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Spotlight

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

Aquatic CO_2 assimilation results in storage in the oceans of ~24% of anthropogenic CO_2 (~40 petagrams per annum) released into the atmosphere and makes significant contributions to the 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.

Recent studies on the BicA and SbtA/B transporters and the NDH-1_3 complex from photoautotrophic β-cyanobacteria (Selim et al., 2018; Kaczmarski et al., 2019; Wang et al., 2019; Schuller et al., 2020), as well as the DAB transporters from proteobacteria (Desmarais et al., 2019) and BST1–BST3 transporters from microalgae (Mukherjee et al., 2019), have advanced our understanding of the structures and functions of diverse C_i pumping systems.

Cyanobacterial Bicarbonate Transporters

Cyanobacteria have three C_i transporters, BCT1, SbtA, and BicA. BicA is a Na^+-dependent, low-affinity HCO_3^- transporter belonging to the SulP family of anion transporters and is present in almost all cyanobacteria (Price et al., 2004). BicA contains an N-terminal transmembrane domain ($BicA^{TM}$) and a C-terminal STAS domain ($BicA^{STAS}$). Wang et al. (2019) reported the crystal structures of $BicA^{TM}$ and $BicA^{STAS}$ domains from *Synechocystis* sp. PCC 6803 (Figure 1). $BicA^{TM}$ showed a cytoplasm-facing conformation with 14 transmembrane helices forming the "7 + 7" fold inverted-topology repeats. The atomic structure indicated a putative HCO_3^-/Na^+-binding hydrophilic pocket in $BicA^{TM}$, facing the cytoplasm. $BicA^{STAS}$ contains five β strands and five α helices; two $BicA^{STAS}$ domains form a homodimer and mediate dimerization of BicA, essential for BicA membrane localization and activity. Biochemical analysis and cryoelectron microscopy (cryo-EM) further corroborated that BicA is dimeric in solution, a common feature of the SLC26-family transporters. The cytoplasm-facing structure of BicA and previously reported extracellular-facing structures of other SLC26 transporters indicated the conformational dynamics of SLC26-family transporters during HCO_3^- transport, allowing to propose an HCO_3^--transport mechanism of BicA (Wang et al., 2019).

SbtA is a Na^+-dependent, high-affinity transporter. SbtB is a P_{II}-like signaling protein highly conserved within cyanobacteria. It inhibits SbtA activity via direct interactions. SbtA and SbtB are encoded in a bicistronic operon that is co-upregulated by C_i-limiting conditions. Selim et al. (2018) showed that SbtB from *Synechocystis* sp. PCC6803 (*Sc*SbtB) could bind different adenosine nucleotides (ATP, ADP, AMP, and cyclic AMP [cAMP]) and has the highest binding affinity to cAMP, whereas other P_{II} proteins could bind only ATP or ADP and 2-oxoglutarate. However, the pocket location and residues forming the binding pockets are conserved among P_{II} family members, suggesting that these structurally conserved binding pockets bear the flexibility of sensing different adenyl nucleotides. Moreover, the *Sc*SbtB-deficient mutants were highly sensitive to rapid changes in CO_2 levels, indicating its regulatory role in C_i acclimation. Deletion of *sbtB* downregulated BicA expression but did not affect SbtA expression (Price et al., 2004), suggesting a more general role of SbtB in C_i accumulation in addition to directly regulating SbtA activity. Likewise, SbtB from *Cyanobium* sp. PCC7001 (SbtB7001) has also been revealed to bind to AMP, ADP, ATP, and cAMP (Kaczmarski et al., 2019). Distinct from *Sc*SbtB, SbtB7001 possesses a greater affinity of ATP than AMP, ADP, and cAMP by 5- to 10-fold. AMP, ADP, and cAMP have little effect on the crystallized SbtB structures, whereas ATP/Ca^{2+}ATP binding induced allosteric rearrangements of the SbtB7001 T loops, implying a possible mechanism for SbtB–SbtA formation and regulation in

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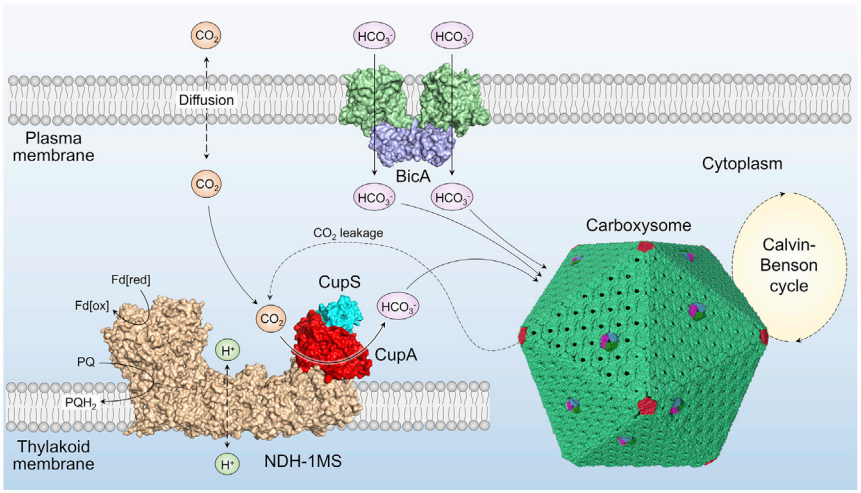

Figure 1. Schematic Model of Cyanobacterial CO_2-Concentrating Mechanisms.
Cyanobacterial CCM consists of three bicarbonate transporters BicA, SbtA, and BCT1 in the plasma membrane (BicA is shown, PDB: 6KI1 and 6KI2) (Wang et al., 2019), the NDH-1MS complex (PDB: 6TJV) in thylakoid membranes (Schuller et al., 2020), and carboxysomes in the cytoplasm. PQ, plastoquinone; PQH_2, plastoquinol.

cyanobacteria in response to varying adenylate charge ratios in the ecological niches.

Photosynthetic Complex I

Cyanobacterial NDH-1 complexes located in the thylakoid membranes exhibit different isoforms varying in function: (1) NDH-1L and NDH-1L′ participate in the cyclic electron flow around Photosystem I to balance the ATP/NADPH ratio; (2) NDH-1MS and NDH-1MS′ convert CO_2 into HCO_3^- to accumulate HCO_3^- in the cytosol and prevent CO_2 leakage. The recently published cryo-EM structure revealed that NDH-1MS (NDH-1_3) from *Thermosynechococcus elongatus* contains a CO_2-converting CupA/S module on the cytoplasmic side of NDH-1MS via binding with the transmembrane domain NdhF3, confirming the function of NDH-1MS for CO_2 conversion resembling a primarily directional CA (Schuller et al., 2020) (Figure 1). The cryo-EM structure suggested a putative Zn^{2+}-binding pocket in the CupA active site and a CO_2 channel from NdhF3 to the Zn^{2+}-binding site to direct CO_2 from the luminal side across the thylakoid membrane to the CupA CO_2-hydration site, although these need to be confirmed by a higher-resolution structure and physiological measurements. Computational simulations further indicated that CO_2 conversion in NDH-1MS is coupled with a redox-driven H^+-pumping process across thylakoid membranes powered by cyclic electron flow.

New C_i Pumps in Proteobacteria

Desmarais et al. reported the first C_i pumps in the γ-proteobacterium *Halothiobacillus neapolitanus* (Desmarais et al., 2019). Two DAB operons, DAB1 and DAB2, were identified near the carboxysome gene operon, each representing a two-gene locus that encodes DabA1–DabB1 and DabA2–DabB2, respectively. DabB2 is a cation transporter (Pfam: PF00361) and contains 12–13 transmembrane helices, with distant similarity to the NdhF subunit of cyanobacterial NDH-1. DabA2 is a soluble, cytoplasmic protein (Pfam: PF10070) and comprises a Zn^{2+}-binding pocket and a β-CA active site that unidirectionally hydrates CO_2 to HCO_3^-. DabA2 and DabB2 assemble into heterodimers, energetically coupling the CA activity to a cation gradient across the plasma membrane to facilitate C_i accumulation in the cytosol. The DAB operons are widespread in bacteria and archaea, including human pathogens.

New Bicarbonate Transporters in Microalgae

Apart from prokaryotic C_i pumps, the recent study by Mukherjee et al. (2019) reported three bestrophin-like anion transporters (BST1–BST3) functioning as putative bicarbonate transporters in chloroplast thylakoids of the green alga *Chlamydomonas reinhardtii*. Although their exact functions remained to be further determined, BST1–BST3 appear to transport HCO_3^- to carbonic anhydrase 3 (CAH3) inside the lumen of pyrenoid-traversing thylakoids. Together with LCI1 and HLA3 transporters in the plasma membrane, and NAR1.2/LCIA in the chloroplast envelope, these C_i transporters may constitute a route of bicarbonate transport toward the pyrenoid, which accommodates Rubisco and CAH3, in algae.

Perspectives

Recent studies on the structures and functions of bicarbonate transporters and determination of new C_i transporters provide insight into the mechanisms underlying C_i transport, accumulation, activation, and regulation in the cell to power carbon assimilation. With the rapid growth in the world's population, 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 a promising strategy to improve agricultural yields (Rae et al., 2017; Hennacy and Jonikas, 2020). Comprehensive studies on diverse C_i 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 C_i transporters as well as their interplay with other CCM components and metabolic networks in the native hosts and transgenic plant chloroplasts.

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