Trends in Plant Science

CellPress REVIEWS

Review

Carbon/Nitrogen Metabolic Balance: Lessons from Cyanobacteria

Cheng-Cai Zhang,[1,5,*] Cong-Zhao Zhou,[2] Robert L. Burnap,[3] and Ling Peng[4]

Carbon and nitrogen are the two most abundant nutrient elements for all living organisms, and their metabolism is tightly coupled. What are the signaling mechanisms that cells use to sense and control the carbon/nitrogen (C/N) metabolic balance following environmental changes? Based on studies in cyanobacteria, it was found that 2-phosphoglycolate derived from the oxygenase activity of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) and 2-oxoglutarate from the Krebs cycle act as the carbon- and nitrogen-starvation signals, respectively, and their concentration ratio likely reflects the status of the C/N metabolic balance. We will present and discuss the regulatory principles underlying the signaling mechanisms, which are likely to be conserved in other photosynthetic organisms. These concepts may also contribute to developments in the field of biofuel engineering or improvements in crop productivity.

Highlights

Cyanobacteria are simple models among photosynthetic organisms for studying the metabolic regulation of carbon/nitrogen (C/N), the two most abundant nutrient elements for all living organisms.

2-Oxoglutarate (2-OG), an intermediate from the Krebs cycle, serves as a carbon skeleton for nitrogen assimilation and as a signal of nitrogen limitation, whereas 2-phosphoglycolate (2-PG), an intermediate from photorespiration, acts as a signal of inorganic carbon limitation.

The levels of 2-PG and 2-OG are inversely correlated, and their ratio reflects the C/N metabolic balance.

The transcriptional activator NtcA is a sensor of 2-OG, and the transcriptional repressor NdhR is a sensor of both 2-OG and 2-PG. These regulators together balance C/N metabolic networks by switching on or off the expression of genes involved mainly in the uptake and assimilation of carbon and nitrogen sources.

The signaling role of 2-OG and 2-PG in C/N balance is likely conserved in other photosynthetic organisms.

Importance of Carbon/Nitrogen (C/N) Balance

Carbon and nitrogen metabolism is essential for every biological system, since all major cellular components, including genetic materials, proteins, pigments, energy carrier molecules, etc., are derived from these activities. Carbon and nitrogen metabolism are tightly coupled in different living organisms. In prokaryotes and plants, such a coupling mechanism can be attributed to two major factors. First, the two elements are the most abundant in cells, intensifying the requirement for coordination mechanisms to avoid metabolic inefficiencies; second, nitrogen assimilation depends on the availability of a carbon skeleton for biosynthesis, and consequently the limitation or oversupply of one element strongly affects the metabolism of the other. Therefore, the C/N stoichiometry in different organisms varies within a relatively narrow range; for example, the mass ratio is C/N = 31/4 in phytoplankton [1].

Because properly balanced metabolism of carbon and nitrogen is necessary for optimal growth, different levels of regulation exist in cells in order to control the uptake and assimilation of various nitrogen and carbon sources whose supply may vary under different environmental conditions [2–4]. Such regulation may occur at various levels of control, ranging from the allosteric modulation of the activity of nutrient assimilation proteins to a variety of mechanisms controlling the expression of genes encoding these structural proteins. The latter includes a rich variety of transcriptional and post-transcriptional mechanisms, some of which are still being experimentally clarified [5–7].

Whatever the type of regulation involved, two major questions arise: how do cells sense the metabolic status, and what signal transduction mechanisms do cells use to maintain a proper balance of nitrogen and carbon metabolism? Recently, tremendous progress has been made towards answering these questions by using cyanobacteria as a model for photosynthetic organisms, and this review serves to summarize the data and highlight the mechanisms

[1]Key Laboratory of Algal Biology, Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan, Hubei 430072, People's Republic of China
[2]School of Life Sciences and Hefei National Laboratory for Physical Sciences at the Microscale, University of Science and Technology of China, Hefei, Anhui, 230027, People's Republic of China
[3]Department of Microbiology and Molecular Genetics, Henry Bellmon Research Center, Oklahoma State

© 2018 Elsevier Ltd. All rights reserved.

underlying the signaling and transcriptional regulation involved in carbon and nitrogen metabolic control. We will also discuss the concept for controlling C/N metabolic balance. Cyanobacteria are the ancestors of plastids [8]; therefore, cyanobacteria and plants may share common features, especially in terms of the signaling mechanisms, for balancing C/N metabolism.

Coupling between Carbon and Nitrogen Metabolic Pathways

To facilitate understanding, we briefly summarize here the metabolic coupling of nitrogen and carbon assimilation in cyanobacteria (Figure 1). As for other autotrophic photosynthetic organisms, CO_2 is the primary carbon source for cyanobacteria, although some cyanobacterial strains can also use a sugar or other organic carbon as the carbon source [4,9,10]. However, as with eukaryotic algae, the availability of CO_2 is often growth-limiting because of specific physical factors, such as the low diffusion rates in the aqueous environment. Moreover, dissolved CO_2 chemically speciates into several forms of inorganic carbon, according to the general reaction describing the inorganic carbon pool:

$$\mathbf{CO_2} + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons \mathbf{HCO_3^-} + H^+ \rightleftharpoons CO_3^{2-} + 2H^+ \quad [1]$$

Note that the equilibrium of the reaction is strongly pH dependent such that the fraction of the total inorganic pool existing as CO_2 is favored by acidic conditions, whereas bicarbonate HCO_3^- dominates under neutral and alkaline conditions. CO_3^{2-} can be ignored in general as it

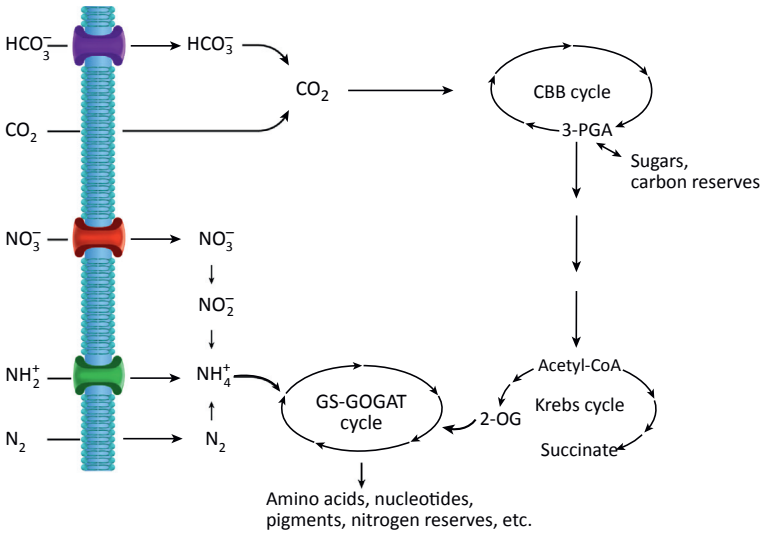


Figure 1. Coupling between Carbon and Nitrogen Metabolism. Inorganic carbon, as CO_2 or HCO_3^-, enters the cells and it is in the form of CO_2 that is assimilated into organic carbons through the Calvin-Benson-Bassham (CBB) cycle. Note that the uptake of CO_2 is energetically driven by specialized forms of the NDH-1 complex in thylakoid membranes and results in the formation of HCO_3^- which is transported into Rubisco-containing carboxysomes before being reconverted to CO_2 (see text for details). Fixed carbon through the CBB cycle is further used for biosynthesis or to feed the Krebs cycle incomplete in cyanobacteria in general due to the lack of the 2-oxoglutarate (2-OG) dehydrogenase], which produces intermediates for a variety of carbon compounds, including lipids. Different forms of inorganic nitrogen can be used by cyanobacteria, and are assimilated in the form of NH_4^+ through the glutamine synthetase-glutamate synthase (GS-GOGAT) cycle using 2-OG as a carbon skeleton. Glu and Gln, the two amino acids produced from the GS-GOGAT cycle are also important nitrogen donors for the synthesis of a variety of nitrogen-containing compounds. 3-PGA, 3-Phosphoglycerate.

University, Stillwater, OK 74078, USA
[4]Aix-Marseille Université, CNRS, Centre Interdisciplinaire de Nanoscience de Marseille, Equipe Labellisée Ligue Contre le Cancer, CINaM UMR 7325, 13288 Marseille, France
[5]Aix-Marseille Université, CNRS, LCB, France

*Correspondence:
cczhang@ihb.ac.cn (C.-C. Zhang).

exists only in strong alkaline condition, which is seldom the case within normal cells. Cyanobacteria and algae are typically capable of acquiring either CO_2 or HCO_3^-. Because both forms may be acquired and are rapidly interchanged within the cell, the symbol C_i is often utilized to refer to total inorganic carbon (i.e., $C_i = CO_2 + HCO_3^-$) (Figure 1). CO_2 fixation is catalyzed by Rubisco in the Calvin-Benson-Bassham (CBB) cycle. The reaction involves the carboxylation, using CO_2 and ribulose 1,5-biphosphate (RuBP), yielding two molecules of the three carbon 3-phosphoglycerate (3-PGA) [11–13]. However, Rubisco has a notoriously low affinity for CO_2 ($Km > 150$ μM) and, problematically, the enzyme also catalyzes the competing and wasteful oxygenase reaction between RuBP and O_2, which yields one molecule of the two-carbon 2-phosphoglycolate (2-PG) and one 3-PGA rather the two 3-PGA molecules of the carboxylation reaction. The oxygenase reaction has resulted in the evolution of scavenging photorespiratory pathways, which recover and recycle the carbon that would otherwise be lost [11,13]. As a consequence of these factors, carbon fixation in cyanobacteria critically depends upon the so-called CO_2-concentrating mechanism (CCM) [4,14,15]. Cyanobacteria actively acquire C_i, either as HCO_3^- or as CO_2, using a complex set of transporters and uptake enzymes. Bicarbonate uptake involves active membrane transporters, such as the sodium-dependent bicarbonate transporter, SbtA or BicA. Energized CO_2 uptake occurs via the enzymatic hydration of CO_2 catalyzed by specialized forms of the NDH-1 complex located in thylakoid membrane. These complexes use redox energy to drive the conversion of CO_2 to HCO_3^- in the cytoplasm and simultaneously draw in CO_2 from the environment. Accordingly, these are not simple carbonic anhydrases, but enzymes capable of using metabolic energy to create a disequilibrium in favor of HCO_3^- accumulation. Together, these systems produce and maintain high concentrations of HCO_3^- in the cytoplasm to supply the carboxysome, which encloses the entire cellular complement of Rubisco within a protein shell. The carboxysome is the first identified member of a large class of prokaryotic structures called 'bacterial microcompartments', which generally partition specific metabolic reactions within the cytoplasm of the bacterial species that possess them. In the case of the carboxysome, RuBP and HCO_3^- are imported through specific pores in the protein shell and 3-PGA is exported through pores in the shell to supply the CBB cycle, although the details of these transport process are only beginning to emerge. Similarly, the oxygenase reaction product, 2-PG, must also be able to diffuse out of the carboxysome, although again, the precise mechanisms for passage through the protein shell of the carboxysome remain to be elucidated [14]. Importantly, the carboxysome also contains a carbonic anhydrase with functions to convert imported HCO_3^- into CO_2 in the proximity of Rubisco. Therefore, given an abundant supply of HCO_3^-, the active sites of Rubisco with the carboxysome are saturated with CO_2, which enables high rates of CO_2 fixation. This enormously diminishes the competing reaction with O_2; however under conditions of C_i limitation or in mutants that fail to accumulate sufficient HCO_3^- in the cytoplasm, significant rates of the oxygenase reaction have been observed to occur [11,13]. Thus, the CCM relies on the ability of the C_i-uptake systems to concentrate high concentrations of HCO_3^- within the cytoplasm in order to create a steep concentration gradient that promotes rapid net diffusion of HCO_3^- into the carboxysome. A substantial proportion of 3-PGA, produced by the CBB cycle, is converted to acetyl-CoA, which feeds the essential central metabolic pathway, the Krebs cycle (also known as the citric acid cycle, tricarboxylic acid cycle, or TCA cycle), thus leading to the production of intermediates for biosynthesis [4,13,16,17] (Figure 1). As discussed below, the coordination of N and C metabolism depends, in part, upon cytoplasmic concentrations of several metabolites affected by the efficiency of the CO_2-fixation reactions.

Cyanobacteria can use different inorganic nitrogen sources, mostly in the form of nitrate or ammonium. Many cyanobacterial strains can synthesize the enzyme nitrogenase for

N_2 fixation [3,9]. Whatever the form of inorganic nitrogen that is taken up into the cells, it is always converted into ammonium, the least costly nitrogen form for assimilation (Figure 1). Nitrate requires two additional enzymes, nitrate reductase and nitrite reductase, and reduced cofactors, in order to be reduced to ammonium, whereas N_2 fixation is even more energetically expensive, requiring both reduced cofactors and up to 16 ATP molecules per ammonium. Moreover, it depends on the nitrogenase complex which needs to operate under micro-oxic conditions, thereby requiring special adaptations. Some filamentous cyanobacteria, such as *Anabaena*/*Nostoc* PCC 7120, can form heterocysts, intercalated among vegetative cells, to provide a micro-oxic intracellular environment for N_2 fixation; alternatively, N_2 fixation and photosynthetic O_2 evolution occur in the same cell, but the activities are diurnally regulated using a circadian clock so that N_2 fixation is restricted to the night [3,18–20]. Hierarchically, ammonium represses the use of alternative nitrogen sources, and heterocyst differentiation and N_2 fixation take place only when neither ammonium nor nitrate is available in the growth medium.

Two ammonium-assimilation pathways are known, the glutamine synthetase-glutamate synthase (GS-GOGAT) cycle, and direct assimilation through the reaction catalyzed by glutamate dehydrogenase (GDH). Both pathways require the carbon skeleton 2-oxoglutarate (2-OG, also known as α-ketoglutarate), one of the intermediates of the Krebs cycle [21,22] (Figure 1). Therefore, ammonium assimilation using 2-OG provides the metabolic basis for coupling between nitrogen and carbon metabolism. In the GS-GOGAT cycle, GS assimilates ammonium using glutamate and ATP to synthesize glutamine, while GOGAT catalyzes glutamate formation using glutamine, 2-OG, and NADPH. The second route for ammonium assimilation, catalyzed by GDH using 2-OG, does not play a major role in cyanobacteria, and the majority of cyanobacterial species whose genomes have been sequenced do not even contain the gene encoding GDH [21,23]. Thus, the GS-GOGAT cycle constitutes the major pathway for ammonium assimilation in cyanobacteria, and hence the central crossroad for carbon and nitrogen metabolism (Figure 1). Glutamine and glutamate are also the major intracellular amino group donors for the synthesis of several other amino acids, and for purine and pyrimidine nucleobases, as well as other nitrogen-containing compounds [24,25].

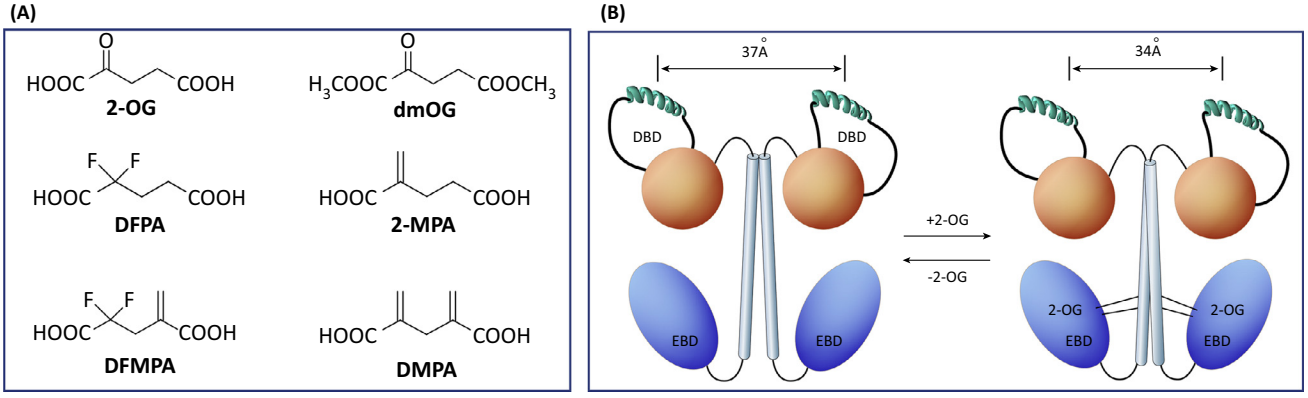
Both carbon and nitrogen assimilation are under multiple control mechanisms depending on the availability or the nature of the nutrients in the environment. To understand how cyanobacteria regulate nitrogen and carbon metabolism, we will first focus on the nature of the signals that allow cells to sense the intracellular metabolic status when nitrogen and carbon metabolism becomes unbalanced, and then we will review the signaling mechanisms that allow cells to regain their metabolic balance in order to better adapt to environmental changes. While the focus of this analysis is based upon the transcriptional regulation, it is important to note that researchers performing global transcriptome and proteome analyses have observed significant differences between fold-changes in transcript and the corresponding protein levels in cyanobacteria [26,27]. This indicates the existence of post-transcriptional factors in the control of gene expression in cyanobacteria. However, it is also important to note that there is generally a good qualitative correspondence between the levels of transcripts and proteins, with the latter typically exhibiting more muted fold changes in response to environmental changes.

The Nitrogen-Starvation Signal 2-OG and Its Receptors

It has been postulated for a while that 2-OG acts as a signal in the control of nitrogen metabolism because of its role as a carbon skeleton in nitrogen assimilation and because its levels can vary according to nitrogen availability [28,29] (Figure 1). Indeed, in both the filamentous heterocyst-forming diazotrophic cyanobacterium *Anabaena* PCC 7120 and the

unicellular non-diazotrophic strain *Synechocystis* PCC 6803, 2-OG accumulates rapidly upon limitation of nitrogen supply in the growth medium [28,29]. A signaling function for 2-OG has also been proposed in a variety of biological systems, including bacteria, animals, and humans [2,25,30–35]. In proteobacteria, for example, 2-OG is involved in the control of metabolism [25,36], although the signaling mechanism is in many ways different from that in cyanobacteria, as will be discussed later in this review.

Because 2-OG is a critical metabolite rapidly incorporated into a variety of N-containing compounds, one crucial question is how to experimentally discriminate its metabolic function from its signaling function, and how its direct effects are distinguished from the indirect effects of its metabolic derivatives. One way to tackle this problem is through the use of non-metabolizable analogs, in which a stable bioisostere function replaces the keto group in 2-OG, the site of amidation in ammonium assimilation in the GS-GOGAT cycle. Specifically, several nonmetabolizable analogs of 2-OG were chemically synthesized and tested in *Anabaena* PCC 7120 [29,37–40]. This organism was chosen for these tests because it has, under nitrogen-limited conditions, a phenotype (formation of N_2-fixing heterocysts) which is easily observable under a light microscope [3,18]. Among the synthesized non-metabolizable analogs of 2-OG, 2,2-difluoropentanedioic acid (DFPA) is particularly interesting (Figure 2). DFPA was designed to mimic 2-OG by replacing the keto group with the stable and nonmetabolizable fluorinated methylene entity [29]. Since fluorine is in general absent in biological systems and cells, fluorinated compounds can be readily tracked in intact cells using ^{19}F-NMR, with little or no background interference [41,42]. Accumulation of DFPA in filaments of *Anabaena* PCC 7120 acts as a trigger for heterocyst differentiation, even when ammonium, which normally represses heterocyst differentiation, is present in the growth medium. DFPA can be recognized by both a 2-OG permease, KgtP, and a 2-OG receptor, NtcA [29,43]. These results provided the first *in vivo* evidence for a direct role of 2-OG as a nitrogen starvation signal in cyanobacteria.



Trends in Plant Science

Figure 2. 2-Oxoglutarate (2-OG), Its Analogs, and Action of Mechanism on NtcA, a Transcription Factor. (A) 2-OG and its analogs that can be used for the study of 2-OG regulation. DFPA, 2,2-Difluoropentanedioic acid, a fluorinated nonmetabolizable analog of 2-OG, which can be traced *in vivo* by ^{19}F-NMR; DFMPA, a hybrid of DFPA and 2-MPA; DMPA, an analog of 2-OG that can be conjugated to resins for enrichment of 2-OG-binding proteins; dmOG, dimethyl 2-oxoglutarate, a membrane permeable analog of 2-OG; 2-MPA, a mimic of the ketone form of 2-OG; 2-OG, 2-oxoglutarate. (B) Schematic representation on the signaling mechanism of NtcA based on structural studies. NtcA is a homodimer and a transcriptional activator, each subunit containing an effector-binding domain (EBD) and a DNA-binding domain (DBD). The two helices in the DBDs (in green) interact with target DNA. When 2-OG binds to NtcA at the EBDs, structural conformation changes are induced, shortening the distance between the two DNA-binding helices from 37 to 34 Å, optimal for interaction with DNA and thus transcriptional control. DFMPA, 2-difluoromethylene-4-methylenepentanoic acid; DMPA, 2,4-dimethylenepentanedioic acid; 2-MPA, 2-methylenepentanoic acid.

Two 2-OG receptors have been known for a while in cyanobacteria, NtcA and PII [2,3]. The third, NdhR, was more recently established as a 2-OG receptor [44–46]. Here we will first focus on NtcA and PII, because NdhR is also a receptor of 2-PG [46–48], and will be discussed later in this review as a key element in C/N metabolic balance.

PII is a trimeric regulatory protein that is highly conserved in both bacteria and plants [2,35,49]. PII exerts its function mainly through protein–protein interactions mediated by the flexible and outward-extended T-loop present on each monomer. The binding of 2-OG determines the conformation of the T-loop, and hence the interaction with the partners in nitrogen metabolism [50]. Among the interacting partners of PII are PipX, which is involved in NtcA-mediated transcriptional control by acting as a coactivator of NtcA, and NAGK (N-acetyl-L-glutamate kinase), a key enzyme for arginine synthesis [51,52]. The interaction of PII with either PipX or NAGK occurs under nitrogen sufficiency, and thus when the intracellular 2-OG level is low [51–54]. PII relieves the feedback inhibition of Arg on the activity of NAGK, resulting in an increase of Arg for cyanophycin synthesis as a nitrogen reserve as a consequence of nitrogen sufficiency [52,53]; at the same time, PII sequesters PipX, preventing its role as a coactivator of NtcA [51,54]. Several reviews dealing with the detailed mechanism of PII signaling are available for readers interested in more details of this topic [2,35,49]. Importantly, additional proteins in the PII-family are recently reported as being involved in carbon assimilation [7,55]. The small PII-type protein StbB is physically linked and coexpressed with the bicarbonate uptake transporter, StbA. Structural and physiological analyses indicate that this protein is involved in cAMP sensing and responds to cyanobacterial inorganic carbon status. This indicates the existence of a direct regulatory connection between inorganic carbon availability and broader carbon metabolism. These findings resonate with the fact that C/N balance in other bacteria, such as *Escherichia coli*, involves cAMP [25,33]; indeed, cAMP participates in carbon catabolite repression and the cAMP levels are themselves regulated by 2-OG. While it remains to be seen whether this also applies to cyanobacteria, it points to the possibility that new modes of regulation affecting C/N balance may still be found.

NtcA is a dimeric global transcriptional factor belonging to the CRP/CAP family [3]. NtcA activates the expression of genes required for heterocyst development in *Anabaena* PCC 7120 and the use of alternative nitrogen sources under nitrogen deprivation. Recent structural studies of NtcA from both *Anabaena* PCC 7120 and *Synechocystis* PCC 6803 provide further insight into the molecular mechanism of 2-OG signaling mediated through NtcA [43,56]. Specifically, each NtcA monomer harbors a 2-OG binding pocket, and 2-OG binding generates two allosteric effects in NtcA: first, there is an increase in the contact area between the two monomers through the two long C-helices, and then this change propagates towards the DNA-binding domain by shortening the distance between the two helices responsible for DNA binding (Figure 2). In the native or apo form of NtcA, the distance between the two DNA-binding helices is about 37 Å, whereas in the 2-OG-bound form, it becomes 34 Å, which corresponds to the distance between successive DNA major grooves [43]. Furthermore, transcriptional activity assays *in vitro* also reveal that interaction of 2-OG-free NtcA with its target promoters is not sufficient for activation of gene transcription, and 2-OG binding to NtcA is necessary to initiate the process of transcriptional control [57].

Based on all information available so far, a molecular model of the signaling mechanism in response to nitrogen starvation can be proposed. Upon nitrogen deprivation, 2-OG accumulates rapidly in cells; it binds to PII and regulates nitrogen metabolism by protein–protein interactions on the one hand, and on the other hand it interacts with NtcA, which activates genes involved in the uptake and assimilation of alternative nitrogen sources (Figure 2). For

those cyanobacteria able to form heterocysts, such as *Anabaena* PCC 7120, 2-OG also serves as a trigger for the initiation of heterocysts, which fix atmospheric N_2 using nitrogenase [29,58].

Evidence for 2-PG as a Signal of Carbon Starvation

CO_2 assimilation is catalyzed by Rubisco, the most abundant enzyme on earth. As mentioned above, CO_2 and O_2 are competitive substrates for the reaction with RuBP in the active site of Rubisco [11–13] (Figure 3). Through the carboxylase activity of Rubisco, CO_2 is combined with RuBP to produce 3-PGA; the remainder of the CBB cycle serves to regenerate RuBP and consumes ATP as energy and NADPH as reducing power. Part of the 3-PGA pool is recycled back to RuBP through the CBB pathway, while the rest of the 3-PGA is used to make sugar to feed carbon metabolism and the Krebs cycle (Figure 3). Through the oxygenase activity of Rubisco, each RuBP molecule is converted to one molecule of 2-PG and one molecule of 3-PGA. 2-PG, being toxic, is then metabolized by the so-called photorespiration pathway [11–13,59]. Thus, the activity of Rubisco is dependent on the relative ratio of CO_2 and O_2.

Two transcriptional regulators have been extensively studied in cyanobacteria for the control of genes involved in carbon acquisition and/or metabolism: NdhR (CcmR) and CmpR [45,60].

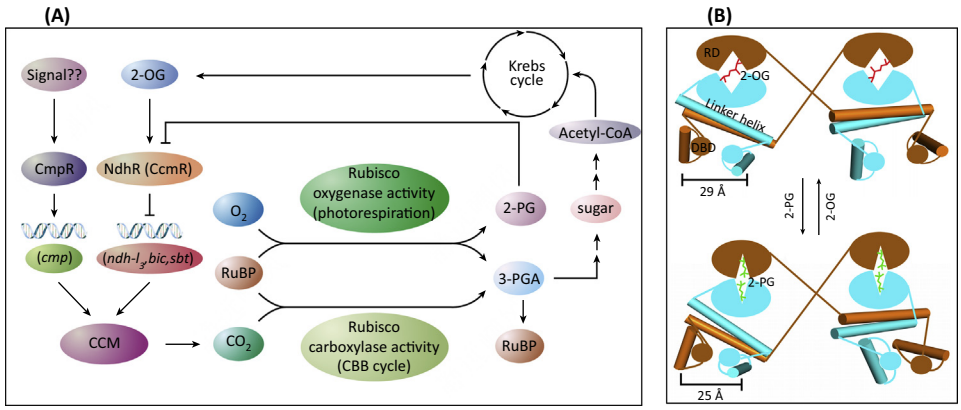


Figure 3. Mechanism of Carbon Metabolic Control and Mechanism of Action of NdhR. (A) Outline of the transcriptional control. The activator CmpR and the repressor NdhR are the two major transcriptional factors for the regulation of CO_2-concentrating mechanism (CCM). CmpR controls the *cmp* operon, encoding a high-affinity bicarbonate transport system, whereas NdhR represses several inorganic carbon uptake systems, including Ndh-1_3, a high-affinity CO_2 uptake system; SbtA, a high-affinity bicarbonate transport system; and BicA, a low-affinity bicarbonate transport system. The signal that regulates CmpR is not yet fully understood. NdhR is under the dual control of 2-phosphoglycolate (2-PG) and 2-oxoglutarate (2-OG), acting as an antirepressor and co-repressor, respectively. When CO_2 is limiting, 2-PG is produced by the oxygenase activity of Rubisco and binds to NdhR. The 2-PG/NdhR complex is unable to fix to DNA, thus the CCM-related operon is expressed, leading to enhanced CO_2/bicarbonate uptake. If CO_2 level is sufficient, the carboxylase activity of Rubisco is favored, leading to CO_2 fixation. Under such a condition, the 2-PG level is low, while that of 2-OG is high, leading to the formation of the 2-OG/NdhR complex and a stronger repression of the CCM-related gene expression. (B) NdhR is a tetramer and a repressor, with two compact subunits (in cyan) and two extended subunits (in orange). Under nitrogen starvation, 2-OG binds at the interdomain cleft between the regulatory domains (RDs). In the 2-OG bound form, the distance between the two helices at the corresponding DNA-binding domains (DBDs) is 29 Å, favorable for DNA binding, thus transcriptional repression. Under a carbon starvation, 2-PG binds at the intradomain cleft of the RDs, leading to shortening of the distance between the two DNA-binding helices to 25 Å, unfavorable for DNA binding, thus relieving transcriptional repression. CBB cycle, Calvin-Benson-Bassham cycle; 3-PGA, 3-phosphoglycerate; RuBP, ribulose 1,5-biphosphate.

A third one, an AbrB-type regulator, is also involved in the control of gene expression according to carbon availability in *Synechocystis* PCC 6803, but the underlying mechanism remains largely unknown [61]; the same is true for PacR, a LysR-type transcriptional regulator identified in *Anabaena* PCC 7120 [62]. Both NdhR and CmpR are LysR-type transcription factors, and their function in cyanobacteria has been well documented [4]. Briefly, CmpR is an activator of the *cmp* operon; this operon encodes the high-affinity bicarbonate transporter BCT1, which is induced under low-CO_2 conditions [60,63] (Figure 3). NdhR is a repressor, whose target genes, among others, encode several CO_2 or bicarbonate uptake systems located on either the cytoplasmic (Na^+-NDH-1, Sbt) or the thylakoid membranes (Ndh-1_3) [4,44–46,48,64,65] (Figure 3). During carbon sufficiency, genes encoding CCM-related functions are therefore repressed by NdhR. LysR-type transcription factors are usually under allosteric control; thus, the existence of effectors that interact with either CmpR or NdhR was expected. Metabolites from photorespiration or CBB, whose levels could oscillate according to carbon availability, have been tested in various studies [45,47,48,63,64,66]. DNA gel-shift assays indicate that RuBP and 2-PG can both bind to CmpR to activate the *cmp* operon and enhance CO_2 uptake under carbon-limiting conditions [48,63]. However, the effect of 2-PG on the transcription of the *cmp* operon mediated by CmpR [47,66] will need to be confirmed *in vivo*, and the effect of both RuBP and 2-PG on CmpR has yet to be demonstrated from structural studies. In contrast, much progress has been made in identifying the effectors of NdhR, which is considered as the most critical regulator of cyanobacterial CCM [4]. Using surface plasmon resonance, it was shown that 2-OG and $NADP^+$ could both interact with NdhR and enhance its DNA binding activity [48]. In a more recent study, four metabolites, 2-OG, 2-PG, RuBP, and NADP+, were investigated, among which 2-OG and 2-PG were found to interact with NdhR: 2-OG enhances the DNA-binding activity, and is therefore a co-repressor, while 2-PG prevents NdhR from DNA binding, thus acting as an antirepressor or inducer [46]. These observations were further confirmed *in vivo* by following the effects on gene expression after changes in the 2-OG or 2-PG levels. Indeed, increasing the cellular concentration of 2-OG by adding the membrane-permeable precursor dimethyl 2-oxoglutarate (dmOG) (Figure 2A), enhanced the repressive effect of NdhR [46], whereas decreasing the 2-PG pool in the cells by overexpressing a gene encoding phosphoglycolate phosphatase brought down the expression levels of the NdhR regulon [46,47].

Crystal structure analyses and structural simulation have provided a mechanism whereby 2-PG and 2-OG effectors control the activity of the NdhR repressor [46] (Figure 3B). NdhR is a tetramer composed of two compact subunits and two extended subunits. Each subunit contains a DNA-binding domain (DBD) and a regulatory domain (RD), bridged by a long α-helical linker. The NdhR tetramer contains two 2-OG binding pockets formed at the interface between two RD domains. In the 2-OG/NdhR complex, the distance between the DNA-recognition helices in the two neighboring DBDs is 29 Å, which matches a continuous DNA major groove and is thus favorable for DNA binding. Therefore, 2-OG is a co-repressor that keeps NdhR bound to its DNA targets, providing a rationale for the effect of 2-OG on the repression of the NdhR regulon. 2-PG binds to NdhR at the intradomain cleft of each RD pair, and the distance between the two DNA-binding helices becomes 25 Å, which is too short to fit the DNA major groove. Thus, 2-PG acts as an antirepressor or inducer of the NdhR regulon. Although 2-PG and 2-OG bind to different sites in NdhR with a similar affinity, their binding is mutually exclusive because of the structural incompatibility between the two-bound forms [46].

All the data summarized above, together with the fact that the 2-PG level transiently increases in cells upon carbon limitation [44], demonstrate that 2-PG acts as a carbon starvation signal, as

already suggested by the effect of reduced 2-PG on the expression of genes related to CCM [47]. Furthermore, NdhR is another 2-OG receptor in addition to PII and NtcA in cyanobacteria.

C/N Metabolic Balance through the Integration of 2-OG and 2-PG Signals

Based on all the studies using different approaches, a model can be proposed to describe how cyanobacteria achieve C/N metabolic balance by transcriptional regulation (Figure 4). Most of the data have been derived using three model cyanobacterial strains, namely *Synechococcus elongatus* PCC 7942, *Synechocystis* PCC 6803, and *Anabaena* PCC 7120.

Firstly, let us consider how changes in the levels of 2-OG and 2-PG are related to carbon and nitrogen metabolism. Cyanobacteria in general lack 2-OG dehydrogenase, thus have an incomplete Krebs cycle [16,17,67]; in such a case, 2-OG can be considered as an end-product of this metabolic pathway (Figure 1). The intracellular pool of 2-OG, derived from the Krebs cycle, accumulates transiently upon, and serves as a signal for, nitrogen deprivation [28,29]. This is consistent with its role as a carbon skeleton for nitrogen assimilation through the GS-GOGAT cycle (Figure 1). The level of 2-PG, a metabolite of photorespiration, increases transiently in cells following carbon limitation [44,47], and serves as a carbon starvation signal, which is directly linked to the activity of Rubisco (Figure 3). Indeed, when the level of CO_2 is high, the higher CO_2/O_2 ratio favors the carboxylase activity of Rubisco, and thus the production of 3-PGA. In contrast, when CO_2 becomes limiting, the lower ratio of CO_2/O_2 favors the oxygenase activity of Rubisco, leading to the production of 2-PG. Because of the strong coupling between carbon and nitrogen metabolism, especially through the GS-GOGAT cycle (Figure 1), nitrogen limitation can also be considered as carbon oversupply, and nitrogen oversupply also correlates with carbon limitation in cells. Altogether, our current knowledge suggests that the levels of 2-OG and 2-PG are somehow inversely correlated in cells, and their ratio may reflect the carbon and nitrogen metabolic status in cyanobacteria. The transient increase in 2-OG or 2-PG observed under a particular condition is characteristic of many signaling molecules, such as Ca^{2+}, cAMP, etc. [68,69].

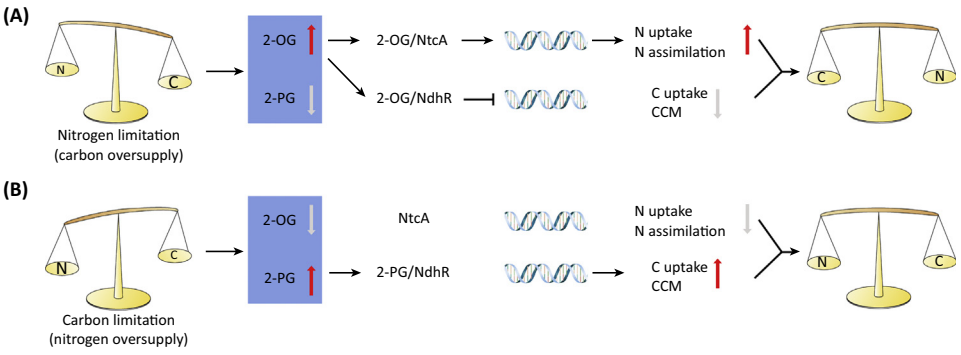


Figure 4. A Model on the Mechanism of Carbon/Nitrogen (C/N) Metabolic Balance in Cyanobacteria. (A) Under nitrogen limitation or carbon oversupply, the 2-oxoglutarate (2-OG) level increases while that of 2-phosphoglycolate (2-PG) decreases. The 2-OG/NtcA complex activates genes involved in nitrogen uptake and assimilation, while the 2-OG/NdhR complex represses the CO_2-concentrating mechanism (CCM)-related gene expression so that carbon uptake decreases. The joint actions of the two transcriptional complexes help the cells to regain C/N balance. (B) Under carbon limitation or nitrogen oversupply, the 2-OG level drops while that of 2-PG increases. The apo form of NtcA has a much lower DNA binding activity, thus leading to a decrease in nitrogen uptake and assimilation. Meanwhile, the 2-PG/NdhR complex cannot act as a repressor, so that genes related to CCM are expressed. Consequently, the C/N balance is achieved.

Secondly, combining with previously published proposal and hypothesis with recent data based on genetic, biochemical, and structural studies [4,28,29,43,45–47,56,64,70], we suggest a model for the transcriptional control of C/N balance, which is illustrated in Figure 4. When C/N metabolism is unbalanced because of nitrogen limitation (or carbon oversupply) (Figure 4A), the 2-OG pool increases while the 2-PG pool decreases for the reasons explained above. Consequently, 2-OG binds to both NtcA and NdhR. The 2-OG/NtcA complex upregulates genes involved in the uptake of alternative nitrogen sources such as nitrate, or heterocyst differentiation and N_2 fixation for diazotrophic heterocyst-forming strains such as *Anabaena* PCC 7120 [3,18]; meanwhile, the 2-OG/NdhR complex downregulates CCM so that bicarbonate uptake goes down. PII also forms a complex with 2-OG and regulates nitrogen assimilation accordingly through protein–protein interaction [2]. Together, the increased uptake and assimilation of nitrogen sources and the decreased uptake of inorganic carbon help the cells to regain balanced C/N metabolism. Similarly, when cells face carbon limitation (or nitrogen oversupply), Rubisco switches towards its oxygenation activity, leading to accumulation of a 2-PG pool, while 2-OG levels decrease because less carbon will go to feed the Krebs cycle (Figures 3 and 4 B). In such a case, NtcA exists in its apo form, which is unfavorable for DNA binding and activation of genes involved in the uptake and assimilation of nitrogen sources; at the same time, 2-PG forms a complex with NdhR, making it unable to bind to DNA, which relieves the repression of genes involved in carbon acquisition. Thus, decreased uptake and assimilation of nitrogen, alongside increased uptake of inorganic carbon, helps cells to rebalance their carbon and nitrogen metabolism.

Some aspects of this model still require further experimental studies. For example, the model assumes concomitant changes in the 2-OG and 2-PG pools upon changes in the availability of carbon or nitrogen sources, consistent with the fact that 2-OG and 2-PG cannot bind to the same molecule of NdhR at the same time [46]. Although transient changes in the pools of these two metabolites have been observed [28,29,44,47], it remains unknown to what extent and on what time-scale their changes are correlated, and the current data are sometimes even contradictory [4]. It will also be relevant to identify the input of other potential signals and regulators involved in C/N metabolism, as well as their interaction with the 2-OG and 2-PG signaling pathways. For example, could RuBP or 3-PGA act as effectors during carbon metabolism as suggested by some studies [4]?

The 2-OG and 2-PG signals control the uptake of nitrogen and carbon sources or the initiation step of heterocyst differentiation when N_2 fixation is needed, and 2-OG also extensively regulates later steps in nitrogen and carbon metabolism through PII and NtcA. Indeed, NtcA is not just required for nitrogen control, it is also a global regulator directly involved in the modulation of carbon metabolism, photosynthesis, and oxidative stress [3]. For example, *cmpR* is possibly a target of NtcA in *Anabaena* PCC 7120 [71], and *ccmK2* encoding a CCM-function protein was also identified by ChIP-seq as a direct target of NtcA in *Synechocystis* PCC 6803 following nitrogen starvation [72]. Although these results still require genetic confirmation, they do point out a much broader role of NtcA in the control of C/N metabolic balance than that summarized in Figure 4. In a regulatory perspective, by switching the early steps in C/N metabolism on and off according to the availability of one nutrient relative to another, cyanobacterial cells ensure rapid responses to environmental changes, so that the C/N balance can be maintained while saving resources that would have to be spent to control later metabolic steps. In principle, 2-OG could regulate the C/N balance alone, as it controls the early steps of both carbon and nitrogen acquisition through its action on NdhR and NtcA (Figure 4). Why then do cyanobacterial cells require multiple signals to control the C/N metabolic balance? The answer may be linked to the response efficiency and the sensitivity

of the control system. While the intracellular concentration of 2-OG, which acts as a carbon skeleton for nitrogen assimilation, may change rapidly following shifts in nitrogen availability, its variation may be slower and of a smaller amplitude following changes in carbon availability because the Krebs cycle is situated far downstream of inorganic-carbon assimilation steps (Figures 1 and 3). By also using 2-PG, the levels of which are directly dependent on carbon availability through the activity of Rubisco, cyanobacterial cells can ensure a proper and timely balance of C/N metabolism.

In the proposed model, we concentrated on 2-PG and 2-OG as signals, and NtcA and NdhR as transcription factors, because these elements are the most thoroughly studied so far using biochemical, genetic, and structural approaches. Other metabolites involved in carbon fixation or photorespiration are also potential signals for carbon metabolism, and other regulatory proteins may also exist [4]. For example, additional robustness in the regulatory systems may be afforded by linkage with cAMP levels as discussed above, although the evidence for such linkages are, for cyanobacteria, largely based upon circumstantial evidence and remain to be fully validated experimentally. In addition, the role of small, noncoding, regulatory RNA in controlling cyanobacterial gene expression is only beginning to be appreciated and, potentially, these could also influence the regulation of C/N balance. These include the recent discovery of a glutamine-responsive riboswitch that enables fast regulation of glutamine synthase, the key enzyme in the 2-OG utilizing GS-GOGAT cycle [5]. In the future, it will also be interesting to study how the 2-PG and 2-OG signaling pathways are modulated by, or respond to, environmental factors other than carbon and nitrogen availability, such as light/dark transition, and light quantity or quality. Other nutritional, developmental, or environmental factors may also shape C/N balance and the underlying signaling pathways. As an illustration to this idea, the C/N ratio differs in vegetative cells and heterocysts in *Anabaena* PCC 7120, with heterocysts accumulating a higher level of nitrogen reserves, consistent with their dedicated function of N_2 fixation for the whole filaments [73].

Cyanobacteria are a diverse group of prokaryotes. Marine cyanobacteria from oligotrophic oceans live in a very stable environment, with less nutritional variations than those from freshwater habitats. Consequently, many of these marine strains, such as *Prochlorococcus* species, have evolved streamlined genomes, including the loss of many regulatory features [74]. Similarly, the interaction between NtcA and its target DNA in these strains become much less responsive to 2-OG, as a consequence of their long-term adaptation to the rather stable environment [75,76].

Tools Used for and Learned from the Study of 2-OG and 2-PG Signaling

The study of 2-OG and 2-PG signaling benefited and will continue to benefit from various tools established in the past. For example, a series of 2-OG analogs have been designed and synthesized for investigating the signaling role of 2-OG [29,37,39,40] (Figure 2). In addition to the nonmetabolizable analog DFPA (described above), which gives an F-NMR signal with little background in living organisms, other 2-OG analogs include those that can mimic either the ketone form or the ketal form of 2-OG [37], which exist in equilibrium in living organisms. It was found that 2-MPA (Figure 2A), a mimic of the ketone form of 2-OG, can play the same signaling role as 2-OG in the cyanobacterium *Anabaena* PCC 7120, while other analogs corresponding to mimics of the ketal form of 2-OG were unable to do so [37]. Attempts were also made to develop an affinity column to enrich or purify 2-OG receptors and binding proteins [77]. To that end, the structure–activity relationship of 2-OG signaling has been explored in order to determine to what extent 2-OG can be modified without affecting its signaling functions [37–40,77]. Two analogs, DFMPA and DMPA, obtained after modification of 2-OG at the

C2 and C4 positions at the same time (Figure 2A), still retained a signaling function in *Anabaena* PCC 7120, like 2-OG [38,39]. Based on this information, DMPA was conjugated to a solid resin using click chemistry, and the affinity resin obtained was used to purify the 2-OG receptor NtcA, which was overexpressed in *E. coli* [77]. Given the fact that 2-OG binding proteins, such as PII, NtcA, and NdhR, do not share a common motif for 2-OG recognition [43,46,50,56], different analogs of 2-OG may be conjugated to a solid resin for identification of new 2-OG binding proteins or receptors.

When 2-OG or its analogs are used, one problem is how to get them into the cells so that the intracellular pool of 2-OG can be manipulated. So far, two methods have proved successful. The first relies on the expression of the *kgtP* gene from *E. coli*, which encodes a permease for C5-compounds, including 2-OG [29,58,78]. The second is the use of the membrane-permeable analog of 2-OG, dmOG (Figure 2A), which is commercially available. Under the action of esterases, dmOG is converted to 2-OG *in vivo*, and hence the level of 2-OG can be increased within the cells of either *E. coli* or *Synechocystis* PCC 6803 after treatment with dmOG [30,46].

Changes in the 2-OG pool in cells may be fast and may vary on an individual cell basis according to cell age or cell cycle progression. Thus, it is interesting to measure the 2-OG level and follow its changes in live single cells, and in real time. Several 2-OG biosensors based on the technique of fluorescence resonance energy transfer (FRET) have been developed [79–82]. These 2-OG biosensors rely on either 2-OG-dependent protein–protein interaction or protein conformational changes induced by 2-OG binding. At least *in vitro*, FRET efficiency of these 2-OG biosensors is quantitatively correlated to 2-OG concentrations.

Compared with the variety of tools available for studies on 2-OG signaling, far fewer have been developed for 2-PG. A 2-PG transporter system has been described in *Arabidopsis thaliana* [83], which may be used to increase the 2-PG pool in the cells. Overexpression of a gene encoding a phosphoglycolate phosphatase was proven to be successful for decreasing the internal pool of 2-PG in cyanobacteria [46,47].

A number of studies, including those reviewed here, revealed that 2-OG is not only an essential metabolite for biosynthesis, but also a signaling molecule in bacteria, plants, animals, and humans [2,25,29–31,43,46]. Therefore, tools developed to study 2-OG signaling are of considerable interest to the scientific community in general.

Concluding Remarks and Future Perspectives

Cyanobacteria are the ancestors of plastids, and they thus have much in common with algae and higher plants in terms of C/N metabolic coupling. Although the sensors/receptors involved may differ, the signaling nature of 2-OG and 2-PG is very likely a common feature in photosynthetic organisms. The signaling function of 2-OG is already well established in bacteria, plants, animals, and humans, although the detailed mechanism in most cases remains poorly understood, except in the conservation of the PII pathway in bacteria and plants, although distinct features have evolved for PII in algae and plants [2,35]. Several metabolites and amino acids affect nitrogen metabolism in plants, but it remains unclear whether they are directly involved in signaling in nitrogen control [84]. The signal for carbon limitation in plants is also not clear, but 2-PG and other metabolites from photorespiration and the CBB cycle are the best candidates in light of the current studies. Indeed, it has been shown in *A. thaliana* that a *pglp1* mutant defective in the gene encoding 2-PG phosphatase accumulates a high level of 2-PG but much less starch than the wild type [85]. These results are consistent with a possible role of

Outstanding Questions

How do the 2-phosphoglycolate (2-PG) and 2-oxoglutarate (2-OG) signaling pathways, and hence the C/N balance, respond to changes other than C/N availability, such as light intensity?

Are there other signals involved in C/N balance in cyanobacteria? If there are, how are different signals integrated to coordinate C/N metabolic balance?

To what extent are changes in 2-PG and 2-OG levels correlated in cells? How do 2-PG and 2-OG signaling affect other regulations required for C/N balance, such as redirection of metabolic fluxes or enzymatic activities?

To what extent are the signaling mechanisms identified from cyanobacteria conserved in eukaryotic algae and plants?

2-PG in signaling a carbon limitation. It is also reported recently that the Krebs cycle display a strikingly coordinated regulation upon nitrogen starvation in the diatom *Phaeodactylum tricornutum*, and this control is mediated through a conserved transcription factor bZIP14 [86]. These data clearly establish a certain parallel between eukaryotic algae and cyanobacteria in terms of nitrogen control. It would therefore be interesting to identify the receptors and the corresponding signaling pathways in plants. The concepts developed so far based on studies in cyanobacteria, and the various tools that are available, could help to stimulate research in this direction in plants.

As we commented in this review, increasing numbers of studies have provided evidence that photorespiration and the Krebs cycle are not only important metabolic pathways but also critical players in signaling in C/N metabolic control. Understanding the underlying mechanism of C/N balance is important not only for fundamental research but also for applied research. For example, bioenergy research largely consists of shifting the C/N balance towards the production of carbon-storage compounds for use as biofuel, and understanding different control points in C/N balance could therefore be instrumental in increasing the efficiency of biofuel production. Better understanding of the C/N balance could also be instructive for improving crop production, because properly engineering the control points of C/N balance could optimize the efficiency of the uptake, assimilation, and metabolism of carbon and nitrogen nutrients. Remarkably, a recent study demonstrates that two protein partners, GRF4 and DELLA, coordinate pant growth and C/N metabolism, and points to new breeding strategies for agriculture [87]. The broad scope of and the general interest in this field, combined with the essential function of C/N metabolic control, will promote further investigations and allow us to answer challenging questions in the future (see Outstanding Questions).

Acknowledgements

Work related to the topics in the authors' laboratories are supported by the Key Research Program of Frontier Sciences of the Chinese Academy of Sciences Grant QYZDJ-SSW-SMC016, the National Natural Science Foundation of China (www.nsfc.gov.cn) Grants 31630001, and the Strategic Priority Research Program of the Chinese Academy of Sciences Grant XDB08020300, La Ligue National Contre le Cancer Grant EL2016.LNCC/LPP and United States Department of Energy (DOE), Office of Science, Basic Energy Sciences, grant no. DE-FG02-08ER15968. We thank Isabel Hanson for English corrections.

References

1. Giordano, M. (2013) Homeostasis: an underestimated focal point of ecology and evolution. *Plant Sci.* 211, 92–101
2. Chellamuthu, V.R. *et al.* (2013) From cyanobacteria to plants: conservation of PII functions during plastid evolution. *Planta* 237, 451–462
3. Herrero, A. *et al.* (2004) Cellular differentiation and the NtcA transcription factor in filamentous cyanobacteria. *FEMS Microbiol. Rev.* 28, 469–487
4. Burnap, R. *et al.* (2015) Regulation of CO_2 concentrating mechanism in cyanobacteria. *Life* 5, 348–371
5. Klähn, S. *et al.* (2018) A glutamine riboswitch is a key element for the regulation of glutamine synthetase in cyanobacteria. *Nucleic Acids Res.* Published online August 2, 2018. http://dx.doi.org/10.1093/nar/gky709
6. Du, J. *et al.* (2014) Characterisation of cyanobacterial bicarbonate transporters in *E. coli* shows that SbtA homologs are functional in this heterologous expression system. *PLoS One* 9, e115905
7. Selim, K.A. *et al.* (2018) P_{II}-like signaling protein SbtB links cAMP sensing with cyanobacterial inorganic carbon response. *Proc. Natl. Acad. Sci. U. S. A.* 115, 4861–4869
8. Ponce-Toledo, R.I. *et al.* (2017) An early-branching freshwater cyanobacterium at the origin of plastids. *Curr. Biol.* 27, 386–391
9. Rippka, R. *et al.* (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* 111, 1–61
10. Zhang, C.C. *et al.* (1989) Molecular and genetical analysis of the fructose-glucose transport system in the cyanobacterium *Synechocystis* PCC6803. *Mol. Microbiol.* 3, 1221–1229
11. Bauwe, H. *et al.* (2010) Photorespiration: players, partners and origin. *Trends Plant Sci.* 15, 330–336
12. Moroney, J.V. *et al.* (2013) Photorespiration and carbon concentrating mechanisms: two adaptations to high O_2, low CO_2 conditions. *Photosynth. Res.* 117, 121–131
13. Hagemann, M. and Bauwe, H. (2016) Photorespiration and the potential to improve photosynthesis. *Curr. Opin. Chem. Biol.* 35, 109–116
14. Turmo, A. *et al.* (2017) Carboxysomes: metabolic modules for CO_2 fixation. *FEMS Microbiol. Lett.* 364, fnx176
15. Price, G.D. *et al.* (2013) The cyanobacterial CCM as a source of genes for improving photosynthetic CO_2 fixation in crop species. *J. Exp. Bot.* 64, 753–768
16. Young, J.D. *et al.* (2011) Mapping photoautotrophic metabolism with isotopically non stationary ^{13}C flux analysis. *Metab. Eng.* 13, 656–665
17. Xiong, W. *et al.* (2015) The plasticity of cyanobacterial metabolism supports direct CO_2 conversion to ethylene. *Nat. Plants* 1, 15053

18. Zhang, C.C. *et al.* (2006) Heterocyst differentiation and pattern formation in cyanobacteria: a chorus of signals. *Mol. Microbiol.* 59, 367–375
19. Herrero, A. *et al.* (2016) The multicellular nature of filamentous heterocyst-forming cyanobacteria. *FEMS Microbiol. Rev.* 40, 831–854
20. Schneegurt, M.A. *et al.* (2000) Metabolic rhythms of a diazotrophic cyanobacterium, *Cyanothece* sp strain ATCC 51142, heterotrophically grown in continuous dark. *J. Phycol.* 36, 107–117
21. Muro-Pastor, M.I. *et al.* (2005) Ammonium assimilation in cyanobacteria. *Photosynth. Res.* 83, 135–150
22. Ohashi, Y. *et al.* (2011) Regulation of nitrate assimilation in cyanobacteria. *J. Exp. Bot.* 62, 1411–1424
23. Shih, P.M. *et al.* (2013) Improving the coverage of the cyanobacterial phylum using diversity-driven genome sequencing. *Proc. Natl. Acad. Sci. U. S. A.* 110, 1053–1058
24. Reitzer, L. (2003) Nitrogen assimilation and global regulation in *Escherichia coli*. *Annu. Rev. Microbiol.* 57, 155–176
25. Huergo, L.F. and Dixon, R. (2015) The emergence of 2-oxoglutarate as a master regulator metabolite. *Microbiol. Mol. Biol. Rev.* 79, 419–435
26. Fulda, S. *et al.* (2006) Proteome analysis of salt stress response in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *Proteomics* 6, 2733–2745
27. Stöckel, J. *et al.* (2011) Diurnal rhythms result in significant changes in the cellular protein complement in the cyanobacterium *Cyanothece* 51142. *PLoS One* 6, e16680
28. Muro-Pastor, M.I. *et al.* (2001) Cyanobacteria perceive nitrogen status by sensing intracellular 2-oxoglutarate levels. *J. Biol. Chem.* 276, 38320–38328
29. Laurent, S. *et al.* (2005) Nonmetabolizable analogue of 2-oxoglutarate elicits heterocyst differentiation under repressive conditions in *Anabaena* sp. PCC 7120. *Proc. Natl. Acad. Sci. U. S. A.* 102, 9907–9912
30. Doucette, C.D. *et al.* (2011) α-Ketoglutarate coordinates carbon and nitrogen utilization via enzyme I inhibition. *Nat. Chem. Biol.* 7, 894–901
31. He, W. *et al.* (2004) Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature* 429, 188–193
32. Haas, R. *et al.* (2016) Intermediates of metabolism: from bystanders to signalling molecules. *Trends Biochem. Sci.* 41, 460–471
33. You, C. *et al.* (2013) Coordination of bacterial proteome with metabolism by cyclic AMP signalling. *Nature* 500, 301–306
34. Araujo, W.L. *et al.* (2014) 2-Oxoglutarate: linking TCA cycle function with amino acid, glucosinolate, flavonoid, alkaloid, and gibberellin biosynthesis. *Front. Plant Sci.* 5, 552
35. Uhrig, R.G. *et al.* (2009) PII in higher plants: a modern role for an ancient protein. *Trends Plant Sci.* 14, 505–511
36. Rabinowitz, J.D. and Silhavy, T.J. (2013) Systems biology: metabolite turns master regulator. *Nature* 500, 283–284
37. Chen, H. *et al.* (2006) Studying the signaling role of 2-oxoglutaric acid using analogs that mimic the ketone and ketal forms of 2-oxoglutaric acid. *Chem. Biol.* 13, 849–856
38. Liu, X. *et al.* (2013) Structural requirements of 2-oxoglutaric acid analogues to mimic its signaling function. *Org. Lett.* 15, 4662–4665
39. Liu, X. *et al.* (2011) 2-Difluoromethylene-4-methylenepentanoic acid, a paradoxical probe able to mimic the signaling role of 2-oxoglutaric acid in cyanobacteria. *Org. Lett.* 13, 2924–2927
40. Wang, Y. *et al.* (2014) Mimicking the 2-oxoglutaric acid signalling function using molecular probes: insights from structural and functional investigations. *Org. Biomol. Chem.* 12, 4723–4729
41. Ziarelli, F. *et al.* (2012) High resolution magic angle spinning NMR to investigate ligand-receptor binding events for mass-limited samples in liquids. *J. Pharm. Biomed. Anal.* 59, 13–17
42. Chen, H. *et al.* (2013) ^{19}F NMR: a valuable tool for studying biological events. *Chem. Soc. Rev.* 42, 7971
43. Zhao, M.-X. *et al.* (2010) Structural basis for the allosteric control of the global transcription factor NtcA by the nitrogen starvation signal 2-oxoglutarate. *Proc. Natl. Acad. Sci. U. S. A.* 107, 12487–12492
44. Klähn, S. *et al.* (2015) Integrated transcriptomic and metabolomic characterization of the low-carbon response using an ndhR mutant of *Synechocystis* sp. PCC 6803. *Plant Physiol.* 169, 1787–1806
45. Wang, H.L. *et al.* (2004) Alterations in global patterns of gene expression in *Synechocystis* sp. PCC 6803 in response to inorganic carbon limitation and the inactivation of *ndhR*, a LysR family regulator. *J. Biol. Chem.* 279, 5739–5751
46. Jiang, Y.-L. *et al.* (2018) Coordinating carbon and nitrogen metabolic signaling through the cyanobacterial global repressor NdhR. *Proc. Natl. Acad. Sci. U. S. A.* 115, 403–408
47. Haimovich-Dayan, M. *et al.* (2015) Does 2-phosphoglycolate serve as an internal signal molecule of inorganic carbon deprivation in the cyanobacterium *Synechocystis* sp. PCC 6803? *Environ. Microbiol.* 17, 1794–1804
48. Daley, S.M.E. *et al.* (2012) Regulation of the cyanobacterial CO2-concentrating mechanism involves internal sensing of NADP+ and α-ketogutarate levels by transcription factor CcmR. *PLoS One* 7, e41286
49. Huergo, L.F. *et al.* (2013) PII signal transduction proteins: nitrogen regulation and beyond. *FEMS Microbiol. Rev.* 37, 251–283
50. Fokina, O. *et al.* (2010) Mechanism of 2-oxoglutarate signaling by the *Synechococcus elongatus* PII signal transduction protein. *Proc. Natl. Acad. Sci. U. S. A.* 107, 19760–19765
51. Espinosa, J. *et al.* (2014) PipX, the coactivator of NtcA, is a global regulator in cyanobacteria. *Proc. Natl. Acad. Sci U. S. A.* 111, E2423–E2430
52. Llácer, J.L. *et al.* (2007) The crystal structure of the complex of PII and acetylglutamate kinase reveals how PII controls the storage of nitrogen as arginine. *Proc. Natl. Acad. Sci. U. S. A.* 104, 17644–17649
53. Maheswaran, M. *et al.* (2004) Complex formation and catalytic activation by the PII signaling protein of N-acetyl-L-glutamate kinase from *Synechococcus elongatus* strain PCC 7942. *J. Biol. Chem.* 279, 55202–55210
54. Espinosa, J. *et al.* (2006) Interaction network in cyanobacterial nitrogen regulation: PipX, a protein that interacts in a 2-oxoglutarate dependent manner with PII and NtcA. *Mol. Microbiol.* 61, 457–469
55. Wheatley, N.M. *et al.* (2016) A PII-like protein regulated by bicarbonate: structural and biochemical studies of the carboxysome-associated CPII protein. *J. Mol. Biol.* 428, 4013–4030
56. Llácer, J.L. *et al.* (2010) Structural basis for the regulation of NtcA-dependent transcription by proteins PipX and PII. *Proc. Natl. Acad. Sci. U. S. A.* 107, 15397–15402
57. Tanigawa, R. *et al.* (2002) Transcriptional activation of NtcA-dependent promoters of *Synechococcus* sp. PCC 7942 by 2-oxoglutarate *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.* 99, 4251–4255
58. Li, J.H. *et al.* (2003) An increase in the level of 2-oxoglutarate promotes heterocyst development in the cyanobacterium *Anabaena* sp. strain PCC 7120. *Microbiology* 149, 3257–3263
59. Eisenhut, M. *et al.* (2008) The photorespiratory glycolate metabolism is essential for cyanobacteria and might have been conveyed endosymbiontically to plants. *Proc. Natl. Acad. Sci. U. S. A.* 105, 17199–17204
60. Takahashi, Y. *et al.* (2004) Roles of CmpR, a LysR family transcriptional regulator, in acclimation of the cyanobacterium *Synechococcus* sp. strain PCC 7942 to low-CO_2 and high-light conditions. *Mol. Microbiol.* 52, 837–845
61. Lieman-Hurwitz, J. *et al.* (2009) A cyanobacterial AbrB-like protein affects the apparent photosynthetic affinity for CO_2 by modulating low-CO_2-induced gene expression. *Environ. Microbiol.* 11, 927–936

62. Picossi, S. *et al.* (2015) The LysR-type transcription factor PacR is a global regulator of photosynthetic carbon assimilation in *Anabaena*. *Environ. Microbiol.* 17, 3341–3351

63. Nishimura, T. *et al.* (2008) Mechanism of low CO_2-induced activation of the *cmp* bicarbonate transporter operon by a LysR family protein in the cyanobacterium *Synechococcus elongatus* strain PCC 7942. *Mol. Microbiol.* 68, 98–109

64. Figge, R.M. *et al.* (2001) Characterization and analysis of an NAD(P)H dehydrogenase transcriptional regulator critical for the survival of cyanobacteria facing inorganic carbon starvation and osmotic stress. *Mol. Microbiol.* 39, 455–468

65. Woodger, F.J. *et al.* (2007) Transcriptional regulation of the CO_2-concentrating mechanism in a euryhaline, coastal marine cyanobacterium, *Synechococcus* sp. strain PCC 7002: role of NdhR/CcmR. *J. Bacteriol.* 189, 3335–3347

66. Hackenberg, C. *et al.* (2012) Low-carbon acclimation in carboxysome-less and photorespiratory mutants of the cyanobacterium *Synechocystis* sp. strain PCC 6803. *Microbiology* 158, 398–413

67. Stanier, R.Y. and Cohen-Bazire, G. (1977) Phototrophic prokaryotes: the cyanobacteria. *Annu. Rev. Microbiol.* 31, 225–274

68. Conrad, M. *et al.* (2014) Nutrient sensing and signaling in the yeast *Saccharomyces cerevisiae*. *FEMS Microbiol. Rev.* 38, 254–299

69. Berridge, M.J. *et al.* (2003) Calcium signalling: dynamics, homeostasis and remodelling. *Nat. Rev. Mol. Cell Biol.* 4, 517–529

70. Kaplan, A. and Reinhold, L. (1999) CO_2 concentrating mechanisms in microorganisms. *Transport* 50, 539–570

71. Lopez-Igual, R. *et al.* (2012) N and C control of ABC-type bicarbonate transporter Cmp and its LysR-type transcriptional regulator CmpR in a heterocyst-forming cyanobacterium, *Anabaena* sp. *Environ. Microbiol.* 14, 1035–1048

72. Giner-Lamia, J. *et al.* (2017) Identification of the direct regulon of NtcA during early acclimation to nitrogen starvation in the cyanobacterium *Synechocystis* sp. PCC 6803. *Nucleic Acids Res.* 45, 11800–11820

73. Teramoto, T. *et al.* (2018) Soft X-ray imaging of cellular carbon and nitrogen distributions in heterocystous cyanobacterium. *Plant Physiol.* 177, 52–61

74. Scanlan, D.J. *et al.* (2009) Ecological genomics of marine picocyanobacteria. *Microbiol. Mol. Biol. Rev.* 73, 249–299

75. Domínguez-Martín, M.A. *et al.* (2018) Differential NtcA responsiveness to 2-oxoglutarate underlies the diversity of C/N balance regulation in *Prochlorococcus*. *Front. Microbiol.* 8, 1–16

76. Domínguez-Martín, M.A. *et al.* (2018) Distinct features of C/N balance regulation in *Prochlorococcus* sp. strain MIT9313. *FEMS Microbiol. Lett.* 365, fnx278

77. Wang, Y. *et al.* (2014) A "click" chemistry constructed affinity system for 2-oxoglutaric acid receptors and binding proteins. *Org. Biomol. Chem.* 12, 6470–6475

78. Seol, W. and Shatkin, A.J. (1991) *Escherichia coli kgtP* encodes an α-ketoglutarate transporter. *Proc. Natl. Acad. Sci. U. S. A.* 88, 3802–3806

79. Chen, H.L. *et al.* (2014) Fluorescence resonance energy transfer based on interaction of PII and PipX proteins provides a robust and specific biosensor for 2-oxoglutarate, a central metabolite and a signalling molecule. *FEBS J.* 281, 1241–1255

80. Zhang, C. *et al.* (2013) Quantitative monitoring of 2-oxoglutarate in *Escherichia coli* cells by a fluorescence resonance energy transfer-based biosensor. *Appl. Microbiol. Biotechnol.* 97, 8307–8316

81. Lüddecke, J. *et al.* (2017) PII protein-derived FRET sensors for quantification and live-cell imaging of 2-oxoglutarate. *Sci. Rep.* 7, 1–13

82. Lüddecke, J. and Forchhammer, K. (2013) From PII signaling to metabolite sensing: a novel 2-oxoglutarate sensor that details PII - NAGK complex formation. *PLoS One* 8, 1–11

83. South, P.F. *et al.* (2017) Bile acid sodium symporter BASS6 can transport glycolate and is involved in photorespiratory metabolism in *Arabidopsis thaliana*. *Plant Cell* 29, 808–823

84. Gent, L. and Forde, B.G. (2017) How do plants sense their nitrogen status? *J. Exp. Bot.* 68, 2531–2540

85. Timm, S. *et al.* (2012) High-to-low CO_2 acclimation reveals plasticity of the photorespiratory pathway and indicates regulatory links to cellular metabolism of *Arabidopsis*. *PLoS One* 7, e42809

86. Matthijs, M. *et al.* (2017) The transcription factor bZIP14 regulates the TCA cycle in the diatom *Phaeodactylum tricornutum*. *EMBO J.* 61, 316–324

87. Li, S. *et al.* (2018) Modulating plant growth – metabolism coordination for sustainable agriculture. *Nature* 560, 595–600