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Is the structure of the CO_2-hydrating complex I compatible with the cyanobacterial CO_2-concentrating mechanism?

Dear Editor,

Recently, Schuller et al. (2020) published the first structure of a cyanobacterial CO_2-hydrating photosynthetic complex I, which plays a fundamental role in oxygenic photosynthesis. This type of photosynthesis is by far the most important process for organic carbon acquisition on Earth. Oxygenic photosynthesis evolved in cyanobacteria about 2.7 billion years ago. Photosynthetic CO_2 fixation is primarily done by the enzyme ribulose 1,5 bisphosphate (RuBP) carboxylase/oxygenase (Rubisco), producing two molecules of 3-phosphoglycerate. Those are then reduced and converted into various organic carbon compounds via the Calvin-Benson-Bassham cycle that predated oxygenic photosynthesis (Hohmann-Marriott & Blankenship, 2011). The rising level of molecular oxygen introduced two main problems to Rubisco activity.

First, O_2 competes with CO_2 in the cleavage of RuBP and thus slows the carboxylase reaction. Binding of O_2 in the oxygenase reaction forms the toxic product 2-phosphoglycolate, which needs to be salvaged via photorespiratory metabolism (that probably co-evolved with oxygenic photosynthesis in ancient cyanobacteria; Eisenhut et al., 2008). Second, the tremendous success of oxygenic phototrophs, particularly after the appearance of eukaryotic algae and plants resulted in a strong decline of atmospheric CO_2, slowing Rubisco activity due to its low affinity to CO_2 and poor specificity (Tcherkez et al., 2006). Cyanobacteria like many other photosynthetic organisms solved these problems, at least partly, with the evolution of inorganic carbon- or CO_2-concentrating mechanisms (CCMs; Raven et al. 2012).

By concentrating CO_2 in close proximity to Rubisco, the CCM raises the apparent photosynthesis affinity for extracellular CO_2 well above that of the enzyme. Hence, in addition to saturating the carboxylase activity, the CCM significantly reduces the oxygenase reaction. Among the various CCMs, the cyanobacterial type is considered the best understood (Raven et al., 2012). Here, light energy is used to fuel various uptake systems for CO_2 and bicarbonate, leading to the accumulation of high concentrations of the latter in the cytoplasm. Bicarbonate enters bacterial micro-

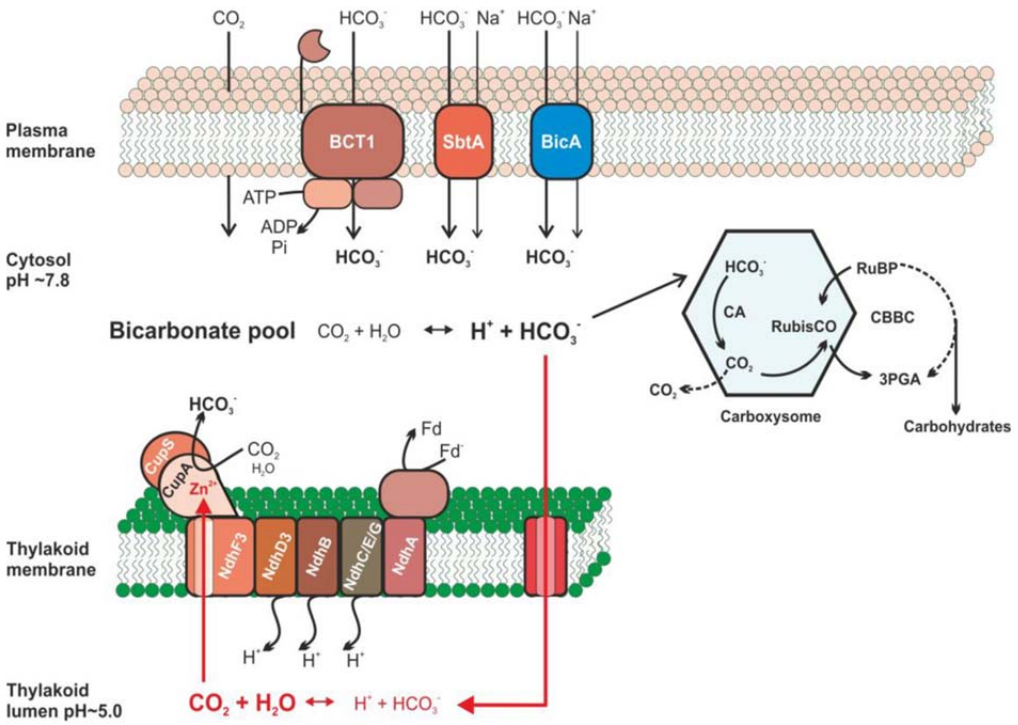

Figure 1: Proposed model of the cyanobacterial CO_2-concentrating mechanism (CCM, classical cycle in black arrows) including the structure-predicted bicarbonate/CO_2 cycling in the thylakoid lumen (red arrows).

The cyanobacterial CCM utilizes three bicarbonate transporters: BCT1, SbtA and BicA located in plasma membrane. The thylakoid embedded CO_2 hydration system (special CO_2-hydrating photosynthetic complex I or $NDH1_3$ comprising the cyanobacteria-specific small subunits NdhD3, NdhF3 and CupA/B) converts cytoplasmic (classical view) or rather luminal (new prediction) CO_2 to bicarbonate. The action of these uptake systems constitutes a large cytoplasmic bicarbonate pool, which penetrates into carboxysomes. There, bicarbonate is dehydrated back to CO_2 by carbonic anhydrase (CA) in proximity to Rubisco and bound to ribulose 1,5-bisphosphate (RuBP) leading to 3-phosphoglycerate (3PGA) that enters carbohydrate synthesis through the Calvin-Benson-Bassham cycle (CBBC). According to the newly resolved structure of the special CO_2-hydrating photosynthetic complex I, the CA side is buried inside the CupA subunit (depicted as red Zn^{2+}), which is connected to the luminal side of the thylakoids via a putative CO_2-channel (Schuller et al., 2020). The most probable source for CO_2 inside the lumen is a leakage of bicarbonate into the luminal space, where it becomes converted into CO_2 due to the acidic pH.

compartments, the carboxysomes, where carbonic anhydrase (CA) converts it to CO_2, thereby raising its concentration in close proximity to Rubisco mostly located in these

bodies (Fig. 1). This enables efficient CO_2 fixation into RuBP and a marked decline in the oxygenase activity of the enzyme (Kaplan & Reinhold, 1999; Rae et al., 2013; Burnap et al., 2015). Three different bicarbonate transport complexes were identified at the cytoplasmic membrane of model cyanobacteria (Fig. 1), the ABC-type bicarbonate transporter BCT1 and two bicarbonate/sodium antiporters, SbtA and BicA (Omata et al., 1999; Shibata et al., 2002; Price et al., 2004). In addition, specialized NDH1 complexes residing in the thylakoid membrane have been shown to act as low ($NDH1_4$) or high affinity ($NDH1_3$, Fig. 1) CO_2-uptake systems (Shibata et al., 2001). They convert cytoplasm-located CO_2 to bicarbonate thereby forming an inward concentration gradient for CO_2 diffusion into the cells and also minimizing the leak of CO_2 formed either by carboxysomal CA activity or dehydration of the cytoplasmic bicarbonate pool. These specific NDH1 complexes recruit the subunits CupA and CupS that form a cytoplasm-exposed structure visible in single particle analyses (Shibata et al., 2001; Folea et al., 2008). It has been proposed that this part of the specialized NDH1 complexes is particularly responsible for the CO_2 hydration; however, the mechanism remained elusive. Because cyanobacterial NDH1 complexes receive electrons from ferredoxin (unlike the mitochondrial NDH complex I, where NADH serves as electron donor), it was recently renamed as photosynthetic complex I (PCI) (Schuller et al., 2019; Laughlin et al., 2019).

Tremendous progress has been made in our understanding of the cyanobacterial CCM with the emergence of structures of carboxysomal proteins and the BicA transporter mainly by cryo-electron microscopy (Kerfeld et al., 2018; Wang et al., 2019). The most recent hallmark in this development represents the structural analysis of the high-affinity CO_2-hydrating PCI (formerly known as $NDH1_3$) from the cyanobacterium *Thermosynechococcus elongatus* (Schuller et al., 2020). The authors isolated a tagged version of this low CO_2-induced complex and resolved its structure by cryo-electron microscopy at 3.2 Å resolution. The obtained structure allowed assignment of a Zn^{2+} atom bound to two helices of CupA near the NdhF3 subunit that is stabilized by CupS. The Zn^{2+} is a reactive metal center in most types of CAs (DiMario et al., 2018). Although the coordination of Zn^{2+} in the CO_2-hydrating PCI differs from canonical CAs, the authors provided a strong theoretical evidence based upon computational simulations

that it is able to catalyze the conversion of CO_2 into bicarbonate (Schuller et al., 2020), a thermodynamically unfavorable reaction due to the high cytoplasmic bicarbonate level. Hence, the structure analysis and the coupled theoretical calculations verified that the CupA/S-formed cytoplasm-exposed structure at NdhF3 represents the expected CA part of this PCI. It is proposed that the CO_2-hydration process is closely coupled to a redox-driven mechanism including electron transport from ferredoxin toward plastoquinone and three proton extrusion steps from the cytoplasmic into the luminal side across the thylakoid membrane, which energizes the CO_2 conversion (Schuller et al., 2020). The proton extrusion mechanism further explains how the predicted “alkaline pocket” is formed (Kaplan & Reinhold, 1999), which drives the CO_2-hydration by the Zn^{2+}-containing active site (Schuller et al., 2020). Contrary to previous models, the CA site appears inside the protein and not at its cytoplasm-exposed surface, where it was tentatively predicted to be, supported by the CA-like EcaB protein (Sun et al., 2019).

Summarizing, the proposed structure nicely explains the biochemical and molecular mechanisms of CO_2 hydration by PCI as part of large bicarbonate accumulation inside the cyanobacterial cell. However, the presented structure is not easily compatible with the traditional view of how this complex is embedded and functions in the cyanobacterial CCM. Most pronounced and surprising is the proposed occurrence of a putative CO_2 channel in the structure. The structure revealed a non-polar tunnel that is surrounded by hydrophobic and bulky amino acid residues, which connects the reactive Zn^{2+} inside CupA with the luminal side of the thylakoids. Molecular dynamic simulations made it possible to conclude that this channel might be the path by which CO_2 reaches the CA site (Schuller et al., 2020). The existence of this channel in the structure implies that CO_2 mostly reaches the CA site from the thylakoid lumen instead of from the cytoplasmic space, which is contrary to the classical view of CO_2 appearance in the cytoplasm but not in the thylakoid lumen. Furthermore, the CA site with the bound Zn^{2+} is densely packed and surrounded by charged amino acid residues, which seem to shield it from cytoplasmic CO_2 (Schuller et al., 2020). These findings are puzzling, since they are not compatible with the current CCM model for cyanobacteria (Rae et al., 2013; Burnap et al., 2015). This model assumes that the substrate CO_2 used by photosynthetic complexes I arises only in the cytoplasm from three different sources (Fig. 1): 1. CO_2

uptake from the medium, the diffusion of which is accelerated due to aquaporin-gating and rapid conversion of dissolved CO_2 into bicarbonate in the cytoplasm; 2. Leakage of non-fixed CO_2 from the carboxysome into the cytoplasm; and 3. pH-dependent physicochemical conversion of the cytoplasmic bicarbonate into CO_2. Hence, according to the existing CCM model the cytoplasmic CO_2 pool should have direct access to the CA site inside CupA of this special PCI, which is obviously not the case in the newly proposed structural model (Schuller et al., 2020).

Can we reconcile the structural with the physiological interpretations? If the predicted CO_2 channel is working as postulated and CO_2 is mostly reaching the CA site from the luminal side, then we must assume that the cytoplasm-accumulated bicarbonate is the most likely source of CO_2 inside the lumen. The negatively charged bicarbonate ion could be driven into the lumen by its electrochemical gradient, using some pores or transporters (Fig. 1), and rapidly converted into CO_2 due to the acidic lumen pH, which is built up by the photosynthetic electron transport. This is a vicious inorganic carbon cycle, breaking and forming bicarbonate and on the way dissipating the CCM. Further, as the magnitude of the required CO_2/HCO_3^- flux across the thylakoid is very high it would be expected to dissipate the light-driven electrochemical proton gradient essential for ATP formation. If this scenario is correct, the cyanobacterial CCM includes a bicarbonate/CO_2 cycle at the thylakoid membrane. This is reminiscent of the function of algal CCMs, where CO_2 is released inside specific thylakoids traversing the Rubisco-containing structure, the pyrenoid (Matsuda et al., 2017). However, in cyanobacteria, Rubisco is located in the carboxysomes, which are not directly attached to the thylakoids.

According to the new proposed structure (Schuller et al., 2020), CO_2 access is predicted to occur mainly from the luminal side of thylakoids, which would severely diminish the photosynthetic efficiency of cyanobacteria. How to solve this puzzling finding? First, the accessibility of the CupA-localized CA site to cytoplasmic CO_2 is still possible, which is in agreement with the traditional view of the function of the CO_2-hydrating PCI within the cyanobacterial CCM. Moreover, the putative CO_2 channel still needs experimental validation. However, if the channel is really functional this finding may suggest that the CO_2-hydrating PCI might have additional/alternative functions. For example: Can it also function to dissipate excess light energy or to dissipate the internal Ci pool upon

126 darkening? In this case, the conversion of cytoplasmic CO_2 may be of secondary
127 importance. Clearly, more function-related studies with isolated CO_2-hydrating PCI
128 preparations are needed; these should include site-specific variants affecting the CO_2
129 channel and the accessibility of the CA site.

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132

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139 **Author Contributions:**

140 MH and AK discussed the issue and wrote the letter.

141 **One-Sentence Summary**: The structure of CO2 hydration complex I is not compatible
142 with the view on how this complex is embedded and functions in the cyanobacterial
143 carbon-concentrating mechanism.

144 **FIGURE LEGENDS**

145 **Figure 1: Proposed model of the cyanobacterial CO_2-concentrating mechanism**
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Parsed Citations

Burnap RL, Hagemann M & Kaplan A (2015) Regulation of CO2 concentrating mechanism in cyanobacteria. Life 5: 348–371
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

DiMario RJ, Machingura MC, Waldrop GL & Moroney JV (2018) The many types of carbonic anhydrases in photosynthetic organisms. Plant Sci 268: 11–17
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Eisenhut M, Ruth W, Haimovich M, Bauwe H, Kaplan A & Hagemann M (2008) The photorespiratory glycolate metabolism is essential for cyanobacteria and might have been conveyed endosymbiontically to plants. Proc Natl Acad Sci USA 105: 17199-17204
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Folea IM, Zhang P, Nowaczyk MM, Ogawa T, Aro EM & Boekema EJ (2008) Single particle analysis of thylakoid proteins from Thermosynechococcus elongatus and Synechocystis 6803: localization of the CupA subunit of NDH-1. FEBS Lett 582: 249-254
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Hohmann-Marriott MF & Blankenship RE (2011) Evolution of photosynthesis. Ann Rev Plant Biol. 62: 515–548
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kaplan A & Reinhold L (1999) The CO2 concentrating mechanisms in photosynthetic microorganisms. Annu Rev Plant Physiol Plant Mol Biol 50: 539-570
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kerfeld CA, Aussignargues C, Zarzycki J, Cai F & Sutter M (2018) Bacterial microcompartments. Nat Rev Microbiol 6: 277-290
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Laughlin TG, Bayne AN, Trempe JF, Savage DF & Davies KM (2019) Structure of complex I-like molecule NDH of oxygenic photosynthesis. Nature 566: 411-414
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Matsuda Y, Hopkinson BM, Nakajima K, Dupont CL & Tsuji Y (2017) Mechanisms of carbon dioxide acquisition and CO2 sensing in marine diatoms: a gateway to carbon metabolism. Philos Trans R Soc Lond B Biol Sci 372: 1728
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Omata T, Price GD, Badger MR, Okamura M, Gohta S & Ogawa, T (1999) Identification of an ATP-binding cassette transporter involved in bicarbonate uptake in the cyanobacterium Synechococcus sp. strain PCC 7942. Proc Natl Acad Sci USA 96: 13571–13576
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Price GD, Woodger FJ, Badger MR, Howitt SM & Tucker L (2004) Identification of a SulP-type bicarbonate transporter in marine cyanobacteria. Proc Natl Acad Sci USA 101: 18228–18233
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Rae BD, Long BM, Badger MR & Price GD (2013) Functions, compositions, and evolution of the two types of carboxysomes: polyhedral microcompartments that facilitate CO2 fixation in cyanobacteria and some proteobacteria. Microbiol Mol Biol Rev 77: 357-379
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Raven, J. A., Giordano, M., Beardall, J. & Maberly, S. C. (2012) Algal evolution in relation to atmospheric CO2: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles. Philos Trans R Soc Lond B Biol Sci 367: 493-507
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Schuller JM, et al. (2019) Structural adaptations of photosynthetic complex I enable ferredoxin-dependent electron transfer. Science 363: 257–260
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Schuller JM, et al. (2020) Redox-coupled proton pumping drives carbon concentration in the photosynthetic complex I. Nat Commun 11: 494
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Shibata M, et al. (2001) Distinct constitutive and low-CO2-induced CO2 uptake systems in cyanobacteria: genes involved and their phylogenetic relationship with homologous genes in other organisms. Proc Natl Acad Sci USA 98: 11789–11794
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Shibata M, et al. (2002) Genes essential to sodium-dependent bicarbonate transport in cyanobacteria: function and phylogenetic analysis. J Biol Chem 277: 18658–18664
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Sun, N., Han, X., Xu, M., Kaplan, A., Espie, G. S. & Mi, H. (2019) A thylakoid-located carbonic anhydrase regulates CO2 uptake in the cyanobacterium Synechocystis sp. PCC 6803. New Phytol 222: 206-217
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Tcherkez GGB, Farquhar GD & Andrews TJ (2006) Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. Proc Natl Acad Sci USA 103: 7246–7251
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wang C, et al. (2019) Structural mechanism of the active bicarbonate transporter from cyanobacteria. Nat Plants 5: 1184-1193
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title