Review

Algal evolution in relation to atmospheric CO_2: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles

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Oxygenic photosynthesis evolved at least 2.4 Ga; all oxygenic organisms use the ribulose bisphosphate carboxylase-oxygenase (Rubisco)–photosynthetic carbon reduction cycle (PCRC) rather than one of the five other known pathways of autotrophic CO_2 assimilation. The high CO_2 and (initially) O_2-free conditions permitted the use of a Rubisco with a high maximum specific reaction rate. As CO_2 decreased and O_2 increased, Rubisco oxygenase activity increased and 2-phosphoglycolate was produced, with the evolution of pathways recycling this inhibitory product to sugar phosphates. Changed atmospheric composition also selected for Rubiscos with higher CO_2 affinity and CO_2/O_2 selectivity correlated with decreased CO_2-saturated catalytic capacity and/or for CO_2-concentrating mechanisms (CCMs). These changes increase the energy, nitrogen, phosphorus, iron, zinc and manganese cost of producing and operating Rubisco–PCRC, while biosphere oxygenation decreased the availability of nitrogen, phosphorus and iron. The majority of algae today have CCMs; the timing of their origins is unclear. If CCMs evolved in a low-CO_2 episode followed by one or more lengthy high-CO_2 episodes, CCM retention could involve a combination of environmental factors known to favour CCM retention in extant organisms that also occur in a warmer high-CO_2 ocean. More investigations, including studies of genetic adaptation, are needed.

Keywords: inorganic carbon; mixing depth; photosynthetically active radiation; Rubisco; temperature; nutrients; UV radiation

1. INTRODUCTION

Algae are oxygenic photosynthetic organisms other than embryophytic plants, and by this definition include the cyanobacteria as well as a wide range of eukaryotic lineages. Cyanobacteria, as indicated by the occurrence of oxygenic photosynthesis, evolved at least 2.4 Ga, although fossil (including chemical biomarker) evidence for cyanobacteria does not go back beyond 2.1 Ga [1]. Eukaryotic algae have occurred since at least 1.2 Ga [2–4] and from freshwaters, and possibly lake margins, since 1.1 Ga [5] (table 1). Since 2.4 Ga, the biosphere has become increasingly oxygenated [1], reflecting the colonization of the oceans by cyanobacteria after their origin in freshwater habitats, with a corresponding

increase in the capacity of these organisms to have global biogeochemical influence [15,16] (table 1). A significant increase in oxygen, with oxygenation of the deep ocean, occurred in the Neoproterozoic 0.54–1 Ga [32], with variations in the Phanaerozoic including the highest known level in the Permo-Carboniferous glaciation [33]. Carbon dioxide varied with a general downward trend, with minima generally related to glaciations [10,34]. CO_2 was relatively constant at about 23 (range of estimates 10–100) times the present level between 2.5 and 1.8 Ga, with a very significant decrease between 1.8 and 1.1 Ga [35], further variations in the Neoproterozoic [36–38] and relatively well-established changes in the Phanaerozoic with minima in the Permo-Carboniferous and in the Pleistocene glaciations [33,39]. In this article, we consider how these environmental changes have influenced algal evolution, both through direct effects of the concentrations of CO_2 and O_2 on photosynthesis and related metabolism, and through indirect

Table 1. Inorganic carbon acquisition characteristics of cyanobacteria and algae related to earliest known occurrence of the taxon. General references on earliest known occurrence of algae: [6–8]. References on presence or absence of CO$_2$-concentrating mechanisms (CCMs): [9–14].

taxon	occurrence of CCM in extant organisms	oldest known fossil	references
Cyanobacteria	CCM ubiquitous	2.15 Ga (biomarkers); 2.45 Ga (O$_2$)	[1,15–17]
Chlorophyta: Prasinophyceae	CCM ubiquitous	1.3 Ga	[18,19]
Chlorophyta: Chlorophyceae	CCM present in all?	(450 Ma)[32]	[19,20]
Chlorophyta: Trebouxiophyceae	CCM present or absent	450 Ma	[19–23]
Chlorophyta: Ulvophyceae	CCM usually present; absent in some; C$_4$ in one	540 Ma	[6,19,20]
Streptophyta: Charophyceae	CCM present in all?	450 Ma	[6,20]
Streptophyta: Embryophytes	CCM usually absent; pyrenoid-based CCM in some anthocerophytes, C$_4$ or CAM in some freshwater tracheophytes, CCMs not involving C$_4$ and CAM in some freshwater and all marine tracheophytes	475 Ma	[6,24,25]
Rhodophyta: Bangiophyceae	CCM in all?	1.2 Ga	[2]
Rhodophyta: Florideophyceae	CCM in many, absent from some marine, many freshwater	600 Ma	[26,27]
Ochrista: Bacillariophyceae	CCM in all?	120 Ma	[28,29]
Ochrista: Fucophyceae	CCM in all?	(570 Ma?)	[27,30]
Ochrista: Tribophyceae	CCM in all?	600 Ma	[3,31]
Ochrista: Chrysophyceae and Synurophyceae	CCM absent in all	?	[9]

effects of changes in temperature (CO$_2$) and the availability of combined nitrogen, phosphorus and iron (CO$_2$ and O$_2$).

2. AUTOTROPHIC CARBOXYLASES

Six pathways of autotrophic CO$_2$ fixation are known in extant organisms, including ribulose bisphosphate carboxylase-oxygenase carboxylase activity in the photosynthetic carbon reduction cycle (Rubisco–PCRC), using CO$_2$ as the inorganic carbon species assimilated, which is at the core of inorganic carbon assimilation in extant oxygenic photosynthetic organisms [40–45]. These pathways are summarized in table 2 with respect to their stoichiometric requirement for reductant and ATP, their affinities for inorganic carbon expressed in terms of the half-saturation value for CO$_2$ and the influence of oxygen on their functioning.

Converting one CO$_2$ to the oxidation–reduction level of carbohydrates (CH$_2$O) requires four reducing equivalents at, or lower than, the midpoint redox potential of the NADPH.NADP$^+$ couple. The values given in table 2 indicate the minimal stoichiometry, assuming no redox side reactions or futile cycles. An example of a side reaction for the Rubisco–PCRC pathway is the Rubisco oxygenase activity in the photosynthetic carbon oxidation cycles(s) (Rubisco–PCOC), which occurs at a relatively low [CO$_2$]/[O$_2$] ratio. All of the autotrophic CO$_2$ fixation pathways also require ATP (table 2), with a range of stoichiometries from one to three ATPs for CO$_2$ converted to carbohydrate. The Rubisco–PCRC pathway has the equal highest energy cost of converting CO$_2$ to carbohydrate, with the further energy cost of the side reaction of the Rubisco–PCOC at low [CO$_2$]/[O$_2$] ratios.

The Rubisco–PCRC pathway seems more appropriate to CO$_2$ fixation at the present atmospheric CO$_2$ level when the half-saturation value for CO$_2$ is considered. The forms of Rubisco with the highest affinities for CO$_2$ (Form IB from some algae and vascular plants relying on CO$_2$ diffusion to Rubisco; Form ID from other algae) have half-saturation values for CO$_2$ almost as low as those of two other pathways, while the values for the other three pathways are considerably higher (table 2). The final criterion in table 1 is the effect of oxygen on the pathways. While the Rubisco–PCRC pathway is competitively (with CO$_2$) inhibited by O$_2$, the enzymes of this pathway are not damaged by O$_2$: some of the other pathways have one or more enzymes that are subject to irreversible inhibition by O$_2$ (table 2). For lack of accurate information, the resource cost of synthesizing the enzymic machinery needed to fix one mole of CO$_2$ per second from the present atmospheric CO$_2$ concentration is not shown in table 2. This value is a function of the stoichiometry of the enzymic protein components in the pathway, with that of the carboxylase(s) set by their CO$_2$ affinity, and the M_r (relative molecular mass) values of the enzymes [40,41]. Here, the relatively low-specific reaction rate and high M_r of Rubisco would tend to make the Rubisco–PCRC pathway expensive at present CO$_2$ concentrations, even without the O$_2$ effect, though some other pathways are probably at least as expensive as a result of the low CO$_2$ affinity of their carboxylases and the consequent large quantity of carboxylase needed to fix one mole of CO$_2$ per second from the present atmospheric CO$_2$ concentration.

Table 2. Energy (NADPH and ATP) stoichiometry, affinity for inorganic carbon expressed as the half-saturation concentration for CO$_2$, competitive inhibition by O$_2$ and damage by O$_2$ for six autotrophic inorganic carbon assimilation pathways. Based on [40–45].

pathway from inorganic carbon to carbohydrate	NAD(P)H per CO$_2$	ATP per CO$_2$	K$_{1/2}$ CO$_2$ mmol m^{-3}	O$_2$ competitive inhibition	O$_2$ damage to one or more enzymes
Rubisco–PCRC, saturating CO$_2$ no O$_2$	2	3	$\geq$10	yes	no
reverse TCAC	2	1.67	>1500	no	yes[a]
3–hydroxypropionate	2	2	10	no	no[b]
3-hydroxypropionate–4-hydroxybutyrate	2	3	>2000	no	O$_2$-insensitive pathway in some organisms living in microaerobic habitats
dicarboxylate–4-hydroxybutyrate	2	2.67	>2000	no	no; some organisms live in microaerophilic habitats
Wood–Ljungdahl pathway	2	1	40 000[c]	no	yes

[a]The reverse tricarboxylic acid cycle (TCAC) can occur in thermophilic aerobic chemolithotrophs as a result of low O$_2$ solubility and high respiratory rates maintaining a low O$_2$ concentration inside the cells, and expression of an O$_2$-insensitive version of the 2-oxoglutarate–ferredoxin oxidoreductase which has at least a fivefold lower specific activity than the O$_2$-sensitive enzyme [43].
[b]The most oxygen-sensitive enzyme, methylmalonyol-CoA mutase, can be assayed and even purified at atmospheric equilibrium O$_2$ concentrations; it may not be sufficiently O$_2$-tolerant to function in illuminated cells of oxygenic photosynthetic organisms [43].
[c]Although acetogens live in habitats with higher CO$_2$ concentrations than correspond to atmospheric equilibrium [46], the *in vitro* K$_{1/2}$ value cited does seem very high.

The occurrence of Rubisco–PCRC as the core carboxylase in oxygenic photosynthetic organisms can be related to opportunity and functionality. By opportunity is meant the occurrence of the pathway at the time (about 2.4 Ga) at which the earliest evidence for oxygenic photosynthetic organisms is known. The Rubisco–PCRC pathway originated before oxygenic photosynthesis evolved (see below). By functionality is meant the carboxylase CO$_2$ affinity and carboxylase M_r values as determinants of the quantity of carboxylase (mass of protein) needed to fix one mole of CO$_2$ per second from the current atmosphere, the extent to which O$_2$ competitively inhibits or damages the enzymes of the pathway, and the ATP cost of the pathway per CO$_2$ assimilated (table 2). The CO$_2$ affinity criterion apparently rules out three of the five pathways (two of which are also very oxygen-sensitive), leaving the 3-hydroxypropionate pathway and the Rubisco–PCRC pathway. While not oxygen inhibited in the manner found for Rubisco, the 3-hydroxypropionate pathway may be sufficiently O$_2$-sensitive to restrict its functionality in oxygenic organisms once O$_2$ had begun to accumulate in the part of the biosphere occupied by oxygenic photosynthetic organisms (tables 1 and 2). In such ways can the role of the Rubisco–PCRC pathway in all known oxygenic photosynthetic organisms be rationalized.

3. RUBISCO CARBOXYLASE ACTIVITY AND THE PHOTOSYNTHETIC CARBON REDUCTION CYCLE

Rubisco evolved before the origin of oxygenic photosynthesis [47–49]. The Rubisco gene family not only contains the forms I, II and III Rubiscos that catalyse the Rubisco carboxylase and Rubisco oxygenase reactions, but also the form IV Rubisco-like protein (RLP) which does not catalyse the typical Rubisco reactions and which is involved in methionine salvage in some bacteria [47–49]. Molecular phylogenetic analysis [47–49] suggests that an ancestral form III Rubisco

arose in a methanogen (i.e. a member of the Archaea) and gave rise, by two vertical transmissions, to all other form III Rubiscos and to form IV. Horizontal gene transfer then moved form III and form IV Rubiscos to an ancestral bacterium, where form III gave rise to the ancestors of forms I and II, and hence by vertical transmission to the form IV RLP of bacilli and to forms I, II and IV of an ancestral proteobacterium.

Vertical descent and further diversification gave rise to the forms IA, IC, ID and II, and the form IV RLP in extant Proteobacteria. Horizontal gene transfer from the Proteobacteria transferred form IA to cyanobacteria, where form IB evolved from form IA [47–50]. However, there is also evidence that extant α-cyanobacteria with form 1A Rubisco (α-cyanobacteria) acquired their form IA Rubisco (and other α-carboxysomal proteins) from a proteobacterium, displacing the form IB Rubisco and associated β-carboxysomal proteins [51–56]. Endosymbiosis of a heterocystous β-cyanobacterium [57] gave rise to the plastids of the Archaeoplastida (i.e. Plantae), where the form IB Rubisco of the endosymbiont was retained by glaucocystophyte and green algae and hence by vertical transfer to embryophytic ('higher') plants, and was moved by secondary endosymbiosis to the (rhizarian) chlorarachniophytes and the (excavate-discicristate) euglenoids. Horizontal gene transfer from a proteobacterium accounts for the presence of form ID Rubisco in red algae and in the chromistan algae (ochrista or heterokontophytes, cryptophytes and haptophytes) whose plastids arose by secondary endosymbiosis of a red alga. The peridinin-containing dinoflagellate and basal apicomplexans (represented today by the photosynthentically competent *Chromera velia* and an as yet un-named close relative) also obtained their plastids by secondary endosymbiosis from a red alga, but horizontal transfer of form II Rubisco from a proteobacterium replaced the form ID Rubisco [58]. Other dinoflagellates have other forms of Rubisco as a result of tertiary endosymbioses. Finally, a second

primary endosymbiosis involving an α-cyanobacterium with form IA Rubisco accounts for the presence of plastids and form IA Rubisco in the (rhizarian) euglyphid amoeba *Paulinella* [54,55].

Some of the enzymes of the PCRC of vascular plants are derived from cyanobacteria in the primary endosymbiosis leading to the Archaeoplastida (i.e. Plantae), while others are of host origin [59]. This work has now been extended to include the glaucocystophyte and red algal members of the Archaeoplastida [60] and the diatoms whose plastids arose from secondary endosymbiosis of a red alga [61] following an earlier secondary endosymbiosis of a green alga [62]. There are also differences among algae in the regulation of enzymes of the PCRC [63]. Further work is needed to establish the relevance, if any, of atmospheric changes to these differences in the regulation of enzymes of the PCRC [63,64], and also the absence of Rubisco activase from algae with the form ID Rubisco in the few cases examined [61].

It is likely that the earliest Rubiscos in chemolithotrophic and anoxygenic photosynthetic Archaea and Bacteria were operating in a high-CO_2 environment and, because there was no oxygenic photosynthesis, in the essential absence of O_2. Such an environment would have provided little or no selective pressure for a high CO_2 affinity or a high CO_2/O_2 selectivity relative to a high CO_2-saturated maximum catalytic rate, using the mechanistic arguments of Tcherkez *et al.* [65] that a high maximum catalytic rate is incompatible with high CO_2 affinity and higher CO_2/O_2 selectivity.

The early organisms using Rubisco as a carboxylase would, then, be able to use diffusive entry of CO_2, with none of the resource costs associated with Rubisco operating at below the CO_2-saturated rate in the presence of O_2 when Rubisco oxygenase activity is expressed. The additional resource costs can be considered as capital (synthetic) costs and running costs. The capital costs are those of synthesizing additional Rubisco enzyme per cell if the per cell rate of CO_2 assimilation is to be retained, as well as the costs of making the enzymes and transporters related to the operation of a PCOC and/or a CO_2-concentrating mechanism (CCM), and include energy, carbon and nitrogen as well as phosphorus for any additional ribosomes that are required [66]. The running costs are in terms of energy, both for synthesizing 2-phosphoglycolate and metabolizing it back to sugar through a PCOC and for operating a CCM.

The early Rubisco-containing organisms could still permit CO_2 saturation of a high specific reaction rate Rubisco, with corresponding savings of resources in constructing the enzymic machinery able to assimilate one mole of CO_2 per second. In other words, the conditions described would give the lowest possible energy, carbon and nitrogen costs of producing the amount of Rubisco capable of fixing one mole of CO_2 per second, and also give the lowest possible energy cost of operating Rubisco, i.e. two NADPH and three ATP per CO_2 converted to carbohydrate. A correlated saving associated with this minimal requirement for NADPH and ATP in autotrophic CO_2 assimilation is in the quantity of redox and ATP synthesis machinery needed for NADPH and ATP production. Minimizing

the protein requirements for autotrophy also decreases the phosphorus requirement for mRNA in ribosomes and in tRNA, and mRNA needed to produce the Rubisco and associated enzymes. These characteristics would minimize the nitrogen needed to produce the machinery associated with a given rate of CO_2 fixation, and thus the phosphorus in RNA needed to synthesize the proteins [66]. Costs in energy, nitrogen, phosphorus, iron and manganese will be considered below in the context of decreasing CO_2 and increasing O_2 [66–74].

The mechanistically constrained covariation in Rubisco kinetics mentioned above, i.e. a high CO_2-saturated specific reaction rate correlated with low CO_2 affinity and CO_2/O_2 selectivity and vice versa [65] has parallels with the growth strategies of vascular plants [75] and of algae [76] according to the competitive–stress-tolerant–ruderal (CSR) paradigm, for which there are also mechanistic bases in terms of having high rates of metabolism and growth in ruderals, and lower rates but perhaps a more effective use of resources in stress-tolerators [77–79].

4. OXYGEN ACCUMULATION, RUBISCO OXYGENASE AND THE METABOLISM OF PHOSPHOGLYCOLATE

The build-up of O_2 has had many effects on algal evolution, permitting respiration and, via the occurrence of stratospheric ozone, a decreased UV-B flux, and production of reactive oxygen species from O_2 rather than UV-B action on cell constituents [79,80]. The presence of O_2 does not, however, inhibit O_2 production by oxygenic photosynthetic organisms [81,82]. The accumulation of O_2 in the habitats of oxygenic photosynthetic organisms using Rubisco as their autotrophic carboxylase permits Rubisco oxygenase activity to occur, provided the CO_2 concentration is below saturation for Rubisco. Such decreased CO_2 concentrations, combined with at least local O_2 accumulation, probably occurred about 2 Ga, when there is evidence of an ice age extending to low palaeolatitudes [35,83].

The product of the Rubisco oxygenase activity is, as well as one 3-phosphoglycerate per O_2 consumed, one 2-phosphoglycolate. In addition to sequestering the often limiting resource phosphorus if 2-phosphoglycolate continues to accumulate, 2-phosphoglycolate is also an inhibitor of some reactions involving phosphate esters, including some in the PCRC [84]. Accordingly, all organisms using Rubisco in the presence of O_2, i.e. oxygenic photolithotrophs and some chemolithotrophs, have 2-phosphoglycolate phosphatase [84,85]. This enzyme would not have been needed to deal with 2-phosphoglycolate from Rubisco oxygenase activity in anoxygenic photosynthetic proteobacteria, or in any anaerobic chemolithtrophs, using the Rubisco–PRCR pathway before build-up of O_2 in the biosphere. 2-Phosphoglycolate phosphatase also occurs in some non-autotrophic bacteria that have no Rubisco, where it is thought to be involved in some forms of DNA repair [86,87]. Since DNA damage and its repair must have occurred before oxygenic photosynthesis, the 2-phosphoglycolate phosphatase in oxygenic photosynthetic organisms could have been recruited from bacteria

lacking autotrophic 2-phosphoglycolate synthesis. However, the 2-phosphoglycolate phosphatase from eukaryotes does not seem to have been derived from the 2-phospho-glycolate phosphatase of cyanobacteria [88].

The organic carbon product of 2-phosphoglycolate phosphatase is glycolate. This can be excreted, with loss from the organism of the energy and carbon used in its synthesis. Alternatively, glycolate can be salvaged by metabolism to 3-phosphoglycerate, and hence triose phosphate, which occur in the PCRC, albeit with the input of energy and the release of CO$_2$ [85]. The cyanobacteria have two variants on pathways converting glycolate to 3-phosphoglycerate.

One means of converting glycolate to 3-phosphoglycerate is the pathway via glycine and serine, with recycling of ammonia, as in the classic PCOC of embryophytic plants and at least some eukaryotic algae [85,88–92]. The pathway through glycine and serine seems to have been gained by eukaryotic photosynthetic organisms during the primary endosymbiosis yielding the plastids of the Archaeplastida (i.e. Plantae), although some of the genes in eukaryotes came from α-proteobacteria rather than cyanobacteria [88]. The β-cyanobacterial plastid ancestor gave rise to the glycolate oxidase, glycerate kinase and hydroxypyruvate reductase of algae and embryophytes, while serine hydroxymethyl transferase and the L, P and T subunits of glycine decarboxylase came from α-proteobacteria by horizontal gene transfer [88]. The origin of the other eukaryotic PCOC genes, i.e. those encoding the H subunit of glycine decarboxylase, glutamate–glyoxylate aminotransferase and serine–glyoxylate aminotransferase, has not yet been established [88].

The other pathway from glycolate to 3-phosphoglycerate involves tartronic semialdehyde, and is called the tartronic semialdehyde pathway by phycologists; bacteriologists call it the glycerate pathway, even though glycerate is also an intermediate of the PCOC [85,88–90]. Parts of the PCOC (glycerate dehydrogenase, serine transminases and serine hydroxymethyltransferase) could have been recruited from core metabolism synthesizing serine and glycine from glycolytic intermediates, while others (glycolate dehydrogenase/oxidase, glycine decarboxylase and glycerate kinase) have no known roles other than in the metabolism of glycolate to PCRC intermediates [93,94].

It seems likely that at least one of the metabolic pathways from glycolate to 3-phosphoglycerate evolved in oxygenic photosynthetic organisms relying on diffusive CO$_2$ entry before CCMs evolved. Not only is there at least a minimal flux through Rubisco oxygenase and thence to intermediates of a glycolate metabolism pathway despite high levels of expression of CCMs [85,89,90,92,95], but elimination of the pathways of glycolate metabolism is fatal to the organism [85,90]. Previous misgivings [91,92] about the occurrence of the complete PCOC in diatoms have now been largely overcome, although there are still doubts as to the glycerate kinase step [61,88].

The changes to CO$_2$ fixation in oxygenic photolithotrophs in relation to decreasing CO$_2$ and, especially, increasing O$_2$ is part of a wider range of resource cost increases as the biosphere becomes oxygenated. Falkowski & Godfrey [72] point out that not only is Rubisco impacted by increasing O$_2$ with decreasing CO$_2$, but that the potential for oxygen damage to nitrogenase becomes manifest, and the very source of the O$_2$, the reaction centre of photosystem II, is itself subject to photodamage both directly through excitation energy transfer to the reaction centre, but also indirectly through the accumulated O$_2$ forming reactive O$_2$ species ([66]). As Raven [66,68–70] points out, the effects on Rubisco demand additional nitrogen in the enzyme itself and in related enzymes, more iron and manganese in additional thylakoid redox agents, and more phosphorus in the RNA needed to make the additional protein, if the rate of photosynthesis is to be maintained. More energy input as NADPH and ATP is also needed to run CO$_2$ assimilation [66,69,70]. For oxygen damage to nitrogenase, there is generally synthesis of 'reserve' nitrogenase in addition to what is needed in the absence of oxygen damage to satisfy the combined nitrogen requirements of cell growth. Synthesis of the 'reserve' nitrogenase requires the nitrogen and energy needed for the synthesis of any protein, but also the iron and (in almost all cases) molybdenum used in the nitrogenase cofactors. When oxygen damage does occur and reserve nitrogenase is used catalytically, more energy (but not nitrogen, iron and molybdenum) is needed to synthesize nitrogenase to replace what is damaged. In both cases, the production of more nitrogenase than would be required in the absence of oxygen involves the use of more phosphorus for the RNA required for the additional protein synthesis. In the case of photoinhibition, more nitrogen and energy are needed to synthesize reserve photosystem II reaction centres, more energy is needed to synthesize replacement photosystem II reaction centres, and more phosphorus is needed for the RNAs needed for the extra protein synthesis. A further aspect of damage to proteins by O$_2$ concerns the absence of any core autotrophic CO$_2$ assimilation pathway other than Rubisco–PCRC from oxygenic photosynthetic organisms. In addition to the CO$_2$ affinity problems outlined for some of the alternative pathways, there would also be the requirement for additional resources (photosynthetically active radiation (PAR), nitrogen and phosphorus) to make and use additional RNA needed for the additional resynthesis of O$_2$-damaged protein (see [66] and above, for other cases).

Compounding this need for additional nitrogen and phosphorus per unit CO$_2$ or N$_2$ assimilated and photons used in photochemistry is the effect of increased O$_2$ on the availability of nitrogen and phosphorus. Falkowski & Godfrey [72] point out that oxygenation of the biosphere not only decreases the potential for diazotrophy, but allows nitrifying microbes to convert NH$_4^+$ to NO$_3^-$, which in hypoxic or anoxic micro-habitats can be denitrified to produce N$_2$O and N$_2$. This nitrification–denitrification sequence decreases the availability of combined nitrogen to non-diazotrophic primary producers. In the case of phosphorus, the availability of O$_2$ converts Fe(II) to oxidized iron (Fe(III)), which binds phosphate and thus decreases global phosphorus availability [96,97].

Phosphorus is one of the biogeochemical regulators of the O_2 content of the atmosphere [96,97]. This topic will be returned to below in the context of ocean deoxygenation as a function of increases in CO_2 and temperature.

5. INTRODUCTION TO CO_2-CONCENTRATING MECHANISMS

Diffusive entry of CO_2 to Rubisco was presumably the ancestral mechanism of autotrophic CO_2 assimilation in oxygenic photosynthetic organisms. Entry of CO_2 to Rubisco by diffusion is found today in the majority, by species number and contribution to global primary productivity, of terrestrial oxygenic photosynthetic organisms, but in a minority of oxygenic photosynthetic organisms in aquatic environments where photolithotrophs with CCMs predominate [11–13,34,67,79, 98–101] (table 1). The references just cited show that CCMs are very widely distributed among algae, both phylogenetically and geographically, although they seem to be absent from chrysophycean and synurophycean algae [9]. The mechanistic, including molecular, details of the CCMs of cyanobacteria are now known [51–53,56,102]. The CCMs of eukaryotic algae are less clearly understood at both the molecular and the mechanistic levels, although they are clearly polyphyletic [10,13,34,67,79,103,104].

As to the evolutionary origin of CCMs, the selective factors were presumably decreasing CO_2 and increasing O_2. The variability of other gases over the last 2.4 Gyr suggests that there were several periods at which CCMs could have been resource-effective (energy, nitrogen, phosphorus, iron, zinc and manganese) alternatives to diffusive CO_2 entry to Rubisco with attendant high activity of Rubisco oxygenase and expression of high levels of enzymes converting 2-phosphoglycolate to sugar phosphate [10,67–71,74,105,106]. We shall return to the question of the timing(s) of the origin(s) of CCMs and how, if they originated early, they were retained through intervening high-CO_2 episodes. First, we consider what the transition from diffusive CO_2 entry to CCM-based delivery of CO_2 to Rubisco involves.

6. THE FUNCTIONING OF CO_2-CONCENTRATING MECHANISMS IN COMPARISON WITH THE DIFFUSIVE ENTRY OF CO_2

One factor in the evolution of a CCM in an organism previously relying on diffusive CO_2 movement from the medium to Rubisco as CO_2 availability decreases is the kinetics of the Rubisco used by the organism. A Rubisco with a high CO_2-saturated catalytic capacity, and a correspondingly low CO_2 affinity and CO_2/O_2 selectivity, such as occurs in the form IA and form IB Rubiscos of extant cyanobacteria, would be expected to present a stronger evolutionary case for a CCM at a given CO_2 concentration and CO_2/O_2 ratio than would eukaryotic form IB and form ID Rubisco with higher CO_2 affinity and CO_2/O_2 selectivity but a lower CO_2-saturated maximum catalytic rate [65]. Young *et al.* [107] have used molecular phylogenetic evidence to show that there was positive selection of the form

ID Rubiscos in some eukaryotes that correspond to low-CO_2 and low-CO_2/O_2 episodes in the geological record, and that these episodes of positive selection could have corresponded to the time of evolution of CCMs. To be effective, the CCM must maintain a higher CO_2 concentration at the site of Rubisco than would be possible by CO_2 diffusion alone [10].

The essential component of a CCM is the accumulation of CO_2 in the compartment containing Rubisco to a higher steady-state concentration than occurs in the growth medium, and hence even higher than the steady-state concentration near Rubisco which could occur with diffusive CO_2 entry. For algae, one mechanism of accumulation could involve C_4-like photosynthetic metabolism with an ATP-dependent ($C_3 + C_1$) carboxylation in the cytosol, using HCO_3^- obtained directly or indirectly (via CO_2 entry from the medium followed by carbonic anhydrase catalysis) from the medium, followed by a ($C_4–C_1$) decarboxylation in the chloroplast [104]. The alternative mechanisms do not involve the inorganic carbon transferred from the medium to Rubisco forming organic intermediates. These alternative mechanisms involve transmembrane-active transport mechanisms which move an inorganic carbon species (CO_2 or HCO_3^-), or H^+, against a free-energy gradient. Such transporters could be (and have been) derived from transporter gene families by change of specificity of the transported substrate (to CO_2 or HCO_3^-), with changes in regulation and, perhaps, changes in intracellular targeting [51–53,56]. An exception to the need for active transport across a membrane is CO_2 use in the cyanobacterial CCMs, where diffusive CO_2 entry across the plasmalemma is followed by energized conversion to HCO_3^- by the NAD(P)H—PQ oxidoreductase of the thylakoid membrane [51–53,56]. Here, a high CO_2 permeability of the plasmalemma is required. Such a high membrane permeability to CO_2 is needed for diffusive CO_2 entry all the way to Rubisco in organisms lacking a CCM, and (as noted above) in CO_2 'active transport' in cyanobacteria. A high CO_2 permeability is also necessary in organisms with a CCM mechanism involving CO_2 production from HCO_3^- in a compartment acidified by a H^+ pump followed by transmembrane movement of CO_2 into the compartment containing Rubisco [13]. The energized conversion of CO_2 to HCO_3^- in the cyanobacterial cytosol could increase the chance of any CO_2 that leaks out of the carboxysomes being trapped as HCO_3^- in the cytosol. In all other cases, a CCM is most energetically efficient with minimal CO_2 flux from the compartment in which it is accumulated back to the medium, i.e. with very low membrane permeabilities to CO_2.

The energetic savings that could result from a low CO_2 permeability of the plasmalemma, and of the inner plastid envelope (if that is the membrane involved in active transport of inorganic carbon), in eukaryotes with CCMs based on active transport of an inorganic carbon species would, if verified, suggest a phylogenetic, and in many cases, an acclimatory (changes from growth in high to low CO_2) decrease in CO_2 permeability. This question has been addressed by a number of workers [103,108], who found relatively high CO_2 permeability in the eukaryotic algal membranes examined, regardless of

whether the algae had been cultured in high (CCM repressed) or low (CCM expressed) inorganic carbon concentrations. The permeability values for CO_2 of the plasmalemma of high-CO_2 and low-CO_2-grown cells of *Chlamydomonas reinhardtii* range from 0.76 to $1.8.10^{-3}$ cm s^{-1} [108], while for four species of diatom the range is $15-56.10^{-3}$ cm s^{-1} [103], with the range of values probably related to methodological as well as phylogenetic differences. While models for CCMs in diatoms consistent with the available data show relatively modest energy losses during CCM operation, they do involve constraints such as the membrane(s) at which active inorganic carbon transport occurs, and the chemical species involved in this active transport [103]. The same argument applies to the protein shell of the carboxysome, for which a restriction on CO_2 diffusion, but not on the diffusion of anionic substrates (HCO_3^- and ribulose-1,5-bisphosphate) and product (3-phosphoglycerate) has been demonstrated [109].

Most CCMs also involve one or more carbonic anhydrase enzymes: an exception is a C_4 mechanism which involves, as indicated above, HCO_3^- entry to the cytosol, $(C_3 + C_1)$ carboxylation using HCO_3^-, and $(C_4 - C_1)$ decarboxylation in the plastid stroma with CO_2 as the inorganic carbon product [13,92,95,104,110]. All other well-investigated CCMs seem to involve 'normal' carbonic anhydrases, i.e. those catalysing the equilibration of CO_2 and HCO_3^- [11,67,111]: this is the case for 'active CO_2 influx' in cyanobacteria, which involves a carbonic anhydrase in the carboxysome as well as the energized conversion of CO_2 to HCO_3^- at the thylakoid membrane, which is effectively a unidirectional carbonic anhydrase [56].

The functioning of CCMs is influenced by a number of factors other than the availability of inorganic carbon (and, in some cases, O_2), e.g. photosynthetically active radiation, UV-B radiation, the form and the concentration of combined nitrogen, the phosphate concentration and the iron concentration [66,67,74,105]. The influence of these factors on the expression and functioning of CCMs is presented in table 3 [66,67,74]. There are also predicted effects of expression of CCMs rather than reliance on diffusive entry of CO_2 on the resource costs of synthesis of the photosynthetic apparatus, and of its operation; these are discussed below. We next discuss the possible influence of these interactions on the evolution of CCMs, their retention through any high-CO_2 episodes between their origin in a low-CO_2 habitat and today, and their fate in a future higher CO_2 and warmer world.

7. THE ORIGINS OF CO$_2$-CONCENTRATING MECHANISMS

The 'why' of the origin of CCMs presumably concerns the occurrence of low CO_2, both in absolute terms and in relation to O_2, which was indicated above as requiring additional protein (hence RNA and phosphorus) in more Rubisco and in enzymes metabolizing 2-phosphoglycolate to sugar phosphate, as well as additional energy input per net CO_2 assimilated by diffusive CO_2 entry of CO_2 to Rubisco and metabolism of 2-phosphoglycolate. Depending on the circumstances, e.g. the form of Rubisco present and the environmental

conditions as well as the mechanism of the particular CCM [10,34,68–71,74,79,91,103,105], a CCM could require less energy, nitrogen, phosphorus, zinc and iron for its synthesis, and less energy for its operation, than diffusive CO_2 entry with metabolism of 2-phosphoglycolate (table 2). Other factors that could have influenced the evolution of CCMs include the decreasing UV-B flux with the increased stratospheric O_3 resulting from the build-up of O_2, which is itself an influence on the evolution of CCMs [79]. UV-B radiation causes damage to Rubisco and to photosystem II, but has less effect on photosystem I. The limited data available suggest that UV-B has little effect on CCM activity in the green alga *Dunaliella tertiolecta* [122] but elevated CO_2 can increase the sensitivity of microalgae to UV-B [123,124]. There is no information about the possibility of a differential impact of UV-B on the various forms of Rubisco, though it would be interesting to know if changes in UV-B in the past relate to the evolution of different Rubiscos.

The 'how' of the origin of CCMs concerns the ancestry of the various components of the pathway. For the active transport components, H^+ pumps are ubiquitous and anion transporters/pumps (hence HCO_3^- transporters/pumps) are also widespread, as could be the ancestors of CO_2 pumps [10,13,34,51–53,56,79,102,104]. Facilitators of downhill transmembrane CO_2 transport, yielding permeabilities in excess of those owing to the lipid phase alone, are required for cyanobacterial active transporters and for the mechanism involving an acidified compartment generating CO_2 from HCO_3^- with subsequent transmembrane CO_2 diffusion to Rubisco. Such facilitators would presumably have originally been components of the diffusive pathway for CO_2 from the medium to Rubisco. Carbonic anhydrases could have had a number of roles prior to their co-option into CCMs, including that of facilitating diffusion of CO_2 (as HCO_3^-) in the diffusive entry of CO_2 from the medium to Rubisco. Cyanobacterial carboxysomes are part of a larger family of bacterial micro-compartments [125]. This brief view may be over-optimistic as to the ease of co-opting existing mechanisms into CCMs [34], and does not address the origin of the eukaryotic pyrenoid [14]; however, it does indicate some possibilities.

There are a number of options as to the 'when' of the evolution of CCMs. We initially consider times of relatively low CO_2, based on low palaeotemperatures (with the requirement that greenhouse gases corrected for the faint young Sun) or on biogeochemical or biological proxies. Glacial/low CO_2 episodes occurred 2.4–2.1 Ga and at 750, 650 and 320–270 Ma, as well as the Pleistocene (last 2.4 Myr) [10]. All but the earliest of these times would have been relevant to at least some of the eukaryotic as well as cyanobacterial oxygenic photosynthetic organisms (table 1). There is no direct fossil evidence as to the origin of CCMs, and little help from molecular clocks [10,67,79], although recent work by Young *et al.* [107] shows episodes of positive selection of form ID Rubisco in diatoms and haptophytes which correspond to low-CO_2 episodes and hence possibly relate to the origin of CCMs. Assuming, as seems very likely, that cyanobacterial and at least some algal CCMs evolved before the

Table 3. Influence of environmental conditions on the expression of CCMs and the resource cost of CCM operation versus diffusive entry of CO_2. Effects on CCMs of environmental factors, and the direction of change of these environmental factors in algal and aquatic plant habitats with global environmental change between icehouse episodes. Modified from [10]. Further details and references are given in the text and in [10–12,101,105,106,110,112–120]. Predicted resource costs of synthesizing and operating a photosynthetic apparatus using a CCM relative to one relying on entry of CO_2 by diffusion [66,68–71,73,91,105,121]. DOC, dissolved organic carbon; n.a., not applicable.

factor	change to algal environment caused by variation in the factor	effects on expression of CCMs and on their affinity for CO_2	predicted effect of CCM expression on resource costs of synthesis and (for PAR) operation of the photosynthetic apparatus
CO_2	increase in CO_2 in essentially all environments, although less predictable effect in freshwaters which can be out of equilibrium with the atmosphere	decreased inorganic carbon affinity with growth at high CO_2; can be a switch to diffusive CO_2 entry in some eukaryotes	no general effect on carbon cost of synthesis of the photosynthetic apparatus
temperature	increase in temperature in all environments	prediction of increased CCM expression, or increased fraction of organisms with CCMs, at higher temperatures as a result of lower CO_2 solubility, is not uniformly supported by the available data.	inapplicable: temperature (Boltzmann energy) is not resource consumed in the synthesis of the photosynthetic apparatus, in its operation or in its maintenance
PAR	increase in PAR in pelagic planktonic environments	decreased inorganic carbon affinity with growth at low PAR	possible decreased energy needed in synthesis of photosynthetic apparatus which uses a CCM. Energy saving in operation of a CCM if there is low CO_2 leakage and a low CO_2 affinity and low $CO_2:O_2$ selectivity
nitrogen	decrease in combined nitrogen in upper mixed layer of lotic environments	generally increased inorganic carbon affinity with growth at low NO_3^-. One example each of decreased carbon affinity with growth at lowest NO_3^- concentration tested, and with growth over entire NH_4^+ range tested.	decreased nitrogen cost of synthesis of the photosynthetic apparatus incorporating a CCM if the savings in the synthesis of smaller amounts per cell of Rubisco and of the PCOC enzymes are not offset by the nitrogen cost of the synthesis of CCM components. No consumption of nitrogen in the operation of the CCM
phosphorus	decrease in phosphate in upper mixed layer of lotic environments	two examples of increased inorganic carbon affinity, two examples of decreased inorganic carbon affinity, with growth at low phosphate	where there is a decreased protein content in the photosynthetic apparatus there could be a corresponding decrease in the need for phosphorus needed to synthesize the photosynthetic apparatus as a result of decreased requirement for RNA
iron	probable decrease in iron in upper mixed layer of lotic environments	one example of increased inorganic carbon affinity with growth at low iron, one example of no effect	decreased Fe content of the photosynthetic apparatus if the decreased requirement for NADPH in the near-absence of Rubisco oxygenase activity, and the PCOC, with correspondingly lower content of non-cyclic electron transport components, is not offset by the additional thylakoid components needed for the additional ATP requirement for CCM operation, especially if this additional ATP is made using cyclic photophosphorylation using only phosystem I with its high Fe content
zinc	as for iron	no direct measurements	variable predictions of relative zinc requirements depending on CCM mechanism

(*Continued.*)

Table 3. (*Continued.*)

factor	change to algal environment caused by variation in the factor	effects on expression of CCMs and on their affinity for CO$_2$	predicted effect of CCM expression on resource costs of synthesis and (for PAR) operation of the photosynthetic apparatus
UV-A	increase in UV-A in lotic planktonic environments, but decrease with higher concentration of DOC	no data	n.a.
UV-B	increase in UV-B in lotic planktonic environments, but decrease with higher concentration of DOC	variable responses of CCMs with increased UV-B flux for growth	n.a.

Pleistocene, they would have had to have survived intervening period(s) of higher CO$_2$ and higher temperatures. The mechanisms of retention of CCMs in these apparently unfavourable environments are now considered in the context of the response of present day CCMs to such environments.

8. RETENTION OF CO$_2$-CONCENTRATING MECHANISMS IN HIGH-CO$_2$ EPISODES

It is possible to argue for long-lasting low-CO$_2$ microhabitats, e.g. in benthic microbial mats, including stromatolites, where inorganic carbon diffusion from the bulk medium into the cells is restricted by thick diffusion boundary layers [79]. Biogeochemically more important are planktonic habitats where such low CO$_2$ refuges are less plausible. CCM retention is considered in the context of present work on the response of phytoplankton to current and expected environmental change, and especially increasing CO$_2$ and the associated warming.

More widespread (planktonic) retention of CCMs in relatively high CO$_2$ concentrations has been argued by Raven *et al.* [10] in the context of what might happen with increasing CO$_2$ and temperature over the next several decades. The argument here is that the increased buoyancy of warmer surface waters will lead to a shoaling of the thermocline, which will decrease fluxes of combined nitrogen and of phosphorus from the deeper ocean where mineralization of sinking particulate organic matter occurs, which have been modelled as decreasing global marine primary productivity [126–128]. This decreased nutrient supply, with the increased mean flux of photosynthetically active radiation (and UV-B) incident on the cells in the shallower upper mixed layer will, on the basis of observations on extant phytoplankton (table 3), favour retention of CCMs despite higher CO$_2$ concentrations [10].

A further relevant consideration which was not mentioned by Raven *et al.* [10] is that a warmer upper mixed layer has decreased oxygen solubility. For the same rate of net oxygen production at two temperatures, there will be greater degree of oxygen superaturation and hence a greater loss of oxygen to the atmosphere at the higher temperature. Together with the decreased solute transfer between the upper mixed layer and deeper ocean, there is less transfer of oxygen below the

thermocline [129–131]. The widespread, but not universal, decrease in calcification by calcified plankton [132–134], and the much smaller effect on silicification by diatoms [135] in a higher CO$_2$, warmer ocean means less ballasting of sinking particulate organic matter, hence slower sinking and more microbial mineralization just below the thermocline ([136], cf. [137]). While this higher nutrient concentration just below the thermocline might be expected to partly offset the lower rate constant for nutrient transfer to the upper mixed layer, another factor must be considered. The combination of more microbial respiration and increased oxygen supply can lead to hypoxia and even anoxia in certain sub-surface waters, with implications for loss of the nitrate and nitrite forms of combined nitrogen produced from organic matter by mineralization and nitrification in less deoxygenated zones followed by denitrification or the anammox reaction in more deoxygenated places [131], with a decrease in the nitrogen : phosphorus ratio in the nutrients reaching the upper mixed layer. Although, in the long term (thousands of years and more), a warmer world would heat the ocean interior as well as the upper mixed layer and potentially decrease the extent to which the thermocline shoals, there is a well-established correlation of widespread deep-ocean anoxia (and even euxinia) with warmer, high-CO$_2$ episodes in Earth history [138], so at least a decreased upward flux of combined nitrogen across the thermocline would continue, favouring retention of CCMs.

These arguments are based on the response of extant algae to the changes occurring, and predicted, in their environment as a result of increased CO$_2$ and temperature, with downstream effects on the marine and inland water inorganic system, the mixed layer depth and water body oxygenation. This can be used to inform us of how CCMs were retained in past episodes of high CO$_2$. Dealing first with the organisms studied, almost all of the work has been carried out with organisms which have only been exposed to the increased CO$_2$ and associated changes in temperature and the availability of other resources (table 3) for time periods of days to weeks. This time period allows 1–100 generation, meaning that only regulatory (altering the existing proteome by post-translational modification, and changes in the metabolome) and acclimatory (altering the expressed proteome based on the existing genome) [139] responses of extant

algae can be expressed. In very few cases has relevant evolutionary evidence been sought in laboratory experiments for increased CO_2 [139–146] and, using different methods, higher temperatures [147]. An example of where evolution has been unable to cope with natural CO_2 enrichment present for several decades concerns calcified red algae growing on seagrass leaves near an underwater vent in the Mediterranean [148]. Even for studies of regulation and acclimation there can be problems with the length of time that an alga has been in culture and frequently exposed to CO_2, nitrogen, phosphorus, PAR, UV and temperature which has little relevance to their natural environment [149]. Furthermore, there is a relative lack of studies in which changes in CO_2 have been combined with other relevant environmental changes: table 3 and Raven *et al.* [10] analyse and give references to the important work in which such interactions have been studied. Finally, there are some relatively poorly constrained factors of ocean chemistry in the past [80,150–154], and until recently little consensus as to the appropriate methodology [134,155–158] for laboratory and mesocosm experimentation on increased CO_2.

Despite these reservations, which also apply to other models of past and future atmosphere–ocean–organism interactions, the suggestions of Raven *et al.* [27] provide a lead into further studies in how CCMs could be retained in lengthy episodes between shorter low-CO_2 episodes. Any retention of CCMs in high-CO_2 episodes would be a further complication in the use of stable carbon isotope ratios of phytoplankton-derived organic carbon from marine sediments as a palaeobarometer for CO_2, since most marine phytoplankton today have CCMs [67,98,159,160]. Organisms lacking CCMs, e.g. terrestrial liverworts and mosses, do not suffer from this problem when used in palaeobarometry of CO_2 [161].

9. CONCLUSIONS

The changing CO_2 and O_2 concentration over the last 2.4 Gyr have had significant effects on the physiology and ecology of cyanobacteria and algae. From the presumed ancestral diffusive CO_2 entry to Rubisco, all extant cyanobacteria have CCMs, Rubiscos with high CO_2-saturated catalytic activity and low CO_2 affinity and CO_2/O_2 selectivity, and an essential role for the capacity to convert the 2-phosphoglycolate formed as a very small fraction of the total carbon flux into triose phosphates. Most eukaryotic algae have CCMs: a greater fraction has CCMs in the sea than in freshwaters, and there is no strong relationship to water temperatures. The evolution of CCMs can apparently be related to decreased CO_2 availability and to the presence of oxygen, modulated by the kinetics of the form of Rubisco in the organisms, with some components of the CCMs adapted in evolution from the roles in other pathways. The retention of CCMs during the high-CO_2 episodes predominant through Earth history could have been related in part to the interaction of CCM expression with other environmental factors which change in high-CO_2 water bodies.

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