Photosynthesis in Phytoplankton: Insights from the Newly Discovered Biological Inorganic Carbon Pumps

Na Sui[1,2,#], Fang Huang[2,#,*], Lu-Ning Liu[2,3,*]

[1]Shandong Provincial Key Laboratory of Plant Stress, College of Life Sciences, Shandong Normal University, Jinan 250014, China.
[2]Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, United Kingdom
[3]College of Marine Life Sciences, and Frontiers Science Center for Deep Ocean Multispheres and Earth System, Ocean University of China, Qingdao 266003, China
[#]N.S. and F.H. contribute equally to this work

*Corresponding authors: luning.liu@liverpool.ac.uk (L.-N.L.) or fang.huang@liverpool.ac.uk (F.H.)

Aquatic CO_2 assimilation results in storage of ~24% of anthropogenic CO_2 (~40 petagrams per annum) released into the atmosphere in the oceans and make significant contributions to global carbon cycle. These processes are executed predominantly in phytoplankton in the oceans (including cyanobacteria), which account for nearly 50% of global primary productivity (~50 gigatons per annum) (Field et al., 1998).

Powered by sunlight, the Calvin–Benson-Bassham cycle converts CO_2 into organic compounds to drive life in the biosphere. The key enzyme for assimilating inorganic carbon (C_i) is ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the most abundant protein on Earth (Bar-On and Milo, 2019). However, Rubisco is surprisingly inefficient, given its slow catalytic rate and poor capability in discriminating between CO_2 and O_2. To cope with ancient changes in atmospheric CO_2 and O_2 levels, phytoplankton have evolved efficient CO_2-concentrating mechanisms (CCMs) to accumulate CO_2 around Rubisco. Cyanobacterial CCMs comprise bicarbonate transporters in the plasma membranes and CO_2-converting complexes in thylakoid membranes to accumulate bicarbonate in the cytoplasm and prevent diffusive CO_2 leakage from the cell, as well as the CO_2-fixing organelles in the cytoplasm—carboxysomes—that encapsulate Rubisco and carbonic anhydrase (CA) by a virus-like shell (Sun et al., 2019)(Figure 1). Elevated bicarbonate then diffuses into the carboxysome through the shell and is dehydrated to CO_2 by CA. Overall, this CCM system concentrates CO_2 around Rubisco up to 1000-fold, promoting Rubisco carboxylation and competitively inhibiting oxygenation.

38 Recent studies on the BicA and SbtA/B transporters and the NDH-1_3 complex from photoautotrophic
39 β-cyanobacteria (Kaczmarski et al., 2019; Schuller et al., 2020; Selim et al., 2018; Wang et al., 2019),
40 as well as the DAB transporters from proteobacteria (Desmarais et al., 2019) and BST1–3
41 transporters from microalgae (Mukherjee et al., 2019) have advanced our understanding of the
42 structures and functions of diverse C_i pumping systems.
43
44 **Cyanobacterial bicarbonate transporters**
45 Cyanobacteria have three C_i transporters, BCT1, SbtA, and BicA. BicA is a Na^+-dependent, low-
46 affinity HCO_3^- transporter belonging to the SulP family of anion transporters and is present in almost
47 all cyanobacteria (Price et al., 2004). BicA contains an N-terminal transmembrane domain (BicA^{TM})
48 and a C-terminal STAS domain (BicA^{STAS}). Wang *et al.* reported the crystal structures of BicA^{TM} and
49 BicA^{STAS} domains from *Synechocystis* sp. PCC 6803 (Wang et al., 2019)(Figure 1). BicA^{TM} showed
50 a cytoplasm-facing conformation with 14 transmembrane helices forming the "7 + 7" fold inverted-
51 topology repeats. The atomic structure indicated a putative HCO_3^-/Na^+−binding hydrophilic pocket
52 in BicA^{TM}, facing the cytoplasm. BicA^{STAS} contains 5 β-strands and 5 α-helices; two BicA^{STAS} domains
53 form a homodimer and mediate dimerisation of BicA, essential for BicA membrane localisation and
54 activity. Biochemical analysis and cryo-electron microscopy further corroborated that BicA is dimeric
55 in solution, a common feature of the SLC26-family transporters. The cytoplasm-facing structure of
56 BicA and previously reported extracellular-facing structures of other SLC26 transporters indicated
57 the conformational dynamics of SLC26-family transporters during HCO_3^- transport, allowing to
58 propose an HCO_3^--transport mechanism of BicA (Wang et al., 2019).
59
60 SbtA is a Na^+-dependent, high-affinity transporter. SbtB is a P_{II}-like signalling protein highly
61 conserved within cyanobacteria. It inhibits SbtA activity via direct interactions. SbtA and SbtB are
62 encoded in a bicistronic operon that is co-upregulated by Ci-limiting conditions. Selim et al. showed
63 that SbtB from *Synechocystis* sp. PCC6803 (*Sc*SbtB) could bind different adenosine nucleotides
64 (ATP, ADP, AMP, and cAMP) and has the highest binding affinity to cAMP (Selim et al., 2018),
65 whereas other P_{II} proteins could bind only ATP or ADP and 2-oxoglutarate. However, the pocket
66 location and residues forming the binding pockets are conserved among P_{II} family members,
67 suggesting these structurally conserved binding pockets bear the flexibility of sensing different
68 adenyl nucleotides. Moreover, the *Sc*SbtB-deficient mutants were highly sensitive to rapid changes
69 in CO_2 levels, indicating its regulatory role in Ci acclimation. Deletion of *sbtB* down-regulated BicA
70 expression but did not affect SbtA expression (Price et al., 2004), suggesting a more general role of
71 SbtB in Ci accumulation in addition to directly regulating SbtA activity. Likewise, SbtB from
72 *Cyanobium* sp. PCC7001 (SbtB7001) has also been revealed to bind to AMP, ADP, ATP, and cAMP
73 (Kaczmarski et al., 2019). Distinct from *Sc*SbtB, SbtB7001 possesses a greater affinity of ATP than
74 AMP, ADP, and cAMP by 5-10 folds. AMP, ADP, and cAMP have little effect on the crystallised SbtB
75 structures, whereas ATP/Ca^{2+}ATP binding induced allosteric rearrangements of the SbtB7001 T-

76 loops, implying a possible mechanism for SbtB−SbtA formation and regulation in cyanobacteria in
77 response to varying adenylate charge ratios in the ecological niches.
78
79 **Photosynthetic Complex I**
80 Cyanobacterial NDH-1 complexes located in the thylakoid membranes exhibit different isoforms
81 varying in functions: 1) NDH-1L and NDH-1L' participate in the cyclic electron flow around
82 Photosystem I to balance the ATP/NADPH ratio; 2) NDH-1MS and NDH-1MS' convert CO_2 into
83 HCO_3^- to accumulate HCO_3^- in the cytosol and prevent CO_2 leakage. The recent cryo-EM structure
84 revealed that NDH-1MS (NDH-1_3) from *Thermosynechococcus elongatus* contains a CO_2-converting
85 CupA/S module on the cytoplasmic side of NDH-1MS via binding with the transmembrane domain
86 NdhF3, confirming the function of NDH-1MS for CO_2 conversion resembling a primarily directional
87 CA (Schuller et al., 2020)(Figure 1). The cryo-EM structure suggested a putative Zn^{2+}-binding pocket
88 in the CupA active site and a CO_2 channel from NdhF3 to the Zn^{2+}-binding site to direct CO_2 from
89 the luminal side across the thylakoid membrane to the CupA CO_2-hydration site, although they need
90 to be confirmed by a higher-resolution structure and physiological measurements. Computational
91 simulations further indicated that CO_2 conversion in NDH-1MS is coupled with a redox-driven H^+-
92 pumping process across thylakoid membranes powered by cyclic electron flow.
93
94 **New Ci pumps in proteobacteria**
95 Desmarais et al. reported the first Ci pumps in the γ-proteobacterium *Halothiobacillus neapolitanus*
96 (Desmarais et al., 2019). Two DAB operons, DAB1 and DAB2, were identified near the carboxysome
97 gene operon, each representing a two-gene locus that encodes DabA1-DabB1 and DabA2-DabB2,
98 respectively. DabB2 is a cation transporter (Pfam:PF00361) and contains 12-13 transmembrane
99 helices, with distant similarity to the NdhF subunit of cyanobacterial NDH-1. DabA2 is a soluble,
100 cytoplasmic protein (Pfam:PF10070) and comprises a Zn^{2+}-binding pocket and a β-CA active site
101 that unidirectionally hydrates CO_2 to HCO_3^-. DabA2 and DabB2 assemble into heterodimers,
102 energetically coupling the CA activity to a cation gradient across the plasma membrane to facilitate
103 Ci accumulation in the cytosol. The DAB operons are widespread in bacteria and archaea, including
104 human pathogens.
105
106 **New bicarbonate transporters in microalgae**
107 Apart from prokaryotic Ci pumps, the recent study by Mukherjee et al. reported three bestrophin-like
108 anion transporters (BST1–3) functioning as putative bicarbonate transporters in chloroplast
109 thylakoids of the green alga *Chlamydomonas reinhardtii* (Mukherjee et al., 2019). Although their
110 exact functions remained to be further determined, BST1–3 appear to transport HCO_3^- to carbonic
111 anhydrase 3 (CAH3) inside the lumen of pyrenoid-traversing thylakoids. Together with LCI1 and
112 HLA3 transporters in the plasma membrane, and NAR1.2/LCIA in the chloroplast envelope, these

Ci transporters may constitute a route of bicarbonate transport towards the pyrenoid, which accommodates Rubisco and CAH3, in algae.

Conclusion and perspectives

Recent studies on the structures and functions of bicarbonate transporters and determining new Ci transporters provide insight into the mechanisms underlying Ci transport, accumulation, activation and regulation in the cell to power carbon assimilation. With the rapid world population growth, improving the efficiency of photosynthetic CO_2 fixation to enhance crop productivity has received increasing attention. As most crop plants lack CCMs, engineering efficient CCM systems into crops is considered as the strategies to improve agricultural yields (Hennacy and Jonikas, 2020; Rae et al., 2017). Comprehensive studies on diverse Ci transporters will offer a range of engineering options, along with carboxysomes, for supercharging crop photosynthesis. Future work will focus on the activation and regulation of Ci transporters as well as their interplay with other CCM components and metabolic networks in the native hosts and transgenic plant chloroplasts.

References

Bar-On, Y.M., and Milo, R. (2019). The global mass and average rate of rubisco. Proceedings of the National Academy of Sciences 116:4738-4743.

Desmarais, J.J., Flamholz, A.I., Blikstad, C., Dugan, E.J., Laughlin, T.G., Oltrogge, L.M., Chen, A.W., Wetmore, K., Diamond, S., Wang, J.Y., et al. (2019). DABs are inorganic carbon pumps found throughout prokaryotic phyla. Nat Microbiol 4:2204–2215.

Field, C.B., Behrenfeld, M.J., Randerson, J.T., and Falkowski, P. (1998). Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. Science 281:237-240.

Hennacy, J.H., and Jonikas, M.C. (2020). Prospects for Engineering Biophysical CO2 Concentrating Mechanisms into Land Plants to Enhance Yields. Annu Rev Plant Biol.

Kaczmarski, J.A., Hong, N.S., Mukherjee, B., Wey, L.T., Rourke, L., Forster, B., Peat, T.S., Price, G.D., and Jackson, C.J. (2019). Structural Basis for the Allosteric Regulation of the SbtA Bicarbonate Transporter by the PII-like Protein, SbtB, from Cyanobium sp. PCC7001. Biochemistry 58:5030-5039.

Mukherjee, A., Lau, C.S., Walker, C.E., Rai, A.K., Prejean, C.I., Yates, G., Emrich-Mills, T., Lemoine, S.G., Vinyard, D.J., Mackinder, L.C.M., et al. (2019). Thylakoid localized

156 bestrophin-like proteins are essential for the CO_2 concentrating mechanism of
157 *Chlamydomonas reinhardtii*. Proc Natl Acad Sci U S A 116:16915-16920.
158 Price, G.D., Woodger, F.J., Badger, M.R., Howitt, S.M., and Tucker, L. (2004). Identification of a
159 SulP-type bicarbonate transporter in marine cyanobacteria. Proc Natl Acad Sci U S A
160 101:18228-18233.
161 Rae, B.D., Long, B.M., Forster, B., Nguyen, N.D., Velanis, C.N., Atkinson, N., Hee, W.Y.,
162 Mukherjee, B., Price, G.D., and McCormick, A.J. (2017). Progress and challenges of
163 engineering a biophysical CO_2-concentrating mechanism into higher plants. J Exp Bot
164 68:3717-3737.
165 Schuller, J.M., Saura, P., Thiemann, J., Schuller, S.K., Gamiz-Hernandez, A.P., Kurisu, G.,
166 Nowaczyk, M.M., and Kaila, V.R.I. (2020). Redox-coupled proton pumping drives carbon
167 concentration in the photosynthetic complex I. Nature Communications 11:494.
168 Selim, K.A., Haase, F., Hartmann, M.D., Hagemann, M., and Forchhammer, K. (2018). PII-like
169 signaling protein SbtB links cAMP sensing with cyanobacterial inorganic carbon response.
170 Proc Natl Acad Sci U S A 115:E4861-E4869.
171 Sun, Y., Wollman, A.J.M., Huang, F., Leake, M.C., and Liu, L.N. (2019). Single-organelle
172 quantification reveals the stoichiometric and structural variability of carboxysomes
173 dependent on the environment. Plant Cell 31:1648-1664.
174 Wang, C., Sun, B., Zhang, X., Huang, X., Zhang, M., Guo, H., Chen, X., Huang, F., Chen, T., Mi,
175 H., et al. (2019). Structural mechanism of the active bicarbonate transporter from
176 cyanobacteria. Nature Plants 5:1184-1193.
177
178

Figure Legend
Fig. 1. Schematic model of cyanobacterial CO$_2$-concentrating mechanisms. Cyanobacterial

CCM consists of three bicarbonate transporters BicA, SbtA, and BCT1 in the plasma membrane

(BicA is shown, PDB ID: 6KI1 and 6KI2)(Wang et al., 2019), the NDH-1MS complex (PDB ID: 6TJV)

in thylakoid membranes (Schuller et al., 2020), as well as carboxysomes in the cytoplasm.

Figure 1