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Review

A basal carbon concentrating mechanism in plants?

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ABSTRACT

Many photosynthetic organisms have developed inorganic carbon (Ci) concentrating mechanisms (CCMs) that increase the CO_2 concentration within the vicinity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). Several CCMs, such as four carbon (C4) and crassulacean acid metabolism (CAM), bicarbonate accumulation systems and capsular structures around RubisCO have been described in great detail. These systems are believed to have evolved several times as mechanisms that acclimate organisms to unfavourable growth conditions. Based on recent experimental evidence we propose the occurrence of another more general CCM system present in all plants. This basal CCM (bCCM) is supposed to be composed of mitochondrial carbonic anhydrases (a β-type carbonic anhydrase and the γ-type carbonic anhydrase domain of the mitochondrial NADH dehydrogenase complex) and probably further unknown components. The bCCM is proposed to reduce leakage of CO_2 from plant cells and allow efficient recycling of mitochondrial CO_2 for carbon fixation in chloroplasts.

Contents

1. Introduction

Carboxylation of ribulose-1,5-bisphosphate by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO, E.C. 4.1.1.39) is the main source of organic carbon for most living organisms. However, due to a side activity of RubisCO, oxygenation of ribulose-1,5-bisphosphate also can take place. The ratio of the carboxylation and oxygenation reactions catalysed by RubisCO depends on the CO_2 and O_2 concentration in the vicinity of the enzyme [1]. On a global scale, one out of four reactions catalysed by RubisCO leads to oxygenation of ribulose-1,5-bisphosphate. However, oxygenation can be much higher under certain conditions, for example high temperatures or aridity. As a consequence of the oxygenation of ribulose-1,5-bisphosphate, one molecule of 3-phosphoglycerate and one of 2-phosphoglycolate are formed in chloroplasts.

Since 2-phosphoglycolate represents a potent inhibitor of photosynthesis, it has to be converted into other compounds. This mainly takes place by the photorespiration pathway: 2-phosphoglycolate is first dephosphorylated in the chloroplast, and

Abbreviations: C3, three carbon; C4, four carbon; CA, carbonic anhydrase; CAL, carbonic anhydrase like; Cam, carbonic anhydrase of *Methanosarcina* spp.; CAM, crassulacean acid metabolism; CCM, carbon concentrating mechanism; Ci, inorganic carbon; CMS, cytoplasmic male sterility; DiT, dicarboxylic acid translocase; EZA, ethoxzolamide; gcvT, cyanobacterial plant-like glycine decarboxylase; glc, cyanobacterial glycolate dehydrogenase; HCR, high carbon requiring; HPR, hydroxypyruvate reductase; LCI, limiting-CO_2-inducible; odc, cyanobacterial oxalate decarboxylase; PEP, phosphoenolpyruvate; RubisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; SSU, small subunit; SHMT, serine hydromethyl transferase; TCA, tricarboxylic acid cycle; tsr, cyanobacterial tartronic semialdehyde reductase.

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the resulting glycolate is subsequently transported into the peroxisome where it is oxidised to glyoxylate. This reaction is linked to hydrogen peroxide (H_2O_2) formation, which is later detoxified by a peroxisomal catalase. Subsequently, glyoxylate is aminated into glycine which is then transported into the mitochondrion. In mitochondria, two molecules of glycine are converted into one molecule of serine by glycine decarboxylase (E.C. 2.1.2.10) and serine hydromethyl transferase (E.C. 2.1.2.1-SHMT) enzymes. This conversion is linked to reduction of NAD^+ and liberation of CO_2 and NH_4^+. Based on this conversion, three out of four carbon atoms are recovered for primary carbon metabolism. Next, the serine formed in mitochondria is transported back into the peroxisome where it is deaminated to form hydroxypyruvate which is subsequently reduced to glycerate by hydroxypyruvate reductase (E.C. 1.1.1.81-HPR). A second but cytosolic HPR was recently discovered and proposed to allow a bypass of this part of photorespiration pathway. This enzyme was also observed to be important in the context of a metabolic overflow protection mechanism [2]. Finally, glycerate is phosphorylated by glycerate kinase (E.C. 2.7.1.31) in the chloroplast to form 3-PGA, which can be fed back into the Calvin cycle. Because the whole metabolic pathway only takes place in the light and leads to liberation of CO_2 it is designated "photo-respiration". The pathway of course co-exists with classical respiration (termed "dark respiration" in plants). Due to the oxygenase activity of RubisCO, it is estimated that C3 plants such as wheat or rice only could fix about 55% CO_2 of what they theoretically could fix if RubisCO was solely a carboxylase. However, indirect measurements of photorespiration indicate a reduction in Ci assimilation within the range of 25–35% under present atmospheric conditions [3].

The CO_2 concentration surrounding RubisCO is crucial for proper carbon fixation to sustain plant growth and ultimately all life on earth. During evolution, especially during periods of low CO_2 in the atmosphere, different biochemical and even anatomical strategies have independently emerged that overcome limiting carbon fixation that are designated as inorganic "carbon concentrating mechanisms" (CCMs).

In addition to the previously described carbon concentrating mechanisms, we here propose, based on recent experimental findings, that all eukaryotic photosynthetic organisms contain a basal CCM (bCCM). The proposed bCCM allows recycling of mitochondrial CO_2 for carbon fixation in chloroplasts. Besides CO_2 diffusion between mitochondria and chloroplasts, we propose an active CO_2 transfer mechanism based on the presence of carbonic anhydrases (E.C. 4.2.1.1) and bicarbonate transporters in the two involved organelles. Carbonic anhydrases are Zn-metalloenzymes that catalyse the reversible hydration of carbon dioxide into bicarbonate. There are five types of enzymes (α, β, γ, δ and ε) which are not evolutionary related and represent a case of convergent evolution. We propose that this basal CCM system probably evolved from endosymbionts (α proteobacteria) during the process of chloroplast adaptation in the modern green eukaryotic cell. In this review, the previously described CCM systems are briefly introduced and compared and experimental evidence for a bCCM is summarised.

2. Types of CCMs

2.1. C4 and CAM mechanisms

Four-carbon (C4) photosynthesis and crassulacean acid metabolism (CAM) in terrestrial higher plants were the first photosynthetic CCM to be described in detail. Both systems are based on a carbon pre-fixation step (binding of bicarbonate to phosphoenolpyruvate [PEP] by PEP carboxylase – E.C. 4.1.1.31). The carbon pre-fixation and the final CO_2 fixation steps by RubisCO are spatially (C4) or temporally (CAM) separated.

In CAM plants, which often grow in very hot and dry locations, oxaloacetate is formed by PEP carboxylation at night when stomata are open and allow gas exchange. Oxaloacetate is subsequently converted into malate, which can be stored in the vacuole. When stomata are closed during the day preventing the loss of water, malate is decarboxylated, providing CO_2 for final carbon fixation by the Calvin cycle [4].

In C4 plants, PEP carboxylation takes place within specialised mesophyll cells that also carry out the complete light reaction of photosynthesis. Malate or aspartate is transported from mesophyll cells to another type of leaf cells termed bundle sheath cells (Kranz anatomy). At this location the pre-fixed carbon is released for final CO_2 fixation by RubisCO. Due to this process, the CO_2 concentration in the bundle-sheath cells is approximately 10-fold higher than in normal air. Thereby, photorespiration is much reduced. Simultaneously, most bundle sheath cells do not carry out the complete photosynthetic light reaction [1].

The primary CO_2 fixation step catalysed by PEP carboxylase takes place in the cytosol of mesophyll cells using HCO_3^- as a substrate. Therefore, carbon dioxide coming from the external surroundings must be rapidly hydrated by a β type carbonic anhydrase (βCA) and converted into HCO_3^-. Thus, carbonic anhydrase activity is mainly found in the cytosol of mesophyll cells whereas in C3 plants, the highest carbonic anhydrase activity is detected within the chloroplast stroma [5].

C4 metabolism has been observed in several important crop species such as maize, sorghum and sugar cane [6] and also in submerged aquatic plants and macroalgae such as *Udotea* spp. [7] or the planktonic diatom *Thalassiosira weissflogii* [8,9] whereas CAM-like metabolism has been found mainly in terrestrial xerophytic species such as cacti, yucca and aloe but also in many aquatic plants and brown macroalgae [4,10]. In some species of *Flaveria* spp. and in *Heliotropium* spp. intermediate "C3–C4 metabolism" takes place [11]. Half of the species of *Flaveria* express intermediate traits between C3 and C4-like forms [12]. Some C3–C4 intermediate species restrict glycine decarboxylation to the bundle sheath compartment, thereby concentrating CO_2 only in this cell type [11]. This process was also named 'C2 photosynthesis' because the decarboxylation of photorespiratory metabolites concentrates CO_2 around RubisCO, thereby increasing photosynthetic efficiency. Some genera only have a small number of confirmed C3–C4 intermediate species (*Brassica*, *Alternanthera*, *Parthenium*, *Neurachne*, *Salsola*, *Cleomaceae*) [11]. Interestingly, certain amphibious species of *Eleocharis*, particularly *Eleocharis vivipara*, express C4 and C3 characteristics under terrestrial and submerged aquatic conditions, respectively [13].

Three species in the Chenopodiaceae family perform single-cell C4 photosynthesis without Kranz anatomy [14,15]. These species have two chloroplast types (dimorphic chloroplasts), which are biochemically and morphologically different. Each type is located in a distinct cytoplasmic domain within individual photosynthetic cells. Single cell C4 metabolism highly resembles C4 metabolism in C4 plants with Kranz-type leaf anatomy [16]. This metabolism also is based on a CO_2 pre-fixation step catalysed by PEP carboxylase, the occurrence of separate photosynthetic compartments domains and physiological responses typical of C4 plants.

Photorespiration is much reduced in C4 plants compared to C3 plants. However, photorespiration is an essential metabolic process because mutants affecting enzymes of the 2-phosphoglycolate metabolism, e.g. glycolate oxidase (E.C. 1.1.3.15) of maize, are not viable in normal air but completely rescued at high CO_2 conditions. A maize *gox1* mutant rapidly accumulates glycolate when transferred to normal air, which greatly decreases the efficiency of net carbon assimilation. The existence of such kind of mutants indicates

that a functional photorespiratory pathway is essential for maize seedling development, most likely for detoxification of glycolate [17,18].

2.2. CCM in Chlamydomonas

Eukaryotic green algae such *Chlamydomonas* spp. have a similar but inducible CCM in which bicarbonate is concentrated in the chloroplast stroma via several Ci transporters and α and βCAs. RubisCO is located in a specialised micro-compartment, the "pyrenoid", where Ci fixation occurs [19].

An αCA, CAH3, is localised in the thylakoid lumen and is enriched in tubules that penetrate the pyrenoid. This enzyme plays an essential role in the rapid dehydration of the accumulated HCO_3^- and thereby releases CO_2 into the pyrenoid [20]. Mutants lacking CAH3 have a non-functional CCM in which HCO_3^- accumulates intracellularly. However, the mutant cells cannot grow at low levels of CO_2 [21]. Furthermore, limiting-CO_2-inducible (LCI) B and C proteins are involved in CO_2 metabolism. These proteins form a complex of 350 kDa localised around the pyrenoid in the light [22] and were initially thought to represent Ci transporters [23,24]. However, upon genetic analyses, these proteins were proposed to trap CO_2 released by CAH3, thereby reducing CO_2 leakage [22,25]. These gene products are members of a small gene family that, to date, have only been found in a few microalgae species [26]. Mutants with defects in B gene expression cannot grow in normal air but survive at very low CO_2 indicating the existence of multiple Ci transporters in different CO_2 conditions [23,25,27]. In green algae, there is considerable evidence of a role in carbon capture of the plastid envelope via the active transport of Ci. Photosynthetically active chloroplasts as well as intact cells grown at high or low CO_2 have low- or high-affinity Ci uptake systems, respectively [27].

Based on mathematical modelling of *Chlamydomonas* metabolism, it was proposed that an additional mitochondrial βCA could be involved in converting CO_2 from the TCA cycle or derived from photorespiration into HCO_3^- which is transported via anaplerotic reactions (i.e. PEP carboxylase reaction to form four carbon acids) back into chloroplasts for carbon fixation by RubisCO, thereby potentially limiting CO_2 leakage from mitochondria [28]. Such a role requires the presence of a bicarbonate translocase in the inner membrane of the mitochondrion, which has not yet been identified. This bicarbonate translocase would allow a controlled carbon efflux to the cytosol.

2.3. CCM in cyanobacteria

The cyanobacterial CCM is based on a system that concentrates HCO_3^- in the matrix using a series of carbonic anhydrases and HCO_3^- transporters. Cyanobacterial RubisCO shows lower affinity for CO_2 than the enzyme present in C3 plants. The higher K_M of cyanobacterial RubisCO to CO_2 is compensated by an efficient CCM that increases the CO_2 concentration surrounding the carboxylating enzyme. Furthermore, RubisCO is localised within an icosahedral proteinaceous compartment called carboxysome (α or β-type carboxysomes, see Ref. [29] for review). Mutants impaired in functional CCM components, such as the carboxysome shell proteins or bicarbonate transporters show very low photosynthetic affinity for external Ci. Thus, mutants have a high CO_2 requiring phenotype. These observations clearly demonstrate the importance of CCM for cyanobacterial survival in present atmospheric conditions. Both types of carboxysomes are composed of several shell proteins with different proposed functions. The main difference between α and β carboxysomes is the RubisCO type (form A or B, respectively) and the operon encoding the shell proteins (the *cso* operon – *csoS123AB* – or by the *ccm* operon – *ccmKLMN* –, respectively) [30]. Bicarbonate dehydratation is catalysed by a specific carboxysomal carbonic anhydrase that, in the case of α-carboxysome, is an εCA (encoded by *csoS3* gene [31]) and in β-carboxysome, a βCA, named CcaA, not integrated in the ccm operon [32]. One of the shell proteins of β-carboxysome, the CcmM subunit, consists of an N-terminus that resembles gamma type carbonic anhydrases (γCAs) and a C-terminus which includes RubisCO small subunit-like repeats [33]. CcmM is an important component of a multiprotein bicarbonate dehydration complex along with CcaA, and CcmN (similar to CcmM). This complex, through CcmM, also interacts with CcmK and CcmL, the major shell proteins. CcmM is inactive as a carbonic anhydrase but has been proposed to be involved in the transport of bicarbonate to the inside of carboxysomes where it is converted by CcaA into CO_2 near RubisCO [34]. However, some β cyanobacteria lack a functional *ccaA* gene. In these species, CcmM is an active carbonic anhydrase and its activity depends on an essential disulphide bond, which is not conserved in other γCA homologues [35]. *Synechocystis* spp. (strain PCC 6803) contains a third structurally related protein with a high similarity to bacterial and plant γCAs. However, neither its function nor localisation is known.

In addition, there is evidence for the association of CA-like proteins (Chp X and Y/Cup A and B) to the NADH dehydrogenase complex (termed NDH-1 complex) of the thylakoid and plasma membranes of cyanobacteria. These polypeptides are involved in catalysing active CO_2 uptake by converting CO_2 into bicarbonate within the cell linked to electron transport and proton translocation associated with the NDH-1 complex. Although Chp/Cup proteins have no homologies with known CA protein families, two conserved histidine residues and one conserved cysteine residue, which could act as a potential Zn coordination site, have been identified (a novel CA class?). Electron donation to the complex by donors such as NAD(P)H produces a reduced intermediate within the NDH-1 complex that could oxidise the Zn–H_2O to Zn–OH^- and H^+. In the second step, Zn–OH could react with CO_2 to form HCO_3^- which together with the released proton are translocated across the plastidial membrane to the lumen via a proton shuttle path within the hydrophobic proton channel subunits of the NDH-1 complex [36].

Because of the presence of efficient CCM systems, the identification of a high CO_2 requiring phenotype, which completely lacks 2-phosphoglycolate metabolism (the following three routes inactivated: oxalate decarboxylase – *odc* –, tartronic semialdehyde reductase – *tsr* – and the plant-like glycine decarboxylase – *gcvT* – or a double mutant in glycolate dehydrogenases – *glcD1* and *D2*) was unexpected and suggests that photosynthesis requires a functional CCM as well as a fully active glycolate detoxifying system [37]. Based on pulse labelling experiments using ^{13}C $NaHCO_3$, ^{13}C glycolate was detected under conditions thought to suppress photorespiration [38]. Therefore, both CCM and 2-phosphoglycolate metabolism appear to be crucial for the viability of all organisms performing oxygenic photosynthesis (cyanobacteria and plants) that grow under normal CO_2 conditions.

3. Proposed basal CCM

The discovery of γCAs in mitochondria of almost all photosynthetic eukaryotic organisms analysed so far but not in animals or fungi has led to hypothesise concerning their physiological role. The mitochondrial γCAs are attached to complex I (CI) of the respiratory chain and form a spherical extra domain (named CA domain) on the matrix exposed side of its membrane arm [39].

A possible hypothesis extends a proposition originally made by Raven [28] that bicarbonate translocases possibly present in the inner mitochondrial membrane in *Chlamydomonas* spp. are involved in recapturing Ci from mitochondrial decarboxylation reactions. We propose that the CA domain of complex I forms

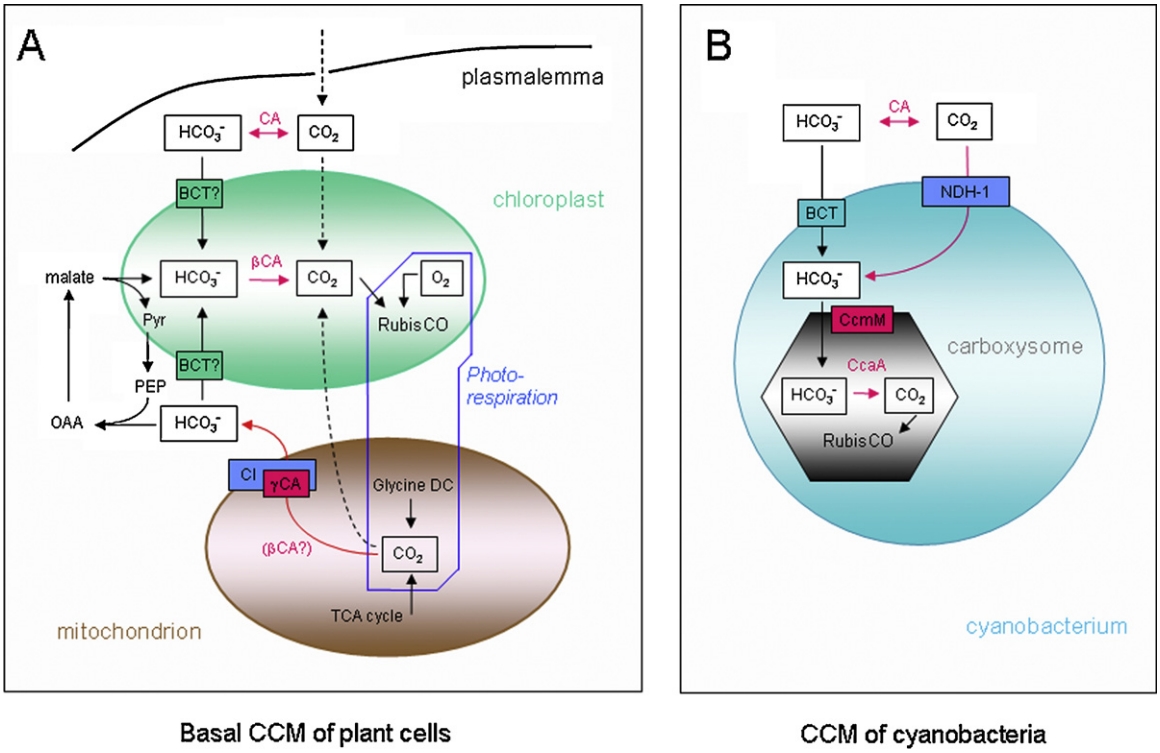

Fig. 1. Model for carbon concentrating mechanism in plants and cyanobacteria. (A) Proposed basal CCM (bCCM) in plants. The CO_2 concentration in chloroplasts is often low due to carbon fixation by RubisCO, especially if plants are cultivated under high light or at high temperatures. At the same time, large amounts of CO_2 are liberated in mitochondria due to the reactions of the TCA cycle and photorespiration. Experimental evidence indicates that mitochondrial CO_2 is recycled for carbon fixation in chloroplasts. Transfer of CO_2 could be based on diffusion but would be more efficient via an active HCO_3^- transfer mechanism. The mitochondrial CA domain (given in red) of complex I (blue) is proposed to play a role in converting CO_2 to HCO_3^- and/or transfer HCO_3^- from mitochondria into the cytosol. A matrix localised βCA (At1g58180) might additionally be involved in HCO_3^- formation. Subsequently, HCO_3^- could be transferred from the cytosol into the chloroplast by a putative bicarbonate translocator in the chloroplast envelope or by a C4-like pathway. (B) Carbon concentration mechanism (CCM) of cyanobacteria. Cyanobacteria export a carbonic anhydrase increasing the bicarbonate concentration in their surroundings. Transporters are localised in the plasma membrane of cyanobacteria thereby facilitating the active uptake of bicarbonate or CO_2. Besides other components, cyanobacterial complex I (NDH-1; given in blue) is involved in this process. Bicarbonate subsequently is transferred into a specialised compartment, the "carboxysome", where CO_2 fixation occurs. This translocation is based on the CcmM protein (given in red) and conversion of bicarbonate into CO_2 by CcaA. Designations: CI: mitochondrial complex I, NDH-1: cyanobacterial complex I involved in Ci uptake, BCT: bicarbonate translocase, βCA and γCA: beta- and gamma-type carbonic anhydrases, CcmM and CcaA: carboxysomal proteins involved in Ci delivery for RubisCO, OAA: oxaloacetate, Pyr: pyruvate, PEP: phosphoenolpyruvate.

part of a mitochondrial bicarbonate export system which enables the efficient transfer of Ci from mitochondria to chloroplasts. The CA domain is involved in bicarbonate formation and/or bicarbonate transfer across the inner mitochondrial membrane. Especially in the presence of high light or temperature, CO_2 in chloroplasts is usually low due to ribulose-1,5-bisphosphate carboxylation by RubisCO and due to the fact that stomata can be closed at daytime and consequently import of atmospheric CO_2 into the leaf is restricted. At the same time, mitochondria produce excess CO_2 as a result of the decarboxylation reactions of the TCA cycle but especially due to glycine–serine conversion from photorespiration. Whilst CO_2 is more or less "waste" in animal and fungal mitochondria, in plant cells it is the main substrate for photosynthetic carbon fixation in chloroplasts and, under many conditions, is rate-limiting for plant growth. Part of the mitochondrial CO_2 can be re-cycled for carbon fixation by RubisCO following diffusion to the chloroplast. However, we additionally propose the occurrence of a more targetted carbon transfer system which, besides other elements, is constituted by a bicarbonate export system linked to mitochondrial complex I. Bicarbonate could be transferred from the cytosol into chloroplasts by another bicarbonate translocase or by a metabolic system resembling the carbon pre-fixation steps during C4-metabolism. In the chloroplasts, βCAs facilitate efficient re-conversion of bicarbonate into CO_2 for carbon fixation by RubisCO. Our hypothesis is summarised in Fig. 1. Furthermore, we propose that the CA domain of complex I has a function as carboxysomal CcmM–CcmN proteins. Plant proteins could have evolved from the γCAs of an ancient α proteobacterium during the adaptation of the first endosymbiont to be converted into a mitochondrion. These proteins are only conserved in the plant and basal lineages of Eukarya. This could be regarded as a case of convergent evolution. We propose that all photosynthetic eukaryotic organisms contain this "basal" CCM (bCCM, Fig. 1) which could account for approximately 10–20% of carbon fixation in C3 plants. In the following sections, we present recent experimental results that, according to our interpretation, support the hypothesised basal carbon transfer mechanism within plant cells.

3.1. Evidences for the basal CCM

Mitochondrial γCAs have been described for *Arabidopsis* (C3, dicotyledonous), rice (C3, monocotyledonous), maize (C4 monocotyledonous) as well as for certain green algae such as *Chlamydomonas* (C3 with CCM) [39–46]. Surprisingly, no γCA homologues have been found in the *Ostreococcus tauri* genome, which is the smallest known eukaryotic green alga [47]. Recently, similar proteins have been found in mitochondrial complex I of *Acanthomoeba castellani* and other aplastidic eukaryotes such as Amoebazoa, Chromalveolata and Excavata [48,49]. It can be assumed that the function of γCA proteins is somewhat different in plant/green algae than in aplastidic eukaryotes.

In *Arabidopsis*, the gammaCA family consists of five proteins encoded by five nuclear genes. Three are named γCA1, γCA2 and γCA3 because they very much resemble the "prototype" CA from

Metanosarcina thermophila (Cam) and two are named γCAL1 and γCAL2 ("gamma carbonic anhydrase like") because they have a more derived sequence with respect to Cam with lower conservation of important residues [50]. At the same time, the *Arabidopsis* CA/CAL proteins exhibit sequence similarity to cyanobacterial CcmM proteins. These proteins form the so called "CA domain" of complex I first discovered in *Arabidopsis* [39,44]. The exact composition of this domain is not known in detail. Based on single particle electron microscopy, the CA domain is most likely a trimer [39,45,51], which nicely corresponds to the composition of Cam [52]. Because the interaction between γCALs and γCAs is strong and all photosynthetic organisms analysed thus far contain at least one γCA and one γCAL, the CA domain most likely represents a heterotrimer [50]. It is still unclear whether all five CA/CAL proteins of *Arabidopsis* are simultaneously present in individual complex I particles. All five proteins were detected by mass spectrometry within the isolated complex I or within the 550 kDa membrane arm of this complex upon dissection of the holo-enzyme by low SDS (0.01%) [46]. Moreover, at slightly higher SDS concentrations (0.04%), an ~85 kDa subcomplex detaches from complex I which includes γCA1 and γCA2 and the two γCALs but surprisingly not γCA3. Additional data suggest that γCAL1 and γCAL2 are not simultaneously present within individual CA subcomplexes [46]. Therefore, the CA domain of complex I seems to be composed by three CA/CAL proteins of varying identity. The physiological relevance of this heterogeneity remains a mystery.

It so far has not been possible to measure carbonic anhydrase activity of the mitochondrial γCAs for any plant or eukaryotic green algae although all relevant aminoacids are present and properly arranged in a putative active site similar to Cam or members of a Cam subfamily termed CamH [42]. However, cyanobacterial CcmM as well as *Arabidopsis* γCA2 have been shown to bind Ci at comparable rates [34,53]. Aminoacids essential to bind bicarbonate are also conserved [42]. Thus we propose that the CA domain of complex I could function as a carbonic anhydrase and/or bicarbonate translocator. Bicarbonate could bind the conserved Gln and Tyr residues together with a possible metal ion (Zn^{2+} or Fe^{2+}). How bicarbonate could be transferred across the mitochondrial membrane? This currently is speculative. Interestingly, EM analysis of complex I from *Arabidopsis* revealed presence of a small cavity within its membrane arm in opposite to the point of attachment of the CA domain, possibly indicating a pore like structure and this position [44]. Furthermore, protease protection experiments indicate that the CA subunits of *Arabidopsis* span the membrane once and that their C-termini constitute a small domain on the intermembrane-space exposed side of the membrane arm [39]. These C-termini could form an amphipathic channel which allows bicarbonate transfer possibly helped by proton transfer as it was proposed for Cup proteins in the NDH-1 complexes in cyanobacteria [36].

Gene expression profiling data indicate that mitochondrial γCAs are downregulated when *Arabidopsis* is cultivated at elevated CO_2 concentrations [54]. These data indicate a possible role for CA proteins during photorespiration [51]. Furthermore, ethoxzolamide (EZA), a strong inhibitor of γCAs (and to a lesser extent of βCAs) impairs photosynthetic oxygen evolution in isolated protoplasts but not in isolated chloroplasts. This effect was only observed at low CO_2 concentrations with almost no effect was observed at high CO_2 concentrations [55]. These results strongly suggest that internal CAs (outside the chloroplast) are involved in recycling of mitochondrial CO_2 under photorespiratory conditions.

Arabidopsis null mutant plants lacking the gene encoding γCA2 contain drastically reduced amounts of complex I. A weaker effect was also observed in *ca3* null mutant plants. Moreover, oxygen consumption experiments carried out in the presence of different respiratory chain inhibitors using leaves and flower tissues as well as cell suspensions growing in the dark indicate that these mutant plants have increased alternative respiration. Both mutant lines did not exhibit an altered phenotype with respect to wild-type plants under the conditions tested. However, cell suspensions derived from mutant lines grew more slowly in the darkness than did suspensions derived from wild-type plants [54].

Arabidopsis plants overexpressing γCA2 plants show a male sterile phenotype by the indehiscence of anthers due to a dramatic reduction in ROS content which seems to cause reduced lignin deposition [56]. Furthermore, these plants have significantly longer roots and bigger seeds. A considerable enrichment of γCA2 within Complex I was consistently detected (Villarreal and Zabaleta, unpublished results) suggesting that the overexpression of this protein leads to the replacement of γCA1 and γCA3 within the trimeric CA domain attached to complex I. Homotrimers of γCA2 bind Ci as efficiently as the cyanobacterial CcmM protein [34,53]. Both proteins are assumed to have a similar function in Ci translocation, which is especially important in the context of photorespiration [51].

Complex I abundance and activity is normal in mitochondria of plants overexpressing γCA2. If the CA domain of complex I indeed is involved in mitochondrial bicarbonate export, a possible explanation of the observed bigger sink organs is that increased abundance of γCA2 should allow higher rates of bicarbonate export resulting in higher CO_2 fixation rates by RubisCO and thus increasing plant growth. This increase in plant growth only can take place if γCA2 over-expression does not impair respiration. Indeed, oxygen consumption experiments using an *Arabidopsis* cell suspension culture overexpressing γCA2 revealed elevated oxygen consumption rates and faster growth (approx. 70% more fresh weight per week compared to wild type cells, Villarreal and Zabaleta, unpublished results).

How bicarbonate reaches the chloroplasts is still unknown because a cyanobacterial-like bicarbonate transporter or equivalent has not yet been identified in higher plants. One possibility is that bicarbonate exported from mitochondria reacts with PEP. This reaction is catalysed by a cytoplasmic PEP carboxylase. The resulting oxaloacetate can be converted into malate and transported into chloroplasts as in C4 metabolism (Fig. 1). All necessary enzymes are present in *Arabidopsis* (cytosolic AtPEP carboxylase [57], cytosolic malate dehydrogenase, E.C. 1.1.1.83, antiporter for malate/oxaloacetate exchange, dicarboxylic acid translocase AtDiT family [58] and a chloroplast NADP malic enzyme, E.C. 1.1.1.40 [59,60]). The *Arabidopsis* dicarboxylic acid translocase DiT2 knockout mutant, *dct2* and tobacco DiT1 antisense plants both have a photorespiratory phenotype [57,61]. DiT1 has been shown to have a high affinity for the dicarboxylate oxaloacetate [61]. These evidences are consistent with the interpretation that malate or oxaloacetate transporters are important during photorespiration, according to our hypothesis, for recycling mitochondrial Ci to carbon fixation by RubisCO. The net result is the introduction of CO_2 into the chloroplast. However, recent comparative proteome and transcriptomic analyses of C3 and C4 plants revealed that DiT proteins are specifically enriched in the chloroplast envelopes of C4 plants [62,63]. The DiT family members have been proposed to play a role in central nitrogen metabolism [61] and for core C4 photosynthesis in maize [64]. All members of the DiT family were identified in C4 as well as in C3 plants. A recent investigation clearly showed that all enzymes required for C4 photosynthesis are present in *Arabidopsis* and that cell-specific expression of the corresponding transcripts in C4 species can be explained by as yet an unidentified trans-factor [65]. Additionally, several reports indicate as well that high activities of the C4 enzymes occur around the veins of C3 species and may have facilitated the polyphyletic evolution of C4 photosynthesis [66].

CMS I and II *Nicotiana sylvestris* cytoplasmic male sterile mutants have drastically reduced amounts of complex I, which affects photosynthesis under normal (photorespiratory) conditions [67]. Steady-state photosynthesis in the mutant was reduced by 20–30% at atmospheric CO_2 levels. The inhibition of photosynthesis was alleviated by high CO_2 or low O_2. Based on these results, it was interpreted that a functional complex I is required to ensure a subcellular redox balance [3,68]. However, the importance of complex I for photosynthesis can also be explained by its proposed role in the recycling of mitochondrial Ci.

Taken together, the proposed basal CCM for efficient recycling of mitochondrial CO_2 for carbon fixation in chloroplasts, which is especially important during photorespiratory conditions, is supported by several experimental findings: (i) the CA domain of complex I is present in all plants but absent in animal and fungal mitochondria; (ii) CA proteins can bind Ci efficiently and highly resemble the cyanobacterial CcmM proteins involved in the enrichment of Ci in carboxysomes; (iii) genes encoding CA proteins are downregulated when plants are cultivated at elevated CO_2 concentrations; (iv) mutants with reduced amounts of complex I have reduced photosynthesis rates under photorespiratory but not under non-photorespiratory conditions and (v) other components of the proposed Ci transfer system, which are necessary for Ci transfer into chloroplasts, might represent enzymes of the carbon pre-fixation step of C4 metabolism. All necessary enzymes are also present in C3 plants. Interestingly, some mutants of these enzymes have photorespiratory phenotypes.

4. Outlook

Photorespiration is a metabolic process of great importance. It is one of the main determining factors for biomass production in C3 crops. Recent technological developments have allowed to address important questions concerning the photorespiratory pathway (reviewed in Ref. [3]). However, a number of aspects deserve further investigation, especially the role of metabolite transporters such as the dicarboxylic acid translocases in photorespiration and the role of the photorespiratory enzymes such as PEP carboxylase and NADP malic enzyme in C3 plants. Last but not least, the physiological role of the CA domain of complex I, which not only occurs in C3 plants but also in plants with other photosynthetic metabolism and in green algae, requires further investigations. Specifically, bicarbonate export by complex I has to be analysed, e.g. by reconstitution of plant complex I into artificial liposomes.

The proposed basal CCM involves a number of metabolites that are also part of photorespiratory [69] and other cellular pathways making its investigation a complicated task. RNA profiling and proteomic analyses of isolated organelles [62,70–72] may help to understand this network. Isolation of organelles from different tissues or cell types will also provide interesting data regarding the function of the proposed basal CCM. For example, the CA domain of complex I is present in C4 maize [45]. Do mitochondria of mesophyll as well as bundle sheath cells contain the same arrangement of complex I subunits? Future research should also investigate other organisms with differences in photosynthetic primary metabolism, for example, green algae. The CA domain is also present in organisms that at the same time contain other sophisticated CCM system. All of these investigations should be of fundamental importance to achieve a better understanding of the evolution of cyanobacterial CCM to the compartmentalised basal CCM proposed for plants.

In the past decade, several projects have attempted to improve the photosynthetic performance of C3 crop plants. For example, one approach is to incorporate C4 CCM into C3 crop (mainly rice), thereby elevating CO_2 concentrations around RubisCO [73–76]. A second approach is to transfer cyanobacterial bicarbonate transporters or even other cyanobacterial CCM proteins to C3 chloroplasts in order to provide a significant improvement in photosynthetic performance [77]. Additionally, because the oxygenation reaction of RubisCO cannot be eliminated, a third approach is to improve the degradation of 2-phosphoglycolate via a bacterial glycolate pathway introduced in *Arabidopsis* chloroplasts [78]. In a fourth approach, the complete design of a novel pathway to fully oxidises 2-phosphoglycolate to CO_2 was undertaken based on the incorporation of glycolate oxidase, malate synthase and catalase into *Arabidopsis* chloroplasts [79]. And finally: alterations of the CA domain of complex I may lead to further optimisation of photosynthesis in crops. The combination of more than one approach might one day allow developing crops for feeding tomorrow's world.

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