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Invited Review Article

The many types of carbonic anhydrases in photosynthetic organisms

Robert J. DiMario[a], Marylou C. Machingura[b], Grover L. Waldrop[b], James V. Moroney[b,*]

[a] School of Biological Sciences, Molecular Plant Sciences, Washington State University, Pullman, WA 99164, United States
[b] Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, United States

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ABSTRACT

Carbonic anhydrases (CAs) are enzymes that catalyze the interconversion of CO_2 and HCO_3^-. In nature, there are multiple families of CA, designated with the Greek letters α through θ. CAs are ubiquitous in plants, algae and photosynthetic bacteria, often playing essential roles in the CO_2 concentrating mechanisms (CCMs) which enhance the delivery of CO_2 to Rubisco. As algal CCMs become better characterized, it is clear that different types of CAs are playing the same role in different algae. For example, an α-CA catalyzes the conversion of accumulated HCO_3^- to CO_2 in the green alga *Chlamydomonas reinhardtii*, while a θ-CA performs the same function in the diatom *Phaeodactylum tricornutum*. In this review we argue that, in addition to its role of delivering CO_2 for photosynthesis, other metabolic roles of CA have likely changed as the Earth's atmospheric CO_2 level decreased. Since the algal and plant lineages diverged well before the decrease in atmospheric CO_2, it is likely that plant, algae and photosynthetic bacteria all adapted independently to the drop in atmospheric CO_2. In light of this, we will discuss how the roles of CAs may have changed over time, focusing on the role of CA in pH regulation, how CAs affect CO_2 supply for photosynthesis and how CAs may help in the delivery of HCO_3^- for other metabolic reactions.

1. Introduction – the CA catalyzed reaction

Carbonic anhydrase (CA) is an enzyme that catalyzes the interchange between CO_2 and HCO_3^- in solution as well as other reactions [1]. While the CO_2 and HCO_3^- conversion does take place in the absence of CA, the interconversion is very slow. It is thought that CA is required to ensure a rapid supply of CO_2 and HCO_3^- for various metabolic pathways in organisms.

2. There are multiple, apparently unrelated CA families

There are a surprisingly wide variety of CA proteins that fall into a number of protein families. These families are named by Greek letters and roughly follow the order in which they were discovered. The first class, the α-CA, was discovered in the 1930′s in vertebrates. Work characterizing plant CAs in the early 1990s recognized this second group as a new type of CA [2,3]. A third group was identified in archaebacteria in 1994 [4] and for a brief time the three protein families were referred to as the animal, plant and archaea CA forms. However, the discovery of both α- and β-CAs in *Chlamydomonas reinhardtii* and in terrestrial plants quickly showed that better terminology was needed and the Greek letters were quickly adopted [5]. The latest CA families, the η-CAs [6] and the θ-CAs [7,8], were discovered in 2015 and 2016, respectively. It should be emphasized that each protein family appears to be phylogenetically unrelated to the others unless noted. In other words, when one compares the sequence or structure of the α- and γ-CA classes, there is often little to no sequence similarity or structural similarity [1]. Even the amino acids coordinating the Zn ions in the CA active sites are not conserved. However, in some cases the active site of some CAs are quite similar. For example, ζ and θ-CAs are structurally analogous to β-CAs at the active site while the active site of δ-CAs are similar to α-CAs.

2.1. Alpha CAs

The first CA was isolated from erythrocytes and later became known as a member of the α-CA class [9,10]. The α-CA is found in plants, green algae, diatoms, cyanobacteria and animals and is distinct from all other CA classes in both protein structure and amino acid sequence. The protein structure of α-CAs is dominated by a central β-sheet consisting of ten β-strands surrounded by seven peripheral α-helices [11]. The central β-sheet houses the active site of the α-CA, coordinating the zinc atom with three histidine residues and a water molecule [11]. Historically, the α-CA is regarded as the only CA class to not form multimers, but a few recent studies report the dimerization of α-CA monomers [12–14].

* Corresponding author.
E-mail addresses: robert.dimario@wsu.edu (R.J. DiMario), mmaching@lsu.edu (M.C. Machingura), gwaldro@lsu.edu (G.L. Waldrop), btmoro@lsu.edu (J.V. Moroney).

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2.2. Beta and epsilon CAs

The first report speculating the presence of CA in plants emerged in 1936 [15] but CA activity in plants was not observed in plants until 1939 when Arthur Neish successfully measured CA activity in isolated chloroplasts and whole leaf extracts of *Trifolium pretense*, *Arctium minus*, and *Onoclea sensibilis* [16]. The discovery that the prominent chloroplast CA is distinct from the α-class CA occurred much later via protein sequence analysis, and these CAs are now known as β-CAs [2,3,17]. The β-CAs are found in plants, algae, cyanobacteria, and non-photosynthetic bacteria but are excluded from animals. The β-CA monomer is distinct from α-CAs as it is mainly composed of α-helices that surround a central β-sheet comprised of four parallel β-strands [18]. β-CA monomers oligomerize to dimers to form two active sites consisting of a zinc atom coordinated by two cysteine residues, a histidine residue, and a water molecule [18]. These dimers can further interact to form tetramers and octamers [18,19]. ε-CAs are found in cyanobacteria and are now recognized as being a highly modified β-CA [20,21]. ε-CAs not only have an enzymatic function, they also form a structural part of the carboxysome shell in cyanobacteria [20].

2.3. Gamma CAs

γ-CAs were first discovered in the archaeon *Methanosarcina thermophila* [4]. Plants [22] and photosynthetic bacteria [23,24] also contain γ-CAs, whereas no reports have emerged detailing γ-CAs in animals. The archaebacterial γ-CA possesses a zinc active site coordinated by three histidine residues and one water molecule [25]. To form the γ-CA active site, one monomer provides two zinc-coordinating histidine residues whereas a second monomer provides the third zinc-coordinating histidine residue [25]. Altogether, three monomers interact to form three active sites per homotrimer, the active γ-CA unit. The cyanobacterial protein CcmM is also a γ-CA, although modified. The N-terminus of CcmM has a γ-CA domain while the C-terminus has three to four RbcS domains allowing the protein to coordinate Rubisco packaging in the carboxysome. Espie and colleagues have shown that some CcmM proteins are enzymatically active CAs while others lack CA activity although they are still important in carboxysome packaging [26]. Those cyanobacteria with inactive CcmM proteins always have a β-CA in the carboxysome to convert HCO_3^- to CO_2 for Rubisco. In terrestrial plants, γ-CAs and γ-like CAs have been shown to be part of complex I of the mitochondria. Disruption of two γ-CA genes leads to loss of complex I. Green algae and diatoms also have γ-CAs localized to the mitochondria. However none of the CAs from eukaryotic algae or plants have been shown to have CA activity at this time.

2.4. Delta and zeta CAs

To date, δ-CAs and ζ-CAs have only been reported in diatoms and coccoliths [27,28]. The δ-CA, TWCA1 was reported in 1997 while the ζ-CA, CDCA1 was reported a few years later [29]. To date these CAs have not been found in other algae, even pennate diatoms. The Zn-binding region of CDCA1 is repeated three times and its structure was reported in 2012 [30]. An unusual aspect of the CDCA1 is its ability to use other metals besides Zn. Activity has been reported for this ζ-CA when binding others metals, most notably Cd. This ability to bind other metals might be an evolutionary adaptation to the low Zn levels often found in oceanic environments.

2.5. Eta CAs

Another new type of CA was reported in 2015. This CA, named an η-CA, was reported in the protozooan *Plasmodium falciparum* [31]. The structure of this η-CA has not been resolved yet, although the Zn coordination pattern is reported to be distantly related to that of α-CAs [32].

2.6. Theta CAs

θ-CAs are the most recent group of CAs reported. The θ class of CA has recently been described in the diatom *Phaeodactylum tricornutum* [7], the chlorophyte, *C. reinhardtii* [8] and the cyanobacterium *Chlorothece* [8]. In the diatom *P. tricornutum,* at least one of the θ-CAs, Pt43233, is localized to the thylakoid lumen [7]. Its function appears to be to catalyze the formation of CO_2 from the HCO_3^- pool in the diatom chloroplast. Since the pH of the thylakoid lumen is below the pKa of the CO_2 to HCO_3^- interconversion, this enzyme would tend to produce CO_2 from any HCO_3^- transported into the thylakoid. The loss of this protein results in a diatom growing slowly on air levels of CO_2 and showing a reduced affinity for inorganic carbon [7]. The other well-studied member of this CA family is the LCIB/LCIC complex of *C. reinhardtii* [8]. The *LCIB* gene (previously referred to as *Pmp*) encodes a θ-CA which is a chloroplast stromal protein surrounding the chloroplast pyrenoid [33]. Loss of LCIB results in a *C. reinhardtii* strain with a disabled CCM that requires high CO_2 concentrations for growth and photosynthesis [34]. LCIB/LCIC is thought to recapture CO_2 leaking out of the pyrenoid and possibly direct the HCO_3^- formed back into the pyrenoid [35].

3. CAs have a broad distribution in plants and algae

The CA enzyme is ubiquitous in nature although only certain classes of CA may be present in a particular organism. A distribution of the various CA classes is shown in Table 1. The focus of this table is to show whether a CA class is present in a group of plants or algae, but other selected model organisms are also shown for comparison. Since the sampling of many of the algal groups is limited to one or two genomes at this time, the absence of a specific class of CA in a specific lineage may not mean that all algae in that group lack that CA class. The origins of the α-, β- and γ-CA classes are ancient with all three CA types being found in bacteria, plants and many eukaryotic algal lineages. The ε-CA,

Table 1
Distribution of CAs in different lineages. One or more protein from each CA group was used to probe the genome sequences of each species using tBLASTn. Each species is only listed as having (+) or not having (−) a gene that encodes a protein related to that CA type. A species that is positive for a CA type may have multiple genes.

Taxa	Species	αCA	βCA	γCA	δCA	ζCA	θCA
Land plants	*Arabidopsis thaliana*	+	+	+	–	–	–
	Oryza sativa	+	+	+	–	–	–
Chlorophytes	*Chlamydomonas reinhardtii*	+	+	+	–	–	+
	Coccomyxa subellipsoidea	+	+	+	–	–	+
Prasinophytes	*Ostreococcus lucimarinus*	–	+	–	+	–	+
	Micromonas pusilla	–	+	+	–	+	+
Rhodophytes	*Chondrus crispus*	+	+	+	–	–	–
	Galdieria sulphuraria	+	–	+	–	–	–
Haptophyte	*Emiliania huxleyii*	+	–	+	+	–	+
Bacillariophytes	*Thalassiosira pseudonana*	+	–	+	+	+	+
	Phaeodactylum tricornutum	+	+	+	–	–	+
Phaeophyte	*Saccharina japonica*	+	+	–	–	–	–
Cryptomonad	*Guillardia theta*	+	+	+	–	–	+
Heterokont	*Aureococcus anophagefferens*	–	–	+	+	–	–
Eubacteria	*Escherichia coli*	–	+	+	–	–	–
Vertebrates	*Homo sapiens*	+	–	–	–	–	–
	Mus musculus	+	–	–	–	–	–
Insect	*Drosophila melanogaster*	+	–	–	–	–	–
	Dictyostelium discordeum AX4	–	+	+	–	–	–
Fungi	*Saccharomyces cervisiae*	–	+	–	–	–	–
Oomycete	*Aphanomyces invadans*	+	+	+	–	–	–
Cyanobacteria	*Synechocystis* sp. PCC 6803	–	+	+	–	–	–
	Cyanothece sp. PCC8801	+	+	+	–	–	+
Archaea	*Sulfolobus acidocaldarius* DSM 639	–	–	+	–	–	–

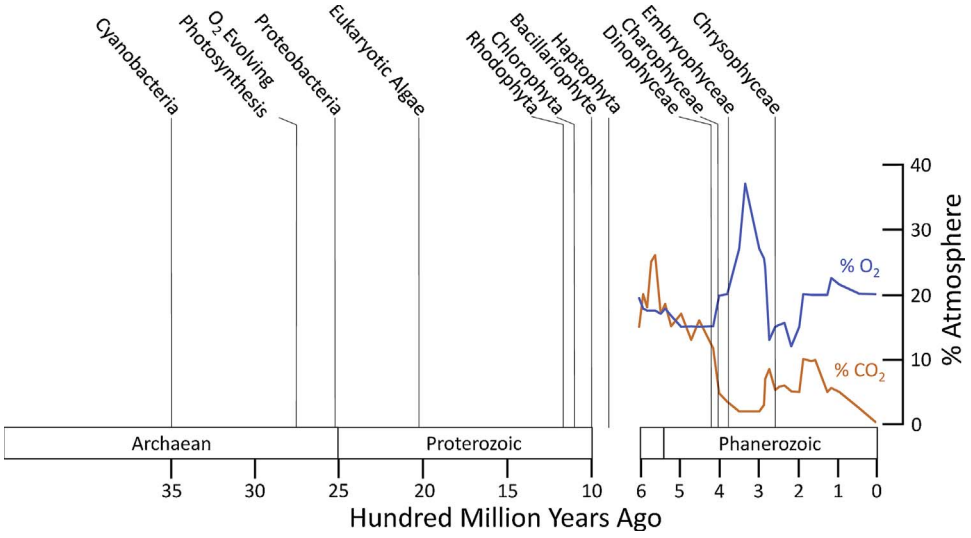

Fig. 1. The origins of different plant and algal lineages along with the historic atmospheric CO_2 and O_2 concentrations, based on Badger and Price [44] and Sage [45]. The CO_2 data are from Berner and Kothavala [43], the O_2 data from Berner [44] and the estimated ages of the algal lineages from Yoon et al. [50] and Bhattacharya et al. [51].

a variation on the β-CA class, is found in bacterial carboxysomes (Section 2.2). To date, the distribution of δ-CAs, ζ-CAs and η-CAs also appears to be more limited. It is interesting that genes encoding proteins with significant homology to the δ- and ζ-CAs can be seen in Prasinophytes, so these CAs are probably not limited to diatoms and coccoliths. In contrast, the newly discovered θ-CA class appears to be broadly distributed with paralogs exhibiting significant homology found in bacteria, cyanobacteria as well as eukaryotic algae (Table 1 and [8]).

It is important to note that the α-, β-, γ- and θ-CA classes are all found in organisms that diverged well over 1 billion years ago. The chlorophyte *C. reinhardtii* and the diatom *P. tricornutum,* diverged about 1.5 billion years ago, and both algal species have the α-, β-, γ- and θ-CA classes present. In addition, the α-, β-, γ- and θ-CA classes are all present in one or more types of bacteria, with cyanobacteria having representatives of all four CA classes. So these classes of CA are quite ancient although it is not clear why so many different CA families evolved in the first place.

The fact that there are so many classes of CAs raises the question why some organisms like vertebrates have only one type of CA (α) while photosynthetic organisms often have three or more CA families. There may be a number of reasons that many CA families have persisted in plants and algae. One reason is that a specific type of CA might be needed to interact with a larger complex. For example plant γ-CAs are part of complex 1 of the mitochondria and also play an intricate role, as CcmM, in the organization of Rubisco in the carboxysome in cyanobacteria. Another example would be CcaA, a β-CA also found in the carboxysome which has an unusual dimer of trimer structure and also interacts with CcmM [36]. It is possible that other CAs interact specifically with one or more enzymes thus ensuring that they are retained by the organism. Similarly, CAs are found in a variety of intracellular locations and in some cases bound to a specific cell membrane. For example, some of the CAs of plants and algae are found in the cell wall region. These are often α-CAs and perhaps they survive the environmental changes better than other CAs. Finally, some CAs are more active than others with α-CAs often having very high k_{cat}'s while β- and γ-CAs are less active. It is likely that many are under post-translational control as has been reported for CAH3 of *C. reinhardtii* [37].

4. Plants and many eukaryotic algae have a large number of CA isoforms

CAs have been studied in many plants and a limited number of eukaryotic algae. Plants, diatoms, and green algae all have many CA isoforms and all have α-, β-, and γ-CAs [38–41]. To date, 19 CA genes have been identified in *Arabidopsis thaliana* (8 alpha, 6 beta, and 5 gamma genes) [38,40], 19 CA genes have been identified in *C. reinhardtii* (3 alpha, 6 beta, 4 gamma, and 4 theta-CAs) [41], and the diatom *P. tricornutum* has 13 genes (5 alpha, 2 beta, 2 gamma, and 4 theta-CAs) [42]. Notably, these CA genes encode proteins that localize to many compartments within the cells of these eukaryotic organisms. Arabidopsis has CA isoforms in the plasma membrane, cytoplasm, mitochondria, and chloroplast stroma [38,40]. Chlamydomonas has isoforms in the cell wall, plasma membrane, cytosol, mitochondria, chloroplast stroma, and chloroplast thylakoid lumen [41]. Most of this work has been done using CA-GFP fusions and it has not been determined whether the CA active site is on the cytoplasmic or exoplasmic side of the membrane. *P. tricornutum* has CA isoforms in most of the same locations. In addition, some of the *P. tricornutum* CAs are in intermembrane spaces within the chloroplast as diatom chloroplasts are surrounded by four sets of membranes instead of two [7,42]. While the CA isoforms of other algae have not been localized, a check of the available genomes indicates they also have a similarly large number of CA genes and these genes likely encode CAs that go to many locations within the cell.

5. CAs may have taken on new roles when the atmospheric CO_2 concentration decreased

The Earth's atmospheric CO_2 level decreased significantly starting around 400 million years ago and then decreased to new low levels during the past 50 million years (Fig. 1) [43–46]. This decrease in CO_2 occurred well after most of the algal lineages listed in Table 1 diverged [47–51]. Therefore, terrestrial plants as well as the different types of algae had to independently adapt to the changes in the Earth's atmospheric composition. Evidence for this independent adaption comes from the different types of CCMs and how the various CAs are used in the CCMs of different algae. For example, the α-CAs CAH3 [52] and CAH1 [53], appear to be responsible for the dehydration of HCO_3^- inside the lumen of the thylakoid in *C. reinhardtii* and *Nannochloropsis oceanica*, respectively. In contrast, the θ-CA Pt43233 is localized to the thylakoid lumen of *P. tricornutum* [7] and is carrying out the same role as the α-CAs CAH3 and CAH1, namely supplying CO_2 from accumulated HCO_3^- thereby increasing the CO_2 supply for Rubisco.

This is similar to what is seen with C_4 plants, although perhaps on a longer timescale. There is good evidence that C_4 photosynthesis has evolved independently at least 60 times in the past 40 million years [45,54]. Algal CCMs may have evolved even earlier as algae have dealt with perhaps two low CO_2 events over the past 400 million years (Fig. 1). In addition, since CO_2 diffuses 10,000 times slower in water

Table 2
There are multiple enzymes in plant cells that use either CO_2 or HCO_3^- to carboxylate their substrate. The measured K_m (CO_2 or HCO_3^-) for the enzymes is given as values in the mM range.

Pathway	Enzyme	Form of C_i Used	Cofactor	K_m (mM)	References
Photosynthesis	Rubisco	CO_2	None	0.009- 0.120	[66,67]
Photosynthesis (C_4)/Amino acid metabolism	Phosphoenolpyruvate Carboxylase (C_4)	HCO_3^-	Mg^{2+}	0.027–0.180	[68–72]
Fatty Acid Synthesis	Acetyl-CoA Carboxylase	HCO_3^-	Biotin	0.9–2.5	[73–75]
Leucine Catabolism	3-methyl Crotonyl-CoA Carboxylase	HCO_3^-	Biotin	0.8–2.0	[76]
Unknown	Geranoyl-CoA Carboxylase	HCO_3^-	Biotin	0.6	[76]
Urea Catabolism	Urea Amidolyase	HCO_3^-	Biotin	2.0	[77]
Pyrimidine and Arginine Synthesis	Carbamoyl Phosphate Synthase	HCO_3^-	None	1.7	[78]
Purine Synthesis	5-aminoimidazole Ribonucleotide Carboxylase	HCO_3^-	None	23.0	[79]

than air, algae have experienced a stronger selective pressure towards the use of a CCM. In light of this, perhaps it is not surprising to see different CA proteins taking on similar metabolic functions in different algal lineages as the CO_2 concentration dropped. In addition, as the atmospheric CO_2 concentration dropped, the supply of both CO_2 and HCO_3^- decreased. The result is obvious in algae as the vast majority of aquatic photosynthetic organisms possess a CCM and all of these CCMs rely on one or more CAs. However, the effect of the decreases in atmospheric CO_2 has had on HCO_3^- is less well understood. As discussed in the next section, many enzymes utilizing HCO_3^- have relatively poor affinity for HCO_3^- and the supply of HCO_3^- for these reactions might be problematic in the absence of CA activity. Finally, the current low CO_2 concentration raises the question of whether the CO_2 and HCO_3^- buffering system is actually important in plants.

5.1. CAs play essential roles in the CO_2 concentrating mechanism of algae and plants

One of the best understood roles of CA in cyanobacteria and algae is in the delivery of CO_2 to Rubisco. In cyanobacteria, a CA is always localized to the carboxysome, where it converts HCO_3^- entering the carboxysome to CO_2 for use by Rubisco. In some cyanobacteria like *Synechocccus* PCC7942, this CA is a β-CA [55], in others such as *Prochlorococcus*, it is a ε-CA [20], while in the cyanobacteria *Nostoc*, the CA is a modified γ-CA [26]. In eukaryotic algae, the CA responsible for converting accumulated HCO_3^- to CO_2 for Rubisco is found in the chloroplast. This is accomplished by an α-CA, CAH3, in *C. reinhardtii* [52]. *Nannochloropsis oceanica* also has an α-CA, CAH1 that supplies CO_2 for Rubisco from accumulated HCO_3^-. However, in *P. tricornutum*, the θ-CA, Pt43233, appears to perform the same function [7]. These proteins are all localized in the thylakoid lumen. Another protein complex, LCIB/C also plays a key role in the CCM of *C. reinhardtii*. This θ-CA-like protein is found in the stroma and has a close association with the chloroplast pyrenoid. It is thought that it may recapture CO_2 leaking out of the pyrenoid [35].

Another role of CA in the CCMs of cyanobacteria and algae is to facilitate the entry of CO_2 from the environment. These CAs are found in the cell walls or periplasmic spaces of the organism and convert HCO_3^- to CO_2 increasing the CO_2 availability to the alga. Again a variety of CAs are found in the walls of different algae. In *C. reinhardtii*, α-CAs are found in the cell wall, helping deliver C_i across the plasma membrane [41,56–58]. In some species of diatoms, β-CAs are found in the cell walls, while in other species the cell wall CAs are of the δ and/or ζ variety [59,60].

In terrestrial C_4 plants, a β-type CA in the cytosol of leaf mesophyll cells converts incoming CO_2 to HCO_3^- to be used by PEP carboxylase [61] . The role of CA in terrestrial C_3 plants is less understood seemingly due to the compensatory effect of multiple CA isoforms. Studies of single knockout CA lines of C_3 plants do not show apparent phenotypic responses under normal growth conditions [62–65], whereas removing multiple CAs have generated a measurable response to various environmental conditions [62–65]. Considering the large number of CA genes potentially coding for active CA proteins, it is possible that removing a single CA isoform does not reduce CA activity nor produce an observable phenotypic response because another CA may compensate for the loss of the other CA. The fact that multiple CA genes may be expressed in a certain cell type [62] or multiple CA isoforms may be present in the same subcellular location [44,64] calls for additional studies looking at multi-knockout CA lines to characterize CA functionality in plants.

5.2. As atmospheric CO_2 dropped, HCO_3^- became a limiting substrate

When we think of the role of CA in plants and algae we tend to think of photosynthesis and to a lesser extent respiration and photorespiration. To be sure, these metabolic pathways are the largest consumers and producers of CO_2 in the plant cell. However, other pathways also require C_i, usually in the form of HCO_3^-. Some of these pathways and the enzymes that use HCO_3^- are shown in Table 2.

There are seven enzymes in Table 2 that utilize HCO_3^- as a source of C_i in carboxylation reactions. All of the enzymes that have HCO_3^- as a substrate have been found in a variety of plants, except urea amidolyase, which so far has only been found in green algae [80]. While PEP carboxylase is found in the cytoplasm of plants, most of the enzymes listed in Table 2 have been shown to be in plastids or are thought to be in plastids as they have a chloroplast leader sequence. Four of the six enzymes that use HCO_3^- are biotin-dependent carboxylases [76]. The first step in biotin-dependent carboxylation reactions is the carboxylation of biotin. The second step in the reaction is the transfer of the carboxyl group from biotin to an acceptor molecule which gives rise to the name of the enzyme. For example, for acetyl-CoA carboxylase the acceptor molecule is acetyl-CoA. In all biotin-dependent carboxylases the protein entity (domain or subunit) that catalyzes the carboxylation of biotin has a three-dimensional structure that places it in the ATP grasp superfamily of enzymes [81]. Enzymes in the ATP grasp superfamily catalyze reactions involved in coupling of a carboxylate to an amino (or thiol) group. The ATP is used to activate the carboxylate for nucleophilic attack by formation of an acylphosphate intermediate (carboxyphosphate where HCO_3^- is the carboxyl group) [81]. The nucleophile for the biotin-dependent carboxylases is the N1′ of biotin. The molecular architecture of the ATP grasp superfamily of enzymes is also used for two HCO_3^- dependent carboxylases that do not use biotin as the nucleophile. Carbamoyl phosphate synthetase and 5-aminoimidazole ribonucleotide carboxylase use ammonia and a primary amine as the nucleophiles, respectively [82].

A question that arises after compiling a list of these enzymes is whether CA is required to supply HCO_3^- in high enough concentrations to efficiently run these reactions. Would the HCO_3^- concentration be

high enough in the absence of CA activity to maintain growth and viability of the cell? Photosynthetic organisms have faced very low atmospheric CO_2 concentrations for long periods of time over the past 600 million years (Fig. 1). At 200 ppm the concentration of CO_2 dissolved in water at 25 °C is approximately 6 μM. If this solution is buffered at pH 7.2-7.3, typical of the cytoplasm, the HCO_3^- concentration at equilibrium would be 60 μM. At the current atmospheric CO_2 concentration those numbers would be about double, 12 μM for CO_2 and 120 μM for HCO_3^-. Nevertheless, if one compares the expected historical concentrations of CO_2 and HCO_3^- to the $K_m(HCO_3^-)$ for the various carboxylating enzymes (Table 2), it would appear that the carboxylating enzymes were often operating below their $K_m(HCO_3^-)$ or $K_m(CO_2)$. In the absence of CA activity, the HCO_3^- supply for the reactions may be even lower. In fact, even in the presence of CA those enzymes that utilize HCO_3^- as a source of CO_2 would only be about 0.5 − 17% saturated at 120 μM HCO_3^- and, as a result, most of the catalytic power of the carboxylating enzymes (Table 2) would be wasted. Again, the situation would be worse in the absence of CA. It is important to note that the low degree of saturation with HCO_3^- is particularly acute for the two enzymes involved in nucleotide synthesis, an essential pathway for all organisms. At a concentration of 120 μM HCO_3^-, 5-aminoimidazole ribonucleotide carboxylase and carbamoyl phosphate synthetase would only be 0.5 and 6.6% saturated with HCO_3^-, respectively. Therefore, CA could play a significant role in supplying the HCO_3^- needed for these reactions, resulting in increased enzymatic activity and ultimately more robust cellular growth.

In the case of Rubisco, which is clearly operating at very poor efficiency, plants and algae have evolved ways to increase the CO_2 concentration to maintain photosynthesis. The fact that most aquatic photosynthetic organism have a CCM and that C4 photosynthesis has independently arisen over 60 times strongly supports the idea that the low atmospheric CO_2 level put plants and algae under enormous selective pressure. What about the other carboxylating enzymes in plants and algae? Part of the answer to that question might be found in the study of yeast and *Escherichia coli* CA knockout strains.

Yeast has one known gene NCE103, encoding a CA. This CA has 60% sequence similarity to the β-type CAs in plants [83,84]. While the gene is nuclear encoded, the protein was first localized to the cytosol [85] where it has been reported to co-localize with several carboxylating enzymes. However, a recent proteomic study localized this protein to the intermembrane space (IMS) of the mitochondria [86]. Analysis of the N-terminal sequence of the protein using a prediction program (YLOC) reveals no mitochondrial target sequence, which is consistent with a targeting to the IMS or to the cytosol. If the single CA gene in yeast is mutated, the mutant (*Δnce103*) cannot grow on air levels of CO_2, a phenotype rescued by growing the cultures on elevated CO_2 [83,84,87]. Further studies with this *Δnce103* mutant confirmed that the basis for this phenotype was that the CA provided HCO_3^- for various carboxylation reactions such as those catalyzed by pyruvate carboxylase, acetyl-CoA carboxylase, and carbamoyl-phosphate synthase [84,88]. Some of these reactions are shown in Table 2. Since CO_2 can readily cross cell membranes, it appears that the CO_2 concentration in the cytosol remains fairly low when the external CO_2 concentration is at ambient levels or below, and the CO_2 generated in metabolic reactions is not sufficient to support growth.

E. coli also require a continuous supply of inorganic carbon for growth, and, like yeast, the source of HCO_3^- needed for these reactions is from CO_2 hydration catalyzed by CAs. Two genes, *Can* and *CynT* encode β-type CAs in *E. coli* [89,90]. Single knockout mutants of these two genes have provided insight into the functions of the CAs in *E. coli*. The *Can* gene is constitutively expressed and mutant cells cannot grow normally on air levels of CO_2. In contrast, the *CynT* gene is induced only during cyanate metabolism, and the mutant requires high CO_2 to grow in the presence of cyanate [90]. The growth of both mutants is poor due to inhibition of metabolic functions involving HCO_3^- [91], implying the CAs function to recapture the released CO_2, and converting it to HCO_3^- [92] . A high reversion rate has however, been reported for the *can* mutant due to expression of *CynT* [90]. As expected, if the two genes are silenced, the double mutant cannot grow on ambient CO_2. Reintroduced CAs have been able to increase the flux of HCO_3^- in *E. coli,* restoring growth on air [92]. In a complementation assay for HCO_3^- uptake, cyanobacteria transporters were also able to rescue the growth phenotype of a high CO_2 requiring mutant in *E. coli* [93], confirming the main role of the CAs is for HCO_3^- supply. That yeast and *E. coli* CA knockout strains must be grown on elevated CO_2 concentrations to survive is almost counter-intuitive considering that they both are producing CO_2 from respiration. That coupled with the loss of CA means the production of HCO_3^- is greatly reduced and it appears likely that it is the low HCO_3^- concentration that leads to the reduced growth phenotype on air.

5.3. Do CAs help control the internal pH in cells at low atmospheric CO_2?

One function often assigned to CAs is regulation of pH. Is this the case in plant cells? To some extent, plant scientists have been overly influenced by medical researchers and vertebrate physiologists regarding the importance of CO_2 and HCO_3^- in controlling pH. To be sure, CA plays an important role in buffering blood pH and probably the cytoplasm and intracellular compartments for most animals. The CO_2 partial pressure (4.7–6.0 kPa) in the blood is typically over 100 times the atmospheric CO_2 partial pressure (40 Pa). At pH 7.4, the typical pH of the blood, the HCO_3^- concentration averages about 24 mM while the dissolved CO_2 concentration is close to 1.5 mM. Thus, in the blood, CO_2 and HCO_3^- are crucial to buffering the system. Likewise, in the oceans, CO_2 and HCO_3^- also play a major role in determining the pH of the seas. Of course, the CO_2 concentration of the ocean is much lower than blood by a factor of about 100, but since the pH of seawater is almost one unit higher than blood, the HCO_3^- concentration remains in the millimolar range. In addition, there are few other ions in seawater with pKas close to 8.2 that are present in high enough concentrations to strongly buffer the ocean.

What about plant cells and freshwater algae? In those organisms the CO_2 concentration is very low, about 12 μM. At the typical pH of the cytoplasm, the HCO_3^- concentration would only be about 110 μM. Even in the chloroplast stroma, the HCO_3^- concentration would only be a bit over 1 mM at equilibrium. Thus, the buffering capacity of CO_2 and HCO_3^- is only about 1/100 of the buffering capacity of the same system in the blood. Since many metabolites of the cytoplasm and the other organelles are in the 1–10 mM range and often phosphorylated, it would appear that CO_2 and HCO_3^- are unlikely to be the major buffering metabolites in plant cells or freshwater algae. A possible exception might be fresh-water cyanobacteria which can concentrate HCO_3^- in the cytoplasm a thousand fold. However, they don't have a CA in the cytoplasm which would appear to reduce the role of HCO_3^-/CO_2 as a cytoplasmic buffer.

6. Conclusions

Progress has been made in elucidating physiological roles for CA isoforms in green algae, photosynthetic bacteria, and C_4 plants, all organisms which contain CCMs. Characterizing roles for CA in C_3 plants which lack a recognizable CCM has proven to be more difficult, albeit researchers have reported some non-photosynthetic roles for CAs in C_3 plants. Hopefully increased utilization of T-DNA libraries for Arabidopsis and new techniques such as the CRISPR- Cas-9 system can yield a new wave of CA research in C_3 plants.

A recent topic of considerable interest is the introduction of an algal or cyanobacterial CCM in higher plants to increase their photosynthetic rates. Researchers are progressing with these projects [94,95] although there is still a ways to go before an algal- or cyanobacterial-like CCM can be realized in a C_3 plant. Native CA activity in C_3 chloroplasts has the potential to disrupt the functionality of an imported algal or

cyanobacterial CCM in the same way as overexpressing human CA in the cytosol impacted the cyanobacterial CCM [96]. The pertinent solution is to remove native C_3 chloroplast CA activity to prevent futile cycling of the different C_i species [97] as most studies that reduced C_3 chloroplast CA activity displayed no negative effects in the plants. Since CAs are referred to as some of the fastest enzymes known, reducing CA activity by 90% may still yield a high enough CA activity to maintain physiological processes. Since CA activity in C_3 plants is not well understood, a greater understanding of the roles of CA in different metabolic reactions is needed before any CA isoform is removed for the implementation of a CCM. With the many CA isoforms in C_3 plants, there is great opportunity for the discovery of new roles for CA.

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References

[1] C.T. Supuran, Structure and function of carbonic anhydrases, Biochem. J. 473 (2016) 2023–2032.
[2] J.N. Burnell, M.J. Gibbs, J.G. Mason, Spinach chloroplastic carbonic anhydrase nucleotide sequence analysis of cDNA, Plant Physiol. 92 (1990) 37–40.
[3] T. Fawcett, J. Browse, M. Volokita, S. Bartlett, Spinach carbonic anhydrase primary structure deduced from the sequence of a cDNA clone, J. Biol. Chem. 265 (1990) 5414–5417.
[4] B.E. Alber, J.G. Ferry, A carbonic anhydrase from the archaeon *Methanosarcina thermophile*, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 6909–6913.
[5] D. Hewett-Emmett, R.E. Tashian, Functional diversity, conservation, and convergence in the evolution of the α-, β-, and γ-carbonic anhydrase gene families, Mol. Phylogenet. Evol. 5 (1996) 50–77.
[6] C.T. Supuran, C. Capasso, The η-class carbonic anhydrases as drug targets for antimalarial agents, Expert Opin. Ther. Targets 19 (2015) 551–563.
[7] S. Kikutani, K. Nakajima, C. Nagasato, Y. Tsuji, A. Miyatake, Y. Matsuda, Thylakoid luminal θ-carbonic anhydrase critical for growth and photosynthesis in the marine diatom *Phaeodactylum tricornutum*, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 9828–9833.
[8] S. Jin, J. Sun, T. Wunder, D. Tang, A.B. Cousins, S.K. Sze, O. Mueller-Cajar, Y. Gao, Structural insights into the LCIB protein family reveals a new group of β-carbonic anhydrases, Proc. Natl. Acad. Sci. U. S. A. (2016) 14716–14721.
[9] R. Brinkman, R. Margaria, N. Meldrum, F. Roughton, The CO_2 catalyst present in blood, J. Physiol. 75 (1932) 3–4.
[10] N. Meldrum, F. Roughton, Some properties of carbonic anhydrase, the CO 2 enzyme present in blood, J. Physiol. 75 (1932) 15.
[11] A. Liljas, S. Lovgren, P.C. Bergsten, U. Carlbom, M. Petef, I. Waara, B. Strandberg, K. Fridborg, L. Jarup, K.K. Kannan, Crystal-structure of human carbonic anhydrase-C, Nat. New Biol. 235 (1972) 131–137.
[12] D.A. Whittington, A. Waheed, B. Ulmasov, G.N. Shah, J.H. Grubb, W.S. Sly, D.W. Christianson, Crystal structure of the dimeric extracellular domain of human carbonic anhydrase XII, a bitopic membrane protein overexpressed in certain cancer tumor cells, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 9545–9550.
[13] K. Suzuki, S.-Y. Yang, S. Shimizu, E.C. Morishita, J. Jiang, F.M.M. Zhang Hoque, Y. Sato, M. Tsunoda, T. Sekiguchi, The unique structure of carbonic anhydrase CA1 from Chlamydomonas reinhardtii, Acta Crystal. Sect. D 67 (2011) 894–901.
[14] J.A. Cuesta-Seijo, M.S. Borchert, J.-C. Navarro-Poulsen, K.M. Schnorr, S.B. Mortensen, L.L. Leggio, Structure of a dimeric fungal α-type carbonic anhydrase, FEBS Lett. 585 (2011) 1042–1048.
[15] G.O. Burr, Carbonic anhydrase and photosynthesis, Proc. Royal Soc. Series B-Biol. Sci. 120 (1936) 42–47.
[16] A.C. Neish, Studies on chloroplasts. I. Separation of chloroplasts, a study of factors affecting their flocculation and the calculation of the chloroplast content of leaf tissue from chemical analysis, Biochem. J. 33 (1939) 293–299.
[17] C.A. Roeske, W.L. Ogren, Nucleotide sequence of pea cDNA encoding chloroplast carbonic anhydrase, Nucl. Acids Res. 18 (1990) 3413.
[18] M.S. Kimber, E.F. Pai, The active site architecture of *Pisum sativum* beta-carbonic anhydrase is a mirror image of that of alpha-carbonic anhydrases, EMBO J. 19 (2000) 1407–1418.
[19] R.S. Rowlett, Structure and catalytic mechanism of the β-carbonic anhydrases, Biochim. Biophys. Acta 2010 (1804) 362–373.
[20] A.K. So, G.S. Espie, E.B. Williams, J.M. Shively, S. Heinhorst, G.C. Cannon, A novel evolutionary lineage of carbonic anhydrase (epsilon class) is a component of the carboxysome shell, J. Bacteriol. 186 (2004) 623–630.
[21] M.R. Sawaya, G.C. Cannon, S. Heinhorst, S. Tanaka, E.B. Williams, T.O. Yeates, C.A. Kerfeld, The structure of beta-carbonic anhydrase from the carboxysomal shell reveals a distinct subclass with one active site for the price of two, J. Biol. Chem. 281 (2006) 7546–7555.
[22] G. Parisi, M. Perales, M.A. Fornasari Colaneri, N. Schain, D. Casati, S. Zimmermann, A. Brennicke, A. Araya, J. Ferry, Gamma carbonic anhydrases in plant mitochondria, Plant Mol. Biol. 55 (2004) 193–207.
[23] G.D. Price, S. Howitt, K. Harrison, M. Badger, Analysis of a genomic DNA region from the cyanobacterium *Synechococcus* sp. strain PCC7942 involved in carboxysome assembly and function, J. Bacteriol. 175 (1993) 2871–2879.
[24] K.L. Peña, S.E. Castel, C. de Araujo, G.S. Espie, M.S. Kimber, Structural basis of the oxidative activation of the carboxysomal gamma-carbonic anhydrase, CcmM, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 2455–2460.
[25] C. Kisker, H. Schindelin, B.E. Alber, J.G. Ferry, D.C. Rees, A left-handed beta-helix revealed by the crystal structure of a carbonic anhydrase from the archaeon *Methanosarcina thermophile*, EMBO J. 15 (1996) 2323–2330.
[26] C. De Araujo, D. Arefeen, Y. Tadesse, B.M. Long, G.D. Price, R.S. Rowlett, M.S. Kimber, G.S. Espie, Identification and characterization of a carboxysomal γ-carbonic anhydrase from the cyanobacterium *Nostoc* sp. PCC 7120, Photosyn. Res. 121 (2014) 135–150.
[27] S.B. Roberts, T.W. Lane, F.M.M. Morel, Carbonic anhydrase in the marine diatom *Thalassiosira weissflogii* (Bacillariophyceae), J. Phycol. 33 (1997) 845–850.
[28] A.R. Sotoj, H. Zheng, D. Shoemaker, J. Rodriguez, B.A. Read, T.M. Wahlund, Identification and preliminary characterization of two cDNAsencoding unique carbonic anhydrases from the marine alga *Emiliania huxleyi*, Appl. Environ. Microbiol. 72 (2006) 5500–5511.
[29] Y. Xu, L. Feng, P.D. Jeffrey, Y. Shi, F.M.M. Morel, Structure and metal exchange in the cadmium carbonic anhydrase of marine diatoms, Nature 452 (2008) 56–61.
[30] V. Alterio, E. Langella, F. Viparelli, D. Vullo, G. Ascione, N.A. Dathan, F.M. Morel, C.T. Supuran, G. De Simone, S.M. Monti, Structural and inhibition insights into carbonic anhydrase CDCA1 from the marine diatom *Thalassiosira weissflogii*, Biochimie 94 (2012) 1232–1241.
[31] S. Del Prete, D. Vullo, G.M. Fisher, K.T. Andrews, S.A. Poulsen, C. Capasso, C.T. Supuran, Discovery of a new family of carbonic anhydrases in the malaria pathogen *Plasmodium falciparum* –the η-carbonic anhydrases, Bioorg. Med. Chem. Lett. 24 (2014) 4389–4396.
[32] G. De Simone, A. Di Fiore, C. Capasso, C.T. Supuran, The zinc coordination pattern in the η-carbonic anhydrase from *Plasmodium falciparum* is different from all other carbonic anhydrase genetic families, Bioorg. Med. Chem. Lett. 25 (2015) 1385–1389.
[33] T. Yamano, T. Tsujikawa, K. Hatano, S. Ozawa, Y. Takahashi, H. Fukuzawa, Light and Low-CO_2-dependent LCIB-LCIC complex localization in the chloroplast supports the carbon-concentrating mechanism in *Chlamydomonas reinhardtii*, Plant Cell Physiol. 51 (2010) 1453–1468.
[34] Y.J. Wang, M.H. Spalding, An inorganic carbon transport system responsible for acclimation specific to air levels Of CO_2 in *Chlamydomonas reinhardtii*, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 10110–10115.
[35] Y.J. Wang, M.H. Spalding, Acclimation to very low CO_2: contribution of limiting CO_2 inducible proteins, LCIB and LCIA, to inorganic carbon uptake in *Chlamydomonas reinhardtii*, Plant Physiol. 166 (2014) 2040–2050.
[36] L.D. McGurn, M. Moazami-Goudarzi, S.A. White, T. Suwal, B. Brar, J.Q. Tang, G.S. Espie, M.S. Kimber, The structure, kinetics and interactions of the β-carboxysomal β-carbonic anhydrase, CcaA Biochem. J. 473 (2016) 4559–4572.
[37] A. Blanco-Rivero, T. Shutova, M.J. Román, A. Villarejo, F. Martinez, Phosphorylation Controls the Localization and Activation of the Lumenal Carbonic Anhydrase in *Chlamydomonas reinhardtii*, PLoS One (2012), http://dx.doi.org/10.1371/journal.pone.0049063.
[38] R.J. DiMario, H. Clayton, A. Mukherjee, M. Ludwig, J.V. Moroney, Plant carbonic anhydrases: structures, locations, evolution, and physiological roles, Mol. Plant 10 (2017) 30–46.
[39] J.V. Moroney, S.G. Bartlett, G. Samuelsson, Carbonic anhydrases in plants and algae, Plant Cell Environ. 24 (2001) 141–153.
[40] N. Fabre, I.M. Reiter, N. Becuwe-Linka, B. Genty, D. Rumeau, Characterization expression analysis of genes encoding alpha and beta carbonic anhydrases in Arabidopsis, Plant Cell Environ. 30 (2007) 617–629.
[41] J.V. Moroney, Y. Ma, W.D. Frey, K.A. Fusilier, T.T. Pham, T.A. Simms, R.J. DiMario, J. Yang, B. Mukherjee, The carbonic anhydrase isoforms of *Chlamydomonas reinhardtii*: intracellular location, expression, and physiological roles, Photosyn. Res. 109 (2011) 133–149.
[42] Y. Tsuji, K. Nakajima, Y. Matsuda, Molecular aspects of the biophysical CO_2-concentrating mechanism and its regulation in marine diatoms, J. Exp. Bot. 68 (2017) 3949–3958, http://dx.doi.org/10.1093/jxb/erx173.
[43] R.A. Berner, Z. Kothavala, GEOCARB III: a revised model of atmospheric CO_2 over Phanerozoic time, Am. J. Sci. 301 (2001) 184–204.
[44] M.R. Badger, G.D. Price, CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution, J. Exp. Bot. 54 (2003) 609–622.
[45] R.F. Sage, The evolution of C_4 photosynthesis, New Phytol. 161 (2004) 341–370.
[46] R.A. Berner, Modeling atmospheric O_2 over phanerozoic time, Geochim. Cosmochim. Acta 65 (2001) 685–694.
[47] C.E. Blank, Origin and early evolution of photosynthetic eukaryotes infreshwater environments: reinterpreting proterozoic paleobiology andbiogeochemical processes in light of trait evolution, J. Phycol. 49 (2013) 1040–1055.
[48] L.W. Parfrey, D.J. Lahr, A.H. Knoll, L.A. Katz, Estimating the timing of early

eukaryotic diversification with multigenemolecular clocks, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 13624–13629.
[49] H.S. Yoon, J.D. Hackett, C. Ciniglia, G. Pinto, D. Bhattacharya, A molecular timeline for the origin of photosynthetic eukaryotes, Mol. Biol. Evol. 21 (2004) 809–818.
[50] S.B. Hedges, J.E. Blair, M.L. Venturi, J.L. Shoe, A molecular timescale of eukaryote evolution and the rise of complexmulticellular life, BMC Evol. Biol. 4 (2) (2004).
[51] D. Bhattacharya, J.M. Archibald, A.P. Weber, A. Reyes-Prieto, How do endosymbionts become organelles? Understanding early events in plastid evolution, Bioessays 29 (2007) 1239–1246.
[52] J. Karlsson, A.K. Clarke, Z.-Y. Chen, S.Y. Hugghins, Y.-I. Park, H.D. Husic, J.V. Moroney, G. Samuelsson, A novel α-type carbonic anhydrase associated with the thylakoid membrane in *Chlamydomonas reinhardtii* is required for growth at ambient CO_2, EMBO J. 17 (1998) 1208–1216.
[53] C.W. Gee, K.K. Niyogi, The carbonic anhydrase CAH1 is an essential component of the carbon-concentrating mechanism in *Nannochloropsis oceanica*, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 4537–4542.
[54] R.F. Sage, T.L. Sage, F. Kocacinar, Photorespiration and the evolution of C-4 photosynthesis, Ann. Rev. Plant Biol. 63 (2012) 19–47.
[55] J.W. Yu, G.D. Price, L. Song, M.R. Badger, Isolation of a putative carboxysomal carbonic anhydrase gene from the cyanobacterium *Synechococcus* PCC7942, Plant Physiol. 100 (1992) 794–800.
[56] S. Fujiwara, H. Fukuzawa, A. Tachiki, S. Miyachi, Structure and Differential Expression of 2 Genes Encoding Carbonic Anhydrase in *Chlamydomonas reinhardtii*, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 9779–9783.
[57] M. Rawat, J.V. Moroney, Partial characterization of a new isoenzyme of carbonic anhydrase isolated from *Chlamydomonas reinhardtii*, J. Biol. Chem. 266 (1991) 9719–9723.
[58] A. Tachiki, H. Fukuzawa, S. Miyachi, Characterization of Carbonic Anhydrase Isozyme CA2, Which Is the CAH2 Gene Product, in *Chlamydomonas reinhardtii* Biosci, Biotechnol. Biochem. 56 (1992) 794–798.
[59] B.M. Hopkinson, C. Meile, C. Shen, Quantification of extracellular carbonic anhydrase activity in two marine diatoms and investigation of its role, Plant Physiol. 162 (2013) 1142–1152.
[60] M. Lapointe, T.D.B. MacKenzie, D. Morse, An external δ-carbonic anhydrase in a free-living marine dinoflagellate may circumvent diffusion-limited carbon acquisition, Plant Physiol. 147 (2008) 1427–1436.
[61] S. von Caemmerer, V. Quinn, N.C. Hancock, G.D. Price, R.T. Furbank, M. Ludwig, Carbonic anhydrase and C_4 photosynthesis: a transgenic analysis, Plant Cell Environ. 27 (2004) 697–703.
[62] H.H. Hu, A. Boisson-Dernier, M. Israelsson-Nordstrom, M. Bohmer, S.W. Xue, A. Ries, J. Godoski, J.M. Kuhn, J.I. Schroeder, Carbonic anhydrases are upstream regulators of CO_2-controlled stomatal movements in guard cells, Nat. Cell Biol. 12 (2010) 87–93.
[63] H. Hu, W.-J. Rappel, R. Occhipinti, A. Ries, M. Böhmer, L. You, C. Xiao, C.B. Engineer, W.F. Boron, J.I. Schroeder, Cellular locations of carbonic anhydrases mediate CO_2 control of stomatal movements, Plant Physiol. 169 (2015) 1168–1178.
[64] R.J. DiMario, J.C. Quebedeaux, D.J. Longstreth, M. Dassanayake, M.M. Hartmann, J.V. Moroney, The cytoplasmic carbonic anhydrases beta CA2 and beta CA4 are required for optimal plant growth at low CO_2, Plant Physiol. 171 (2016) 280–293.
[65] J. Huang, Z. Li, G. Biener, E. Xiong, S. Malik, N. Eaton, C.Z. Zhao, V. Raicu, H. Kong, D.D. Zhao, Carbonic anhydrases function in anther cell differentiation downstream of the receptor-like kinase EMS, Plant Cell 29 (2017) 1335–1356.
[66] D.B. Jordan, W.L. Ogren, Species variation in the specificity of ribulose-bisphosphate carboxylase-oxygenase, Nature 291 (1981) 513–515.
[67] D.J. Orr, A. Alcantara, M.V. Karpralov, P.J. Andralojc, E. Carmo-Silva, M.A.J. Parry, Surveying rubisco diversity and temperature response to improve crop photosynthetic efficiency, Plant Physiol. 172 (2016) 707–717.
[68] P.H. Reibach, C.R. Benedict, Fractionation of stable carbon isotopes by phosphoenolpyruvate carboxylase from C_4 plants, Plant Physiol. 59 (1977) 564–568.
[69] H. Bauwe, An efficient method for the determination of K_m values for HCO_3^- of phosphoenolpyruvate carboxylase, Planta 169 (1986) 356–360.
[70] J.W. Janc, M.H. Oleary, W.W. Cleland, A kinetic investigation of phosphoenolpyruvate carboxylase from *Zea-mays*, Biochemistry 31 (1992) 6421–6426.
[71] K. Parvathi, A.S. Bhagwat, Y. Ueno, K. Izui, A.S. Raghavendra, Illumination increases the affinity of phosphoenolpyruvate carboxylase to bicarbonate in leaves of a C-4 plant, *Amaranthus hypochondriacus*, Plant Cell Physiol. 41 (2000) 905–910.
[72] R.A. Boyd, A. Gandin, A.B. Cousins, Temperature responses of C-4 photosynthesis: biochemical analysis of rubisco, phosphoenolpyruvate carboxylase, and carbonic anhydrase in *Setaria viridis*, Plant Physiol. 169 (2015) 1850–1861.
[73] B.J. Nikolau, J.C. Hawke, Purification and characterization of maize leaf acetyl-coenzyme a carboxylase, Arch. Biochem. Biophys. 228 (1984) 86–96.
[74] C. Alban, P. Baldet, R. Douce, Localization and characterization of two structurally different forms of acetyl-CoA carboxylase in young pea leaves, of which one is sensitive to aryloxyphenoxypropionate herbicides, Biochem. J. 300 (1994) 557–565.
[75] D. Herbert, C. L.J.Price, L. Alban, D. Dehaye, D.J. Job, K.E. Cole, J.L. Pallett, J.L. Harwood, Kinetic studies on two isoforms of acetyl-CoA carboxylase from maize leaves, Biochem. J. 318 (1996) 997–1006.
[76] B.J. Nikolau, J.B. Ohlrogge, E.S. Wurtele, Plant biotin-containing carboxylases, Arch. Biochem Biophys. 414 (2003) 211–222.
[77] S. Kimura, H. Yamanishi, S. Iyama, Y. Yamaguchi, Y. Kanakura, Enzymatic assay for determination of bicarbonate ion in plasma using urea amidolyase, Clin. Chim. Acta 328 (2003) 179–184.
[78] F. Javid-Majd, M.A. Stapleton, M.F. Harmon, B.A. Hanks, L.S. Mullins, F.M. Raushel, Comparison of the functional differences for the homologous residues within the carboxy phosphate and carbamate domains of carbamoyl phosphate synthetase, Biochemistry 35 (1996) 14362–14369.
[79] S.M. Firestine, V.J. Davisson, Carboxylases in de novo purine biosynthesis. characterization of the *Gallus gallus* bifunctional enzyme, Biochemistry 33 (1994) 11917–11926.
[80] P.K. Strope, K.W. Nickerson, S.D. Harris, E.N. Moriyama, Molecular evolution of urea amidolyase and urea carboxylase in fungi, BMC Evol. Biol. 11 (2011) 80.
[81] G.L. Waldrop, H.M. Holden, M. St Maurice, The enzymes of biotin dependent CO_2 metabolism: what structures reveal about their reaction mechanisms, Protein Sci. 21 (2012) 1597–1619.
[82] R. Zrenner, M. Stitt, U. Sonnewald, R. Boldt, Pyrimidine and purine biosynthesis and degradation in plants, Ann. Rev. Plant Biol. 57 (2006) 805–836.
[83] R. Götz, A. Gnann, F.K. Zimmermann, Deletion of the carbonic anhydrase-like gene NCE103 of the yeast *Saccharomyces cerevisiae* causes an oxygen-sensitive growth defect, Yeast 15 (1999) 855–864.
[84] R. Lehneck, S. Pöggeler, A matter of structure: structural comparison of fungal carbonic anhydrases, Appl. Microbiol. Biotechnol. 98 (2014) 8433–8441.
[85] A. Kumar, S. Agarwal, J.A. Heyman, S. Matson, M. Heidtman, S. Piccirillo, L. Umansky, A. Drawid, R. Jansen, Y. Liu, K.H. Cheung, P. Miller, M. Gerstein, G.S. Roeder, M. Snyder, Subcellular localization of the yeast proteome, Genes Dev. 16 (2002) 707–719.
[86] F.-N. Vögtle, J.M. Burkhart, S. Rao, C. Gerbeth, J. Hinrichs, J.-C. Martinou, A. Chacinska, A. Sickmann, R.P. Zahedi, C. Meisinger, Intermembrane space proteome of yeast mitochondria, Mol. Cell. Proteomics 11 (2012) 1840–1852.
[87] G. Amoroso, L. Morell-Avrahov, D. Müller, K. Klug, D. Sültemeyer, The gene NCE103 (YNL036w) from *Saccharomyces cerevisiae* encodes a functional carbonic anhydrase and its transcription is regulated by the concentration of inorganic carbon in the medium, Mol. Microbiol. 56 (2005) 549–558.
[88] J. Aguilera, J.P. Van Dijken, J.H. De Winde, J.T. Pronk, Carbonic anhydrase (Nce103p): an essential biosynthetic enzyme for growth of *Saccharomyces cerevisiae* at atmospheric carbon dioxide pressure, Biochem. J. 391 (2005) 311–316.
[89] B. Kusian, D. Sültemeyer, B. Bowien, Carbonic anhydrase is essential for growth of *Ralstonia eutropha* at ambient CO_2 concentrations, J. Bacteriol. 184 (2002) 5018–5026.
[90] C. Merlin, M. Masters, S. McAteer, A. Coulson, Why is carbonic anhydrase essential to *Escherichia coli*? J. Bacteriol. 185 (2003) 6415–6424.
[91] S. Isik, F. Kockar, O. Arslan, O.O. Guler, A. Innocenti, C.T. Supuran, Carbonic anhydrase inhibitors. Inhibition of the β-class enzyme from the yeast *Saccharomyces cerevisiae* with anions, Med. Chem. Letts. 18 (2008) 6327–6331.
[92] S. Park, J.-U. Lee, S. Cho, H. Kim, H.B. Oh, S.P. Pack, J. Lee, Increased incorporation of gaseous CO_2 into succinate by *Escherichia coli* overexpressing carbonic anhydrase and phosphoenolpyruvate carboxylase genes, J. Biotechnol. 241 (2017) 101–107.
[93] J. Du, B. Förster, L. Rourke, SM, G.D. Howitt, Price characterisation of cyanobacterial bicarbonate transporters in *E. coli* shows that SbtA homologs are functional in this heterologous expression system, PLoS One 9 (2014) e115905.
[94] J.J.L. Pengelly, B. Forster, S. von Caemmerer, M.R. Badger, G.D. Price, S.M. Whitney, Transplastomic integration of a cyanobacterial bicarbonate transporter into tobacco chloroplasts, J. Exp. Bot. 65 (2014) 3071–3080.
[95] M.T. Lin, A. Occhialini, P.J. Andralojc, J. Devonshire, K.M. Hines, M.A.J. Parry, M.R. Hanson, Beta-carboxysomal proteins assemble into highly organized structures in Nicotiana chloroplasts, Plant J. 79 (2014) 1–12.
[96] G.D. Price, M. Badger, Expression of human carbonic anhydrase in the cyanobacterium synechococcus PCC7942 creates a high CO_2-requiring phenotype evidence for a central role for carboxysomes in the CO_2 concentrating mechanism, Plant Physiol. 91 (1989) 505–513.
[97] J.M. McGrath, S.P. Long, Can the cyanobacterial carbon-concentrating mechanism increase photosynthesis in crop species? A theoretical analysis, Plant Physiol. 164 (2014) 2247–2261.