

1 **Photosynthesis in Phytoplankton: Insights from the Newly** 2 **Discovered Biological Inorganic Carbon Pumps**

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17 Aquatic CO₂ assimilation results in storage of ~24% of anthropogenic CO₂ (~40 petagrams per
18 annum) released into the atmosphere in the oceans and make significant contributions to global
19 carbon cycle. These processes are executed predominantly in phytoplankton in the oceans
20 (including cyanobacteria), which account for nearly 50% of global primary productivity (~50 gigatons
21 per annum) (Field et al., 1998).

22
23 Powered by sunlight, the Calvin–Benson-Bassham cycle converts CO₂ into organic compounds to
24 drive life in the biosphere. The key enzyme for assimilating inorganic carbon (C_i) is ribulose-1,5-
25 bisphosphate carboxylase/oxygenase (Rubisco), the most abundant protein on Earth (Bar-On and
26 Milo, 2019). However, Rubisco is surprisingly inefficient, given its slow catalytic rate and poor
27 capability in discriminating between CO₂ and O₂. To cope with ancient changes in atmospheric CO₂
28 and O₂ levels, phytoplankton have evolved efficient CO₂-concentrating mechanisms (CCMs) to
29 accumulate CO₂ around Rubisco. Cyanobacterial CCMs comprise bicarbonate transporters in the
30 plasma membranes and CO₂-converting complexes in thylakoid membranes to accumulate
31 bicarbonate in the cytoplasm and prevent diffusive CO₂ leakage from the cell, as well as the CO₂-
32 fixing organelles in the cytoplasm—carboxysomes—that encapsulate Rubisco and carbonic
33 anhydrase (CA) by a virus-like shell (Sun et al., 2019)(Figure 1). Elevated bicarbonate then diffuses
34 into the carboxysome through the shell and is dehydrated to CO₂ by CA. Overall, this CCM system
35 concentrates CO₂ around Rubisco up to 1000-fold, promoting Rubisco carboxylation and
36 competitively inhibiting oxygenation.

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38 Recent studies on the BicA and SbtA/B transporters and the NDH-1₃ complex from photoautotrophic
39 β-cyanobacteria (Kaczmarek 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 HCO₃⁻ 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 (BicATM)
48 and a C-terminal STAS domain (BicA^{STAS}). Wang *et al.* reported the crystal structures of BicATM and
49 BicA^{STAS} domains from *Synechocystis* sp. PCC 6803 (Wang et al., 2019)(Figure 1). BicATM 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 HCO₃⁻/Na⁺-binding hydrophilic pocket
52 in BicATM, 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 HCO₃⁻ transport, allowing to
58 propose an HCO₃⁻-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 C_i-limiting conditions. Selim et al. showed
63 that SbtB from *Synechocystis* sp. PCC6803 (ScSbtB) 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 adenylylated nucleotides. Moreover, the ScSbtB-deficient mutants were highly sensitive to rapid changes
69 in CO₂ levels, indicating its regulatory role in C_i 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 C_i 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 (Kaczmarek et al., 2019). Distinct from ScSbtB, 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/Ca²⁺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 CO₂ into
83 HCO₃⁻ to accumulate HCO₃⁻ in the cytosol and prevent CO₂ leakage. The recent cryo-EM structure
84 revealed that NDH-1MS (NDH-1₃) from *Thermosynechococcus elongatus* contains a CO₂-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 CO₂ conversion resembling a primarily directional
87 CA (Schuller et al., 2020)(Figure 1). The cryo-EM structure suggested a putative Zn²⁺-binding pocket
88 in the CupA active site and a CO₂ channel from NdhF3 to the Zn²⁺-binding site to direct CO₂ from
89 the luminal side across the thylakoid membrane to the CupA CO₂-hydration site, although they need
90 to be confirmed by a higher-resolution structure and physiological measurements. Computational
91 simulations further indicated that CO₂ 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 Zn²⁺-binding pocket and a β-CA active site
101 that unidirectionally hydrates CO₂ to HCO₃⁻. 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 HCO₃⁻ 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

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

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116

117 **Conclusion and perspectives**

118 Recent studies on the structures and functions of bicarbonate transporters and determining new Ci
119 transporters provide insight into the mechanisms underlying Ci transport, accumulation, activation
120 and regulation in the cell to power carbon assimilation. With the rapid world population growth,
121 improving the efficiency of photosynthetic CO₂ fixation to enhance crop productivity has received
122 increasing attention. As most crop plants lack CCMs, engineering efficient CCM systems into crops
123 is considered as the strategies to improve agricultural yields (Hennacy and Jonikas, 2020; Rae et
124 al., 2017). Comprehensive studies on diverse Ci transporters will offer a range of engineering options,
125 along with carboxysomes, for supercharging crop photosynthesis. Future work will focus on the
126 activation and regulation of Ci transporters as well as their interplay with other CCM components
127 and metabolic networks in the native hosts and transgenic plant chloroplasts.

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179 **Figure Legend**

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181 **Fig. 1. Schematic model of cyanobacterial CO₂-concentrating mechanisms.** Cyanobacterial
182 CCM consists of three bicarbonate transporters BicA, SbtA, and BCT1 in the plasma membrane
183 (BicA is shown, PDB ID: 6KI1 and 6KI2)(Wang et al., 2019), the NDH-1MS complex (PDB ID: 6TJV)
184 in thylakoid membranes (Schuller et al., 2020), as well as carboxysomes in the cytoplasm.

Figure 1

