sustain CO₂ fixation make it imperative that cells possess several effective means of cytoplasmic pH control. During the initial illumination phase, CO₂ is the main substrate for uptake and accumulation, and this CO₂ uptake is strongly activated within a few seconds of illumination and, depending on the CO₂ supply rate, usually persists for up to 30 s (Badger and Andrews, 1982; Price and Badger, 1989a). Thereafter, HCO₃⁻ uptake is slowly activated (within 20–60 s) and becomes a major fraction of total uptake.

![Fig. 1](https://academic.oup.com/jxb/article-abstract/59/7/1441/636484)
Ci uptake within 2 min. During the initial period of strong CO₂ uptake the internal Ci pool can reach up to 20 mM (or higher), and since CO₂ uptake requires a conversion of CO₂ to HCO₃⁻ (1 OH⁻ per CO₂ hydrated) during the uptake process, it follows that the equivalent of 20 mM H⁺ needs to be neutralized or ejected from the cytoplasm to maintain internal pH, especially if CO₂ fixation is still inactive or has been inhibited by glycolaldehyde, or is inoperative due to a carboxysome mutation. An apparent 1:1 stoichiometry of H⁺ efflux per CO₂ uptake has been detected in this initial CO₂-uptake phase using a carboxysome defective mutant, with alkalization of a similar magnitude observed during CO₂ efflux on darkening (Ogawa and Kaplan, 1987). The gene product of pxcA has been identified in Synechocystis PCC6803 as being essential for H⁺ extrusion during this initial CO₂ uptake phase (Sonoda et al., 1998); PxcA function is also dependent on PSII electron transport, and appears to have a role in Na⁺-dependent HCO₃⁻ uptake and nitrate uptake. Whether primary H⁺ extrusion via PxcA has a direct role in generating an electrochemical gradient for Na⁺, perhaps via H⁺/Na⁺ antiport activity, which could be used to support Na⁺-dependent HCO₃⁻ is still unclear. CO₂ uptake during steady-state CO₂ fixation will tend to be pH-neutral due to the net H⁺ generated during uptake being neutralized by the H⁺ consumed in converting HCO₃⁻ to CO₂ in the carboxysomes. However, during steady-state photosynthesis when HCO₃⁻ is the predominant substrate the H⁺ consumed in the conversion of HCO₃⁻ to CO₂ in the carboxysomes will lead to a net OH⁻ inside the cell that will either need to be ejected or neutralized by H⁺ uptake from the external medium.

**A compartment for CO₂ elevation**

Accumulated HCO₃⁻ is used in the generation of elevated CO₂ levels within specialized, Rubisco-containing, micro-compartments known as carboxysomes (Fig. 1). Carboxysomes contain most, if not all, of the cellular Rubisco content. These structures (90–250 nm in diameter, depending on the cyanobacterial spp.) are proteinaceous, polyhedral bodies featuring tightly packed Rubisco molecules (L₈S₈) bounded by a thin protein shell of about 3 nm. In an analogy with the bundle sheath of C₄ plants, the carboxysomes are essential for efficient operation of the CCM and CO₂ leakage from this micro-compartment must be effectively minimized for efficient CO₂ fixation (see below). Two types of carboxysomes are known in cyanobacteria, each apparently having evolved by parallel evolution to a convergent evolutionary function. The oceanic cyanobacteria possess what have been referred to as α-carboxysomes whilst freshwater species and estuarine species possess β-carboxysomes (Badger et al., 2002; Badger, 2003). More details on carboxysomes are provided below.

**Carbonic anhydrase**

Both types of carboxysomes contain a specific carbonic anhydrase (CA) for catalysis of the conversion of HCO₃⁻ to CO₂. In the case of β-carboxysomes the CA function is carried out by CcaA/IcfA (Fukuzawa et al., 1992; Yu et al., 1992; So et al., 2002), whilst in α-carboxysomes the shell protein CsoS3 (CsoSCA) is required (So et al., 2004). For optimal efficiency the carboxysomal CA activity needs to match, as closely as possible, the maximal rate of CO₂ fixation (Price and Badger, 1989b; Reinhold et al., 1987, 1991).

**A leak barrier**

Another basic requirement of a CCM is the existence of an effective mechanism for minimizing the leakage of CO₂ from the site of elevation in the carboxysome; this feature is envisaged to be associated with the shell of the carboxysosome or with the molecular arrangement within the polyhedral structure: both scenarios have been theoretically modelled (Reinhold et al., 1987, 1991). Recent evidence for a possible leak barrier to CO₂ diffusion has come from analysis of the CsoS3 (CsoSCA) carboxysomal carbonic anhydrase activity present in isolated intact carboxysomes from Halothiobacillus neapolitanus compared with broken carboxysomes. Here the decrease in the rate of HCO₃⁻ dehydration in intact carboxysomes was taken to indicate the existence of a leak barrier to CO₂ (Heinhorst et al., 2006). This presence of a leak barrier associated with the carboxysome shell is highly likely to be supplemented by an efficient CO₂ uptake mechanism that can recycle CO₂ leaking from the carboxysomes back into HCO₃⁻ before it leaves the cell (see below).

**Recycling of leaked CO₂**

An unavoidable feature of any compartment that requires an elevated CO₂ level to support CO₂ fixation is the problem of CO₂ leakage. In addition to intrinsic properties of carboxysomes in the minimization of CO₂ leakage, it is apparent that the CO₂ uptake systems aid in the recapture or recycling of leaked CO₂ before it escapes from the cell (Price and Badger, 1989b; Espie et al., 1991; Fridlyand et al., 1996; Kaplan and Reinhold, 1999; Price et al., 1998, 2002). CO₂ uptake systems will possess this recycling function essentially by default, but particularly if such systems are located on the thylakoids where CO₂ leakage originating from the carboxysomes could be effectively intercepted before leaving the outer cytosolic layer. The best experimental evidence for recycling by the CO₂ pumps comes from two lines of evidence. Firstly, it has been observed that a high-CO₂-requiring mutant of Synechococcus PCC7942 with extremely low carboxysomal CA levels, mutant 68, under illumination exchanges ¹⁸O out of labelled CO₂ at rates approaching that of WT
cells (Price and Badger, 1989b; Price et al., 1992); such exchange is normally slow unless the labelled CO$_2$ encounters CA activity or CA-like catalysis. The rapid exchange seen in the mutant is consistent with the view that CO$_2$ uptake systems can efficiently recycle leaked CO$_2$, even in the absence of appreciable CO$_2$ fixation (Price et al., 2002). The second line of evidence involves analysis of a chpX/chpY double mutant in *Synechococcus* sp. PCC7942. This mutant, completely lacking active CO$_2$ uptake, but still possessing two modes of HCO$_3^-$ uptake, showed abnormally low exchange of $^{18}$O label from doubly-labelled CO$_2$ in the light (Maeda et al., 2002; Price et al., 2002). Significantly, this mutant also shows a large net efflux of CO$_2$ during steady-state photosynthesis. Both observations are consistent with the view that the CO$_2$ pumps also play a role in the recycling of leaked CO$_2$ originating from the carboxysomes.

**Transcriptional regulation**

All cyanobacteria so far examined exhibit a basal, low transporter affinity form of the CCM when grown under conditions of Ci sufficiency, indicating that many of the CCM genes coding for Rubisco, carboxysomes proteins, and low affinity Ci transporters are essentially under constitutive expression with limited plasticity (Kaplan and Reinhold, 1999; Price et al., 1998, 2002). However, under Ci-limited conditions, β-cyanobacteria in particular, are able to up-regulate the expression of high affinity Ci transporters such as high affinity CO$_2$ uptake (NDH-1), SbtA, BCT1, or BicA. The expression of these CO$_2$-responsive genes is controlled by one or two LysR-type transcription regulators known as CcmR (NdhR) or CmpR (Omata et al., 2001; Wang et al., 2004; Woodger et al., 2007). The precise signal transduction pathway through CcmR or CmpR is still unknown, however, after considering a number of potential signalling conditions, it was found that expression of CO$_2$-responsive genes appears to respond inversely to the size of the internal Ci pool during illumination (Woodger et al., 2003, 2005a, b). One suggestion is that CcmR itself may be able to respond directly to the HCO$_3^-$ accumulation level in the cell (Woodger et al., 2005b, 2007); alternatively a signal transduction pathway may transduce a signal to CcmR or CmpR. See the section on Regulation of the CCM for more details.

**Allosteric or post-translational regulation**

One significant gap in our understanding of the regulation of Ci uptake processes concerns the nature of the molecular events controlling post-translational or allosteric activation of Ci transporters. It is known that cyanobacterial Ci transporters are inactive in darkness but are quickly activated upon illumination, with CO$_2$ uptake being very rapid and HCO$_3^-$ uptake apparent within 20–60 s post-illumination. Inactivation of these transporters in the dark is probably required to prevent futile cycling (pump and leak) and unnecessary depletion of metabolic energy. There is some evidence that activation of HCO$_3^-$ transporters might involve a redox signal (Kaplan et al., 1987), while other evidence suggests that full activation of HCO$_3^-$ transport might involve phosphorylation via a Ser/Thr protein kinase (Sültmeyer et al., 1998a). It is highly probable that activation involves specific phosphorylation events, but this is not yet proven.

**Carboxysomes**

As pointed out in the Introduction there are two types of carboxysomes present in cyanobacteria, namely the α-carboxysomes and the β-carboxysomes, with the former present mainly in oceanic strains (and sulphur proteobacteria such as *Thiobacillus* spp.), whereas the latter are found in freshwater and estuarine spp. (the β-cyanobacteria). These two carboxysome forms appear to have arisen by parallel evolution, although some of the small carboxysome shell proteins are clearly related.

The majority of studies on carboxysome function have been centred on β-carboxysomes in model laboratory species (Price et al., 1993, 1998; Kaplan and Reinhold, 1999). Mutant studies in β-cyanobacteria such as *Synechococcus* PCC7942, have demonstrated that the ccmKLMNO operon (although this operon structure may be partly fragmented on other species, and two copies of ccmK may be present), and the gene for carboxysomal CA, ccaA (icfA) are essential for carboxysome functionality. By contrast with the situation with microalgal pyrenoids, carboxysomes do not appear to be disassembled during the final stage of cell division; they are simply apportioned between the two daughter cells (GD Price, unpublished data). Experimental data indicate that a clear majority of cellular Rubisco in cyanobacteria is located within the carboxysomes (Beudeker et al., 1980; Price et al., 1992; McKay et al., 1993). The Rubisco within these bodies appears to be packed into para-crystalline arrays (Shively et al., 1973; Holthuijsen et al., 1986). It is proposed that HCO$_3^-$ diffuses through the proteinaceous shell of the carboxysome where a low activity of carbonic anhydrase inside the structure acts to catalyse the formation of CO$_2$ from HCO$_3^-$ at rates high enough to saturate the carboxylation reaction of Rubisco. The proposed role of the carboxysome as a site for CO$_2$ elevation was forwarded in a theoretical model (Reinhold et al., 1987) that postulated that some property of the carboxysome shell acts to reduce CO$_2$ leakage. Since the shell is composed of protein, it is feasible to envisage a situation where charged species have relatively good permeability relative to uncharged species such as CO$_2$.

In contrast to functional studies, much of the information on the structure and composition of α-carboxysomes...
has arisen from the study of *Thiobacillus* species (Cannon et al., 2001, 2003), plus one recent study on carboxysome protein:protein interactions for an α-cyanobacterium, *Synechococcus* WH8102 (Gonzales et al., 2005). The protein coat is around 3 nm in diameter and is perhaps the least understood part of the carboxysome, and arguably the most important. The α-carboxysomes in *Thiobacillus* (Cannon et al., 2001) have a shell consisting of at least four different types of polypeptides. A number of small polypeptides (8–12 kDa), all related to each other, have been identified and named CsoS1, peptides A and B. Frequently there are several members of each polypeptide type. Homologues of these small peptides have been identified in β-cyanobacterial genomes and include CcmK, L, and O. Together, these proteins are related to each other by containing one or more regions of homology that have become known as bacterial micro-compartment domains (PROSITE pfam09936). These conserved structural domains have been identified by comparison of CcmK, CcmL, CcmO, or CsoS1-like proteins involved in carboxysome formation in α- and β-cyanobacteria and β-proteobacteria (Price et al., 1998; Shively et al., 1998; Cannon et al., 2001), as well as more recently discovered genes associated with enteric proteobacteria containing carboxysome-like micro-compartmentalized in both proaneprodiol and ethanolamine metabolism and detoxification (Bobik et al., 1999; Kofoid et al., 1999). A recent cryo-electron tomographic study of carboxysomes isolated from *Halothiobacillus neapolitanus* indicates that these carboxysomes best approximate an icosaedral shape (Schmid et al., 2006). It is interesting to note that a medial section through a icosaederon yields a slightly flattened hexagonal profile; such profiles are often seen in medial electron microscopic images of carboxysomes from α- and β-carboxysomes (McKay et al., 1993; Schmid et al., 2006). The icosaederal shape, consisting of 20 identical triangular facets (equilateral triangles) is appealing from an assembly perspective since it indicates a simplicity of design, in analogy with some virus particles, that could incorporate a level of self-assembly once each facet is produced.

Carboxysome shells of α-carboxysomes and β-carboxysomes also contain two or three other larger polypeptides that bear no homology to each other and appear to have evolved by parallel evolution. In α-carboxysomes, they are CsoS2 (80–90 kDa) and CsoS3 (55–65 kDa), while in β-carboxysomes these appear to be replaced by CcmM (55–70 kDa) and CcmN (26 kDa) together with CcaA (30 kDa), the latter being a carboxysomal β-carbonic anhydrase (CA) (Price et al., 1998; Cannon et al., 2001; So and Espie, 2005). For some time there was no identified candidate for a carbonic anhydrase in α-carboxysomes, but recently the CsoS3 gene has been identified as a new class of carbonic anhydrase that apparently serves both a structural role in the carboxysome coat as well as a catalytic role in the conversion of HCO$_3^-$ to CO$_2$ (So et al., 2004). Interestingly, CsoS3 has a crystal structure that is similar to β-CAs, although it has no obvious protein sequence homology (Sawaya et al., 2006). A number of the larger carboxysome proteins, such as CcmM, N, CsoS2, and CsoS3 have no obvious functional homologues in other bacterial systems, although the CcmM protein has an N-terminal domain that is homologous to γ-CA from the archaeabacterium, *Methanosarcina thermophila* (Kisker et al., 1996); CcmM also has an L-terminal domain that has 3–4 repeated motifs with homology to the Form 1B Rubisco small subunit protein (Price et al., 1998; Ludwig et al., 2000; So and Espie, 2005). It has been speculated that the SSU-like repeats could have a role in the binding of CcmM to the L$_8$ cores of L$_8$S$_8$ Rubisco, possibly allowing the formation of protein associations in the carboxysome shell (Price et al., 1993; Ludwig et al., 2000). CcmM has been confirmed to be a structural component of the carboxysome (Price et al., 1998; Long et al., 2005), and recent evidence confirms that there is a strong interaction between Rubisco haloenzymes and both CcmM 58 kDa and 35 kDa forms, potentially acting as a basis for the initial assembly of the triangular facets of carboxysomes (B Long, S Whitney, M Badger, GD Price, unpublished data). It is possible that CsoS2 could play a similar role in α-carboxysomes, but evidence is so far lacking.

A protein structure has been determined for one of the small bacterial micro-compartment proteins, namely CcmK (sourced from *Synechocystis* PCC6803). Interestingly, this protein can assemble into flattened, hexamer plates of about 7 nm diameter, and may be able to assemble further into larger sheets of about 1.5 nm thick (Kerfeld et al., 2005). Potentially, it is feasible for this protein, along with other minor proteins (CcmL, N and/or O) to form the outer coat of the carboxysome, possibly with CcmM:Rubisco associations underlying this. Another interesting feature of the CcmK hexamers is that they possess a small hole (~0.4 nm) in the centre of each plate that could pass small negatively charged molecules such as HCO$_3^-$, ribulose bisphosphate (RuBP), phosphoglycerate (PGA), and OH$, all of these molecules need to pass across the carboxysome coat (Kerfeld et al., 2005). Given the similarity between CcmK and CsoS1 (A, B, C) it is probable that CsoS2 could form similar hexamers and extended sheets in α-carboxysomes.

The existence of two distinctly different types of carboxysomes within the different cyanobacterial groups suggests that these two carboxysome types have either been differentially inherited or evolved in parallel, in association with the Form 1A and Form 1B Rubiscos found in each cyanobacterial group. Since in many cyanobacteria both Rubisco and carboxysome genes are often found organized on contiguous regions of the chromosome (Badger et al., 2002; Cannon et al., 2002), it has previously been suggested...
Ci transporters: types and diversity

There are five modes of Ci uptake so far discovered in cyanobacteria, however, since this work is largely based on common laboratory strains such as *Synechococcus* PCC7942, *Synechocystis* PCC6803, and *Synechococcus* PCC7002, there is scope for the discovery of variants or new transporters in cyanobacteria from more extreme habitats. In general, freshwater and estuarine species have the most Ci transporters, whereas the oceanic strains tend to have the fewest. The five Ci uptake systems are explained in more detail in the subsequent sections and in Figs 2 and 3, but in brief, the five systems are described below.

(i) BCT1, an inducible high affinity HCO$_3^-$ transporter encoded by the *cmpABCD* operon and belonging to the traffic ATPase family (Omata et al., 1999).

(ii) SbtA, an inducible, high affinity Na$^+$-dependent HCO$_3^-$ transporter (Shibata et al., 2002a). It is presumed that SbtA is a Na$^+$/HCO$_3^-$ symporter, but this has not been clearly established, nor has the Na$^+$/HCO$_3^-$ transport ratio.

(iii) BicA, a low affinity, high flux, Na$^+$-dependent HCO$_3^-$ transporter belonging to the widespread SulP family (Price et al., 2004). BicA might also be a Na$^+$/HCO$_3^-$ symporter, but as with SbtA, this is still unproven.

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**Fig. 2.** Schema showing the probable structure and membrane location of the five known modes of Ci transport in cyanobacteria. Ancillary components such as porins and PxcA are also shown.

**Fig. 3.** Depiction of the CCM components present during the two extreme states of the CCM in *Synechococcus* PCC7942.
BCT1 HCO$_3^-$ transporters

The high affinity HCO$_3^-$ transporter, BCT1 (Omata et al., 1999), belongs to the ATP binding cassette (ABC) transporter family, also known as traffic ATPases since family members are usually energized by ATP (Higgins, 2001). However, direct confirmation that BCT1 uses ATP is still lacking. To date, BCT1 has been physiologically characterized in just one cyanobacterium, namely Synechococcus PCC7942, although close homologues have been detected in 10 other species (Table 1). In Synechococcus PCC7942, and other species, BCT1 is encoded by the cmpABCD operon which is only expressed under Ci limitation (Omata et al., 1999). In gain-of-function analysis the transporter displayed a high photosynthetic affinity for HCO$_3^-$ of around 15 μM, and supported a medium flux rate (Omata et al., 2002). Gene transcript analyses show that the cmpABCD operon is strongly induced under conditions of relatively severe Ci limitation (McGinn et al., 2003, 2004; Woodger et al., 2003, 2005b; Wang et al., 2004). High light stress can also lead to expression (Reddy et al., 1989), although high light conditions can also exacerbate the degree of Ci limitation (Woodger et al., 2003; McGinn et al., 2004).

BCT1 is a multimeric complex composed of four different subunits and appears to be the only cyanobacterial example of a primary transporter (uniporter) for HCO$_3^-$ (Fig. 2). The CmpA lipoprotein (42 kDa mature size) is a protein that is bound to the periplasmic face of the plasma membrane where it acts as a mobile collector or binding protein for HCO$_3^-$ The CmpA protein from Synechococcus PCC7942 has been over-expressed in E. coli, purified, and shown specifically to bind HCO$_3^-$ with a $K_D$ of around 5 μM (Maeda et al., 2000). CmpA has a role in collecting substrate and passing it onto the transporter, and as such the abundance of CmpA can significantly exceed that of the other BCT1 components (Higgins, 2001). The crystal structure of CmpA has recently been determined, and unexpectedly it has been found that the HCO$_3^-$ binding site also binds Ca$^{2+}$ as a cofactor (Koropatkin et al., 2007); it is not clear yet whether Ca$^{2+}$ is also involved in the actual transport steps. CmpB is a hydrophobic membrane protein that probably exists as a dimer within the membrane, by analogy with other ABC transporters, thereby forming a transport path through the membrane. Both CmpC and CmpD are extrinsic proteins located on the cytoplasmic face of CmpB, and both possess consensus binding sites for ATP. However, CmpC has an extra domain not present in CmpD. It has been speculated that this extra domain is involved in allosteric regulation of the BCT1 transporter since a similar domain in the analogous NrtC protein of NRT1 has been shown to have a role in inactivating the transporter when NH$_4^+$ is present (Maeda and Omata, 1997). Potentially, CmpC could have a role in dark inactivation of the BCT1 transporter.

Until very recently (Badger et al., 2006), a comparative analysis of available genome databases (Table 1), indicated that BCT1 was only present in β-cyanobacteria and that marine cyanobacteria did not appear to possess the BCT1 transporter (or the related NRT1 transporter). The reason for the general absence of BCT1 in marine cyanobacteria is unclear, but it may be related to a potential strategy of employing the electrochemical driving force that is associated with maintaining a mandatory standing Na$^+$ gradient (inwardly directed) for energization of uptake, rather than using ATP as a direct energy source for pumping (Bryant, 2003). The arrival of the Synechococcus WH5701 genome (draft assembly coordinated by Dr Dave Scanlan and the Moore Foundation, www.moore.org) changes this view somewhat. This organism is a coastal α-cyanobacterium, but it possesses close homologues of all five transporters present in β-cyanobacteria, including both CO$_2$ uptake systems, BCT1, SbtA, and BicA (see below). However, the organism is described as being able to survive under freshwater or saltwater conditions, and may be capable of growing in waters with variable Ci content. It is interesting to speculate that Synechococcus WH5701 could potentially represent an α-cyanobacterium that has acquired Ci transporters from a β-cyanobacterium, possibly in recent evolutionary time.

SbtA HCO$_3^-$ transporters

The Na$^+$-dependent SbtA transporter was originally identified in Synechocystis PCC6803 as a single subunit type transporter (Shibata et al., 2002a). From this study the SbtA transporter was estimated to have a transport affinity for HCO$_3^-$ of a round 16 μM, but it now appears that Synechocystis PCC6803 possesses a third HCO$_3^-$ transporter, called BicA (Price et al., 2004) that was missed during the analysis (Shibata et al., 2002a). The
likely effect of this third transporter would be to skew the flux rate and affinity estimates for SbtA. In fact, in Synechococcus PCC7002 the SbtA transporter has been estimated to have a photosynthetic affinity for HCO$_3^-$ (under pH 9.3 conditions) of around 2 μM and was only capable of supporting a relatively low flux rate (Price et al., 2004). Potentially, the SbtA transporter from Synechocystis PCC6803 has a stronger uptake affinity closer to 2–5 μM and a lower than expected flux rate, but this needs to be re-evaluated.

In Synechocystis PCC6803, the HCO$_3^-$ uptake capacity attributable to SbtA was shown to be Na$^+$-dependent, requiring around 1 mM Na$^+$ for half-maximal HCO$_3^-$ uptake activity (Shibata et al., 2002a). This finding was in keeping with previous physiological studies suggesting that cyanobacteria might possess a Na$^+$/HCO$_3^-$ symporter driven by the standing electrochemical gradient for Na$^+$ (inwardly directed), in turn, maintained by Na$^+$/H$^+$ antiporter activity (Espie and Kandasamy, 1994); however, a role for Na$^+$ in pH regulation was also suggested. It is likely that the Na$^+$ requirement for SbtA indicates that the transporter is a Na$^+$/HCO$_3^-$ symporter driven by the electrochemical Na$^+$ gradient, but confirmation of this requires flux studies with SbtA reconstituted into artificial

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### Table 1. Variation in the suites of genes for Ci transporters and carboxysomes found in the genomes of sequenced cyanobacteria

<table>
<thead>
<tr>
<th>Habitat$^a$</th>
<th>Species</th>
<th>Ci transporters</th>
<th>CO$_2$ Carboxysomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bicarbonate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCT1</td>
<td>SbtA$^b$</td>
</tr>
<tr>
<td>B1</td>
<td><em>Synechocystis</em> PCC6803</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>B1</td>
<td><em>Synechococcus elongatus</em> PCC7942 (or PCC6301)</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>B2</td>
<td><em>Nostoc</em> PCC7120</td>
<td>+</td>
<td>+++, ?</td>
</tr>
<tr>
<td>B1,D1/2</td>
<td><em>Anaibaena variabilis</em></td>
<td>+</td>
<td>+++, ?</td>
</tr>
<tr>
<td>B1,D1</td>
<td><em>Nostoc punctiforme</em></td>
<td>+</td>
<td>+++, ?</td>
</tr>
<tr>
<td>C3</td>
<td><em>Thermosyphococcus BP1</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C3</td>
<td><em>Synechococcus</em> sp. JA-2-3B</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C3</td>
<td><em>Synechococcus</em> sp. JA-3-3A</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C4</td>
<td><em>Gloeobacter violaceus</em></td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>A2,A3</td>
<td><em>Synechococcus</em> PCC7002</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>A2, A3</td>
<td><em>Nodularia spumigena</em></td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>A2, A3</td>
<td><em>Lyngbya sp. PCC 8106</em></td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>A1,A2</td>
<td><em>Trichodesmium erythraeum</em></td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>A1,A2</td>
<td><em>Crocosphaera watsonii</em></td>
<td>–</td>
<td>++</td>
</tr>
</tbody>
</table>

| A1 | *Synechococcus* WH8102 | – | – | +++, ? | + | – | + |
| A1 | *Synechococcus* sp. WH 7805 | – | – | +++, ? | + | – | + |
| A1 | *Synechococcus* CC9605 | – | – | +++, ? | + | – | + |
| A1 | *Synechococcus* CC9902 | – | – | +++, ? | + | – | + |
| A2,B1 | *Synechococcus* sp. WH 5701 | + | +++, ? | + | + | + |
| A1,A2 | *Synechococcus* sp. RS9917 | – | – | +++, ? | + | – | + |
| A1,A2 | *Synechococcus* sp. RS9916 | – | – | +++, ? | + | – | + |
| A1 | *Synechococcus* sp. BL107 | – | – | +++, ? | + | – | + |

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$^a$ Habitat refers to the classification shown in Table 1 of Badger et al. (2006). Note than marine habitat species (A; marine/oceanic) are shaded.

$^b$ For SbtA, there may be multiple homologues in each genome. Strong homology is indicated by ++ (% identity with PCC6803 sequence >60%); weak homology (% identity 20–35%) is indicated by ?.

$^c$ For BicA, there are often multiple homologues in each genome. Strong homology is indicated by ++ (% identity with PCC7002 sequence >60%); moderate homology (+% identity 35–60%); and weak homology (?% identity 20–35%).

$^d$ For the NDH-1$_3$ complex, there is some ambiguity in *Crocosphaera watsonii* as to its presence. A shortened form of ChpY seems to be coded for, together with the smaller ORF133 peptide associated with these complexes (Herranen et al., 2004). However, clear NdhD3/F3 homologues appear to be absent from the current unfinished sequence.

$^e$ All β-carboxysomes contain Form 1B Rubisco whereas α-carboxysomes contain Form 1A.
liposomes. SbtA could be a single subunit transporter, but a gain-of-function approach is needed to establish if this is the case. Interestingly, a small gene downstream of sbtA in *Synechocystis* PCC6803, sbtB, is co-expressed under Ci limitation (Wang et al., 2004). The SbtA protein has been detected in cytoplasmic membranes isolated from *Synechocystis* PCC6803 with an apparent complex size of around 160 kDa (Zhang et al., 2004), indicating that SbtA probably exists in the membrane as a functional tetramer. This proteomic study also confirmed that the abundance of SbtA is dramatically increased under Ci limitation.

SbtA is also present in *Synechococcus* PCC7942 and topology predictions (Fig. 4) indicate that the 39.5 kDa protein could possess nine membrane spanning domains; two centrally located hydrophilic regions (60 and 34 amino acids) could potentially face the cytoplasm and be involved in allosteric regulation of the transporter. Previous studies have shown that a constitutive HCO$_3^-$ transport capacity in high-CO$_2$-grown cells of *Synechococcus* PCC7942 and *Synechococcus* PCC7002 can be rapidly activated under severe Ci limitation and that this response could involve protein phosphorylation via a serine–threonine protein kinase (Sültemeyer et al., 1998a); this rapid induction of HCO$_3^-$ transport is also dependent on Na$^+$ (Amoroso et al., 2003) suggesting that SbtA could be a target for this activation response.

Strong homologues of SbtA are present in many β-cyanobacteria (Badger et al., 2002, 2006; Badger and Price, 2003) but appear to be lacking from species such as *Thermosynechococcus*, *Gloeobacter violaceus*, and *Trichodesmium erythraeum* (Table 1). To date, SbtA from *Synechocystis* PCC6803 (Shibata et al., 2002a) and *Synechococcus* PCC7002 (Price et al., 2004) are the only β-cyanobacterial forms that have been confirmed by genetic and physiological analysis. There are weak homologues of SbtA present in most of the α-cyanobacteria, except *Synechococcus* WH8102 and related *Synechococcus* species, but it is not yet clear whether any of these divergent forms are able to transport HCO$_3^-$ or if so, whether the kinetic properties differ.

**BicA HCO$_3^-$ transporters**

The BicA transporter is another Na$^+$-dependent HCO$_3^-$ transporter, but with no obvious amino acid sequence similarity to SbtA. Discovered in the coastal marine cyanobacterium *Synechococcus* PCC7002 (Price et al., 2004), it is important because although it has a relatively low photosynthetic transport affinity in this organism (around 38 μM HCO$_3^-$), it is nonetheless able to support a high photosynthetic flux rate. BicA belongs to a large family of eukaryotic and prokaryotic transporters often annotated as sulphate transporters in many bacteria (the SulP family), and BicA homologues are widespread in both β- and α-cyanobacteria. Gain-of-function experiments in the freshwater cyanobacterium, *Synechococcus* PCC7942, were used to show that bicA expression alone was sufficient to confer Na$^+$-dependent, HCO$_3^-$ uptake activity. In this system, HCO$_3^-$ uptake via BicA required around 1.7 mM Na$^+$ for half maximal HCO$_3^-$ uptake activity and reached saturation in the presence of 20 mM Na$^+$ (Price et al., 2004). Gain-of-function experiments allowed two other BicA transporters to be identified and characterized, including one from the ecologically-important oceanic strain, *Synechococcus* WH8102; the other was from *Synechocystis* PCC6803. Here, the three BicA transporters had transport affinities for HCO$_3^-$ that ranged from 74 μM to 353 μM, with the *Synechocystis* PCC6803 form having the lowest affinity, the WH8120 form having the highest affinity and the PCC7002 form having an affinity of 217 μM.

In *Synechococcus* PCC7002, BicA expression is highly inducible under Ci limitation, but also appears to be expressed at low levels in cells grown at high CO$_2$ (Price et al., 2004). However, in *Synechocystis* PCC6803 the BicA gene appears to be constitutively expressed (see supplementary data in Wang et al., 2004), whereas in *Synechococcus* WH8102 and in other oceanic strains the

![Fig. 4. Topology predictions for BicA (*Synechococcus* PCC7002) and SbtA (*Synechococcus* PCC7942); predictions generated by TopPred (http://bioweb.pasteur.fr/seqanal/interfaces/toppred.html).](https://academic.oup.com/jxb/article-abstract/59/7/1441/636484)
expression characteristics have yet to be determined, although there is a strong likelihood that expression is largely constitutive in nature. The predicted $bicA$ gene product from *Synechococcus* PCC7002 is around 59.6 kDa and topology predictions indicate that it could possess up to 12 membrane spanning domains (Fig. 4). Interestingly, the C-terminus includes a hydrophilic region of about 102 amino acids that is likely to face the cytoplasm and be involved in allosteric regulation of the transporter. Indeed, protein functional domain searches indicate that this region contains a domain designated as STAS (sulphate transporter anti-sigma factor-like domain) because it has significant similarity to bacterial anti-sigma factor antagonists and is found in plant sulphate transporters. The STAS domain of SulP transporters is known to be a regulatory domain in some mammalian homologues (Ko *et al.*, 2004) and has some functional importance in *Arabidopsis* but a definite role has not been determined (Shibagaki and Grossman, 2004).

$BicA$ homologues are well represented in both $\alpha$- and $\beta$-cyanobacteria with most of the 33 sequenced cyanobacterial genomes having close or easily recognizable homologues; exceptions are *Gloeobacter* and *Synechococcus* PCC7942. In the case of *Prochlorococcus* spp. $BicA$ homologues are the only easily recognizable candidates for Ci uptake; these species have no recognizable predicted proteins for CO$_2$ uptake, but they also have a distant form of SbtA. There has been no physiological analysis of *Prochlorococcus* spp. but it is highly probable that such species are heavily dependent on HCO$_3^-$ uptake via $BicA$. Potentially, $BicA$ is an important mode of Ci uptake in many oceanic species of cyanobacteria, however, physiological studies on these species are still lacking.

**$CO_2$-uptake systems based on specialized NDH-1 complexes**

Two types of CO$_2$-uptake systems have been identified in cyanobacteria and in a sense they are not true transporters, but could be termed active facilitators since the uptake process involves passive entry of CO$_2$ into the cell followed by conversion of CO$_2$ to HCO$_3^-$. Both are based on modified NADPH dehydrogenase (NDH-1) complexes, with one system (NDH-1$_4$) being constitutively expressed, whilst the other (NDH-1$_3$) is inducible under Ci limitation and is capable of higher uptake affinity. Early studies implicated NDH-1 in active CO$_2$ uptake by cyanobacteria (Ogawa, 1992), and initially it was thought that this involved a supply of ATP generated by NDH-1 cyclic electron flow. However, within $\beta$-cyanobacteria species, initially typified by genome data from *Synechocystis* PCC6803, it became clear that there may be a number of distinct types of NDH-1 complexes with different roles within the cell (Ohkawa *et al.*, 1998, 2000a, b; Price *et al.*, 1998; Klughammer *et al.*, 1999). For instance, in the genome of *Synechocystis* PCC6803 there are a number of NDH-1 genes that are present as single copies in the genome; these are: $ndhAIGE$, $ndhB$, $ndhCJK$, $ndhH$, and $ndhL$. However, importantly, there are multiple copies of $ndhD$ (six homologues) and $ndhF$ (three homologues). Homology relationships reveal a large diversity in $NdhD$ and $NdhF$ proteins, with up to three groupings of $NdhD$ being identifiable, as well as two groups of $NdhF$ (Price *et al.*, 1998).

Some of the first data indicating that cyanobacteria possess specific NDH-1 complexes specialized for CO$_2$ uptake came from the finding that the gene cluster $ndhF3$-$ndhD3$-$chpY$ ($chpY$ then known as *Orf427*) is necessary for inducible, high-affinity CO$_2$ uptake in *Synechococcus* PCC7002, but without any effect on light-to-dark re-reduction rates of P700 (Sültemeyer *et al.*, 1997; Klughammer *et al.*, 1999). Another key finding was that $ndhD1$/$D2$ gene products, together with $ndhF1$, are involved in forming a conventional respiratory NDH-1 complex, oxidizing NADPH/NADH and reducing plastoquinone, thus enabling cyclic electron transport around PSI (Ohkawa *et al.*, 2000a, b). $NdhD3/D4$ proteins, and $NdhF3/F4$ proteins were proposed to form essential components of two specialized NDH-1 complexes involved in catalysing active CO$_2$ uptake by converting CO$_2$ to HCO$_3^-$ within the cell (Ohkawa *et al.*, 2000b; Shibata *et al.*, 2001; Maeda *et al.*, 2002). The exact role of $NdhD5/D6$ polypeptides (alternatively known as $MnhD1$ and $MnhD2$) is still unclear, but they do have similarity to another specialized NDH-1 complex ($Mnh$ complex) that is thought to be involved in Na$^+$/H$^+$ antiport in bacteria (Hiramatsu *et al.*, 1998), but could also be involved in primary Na$^+$ extrusion (generation of an electrochemical Na$^+$ gradient). An $Mnh$-like operon is known to be Ci-responsive in *Synechocystis* PCC6803 (Wang *et al.*, 2004) and could potentially aid in Na$^+$-dependent energization of HCO$_3^-$ uptake due to SbtA and BicA. Interestingly, an $Mnh$ cluster, including $mnhD1$ and $mnhD2$, is expressed as part of the $bicA$ operon in *Synechococcus* PCC7002, and is responsible for improving the efficiency of HCO$_3^-$-dependent photosynthesis (Woodger *et al.*, 2007).

As part of the two CO$_2$ uptake systems, two other proteins are involved in enabling the CO$_2$-uptake activity of the NDH-1 complex, and these are referred to here as ChpX and ChpY (Maeda *et al.*, 2002) for CO$_2$ hydration proteins; note that these proteins are also known as $CupB$ and $CupA$ (Shibata *et al.*, 2001). Available phenomic evidence (Shibata *et al.*, 2001; Maeda *et al.*, 2002) indicates that the $ndhF3/ndhD3/chpY$ orf133 genes code for polypeptides that are part of a high-affinity CO$_2$ uptake NDH-1$_3$ complex, while the $ndhF4/ndhD4/chpX$ genes code for a constitutively expressed NDH-1$_4$ complex involved in low-affinity CO$_2$ uptake. Proteomic studies in *Synechocystis* PCC6803 have confirmed the presence of $NdhF4/2/ChpY/11735$ (Herranen *et al.*, 2004; Prommeenate *et al.*, 2004; Zhang *et al.*, 2004) as...
being present in thylakoid membranes as a subcomplex that is induced under Ci limitation. Paradoxically, such studies have so far failed to detect the constitutively expressed NdhF4/NdhD4/ChpX proteins in thylakoid or plasma membranes. One explanation could be that the NDH-I complex is located on the plasma membrane and is unintentionally lost during cell fractionation procedures. Inhibitor studies with *Synechococcus* PCC7942 mutants lacking one or other CO₂ uptake system have unearthed a differential sensitivity to DCMU, with the NDH-I₄ system being largely unaffected by DCMU, whereas the thylakoid-based NDH-I₃ system is sensitive (Maeda *et al.*, 2002). These data suggest that the NDH-I₄ system could be located on the plasma membrane (i.e. not closely dependent on PSII activity). Recent evidence from *Synechococcus* PCC7942 indicates that ChpX can be detected in isolated plasma membrane fractions (GD Price, unpublished data).

It has previously been speculated that the ChpX and ChpY polypeptides may be an integral part of the NDH-1 CO₂-uptake complex (NDH-1/3/4) and involved directly in the unidirectional conversion of CO₂ to HCO₃⁻, linked to electron transport and proton translocation functions associated with the NDH-1 complex (Maeda *et al.*, 2002; Price *et al.*, 2002). As noted above, proteomic data indicate that NDH-I₄ complexes are restricted to the thylakoid membrane thus linking them directly to the photosynthetic electron transport chain. A model of the operation of such an NDH-I₃/4 CO₂-uptake complex has been presented previously (Price *et al.*, 2002; Badger and Price, 2003) based on the generation a nuclephilic Zn-OH⁻ species within the ChpX or ChpY proteins of specialized NDH-1 complexes.

Considering the present availability of 33 cyanobacterial genome sequences it is clear that there is significant diversity in NDH-1/3/4 gene content across the α- and β-cyanobacteria, as previously proposed (Badger *et al.*, 2002, 2006; Badger and Price, 2003). The sequenced genomes of all 14 β-cyanobacteria contain single copies of the specific ndhF4, ndhD4, and chpY genes required for low-affinity CO₂ uptake via NDH-I₄ complexes (Table 1). Of these cyanobacteria, all possess the specific genes for high-affinity CO₂-uptake (NDH-1) except for the marine β-cyanobacterium *Trichodesmium erythraeum* which has only the low-affinity uptake forms; there is uncertainty if *Crocosphaera watsonii* has a full set of NDH-1₄-specific genes. In the oceanic α-cyanobacteria, *Synechococcus* spp. such as *Synechococcus* WH8102, are the only oceanic species, so far, that possess single copies of the *ndhF4*, *ndhD4*, and *chpX* genes coding for a putative low-affinity CO₂-uptake system, i.e. they lack the specific genes for the high-affinity uptake of CO₂. However, an exception is the peculiar *Synechococcus* WH5701 strain that has specific genes for both types of CO₂ uptake, as well as genes for the three types of HCO₃⁻ uptake. As noted previously (Badger *et al.*, 2002; Badger and Price, 2003) none of the 10 sequenced *Prochlorococcus* species have homologues for either the low-affinity or high affinity NDH-1/3/4 specific genes. This absence leads to the prediction that *Prochlorococcus* species lack the capacity for active CO₂ uptake, unless they possess another active CO₂-uptake system that is presently unidentified. It should be noted that the genetic and physiological characterization of CO₂ uptake has been achieved mainly in three common β-cyanobacterial strains, *Synechococcus* PCC7002, *Synechococcus* PCC7942, and *Synechocystis* PCC6803 (Klughammer *et al.*, 1999; Shibata *et al.*, 2001; Maeda *et al.*, 2002; Price *et al.*, 2004) and so the comparative diversity in transporter kinetics and gene expression are not known in many species.

### Regulation of the CCM

#### The constitutive state of the CCM

A notable feature of the cyanobacterial CCM is the existence of a basic, fully functional form of the CCM when cells are grown at non-limiting levels of Ci. This constitutive level of expression of CCM activity is present even in cells grown at hyper-normal levels of CO₂ (e.g. 2% CO₂) and it is easy to demonstrate that such cells generally use both CO₂ and HCO₃⁻ as substrates for Ci accumulation at moderate uptake affinity (Fig. 3); such cells are often termed high-Ci cells, and this basal CCM state can even be produced in cultures vigorously bubbled with air (Yu *et al.*, 1994). In freshwater strains such as *Synechococcus* PCC7942, uptake of CO₂ through the constitutive CO₂-uptake system (NDH-I₄) is possibly the predominant form of Ci uptake in high-Ci cells provided the CO₂ supply rate can be maintained. One demonstration of the dominance of CO₂ uptake is that carefully managed mutants lacking ChpX have a pronounced high-CO₂-requiring (HCR) physiological phenotype characterized by requiring more than 100 mM external Ci to reach near maximal rates of photosynthesis (Woodger *et al.*, 2005b). Similarly, high-Ci cells employ a fully functional carboxysome system as demonstrated by a number of carboxysome mutations that produce HCR growth phenotypes that are unable to survive at air levels of CO₂ (0.038% CO₂) or even 0.14% CO₂ in air (Friedberg *et al.*, 1989; Price and Badger, 1989b; Emlyn-Jones *et al.*, 2006).

#### The fully induced state of the CCM

When exposed to Ci limitation β-cyanobacteria have the ability to express an enhanced level of CCM activity. This change is accompanied by increases in Rubisco activity (Price *et al.*, 1992), and up to 2-fold increases in carboxysome content in some species (Turpin *et al.*, 1984; McKay *et al.*, 1993) and increases in the affinities for CO₂ and HCO₃⁻ uptake activities (Badger and Price, 1992; Kaplan *et al.*, 1994; Kaplan and Reinhold, 1999;
Price et al., 1998). Typically a 20-fold decrease in $K_{\text{a}}$(Ci) is seen when cells are grown at 30 ppm CO$_2$ levels (Fig. 3) and more than half of this rise in affinity is due to a lift in the affinity for HCO$_3^-$ uptake in Synechococcus PCC7942 (Yu et al., 1994), and considerably more than half in the estuarine Synechococcus PCC7002 strain (Sülttemeyer et al., 1995). The induction of enhanced uptake capabilities can approach completion within 4 h of transfer from high Ci conditions to 20 ppm (Yu et al., 1994) or air (Omata and Ogawa, 1986). Notably, if instead of simply switching cultures from high CO$_2$ to low CO$_2$, the cells are collected by centrifugation and washed in to low-Ci media, cells can approach full physiological induction within 60–90 min (Woodger et al., 2003).

Those genes that respond to Ci limitation in β-cyanobacteria are chiefly the high-affinity Ci-uptake systems. In Synechococcus PCC7942 and Synechocystis PCC6803 this includes genes for NDH-I$_4$-specific components, BCT1 and SbtA (Shibata et al., 2002b; McGinn et al., 2003; Woodger et al., 2003). In the estuarine Synechococcus PCC7002 strain, CO$_2$-responsive systems include NDH-I$_4$-specific components, BicA, SbtA, and a probable porin, PorB, that could potentially be involved to HCO$_3^-$ access across the outer membrane (Price et al., 2004; Woodger et al., 2007). There is much less known about adaptation to limiting Ci conditions in α-cyanobacteria. However, judging from the data in Table 1 it seems likely that most oceanic strains lack inducible, high affinity uptake systems. However, one physiological study on Synechococcus WH7803, suggests that photosynthetic affinity for Ci can be improved by growth at air levels of CO$_2$ (Hassidim et al., 1997), further indicating that expression of basal Ci transporters may be at least partly inducible. Also, a study by Palinska et al., (2002) suggests that Prochlorococcus marinus strain PCC9511 (closely related to P. marinus MED4) possesses HCO$_3^-$ uptake with apparent $K_{m}$s of 240 μM and 4 μM; this is consistent with the possibility that the identified homologues for BicA, and distant forms SbtA in Prochlorococcus species, are active in Ci uptake.

**Genetic regulation of the CCM**

From experimental and genomic information it is clear that the β-cyanobacteria have the greatest capacity to respond to Ci limitation by up-regulating the expression of high affinity Ci transporters such as high affinity CO$_2$ uptake (NDH-I$_4$), SbtA, BCT1, and BicA. From an environmental niche perspective this makes sense since Ci availability is most variable in the habitats occupied by β-cyanobacteria (Badger et al., 2006). Only three species of β-cyanobacteria, namely Synechococcus PCC7942, Synechocystis PCC6803, and Synechococcus PCC7002, have been studied in any detail at the physiological level, and combined with data for gene transcription. To achieve a physiological state of the CCM with high uptake affinity for Ci, these cyanobacteria generally add uptake systems with high affinity and low-to-moderate maximum flux rates. CO$_2$-responsive gene transcripts can respond to Ci limitation in as little as 15 min (McGinn et al., 2003; Woodger et al., 2003, 2005a, b, 2007); carboxysome components show relatively little up-regulation under Ci limitation. The inducible Ci uptake systems usually complement the kinetics of the constitutively expressed, low-affinity, high-flux-rate uptake systems (Price et al., 1998). For instance, high-Ci cells of Synechococcus PCC7942 possess a constitutively expressed high-flux low-affinity CO$_2$-uptake system (NDH-I$_4$) and possibly a low level of SbtA, but after the onset of Ci limitation, three high-affinity low-flux systems are added (Fig. 5); these systems are the high-affinity CO$_2$-uptake system (NDH-I$_3$), the high-affinity SbtA and BCT1 HCO$_3^-$ transporters (Maeda et al., 2002; Omata et al., 1999; Woodger et al., 2003, 2005b; Price et al., 2004). The response differs somewhat in Synechococcus PCC7002 (Fig. 5); here high-Ci cells express NDH-I$_4$ and possibly a low level of BicA, and upon Ci limitation, two low-flux high-affinity systems are induced.

![Fig. 5. Transcriptional regulation of CO$_2$-responsive genes in Synechocystis PCC6803 (A) and Synechococcus PCC7002 (B).](https://academic.oup.com/jxb/article-abstract/59/7/1441/636484)
(NDH-13 and SbtA) plus a high-flux low-affinity BicA HCO\(_3^-\) transporter is up-regulated, and a putative HCO\(_3^-\) porin, PorB (Price et al., 2004; Woodger et al., 2007). The induction of CO\(_2\)-responsive genes can be quite rapid, usually within 15 min, if cells are first washed and transferred to low-Ci media in the light (McGinn et al., 2003; Woodger et al., 2003, 2005b).

In β-cyanobacteria, the expression of CO\(_2\)-responsive genes is controlled by one or two LysR-type transcription regulators known as CcmR (NdhR) and CmpR (Omata et al., 2001; Wang et al., 2004; Woodger et al., 2007). The LysR-family is a large family of DNA binding proteins that act to either activate or repress a particular gene by interacting with RNA polymerase at the promoter region of specific genes in proteobacteria (Schell, 1993). Within the LysR family, CcmR and CmpR fall within a subfamily known as the proteobacterial Calvin-cycle regulator, CbbR (Fig. 6). In cyanobacteria, the CbbR subfamily also contains a dominant form known as RbcR (or CbbR) and all strains so far seem to have this member. RbcR is possibly responsible for the expression of constitutive CCM genes such as carboxysomes genes and \(rbcL/X\delta\), however, since gene inactivations appear to be lethal (Figge et al., 2001; Omata et al., 2001) full characterization of the genes regulated by RbcR has been hampered. RbcR forms fall into two subgroups based on α-cyanobacterial and β-cyanobacterial sources (Fig. 6). The more diverse CcmR/CmpR group seems to be restricted to the β-cyanobacteria, or in other words, those strains that regularly experience large fluctuations in Ci availability. Importantly, the presence of CcmR/CmpR correlates with the presence of one or more inducible high-affinity transporters (Table 1; Fig. 6). *Trichodesmium erythraeum* is the only β-cyanobacterium that lacks CcmR/CmpR, but it also lacks any genes associated with high affinity transporters.

In *Synechocystis* PCC6803 and *Synechococcus* PCC7942, CmpR has been shown to regulate the CO\(_2\)-responsive, \(cmpA\beta\theta(B\betaC)D\) operon (BCT1 transporter), where it apparently acts as a transcriptional activator (Omata et al., 2001). Also in *Synechocystis* PCC6803, CcmR (NdhR) acts as a negative regulator of CO\(_2\)-responsive genes (Fig. 6), other than \(cmpA\beta\theta(B\betaC)D\) (Wang et al., 2004). However, in *Synechococcus* PCC7002, Cmr acts as negative regulator of all the known CO\(_2\)-responsive genes (Fig. 6), as a CmpR form is absent from the genome (Woodger et al., 2007). That CcmR is a repressor in *Synechocystis* PCC6803 and

![Fig. 6. A phylogenetic tree of the CbbR family in α- and β-cyanobacteria. The RbcR subfamilies are quite distinct from the more diverse family of CcmR and CmpR proteins that are involved in regulated transcription of CO\(_2\)-responsive genes in several model species β-cyanobacteria, and, possibly, in other species as well. Protocols were aligned using CLUSTALW and the dendrogram was generated in the TreeView program. Abbreviations: Ana-var, Anabaena variabilis ATCC29413; Croco, Crocosphaera watsonii WH8501; Gloeo, Gloeobacter violaceus PCC7421; Npunct, Nostoc punctiforme PCC73102; PCC7120, Nostoc sp. PCC7120; MIT9312 or NATL2A, Prochlorococcus marinus strains MIT9312 or NATL2A; PCC6301 or PCC7942, Synechococcus elongatus PCC6301 or PCC7942; BL107, Synechococcus sp. BL107; CC9902, Synechococcus sp. CC9902; IA-2-3B, Synechococcus sp. IA-2-3B(a(2-13)); JA-3-3A, Synechococcus sp. JA-3-3Ah; RS9916, Synechococcus sp. RS9916; RS9917, Synechococcus sp. RS9917; WH5701, Synechococcus sp. WH5701; WH7805, Synechococcus sp. WH7805; WH1802, Synechococcus sp. WH1802; PCC6803, Synechocystis sp. PCC6803; Thermo, Thermosynechococcus elongatus BP-1; Tricho, Trichodesmium erythraeum IMS101.](https://academic.oup.com/jxb/article-abstract/59/7/1441/636484)
Synechococcus PCC7002 is clearly demonstrated by gene disruption, leading to a spectacular derepressed phenotype where even high-Ci cells possess high affinity Ci uptake (Wang et al., 2004; Woodger et al., 2007). Regulation of CO2-responsive genes in Synechococcus PCC7942 is still unclear, as this species lacks an identifiable ccmR gene; in all probability CmpR should be renamed CcmR, but if so this regulator could be both an activator (cmpABC) and repressor (sbtA, ndhF3-D3-chpY) of gene transcription. Amongst the α-cyanobacteria, the peculiar Synechococcus WH5701 strain has the most likelihood of needing and possessing CcmR or CmpR, however, an oddity at this stage is that ccmR cannot be identified in the draft genome sequence.

The precise signal transduction pathway through CcmR or CmpR is still unknown, however, after considering a number of potential signalling conditions it has been found that expression of CO2-responsive genes best correlates with the size of the internal Ci pool being the primary signal under illuminated conditions. Here, induction of expression would occur when the Ci pool drops below some threshold value, possibly below 5 mM, causing CcmR to unbind from the promoter allowing derepression of transcription (Woodger et al., 2003, 2005a, b). Likewise, CmpR would be postulated to bind to a CO2-responsive promoter when internal Ci levels are low, causing transcriptional activation. Given the cases where LysR transcription factors respond directly to small analytes (Schell, 1993), one suggestion is that CcmR itself may be able to respond directly to the HCO$_3^-$ accumulation level in the cell (Woodger et al., 2005b); a two- or three-component phosphorylation signalling cascade is also possible. It is worth noting that a prerequisite signal for expression of CO2-responsive genes is the presence of light, as Ci limitation in darkness is ineffective for induction (F Woodger, unpublished data; McGinn et al., 2003, 2005). The way in which light is transduced as a signal to ‘unlock’ CO2-responsive gene expression is unknown but could involve redox or phytochrome signals. Ci limitation as a basic induction signal is also accompanied by secondary signals that can enhance the transcriptional response. For instance, high irradiance is known to enhance expression of CO2-responsive genes (Woodger et al., 2003; McGinn et al., 2004) although part of this response could be due to a greater flux through photosynthetic CO2 fixation and an associated drawdown of internal Ci levels. On the basis of limited evidence from PII (glnB) mutants, nitrogen status might also be a secondary signal acting on expression of CO2-responsive genes (Hisbergues et al., 1999).

Over the years a number of theories have been suggested as the primary event for signalling Ci limitation. These include external Ci status, over-reduction of photosystem redox status, increases in photorespiratory intermediates, increases in Calvin cycle precursors, or a rise in reactive oxygen species (ROS) (Kaplan and Reinhold, 1999). Any link between external Ci concentration and induction is easily broken by the addition of Calvin-cycle and PSII inhibitors (Woodger et al., 2003; McGinn et al., 2004). Similarly, the redox status of the photosynthetic electron transport pathway and ROS tend to be unlikely signals since Calvin cycle inhibitors that would enhance light stress and ROS by elimination of CO2 as an ultimate electron acceptor do not lead to the induction of CO2-responsive genes (Woodger et al., 2003, 2005a, b; McGinn et al., 2004). In addition, high light stress under Ci-sufficient conditions does not lead to the induction of CO2-responsive genes (McGinn et al., 2003; Woodger et al., 2003). It has been established that Synechocystis PCC6803 has plant-like and bacterial-type cycles for dealing with the metabolism of photorespiratory phosphoglycolate (Eisenhut et al., 2006), so an increase in photorespiratory intermediates under Ci-limited conditions has long been a favoured primary signal for CCM induction. One inhibitor combination seems to argue against photorespiration as an induction signal; here glycolaldehyde was used to block both CO2 fixation and photorespiration (leading to no CCM induction), yet when an inhibitor of CO2 uptake, ethoxyzolamide, was added, causing a reduction in the internal Ci pool, induction of CO2-responsive genes did occur (Woodger et al., 2005b). This suggests that a low internal Ci pool is more likely to be the primary induction signal than photorespiration. Other evidence in support of internal Ci as the primary signal included the use of a chpX/chpY double mutant that lacks CO2 uptake and has poor Ci accumulation (Woodger et al., 2005b); here a switch from growth at 5% CO2 to 1% CO2 caused a marked induction of CO2-responsive genes relative to WT cells (unaffected). This study also found an effect of O2 levels on the enhancement of induction, but the reason for this enhancement is unclear.

**Allosteric or post-translational regulation of Ci transport systems**

As pointed out in the section on the Energization of Ci uptake, a significant gap in our understanding of the regulation of Ci uptake processes concerns the nature of the molecular events controlling post-translational or allosteric activation of Ci transporters. It is apparent that cyanobacterial Ci transporters are inactive in darkness, possibly to prevent futile cycling, but upon illumination uptake is quickly activated, with CO2 uptake being very rapid and HCO$_3^-$ uptake activated within 20–30 s. There is some evidence that activation of HCO$_3^-$ transporters might involve a redox signal (Kaplan et al., 1987), however, activation due to a protein kinase-mediated phosphorylation of transporters is certainly a possibility (and deactivation by a specific protein phosphorylase). For instance, the putative regulatory domains mentioned on the cytoplasmic faces of SbtA and BicA (Fig. 4) are
regions of future research interest as possible sites for activation of Ci transporters by phosphorylation. Phosphorylation via protein phosphokinases of the Ser/Thr class has already been implicated in the rapid induction of latent HCO$_3^-$ uptake capacity in *Synechococcus* PCC7942 and PCC7002 species subjected to severe Ci limitation (Sültемeyer *et al.*, 1998a, b).

**Prospects for introducing cyanobacterial CCM components into higher plants**

*C*$_3$ crop plants do not appear to possess active uptake systems for the acquisition of CO$_2$ or HCO$_3^-$. There is a case for suggesting that cyanobacteria may have evolved a CCM based on carboxysomes and novel Ci transporters as late as 350 million years (Mya) ago during the dramatic decline in global CO$_2$ levels that occurred around this time (Badger *et al.*, 2002; Badger and Price, 2003; Giordano *et al.*, 2005). If so, this event is well after the first terrestrial plants evolved from eukaryotic algae around ~450 Mya (Kenrick and Crane, 1997). Analysis of the *Arabidopsis* genome makes it clear that cyanobacterial CCM components are absent from plants. However, some plant species such as maize and sugar cane are known to possess a biochemically and anatomically complex CCM known as the C$_4$ cycle, and as a result, use significantly less water and N than *C*$_3$ plants (e.g. wheat and canola); [NB: there are notable variants of the C$_4$ cycle known as ‘single-cell C$_4$’ that occur in land plants (Edwards *et al.*, 2004) and diatoms (Granum *et al.*, 2005) that allow the C$_4$ cycle to proceed in a single cell, but with a level of intracellular compartmentalization of biochemical reactions]. C$_3$ crop plants lose around 500 molecules of water through leaf pores for every CO$_2$ molecule that is acquired from the atmosphere; C$_4$ plants can use as little as half this amount of water per CO$_2$ fixed (von Caemmerer and Furbank, 2003). In an effort to improve CO$_2$ fixation the plant allocates up to 50% of soluble protein reserves to the inefficient Rubisco enzyme: improving CO$_2$ fixation the plant allocates up to 50% of the C4 cycle to proceed in a single cell, but with a level of intracellular compartmentalization of biochemical reactions]. 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. The uptake affinities of SbtA (low flux rate) and BicA (high flux rate) for HCO$_3^-$ in cyanobacteria are 5–15 µM and 40–100 µM, respectively (Shibata *et al.*, 2002a; Price *et al.*, 2004). So there would appear to be sufficient HCO$_3^-$ substrate since at least 250 µM HCO$_3^-$ is present in the cytosol of a leaf cell (Evans and von Caemmerer, 1996) and this appears to be maintained by cytosolic CA activity. Both SbtA and BicA require about 1 mM Na$^+$ for half maximal activity. The leaf cytosol possesses 1–3 mM Na$^+$ (Karley *et al.*, 2000) and recent 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 an inwardly-directed Na$^+$ gradient; the transfer of a cyanobacterial Na$^+$/H$^+$ antiporter could be 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). Other problems in establishing BicA and/or SbtA in C₃ chloroplasts would include correct targeting to the chloroplast envelope and uncertainty about whether these transporters would be post-translationally activated.

It is noteworthy that one published attempt to place a putative cyanobacterial Ci transporter, IctB, in plants has been reported to improve photosynthetic performance (water use-efficiency) in Arabidopsis plants grown under dry air conditions; in particular, a drop in the CO₂ compensation point was observed (Lieman-Hurwitz et al., 2003). The gene in question has been shown to have a role in HCO₃⁻ uptake, and at one stage was thought to be a HCO₃⁻ transporter (Bonfil et al., 1998). However, it is now clear that the gene does not code for a transporter (Shibata et al., 2002a) and its role in cyanobacteria is still unclear; strangely it has not been possible to inactivate ictB insertionaly, and obtain fully segregated mutants, even though all five known Ci transporters in cyanobacteria have been successfully inactivated. Likewise, the basis of the beneficial effects of expressing ictB in plants is still unclear.

A longer-term objective, involving considerable technical hurdles, would be to establish a more elaborate form of the cyanobacterial CCM in the chloroplast. Ideally, this would involve the transfer of a functionally active HCO₃⁻ transporter combined with a CO₂ uptake system; the latter would be particularly important for reducing the inevitable back flux of CO₂ from the chloroplast to the cytosol due to HCO₃⁻ uptake. In addition, it would be necessary to reorganize chloroplastic Rubisco into an effective carboxysome or pseudo-pyrenoid. However, for this to be effective a means of removing the highly abundant chloroplastic CA would need to be devised. Certainly, it has been possible to remove up to 99% of chloroplastic CA activity in tobacco leaves by antisense RNA approaches (Majeau et al., 1994; Price et al., 1994), although a complete removal of CA from the stroma would be more desirable.

It is worth noting that one homologue of a cyanobacterial active Ci acquisition system may already exist in higher plants. It has recently been found that the mitochondrial NDH-I complex (complex I) has a gamma CA subunit that appears to be an integral and extrinsic part of the complex (Sunderhaus et al., 2006). Based on functional homology with the NDH-I CO₂ uptake complex in cyanobacteria, there is speculation that this may play a role in actively converting CO₂ to HCO₃⁻ in the mitochondria (Braun and Zabaleta, 2007). The conversion of CO₂ to HCO₃⁻ in the mitochondria could act as a means of recycling photorespiratory CO₂ to the chloroplast. This proposal is, in part, similar to one suggested for micro-algal photorespiration (Raven, 2001). There is presently a lack of evidence for the recycling of photo-respiratory CO₂ via a gamma-CA/complex I system, but if proven it may suggest an interesting possibility for elevation of the CO₂ level in the chloroplast.

**Concluding remarks**

There have been significant gains in the past 10 years towards understanding the components required to achieve a functional CCM in cyanobacteria. These include the genetic identification and physiological characterization of five distinct modes of active Ci uptake in cyanobacteria. While it has been relatively easy to identify gene homologues in the 33 sequenced cyanobacterial genomes presently available, the characterization of the general properties and uptake kinetics of transporters has mainly been restricted to three model β-cyanobacteria, namely Synechocystis PCC6803, Synechococcus PCC7942, and Synechococcus PCC7002. Similarly, good progress has been made towards understanding the transcriptional regulation of the subset of CCM genes that are CO₂-responsive, with CcmR and CmpR identified as key transcriptional regulators, but once again most of the available data are restricted to the three model systems. Progress has also been made on the task of unravelling the protein:protein interactions that lead to the assembly of carboxysomes, with information available on both α-carboxysomes (chiefly using Thiobacillus as a model system) and β-carboxysomes, chiefly from freshwater model systems. In the area of comparative analysis of transporters, carboxysomes and regulation of the CCM, the details in each strain do certainly differ, and hopefully we are getting to a situation where those who believe that it is sufficient to study just one model system are fading into a minority.

The challenge for the next decade is to engage in the area of comparative phenomics of CCM function and regulation in a wider selection of cyanobacteria, with considerable attention to the highly productive oceanic strains. Particular gaps in our understanding have been highlighted throughout this review. This would include the comparative kinetics of a wider range of transporter homologues, particularly from the α-cyanobacteria; a gain-of-function approach in a model system may be one way of proceeding. The structure and function of the key transporters also need to be determined; this is likely initially to involve topology mapping procedures, and eventually proceed to the determination of crystal structures. A particular gap revolves around the question of how transporters are regulated at the allosteric or post-translational level to produce inactive transporters in the dark, with reactivation during illumination. The nature of the primary signal for the induction of CO₂-responsive genes in response to Ci limitation needs to be resolved, along with the signal transduction mechanism that ultimately regulates transcriptional regulators such as CcmR, CmpR, and RbcR. Secondary signal transduction
pathways, such as light and nitrogen status, that impinge on the primary signal also need to be characterized. There is still much to be determined about the regulation and assembly of carboxysomes, and a comparison of the relative efficiency of β-carboxysomes versus α-carboxysomes. In the longer term, we need to consider strategies for engineering some of the cyanobacterial components into higher plants with an eventual goal of improving water and nitrogen-use efficiencies.

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