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The environmental plasticity and ecological genomics of the cyanobacterial CO_2 concentrating mechanism

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Abstract

Cyanobacteria probably exhibit the widest range of diversity in growth habitats of all photosynthetic organisms. They are found in cold and hot, alkaline and acidic, marine, freshwater, saline, terrestrial, and symbiotic environments. In addition to this, they originated on earth at least 2.5 billion years ago and have evolved through periods of dramatic O_2 increases, CO_2 declines, and temperature changes. One of the key problems they have faced through evolution and in their current environments is the capture of CO_2 and its efficient use by Rubisco in photosynthesis. A central response to this challenge has been the development of a CO_2 concentrating mechanism (CCM) that can be adapted to various environmental limitations. There are two primary functional elements of this CCM. Firstly, the containment of Rubisco in carboxysome protein microbodies within the cell (the sites of CO_2 elevation), and, secondly, the presence of several inorganic carbon (Ci) transporters that deliver HCO_3^- intracellularly. Cyanobacteria show both species adaptation and acclimation of this mechanism. Between species, there are differences in the suites of Ci transporters in each genome, the nature of the carboxysome structures and the functional roles of carbonic anhydrases. Within a species, different CCM activities can be induced depending on the Ci availability in the environment. This acclimation is largely based on the induction of multiple Ci transporters with different affinities and specificities for either CO_2 or HCO_3^- as substrates. These features are discussed in relation to our current knowledge of the genomic sequences of a diverse array of cyanobacteria and their ecological environments.

Key words: Carboxysomes, CO_2 concentrating mechanism, CO_2 transporters, cyanobacteria, HCO_3^- transporters, ecological genomics, photosynthesis.

Introduction

Of all photosynthetic organisms, cyanobacteria probably inhabit the widest range of ecological habitats. They are found in cold and hot, alkaline and acidic, marine, freshwater, saline, terrestrial, and symbiotic environments. This broad habitat range is due to the fact that they evolved a PSII reaction centre that can extract electrons from water and thus are not limited to environments with other scarcer reduced electron donors, as are other non-oxygenic photosynthetic prokaryotes. In fact, cyanobacteria seem to be able to establish competitive growth in almost any environment that has, at least temporarily, liquid water and sunlight. The diversity and adaptability of cyanobacterial species is also in no small part due to the fact that they have withstood the challenges of evolutionary environmental change. Since their appearance at least 2.5 billion years ago (see Giordano *et al*., 2005, for a detailed discussion), the earth has experienced periods of high and low temperatures, high and low CO_2, and low and high O_2 levels. These temporal and spatial variations have been the driving force for the evolution and acquisition of many genes and physiological properties which have enabled successful growth in the diverse range of environments occupied by cyanobacteria today.

The diverse environments occupied by cyanobacterial species vary dramatically with regard to factors that may limit CO_2 fixation. Major challenges to photosynthesis include (i) the restricted diffusion of Ci $(CO_2 + HCO_3^-)$

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species in water, which is only one ten-thousandth of the value in air; (ii) variability in the levels of Ci and the predominant form of Ci, HCO_3^-, or CO_2, which is available for uptake; (iii) wide fluctuations in temperatures and light, and (iv) fluctuations in O_2 levels, which can vary from anaerobic to supersaturated through a daily cycle. The significance of these challenges are realized when one considers that the capture of CO_2 is finally dependent on the CO_2-fixing enzyme Rubisco which has inherent inefficiencies that are exacerbated by many of these extreme environmental conditions. This review focuses on how cyanobacteria have met the challenges posed for photosynthetic CO_2 acquisition by the development of a flexible CO_2 concentrating mechanism (CCM) and particularly addresses what is known about the genomic diversity of the CCM within cyanobacterial species and how this has evolved to match their habitat requirements.

Environments inhabited by cyanobacteria

Table 1 summarizes the various aquatic and terrestrial environments that are commonly inhabited by cyanobacteria. The environmental characteristics that are typical of these various habitats, with particular reference to factors that may influence CO_2 acquisition and fixation by photosynthesis being noted, along with a list of common species.

Marine planktonic environments

Cyanobacteria are an important component of oceanic, coastal, and estuarine marine phytoplankton communities

Table 1. *Ecological habitats occupied by cyanobacteria*

Habitat	Common species	Environmental characteristics	Ci supply conditions
A. Marine planktonic			
A1 Oceanic	*Prochlorococcus, Synechococcus, Trichodesmium, Crocosphaera*	May range from high light, oligotrophic to low light higher nutrients. Environments range from tropical to polar.	Ci is consistently around 2 mM and pH 8.3.
A2 Coastal	*Synechococcus, Oscillatoria, Phormidium, Trichodesmium*	May range from oligotrophic to eutrophic, depending on terrestrial inputs.	Ci generally around 2 mM but may be altered by pulses of freshwater and nutrients from land. Some bloom-forming conditions.
A3 Estuarine	*Anabaena, Aphanizomenon*	More variable changes in salinity, pH, temperature and nutrients	Ci levels and pH will change with freshwater inputs;nutrient pulses and temperature increases will create bloom-forming conditions
B. Freshwater planktonic			
B1 Non-bloom-forming	*Cyanobium, Synechococcus, Cyanothece, Aphanocapsa, Aphanothece, Chroococcus*	Waters are poorly buffered, lakes may stratify in summer favouring growth in high-light surface waters. Nutrients may range from oligotrophic to eutrophic	Dramatic changes in the temperature, pH and nutrient supply will all alter the conditions for growth and Ci acquisition. HCO_3^- becomes dominant species under high photosynthetic conditions and alkaline pH.
B2 Bloom-forming	*Anabaena, Aphanizomenon, Microcystis, Oscillatoria, Spirulina*	Gas vacuoles allow species to control buoyancy and exploit high-light waters in in summer. Stratification and mixing of lakes is important in creating bloom conditions.	Factors outlined above are important, but will be accentuated under high density bloom conditions. pH levels rise, Ci declines, surface temperature rises, and oxygen may build up during peak photosynthetic conditions.
C. Microbial mats			
C1 Coastal mats C2 Hypersaline mats C3 Hot Spring mats C4 Terrestrial-mats	*Microcoleus, Symploca, Schizothrix* *Aphanothece, Microcoleus, Oscillatoria, Spirulina* *Oscillatoria, Chlorogloeopsis, Synechococcus* *Crinalium, Microcoleus, Chroococcus, Nostoc*	Light is strongly attenuated in microbial mats; the photic zone may extend 1–2 mm from the surface. Diffusion is limited, allowing anoxic conditions to occur at night and hyperoxic, high pH and Ci depletion during photosynthetic conditions. High surface temperatures occur.	Strong gradients of light, temperature, nutrients, O_2, pH, and Ci during photosynthesis all present challenges for efficient Ci acquisition.
D. Symbiotic			
D1 Lower and higher lants	*Nostoc*	Occupy small pockets or cavities formed by the host. Aquatic or aerobic environments.	The CO_2 supply is derived either from host respiration or externally. For aerobic environments, Ci may be present around the cyanobacterium predominantly as CO_2, depending on the extracellular pH. The CO_2 supply may be limited in lichens when thalli are water saturated.
D2 Fungi	*Nostoc, Calothrix, Scytonema, Gloeocapsa, Gloeothece*	Are found as an extracellular layer within the lichen thallus. Thalli may be filled with water or air.	
D3 Sponges	*Prochloron, Prochlorothrix*	May exist both inter- and extracellularly with a host living in seawater.	The CO_2 supply can come from host respiration or as CO_2 or HCO_3^- from the seawater.

and contribute significantly to the carbon and nitrogen cycling of these environments (Paerl, 2000). As much of the world's ocean surface waters are nitrogen-deficient, N_2-fixing cyanobacteria can compete well in this environment. In warm waters, the non-heterocystous N_2-fixing cyanobacteria *Trichodesmium* and *Crocosphaera* are vital components of the global nitrogen cycle through the fixation of new nitrogen (Webb *et al.*, 2001). Single-celled cyanobacteria such as *Synechococcus* and *Prochlorococcus* are also common and often dominant in the subsurface waters from 40° S to 40° N where they may account for more than 50% of the photosynthetic biomass (Paerl, 2000; Partensky *et al.*, 1999). These picoplankton forms (<5 μm) generally do not have the capacity to fix N_2, but their small size, high surface-to-volume ratios, and ability to grow at a range of light intensities, particularly nutrient-rich deep waters, may ensure their access to nitrogen and other nutrients (Paerl, 2000). Cyanobacteria that periodically dominate oligotrophic waters employ various strategies to improve photosynthesis and nutrient acquisition, including the ability to regulate buoyancy (e.g. *Trichodesmium*), aggregation in colonies and consortia, and light-harvesting pigment adaptation. In addition to their ability to thrive in oligotrophic waters, many cyanobacteria can exploit more nutrient-rich estuaries and seas, sometimes as persistent nuisance blooms.

In general, marine environments are relatively constant in their pH and inorganic carbon conditions compared with freshwater environments. The Ci levels in open oceans are around 2 mM with a pH of 8.3. The HCO_3^-/CO_2 ratio is over 100 and thus HCO_3^- is the dominant form of Ci. Species growing in coastal and estuarine environments may experience more variable environments with inputs of nutrients and freshwater from the land which may lead to higher growth rates. Under these conditions and particularly in estuaries, it is possible for Ci levels to fall and pH to rise.

Freshwater planktonic environments

Cyanobacteria are common in many freshwater aquatic systems including tropical and temperate lakes, rivers, and estuaries. In general, two major groups of cyanobacteria have been identified, the bloom formers and the non-bloom formers (Oliver and Ganf, 2000; Stockner *et al.*, 2000). The distinguishing feature of the bloom formers is their ability to make gas vacuoles. This allows them to regulate their buoyancy and form persistent assemblages on the surface waters, particularly towards the end of summer. The bloom formers tend to be colonial in nature and vary in form and size, from small filaments to large globular colonies. Many of the filamentous forms such as *Anabaena* and *Aphanizomenon* are also heterocyst-forming N_2-fixers. The non-bloom formers include both single-celled species, such as *Synechococcus*, and colony formers such as *Aphanothece* and *Aphanocapsa*.

Two major environmental variables drive the seasonal development of cyanobacteria in freshwater bodies during summer, namely the changes in stability and stratification of the water column and declining nutrient availability (Oliver and Ganf, 2000). Over the growing season, stratification increases to a maximum in summer, when the water mixing is insufficient to maintain larger and more dense phytoplankton species in the upper layers. The water column separates into an upper epilimnion, where light is high but nutrients are low, and a dark but nutrient-rich hypolimnion. It is during calm weather in summer and autumn that surface blooms develop, associated with reduced nutrients, high light, and higher temperatures. Non-bloom-forming cyanobacteria are generally capable of growth over a wide range of light intensities and both single-celled and colonial forms have been found at different light levels within the upper euphotic zone. The peak abundance is also found during summer and autumn, and the density tends to be less in the high-light surface layers with higher abundance in the lower levels of the euphotic zone where nutrient levels may also be elevated (Stockner *et al.*, 2000).

In general, freshwater environments are much more variable in their pH, Ci and temperature than their marine counterparts. The pH environment is poorly buffered and the depletion of Ci by photosynthetic activity is accompanied by increases in pH and a corresponding increase on the HCO_3^-/CO_2 ratio. Thus, during the progression of summer, the upper layers of the euphotic zone become depleted both in nutrients and Ci and pHs may rise to in excess of pH 9 (Talling, 1985). In surface cyanobacterial blooms, it is also likely that O_2 levels will rise within the scum due to photosynthesis.

Microbial mats

Cyanobacteria are often the key organism in microbial mats. They form dense micro-scale communities in which a wide range of microbes and their metabolism may be present (Stal, 2000). A typical property of microbial mats is their laminated or stratified structure in which different groups of organisms occur in particular vertical layers and cyanobacteria are associated with the upper layers receiving sufficient light for photosynthesis. Cyanobacteria are the most successful mat-building organisms due to a number of factors. They are the only oxygenic phototrophic prokaryotes and as such have the capacity for net carbon fixation using only light and water. In addition, they may fix N_2 and add to the nutrient status of these communities. Finally, as described below, their ability to adapt to the wide fluctuations in environmental conditions experienced by the mats is of great importance to the survival of these communities.

Cyanobacterial mats or crusts are found in a range of aquatic and terrestrial environments including (i) coastal tidal and sand flats, where large areas are covered by water

for only a short period during the tide and where wide fluctuations in water content, salinity, and temperature occur; (ii) hypersaline environments which can occur in shallow and sheltered coastal lagoons with high rates of evaporation and low precipitation, such as the coast of the Red Sea; (iii) thermal hot springs, where the combination of high temperature in combination with H_2S or acidic conditions decreases biodiversity. Cyanobacterial mats are most common in hot springs at near neutral or alkaline conditions, and are characterized by temperatures up to 70 °C; (iv) various terrestrial environments ranging from coastal dunes to desert soil and rock surfaces where cyanobacteria may form surface crusts or layers associated with the surface of rocks. Cyanobacteria inhabiting terrestrial environments are able to withstand cyclical desiccation and rehydration, and high surface temperatures.

The microbial mat environments are the most extreme environments in which cyanobacteria are found. In these habitats, cyanobacteria are exposed to low nutrient conditions, variable light, long periods of desiccation, and fluctuating salinity and temperature. Microbial mats of all kinds are characterized by a number of factors that have great impacts on photosynthesis. There is a shallow surface photic zone (1–2 mm) where light is rapidly attenuated. The diffusion of CO_2 and O_2 is limited allowing O_2 to build up during the day to over 1000 μM, Ci levels to be greatly depleted and high pH conditions to be established (Stal, 2000; Ward and Castenholz, 2000). This is coupled with high surface temperatures, all of which may put extreme pressures on CO_2 acquisition and photosynthesis.

Symbiotic environments

Cyanobacteria are unique in their capacity to form symbiotic associations with a remarkable range of eukaryotic hosts, including plants, fungi, sponges, and protists (Adams, 2000). In most cases the host benefits from the provision of metabolites that contain both nitrogen and carbon. The benefits to cyanobacteria are less clear, but may include protection from environmental extremes such as high-light intensity and desiccation. Many cyanobacterial symbionts are filamentous, are able to fix N_2 in heterocysts, and develop motile hormogonia that serve as the infective agents in many of the symbioses. Their host environments range from small cavities in plants, extracellular layers within a lichen thallus or both inter- and extracellular locations within marine sponges.

The symbiotic photosynthetic environment may be buffered within the host, with the CO_2 supply being derived either from host respiration or from the external environment. For aerobic environments, Ci may reach the cyanobacterium predominantly as CO_2, depending on the extracellular pH. In addition, the CO_2 supply may be limited when thalli or cavities are saturated with water. For marine sponges, the direct supply of HCO_3^- from seawater is also a possibility.

Environmental extremes and their influence on CO_2 acquisition

The environmental extremes experienced by cyanobacteria have significant influence on the strategies that may need to be developed to achieve efficient CO_2 capture during photosynthesis. As a solution to these problems, cyanobacteria have developed a sophisticated CCM which is dependent on a variety of active CO_2 and HCO_3^- uptake systems and an internal micro-compartment (carboxysome) where the CO_2 level is elevated around the active site of Rubisco (see below for more detailed discussion). Table 2 summarizes the occurrence of environmental extremes in various growth habitats and the predicted impact of these extremes on modes of CO_2 acquisition and CCM function. These extremes include:

(i) Temperature extremes, which are found in many cyanobacterial environments. The potential impact of temperatures above 30 °C is primarily through their effect on the kinetic properties of Rubisco (Badger, 1980). Rubisco decreases its affinity for CO_2 at higher temperatures and its oxygenase activity is accentuated. Thus, cyanobacteria actively photosynthesizing at elevated temperatures may be expected to require more effective CCM activity, and, conversely, at lower temperatures the intervention of CCM activity may be less critical.

(ii) Variation in the pH of aquatic medium, particularly in freshwater where the CO_2/HCO_3^- buffer is important in determining the pH. High pHs are associated with a depletion of inorganic carbon by photosynthesis and mean that HCO_3^- becomes a more dominant species. In acidic environments the converse is true, with CO_2 being dominant and often being associated with levels of CO_2 that are at or above atmospheric equilibrium levels. Variation in the levels of inorganic carbon and the predominance of CO_2 and HCO_3^- as Ci species will have implications for the development of specific CO_2 or HCO_3^- uptake systems (see later).

(iii) Wet and dry conditions, often experienced by cyanobacterial stratified communities. When cyanobacterial mats, crusts, and lichen associations are saturated with water, a high diffusive resistance to Ci and O_2 diffusion will be created, and active photosynthesis may create local environments of low Ci and elevated O_2 levels. These conditions will favour the development of an effective CCM.

(iv) High and low light extremes. Generally speaking, high light will be associated with higher rates of photosynthesis and higher CCM activity will be required to secure sufficient Ci.

(v) Variation in O_2 levels, particularly in those environments where there are limitations to gas diffusion and the rate of photosynthesis per unit volume of liquid in high. The impact of this will be through effects on

Table 2. *Environmental extremes experienced by cyanobacteria*

The summaries presented on environments have been derived from the reviews presented in Whitton and Potts (2000), while the predicted implications are derived from our collective knowledge of the CCM in cyanobacteria as presented in recent reviews (Badger *et al*., 1998, 2002; Badger and Price, 2003).

Environmental extreme	Typical environments	Predicted implications for CO_2 fixation and CCM activity
Temperature		
Hot	May occur at the surface of cyanobacterial mats found in hot springs, and on the coast, and in soil crusts and edolithic rock layers	High temperatures reduce the efficiency of Rubisco and promote its interactions with O_2. A greater capacity to concentrate CO_2 may be required.
Cold	For species living in polar regions and in cold deserts, including plankton, soil crusts and lichens.	Conversely, low temperatures improve Rubiscos ability to capture CO_2 and discriminate against O_2 and species are slower growing. There will be a lesser need for CCM activity.
pH		
Alkaline	Most cyanobacteria live in alkaline environments. Particularly accentuated in freshwater environments during high photosynthesis in summer. Also found in carbonate dominated lakes and limestone surfaces.	HCO_3^- is often the major form of Ci available, particularly when Ci starts to be depleted and pH rises. CCM activity using HCO_3^- species for photosynthesis will be required.
Acidic	Some cyanobacteria have been found in acidic soils down to pH 3. Lichen thalli may also be neutral to acidic.	Presumably CO_2 is the dominant Ci species for photosynthesis. Depending on the CO_2 level, a CCM using CO_2 may be required.
Water content		
Wet and dry	Occurs for soil crusts, cyanobacterial mats and lichens. Photosynthesis ceases when dry but initiates rapidly on rewetting with liquid water.	Hydration with water is associated with the creation of high diffusive resistances to CO_2 and O_2 transfer. Photosynthesis may create local environments of low Ci and high O_2. Increased CCM activity will be required under these conditions.
Light		
High light	Occurs for surface plankton species, the surfaces of cyanobacterial mats and crusts and exposed lichens.	High light tolerance will be a prerequisite for effective photosynthesis. An active CCM will be essential.
Low light	Found for deepwater plankton, lower layers of cyanobacterial mats and crusts and shaded lichens	Lower rates of photosynthesis will mean less demand for Ci. Less CCM activity needed.
Oxygen		
High oxygen	Associated with environments with high photosynthesis and reduced diffusion. Includes cyanobacterial mats and surface blooms.	High O_2 is generally associated with Ci depletion and a need for an active CCM.
Low oxygen	Occurs in the lower levels of cyanobacterial mats and benthic situations. Light will be low and photosynthesis limited. Ci will be high due to high respiratory activity.	Due to low light and high Ci (CO_2) there will be reduced need for CCM activity.
Inorganic carbon		
High Ci	Generally associated with environments rich in bicarbonate and carbonate or high respiration, for example, limestone substrates in association with water bodies. Marine environments may be said to be high-Ci relative to freshwater.	Ci species will be high supply, but may be dominated by HCO_3^- and CO_3^{2-}. CCM activities will be reduced.
Low Ci	Generally associated with environments with reduced alkalinity, high photosynthesis and diffusive transfer limitations. Includes cyanobacterial mats and freshwater assemblages in summer.	CCM activity which can acquire Ci species at low concentrations will be present.

Rubisco oxygenase activity and production and excretion of photorespiratory metabolites such as glycolate. This is probably most extreme in cyanobacterial mat communities where the O_2 levels in the top 2–3 mm during the day may reach in excess of 1000 μM (4–5 times atmospheric), pH rises to >9, temperatures increase and Ci levels fall (Stal, 2000; Ward and Castenholz, 2000). Similar effects may also occur in lichens when they are saturated with liquid water; however, temperatures are likely to be less extreme and this will reduce the impacts of increased O_2 levels.

(vi) Ci availability. Both low and high environments are commonly inhabited by cyanobacteria. Obviously high Ci environments, such as waters high in carbonate or bicarbonate (e.g. marine waters and carbonate lakes), will necessitate a less active CCM. Low Ci environments (e.g. freshwater lakes, cyanobacterial mats) will increase the requirement for a strong and flexible CCM.

Evolutionary challenges faced by cyanobacteria

The evolution of cyanobacteria over the past 2.5 billion years and the relationship to changes in atmospheric CO_2 and O_2 levels has been speculated on previously (Badger

et al., 2002; Badger and Price, 2003; Raven, 2003). Past atmospheric CO_2 levels, when cyanobacteria first arose, were probably over 100-fold higher than present day conditions. In combination with the prevailing low O_2 conditions, ancient cyanobacteria would not have required a CCM to achieve effective photosynthesis. The initial development of a CCM in cyanobacteria would have been triggered by changes in CO_2 and O_2 that caused CO_2 to be a limiting resource for photosynthesis and the Rubisco oxygenase reaction to become a significant problem. Clear records for changes in O_2 and CO_2 before about 600 million years ago are lacking, but is has been inferred that O_2 was near present levels by the beginning of the Phanerozoic (600 million years ago) and CO_2 may have been around 15–20 times current atmospheric levels (Berner *et al.*, 2003). Given the properties of current cyanobacterial Rubiscos (Badger *et al.*, 1998), these enzymes should have been able to achieve efficient photosynthesis under these conditions. However, about 400 million years ago there was a large decline in CO_2 levels and an almost doubling in the oxygen concentration. These changes would have placed significant pressures on cyanobacterial photosynthesis. It has been argued that this may have been the first evolutionary pressure for the development of CCMs in photosynthetic organisms (Raven, 1997; Badger *et al.*, 2002). However, other views of an earlier evolutionary appearance have also been expressed (Raven, 2003).

The development of a cyanobacterial CCM

In response to the pressures of both evolutionary climate change and cyclical environmental extremes, cyanobacteria have evolved a sophisticated CCM to help acquire CO_2 for photosynthesis. They have co-evolved a Rubisco which is adapted to optimal performance under the elevated CO_2 conditions produced by this mechanism (Badger *et al.*, 1998). Thus cyanobacterial Rubiscos have much lower affinities for both CO_2 and O_2 than other algal or higher plant counterparts, but have much higher turnover rates per unit protein. The CCM allows Rubisco to operate near V_{max} and a much smaller investment of nitrogen in Rubisco is required to achieve a particular rate of photosynthesis.

The components of the cyanobacterial CCM are shown in Fig. 1. The CCM primarily consists of a number of active Ci transporters which may transport CO_2 or HCO_3^- from the external environment and deliver it as HCO_3^- to the interior of the cell. In the absence of a cytosolic carbonic anhydrase, the internal HCO_3^- pool is able to accumulate well above the external level (Price *et al.*, 1998). The other significant component of the CCM is an internal protein microbody called the carboxysome, which contains the cellular Rubisco. In this compartment the accumulated HCO_3^- pool is converted to CO_2 through the action of specific carboxysomal carbonic anhydrases and CO_2 is elevated due to diffusion restrictions on efflux that are proposed to be present as part of the carboxysome protein shell structure (Kaplan and Reinhold, 1999; Price *et al.*, 1998). Depicted in Fig. 1 are two types of cyanobacterial CCMs, which may be classified according to the nature of the carboxysome structures present in the cell. The α-cyanobacteria possess a Form 1A type Rubisco and distinct α-carboxysomes, while the β-cyanobacteria have Form 1B Rubisco and β-carboxysomes (Badger *et al.*, 2002). The carboxysome structure is covered in more detail below. Cyanobacterial CCMs exhibit species diversity, particularly with regard to the suite of Ci transporters that a particular cyanobacterium may possess, with β-cyanobacteria currently demonstrating a greater array of Ci transport options (see below for more detail).

The first evolutionary steps towards developing a cyanobacterial CCM may have been quite simple and speculation has previously been suggested (Badger *et al.*, 2002). In the initial stages of CO_2 decline, the first step in the development of a CCM would necessarily have been the evolution of a carboxysome structure for Rubisco. This structure is fundamental to enabling the concentration of CO_2 and Ci transporters are ineffectual without it. The evolution of both α- and β-carboxysomes within cyanobacteria is intriguing, and is probably linked to lateral gene transfer between photosynthetic proteobacteria and cyanobacteria (Badger *et al.*, 2002). A carboxysomal carbonic anhydrase would, probably, also have been acquired at this stage as the rate of chemical conversion of HCO_3^- to CO_2 would have been too slow to support photosynthetic CO_2 supply. As CO_2 limitation became more severe, the CCM would probably have been improved by the development of a diverse array of both CO_2 and HCO_3^- uptake systems of varying affinities in order actively to acquire Ci from the surrounding environments.

The elements of the CCM

The carboxysome

Carboxysomes have been found in all cyanobacteria characterized to date and homologous polyhedral bodies are also present in a number of chemoautotrophic bacteria (Cannon *et al.*, 2001). At the genetic level, carboxysomes from all groups share degrees of similarity although there are distinct differences that separate carboxysomes into two groups based on the form of Rubisco associated with the structure. Carboxysomes containing Form 1A Rubisco form one group while those with Form 1B Rubisco form another (Badger *et al.*, 2002). Phylogenetic analysis of the genomes of these two groups has revealed that there are also distinct differences between their carboxysome genes, prompting Badger *et al.* (2002) to propose that carboxysomes containing Form 1A Rubisco and those containing Form 1B Rubisco should be termed α- and β-carboxysomes,

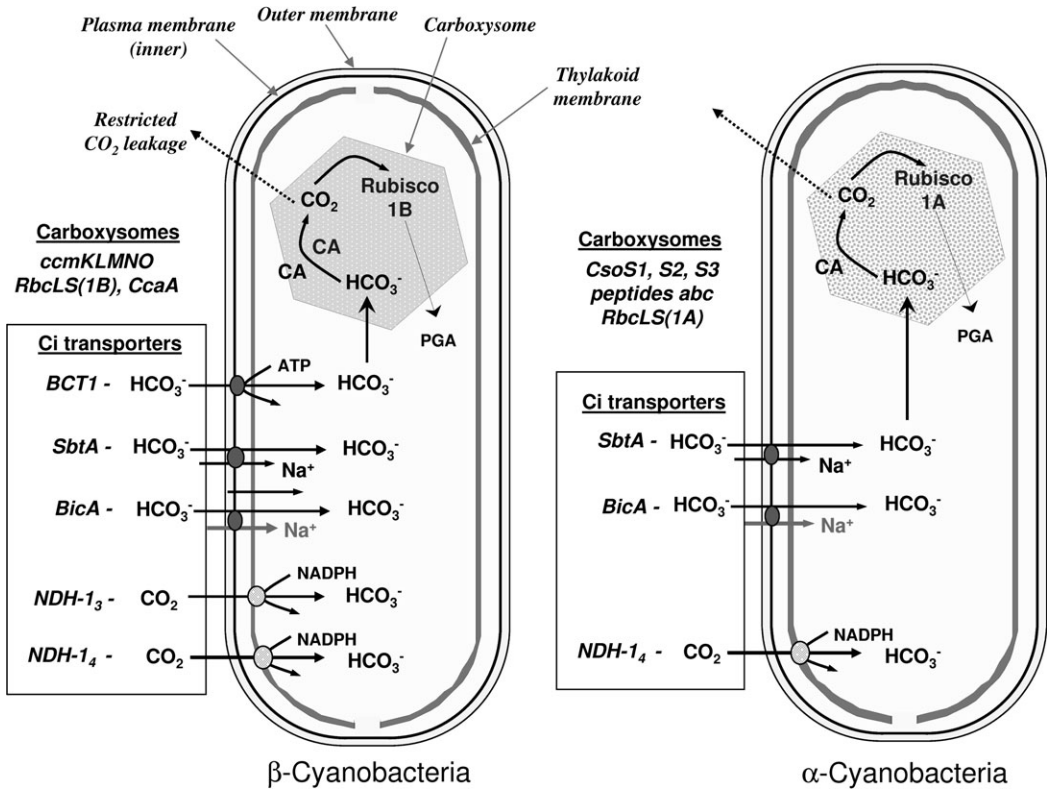

Fig. 1. Characteristic components of the CO_2 concentrating mechanism in α and β-cyanobacteria.

respectively. Under this scheme, the cyanobacteria fall into two groups; α- and β-cyanobacteria producing α- and β-carboxysomes, respectively. Those with α-carboxysomes share carboxysome protein homologies with a number of chemoautotrophic proteobacteria while β-carboxysomes are, to date, confined to the β-cyanobacteria (Cannon *et al.*, 2002; Badger and Price, 2003).

An understanding of the protein composition of carboxysomes, and details of their assembly, is essential to determining the structure and function of these microbodies and their role in CCMs. Carboxysomal protein composition has been studied in greatest detail in the α-carboxysomes, with many studies concentrated on the chemoautotrophic proteobacterium *Halothiobacillus neapolitanus* (Cannon *et al.*, 2001, 2002). The polypeptides making up the protein coat of α-carboxysomes from *H. neapolitanus* are coded for by genes of the cso type (*csoS1A*, *csoS1B*, *csoS1C*, *csoS2*, *csoS3*, *orfA*, and *orfB*) as described by Cannon *et al.* (2002) while the genes *cbbL* and *cbbS* code for Rubisco large and small subunits, respectively (Fig. 1). Together, these genes form a putative carboxysome operon (Cannon *et al.*, 2003). Of the proteins residing in the carboxysomal coat of *H. neapolitanus*, CsoS3 has recently been characterized as an epsilon-class carbonic anhydrase (So *et al.*, 2004), while the CsoS1 proteins are major constituents of the α-carboxysome coat (Cannon and Shively, 1983).

Among the cyanobacteria, little information has yet been gathered to describe the protein composition of either α- or β-carboxysomes. Based on gene content, the α-cyanobacteria share all of the carboxysomal proteins so far described from proteobacteria (Badger *et al.*, 2002). However, the molecular information for β-carboxysomes suggests several unique proteins constitute this type of carboxysome. Unlike α-carboxysomes, β-carboxysome proteins are coded for primarily by a cluster of genes of the ccm type (*ccmKLMNO*), along with the genes for the large and small subunits of Rubisco (*rbcL* and *rbcS*) (Fig. 1). In addition, a carboxysomal CA (CcaA) and sometimes extra CcmK homologues are coded for elsewhere on the genome. Based upon homologies between several α- and β-carboxysomal proteins (namely: CcmK/O and CsoS1; and CcmL and Proteins A/B) it is assumed that these proteins will have common functional and/or structural roles in both carboxysome types (Cannon *et al.*, 2002). Interestingly, however, CcmM and CcmN have no sequence homologues in α-carboxysomes and CsoS2 and CsoS3 have no homologues in β-carboxysomes (Badger *et al.*, 2002; Cannon *et al.*, 2002). Nevertheless, it is suggested that CcmM and CcmN may be functionally similar to CsoS2 and CsoS3 in β-carboxysomes (Cannon *et al.*, 2002). It is also interesting to note that while CsoS3 has now been characterized as an epsilon-CA (So *et al.*, 2004) and that CcmM has

a gamma-CA domain (Ludwig *et al.*, 2000), β-carboxysomal CA activity is currently attributed to CcaA (Price *et al.*, 1992; Yu *et al.*, 1992; Badger *et al.*, 2002).

The models of CO_2 fixation in carboxysomes propose that HCO_3^- diffuses through the proteinaceous shell of the carboxysome where carbonic anhydrase inside the structure acts to catalyse the formation of CO_2. The CO_2 concentration can be elevated within the carboxysome with the aid of some poorly understood diffusion barrier, such as the protein shell, that restricts CO_2 diffusion out of the carboxysome. The CO_2 pump also plays a critical role in recycling leaked CO_2, thereby minimizing CO_2 loss from the carboxysome (Price *et al.*, 2002). Further information on the role of carboxysomes in the cyanobacterial CCM can be found in several reviews (Price *et al.*, 1998, 2002; Kaplan and Reinhold, 1999; Cannon *et al.*, 2001).

Carboxysomal carbonic anhydrases

The carboxysomal model, shown in Fig. 1, clearly requires the presence of a carbonic anhydrase to generate CO_2 from HCO_3^-. However, the presence of a specific carboxysomal CA enzyme was, until recently, only identified in β-carboxysomes from a number of β-cyanobacteria (Fukuzawa *et al.*, 1992; Price *et al.*, 1992; Yu *et al.*, 1992). The emergence of complete genome sequences for a number of α- and β-cyanobacteria has revealed variability in the nature of potential carboxysomal CAs (Table 3). None of the α-cyanobacteria sequenced so far has a recognizable carboxysomal CA homologue (CcaA), although a beta-CA (note that nomenclature is not related to α and β terminology used for carboxysomes and cyanobacteria) is present in the α-*Synechococcus* genomes (Table 3). In addition, some of the β-cyanobacteria appear to contain no identifiable carboxysomal CA homologues. The resolution of this apparent conundrum may lie in the recent discovery that the CsoS3 protein of the shell of α-carboxysomes has CA activity (So *et al.*, 2004). CsoS3 is present in all α-cyanobacteria (Table 3). This raises the distinct possibility that the gamma-CA N-terminal domain of the CcmM protein of β-carboxysomes (Ludwig *et al.*, 2000) may also be active as a CA in some species of β-cyanobacteria, such as *Trichodesmium*, and *Thermosynechococcus*. It thus appears as though the nature of CA function within the carboxysomes is quite variable, with both shell-based and soluble activities being possible in different species. The implications of this for function of the carboxysomes are not yet clear.

An analysis of possible alpha, beta, and gamma carbonic anhydrases (Smith and Ferry, 2000) in cyanobacterial genomes shows that there is a wide diversity in carbonic anhydrase gene content (Badger *et al.*, 2002; So and Espie, 2005; Table 3). The role of these other carbonic anhydrases in cyanobacterial genomes is unclear. However, some species such as *Nostoc* and *Anabaena* species may have a range of beta-CAs other than carboxysomal CA (CcaA) and an alpha-CA (EcaA).

The Ci uptake systems

Experimental evidence thus far indicates that there are at least five distinct modes of active Ci uptake in cyanobacteria. However, since this work is primarily 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. The five Ci uptake systems are explained in more detail in the subsequent sections and in Figs 1 and 2, but, in brief, the systems are: (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.*, 2002); (iii) BicA, a newly discovered low affinity Na^+-dependent HCO_3^- transporter belonging to the widespread SulP family (Price *et al.*, 2004); (iv) NDH-1_4, a constitutive CO_2 uptake system based on a specialized NDH-1 complex that appears to be located on the thylakoid membrane (Maeda *et al.*, 2002; Shibata *et al.*, 2001; Price *et al.*, 2002); and (v) NDH-1_3, a second CO_2 uptake system based on a modified NDH-1 complex that is inducible under Ci limitation (Shibata *et al.*, 2001; Maeda *et al.*, 2002).

The BCT1 HCO_3^- transporter: The high affinity HCO_3^- transporter, BCT1, belongs to the ATP binding cassette (ABC) transporter family, also known as traffic ATPases because family members are usually energized by ATP (Higgins, 2001). BCT1 was the first cyanobacterial Ci transporter to be convincingly identified and characterized. To date, BCT1 has been physiologically characterized in just one cyanobacterium, namely *Synechococcus* PCC7942, although close homologues have been detected in six other species (Table 4). In *Synechococcus* PCC7942, and other species, BCT1 is encoded by the *cmpABCD* operon and it is induced under Ci limitation (Omata *et al.*, 1999; McGinn *et al.*, 2003; Woodger *et al.*, 2003). When overexpressed in high CO_2-grown cells BCT1 displays a photosynthetic affinity for HCO_3^- of around 15 μM, and supports a moderate flux rate (Omata *et al*., 1999). The *cmpABCD* operon codes for a multimeric four subunit complex that is strongly induced under conditions of relatively severe Ci limitation (Omata and Ogawa, 1986; Omata *et al.*, 1999; McGinn *et al.*, 2003, 2004; Woodger *et al.*, 2003; Wang *et al.*, 2004) and also under high light stress (Reddy *et al.*, 1989), although the latter condition can also exacerbate a condition of Ci limitation (Woodger *et al.*, 2003; McGinn *et al.*, 2004). BCT1 appears to be the only cyanobacterial example of a primary transporter (uniporter) for HCO_3^-. This transporter is also closely related to the NRT1 transporter from cyanobacteria, which in turn, acts as a high affinity

Table 3. *Variation in the content of carbonic anhydrase genes found in the genomes of sequenced cyanobacteria listed in Table 5*

Four recognized classes of carbonic anhydrases are currently represented in cyanobacteria. While there are protein homologies within each class there is no relationship between proteins from the different classes.

Species	Carbonic anhydrase classes			
	alpha	beta	gamma	epsilon
Synechocystis PCC6803	–	CcaA +EcaB	CcmM	–
Synechococcus elongatus	EcaA	CcaA	CcmM	–
Nostoc PCC7120	EcaA	1 beta	CcmM	–
Anabaena variabilis	EcaA	CcaA?, 2 betas	CcmM	–
Nostoc punctiforme	–	CcaA + EcaB + 3 betas	CcmM	–
Thermosynechococcus	–	–	CcmM	–
Gloeobacter violaceus		2 betas	CcmM	–
Synechococcus PCC7002	EcaA	CcaA	CcmM	–
Trichodesmium erythraeum	–	–	CcmM	–
Crocosphaera watsonii	–	CcaA	CcmM	–
Synechococcus WH8102	–	1 beta	–	CsoS3
Synechococcus CC9605	–	2 beta	–	CsoS3
Synechococcus CC9902	–	1 beta	–	CsoS3
Synechococcus CC9311	–	1 beta	–	CsoS3
Prochlorococcus MED4	–	–	–	CsoS3
Prochlorococcus MIT9313	–	–	–	CsoS3
Prochlorococcus MIT9312	–	–	–	CsoS3
Prochlorococcus SS120	–	–	–	CsoS3

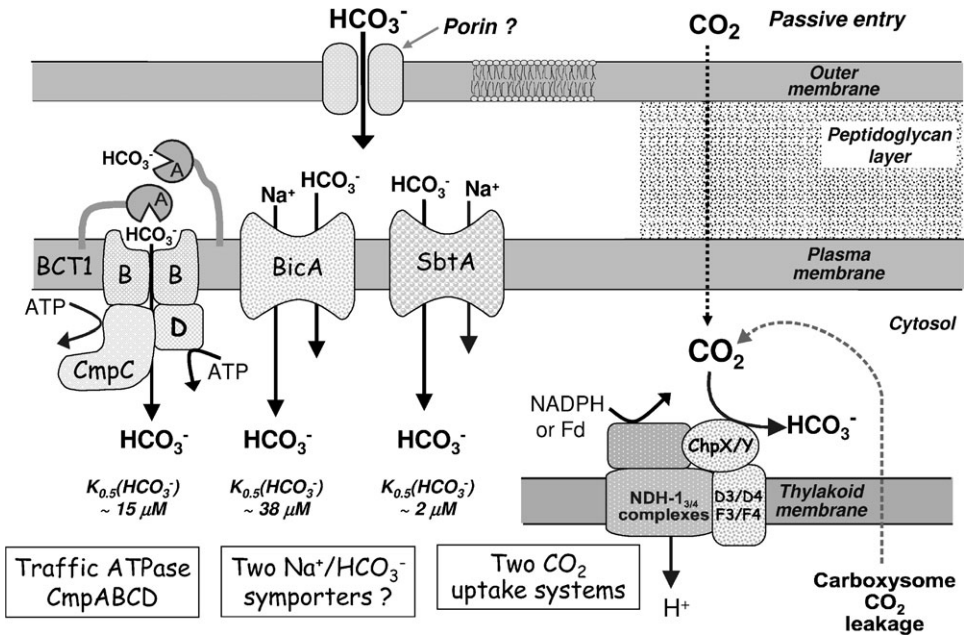

Fig. 2. Five Ci uptake mechanisms in cyanobacteria. Three bicarbonate transporters, BCT1, BicA and SbtA, located on the plasmamembrane and two CO_2 uptake systems, NDH-1_3 and NDH-1_4, on the thylakoids.

nitrate/nitrite transporter and is encoded by the *nrtABCD* operon (Omata *et al.*, 1993; Maeda and Omata, 1997).

The SbtA HCO_3^- transporter: The SbtA transporter was first identified in *Synechocystis* PCC6803 (Shibata *et al.*, 2002). In *Synechocystis* PCC6803, the HCO_3^- uptake capacity that was attributable to SbtA was shown to be Na^+-dependent, requiring around 1 mM Na^+ for half maximal HCO_3^- uptake activity (Shibata *et al.*, 2002). This finding was consistent 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); a role for Na^+ in pH regulation was also mooted. It has been suggested that NtpJ is involved in Ci uptake in *Synechocystis* PCC6803, possibly as a primary Na^+ extrusion pump (Shibata *et al.*, 2002). It is probable that SbtA is a single subunit transporter (although it may reside in the membrane as a

Table 4. *Variation in the suites of genes for Ci transporters and carboxysomes found in the genomes of sequenced cyanobacteria listed in Table 5*

Habitat[a]	Species	Ci transporters					Carboxysome-related			
		Bicarbonate			CO_2		Rubisco form		Carboxysome	
		BCT1	SbtA[b]	BicA[c]	Ndh-1_4	Ndh-1_3[d]	1A	1B	α	β
B1	*Synechocystis* PCC6803	+	++	++,+,?,?	+	+		+		+
B1	*Synechococcus elongatus*	+	++	?,?	+	+		+		+
B2	*Nostoc* PCC7120	+	++,?	++,+,?	+	+		+		+
B1,D1/2	*Anabaena variabilis*	+	++,?	++	+	+		+		+
B1,D1	*Nostoc punctiforme*	+	?	?,?	+	+		+		+
C3	*Thermosynechococcus*	+	−	+	+	+		+		+
C4	*Gloeobacter violaceus*	+	−	−	+	+		+		+
A2,A3	*Synechococcus* PCC7002	−	++	++,+,?	+	+		+		+
A1,A2	*Trichodesmium erythraeum*	−	−	++,+	+	−		+		+
A1,A2	*Crocosphaera watsonii*	−	++	++,++,?	+	?		+		+
A1	*Synechococcus* WH8102	−	−	++,+,?	+	−	+		+	
A1	*Synechococcus* CC9605	−	−	++,+	+	−	+		+	
A1,A2	*Synechococcus* CC9902	−	−	++,+	+	−	+		+	
A1	*Synechococcus* CC9311	−	?	++,+	+	−	+		+	
A1	*Prochlorcoccus* MED4	−	?	+,?	−	−	+		+	
A1	*Prochlorococcus* MIT9313	−	?	+,?	−	−	+		+	
A1	*Prochlorococcus* MIT9312	−	?	+	−	−	+		+	
A1	*Prochlorococcus* SS120	−	?	+,?	−	−	+		+	

[a] Habitat refers to the classification shown in Table 1. Note than marine habitat species (A) 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.

homodimer or homotetramer), but it has not yet been established if this is the case. A gain-of-function approach through overexpression of SbtA is required before this can be concluded with any confidence. The protein has, however, been detected in cytoplasmic membranes isolated from *Synechocystis* PCC6803 with an apparent complex size of around 160 kDa (Zhang *et al.*, 2004). This might indicate that SbtA exists in the membrane as a tetramer. This proteomic study also confirmed that the abundance of SbtA is dramatically increased under Ci limitation in this species.

The BicA HCO_3^- transporter: The BicA transporter is the most recently discovered HCO_3^- transporter present in cyanobacteria, and like SbtA, is also Na^+-dependent (Price *et al.*, 2004). However, it has no obvious sequence similarity to SbtA. BicA was discovered in the coastal marine cyanobacterium *Synechococcus* PCC7002, and is interesting because it has a relatively low transport affinity (around 38 μM), but is able to support high photosynthetic flux rates. BicA belongs to a large family of eukaryotic and prokaryotic transporters presently annotated as sulphate transporters or permeases in many bacteria (SulP family). Through the use of gain-of-function experiments in the freshwater cyanobacterium, *Synechococcus* PCC7942, it was revealed that *bicA* expression alone is sufficient to confer a Na^+-dependent, HCO_3^- uptake activity. 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). Two other BicA transporters were identified and characterized in this manner, including one from the ecologically-important oceanic strain, *Synechococcus* WH8102 and the other from *Synechocystis* PCC6803. In this assay system, the three BicA transporters had transport affinities that ranged from 74–353 μM, with the *Synechocystis* PCC6803 form having the lowest affinity and the WH8102 form having the highest affinity. BicA expression is highly inducible under Ci limitation in *Synechococcus* PCC7002, but appears also to be present 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 (Price *et al.*, 2004; Wang *et al.*, 2004), whereas in *Synechococcus* WH8102 the expression characteristics are unknown.

CO_2 uptake based on specialized NDH-1 complexes: Early studies had shown that the NDH-1 dehydrogenase complex is involved in active CO_2 uptake by cyanobacteria, potentially via supply of ATP generated by NDH-1 generated cyclic electron flow (Ogawa, 1992). However, within β-cyanobacteria species, exemplified in genome data from *Synechocystis* PCC6803, there may be a number of distinct types of NDH-1 complexes with different roles within the cell (Ohkawa *et al.*, 1998; Price *et al.*, 1998). The first evidence that cyanobacteria possess NDH-1 complexes

specialized for CO_2 uptake came from the observation that the gene cluster *ndhF3-ndhD3-chpY* is necessary for inducible, high affinity CO_2 uptake in *Synechococcus* PCC7002, but importantly it was also found that re-reduction of P700 (light to dark) was unaffected by mutation of this cluster (Sültemeyer *et al.*, 1997; Klughammer *et al.*, 1999).

The NdhD3/D4 proteins, together with NdhF3/F4 components were proposed as components of two forms of a specialized NDH-1 complex involved in catalysing active CO_2 uptake by converting CO_2 to HCO_3^- within the cell (Ohkawa *et al.*, 2000*a*, *b*; Shibata *et al.*, 2001; Maeda *et al.*, 2002). In addition to this, two other genes/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* (note that Shibata, Ogawa and colleagues have named these genes as *cupB* and *cupA*, while Price and co-workers have used the *chpX* and *chpY* nomenclature). It is now clear that the *ndhF3*/*ndhD3*/*chpY* 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 NDH-1_4 complex involved in low affinity CO_2 uptake (Shibata *et al.*, 2001; Maeda *et al.*, 2002). Recent proteomic studies have confirmed the presence of NdhF4/NdhD4/ChpY/sll1735 in thylakoid membranes as a sub-complex that is induced under conditions of Ci limitation (Herranen *et al.*, 2004; Prommeenate *et al.*, 2004; Zhang *et al.*, 2004; Battchikova *et al.*, 2005). Curiously, such studies have failed to detect the constitutive NdhF3/NdhD3/ChpX proteins in thylakoids. There is still a possibility that the NDH-1_3 complex is located on the cytoplasmic membrane. Interestingly, *Gloeobacter* possesses specific genes for the NDH-1_3 and NDH-1_4 complexes (Table 4) but does not possess thylakoid membranes; instead the photosystem machinery is located on the plasma membrane, so NDH-I_3 must be able to operate on the plasma membrane, at least in *Gloeobacter*.

Price *et al*. (2002) have speculated that the ChpX and ChpY polypeptides may be an integral part of the NDH-1 CO_2 uptake complex (NDH-$1_{3/4}$) and involved directly in the conversion of CO_2 to HCO_3^-. Recent proteomic data indicates that the NDH-1_4 complex is restricted to the thylakoid membrane (Ohkawa *et al.*, 2001) thus linking them directly to the photosynthetic electron transport chain. A model of the operation of such an NDH-$1_{3/4}$ CO_2-uptake complex has been proposed, where ChpX and Y are speculated to act as specialized unidirectional carbonic anhydrases, allowing proton abstraction associated with the conversion of CO_2 to HCO_3^- that is linked to electron flow through the NDH-1 complex (Price *et al.*, 2002).

Regulation of the CCM by Ci limitation

Early studies of the CCM in cyanobacteria showed that its activity varies dramatically depending on the degree of Ci limitation that the cells were exposed to during growth (Kaplan *et al.*, 1980). Thus cells of model species such as *Synechococcus* PCC7942 and *Synechocystis* PCC6803 grown at low Ci (<200 μM pH 8.0) have a high photosynthetic affinity for Ci ($K_{0.5}$ <20 μM), while those grown at high Ci (>2 mm pH 8.0) have a reduced affinity ($K_{0.5}$ > 200 μM) (McGinn *et al.*, 2003; Woodger *et al.*, 2003). Recent studies have shown that this variation in affinity is almost solely due to the induction of various high and medium affinity HCO_3^- transporters (BCT1, SbtA, BicA) and the high affinity CO_2 NDH-1_3 uptake system (McGinn *et al.*, 2003, 2004; Woodger *et al.*, 2003). However, the production of more numerous and smaller carboxysomes in species grown at low Ci has also been noted (McKay *et al.*, 1993). The changes in CCM function which occur during induction of a high affinity state are summarized in Fig. 3.

Cyanobacteria that induce multiple transporters under Ci limitation appear to utilize pairs of Ci transporters with complementary kinetics for the same Ci species. For instance, there are two HCO_3^- transporters present in *Synechococcus* PCC7002 (Table 4), the BicA transporter has a relatively low transport affinity of around 38 μM but is able to support a high flux rate. Conversely, the SbtA transporter has a high transport affinity of around 2 μM but possesses a lower flux rate. Under steady-state photosynthetic conditions these two transporters achieve a composite photosynthetic affinity of around 6.5 μM and a high flux rate (Price *et al.*, 2004). Likewise, the two CO_2 uptake systems present in *Synechococcus* PCC7942 have been shown to have contrasting kinetics. The constitutive NDH-1_4 ChpX-containing system has an initial uptake affinity of 10 μM with a high flux rate, whereas the inducible NDH-1_3 ChpY-based system has an uptake affinity of around 0.9 μM, but is only able to sustain a relatively low flux rate in isolation. Yet in WT cells, the composite uptake rate for CO_2 is around 0.8 μM, with a high flux rate (Maeda *et al.*, 2002). Whether this apparent strategy of employing a high flux/low affinity transporter with a low flux/high affinity transporter is a general rule in freshwater and estuarine cyanobacteria and whether it leads to more efficient Ci uptake remains to be generally established.

Species diversity in the CCM and the relationship to habitat

With the genomic sequence data for at least 18 cyanobacterial species, representing 8 α and 10 β-cyanobacteria (Tables 3, 4, 5), it is now possible to correlate the occurrence of known CCM components in species with their cognate ecological habitats and to assess how CCM properties may have adapted to environmental factors that influence Ci acquisition and CO_2 fixation (Table 2). The most striking correlation is that all the α-cyanobacteria are open ocean marine species. The reason for this exclusive

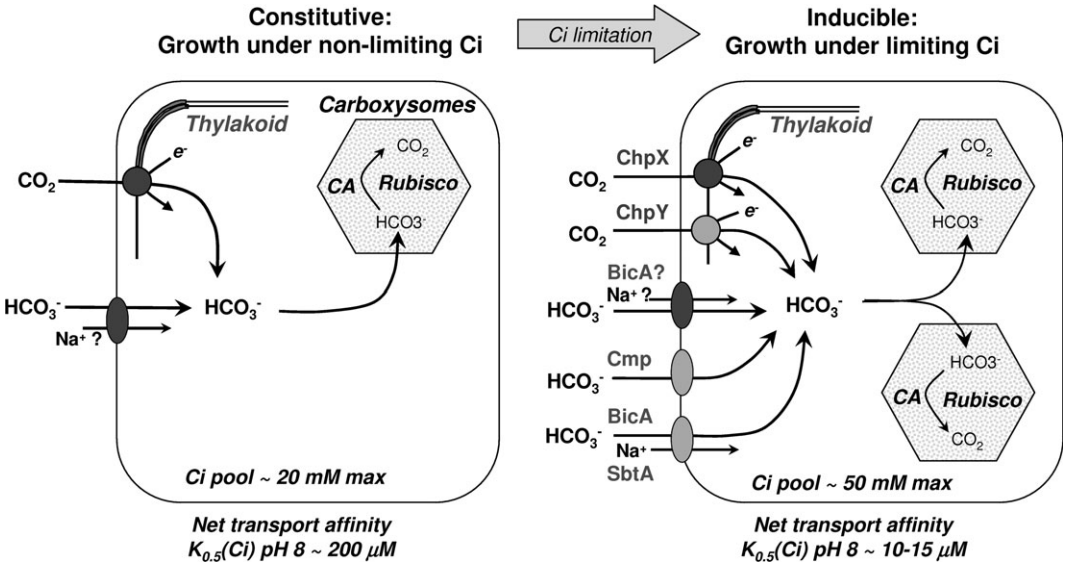

Fig. 3. Changes in the components of the CO_2 concentrating mechanism that occur when cyanobacteria acclimate to limiting Ci conditions. Changes primarily occur in the induction of higher affinity Ci transport systems for the capture of CO_2 and HCO_3^-.

habitat niche is not clear; however, β-cyanobacteria are represented in both freshwater and marine environments. They appear able to adapt to a much wider range of ecological environments except perhaps the deep sea oligotrophic environments such as those inhabited by some *Prochlorococcus* (α-cyanobacteria) species.

Carboxysome diversity

The most obvious CCM diversity between cyanobacteria is in the nature of the carboxysomes that they possess. It appears that a majority of the marine cyanobacterial species contain α-carboxysomes and that α-carboxysomes are found only in marine species. However, it is hard to interpret this observation given the lack of understanding about the physiological differences between the two carboxysome types and the functional attributes they may confer. It is possible that the restriction of α-cyanobacteria to marine environments is solely related to the evolutionary origins of this group and their interchange of genes with proteobacterial species. Alternatively, their marine restriction may be related to other evolved characteristics unrelated to any aspect of their CCM function. However, it may also be possible that a physiology based on α-carboxysomes imposes limits on the range of environments that can be inhabited. Perhaps α-carboxysomes are less effective as CO_2 concentrating compartments, and thus restrict effective photosynthesis to regions where inorganic carbon supplies are constant and high (2 mM, pH 8.3), temperatures are cool to moderate, and O_2 is not elevated. The absence of α-cyanobacteria from estuarine environments where more extreme environmental variation occurs supports this notion.

The presence or absence of a clearly identifiable form of soluble CA associated with carboxysomes is also an interesting variable. Based on work with the β-carboxysomes of *Synechococcus* PCC7942 (Price *et al.*, 1992; Yu *et al.*, 1992) and *Synechocystis* PCC6803 (So and Espie, 1998), carboxysomal beta carbonic anhydrase (CcaA) would appear to be present in six of the β-cyanobacteria listed in Table 3. The apparent absence of CcaA in *Nostoc* PCC7120, *Thermosynechococcus*, *Gloeobacter*, and *Trichodesmium* suggests a different mode of carboxysome function. As discussed earlier, the gamma-CA of the CcmM shell protein may fill this role, but this implies variation in the subtle detail of carboxysome function within the β-carboxysome types, based on the nature of CA function. The consequence of this altered functioning for the habitat distribution of species is unclear and remains to be resolved. While *Prochlorococcus* species clearly lack a CA apart from the CsoS3 shell protein, the α-*Synechococcus* species all have one beta-CA which is very similar between all species. This CA lacks the carboxyl extension characteristic of the CcaA protein of β-carboxysomes (data not shown), but the possibility that it may also be associated with α-carboxysomes cannot be excluded at present.

Ci transporters

As shown in Table 4, there is considerable variation in the suites of Ci transporters that are found in different cyanobacterial species. The association between suites of Ci transporters and species habitat is discussed below.

Marine environments: The BCT1 transporter is only present in freshwater β-cyanobacteria and conversely marine cyanobacteria do not possess the BCT1 transporter (or the related NRT1 nitrate transporter: data not shown). The

Table 5. *Environmental habitats of cyanobacterial species for which genome sequences are available*

Species	Growth and habitat characteristics	Genome web reference
Synechococcus elongatus (sp. PCC7942)	A unicellular rod-shaped freshwater cyanobacterium. Low salt tolerance non-motile. Common laboratory strain. Non-nitrogen-fixing.	http://genome.jgi-psf.org/microbial/
Synechocystis sp. PCC6803	A unicellular coccoid non-nitrogen-fixing cyanobacterium and an inhabitant of fresh water.	http://www.kazusa.or.jp/cyano/cyano.html
Nostoc sp. PCC7120	A filamentous, heterocyst-forming cyanobacterium capable of nitrogen fixation. Freshwater environments, contains gas vacuoles.	http://www.kazusa.or.jp/cyano/cyano.html
Nostoc punctiforme ATCC 29133	A filamentous, heterocyst-forming cyanobacterium capable of nitrogen fixation. The strain was isolated from symbiotic association with the gymnosperm cycad *Macrozamia* sp. It has a complex life cycle in the differentiation of heterocysts, hormogonia and akinetes.	http://genome.jgi-psf.org/microbial/
Anabaena variabilis ATCC 29413	A filamentous heterocyst-forming cyanobacterium that fixes nitrogen. This strain has been studied extensively for the production of hydrogen using solar energy. It has a complex life cycle that includes multiple types of differentiated cells: heterocysts for nitrogen fixation, akinetes and hormogonia and established symbiotic associations with plants and fungi.	http://genome.jgi-psf.org/microbial/
Thermosynechococcus elongatus BP-1	A unicellular thermophilic cyanobacterium isolated at Beppu hot spring in Japan. Non-nitrogen-fixing.	http://www.kazusa.or.jp/cyano/cyano.html
Gloeobacter violaceus PCC7421	A unicellular cyanobacterium with unusual characteristics. This strain lacks thylakoids and phycobilisomes are attached to the plasma membrane. Non-nitrogen-fixing. Isolated from calcareous rock in Switzerland.	http://www.kazusa.or.jp/cyano/cyano.html
Synechococcus sp. PCC7002	A single-celled, rod-shaped cyanobacterium. Isolated from mud sample from fish pens, Magueyes Island, Puerto Rico. It grows in brackish (euryhaline) and/or marine water.	http://www.bmb.psu.edu/faculty/bryant/lab/synechococcus7002genome/
Trichodesmium erythraeum IMS101	Filamentous cyanobacteria that plays a major role in the tropical and subtropical oceans both as primary producers and suppliers of nitrogen through their ability to fix atmospheric N_2. They lack heterocysts but use gas vacuoles for buoyancy and bloom formation.	http://genome.jgi-psf.org/microbial/
Crocosphaera watsonii WH8501	Belongs to a novel genus of marine unicellular (2.5–6 μm diameter) N_2-fixing cyanobacteria that occur in ocean waters warmer than 24 °C. It has been isolated from offshore open ocean oligotrophic waters in the western tropical Atlantic and from the tropical Pacific oceans.	http://genome.jgi-psf.org/microbial/
Prochlorococcus MED4 *Prochlorococcus* MIT9313 *Prochlorococcus* MIT9312 *Prochlorococcus* SS120	A group of small (2–5 μm) unicellular cyanobacteria that dominates the temperate and tropical oceans. It lacks phycobilisomes that are characteristic of cyanobacteria, and contains chlorophyll *b* as their major accessory pigment. MED4 and MIT9312 is are high-light-adapted species and MIT9313 and SS120 come from deeper low-light environments	http://genome.jgi-psf.org/microbial/ http://genome.jgi-psf.org/microbial/ http://genome.jgi-psf.org/microbial/ http://www.sb-roscoff.fr/Phyto/ProSS120/
Synechococcus sp. WH8102 *Synechococcus* sp. CC9605 *Synechococcus* sp. CC9902 *Synechococcus* sp. CC9313	A group of small (2–5 μm) unicellular cyanobacteria that are abundant in the world's oceans and are major primary producers on a global scale. Can co-exist with *Prochlorococcus* species and are generally found in the higher light environments. CC9605 is found in surface oligotrophic waters while CC9902 is a coastal species.	http://genome.jgi-psf.org/microbial/ http://genome.jgi-psf.org/microbial/ http://genome.jgi-psf.org/microbial/ http://www.tigr.org/tdb/mdb/mdbinprogress.html

reasons for the absence of BCT1 in marine cyanobacteria are unclear. 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 Ci uptake, rather than using ATP as a direct energy source for pumping (Bryant, 2003). It is also possible that, in marine environments, where HCO_3^- levels are relatively high, there may be a reduced requirement for a high affinity and energy expensive transport system.

The SbtA transporter has a variable representation. Among the β-cyanobacteria, *Synechococcus* PCC7002 and *Crocosphaera watsonii* have strong homologues while *Trichodesmium erythraeum* lacks it. For the α-cyanobacteria, all *Prochlorococcus* species have a weak homologue (which is not yet proven as a HCO_3^- transporter) while of the *Synechococcus* species only CC9311 has this same weak homologue. The SbtA transporter characterized in β-cyanobacteria has a relatively high affinity for HCO_3^- (Shibata *et al.*, 2002; Price *et al.*, 2004) and may be expected to be present where species are required to grow at significantly reduced Ci. This may be the case for *Synechococcus* PCC7002 which grows at high temperatures on estuarine mud flats. The presence of SbtA in both *Synechococcus* PCC7002 and *Crocosphaera* appears to be correlated with the presence of both low and high affinity CO_2 uptake systems, which may indicate that both these species require a full suite of Ci transport options to respond to the Ci environments which they experience. *Crocosphaera watsonii* is an oligotrophic warm ocean species and should not experience the same extremes as PCC-7002, however, aspects of growth in warm, high-light, and

nutrient-depleted waters may exert certain stresses that favour the development of a fully flexible CCM. The absence of SbtA from *Trichodesmium* is correlated with the absence of a high affinity CO_2 uptake system (NDH-1_3), and indicates a clear difference between these two marine nitrogen-fixing species.

The BicA transporter family appears to be the most widely distributed Ci transport system, although the functionality of all the homologues as HCO_3^- transporters has not yet been established (Price *et al.*, 2004). For the β-cyanobacteria, all three marine species have at least one strong homologue and at least one other moderate homologue. *Crocosphaera* has two strong homologues. For the α-cyanobacteria, all *Synechococcus* species have a strong and a medium homologue, while the *Prochlorococcus* species only have medium and weak homologues. The BicA transporter would appear to be a medium affinity transporter that can sustain high flux rates of HCO_3^- uptake and has been shown to be inducible in *Synechococcus* PCC7002 (Price *et al.*, 2004) although in *Synechococcus* WH8102 it may have a lower flux rate. In the absence of other known HCO_3^- transporters, BicA transporters in α-cyanobacteria may sustain the bulk of the HCO_3^- uptake occurring in the marine environment and may be inducible in response to environmental conditions.

The two CO_2 uptake systems, NDH-$1_{3/4}$, are most variably represented in the marine cyanobacteria. For the β-cyanobacteria, the low-affinity uptake system (NDH-1_4) is present in all three species, while the high-affinity system (NDH-1_3) is only present in *Synechococcus* PCC7002 and *Crocosphaera* (although the genome information is not completely clear for *Crocosphaera*). For the α-cyanobacteria there is considerable variation. The low affinity system is present in the *Synechococcus* species, while *Prochlorococcus* species appear to lack any CO_2 uptake system.

Freshwater environments: The BCT1 HCO_3^- transporter is present in cyanobacteria from all freshwater environments. It is inducible at low Ci levels in *Synechococcus* PCC7942 and *Synechocystis* PCC6803 and would appear to be necessary in all environments where high-affinity uptake of HCO_3^- is required. However, the presence of the other two HCO_3^- transport systems is more variable. Single strong homologues of the SbtA transporter are present in all the species except *Nostoc punctiforme*. Interestingly, a weak SbtA homologue closely related to the *Prochlorococcus* protein is found in the three *Nostoc/Anabaena* species. BicA is also variable in its presence. *Synechocystis* PCC6803 and *Nostoc* PCC7120 have strong and medium homologues, *Anabaena variabilis* a single strong homologue, while *Synechococcus* PCC7942 and *Nostoc punctiforme* have only weak homologues. High and low affinity CO_2 uptake systems are present in all species.

Freshwater cyanobacteria have a diverse array of Ci uptake systems. Species such as *Synechocystis*, *Nostoc* PCC7120, and *Anabaena variabilis* may have 5–6 separate HCO_3^- and CO_2 transport systems which presumably are regulated in their induction to match the external conditions of Ci, pH, O_2, and temperature. Other species such as *Synechococcus* PCC7942 and *Nostoc punctiforme* lack one or more HCO_3^- uptake systems. It would appear that the core transport systems for the operation of the CCM in a freshwater environment are the two CO_2 uptake systems and BCT1. Depending on the species, this core is supplemented with other HCO_3^- transport systems, presumably to meet specific demands of the environment. It is not surprising that *Nostoc* PCC7120 has a full suite of Ci transporters as it forms gas vacuoles and would exist in high-light, low-Ci, and low-nutrient waters. The apparent paucity of Ci transporters in *Nostoc punctiforme* may be related to its symbiotic nature. Although found in free-living forms, its existence in symbiotic associations with cycads may require much less extreme adjustment to environmental variation. The two CO_2 uptake systems and the BCT1 transporter may be sufficient in this habitat.

Other environments: Of the species in Tables 4 and 5, *Gloeobacter violaceus* and *Thermosynechococcus elongatus* occupy the most different habitats. *Gloeobacter* originates from calcareous rock surface environments, while *Thermosynechococcus* is from thermal hot springs. The consequences of these environments for the Ci transporter complement are interesting. *Gloeobacter* contains only the two CO_2 uptake systems and the BCT1 HCO_3^- transporter. The surface of a calcareous rock is presumably more constant in its Ci supply, both as CO_2 from the atmosphere and HCO_3^- from the underlying rock. In addition, this is a slow-growing species and photosynthesis would require low Ci flux rates. It is unclear in this case if there would be environmental variation in Ci availability that would require the induction of the high-affinity CO_2 uptake system and the BCT1 transporter, or if their expression may be more constitutive.

By contrast, one would expect hot springs to be quite an extreme environment for efficient photosynthesis, with its elevated temperatures. However, *Thermosynechococcus* displays a reduced set of HCO_3^- transporters compared with freshwater species. Again, two CO_2 uptake systems, the BCT1 transporter and a single medium homologue of the BicA transporter are present. The chemistry of the Beppu hot springs is variable with waters varying from alkaline to acidic. The alkaline waters are dominated by HCO_3^- and species from these environments may be subjected to fairly constant levels of CO_2 and HCO_3^-. Under these conditions, great flexibility in varying Ci transporter composition may not be necessary. However, there is variation in the pH and HCO_3^- conditions between different thermal pools of this system and widely distributed cyanobacterial species may require flexibility in Ci acquisition strategies. In addition, mat-forming species

which inhabit the edges of the pools may experience extremes of Ci and O_2 during the day, depending on submersion levels and some flexibility in Ci acquisition strategies between free-living and mat community conditions may also be necessary.

Conclusions

Over the last ten years a detailed understanding of the genes and proteins involved in the formation of a cyanobacterial CCM has emerged. These proteins are involved in two primary aspects of CCM function. Firstly, they are involved in the formation of functional carboxysome microbodies within the cell, where CO_2 can be elevated around Rubisco. Secondly, they comprise an array of at least five Ci transport mechanisms, which facilitate flexibility in Ci in diverse environments. During the last five years, genome sequences for at least 18 cyanobacterial species from a range of ecological environments have become available and this has allowed us to view the ecological adaptation of the cyanobacterial CCM from a genomic perspective.

Two types of carboxysomes are found in cyanobacteria and their distribution seems to be correlated with the restriction of some species to oligotrophic open ocean environments where they are often the dominant cyanobacterial species. These are the α-cyanobacteria (with α-carboxysomes) which include *Prochlorococcus* and α-*Synechococcus* species. The β-cyanobacteria (with β-carboxysomes) are much more widespread, occupying both freshwater and marine environments with a greater range of environmental extremes. Combined with carboxysome diversity, there has been evolution of the nature of carbonic anhydrases which are involved in CO_2 generation within the carboxysome. There are at least four classes of CA enzymes represented in cyanobacterial genomes, with a potential for members of at least three of these to be directly involved in carboxysome function (CcaA, CcmM, and CsoS3)

The suites of Ci transporters which are present are also highly variable and perhaps the most highly correlated with nature of the aquatic habitat. Higher affinity Ci transporters are induced under environmental conditions when Ci acquisition for photosynthesis becomes limiting. Open ocean marine species show a restricted complement of transporters, with the extreme represented by *Prochlorococcus* species which have a limited range of HCO_3^- transporters and no CO_2 uptake systems. Variable representation of HCO_3^- and CO_2 uptake systems occurs in other marine species. In general, the maximum complement is present in species which probably experience the most variable environments such as coastal or estuarine environments with fluctuations in Ci levels and temperature. Freshwater species also show a range of Ci transporters. Species which occupy lake environments and peak in their abundance during summer contain the most complete complement of transporters, being correlated with the most extreme environmental fluctuations in Ci, temperature, O_2, and nutrients. Species with reduced sets of transporters are correlated with growth in symbiotic environments, thermal hotsprings and calcareous rock, where environmental fluctuations may be much less extreme.

Several more cyanobacterial species are currently being sequenced and sequence information will become available over the next year or so. This will expand the species representation from various environments, particularly marine symbiotic systems (*Prochloron* and *Prochlorothrix* species) and more freshwater species such as *Microsytis aeruginosa*. However, the ability accurately to interpret the presence of genes and their meaning in terms of ecological performance is still limited by a lack of comparative physiology of the CCM in a wide range of cyanobacterial species. Knowledge to date is largely based on three model species, *Synechocystis* PCC6803, *Synechococcus* PCC7942, and *Synechococcus* PCC7004 and interpretation of function in other species is based on this. Hopefully research in the next few years will discover more about the detailed structure and function of carboxysomes and Ci transporters in wider range of cyanobacterial species, allowing the interpretation of genetic diversity of the CCM in relation to the ecological habitat to be improved.

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