Photosynthesis Research (2005) 83: 117–133 © Springer 2005

Review

Photosynthetic nitrate assimilation in cyanobacteria★

Enrique Flores[1,*], José E. Frías[1], Luis M. Rubio[2] & Antonia Herrero[1]
[1]*Instituto de Bioquímica Vegetal y Fotosíntesis, C.S.I.C.-Universidad de Sevilla, Avda. Américo Vespucio 49, 41092 Sevilla, Spain;* [2]*Department of Plant and Microbial Biology, 111 Koshland Hall, University of California, Berkeley, CA 94720, USA; *Author for correspondence (e-mail: eflores@ibvf.csic.es; fax: +34-954460065)*

Received 26 August 2004; accepted in revised form 2 November 2004

Key words: CnaT, ferredoxin, nitrate permease, nitrate reductase, nitrite reductase, NtcA, NtcB, P_{II} protein, 2-oxoglutarate

Abstract

Nitrate uptake and reduction to nitrite and ammonium are driven in cyanobacteria by photosynthetically generated assimilatory power, i.e., ATP and reduced ferredoxin. High-affinity nitrate and nitrite uptake takes place in different cyanobacteria through either an ABC-type transporter or a permease from the major facilitator superfamily (MFS). Nitrate reductase and nitrite reductase are ferredoxin-dependent metalloenzymes that carry as prosthetic groups a [4Fe–4S] center and Mo-*bis*-molybdopterin guanine dinucleotide (nitrate reductase) and [4Fe–4S] and siroheme centers (nitrite reductase). Nitrate assimilation genes are commonly found forming an operon with the structure: *nir* (nitrite reductase)-permease gene(s)-*narB* (nitrate reductase). When the cells perceive a high C to N ratio, this operon is transcribed from a complex promoter that includes binding sites for NtcA, a global nitrogen-control regulator that belongs to the CAP family of bacterial transcription factors, and NtcB, a pathway-specific regulator that belongs to the LysR family of bacterial transcription factors. Transcription is also affected by other factors such as CnaT, a putative glycosyl transferase, and the signal transduction protein P_{II}. The latter is also a key factor for regulation of the activity of the ABC-type nitrate/nitrite transporter, which is inhibited when the cells are incubated in the presence of ammonium or in the absence of CO_2. Notwithstanding significant advance in understanding the regulation of nitrate assimilation in cyanobacteria, further post-transcriptional regulatory mechanisms are likely to be discovered.

Introduction

The assimilatory reduction of nitrate to ammonium is a key step of the nitrogen cycle in the biosphere. Nitrate reduction to ammonium is carried out by many bacteria, fungi, algae and plants, and takes place through two sequential reactions involving 2-electron and 6-electron reductions that are catalyzed, respectively, by nitrate reductase and nitrite reductase. The ammonium resulting from nitrate reduction is incorporated into carbon skeletons via glutamate dehydrogenase or the glutamine synthetase/glutamate synthase cycle, the latter being the predominant ammonium assimilation pathway in phototrophic organisms. Because nitrate is frequently found in the environment at relatively low concentrations (e.g., in the μM range), specific nitrate uptake systems are required to concentrate this nutrient inside the cells before nitrate reduction can take place. In organisms performing oxygenic photosynthesis, i.e., the cyanobacteria, algae and plants, nitrate reduction can be func-

★ This article is dedicated to Professor Manuel Losada on the occasion of his 75th anniversary.

tionally linked to the photosynthetic process, and this is especially true for the case of nitrate assimilation in cyanobacteria, which is the subject of this review article.

Photosynthesis beyond CO_2 fixation

A nitrate-dependent photosynthetic oxygen evolution was first described in 1920 by OttoWarburg, who, using cell suspensions of the green alga *Chlorella*, also observed that the process was strictly dependent on the presence of CO_2 making him to fervently defend the concept of photosynthesis as the photolysis of CO_2 (see Warburg et al. 1965). Our understanding of photosynthetic nitrate reduction relies however on the modern concept of photosynthesis as developed by Chase B. van Niel, in which photolysis of a hydrogen donor (e.g., H_2O, H_2S) permits the transference of H to an ultimate acceptor that can be, but not necessarily is, CO_2. Among possible alternative acceptors, van Niel himself considered nitrate (van Niel 1941).

Two landmarks in photosynthesis research relevant to our discussion were the finding that isolated chloroplasts could evolve O_2 using ferricyanide as an electron acceptor (Hill 1939) and the identification of ferredoxin (see Arnon 1988). The so-called Hill reaction with ferricyanide or other artificial acceptors corroborated that O_2 evolution is not necessarily coupled to CO_2 fixation and encouraged the search for natural Hill reagents, which led to the discovery of ferredoxin and the photosynthetic reduction of $NADP^+$, in which ferredoxin–$NADP^+$ oxidoreductase has a key role. It is now known that NADPH is the electron donor for CO_2 fixation through the reductive pentose phosphate cycle, but reduced ferredoxin itself is used as an electron donor for a number of reactions that take place in chloroplasts or in cyanobacterial cells (Knaff 1996). Significantly, reduced ferredoxin was soon established as the electron donor for nitrite reductase in chloroplasts (Paneque et al. 1964). The term ferredoxin refers to a family of acidic, low molecular mass (6–12 kDa) proteins possessing iron and inorganic (labile) sulfur that exhibit low redox potentials (Knaff 1996). Although there are different types of ferredoxins, the one that constitutes the terminal electron acceptor at Photosystem I in the oxygenic photosynthetic apparatus is a [2Fe–2S]-containing ferredoxin.

Photosynthetic nitrate uptake and reduction

Stoichiometric nitrate- and nitrite-dependent O_2 evolution has been demonstrated for cells of different cyanobacteria incubated in the absence of CO_2. Thus, cells of the heterocyst-forming species *Anabaena cylindrica* incubated in an atmosphere of argon have been shown to produce 1 mol of ammonium and 1.5 mol of O_2 per mol of nitrite disappeared from the culture medium when incubated in the light (Hattori 1962). This stoichiometry corresponds to the use of six electrons derived from the photolysis of water in the reduction of nitrite to ammonium, but the author could not obtain data adequate to show the stoichiometry with nitrate. This could be achieved with cells of the unicellular cyanobacterium *Synechococcus elongatus* (formerly known as *Anacystis nidulans*) in which ammonium assimilation was inhibited by inactivation of glutamine synthetase with L-methionine-D,L-sulfoximine (MSX). When incubated in the light in the presence of nitrate or nitrite, these treated cells released as ammonium the nitrate or nitrite that was taken up, and evolved oxygen concomitantly (Flores et al. 1983a). Stoichiometries of 1.5 mol of O_2 evolved per mol of nitrite reduced to ammonium and of 2.0 mol of O_2 evolved per mol of nitrate reduced to ammonium could be determined, the latter corresponding to the use of eight electrons derived from the photolysis of water. These results suggest a close functional relationship between nitrate reduction and photosynthesis in cyanobacteria, in which water photolysis can directly provide, without the need for carbon-compounds, the reducing power required for nitrate reduction. Cyanobacterial cells incubated in the absence of an electron acceptor, under an argon atmosphere, show a high level of chlorophyll fluorescence. Quenching of this fluorescence by nitrate or nitrite has been observed in *Anabaena* sp. further suggesting a direct use of photosynthetic reducing power in nitrate and nitrite reduction in these organisms (Serrano et al. 1981).

Thylakoid membrane preparations capable of carrying out a photochemical (Photosystem I)-dependent reduction of nitrate or nitrite have been

obtained from both *A. cylindrica* and *S. elongatus* (Hattori and Myers 1967; Guerrero et al. 1974; Manzano et al. 1976). A role for ferredoxin as an electron carrier in the thylakoid-dependent photoreduction of nitrate and nitrite could be demonstrated (Hattori and Myers 1967; Manzano et al. 1976). Furthermore, reduction of nitrate to nitrite (Ortega et al. 1976) or of nitrate to nitrite and ammonium (Candau et al. 1976) coupled to water photolysis and concomitant oxygen evolution, has been achieved with thylakoid membrane preparations and ferredoxin. Since these reactions involve Photosystems I and II, the results demonstrated a linkage of the cyanobacterial nitrate reduction system to the photosynthetic apparatus, with nitrate and nitrite acting as Hill reagents. Additionally, these results indicated a molecular mechanism for this linkage, which is based on the use of reduced ferredoxin as the direct electron donor for both nitrate reductase and nitrite reductase.

Experiments performed with MSX-treated cells of *S. elongatus* also showed a requirement for ATP in the *in vivo* transformation of externally provided nitrate or nitrite to ammonium (Flores et al. 1983a). Because the reductive reactions do not exhibit any requirement for ATP, it was concluded that an active transporter is involved in nitrate and nitrite uptake by the cells. The same transporter mediates the uptake of nitrate and nitrite (Madueño et al. 1987), and its K_s for any of these anions could be estimated to lie below a value of 10 μM (Flores et al. 1983a). Thus, the first steps of nitrate assimilation in cyanobacteria, namely nitrate uptake and reduction to ammonium, can operate with the use of the photosynthetically generated assimilatory power, which is composed of reducing power and ATP (see Arnon 1984).

The nitrate assimilation genes

The classical studies summarized above identified the three elements of the cyanobacterial nitrate assimilation system: the nitrate/nitrite permease and the ferredoxin-dependent nitrate and nitrite reductases (Figure 1). The remaining of this review will be devoted to the description of the structural and regulatory molecular elements that effect nitrate assimilation in cyanobacteria.

A component of the nitrate permease was first identified as an ammonium-repressible protein of about 48 kDa present in cytoplasmic membranes of *S. elongatus* (Madueño et al. 1988; Omata et al. 1989; Sivak et al. 1989). This is one of the most abundant proteins in the cytoplasmic membrane, and it has also been identified as an abundant protein in proteomic analysis of *Anabaena* cells (Sazuka 2003). Cloning of the corresponding gene, *nrtA* (Omata 1991), and isolation of a genomic DNA fragment from a transposon-induced nitrite reductase mutant of *S. elongatus* (Luque et al. 1992) permitted the identification of a nitrate assimilation gene cluster (Luque et al. 1992; Omata et al. 1993). Polar effects of inactivation of the nitrite reductase gene on the expression of nitrate reductase activity indicated co-transcription of that gene with a downstream putative nitrate reductase gene, thus identifying the gene cluster as an operon (Luque et al. 1992).

The *S. elongatus* nitrate assimilation operon bears the following genes: *nir*, encoding nitrite reductase (Luque et al. 1993; Suzuki et al. 1993); *nrtA*, *nrtB*, *nrtC* and *nrtD*, encoding a nitrate/nitrite transporter (Omata et al. 1993; Luque et al. 1994a); and *narB*, encoding nitrate reductase (Omata et al. 1993; Rubio et al. 1996). A similar gene cluster has been identified as the nitrate assimilation operon in *Anabaena* sp. strain PCC 7120 (Cai and Wolk 1997; Frías et al. 1997). However, a single permease gene, *nrtP*, encoding a different type of nitrate/nitrite permease (see below), is present in some cyanobacteria (Sakamoto et al. 1999; Wang et al. 2000). This conserved gene structure likely ensures production of a balanced amount of the different proteins of the nitrate assimilation system since gene expression declines 5′ to 3′ in the operon (Frías et al. 1997).

The capability to assimilate nitrate appears to be widespread among cyanobacteria, and the recent sequencing of numerous cyanobacterial genomes has permitted a wide analysis of the presence of nitrate assimilation genes in different cyanobacteria. Figure 2 presents schemes of genomic regions carrying nitrate assimilation genes in some cyanobacteria with sequenced genomes. It is evident that gene clusters with the structure nitrite reductase–nitrate/nitrite permease–nitrate reductase are found in numerous cyanobacteria. However, some *Synechococcus* marine strains (see e.g. Bird and Wyman 2003;

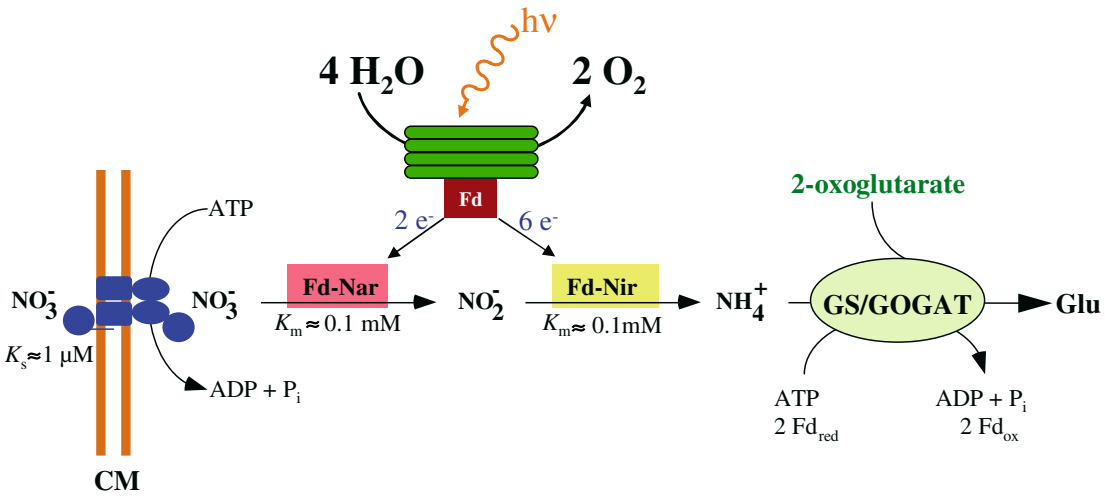

Figure 1. The nitrate assimilation system as found in fresh-water cyanobacteria like *Synechococcus elongatus* or *Anabaena* sp. strain PCC 7120. The structural elements of this system are an ABC-type permease located at the cytoplasmic membrane (CM) and the enzymes ferredoxin–nitrate reductase (Fd–Nar) and ferredoxin–nitrite reductase (Fd–Nir), which make use of ferredoxin (Fd) photosynthetically reduced at the thylakoids as electron donor. The nitrate/nitrite permease is composed of a periplasmic substrate-binding protein that is anchored to the membrane, two transmembrane subunits and two ATPase subunits that are located in the cytoplasmic side of the membrane; one of the ATPase subunits has a C-terminal extension that is homologous to the periplasmic substrate-binding protein. Ammonium resulting from nitrate reduction is incorporated into amino acids by the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway, which consumes ATP and reducing power and uses 2-oxoglutarate as a final N acceptor (Fd_{red}, reduced ferredoxin; Fd_{ox}, oxidized ferredoxin). Approximate values for the affinity constants of the permease (K_s) and the reductases (K_m) are indicated.

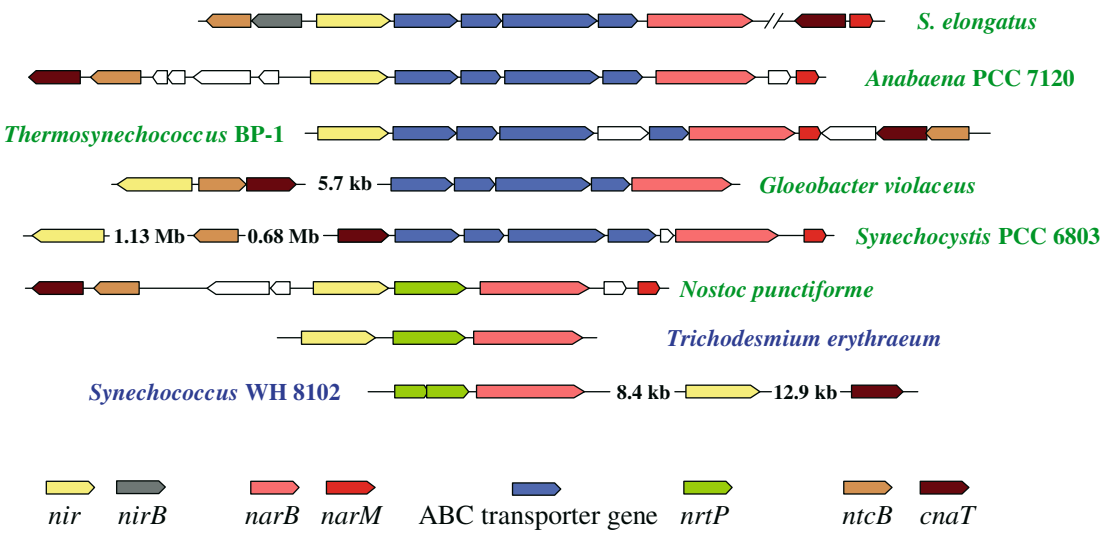

Figure 2. Nitrate assimilation genes and gene clusters in different cyanobacteria. References and sources are as follows: *Synechococcus elongatus* strain PCC 7942 (Luque et al. 1993a; Omata et al. 1993; Suzuki et al. 1993, 1995b); *Anabaena* sp. strain PCC 7120, *Thermosynechococcus elongatus* strain BP-1, *Gloeobacter violaceus* strain PCC 7421 and *Synechocystis* sp. strain PCC 6803 (Kazusa DNA Research Institute, http://www.kazusa.or.jp/cyanobase/); *Nostoc punctiforme* strain ATCC 29133, *Trichodesmium erythraeum* and *Synechococcus* sp. strain WH 8102 (DOE Joint Genome Institute, http://www.jgi.doe.gov/). The orientation with respect to each other and the distance between the two *S. elongatus* gene clusters are unknown. Names in green denote fresh-water cyanobacteria, except for *N. punctiforme*, a soil cyanobacterium, and names in blue denote marine cyanobacteria. White arrows refer to open reading frames encoding proteins of unknown function. The genes encoding the ABC-type permease are arranged in everycase (5′ to 3′): *nrtA*, *nrtB*, *nrtC*, and *nrtD*.

Palenik et al. 2003) carry a different type of nitrate assimilation gene cluster, in which the nitrate assimilation structural genes are clustered with genes encoding proteins involved in prosthetic group biosynthesis (see below). On the other hand, strains unable to use nitrate can be found scattered in the cyanobacterial phylogenetic radiation (Miller and Castenholz 2001), and several marine *Prochlorococcus* strains have been found to lack the nitrate assimilation genes (López-Lozano et al. 2002; Dufresne et al. 2003; Rocap et al. 2003). Finally, an unusual and interesting case is that of *Prochlorococcus* sp. MIT9313, which has been shown to carry a nitrite reductase gene but no gene encoding a nitrate reductase, thus suggesting the capability of this cyanobacterium to assimilate nitrite but not nitrate (Rocap et al. 2003).

The cyanobacterial nitrate/nitrite permeases

ABC-type uptake transporter

The *nrtA*, *nrtB*, *nrtC* and *nrtD* genes encode elements of an ABC-type uptake permease (Omata et al. 1993). ABC-type permeases couple ATP hydrolysis to the transport of solutes across cell membranes. ATP binding and hydrolysis drive conformational changes in the membrane spanning domains of the permease that mediate the transport process (Davidson and Chen 2004). ABC-type uptake permeases are highly efficient transporters. Consistently, K_s values for nitrate of cyanobacterial cells bearing ABC-type nitrate permeases are about 1–4 μM for *S. elongatus* (Tischner and Schmidt 1984; Rodríguez et al. 1992) and close to 30 μM for *Anabaena* sp. strain PCC 7120 (Meeks et al. 1983).

NrtA is a periplasmic substrate-binding lipoprotein of about 440 amino acids that is anchored to the cytoplasmic membrane and can bind both nitrate and nitrite with high affinity (K_d, 0.3 μM for *S. elongatus* NrtA; Maeda and Omata 1997). Two important features of the amino-terminal region of cyanobacterial NrtA are the presence of the twin arginine motif for export through the Tat pathway (discussed in Wu and Stewart 1998) and the presence of a sequence for signal-peptide cleavage by signal peptidase II, which is involved in maturation of lipoproteins and attaches a lipid moiety to the cysteine that constitutes the N-terminal residue of the processed protein (Maeda and Omata 1997).

NrtB is a highly hydrophobic protein of about 280 amino acids that has been suggested to bear six transmembrane segments (Wu and Stewart 1998). However, a prediction from a multiple sequence alignment (TMAP program, Persson and Argos 1996) of several NrtB proteins suggests five transmembrane segments with the N-terminus of the protein located in the cytoplasm. Because there is usually only one such protein encoded in the nitrate assimilation operon of different cyanobacteria, and because two transmembrane proteins are generally found in ABC-type uptake transporters, it is assumed that a homodimer of NrtB forms the substrate translocation pathway through the membrane. The sequence motif known as the EAA loop that is present in numerous transmembrane components of ABC-type uptake transporters is not found in NrtB.

ABC-type uptake transporters also have two cytoplasmic subunits that power the transport reaction through binding and hydrolysis of ATP. These ATP-binding cassette (ABC) subunits or domains are also known as the 'conserved components' of ABC-type transporters because of their very high conservation in sequence throughout the biological world. NrtD, a protein of about 275 amino acids, represents a conserved component for the nitrate/nitrite permease. The NrtD primary structure features a characteristic set of ATPase motifs, including the well-known Walker A (P-loop) and B motifs and the ABC signature motif (LSGGQ), which is strictly characteristic of ABC-type ATPases and is found in NrtD as a (I/L)SGG(M/Q) sequence. Because two ATPase subunits or domains contribute to the binding of the nucleotide (Bass et al. 2003) an ATPase homo- or hetero-dimer is commonly found in ABC-type transporters.

The cyanobacterial *nir* operons that determine ABC-type uptake permeases generally contain a gene, *nrtC*, encoding a protein of about 660 amino acids with two well differentiated domains: an N-terminal domain homologous to NrtD (amino acids 1–260) and a C-terminal domain homologous to NrtA (amino acids 261–660). The N-terminal domain of NrtC from the filamentous cyanobacterium *Phormidium laminosum* has been expressed in *Escherichia coli* and shown to bind

ATP (Nagore et al. 2003). The N-terminal domain of NrtC can thus represent a second ABC-domain of the transporter, while the the C-terminal domain, which provides a cytoplasmic nitrate/nitrite-binding site, may have a regulatory role (see below).

The NrtABCD transporter together with the cyanobacterial CynABD (cyanate) and CmpABCD (bicarbonate) transporters make up a distinctive family within the ABC-type uptake transporters (family 3.A.1.16 of the transporter classification of Busch and Saier 2002).

Major facilitator superfamily permease

Some cyanobacteria carry a single nitrate transport *gene, nrtP* (Sakamoto et al. 1999; termed *napA* by Wang et al. 2000; see Figure 2). NrtP belongs to the nitrate/nitrite porter family (family 2.A.1.8 of the transporter classification of Busch and Saier 2002), which also contains transporters of other bacteria, fungi, algae and plants, including the well-characterized NRT2 transporter (Forde 2000), and is part of the major facilitator superfamily of proteins (MFS). Like some other MFS permeases, NrtP likely contains 12 transmembrane segments in the form of α-helices. The recently determined structure of two MFS proteins, LacY and GlpT, shows that the 12 transmembrane helices are organized in two domains (corresponding to the N- and C-terminal halves of the proteins) with a two-fold symmetry axis (see discussion by Locher et al. 2003). This type of structure supports an 'alternating access' mechanism of transport, in which the substrate binding site is alternatively accessible to one side of the membrane or to the other but not to both sides simultaneously (Locher et al. 2003). Interestingly, the DNA sequences encoding NrtP are split into two overlapping genes in *Synechococcus* sp. strain WH 8102 (Figure 2). It is tempting to speculate that these genes would encode, respectively, the N-terminal and C-terminal halves of a functional NrtP protein.

Like NrtABCD, NrtP is a nitrate/nitrite permease (Sakamoto et al. 1999; Wang et al. 2000). MFS permeases that mediate uniport, symport or antiport are known, but the fueling mechanism for cyanobacterial NrtP has not yet been determined, although Na^+ or H^+ symport has been suggested (Sakamoto et al. 1999; Wang et al. 2000). The NrtP permease, rather than an NrtABCD permease, is commonly found in marine cyanobacteria (Sakamoto et al. 1999; Wang et al. 2000; Bird and Wyman 2003), but NrtP has now been found as part of the nitrate assimilation system of *Nostoc punctiforme*, a soil cyanobacterium that exhibits a great capacity for establishing symbiotic associations with plants (Meeks et al. 2001).

Ferredoxin–nitrate reductase

Cyanobacterial nitrate reductases are enzymes that catalyze the 2-electron reduction of nitrate to nitrite for assimilatory purposes. They are monomers of the product of the *narB* gene with a molecular weight of about 80 kDa, which contain a [4Fe–4S] cluster and a Mo-cofactor (Mikami and Ida 1984; Rubio et al. 2002; Jepson et al. 2004). Cyanobacterial nitrate reductase is partially associated with the thylakoid membrane and can use photosynthetically reduced ferredoxin or flavodoxin as physiological electron donor (Candau et al. 1976; Manzano et al. 1976), although it can also accept electrons from artificial electron donors like methyl viologen. The complex between nitrate reductase and ferredoxin is electrostatically stabilized by the interaction of lysine and arginine residues on nitrate reductase and negatively charged residues on ferredoxin (Hirasawa et al. 2004).

The specific activities reported for purified cyanobacterial nitrate reductases lie between 20 and 300 units (μmol NO_2^- formed/min·mg of NarB) in the ferredoxin-dependent activity assay, and between 700 and 1000 units in the methyl viologen-dependent assay (Mikami and Ida 1984; Rubio et al. 2002). Nitrate reductases from most unicellular cyanobacteria exhibit Michaelis–Menten kinetics with K_m for nitrate between 1 and 10 mM (Martín-Nieto et al. 1992; Rubio et al. 1996), although in a recent study performed with carefully-preserved protein preparations, values of 50–80 μM have been determined (Jepson et al. 2004). Nitrate reductases from filamentous nitrogen-fixing cyanobacteria and the unicellular organisms *Synechocystis* sp. strain PCC 6803 and *Synechococcus* sp. strain RF-1 exhibit biphasic kinetics with K_m for nitrate ranging between 0.05–0.5 mM for the high affinity component and 5–20 mM for the low affinity component (Martín-Nieto et al. 1992; Wang et al. 2003). The

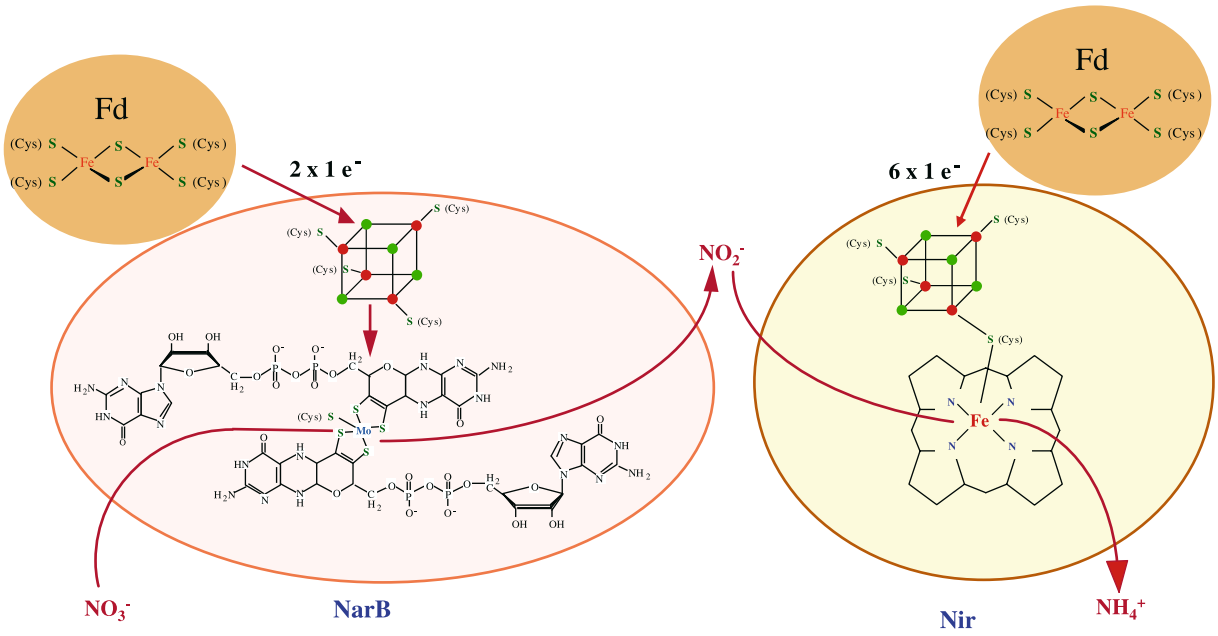

Figure 3. Schematic representation of the *Synechococcus elongatus* nitrate reductase (NarB) and nitrite reductase (Nir) proteins, with indication of their prosthetic groups (iron–sulfur center and molybdenum cofactor for NarB; iron–sulfur center and siroheme for Nir) and their interactions with ferredoxin (Fd) and the nitrogen substrates. Proteins and prosthetic groups are not to scale. In the iron–sulfur centers, iron atoms are represented in red, and sulfur atoms in green.

molecular basis of this dual-affinity behavior is currently unknown.

Biochemical studies on *S. elongatus* nitrate reductase and comparisons to homologous reductases whose molecular structures have been solved, have led to a model in which the Mo-cofactor of nitrate reductase from cyanobacteria would be in the form of Mo-*bis*-molybdopterin guanine dinucleotide (Mo-*bis*-MGD; Figure 3). In this model, the Mo atom is coordinated by at least five thiolate ligands, the dithiolene groups of two MGDs and the thiol group of a Cys residue (Cys^{148} in *S. elongatus* NarB) found to be conserved among cyanobacterial and other nitrate reductases (Rubio et al. 2002; Jepson et al. 2004). The [4Fe–4S] center is coordinated by four Cys residues that form a well-conserved cluster at the N-terminus of NarB (Cys^{9}, Cys^{12}, Cys^{16} and Cys^{56} in *S. elongatus* NarB). The metal centers in *S. elongatus* nitrate reductase have midpoint redox potentials of –190 and –150 mV for $[4Fe–4S]^{1+/2+}$ and $Mo^{6+/5+}$, respectively (Jepson et al. 2004). Interestingly, nitrate reductase activity is highly enhanced at potentials below –200 mV when its metal centers are mostly in the $[4Fe–4S]^{1+}$ and Mo^{5+} states (Jepson et al. 2004). The physiological relevance of this feature is that low potential electron donors are needed to transfer electrons to this enzyme. Electrons from reduced ferredoxin are presumably transferred to the [4Fe–4S] cluster and subsequently to the Mo-cofactor, the site of substrate reduction on nitrate reductase. Ferredoxin–nitrate reductase is thus different from the family of bacterial NADH-dependent assimilatory nitrate reductases, which are heterodimers of a 45-kDa FAD-containing diaphorase and a 95-kDa catalytic subunit that carries a [4Fe–4S] cluster and a MGD cofactor (Moreno-Vivián et al. 1999). Cyanobacterial nitrate reductase is also different from the well characterized fungal and plant NAD(P)H-dependent nitrate reductases, which are modular proteins that carry FAD-binding, heme-binding, and molybdopterin-binding domains (Campbell 1999).

The biosynthesis of the Mo-cofactor of nitrate reductase

As mentioned above, cyanobacterial nitrate reductase contains a Mo-cofactor that constitutes the active site of the enzyme. All Mo-cofactors in

nature, with the exception of FeMo-co of nitrogenase, share a common basic chemical structure, a Mo–molybdopterin (Mo–MPT). Much of what is known about the biosynthesis of Mo-cofactors comes from the pioneering studies on *Escherichia coli* (Rajagopalan and Johnson 1992; Rajagopalan 1996), although significant contributions have been made afterwards by studying plant and human systems (for a review, see Mendel and Hänsch 2002). In brief, the products of at least seven genes (*moaA*, *moaC*, *moaD*, *moaE*, *moeA*, *moeB* and *mobA*) are required for the biosynthesis of Mo-cofactor in all bacteria studied so far. In addition, the products of *moaB*, *mog*, and *mobB* seem to be involved in some organisms.

The biosynthesis of Mo-cofactor for bacterial enzymes proceeds in four steps (see Figure. 4). First, conversion of a guanosine derivative, presumably GTP, to a sulfur-free pterin known as precursor Z; the products of *moaA* and *moaC* are required here. Second, the addition of two sulfur atoms to precursor Z to generate MPT, catalyzed by the heterotetrameric enzyme MPT synthase ($MoaE_2MoaD_2$). The sulfur atoms required to form the dithiolene group of MPT are transferred from the MoaD subunits, which in the active form are thiocarboxylated at the C-terminal Gly residues. The regeneration of the thiocarboxylated MPT synthase is achieved by the concerted action of an activating enzyme (encoded by *moeB*) and a sulfurilase, an activity exhibited by a rhodanase-like C-terminal extension in MoeB proteins from plants and humans, but by a different and unidentified protein in some bacteria (see below for cyanobacteria). The third step in Mo-cofactor biosynthesis is the activation and incorporation of Mo to MPT to generate Mo–MPT; the products of *moeA* and *mog* (or *moaB*) are required at this step. The fourth step is the MobA-dependent attachment of a ribonucleotide to Mo–MPT to synthesize a dinucleotide form of Mo-cofactor. MobB is a GTP-binding protein that facilitates the activity of MobA but does not seem essential for this process. Recent studies indicate that steps three and four of Mo-cofactor biosynthesis in bacteria are likely to proceed simultaneously and are coupled to the insertion of the cofactor into the apoenzyme.

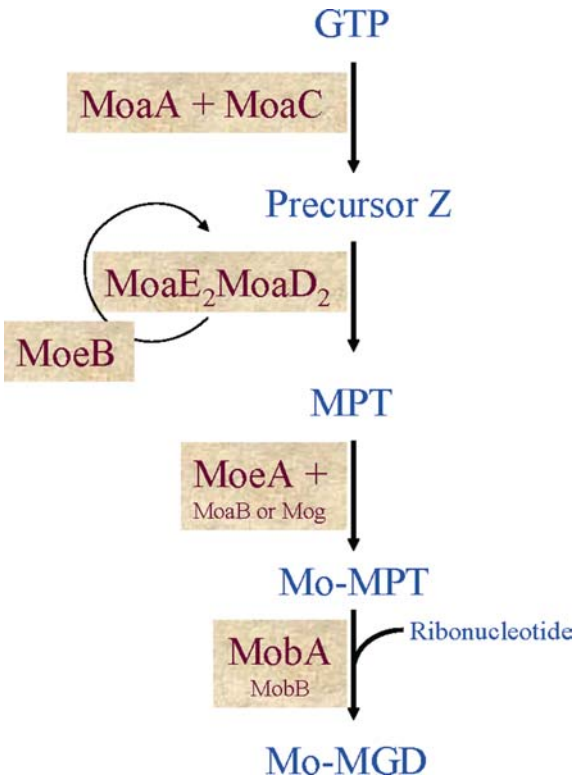

Figure 4. Deduced Mo-molybdopterin guanine dinucleotide (MGD) biosynthesis pathway (see text for details). MoaC is fused to MoaB/Mog in *S. elongatus* and to MobA in *T. elongatus* and *Synechocystis* sp. strain PCC 6803. MoeB is indicated to participate in regeneration of an active $MoaE_2$–$MoaD_2$ complex. Names in small print denote proteins that are not strictly required for the indicated reaction to proceed.

All *narB*-containing cyanobacterial genomes sequenced to-date carry *moaA*, *moaC*, *moaE*, *moeA*, *moeB*, and *mobA* genes. The detection of MoaD proteins by sequence comparison methods is more complicated because MoaD is a small protein (80–90 amino acids) with low conservation in the overall sequence. However, genes encoding MoaD-like proteins can be identified in most cyanobacterial sequenced genomes, and it is likely that all cyanobacterial strains containing *moaE* genes also contain their *moaD* counterparts. Interestingly, all cyanobacterial genomes, including those lacking *narB* genes, carry *moeB* genes. Cyanobacterial MoeB belongs to the eukaryotic type (containing the rhodanase-like extension) and is not capable of complementing an *E. coli moeB* mutant (Rubio et al. 1999). The unfruitful attempts to inactivate *S. elongatus moeB* have been interpreted in terms of MoeB having essential function(s) in addition to the one in MPT biosynthesis (Rubio et al. 1999). The presence of MoeB and MoaD-like proteins in cyanobacterial strains clearly lacking a Mo-cofactor biosynthesis pathway (e.g. some *Prochlorococcus* strains) sug-

gests that the different functions of MoeB would share a similar mechanism. Cyanobacterial nitrate reductase contains an MPT guanine dinucleotide (MGD) as active site. Accordingly, the *mobA* gene has been described in *S. elongatus* (Rubio et al. 1999) and is present in all *narB*-containing genomes. Finally, some cyanobacterial genomes carry either a *mog* gene or the homologous *moaB* gene but not both genes. The absence of *moaB*/*mog* genes in *Synechocystis* sp. strain PCC 6803 and *Thermosynechococcus elongatus*, which bear *narB*, suggests that their function(s) are not essential for Mo-cofactor synthesis. Likewise, MobB, which does not seem essential for the attachment of the dinucleotide, is not found in cyanobacteria.

In *S. elongatus*, the Mo–MPT biosynthesis genes *moaA*, *moaC*, *moaD*, *moaE* and *moeA* are clustered together (Rubio et al. 1998), the first four genes forming an operon (*moaC – moaD – moaE – moaA*), whereas the *moeB* gene, encoding the bifunctional MoeB protein, and *mobA* are in other independent genome locations (Rubio et al. 1999). In some of the cyanobacteria represented in Figure 2, those Mo–MPT biosynthesis genes are linked to other nitrate assimilation genes. A putative *moeA–moaA–moaC–moaD–moaE* operon is found in *T. elongatus* immediately upstream from the *ntcB* gene and in *Synechocystis* sp. strain PCC 6803 closely linked to, downstream from, the *nir* gene. *Synechococcus* sp. strain WH 8102 (and also strain WH 8103; Bird and Wyman 2003) also has some Mo–MPT biosynthesis genes clustered between *narB* and *nir*.

Genes specific for the biosynthesis of the nitrate reductase iron–sulfur center have not been identified by mutagenesis, likely reflecting the involvement of their protein products in different metabolic pathways (Frazon and Dean 2003). On the other hand, the recently reported *narM* gene, identified in *S. elongatus* (Maeda and Omata 2004) and located downstream of *narB* in some cyanobacteria (Figure 2), could encode a protein involved in nitrate reductase maturation or cofactor biosynthesis.

Ferredoxin–nitrite reductase

Cyanobacterial nitrite reductases catalyze the assimilatory 6-electron reduction of nitrite to ammonium (see Knaff and Hirasawa 1991 for a review). They are monomers of the *nir* gene product with a molecular weight of 52–56 kDa, which contain a [4Fe–4S] cluster and a siroheme as prosthetic groups. The physiological electron donor to nitrite reductase is the photosynthetically reduced ferredoxin or flavodoxin (Manzano et al. 1976), although it can also accept electrons from reduced viologens. The interaction of nitrite reductase with ferredoxin seems to be stabilized by electrostatic forces. It has been shown that at least two regions in nitrite reductase that are rich in positively-charged amino acid residues (amino acids 14–22 and 473–479) contribute to the binding of ferredoxin (Curdt et al. 2000). An interesting variation is found in *Plectonema boryanum* nitrite reductase, which contains a C-terminal domain extension similar to plant-type [2Fe–2S]-containing ferredoxins that could be involved in intramolecular electron transfer to the [4Fe–4S] cluster (Suzuki et al. 1995a).

Nitrite reductase enzymes from the cyanobacteria *P. laminosum*, *Anabaena* sp. strain PCC 7119 and *A. cylindrica* have been purified, characterized and shown to have biochemical properties much alike those of plant and green algae assimilatory nitrite reductases, and different from the nitrite reductases of non-photosynthetic organisms (Hattori and Uesugi 1968; Méndez and Vega 1981; Arizmendi and Serra 1990). Highly purified nitrite reductase exhibits specific activities of up to 225 nmol NH_4^+ formed/min·mg of protein at 30 °C with K_m values of 5–22 μM for ferredoxin and 40–50 μM for nitrite. CO, CN^- and thiol reagents are inhibitors of nitrite reductase activity when tested under reducing conditions. Unlike nitrite reductases from non-photosynthetic organisms, cyanobacterial nitrite reductase cannot accept electrons from reduced FAD, FMN, NADH or NADPH. Definitive proof demonstrating that assimilatory nitrite reductases from different oxygenic photosynthetic organisms are indeed very similar enzymes, came when the *nir* gene from *S. elongatus* was sequenced showing that its product presents high amino acid similarity to plant nitrite reductases and to the hemoprotein subunit of sulfite reductase from enteric bacteria (Luque et al. 1993).

The three-dimensional molecular structure of assimilatory nitrite reductase has not yet been solved but, because of their biochemical,

spectroscopic and sequence similarities, it is thought to be similar to that of the hemoprotein subunit of sulfite reductase from *E. coli* (McRee et al. 1986; Crane et al. 1995). This enzyme consists of three domains with an α/β mixed-sheet structure that are arranged forming a trilobal shape. All three domains are involved in the coordination of the prosthetic groups either by providing direct ligands or by contributing to the overall structure around the groups. The siroheme and the [4Fe–4S] center are juxtaposed at the interface of the three protein domains. The iron–sulfur center is bound to the protein through four cysteine residues one of which bridges it to the siroheme representing a common cysteine thiolate ligand. The sequence motif containing the four cysteine residues known to coordinate the prosthetic groups in sulfite reductases are also conserved in the sequence of nitrite reductase (Cys^{396}, Cys^{402}, Cys^{437} and Cys^{441} in *S. elongatus* Nir). Electrons are transferred from ferredoxin to the [4Fe–4S] cluster and subsequently to the siroheme, where nitrite is reduced to ammonium (Figure 3). The enzyme catalytic cycle has been investigated in plant nitrite reductase, and it appears to involve siroheme-bound NO and NH_2OH as intermediates that result from successive 2-electron accumulation steps in the cycle (Kuznetsova et al. 2004).

As mentioned above for nitrate reductase iron–sulfur center, genes specific for the biosynthesis of the nitrite reductase prosthetic groups have not been identified by mutagenesis, likely reflecting the involvement of iron–sulfur center and siroheme biosynthesis proteins in different metabolic pathways (Frazon and Dean 2003). Nonetheless, the *cysG* gene encoding a siroheme-synthesizing enzyme is notably clustered with the *nir* gene in some cyanobacteria (e.g., *Synechococcus* sp. strains WH 8102 and WH 8103; for the latter, see Bird and Wyman 2003). On the other hand, the *nirB* gene, which would encode a protein with no significant similarity to any known protein and appears to be required for maximum expression of nitrite reductase activity, has been identified in *S. elongatus* (Suzuki et al. 1995b). A *Synechococcus nirB* mutant is, however, able to grow using nitrate as nitrogen source. This gene is clustered together with the *ntcB* gene (see below) constituting an operon (*nirB–ntcB*) that is transcribed only in the absence of ammonium and divergently from the *nir* operon (Figure 2).

Regulation of expression of the *nir* operon

Nitrate reductase and nitrite reductase activities are lower in ammonium-grown than in nitrate-grown cyanobacterial cells (Herrero et al. 1981, 1985; Herrero and Guerrero 1986). Expression of these enzyme activities takes place at appreciable levels in the absence of nitrate or nitrite in some cyanobacteria like *S. elongatus*, but not in the heterocyst-forming N_2-fixing cyanobacteria. These observations led to the notion that in the non-N_2-fixing cyanobacteria, the nitrate assimilation system is mainly subjected to ammonium-promoted repression, whereas in the N_2-fixing cyanobacteria, in addition to repression by ammonium, induction by nitrate or nitrite would also be required for expression (Flores and Herrero 1994). Repression by ammonium is not observed in cells treated with MSX, indicating that ammonium assimilation via glutamine synthetase is required for repression (Herrero et al. 1981, 1985). Interestingly, the highest nitrate reductase levels were observed in MSX-treated cells supplemented with nitrate, which cannot assimilate the ammonium produced by the intracellular reduction of nitrate.

Expression of the *nir* operon at the mRNA level has now been examined in a number of cyanobacteria and found to take place upon ammonium withdrawal from the culture medium (Suzuki et al. 1993; Luque et al. 1994b; Merchán et al. 1995; Suzuki et al. 1995a; Cai and Wolk 1997; Frías et al. 1997). However, in *S. elongatus* (Luque et al. 1994b; Kikuchi et al. 1996) and *Anabaena* sp. strain PCC 7120 (Frías et al. 1997), it has been shown that the transcript levels are higher in the presence of nitrate than in its absence. Expression under nitrogen deprivation or in nitrate-supplemented cultures, but not in the presence of ammonium or urea, has also been shown for *Synechococcus* strains in which the permease (*nrtP*) and reductases genes are expressed as independent transcripts (Sakamoto et al. 1999; Bird and Wyman 2003). In *Synechocystis* sp. strain PCC 6803, expression of two nitrate assimilation transcriptional units, *nrtABCD–narB* and *nirA* (Figure 2), has been shown to take place in the absence of ammonium, or in response to inhibition of ammonium assimilation, with a further positive effect of nitrite (Aichi et al. 2001).

The genetic basis for ammonium-promoted repression in cyanobacteria has now been

identified as a requirement for activation of gene expression in the absence of ammonium. NtcA, a transcription factor of the CRP (or CAP) family of bacterial transcriptional regulators, activates expression of the *nir* operon, as well as of other genes involved in nitrogen assimilation, upon ammonium withdrawal, probably responding to the cellular C to N ratio (Vega-Palas et al. 1990, 1992; Frías et al. 1994; Luque et al. 1994b, 2004). This ratio is likely signaled in cyanobacteria by the cellular levels of 2-oxoglutarate (Muro-Pastor et al. 2001). Indeed, exogenously added 2-oxoglutarate has been shown to stimulate the expression of NtcA-dependent genes, including the *nir* operon, in a strain of *S. elongatus* transformed with a heterologous 2-oxoglutarate permease (Vázquez-Bermúdez et al. 2003). NtcA appears to be influenced by 2-oxoglutarate both directly and indirectly. Directly, because *in vitro* binding of NtcA to some NtcA-regulated promoters is stimulated in the presence on 2-oxoglutarate (Vázquez-Bermúdez et al. 2002; Tanigawa et al. 2002), and indirectly, because the 2-oxoglutarate-binding protein P_{II} has been shown to be required for expression of the *nir* operon and other NtcA-regulated genes under nitrogen deprivation, although not in nitrate-containing media (Aldehni et al. 2003; Paz-Yepes et al. 2003). The cyanobacterial P_{II} protein is a trimer of the *glnB* gene product that can be phosphorylated at a Ser residue (Forchhammer 2004). The degree of phosphorylation of P_{II} increases with the cellular C to N ratio, and phosphorylated P_{II} appears more active than the non-phosphorylated form of the protein in promoting NtcA-dependent gene expression (Paz-Yepes et al. 2003).

Structure and function of the nir *operon promoter*

NtcA activates transcription by binding to the promoter region of the regulated genes at a DNA-binding site with the consensus sequence $GTAN_8TAC$, which is frequently found about 22 bp upstream of a canonical –10 promoter box of sequence TAN_3T (Luque et al. 1994b; Herrero et al. 2001). Two *nir* operon promoters, those of *S. elongatus* and *Anabaena* sp. strain PCC 7120, have been analyzed in detail and found to bear a complex structure with several binding sites for NtcA and also for a second transcription factor, NtcB, which belongs to the LysR family of bacterial transcriptional regulators (Suzuki et al. 1995b; Frías et al. 2000). In both *S. elongatus* and *Anabaena* sp. strain PCC 7120, as well as in some other cyanobacteria, the *ntcB* gene is found closely linked to the *nir* operon (Figure 2).

Three NtcA-binding sites are found in the DNA region between the *nir* and *nirB–ntcB* operons of *S. elongatus* (Figure 5a). They are centered at positions −40.5, −109.5, and −180.5 with respect to the transcription start point of the *nir* operon, which is located 32 bp upstream of the translation start of *nir* (Luque et al. 1994b; 29 bp in Suzuki et al. 1993). The binding site centered at position −180.5 appears to be part of an NtcA-activated promoter that directs the expression of the divergent *nirB–ntcB* operon from a transcription start point located 30 bp upstream of *nirB* (Suzuki et al. 1995b; Maeda et al. 1998). The binding site centered at position −40.5 is included in a canonical NtcA-activated promoter structure (Luque et al. 1994b), which can be typified as a bacterial Class II activated promoter (Barnard et al. 2004) and is essential for expression of the *nir* operon (Maeda et al. 1998). The site centered at position −109.5, together with an NtcB-binding site centered at position −70, has been implicated in a nitrite-dependent enhancement of the expression of the *nir* operon that is observed in the presence of inhibitors of ammonium assimilation (Maeda et al. 1998). Consistently, expression of the *Synechococcus nir* operon shows a strict dependency on NtcA (Luque et al. 1994b; Maeda et al. 1998) but not on NtcB (Suzuki et al. 1995b). On the other hand, a negative effect of NtcB in nitrogen-deprived medium (Aichi and Omata 1997) and a repressor role of the −109.5 NtcA-binding site in ammonium-supplemented medium (Maeda et al. 1998) have been observed. The mechanism through which NtcA and NtcB bound to their DNA target sites interact with each other and with RNA polymerase is unknown.

NtcA and NtcB have been shown to bind simultaneously to the *nir* operon promoter of *Anabaena* sp. strain PCC 7120 (Frías et al. 2000), which presents NtcA- and NtcB-binding sites centered at positions −41.5 and −93, respectively, with respect to the transcription start point (Figure 5b). High-level expression of the *Anabaena nir* operon depends on both NtcA and NtcB (Frías et al. 2000). The structure and operation of the *Anabaena nir* operon promoter are therefore similar to

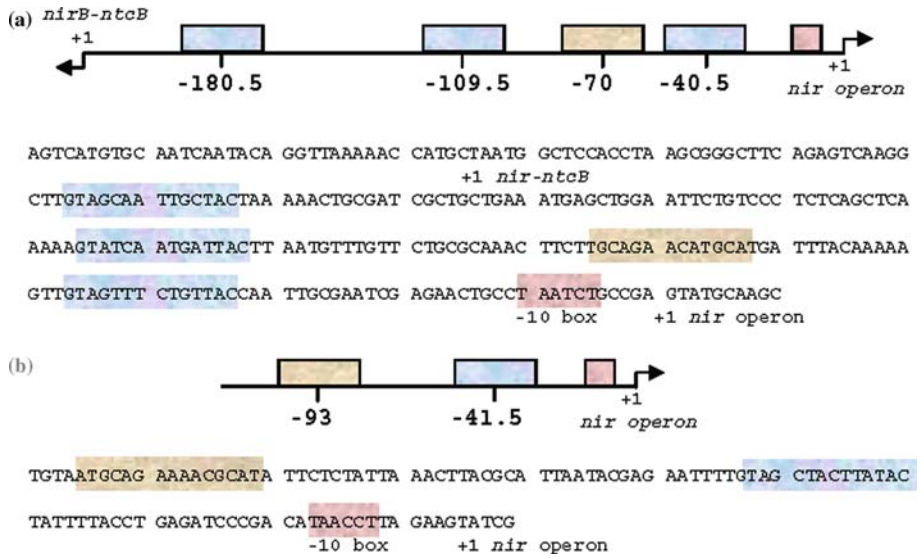

Figure 5. Schematic representation and sequence of the *nir* operon promoter in *S. elongatus* (a) and *Anabaena* sp. strain PCC 7120 (b). NtcA binding sites (blue), NtcB binding sites (light brown) and −10 promoter elements (pink) are boxed. Transcription start points (+1) are also indicated (note that the *S. elongatus nirB–ntcB* operon is divergent from the *nir* operon).

those of some other complex bacterial promoters in which efficient recruitment of RNA polymerase depends on contacts with both a Class I (in this case NtcB) and a Class II (NtcA) activator (Barnard et al. 2004). Whereas NtcA can promote N-regulated expression of the *nir* operon at a low-level in the absence of NtcB, the latter acts as a booster of expression in response to ammonium withdrawal. Expression of *ntcB* itself takes place from an NtcA-dependent promoter (Frías et al. 2000), ensuring that NtcB is produced at appreciable levels only under conditions of lack of ammonium.

In contrast to NtcA that is strictly necessary for expression of the *nir* operon in all investigated cyanobacterial strains, NtcB seems to be involved in regulation with different stringency levels. NtcB is strictly required for nitrate assimilation in *Anabaena* sp. strain PCC 7120, independent of the presence of nitrate or nitrite in the medium. In *S. elongatus,* NtcB has been described to mediate a nitrite-dependent enhancement of *nir* transcription but not to be absolutely required for nitrate assimilation. In *Synechocystis* sp. strain PCC 6803 an intermediate situation has been reported. NtcB is here necessary to accumulate substantial levels of *nirA* and *nrtABCD–narB* transcripts independent of the presence of nitrate and, additionally, it mediates a nitrite-dependent enhancement of the expression of these genes (Aichi et al. 2001). A *Synechocystis ntcB* insertional mutant shows reduced growth with nitrate as the nitrogen source (Aichi et al. 2001).

Contribution of other factors to nir *operon expression*

The *cnaT* gene, which has been recently identified in *Anabaena* sp. strain PCC 7120, encodes a protein required for expression of the *nir* operon (Frías et al. 2003). CnaT shows overall similarity to proteins with glycosyl transferase activity and, in particular, to anthranilate phosphoribosyltransferase (TrpD) of the tryptophan biosynthesis pathway. ORFs of unknown function homologous to *cnaT* are found in other cyanobacterial genomes, and in every case the *cnaT* homologue is located adjacent to *ntcB* or other nitrate assimilation genes (Figure 2). An *Anabaena cnaT* insertional mutant is unable to use nitrate as a nitrogen source due to a defect in activation of transcription of the *nir* operon. However, CnaT does not appear to be a DNA-binding protein and, consequently, the effect of CnaT on *nir* operon expression may be indirect. The *Anabaena cnaT* mutant shows a high reversion frequency to a positive nitrate assimilation phenotype (Nas^+) suggesting the existence of an additional negative factor that acts downstream of CnaT in the regulation of *nir* operon expression. The expression of *cnaT* is constitutive with regard to the nitrogen status, which would ensure the

presence of the CnaT protein under different nitrogen regimes. No effect of inactivation of a *cnaT* homologue on the expression of the *nir* operon has been observed in *S. elongatus* (Maeda and Omata 2004).

In addition to regulation of transcription initiation, mRNA stability could contribute to the determination of transcript levels of nitrate assimilation genes. Consistent with this possibility, the 5′ to 3′ decay of the *nir* operon transcript level that has been observed in *Anabaena* sp. strain PCC 7120 is lower in the presence than in absence of nitrate (Frías et al. 1997). The *Anabaena nir* operon transcript has a large leader sequence consisting of 460 nucleotides (Frías et al. 1997) that might be the target of unknown regulatory factor(s). Finally, in *S. elongatus*, nitrate reductase has been shown to be more stable in the presence than in absence of combined nitrogen in the medium, suggesting a post-translational regulation of this protein (Herrero et al. 1984).

Regulation of nitrate uptake

Nitrate-grown cyanobacterial cells exhibit the ability to take up nitrate when this ion is present at low concentrations in the medium, but nitrate uptake is rapidly inhibited if ammonium is added to the medium (Ohmori et al. 1977; Flores et al. 1980). Thus, in addition to the regulation at the level of gene expression, the nitrate assimilation system appears to be subjected to regulation at the activity level. The short-term inhibition of nitrate uptake might involve an inhibition of nitrate transport into the cell. This notion was corroborated by analyzing the uptake of nitrite, which in cyanobacteria takes place via permease (NrtABCD or NrtP)-mediated active transport and by diffusion of nitrous acid through the cytoplasmic membrane; only the permease-mediated nitrite uptake is inhibited by ammonium (Flores et al. 1987). Additionally, an inhibitory effect of ammonium on the operation of nitrate reductase in *S. elongatus* may take place (Kobayashi et al. 1997).

Inhibition of nitrate uptake by ammonium is not observed in cells treated with MSX indicating that ammonium has to be incorporated into carbon skeletons via glutamine synthetase to exert inhibition (Flores et al. 1980). In order to take up nitrate at appreciable rates, cells of *S. elongatus* require the simultaneous fixation of CO_2, but this requirement can also be overridden by treatment of the cells with MSX (Flores et al. 1983b). These observations suggested that the nitrate permease is subjected to allosteric regulation by negative effectors generated through ammonium assimilation or/and by positive effectors resulting from CO_2 fixation, with a putative key regulatory role of 2-oxoglutarate (Romero et al. 1985).

The short-term inhibition of nitrate (and nitrite) uptake by ammonium is not observed in a *S. elongatus glnB* insertional mutant, implicating the P_{II} protein in regulation of activity of the nitrate/nitrite permease (Lee et al. 1998). The CO_2-dependence of nitrate uptake is also lost in the *glnB* mutant (Forchhammer and Tandeau de Marsac 1995). These observations are consistent with the above-mentioned putative physiological role of 2-oxoglutarate in the regulation of the transport process. The non-phosphorylated form of P_{II}, which accumulates in cells experiencing a low C to N ratio, appears to be always inhibitory for the permease, whereas the phosphorylated form may or may not be inhibitory depending on whether it is free or has bound 2-oxoglutarate, respectively (Lee et al. 2000). The mechanism of inhibition by P_{II} of the permease is unknown, but an interaction with the C-terminal half of the NrtC protein is possible, since deletion of such domain alleviates ammonium inhibition (Kobayashi et al. 1997). In this context, it is of interest that P_{II} can bind to the cytoplasmic membrane of nitrate-grown cells of *Synechocystis* sp. strain PCC 6803 (Huang et al. 2003).

Coming back to the beginning of this article, the CO_2-dependence of nitrate uptake in *S. elongatus* resembles the CO_2-dependence of nitrate reduction observed in *Chlorella* (Warburg et al. 1965). Although molecular details may be different, a regulatory role of the cellular C status in nitrate assimilation, as found for cyanobacteria, may offer the basis to understand the long-standing problem in plant physiology of the requirement of CO_2 for photosynthetic nitrate assimilation.

Concluding remarks

Classical physiological and biochemical research and more recent molecular genetics investigations

have converged to offer an integrated picture of the cyanobacterial nitrate assimilation system, which has also benefited from recent genome analysis that has added information on the nitrate assimilation capabilities and its genetic basis in cyanobacteria. The basic structural components of this system are the nitrate/nitrite permease, which can be either an ABC-type uptake transporter or an MFS permease that concentrates the substrates inside the cell, and the nitrate and nitrite reductases, which make use of photosynthetically reduced ferredoxin as an electron donor in what represents one of the simplest examples of photosynthesis. Genes encoding a nitrate/nitrite permease and both reductases are commonly found in a gene cluster that behaves as an operon. Additionally, closely linked to the nitrate assimilation (*nir*) operon, a set of genes is usually found that encode proteins which influence *nir* operon expression or nitrate assimilation activity. These include the NtcB transcription factor, the CnaT putative glycosyl transferase that affects transcription, and the NirB and NarM proteins that are required for attaining maximum levels of nitrite reductase and nitrate reductase, respectively. Recent research has also provided information on genes that encode proteins involved in the biosynthesis of the nitrate reductase molybdenum cofactor (Mo-*bis*-molybdopterin guanine dinucleotide), which is essential for nitrate reduction. Two general N-control proteins, the NtcA transcription factor and the signal transduction protein P_{II}, play essential roles in nitrate assimilation in cyanobacteria. Whereas the role in nitrate assimilation of NtcA-dependent activation of gene expression is well established, details of the function of NtcB and CnaT, which are required for *Anabaena nir* operon expression, or of the P_{II} protein, which regulates both gene expression and the activity of the nitrate/nitrite permease, remain to be known and represent an important task for future research. Finally, the search for genes encoding protein(s) mediating nitrate effect(s) on *nir* operon expression at different levels, e.g., transcript stability or translation efficiency, would also constitute an important research topic for the future.

Acknowledgements

Use of genomic sequences from the Kazusa DNA Research Institute (Japan) and the DOE Joint Genome Institute (USA) is acknowledged. Work currently supported by research grant BMC 2002-03902 from Ministerio de Educación y Ciencia, Spain.

References

Aichi M and Omata T (1997) Involvement of NtcB, a LysR family transcription factor, in nitrite activation of nitrate assimilation operon in the cyanobacterium *Synechococcus* sp. strain PCC 7942. J Bacteriol 179: 4671–4675

Aichi M, Takatani N and Omata T (2001) Role of NtcB in activation of nitrate assimilation genes in the cyanobacterium *Synechocystis* sp. strain PCC 6803. J Bacteriol 183: 5840–584

Aldehni MF, Sauer J, Spielhaupter C, Schmid R and Forchhammer K (2003) Signal transduction protein P_{II} is required for NtcA-regulated gene expression during nitrogen deprivation in the cyanobacterium *Synechococcus elongatus* strain PCC 7942. J Bacteriol 185: 2582–2591

Arizmendi JM and Serra JL (1990) Purification and some properties of the nitrite reductase from the cyanobacterium *Phormidium laminosum*. Biochim Biophys Acta 1040: 237–244

Arnon DI (1984) The discovery of photosynthetic phosphorylation. Trends Biochem Sci 9: 258–262

Arnon DI (1988) The discovery of ferredoxin: the photosynthetic path. Trends Biochem Sci 13: 30–33

Barnard A, Wolfe A and Busby S (2004) Regulation at complex bacterial promoters: how bacteria use different promoter organizations to produce different regulatory outcomes. Curr Opin Microbiol 7: 102–108

Bass RB, Locher KP, Borths E, Poon Y, Strop P, Lee A and Rees DC (2003) The structures of BtuCD and MscS and their implications for transporter and channel function. FEBS Lett 555: 111–115

Bird C and Wyman M (2003) Nitrate/nitrite assimilation system of the marine picoplanktonic cyanobacterium *Synechococcus* sp. strain WH 8103: effect of nitrogen source and availability on gene expression. Appl Environ Microbiol 69: 7009–7018

Busch W and Saier MH Jr (2002) The transporter classification (TC) system, 2002. Crit Rev Biochem Mol Biol 37: 287–337

Cai Y and Wolk CP (1997) Nitrogen deprivation of *Anabaena* sp. strain PCC 7120 elicits rapid activation of a gene cluster that is essential for uptake and utilization of nitrate. J Bacteriol 179: 258–266

Campbell WH (1999) Nitrate reductase structure, function and regulation: bridging the gap between biochemistry and physiology. Annu Rev Plant Physiol Plant Mol Biol 50: 277–303

Candau P, Manzano C and Losada M (1976) Bioconversion of light energy into chemical energy through reduction with water of nitrate to ammonia. Nature 262: 715–717

Crane BR, Siegel LM and Getzoff ED (1995) Sulfite reductase structure at 1.6 Å: evolution and catalysis for reduction of inorganic anions. Science 270: 59–67

Curdt I, Singh BB, Jakoby M, Hachtel W and Böhme H (2000) Identification of amino acid residues of nitrite reductase from *Anabaena* sp. PCC 7120 involved in ferredoxin binding. Biochim Biophys Acta 1543: 60–68

Davidson AL and Chen J (2004) ATP-binding cassette trasporters in bacteria. Annu Rev Biochem 73: 241–268

Dufresne A, Salanoubat M, Partensky F, Artiguenave F, Axmann IM, Barbe V, Duprat S, Galperin MY, Koonin EV, Le Gall F, Makarova KS, Ostrowski M, Oztas S, Robert C, Rogozin IB, Scanlan DJ, Tandeau de Marsac N, Weissenbach J, Wincker P, Wolf YI and Hess WR (2003) Genome sequence of the cyanobacterium *Prochlorococcus* marinus SS120, a nearly minimal oxyphototrophic genome. Proc Natl Acad Sci USA 100: 10020–10025

Flores E and Herrero A (1994) Assimilatory nitrogen metabolism and its regulation. In: Bryant DA (ed) The Molecular Biology of Cyanobacteria, pp 487–517. Kluwer Academic Publishers, Dordrecht, The Netherlands

Flores E, Guerrero MG and Losada M (1980) Short-term ammonium inhibition of nitrate utilization by *Anacystis nidulans* and other cyanobacteria. Arch Microbiol 128: 137–144

Flores E, Guerrero MG and Losada M (1983a) Photosynthetic nature of nitrate uptake and reduction in the cyanobacterium *Anacystis nidulans*. Biochim Biophys Acta 722: 408–416

Flores E, Romero JM, Guerrero MG and Losada (1983b) Regulatory interaction of photosynthetic nitrate utilization and carbon dioxide fixation in the cyanobacterium *Anacystis nidulans*. Biochim Biophys Acta 725: 529–532

Flores E, Herrero A and Guerrero MG (1987) Nitrite uptake and its regulation in the cyanobacterium *Anacystis nidulans*. Biochim Biophys Acta 896: 103–108

Forchhammer K (2004) Global carbon/nitrogen control by P_{II} si gnal transduction in cyanobacteria: from signals to targets. FEMS Microbiol Rev 28: 319–333

Forchhammer K and Tandeau de Marsac N (1995) Functional analysis of the phosphoprotein P_{II} (*glnB* gene product) in the cyanobacterium *Synechococcus* sp. strain PCC 7942. J Bacteriol 177: 2033–2040

Forde BG (2000) Nitrate transporters in plants: structure, function and regulation. Biochim Biophys Acta 1465: 219–35

Frazon J and Dean DR (2003) Formation of iron–sulfur clusters in bacteria: an emerging field in bioinorganic chemistry. Curr Opin Chem Biol 7: 166–173

Frías JE, Flores E and Herrero A (1994) Requirement of the regulatory protein NtcA for the expression of nitrogen assimilation and heterocyst development genes in the cyanobacterium *Anabaena* sp. PCC 7120. Mol Microbiol 14: 823–832

Frías JE, Flores E and Herrero A (1997) Nitrate assimilation gene cluster from the heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120. J Bacteriol 179: 477–486

Frías JE, Flores E and Herrero A (2000) Activation of the *Anabaena nir* operon promoter requires both NtcA (CAP family) and NtcB (LysR family) transcription factors. Mol Microbiol 38: 613–625

Frías JE, Herrero A and Flores E (2003) Open reading frame *all0601* from *Anabaena* sp. strain PCC 7120 represents a novel gene, *cnaT*, required for expression of the nitrate assimilation *nir* operon. J Bacteriol 185: 5037–5044

Guerrero MG, Manzano C and Losada M (1974) Nitrite photoreduction by a cell-free preparation of *Anacystis nidulans*. Plant Sci Lett 3: 273–278

Hattori A (1962) Light-induced reduction of nitrate, nitrite and hydroxylamine in a blue-green alga, *Anabaena cylindrica*. Plant Cell Physiol 3: 355–369

Hattori A and Myers J (1967) Reduction of nitrate and nitrite by subcellular preparations of *Anabaena cylindrica*. II. Reduction of nitrate to nitrite. Plant Cell Physiol 8: 327–337

Hattori A and Uesugi I (1968) Purification and properties of nitrite reductase from the blue-green alga *Anabaena cylindrica*. Plant Cell Physiol 9: 689–699

Herrero A and Guerrero MG (1986) Regulation of nitrite reductase in the cyanobacterium *Anacystis nidulans*. J Gen Microbiol 132: 2463–2468

Herrero A, Flores E and Guerrero MG (1981) Regulation of nitrate reductase levels in the cyanobacteria *Anacystis nidulans, Anabaena* sp. strain 7119, and *Nostoc* sp. strain 6719. J Bacteriol 145: 175–180

Herrero A, Flores E and Guerrero MG (1984) Regulation of the nitrate reductase level in *Anacystis nidulans*: activity decay under nitrogen stress. Arch Biochem Biophys 234: 454–459

Herrero A, Flores E and Guerrero MG (1985) Regulation of nitrate reductase cellular levels in the cyanobacteria *Anabaena variabilis* and *Synechocystis* sp. FEMS Microbiol Lett 26: 21–25

Herrero A, Muro-Pastor AM and Flores E (2001) Nitrogen control in cyanobacteria. J Bacteriol 183: 411–425

Hill R (1939) Oxygen produced by isolated chloroplasts. Proc Royal Soc B 127: 192–210

Hirasawa M, Rubio LM, Griffin JL, Flores E, Herrero A, Li J, Kim SK, Hurley JK, Tollin G and Knaff DB (2004) Complex formation between ferredoxin and *Synechococcus* ferredoxin: nitrate oxidoreductase. Biochim Biophys Acta 1608: 155–162

Huang F, Parmryd I, Nilsson F, Persson AL, Pakrasi HB, Andersson B and Norling B (2003) Proteomics of *Synechocystis* sp. strain PCC 6803. Identification of plasma membrane proteins. Mol Cell Proteom 1: 956–966

Jepson BJN, Anderson LJ, Rubio LM, Taylor CJ, Butler CS, Flores E, Herrero A, Butt JN and Richardson DJ (2004) Tuning a nitrate reductase for function: the first spectropotentiometric characterization of a bacterial assimilatory nitrate reductase reveals novel redox properties. J Biol Chem 279: 32212–32218

Kikuchi H, Aichi M, Suzuki I and Omata T (1996) Positive regulation by nitrite of the nitrate assimilation operon in the cyanobacteria *Synechococcus* sp. strain PCC 7942 and *Plectonema boryanum*. J Bacteriol 178: 5822–5825

Knaff DB (1996) Ferredoxin and ferredoxin-dependent enzymes. In: Ort DR and Yocum CF (eds) Oxygenic Photosynthesis: the Light Reactions, pp 333–361. Kluwer Academic Publishers, Dordrecht, The Netherlands

Knaff DB and Hirasawa M (1991) Ferredoxin-dependent chloroplast enzymes. Biochim Biophys Acta 1056: 93–125

Kobayashi M, Rodríguez R, Lara C and Omata T (1997) Involvement of the C-terminal domain of an ATP-binding subunit in the regulation of the ABC-type nitrate/nitrite transporter of the cyanobacterium *Synechococcus* sp. strain PCC 7942. J Biol Chem 272: 27197–27201

Kuznetsova S, Knaff DB, Hirasawa M, Lagoutte B and Setif P (2004) Mechanism of spinach chloroplast ferredoxin-dependent nitrite reductase: spectroscopic evidence for intermediate states. Biochemistry 43: 510–517

Lee HM, Flores E, Herrero A, Houmard J and Tandeau de Marsac N (1998) A role for the signal transduction protein P_{II} in the control of nitrate/nitrite uptake in a cyanobacterium. FEBS Lett 427: 291–295

Lee HM, Flores E, Forchhammer K, Herrero A and Tandeau de Marsac N (2000) Phosphorylation of the signal transducer P_{II} protein and an additional effector are required for the P_{II}-mediated regulation of nitrate and nitrite uptake in the cyanobacterium *Synechococcus* sp. PCC 7942. Eur J Biochem 267: 591–600

Locher P, Bass RB and Rees DC (2003) Breaching the barrier. Science 301: 603–604

López-Lozano A, Díez J, El Alaoui S, Moreno-Vivián C and García-Fernández JM (2002) Nitrate is reduced by heterotrophic bacteria but not transferred to *Prochlorococcus* in non-axenic cultures. FEMS Microbiol Ecol 41: 151–160

Luque I, Herrero A, Flores E and Madueño F (1992) Clustering of genes involved in nitrate assimilation in the cyanobacterium *Synechococcus*. Mol Gen Genet 232: 7–11

Luque I, Flores E and Herrero A (1993) Nitrite reductase gene from *Synechococcus* sp. PCC 7942: homology between cyanobacterial and higher-plant nitrite reductases. Plant Mol Biol 21: 1201–1205

Luque I, Flores E and Herrero A (1994a) Nitrate and nitrite transport in the cyanobacterium *Synechococcus* sp. PCC 7942 are mediated by the same permease. Biochim Biophys Acta 1184: 296–298

Luque I, Flores E and Herrero A (1994b) Molecular mechanism for the operation of nitrogen control in cyanobacteria. EMBO J 13: 2862–2869

Luque I, Vázquez-Bermúdez MF, Paz-Yepes J, Flores E and Herrero A (2004) In vivo activity of the nitrogen control transcription factor NtcA is subjected to metabolic regulation in *Synechococcus* sp. strain PCC 7942. FEMS Microbiol Lett 236: 47–52

Madueño F, Flores E and Guerrero MG (1987) Competition between nitrate and nitrite uptake in the cyanobacterium *Anacystis nidulans*. Biochim Biophys Acta 896: 109–112

Madueño F, Vega-Palas MA, Flores E and Herrero A (1988) A cytoplasmic-membrane protein repressible by ammonium in *Synechococcus* R2: altered expression in nitrate-assimilation mutants. FEBS Lett 239: 289–291

Maeda SI and Omata T (1997) Substrate-binding lipoprotein of the cyanobacterium *Synechococcus* sp. strain PCC 7942 involved in the transport of nitrate and nitrite. J Biol Chem 272: 3036–3041

Maeda S and Omata T (2004) A novel gene (*narM*) required for expression of nitrate reductase activity in the cyanobacterium *Synechococcus elongatus* strain PCC7942. J Bacteriol 186: 2107–2114

Maeda SI, Kawaguchi Y, Ohe TA and Omata T (1998) *cis*-Acting sequences required for NtcB-dependent, nitrite-responsive positive regulation of the nitrate assimilation operon in the cyanobacterium *Synechococcus* sp. strain PCC 7942. J Bacteriol 180: 4080–4088

Manzano C, Candau P, Gómez-Moreno C, Relimpio AM and Losada M (1976) Ferredoxindependent photosynthetic reduction of nitrate and nitrite by particles of *Anacystis nidulans*. Mol Cell Biochem 10: 161–169

Martín-Nieto J, Flores E and Herrero A (1992) Biphasic kinetic behavior of nitrate reductase from heterocystous, nitrogen-fixing cyanobacteria. Plant Physiol 100: 157–163

McRee DE, Richardson DC, Richardson JS and Siegel LM (1986) The heme and Fe_4S_4 cluster in the crystallographic structure of *Escherichia coli* sulfite reductase. J Biol Chem 261: 10277–10281

Meeks JC, Wycoff KL, Chapman JS and Enderlin CS (1983) Regulation of expression of nitrate and dinitrogen assimilation by *Anabaena* species. Appl Environ Microbiol 45: 1351–1359

Meeks JC, Elhai J, Thiel T, Potts M, Larimer F, Lamerdin J, Predki P and Atlas R (2001) An overview of the genome of *Nostoc punctiforme*, a multicellular, symbiotic cyanobacterium. Photosynth Res 70: 85–106

Mendel RR and Hänsch (2002) Molybdoenzymes and molybdenum cofactors in plants. J Exp Bot 53: 1689–1698

Méndez JM and Vega JM (1981) Purification and molecular properties of nitrite reductase from *Anabaena* sp. 7119. Physiol Plant 52: 7–14

Merchán F, Kindle KL, Llama MJ, Serra JL and Fernández E (1995) Cloning and sequencing of the nitrate transport system from the thermophilic, filamentous cyanobacterium *Phormidium laminosum*: comparative analysis with the homologous system from *Synechococcus* sp. PCC 7942. Plant Mol Biol 28: 759–766

Mikami B and Ida S (1984) Purification and properties of ferredoxin–nitrate reductase from the cyanobacterium *Plectonema boryanum*. Biochim Biophys Acta 791: 294–304

Miller SR and Castenholz RW (2001) Ecological physiology of *Synechococcus* sp. strain SH-94-5, a naturally occurring cyanobacterium deficient in nitrate assimilation. Appl Environ Microbiol 67: 3002–3009

Moreno-Vivián C, Cabello P, Martínez-Luque M, Blasco R and Castillo F (1999) Prokaryotic nitrate reduction: molecular properties and functional distinction among bacterial nitrate reductases. J Bacteriol 181: 6573–6584

Muro-Pastor MI, Reyes JC and Florencio FJ (2001) Cyanobacteria perceive nitrogen status by sensing intracellular 2-oxoglutarate levels. J Biol Chem 276: 38320–38328

Nagore D, Llarena M, Llama MJ and Serra JL (2003) Characterization of the N-terminal domain of NrtC, the ATP-binding subunit of ABC-type nitrate transporter of the cyanobacterium *Phormidium laminosum*. Biochim Biophys Acta 1623: 143–153

Ohmori M, Ohmori K and Strotmann H (1977) Inhibition of nitrate uptake by ammonia in a blue-green alga, *Anabaena cylindrica*. Arch Microbiol 114: 225–229

Omata T (1991) Cloning and characterization of the *nrtA* gene that encodes a 45-kDa protein involved in nitrate transport in the cyanobacterium *Synechococcus* PCC 7942. Plant Cell Physiol 32: 151–157

Omata T, Ohmori M, Arai N and Ogawa T (1989) Genetically engineered mutant of the cyanobacterium *Synechococcus* PCC 7942 defective in nitrate transport. Proc Natl Acad Sci USA 86: 6612–6616

Omata T, Andriesse X and Hirano A (1993) Identification and characterization of a gene cluster involved in nitrate transport of the cyanobacterium *Synechococcus* sp. PCC7942. Mol Gen Genet 236: 193–202

Ortega T, Castillo F and Cárdenas J (1976) Photolysis of water coupled to nitrate reduction by *Nostoc muscorum* subcellular particles. Biochem Biophys Res Commun 71: 885–891

Palenik B, Brahamsha B, Larimer FW, Land M, Hauser L, Chain P, Lamerdin J, Regala W, Allen EE, McCarren J, Paulsen I, Dufresne A, Partensky F, Webb EA and Waterbury J (2003) The genome of a motile marine *Synechococcus*. Nature 424: 1037–1042

Paneque A, Ramírez JM, del Campo FF and Losada M (1964) Light and dark reduction of nitrite in a reconstituted enzymic system. J Biol Chem 239: 1737–1741

Paz-Yepes J, Flores E and Herrero A (2003) Transcriptional effects of the signal transduction protein P_{II} (*glnB* gene product) on NtcA-dependent genes in *Synechococcus* sp. PCC 7942. FEBS Lett 543: 42–46

Persson B and Argos P (1996) Topology prediction of membrane proteins. Protein Sci 5: 363–371

Rajagopalan KV (1996) Biosynthesis of the molybdenum cofactor. In: Neidhardt FC (ed) *Escherichia coli* and *Salmonella*: cellular and molecular biology, pp 674–679. ASM Press, Washington, DC

Rajagopalan KV and Johnson JL (1992) The pterin molybdenum cofactors. J Biol Chem 267: 10199–10202

Rocap G, Larimer FW, Lamerdin J, Malfatti S, Chain P, Ahlgren NA, Arellano A, Coleman M, Hauser L, Hess WR, Johnson ZI, Land M, Lindell D, Post AF, Regala W, Shah M, Shaw SL, Steglich C, Sullivan MB, Ting CS, Tolonen A, Webb EA, Zinser ER and Chisholm SW (2003) Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. Nature 424: 1042–1047

Rodríguez R, Lara C and Guerrero MG (1992) Nitrate transport in the cyanobacterium *Anacystis nidulans* R2. Kinetic and energetic aspects. Biochem J 282: 639–643

Romero JM, Flores E and Guerrero MG (1985) Inhibition of nitrate utilization by amino acids in intact *Anacystis nidulans* cells. Arch Microbiol 142: 1–5

Rubio LM, Herrero A and Flores E (1996) A cyanobacterial *narB* gene encodes a ferredoxindependent nitrate reductase. Plant Mol Biol 30: 845–850

Rubio LM, Flores E and Herrero A (1998) The *narA* locus of *Synechococcus* sp. strain PCC 7942 consists of a cluster of molybdopterin biosynthesis genes. J Bacteriol 180: 1200–1206

Rubio LM, Flores E and Herrero A (1999) Molybdopterin guanine dinucleotide cofactor in *Synechococcus* sp. nitrate reductase: identification of *mobA* and isolation of a putative *moeB* gene. FEBS Lett 462: 358–362

Rubio LM, Flores E and Herrero A (2002) Purification, cofactor analysis, and site-directed mutagenesis of *Synechococcus* ferredoxin–nitrate reductase. Photosynth Res 72: 13–26

Sakamoto T, Inoue-Sakamoto K and Bryant DA (1999) A novel nitrate/nitrite permease in the marine cyanobacterium *Synechococcus* sp. strain PCC 7002. J Bacteriol 181: 7363–7372

Sazuka T (2003) Proteomic analysis of the cyanobacterium *Anabaena* sp. strain PCC7120 with two-dimensional gel electrophoresis and amino-terminal sequencing. Photosynth Res 78: 279–291

Serrano A, Rivas J and Losada M (1981) Nitrate and nitrite as 'in vivo' quenchers of chlorophyll fluorescence in blue-green algae. Photosynth Res 2: 175–184

Sivak MN, Lara C, Romero JM, Rodríguez R and Guerrero MG (1989) Relationship between a 47-kDa cytoplasmic membrane polypeptide and nitrate transport in *Anacystis nidulans*. Biochem Biophys Res Commun 158: 257–262

Suzuki I, Sugiyama T and Omata T (1993) Primary structure and transcriptional regulation of the gene for nitrite reductase from the cyanobacterium *Synechococcus* PCC 7942. Plant Cell Physiol 34: 1311–1320

Suzuki I, Kikuchi H, Nakanishi S, Fujita Y, Sugiyama T and Omata T (1995a) A novel nitrite reductase gene from the cyanobacterium *Plectonema boryanum*. J Bacteriol 177: 6137–6143

Suzuki I, Horie N, Sugiyama T and Omata T (1995b) Identification and characterization of two nitrogen-regulated genes of the cyanobacterium *Synechococcus* sp. strain PCC7942 required for maximum efficiency of nitrogen assimilation. J Bacteriol 177: 290–296

Tanigawa R, Shirokane M, Maeda Si S, Omata T, Tanaka K and Takahashi H (2002) Transcriptional activation of NtcA-dependent promoters of *Synechococcus* sp. PCC 7942 by 2-oxoglutarate *in vitro*. Proc Natl Acad Sci USA 99: 4251–4255

Tischner R and Schmidt A (1984) Light mediated regulation of nitrate assimilation in *Synechococcus leopoliensis*. Arch Microbiol 137: 151–154

Van Niel CB (1941) The bacterial photosyntheses and their importance for the general problem of photosynthesis. Adv Enzymol 1: 263–328

Vázquez-Bermúdez MF, Herrero A and Flores E (2002) 2-Oxoglutarate increases the binding affinity of the NtcA (nitrogen control) transcription factor for the *Synechococcus glnA* promoter. FEBS Lett 512: 71–74

Vázquez-Bermúdez MF, Herrero A and Flores E (2003) Carbon supply and 2-oxoglutarate effects on expression of nitrate reductase and nitrogen-regulated genes in *Synechococcus* sp. strain PCC 7942. FEMS Microbiol Lett 221: 155–159

Vega-Palas MA, Madueño F, Herrero A and Flores E (1990) Identification and cloning of a regulatory gene for nitrogen assimilation in the cyanobacterium *Synechococcus* sp. strain PCC 7942. J Bacteriol 172: 643–647

Vega-Palas MA, Flores E and Herrero A (1992) NtcA, a global nitrogen regulator from the cyanobacterium *Synechococcus* that belongs to the Crp family of bacterial regulators. Mol Microbiol 6: 1853–1859

Wang Q, Li H and Post AF (2000) Nitrate assimilation genes of the marine diazotrophic, filamentous cyanobacterium *Trichodesmium* sp. strain WH9601. J Bacteriol 182: 1764–1767

Wang TH, Fu H and Shieh YJ (2003) Monomeric NarB is a dual-affinity nitrate reductase, and its activity is regulated differently from that of nitrate uptake in the unicellular diazotrophic cyanobacterium *Synechococcus* sp. strain RF-1. J Bacteriol 185: 5838–5846

Warburg O, Krippahl G and Jetschmann (1965) Widerlegung der Photolyse des Wassers und Beweis der Photolyse der Kohlensäure nach Versuchen mit lebender *Chlorella* und den Hill-Reagentien Nitrat und $K_3Fe(CN)_6$. Z Naturforsch 20b: 993–996

Wu Q and Stewart V (1998) NasFED proteins mediate assimilatory nitrate and nitrite transport in *Klebsiella oxytoca* (*pneumoniae*) M5al. J Bacteriol 180: 1311–1322