Dynamic changes in gene expression of the cyanobacterium Synechocystis sp. PCC 6803 in response to nitrogen starvation

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Chapter 1

General introduction

Contents

1. Cyanobacteria

1.1. Prelude

Cyanobacteria represent a large phylum of Bacteria able to perform photosynthesis. Some of the oldest fossils on Earth, from Australian Archaean rocks dated around 3.5 billion years ago, belong to ancient cyanobacteria (Schopf, 1993 and 2006). Nowadays, cyanobacteria may be found in almost every imaginable habitat on Earth: in oceans and fresh water, on rocks and in soil, and some of them have even been isolated from hot springs and Antarctic ice sheets. They may be single-celled, may form multicellular filaments or aggregate to colonies of various shapes (Fig. 1).

Probably the most striking feature of cyanobacteria is their ability to fix carbon dioxide and produce oxygen in the process of oxygenic photosynthesis. In fact, it is believed that photosynthesis by ancient cyanobacteria has been responsible for the large amounts of oxygen in the atmosphere of our planet. Oxygenic photosynthesis is performed on thylakoid

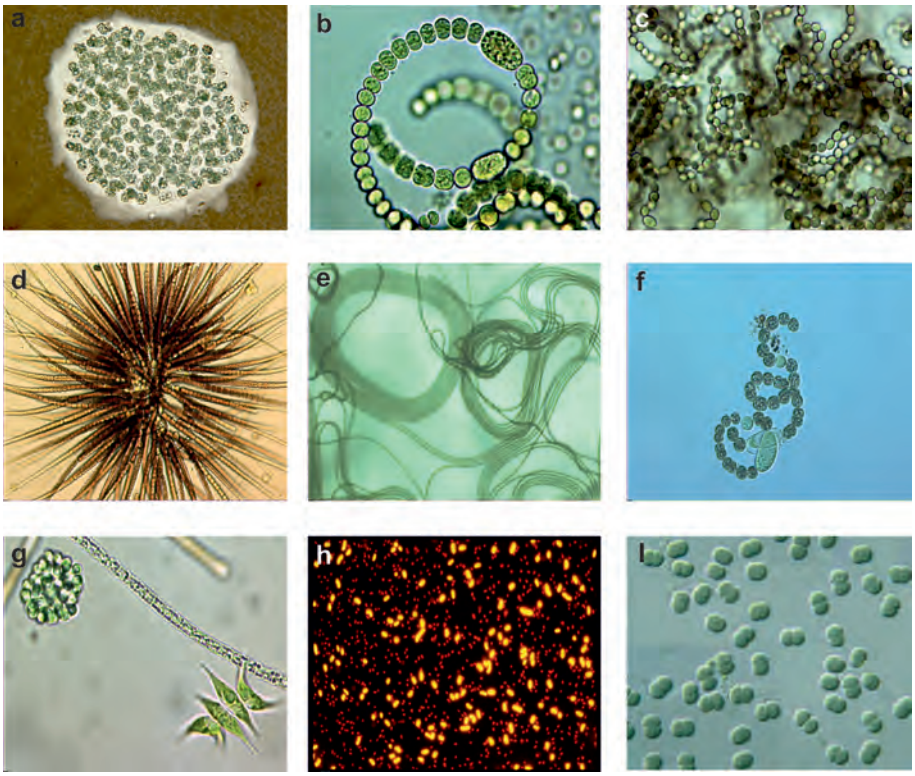

Figure 1. Cyanobacterial biodiversity. A. *Microcystis*, **B**. *Anabaena*, **C**. *Nostoc*, **D**. *Gloeotrichia*, **E**. *Planktothrix*, **F**. *Anabaena*, **G**. *Planktothrix*, *Scenedesmus* and *Gomphosphaeria*, **H**. *Synechococcus* spp. from the Baltic Sea, **I**. *Synechocystis* sp. PCC 6803. Photos are courtesy of the research group Aquatic Microbiology, University of Amsterdam.

membranes. These internal membranes embed sophisticated protein complexes involved in the light reactions and photosynthetic electron transfer by tandem action of Photosystem II and Photosystem I. These protein complexes of cyanobacteria are regarded as the evolutionary origins of those used for oxygenic photosynthesis in chloroplasts of eukaryotic algae and higher plants. Water is used as electron donor for Photosystem II, releasing oxygen as by-product. Some cyanobacteria may also use hydrogen sulfide as electron donor (Cohen *et al*., 1986). This rare relict may indicate how cyanobacteria themselves gained their photosynthetic ability from purple and green sulfur bacteria (Blankenship, 1992), although other analyses suggest that photosynthesis originated in the cyanobacterial lineage and the first phototrophs were anaerobic "procyanobacteria" (Mulkidjanian *et al*., 2006). A full respiratory chain, including a cytochrome aa3 type of terminal oxidase, can provide cyanobacteria with energy in darkness (Matthijs *et al.*, 1984; Hart *et al*., 2005). In some strains fermentation during anaerobic growth conditions has been shown as well (Stal and Moezelaar, 1997). These multiple physiological attributes have provided extreme versatility to cyanobacteria, not only making them evolutionary highly interesting organisms, but also explaining their ubiquitous distribution on Earth till this very day. A schematic representation of the cyanobacterial cell is shown in Figure 2.

The name 'cyano' (blue, in Greek) refers to a typical attribute of the light harvesting antennae of cyanobacteria. Phycobilisomes are attached to the thylakoid membrane, and bear the phycocyanin pigment that is responsible for the blue-green pigmentation of most cyanobacteria. Phycobilisomes play a role in light harvesting for Photosystem II. Other

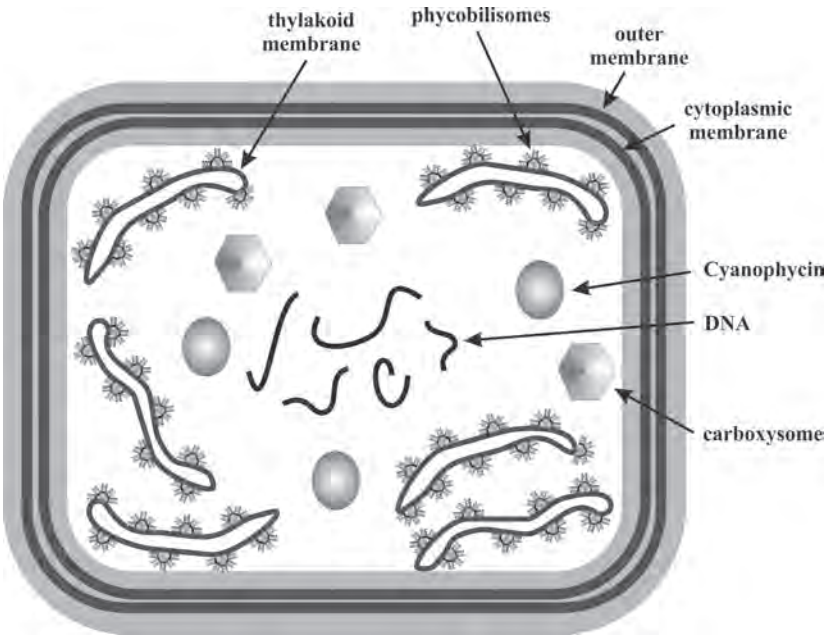

Figure 2. Diagram of the cyanobacterial cell.

pigments may be present as well, such as phycoerythrins (also located in phycobilisomes) and carotenoids, introducing red, brown and yellow palettes. Differences in relative proportion of chlorophyll *a*, phycocyanin, phycoerythrin and other accessory pigments give cyanobacteria a plethora of color variations: yellow, red, violet, pink, green, deep blue and blue-green cyanobacteria are known (Haverkamp *et al.*, 2009). The light spectrum also influences the pigment composition of cyanobacteria, and this way cells can maximize the use of available light for photosynthesis. In particular, through a process known as complementary chromatic adaptation, some cyanobacteria adapt their pigment composition to complement the prevailing light spectrum. Accordingly, these cyanobacteria appear bluish green by accumulating phycocyanin in red light, while cells turn reddish by producing phycoerythrin in green light (Stomp *et al*., 2004 and 2008).

Marine cyanobacteria are the most abundant photosynthetic organisms in the oceans. *Prochlorococcus* and *Synechococcus* are prevalent in the picoplanktonic fraction (with cell diameter less than 2 μm). The *Prochlorococcus* group dominates the nutrient-poor subtropical gyres of the world's oceans, and it is speculated to be the most abundant photosynthetic organism on the planet (Partensky *et al*., 1999). In the subtropical Pacific, for example, it often represents 50% of the total chlorophyll. These tiny cyanobacteria are also the smallest known photosynthetic organisms with a genome size of approximately 2 Mb. While *Synechococcus* is less abundant on a global scale, it has a more cosmopolitan distribution and is both genetically and morphologically more diverse than *Prochlorococcus*. *Synechococcus* cells can bloom in nutrient-rich coastal waters, and are also often found in freshwater ecosystems.

1.2. *Synechocystis* sp. PCC 6803

Model cyanobacterium *Synechocystis* sp. PCC 6803 is a small unicellular picocyanobacterium isolated from a freshwater lake in 1968 (Fig. 1i). The genome of *Synechocystis* sp. PCC 6803 is the first completely sequenced genome of a photosynthetic organism (Kaneko *et al.,* 1996). *Synechocystis* offers the advantage of an easily accessible genomic database with a relatively small genome size. *Synechocystis* can also be grown photo- and chemoheterotrophically, enabling decoupling of the light and dark reactions of photosynthesis. Moreover, *Synechocystis* can integrate foreign DNA into its own DNA via homologous recombination (Shestakov and Khyen, 1970), which makes cloning procedures for *Synechocystis* sp. PCC 6803 straightforward and thus provides researchers with an easy tool for targeted mutagenesis. Hence, *Synechocystis* has been one of the most popular organisms for genetic, biochemical and physiological studies, particularly for the analysis of oxygenic photosynthesis.

2. Current advances in transcriptomics and proteomics of cyanobacteria

2.1. "…omics"

Recently, the biological sciences have been revolutionized by a series of "omes": the genome (the complete genetic information of an organism), the transcriptome (all messenger RNAs expressed at a given time in a cell), the proteome (the protein equivalent), and the latest addition to the family is the metabolome (all small molecules that are the product of enzymatic and chemical activity within the cell). In contrast to the genome, which is fairly inert, the latter three molecular entities are highly dynamic and vary greatly according to endogenous and exogenous conditions; they also vary throughout the life cycle of an organism. The dynamic expression of genes as mRNA (the transcriptome) can be followed in a quantitative and qualitative manner using binding assays based on DNA arrays (Schena *et al*., 1995). Alternatively, sequencing methods based on either differential-display polymerase chain reactions (PCR) (Liang and Pardee, 1992 and 1995) or tagged-DNA approaches for serial analysis of gene expression (Velculescu *et al*., 1995) can be used. DNA-microarrays thus provide the technology to decipher how organisms exploit their genetic makeup in different environments. It has become an indispensable tool for analyzing the information encoded in the genome and transcriptome.

2.2. Cyanobacterial genomic projects

The complete genome sequence of the cyanobacterium *Synechocystis* sp. PCC 6803 was determined in 1996 by the Kazusa DNA Research Institute (http://genome.kazusa.or.jp/cyanobase). It was the first genome completely sequenced in Japan and the fourth in the world following the three genomes (*Haemophilus influenzae*, *Mycoplasma genitalium*, and *Methanococcus jannaschii*) sequenced earlier by TIGR. In comparison to other bacterial species, such as *Escherichia coli* and *Bacillus subtilis*, the *Synechocystis* genome contained a larger proportion of unknown genes, because cyanobacteria and their genes had not been well studied despite their importance in the evolution of life and the maintenance of the biosphere. Thus, with the availability of the genome sequence, gene annotation became a great spurt to cyanobacterial scientists, accelerating their research and resulting in a large number of publications.

Since 1996, more than 50 cyanobacterial genomes have been sequenced including freshwater and marine, unicellular and filamentous model organisms and ecologically relevant species. Some of the most noteworthy sequenced species are enlisted in Table 1.

2.3. Cyanobacterial transcriptomics

Since the appearance in Japan of the first microarray platform for the cyanobacterium *Synechocystis* sp. PCC 6803, in 2001 (Hihara *et al.*, 2001), researchers performed numerous experiments that made use of the transcriptome approach based on cDNA microarray platforms. The first oligo-microarray platform for the nitrogen-fixing cyanobacterium *Anabaena* sp. PCC

Table 1. A selection of published cyanobacterial genomes (http://genome.kazusa.or.jp/cyanobase).

Organism	Genome size *, bp	Year	Description	Reference
Synechocystis sp. PCC 6803	3,573,471	1996	naturally transformable freshwater unicellular cyanobacterium; can be grown photoheterotrophically	Kaneko *et al.* (1996)
Anabaena sp. PCC 7120	6,413,771	2001	freshwater nitrogen-fixing filamentous, heterocyst-forming cyanobacterium; obligate photoautotroph	Kaneko *et al.* (2001) Ohmori *et al.* (2001)
Thermosynechococcus elongatus BP-1	2,593,857	2002	formerly *Synechococcus elongatus*; thermophilic (optimum ca. 55 °C), unicellular	Nakamura *et al.* (2002)
Gloeobacter violaceus PCC 7421	4,659,019	2003	unicellular cyanobacterium, which lacks thylakoids and phycobilisomes are attached to the plasma membrane	Nakamura *et al.* (2003)
Prochlorococcus marinus MED4	1,657,995	2003	ecotype adapted to high light	Rocap *et al.* (2003)
Prochlorococcus marinus MIT9313	2,404,274	2003	ecotype adapted to low light	Rocap *et al.* (2003)
Prochlorococcus marinus SS120	1,751,080	2003	small genome size, marine; ecotype adapted to low light	Dufresne *et al.* (2003)
Synechococcus sp. WH8102	2,434,428	2003	marine, unicellular, swimming motility	Palenik *et al.* (2003)
Prochlorococcus marinus MIT9312	1,709,204	2004	marine high-light adapted ecotype	JGI[a]
Synechococcus elongatus PCC 7942	2,695,905	2004	freshwater unicellular	JGI
Synechococcus sp. CC9605	2,510,659	2004	marine oligotrophic	JGI
Synechococcus sp. CC9902	2,234,828	2004	marine coastal	JGI
Trichodesmium erythraeum IMS101	7,750,108	2004	marine filamentous, nitrogen fixing	JGI
Anabaena variabilis ATCC29413	6,365,727	2005	filamentous heterocyst-forming; intensively studied for its hydrogen production	JGI
Synechococcus sp. CC9311	2,606,748	2006	marine coastal	Palenik *et al.* (2006)
Synechococcus sp. JA-2-3B	3,046,682	2006	thermophilic *Synechococcus*, nitrogen fixing	Allewalt *et al.* (2006)
Synechococcus sp. JA-3-3Ab	2,932,766	2006	thermophilic *Synechococcus*, nitrogen fixing	Allewalt *et al.* (2006)
Microcystis aeruginosa NIES-843	5,842,795	2007	Bloom-forming toxic	Kaneko *et al.* (2007)
Synechococcus elongatus PCC 6301	2,696,255	2007	freshwater unicellular	Sugita *et al.* (2007)
Prochlorococcus marinus NATL2A	1,842,899	2007	marine low-light adapted ecotype	JGI
Prochlorococcus marinus MIT9215	1,738,790	2007	marine high-light adapted ecotype	JGI
Prochlorococcus marinus AS9601	1,669,886	2007	marine high-light adapted ecotype	Moore[b]
Prochlorococcus marinus MIT9515	1,704,176	2007	marine high-light adapted ecotype	Moore
Prochlorococcus marinus MIT9303	2,682,675	2007	marine low-light adapted ecotype	Moore
Prochlorococcus marinus NATL1A	1,864,731	2007	marine low-light adapted ecotype	Moore
Prochlorococcus marinus MIT9301	1,641,879	2007	marine high-light adapted ecotype	Moore
Synechococcus sp. RCC307	2,224,914	2007	marine	Genoscope[c]
Synechococcus sp. WH7803	2,366,980	2007	marine	Genoscope
Synechococcus sp. PCC7002	3,008,047	2008	marine	Penn[d]
Acaryochloris marina MBIC11017	6,503,724	2008	chlorophyll d as major (95%) pigment	TGen[e]
Nostoc punctiforme ATCC29133	8,234,322	2008	freshwater nitrogen-fixing filamentous, heterocyst-forming cyanobacterium	JGI
Prochlorococcus marinus MIT9211	1,688,963	2008	marine low-light adapted ecotype	Moore
Cyanothece sp. ATCC51142	4,934,271	2008	aerobic, unicellular, nitrogen-fixing cyanobacterium	Welsh *et al.* (2008)
Cyanothece sp. PCC7424	5,942,652	2008	unicellular isolated from rice fields	JGI
Cyanothece sp. PCC7425	5,374,574	2008	unicellular isolated from rice fields	JGI
Cyanothece sp. PCC8801	4,679,413	2008	unicellular isolated from rice fields	JGI
Arthrospira platensis NIES-39	6,788,435	2010	filamentous	Fujisawa *et al.* (2010)

***** genome size is given for the main chromosome only excluding plasmids; **a** published online by Joint Genome Institute (www.jgi.doe.gov); **b** The Gordon and Betty Moore Foundation; Marine Microbiology Initiative (www.moore.org); **c** Genoscope (www.cns.fr); **d** published by Penn State University; **e** TGen Sequencing Center.

7120 appeared in 2005 (Ehira *et al.*, 2005) and the first oligo-microarray for *Synechocystis* appeared in 2007 (Eisenhut *et al.*, 2007). Publication of many new cyanobacterial genomes facilitated fast appearance of new microarray platforms for various cyanobacteria, and this promoted more complete transcriptome analysis and the reconstruction of genomic networks in cyanobacteria. These studies investigated how the entire transcriptome responded to, amongst others, salt, osmotic, cold, high light and oxidative stress (Table 2).

Despite these advances, remarkably few studies have investigated the transcriptome response of cyanobacteria to nitrogen limitation. Sato *et al.* (2004) and Flaherty *et al.* (2011) investigated the transcriptome response of *Anabaena* PCC 7120 to nitrogen deprivation. This is a somewhat special case, however, since *Anabaena* can replenish its cellular nitrogen by atmospheric nitrogen fixation. Osanai *et al.* (2006) presented the response of the entire transcriptome of *Synechocystis* PCC 6803 to 4 hours of nitrogen starvation. These authors found induction of many sugar catabolic genes and repression of sugar anabolic genes. They also found induction of several genes related to nitrogen assimilation, and repression of photosynthetic genes and ribosomal protein genes. However, since their study covered only a limited time span of 4 hours, the persistence of these short-term responses or additional long-term responses to nitrogen starvation remained unknown. Hence, a better understanding of nitrogen limitation at the transcriptome level is one of the key challenges to be tackled in this PhD thesis.

2.4. Cyanobacterial proteomics

Although transcriptome studies have gained many new insights, there are several objections against the reduction of biological studies to monitoring of the transcriptome (mRNA) only: (1) the level of mRNA does not allow accurate prediction of the level of protein expression (Anderson and Seilhamer, 1997; Gygi *et al*., 1999); (2) protein function is controlled by many post-translational modifications; and (3) protein maturation and degradation are dynamic processes that dramatically alter the final amount of active protein, independent of the mRNA level. Correlation of the mRNA levels with the expression, modification and activity of proteins requires a systematic method for separating and visualizing the protein components of a cell that allows: (1) extraction and high-resolution separation of protein components, including membrane, extreme-pI and low-copy-number proteins; (2) identification and quantification of each component; and (3) comparison, analysis and visualization of complex changes in expression patterns.

In one of the first proteome studies of *Synechocystis* PCC 6803 proteins have been investigated by two-dimensional gel electrophoresis (2DGE) and *N*-terminal amino acid sequencing. In this study 234 protein spots could be identified (Sazuka *et al*., 1999). 2DGE aided by mass spectrometry identification of resolved spots has been successfully applied in qualitative characterization of the protein content of purified cellular sub-fractions of outer and cytoplasmic membranes (Huang *et al.,* 2002a and 2004), for thylakoid membranes (Wang *et al*., 2000; Herranen *et al.,* 2004), as well as for a total proteome of *Synechocystis*

(Gan *et al*., 2005). Numerous efforts have been undertaken for quantitative proteomics in cyanobacteria. However, only a limited number of proteins were found to be differentially expressed in the acclimation to high salinity (Fulda *et al.,* 2000 and 2006), high light (Choi *et al.,* 2000), or high pH stress (Zhang *et al*., 2009). The proteome of *Synechocystis* acclimated to low CO_2 was measured with the iTRAQ technique (isobaric Tag for Relative and Absolute Quantification; Ross *et al*., 2004). In this study, 19% of the *Synechocystis* proteome was identified and expression changes were quantified for 86% of the identified proteins (Battchikova *et al*., 2010). New trends in high-throughput proteomics of cyanobacteria are rapidly developing (reviewed by Ow and Wright, 2009).

3. Assimilatory nitrogen metabolism and its regulation

3.1. Nitrogen assimilation

Nitrogen is one of the key elements of life, and an important limiting nutrient in many terrestrial, freshwater and marine ecosystems (Vitousek and Howarth, 1991; Sterner and Elser, 2002). Microorganisms and plants incorporate nitrogen through assimilation processes. As sources of nitrogen they may utilize ammonium, nitrate and nitrite ions, sometimes dinitrogen gas, and organic compounds like urea, amino acids and some nitrogen-containing purine and pyrimidine bases. Uptake of these nitrogen-containing compounds takes place through permeases that are located in the cytoplasmic membrane (Fig. 3). For instance, ABC-type uptake transporters have been shown to be involved in the uptake of nitrate and nitrite (Omata *et al.,* 1993; Luque *et al*., 1993 and 1994) in several cyanobacteria. ABC-type permeases are also required for the uptake of amino acids like arginine and glutamine (Quintero *et al*., 2001). These permeases use ATP to drive active nitrogen uptake.

For most microorganisms ammonium is the preferred source of nitrogen, because ammonium can be easily incorporated into amino acids. The transport of ammonium is mediated by secondary permeases of the Amt family (Fig. 3). It has been shown that [^{14}C] methylammonium is accumulated in cells of the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803 to a level that suggests a membrane potential-driven transport (Montesinos *et al*., 1998; Vazquez-Bermudez *et al*., 2002). Intracellular ammonium is incorporated into carbon skeletons through the GS/GOGAT pathway (Fig. 3). In cyanobacteria, 2-oxoglutarate (2-OG) provided by the tricarboxylic acid (TCA) cycle is mainly used as carbon skeleton for the incorporation of nitrogen (Vazquez-Bermudez *et al*., 2000; Muro-Pastor *et al*., 2001). It was generally accepted that cyanobacteria lack 2-oxoglutarate dehydrogenase, an enzyme of the TCA cycle (Stanier and Cohen-Bazire, 1977). However, a recent finding has provided evidence that 2-oxoglutarate may be converted to succinate by an alternative enzyme, thus closing the TCA cycle (Zhang and Bryant, 2011). The enzyme glutamine oxoglutarate aminotransferase (GOGAT) combines one molecule of 2-oxoglurate with one molecule of glutamine to produce two molecules of glutamate. Subsequently, the two glutamate molecules incorporate two ammonium ions and are converted in tandem into two glutamine molecules

Table 2. Key studies featuring cyanobacterial transcriptomics.

Strain	Research question	Reference
Synechocystis sp. PCC 6803 [1]	Acclimation to high light	Hihara *et al.* (2001)
	Cold stress; cold-regulated genes by Hik33 kinase	Suzuki *et al.* (2001)
	Salt stress and hyperosmotic stress	Kanesaki *et al.* (2002)
	Screening for the targets of cAMP receptor protein	Yoshimura *et al.* (2002)
	Mn2+-sensing system	Yamaguchi *et al.* (2002)
	Osmotic stress and cold stress	Mikami *et al.* (2002)
	Salt stress; high light stress	Allakhverdiev *et al.* (2002)
	Cold stress	Inaba *et al.* (2003)
	Analysis of redox-responsive genes	Hihara *et al.* (2003)
	Perception of salt stress	Marin *et al.* (2003)
	Phosphate limitation	Suzuki *et al.* (2004)
	Oxidative stress	Kobayashi *et al.* (2004)
	High salinity stress	Hihara *et al.* (2004)
	High salinity stress	Marin *et al.* (2004)
	Osmotic stress, histidine kinases	Paithoonrangsarid *et al.* (2004)
	Salt stress	Asadulghani *et al.* (2004)
	Response to red and far-red light	Hubschmann *et al.* (2005)
	Salt and hyperosmotic signals	Shoumskaya *et al.* (2005)
	Regulation of sugar catabolic pathways	Osanai *et al.* (2005a)
	Heat shock	Suzuki *et al.* (2005)
	Transcription profiling of the delta-sll1961 mutant	Fujimori *et al.* (2005)
	Glucose sensitivity	Kahlon *et al.* (2006)
	Heat shock response	Suzuki *et al.* (2006)
	Nitrogen starvation	Osanai *et al.* (2006)
	Hydrogen peroxide perception	Kanesaki *et al.* (2007)
	Low-temperature stress	Prakash *et al.* (2010)
Synechocystis sp. PCC 6803 [2]	Light-to-dark transition	Gill *et al.* (2002)
	Response to irradiation with UV-B and white light	Huang *et al.* (2002b)
Synechocystis sp. PCC 6803 [3]	Response to iron deficiency and iron reconstitution	Singh *et al.* (2003)
	High and low light conditions	Tu *et al.* (2004)
	Expression in response to hydrogen peroxide	Li *et al.* (2004)
	Redox control of gene expression	Singh *et al.* (2004)
	Expression in delta-isiA mutant	Singh *et al.* (2005)
	Heat shock	Singh *et al.* (2006)
	Growth at alkaline conditions	Summerfield and Sherman (2008)
	Iron and oxidative stress	Shcolnick *et al.* (2009)
	Low-oxygen conditions	Summerfield *et al.* (2011)
Synechocystis sp. PCC 6803 [4]	Response to inorganic carbon limitation	Eisenhut *et al.* (2007)
	Heat acclimation	Tuominen *et al.* (2008)
	Coordination C/N metabolism	Schriek *et al.* (2008)
	Photorespiration	Hackenberg *et al.* (2009)
	Low-carbon acclimation	Hackenberg *et al.* (2012)
	Nitrogen starvation	This thesis (Chapter 5)
	Transitions from nitrogen to light limitation	This thesis (Chapter 6)
Anabaena sp. PCC 7120 [5]	Gene expression under desiccation	Katoh *et al.* (2004)
	Nitrogen deprivation, low temperature and drought stress	Sato *et al.* (2004)

Table 2 (continued). Key studies featuring cyanobacterial transcriptomics.

Strain	Research question	Reference
Anabaena sp. PCC 7120 [6]	Low-temperature regulated expression	Ehira *et al.* (2005)
	Sodium chloride, cAMP concentration	Imashimizu *et al.* (2005)
	Genes for trehalose metabolism in dehydration tolerance	Higo *et al.* (2006)
	Heterocyst development	Ehira and Ohmori (2006)
Fremyella diplosiphon [7]	Genes regulated by light color	Stowe-Evans *et al.* (2004)
	Chromatic adaptation	Shui *et al.* (2009)
Thermosynechococcus elongatus BP-1 [8]	Circadian clock expression	Kucho *et al.* (2004)
Prochlorococcus MED4 [9]	Light sensing	Steglich *et al.* (2006)
Prochlorococcus MIT9313 [9]	Host-phage interaction	Lindell *et al.* (2007)
	Light-dark cycle	Zinser *et al.* (2009)
	Iron availability	Thompson *et al.* (2011)
Cyanothece sp. ATCC 51142 [10]	Light-dark and continuous light growth	Toepel *et al.* (2008)
	Diurnal oscillation	Stöckel *et al.* (2008)
	Short day/night cycles	Toepel *et al.* (2009)
Microcystis aeruginosa PCC 7806 [11]	Light/dark cycle	Straub *et al.* (2011)
Synechococcus sp. WH7803 [12]	Oxidative stress	Blot *et al.* (2011)

Microarray platform: 1, CyanoCHIP – PCR-amplified DNA microarray (TaKaRa, Japan); 2, PCR-amplified DNA microarray (Gill *et al*., 2002); 3, PCR-amplified DNA microarray (Postier *et al*., 2003); 4, Agilent 60-mer oligonucleotide microarray; 5, Long chromosomal DNA fragments spotted as microarray (Katoh *et al*., 2004); 6, Complete oligo microarray (Ehira *et al*., 2005); 7, Long chromosomal DNA fragments representing half of the genome (Stowe-Evans *et al*., 2004); 8, 45-mer oligonucleotide microarray (Kucho *et al*., 2004); 9, Affymetrix high-density array MD4-9313; 10, Agilent 60-mer oligonucleotide microarray; 11, Agilent 4x44K 60-mer oligomicroarray; 12, 60-mer oligonucleotide.

by the enzyme glutamine synthetase (GS). One of these glutamines goes back into the GS/GOGAT cycle, while the other can be used for further amino acid synthesis. The GS/GOGAT pathway thus plays a key role in the C to N balance of the cells.

Two types of nitrate transport systems prevail in cyanobacteria, a prokaryotic ABC-type transporter known as NrtABCD (Omata *et al.*, 1993) and a nitrate transporter more similar to those found in Eukaryotes known as NrtP (Sakamoto *et al.*, 1999). NrtABCD effectively binds both nitrate and nitrite, whereas the affinity of NrtP is higher for nitrate than nitrite. In addition, a secondary transporter of the major facilitator superfamily has been identified as nitrate-nitrite transporter in some marine cyanobacteria (Sakamoto *et al*., 1999).

The ABC-type transporter NrtABCD is composed of four subunits (Fig. 4): (i) NrtA, a high-affinity periplasmic solute-binding lipoprotein, (ii) NrtB, an integral membrane permease, (iii) NrtC, a unique ATPase solute-binding fusion protein, and (iv) NrtD, a cytoplasmic ATPase (Omata, 1995). The 3D structure of NrtA from *Synechocystis* sp. PCC 6803 was revealed at a resolution of 1.5 Å (Koropatkin *et al*., 2006). NrtA is the most abundant protein in the plasma membrane of cyanobacteria, and necessary for cell survival when nitrate is the primary nitrogen source (Maeda and Omata, 1997). NrtA represents a unique class of transport proteins as it is significantly larger than other oxyanion-binding proteins. Nitrate is scavenged in the periplasmic space by NrtA and then delivered to the cytoplasmic membrane permease NrtB. The transfer of nitrate through the transmembrane pore is assisted

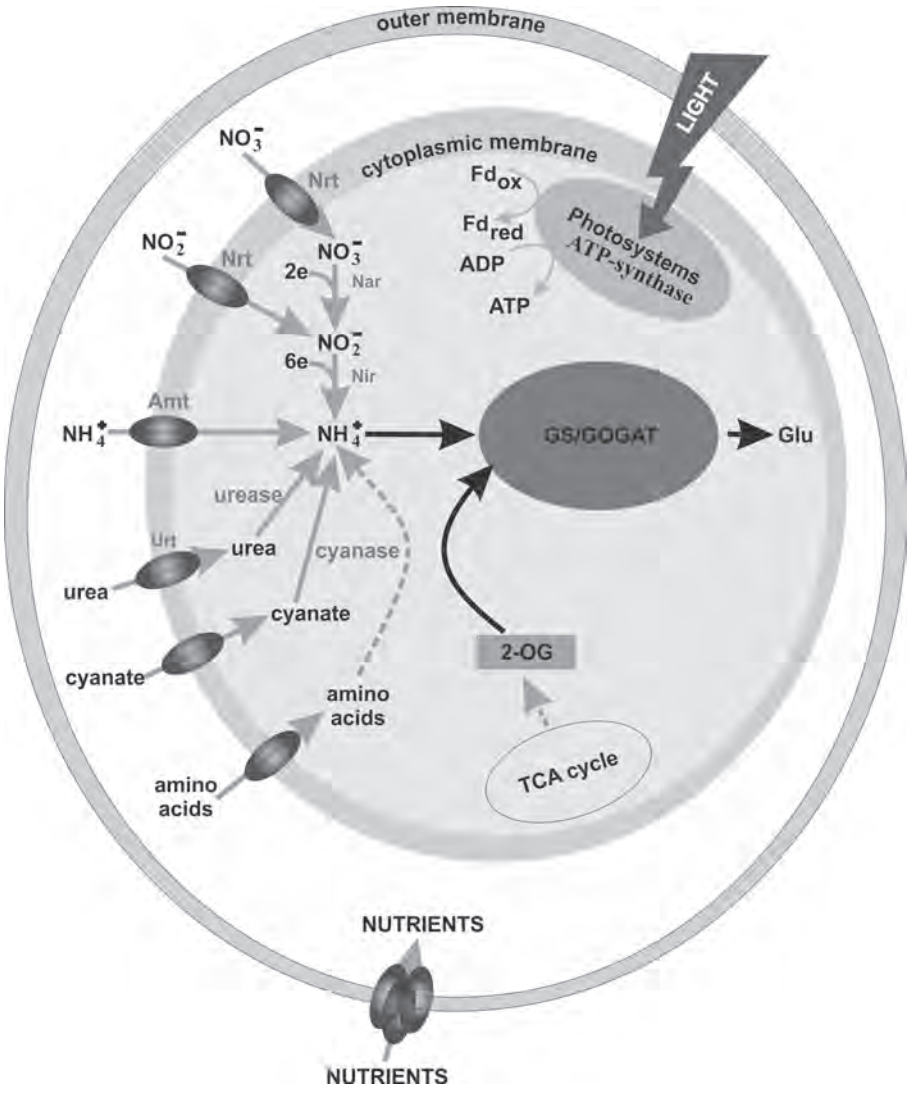

Figure 3. Main nitrogen assimilation pathways in cyanobacteria. Combined nitrogen sources are taken up through permeases and metabolized to ammonium, which is incorporated into carbon skeletons through the glutamine synthetase–glutamate synthase pathway (GS/GOGAT). Nitrogen is then distributed from glutamine or glutamate to the other nitrogen-containing organic compounds. Amt, ammonium permease; Fd_{ox}, oxidized ferredoxin; Fd_{red}, reduced ferredoxin; GOGAT, glutamate synthase; GS, glutamine synthetase; Nar, nitrate reductase; Nir, nitrite reductase; Nrt, ABC-type nitrate/nitrite transporter; 2-OG, 2-oxoglutarate; TCA cycle, tricarboxylic acid cycle; Urt, ABC-type urea transporter.

by ATPase activity of NrtC and NrtD. NrtD consists of a single ATPase domain, whereas NrtC contains both an ATPase domain and a C-terminal solute-binding domain that shares 50% amino acid sequence similarity with NrtA. This domain is required for the ammonium-mediated inhibition of nitrate transport (Kobayashi *et al*., 1997 and 2005). Another feature of the cyanobacterial NrtABCD transporter is its homology to the bicarbonate transporter CmpABCD. For instance, almost 50% of the amino acids in the subunit NrtA, which is the receptor subunit of the transporter, are identical to the subunit CmpA in the bicarbonate transporter. Koropatkin *et al*. (2006) present a model, based on the structure and sequence alignments of bicarbonate and nitrate transporters, that suggests that regulatory domains of both bicarbonate and nitrate transport systems bind nitrate. Comparison of the NrtA and putative NrtC and CmpC binding sites shows that all three proteins might bind nitrate. This similarity might enable cyanobacteria to perform synergetic coordination of their nitrogen and carbon assimilation.

Intracellular nitrate is reduced to nitrite and then to ammonium by nitrate and nitrite reductases (Fig. 3), which are products of the *nar*B and *nir*A genes respectively (Rubio *et al*., 1996; Luque *et al*., 1993). Cyanobacterial nitrate reductase is homologous with Mo-containing bacterial oxidoreductases but is unique in that it uses ferredoxin as an electron donor (Hirasawa *et al*., 2004). In this enzyme system, electrons flow from reduced ferredoxin

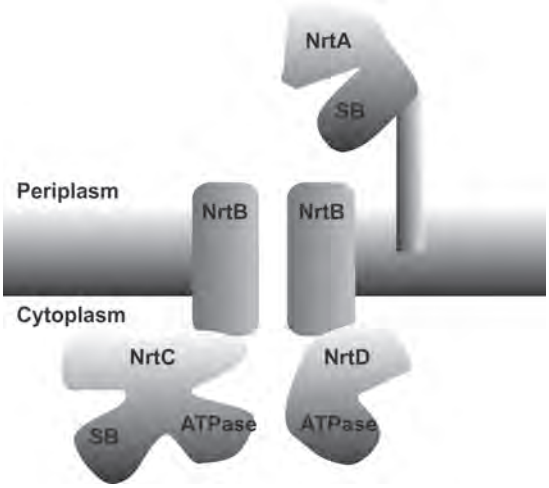

Figure 4. Assembly of the NrtABCD nitrate transporter from *Synechocystis* sp. PCC 6803. NrtA is attached to the periplasmic membrane by a flexible linker. It captures nitrate/nitrite in the periplasm by a solute-binding domain (SB) and subsequently delivers this nitrogen to the transmembrane pore created by the NrtB-dimer. NrtC and NrtD are ATPases that couple ATP hydrolysis to nitrate/nitrite transport through the pore; NrtC contains a C-terminal solute-binding domain (SB) homologous to NrtA.

to the iron-sulphur cluster and then to the Mo cofactor, where nitrate is reduced to nitrite (Rubio *et al*., 1998 and 1999; Jepson *et al*., 2004). Cyanobacterial nitrite reductase is homologous with the ferredoxin-dependent nitrite reductase of plants, and contains a [4Fe-4S] cluster and haem as prosthetic groups. Electrons from reduced ferredoxin are transferred to the iron-sulphur cluster and then to sirohaem, where nitrite is reduced to ammonium. This ammonium can subsequently be incorporated into carbon skeletons through the GS/GOGAT pathway as described above.

Cyanobacteria may also use organic compounds as sources of nitrogen (Fig. 3). For instance, urea is degraded to ammonium and CO_2 by a standard bacterial Ni^{2+}-dependent urease. Another organic nitrogen source is arginine, which is catabolized by an unusual pathway that combines the urea cycle and the arginase pathway rendering ammonium and glutamate as final products (Quintero *et al*., 2000).

3.2. Chlorosis and nitrogen limitation

Lack of nitrogen leads to a series of general responses in cyanobacteria: inhibition of cell division, loss of photosynthetic membranes, accompanied by loss of photosynthetic pigments (chlorophyll, phycobiliproteins, and all carotenoids except zeaxanthin), and accumulation of glycogen and inclusion bodies. The effect of nitrogen depletion on the abundance of pigment molecules has been reported in a wide range of cyanobacterial strains including *Anacystis nidulans* (Allen and Smith, 1969), *Synechococcus* spp. (Yamanaka *et al*., 1978; Sauer *et al.*, 2001), *Anabaena* (Foulds and Carr, 1977; Wood and Haselkorn, 1980) and *Synechocystis* strain PCC 6803 (Elmorjani and Herdman, 1987). The common result is massive and rapid decrease of the chlorophyll and phycobilisome content, which leads to a dramatic change in color from deep blue-green in nitrogen-replete cells to light yellow-green in nitrogen-deplete cells. This process is historically referred to as "chlorosis" (Allen and Smith, 1969). In some cyanobacterial strains, chlorosis also occurs in response to starvation by sulfur (Schmidt *et al*., 1982; Jensen and Rachlin, 1984), phosphorus (Ihlenfeldt and Gibson, 1975), carbon (Miller and Holt, 1977), and iron (Sherman and Sherman, 1983). Schwarz and Grossman (1998) suggested that chlorosis under stress conditions is required to decrease light absorption by the photosynthetic machinery of the cell. The general and specific stress responses of cyanobacteria to macronutrient deficiencies, including modification of the photosynthetic apparatus and phycobilisome degradation, are reviewed by Schwarz and Forchhammer (2005). In cyanobacteria, phycobilisomes can constitute up to 50% of the total cellular protein. Hence, they act as an important internal nitrogen store for the cell, providing building blocks for protein synthesis at times when extracellular nitrogen availability is low. In *Synechococcus* PCC 7942, knock-out mutagenesis has been used to identify genes required for degradation of the phycobilisomes. The knock-out mutants produce a non-bleaching phenotype during nitrogen and sulfur starvation (Schwarz and Grossman, 1998; Dolganov and Grossman, 1999). The *nblB* gene is constitutively expressed, whereas transcription of the *nblA* gene initiates phycobilisome degradation during nutrient starvation (Dolganov and

Grossman, 1999; Baier *et al.*, 2001). It encodes the NblA protein, which is a small polypeptide of 59 amino acids that binds to the phycobilisome and serves as an adaptor to the ClpC-ClpP protease complex, thus inducing proteolytic degradation of the phycobilisomes (Karradt *et al.*, 2008).The *nblR* gene product, which exhibits strong similarity to the response regulator OmpR (Schwarz and Grossman, 1998), controls the induction of expression of the *nbl*A gene. It has been suggested that NblR integrates diverse environmental signals that lead to phycobilisome degradation (Schwarz and Grossman, 1998).

3.3. The nitrate assimilation genes

Recent sequencing of numerous cyanobacterial genomes has permitted an extensive analysis of their nitrate assimilation genes. The distribution of these genes among different cyanobacteria reflects their evolutionary adaptation to various ecological niches (Bird and Wyman, 2003; Palenik *et al*., 2006; Scanlan *et al*., 2009).

Of the active nitrate/nitrite transporters, the prokaryotic ABC-type transporter NrtABCD is encoded by the *nrt*ABCD-gene cluster (Omata *et al*., 1993), while the eukaryotic-like permease NrtP is encoded by the *nrt*P gene (Sakamoto *et al*., 1999). In addition, a putative nitrite transporter encoded by the *focA* gene is present in some cyanobacteria (Martiny *et al.*, 2009; Ohashi *et al.*, 2011). Nitrate reductase (NarB) and nitrite reductase (NirA), the two key enzymes involved in the reduction of nitrate to ammonium, are encoded by the *narB* gene and *nir*A gene, respectively. Other genes related to nitrate assimilation, such as *narM* (Maeda and Omata, 2004), *cnaT* (Frias *et al*., 2003) and *nirB* (Suzuki *et al*., 1995), are often found in the proximity of the nitrate and nitrite transporter and reductases genes. Mutagenesis has shown that these three genes can modulate the activity of the major nitrogen assimilation genes, but their exact functions have remained elusive.

Cyanobacteria are classified in two groups by having Rubisco form 1A and 1B and α- and β-carboxysomes (Badger *et al*., 2002). β-cyanobacteria include diverse freshwater and marine strains, while the α-cyanobacteria group contains solely marine picoplanktonic strains (Ohashi *et al*., 2011). Most freshwater β-cyanobacteria bear the *nrt*ABCD gene cluster and are capable to assimilate nitrate, some freshwater β-cyanobacteria possess additionally the *nrt*P transporter gene (Table 3). Conversely, α-cyanobacteria such as the marine *Synechococcus* strains bear the *nrt*P gene, and some of them also have *foc*A (Palenik *et al*., 2003 and 2006). *Prochlorococcus* strains lack both the *nrt*ABCD and *nrt*P gene, although some of the strains possess *focA* (Table 3). Nitrate and nitrite reductases are present in all β-cyanobacteria and in some α-cyanobacteria. However, *Prochlorococcus* lacks the *nar*B gene, and only some *Prochlorococcus* strains bear the *nir*A gene (Dufresne *et al*., 2003; Rocap *et al*., 2003). Hence, in contrast to most other cyanobacteria, *Prochlorococcus* strains are unable to grow on nitrate.

The nitrate assimilation genes are commonly clustered near each other on the chromosomes of cyanobacteria. They often form a so-called *nirA* operon, with genes arranged in the order *nirA-nrtABCD-narB*, as illustrated for, e.g., *Synechococcus elongatus* PCC 7942 (Omata *et*

Table 3. Nitrate assimilation genes (adapted from Ohashi *et al*., 2011)**.**

	Nitrate/nitrite transporters			Reductases		Regulators				Unknown function		
Strains	***nrtABCD***	***nrtP***	***focA***	***narB***	***nirA***	***ntcB***	***ntcA***	***glnB***	***pipX***	***narM***	***cnaT***	***nirB***
Freshwater α-cyanobacteria												
Gloeobacter violaceus PCC7421	+	-	-	+	+	+	+	+	+	+	+	-
Synechocystis sp. PCC6803	+	-	-	+	+	+	+	+	+	+	+	-
Microcystis aeruginosa NIES-843	+	-	-	+	+	+	+	+	+	+	+	+
Synechococcus elongatus PCC6301	+	-	-	+	+	+	+	+	+	+	+	+
Synechococcus elongatus PCC7942	+	-	-	+	+	+	+	+	+	+	+	+
Synechococcus sp. JA-3-3Ab	+	-	-	+	+	+	+	+	+	+	+	-
Synechococcus sp. JA-2-3B'a	+	-	-	+	+	+	+	+	+	+	+	-
Thermosynechococcus elongatus BP1	+	-	-	+	+	+	+	+	+	+	+	-
Anabaena sp. PCC7120	+	-	-	+	+	+	+	+	+	+	+	+
Anabaena variabilis ATCC29413	+	-	-	+	+	+	+	+	+	+	+	+
Cyanothece sp. PCC7425	+	-	-	+	+	+	+	+	+	+	+	-
Cyanothece sp. PCC7424	+	+	-	+	+	+	+	+	+	+	+	+
Cyanothece sp. PCC8801	+	+	-	+	+	+	+	+	+	+	+	+
Cyanothece sp. PCC8802	+	+	-	+	+	+	+	+	+	+	+	+
Arthrospira platensis NIES-39	+	+	-	+	+	+	+	+	+	+	+	+
Nostoc punctiforme ATCC29133	-	+	-	+	+	+	+	+	+	+	+	+
Marine β-cyanobacteria												
Cyanothece sp. ATCC51142	-	+	-	+	+	+	+	+	+	+	+	+
Trichodesmium erythraeum IMS101	-	+	-	+	+	+	+	+	+	+	+	+
Acaryochloris marina MBIC11017	-	+	-	+	+	+	+	+	+	+	+	+
Synechococcus sp. PCC7002	-	+	+	+	+	+	+	+	+	+	+	+
Marine β-cyanobacteria												
Synechoccocus sp. WH8102	-	+	-	+	+	-	+	+	+	+	+	-
Synechoccocus sp. CC9605	-	+	+	+	+	-	+	+	+	+	+	-
Synechoccocus sp. CC9902	-	+	+	+	+	-	+	+	+	+	+	-
Synechococcus sp. CC9311	-	+	+	+	+	-	+	+	+	+	+	-
Synechoccocus sp. WH7803	-	+	+	+	+	-	+	+	+	+	+	-
Synechococcus sp. RCC307	-	+	+	+	+	-	+	+	+	+	+	-
Prochlorococcus marinus NATL1A	-	-	+	-	+	-	+	+	+	-	+	-
Prochlorococcus marinus NATL2A	-	-	+	-	+	-	+	+	+	-	+	-
Prochlorococcus marinus MIT9303	-	-	+	-	+	-	+	+	+	-	+	-
Prochlorococcus marinus MIT9313	-	-	+	-	+	-	+	+	+	-	+	-
Prochlorococcus marinus MIT9211	-	-	-	-	-	-	+	+	+	-	+	-
Prochlorococcus marinus SS120	-	-	-	-	-	-	+	+	+	-	+	-
Prochlorococcus marinus MED4	-	-	-	-	-	-	+	+	+	-	-	-
Prochlorococcus marinus MIT9515	-	-	-	-	-	-	+	+	+	-	-	-
Prochlorococcus marinus MIT9215	-	-	-	-	-	-	+	+	+	-	-	-
Prochlorococcus marinus MIT9301	-	-	-	-	-	-	+	+	+	-	-	-
Prochlorococcus marinus MIT9312	-	-	-	-	-	-	+	+	+	-	-	-
Prochlorococcus marinus AS9601	-	-	-	-	-	-	+	+	+	-	-	-

al., 1993) and *Anabaena* sp. strain PCC 7120 (Frias *et al*., 1997) in Figure 5. This gene arrangement ensures higher expression levels for upstream genes in the operon, and suggests the production of a balanced amount of the different proteins of the pathway (Frias *et al*., 1997). In several other species, the *nirA* operon contains the *nrtP* gene instead of the *nrtABCD* gene cluster (Fig. 5). Genes *narM*, *cnaT*, *nirB*, and *ntcB* are often found close to the *nir*A operon. Quite often various gene insertions are observed between genes of the *nir*A operon, and in some cases the *nirA* gene is distinctly separated from the other genes. Fast progress in cyanobacterial genomics also revealed other arrangements of the nitrate assimilation genes. For example, *nir*A, *nrt*ABCD and *nar*B genes are scattered on the chromosome of *Microcystis aeruginosa* NIES-843 (Fig. 5).

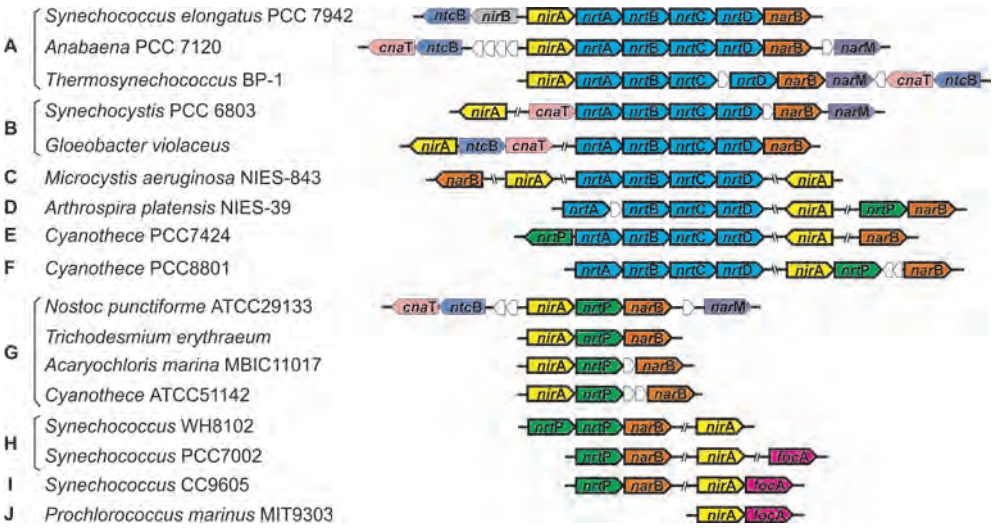

Figure 5. Nitrogen assimilation genes in different cyanobacteria. Bold pentagons represent core nitrate/nitrite assimilation genes (*nrt*ABCD, *nrt*P, *foc*A, *nar*B, and *nir*A). Genes *cna*T, *nar*M, *nir*B and *ntc*B are also shown in some cases, where they are located in close proximity to the core genes. The orientation of genes and relative position on the chromosome with respect to each other is shown. White pentagons refer to open reading frames encoding proteins with unknown function.

3.4. Nitrogen control

The activation or suppression of different pathways for nitrogen assimilation is known as nitrogen control. Nitrogen control systems have been well characterized in various microorganisms, including the NtrB-NtrC two-component regulatory system in proteobacteria (Merrick and Edwards, 1995), the GATA family of global nitrogen control transcription factors in yeast and some fungi (Marzluf, 1997), the GlnR-TnrA system in *Bacillus subtilis* (Fisher, 1999), and the AmtR master regulator of nitrogen assimilation in *Corynebacterium glutamicum* (Jakoby *et al.*, 2000). In cyanobacteria, several key regulators and a number of accessory players tightly control nitrogen assimilation, by exerting control at both the transcriptional and post-transcriptional level.

Transcriptional control is mainly exerted by the NtcA global nitrogen regulator, and is supported by the NtcB transcriptional regulator. NtcA activates transcription of nitrogen assimilation genes when nitrogen becomes limiting. Moreover, NtcA also acts as a sensor for the intracellular 2-oxoglutarate (2-OG) concentration, thus linking the C and N assimilation of cells. NtcA binds to the DNA of nitrogen assimilation genes, and its binding is enhanced by 2-OG (Vazquez-Bermudez *et al*., 2002). The consensus sequence of promoters targeted by NtcA is GTA-N_8-TAC-$N_{22/23}$-TAN_3T, where GTA-N_8-TAC corresponds to the NtcA binding motif and TAN_3T is the -10 element (Luque *et al*., 1994; Herrero *et al*., 2001). NtcA bound to the NtcA-binding site of the promoter recruits RNA polymerase via interaction with its σ subunit, thus facilitating gene transcription (Barnard *et al*., 2004). The transcriptional regulator NtcB is involved in activation of some of the nitrate assimilation genes and requires the presence of nitrite (Aichi and Omata, 1997, Aichi *et al*., 2001). In particular, activation of the *nirA* operon is achieved by dual action of NtcA and NtcB, with nitrite and 2-OG as co-activating factors. While NtcA is essential for the activation of gene transcription, NtcB by itself is not sufficient to promote gene expression (Maeda *et al*., 1998). The molecular basis of the NtcB action remains unclear. However, a conserved pair of the NtcB-binding motifs (ATGC-N_7-GCAT) is essential in promoters of nitrate assimilation genes in β-cyanobacteria (Ohashi *et al*., 2011). The *ntcB* gene is absent in the genomes of α-cyanobacteria, and its binding motifs are absent in the promoters of the associated genes.

Post-transcriptional control of nitrogen assimilation genes in cyanobacteria is mainly provided by the P_{II} protein, which is encoded by the *glnB* gene (Forchhammer 2004). Its key role is highlighted by the fact that the *gln*B gene is present in all cyanobacterial strains sequenced so far. The P_{II} protein binds to the central metabolites ATP, ADP, and 2-oxoglutarate (2-OG), and modulates activities of key enzymes in response to the energetic status and carbon/nitrogen balance of the cyanobacterial cell. Under conditions of nitrogen limitation, cyanobacterial P_{II} is phosphorylated at the conserved Ser^{49} residue. Dephosphorylation in turn is performed by the PphA protein phosphatase (Kloft and Forchhammer, 2005). This covalent modification of P_{II} plays a critical role in the regulation of N-acetylglucomate kinase (NAGK) activity (Burillo *et al*., 2004; Heinrich *et al*., 2004). NAGK is a key enzyme in arginine biosynthesis, and it catalyses conversion of N-acetylglutamate to N-acetylglutamate

phosphate. Low nitrogen content (corresponding to high 2-OG levels) or low energetic status (corresponding to high ADP levels) promotes dissociation of the P_{II}-NAGK complex. Without P_{II}, NAGK is highly sensitive to feedback inhibition by arginine. Conversely, in conditions of nitrogen and energy excess, P_{II} forms a stable complex with NAGK, thus promoting NAGK catalytic activity and an induced level of arginine biosynthesis. High arginine levels lead in turn to intracellular storage of nitrogen in cyanophycin.

Recent studies have highlighted the role of PipX in NtcA activity regulation (Espinosa *et al*., 2006, 2007, 2009). PipX is a small P_{II}-and NtcA-binding protein, and its gene is also present in all cyanobacterial strains. Its binding capacity to NtcA is affected by the nitrogen status of the cell (2-OG levels). When the 2-OG concentration is low, PipX preferentially binds to P_{II}. With increasing 2-OG concentration, PipX dissociates from P_{II}, binds to NtcA and enhances NtcA activity (Fig. 6). Hence, P_{II} perceives the nitrogen, carbon and energetic status of the cell by sensing the 2-OG, ATP and ADP levels and by interaction with its partners PipX, PphA, NAGK and PamA. The latter is a putative membrane protein of unknown function; it interacts with P_{II} in a 2-OG and ATP-sensitive manner (Osanai *et al*., 2005b) and influences transcription levels of several NtcA-dependent genes by a not yet identified mechanism.

The interplay between NtcA, P_{II} and PipX thus yields a remarkable spectrum of regulatory functions in cyanobacterial cells, controlling carbon and nitrogen metabolism at the gene expression level as well as at the protein activity level.

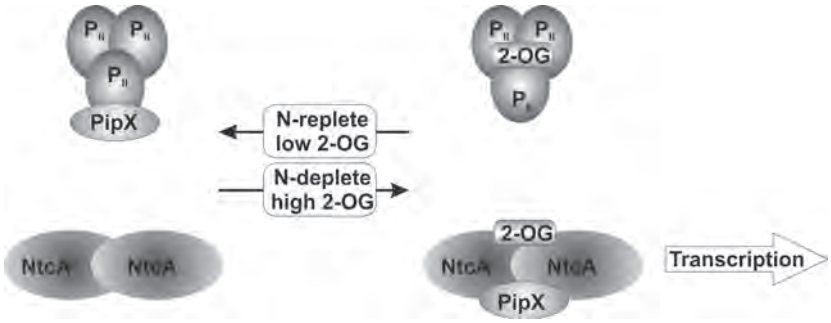

Figure 6. Regulation of NtcA activated transcription by P_{II} and PipX. Under N-replete conditions (low 2-OG levels), PipX binds preferentially to a P_{II}-trimer. In case of N-deplete conditions (high 2-OG levels), PipX dissociates from P_{II} and interacts with NtcA, resulting in enhanced transcription of NtcA-dependent genes.

4. Scope and outline of this thesis

The research presented in this thesis concentrates on the genetic, molecular and physiological aspects of nitrogen assimilation in the model freshwater cyanobacterium *Synechocystis* sp. PCC6803. In particular, I will investigate dynamic changes in gene expression across the entire transcriptome, and will compare this with changes in physiological traits, to elucidate the cascade of genetic and physiological reactions unfolding at different time scales during adaptation to nitrogen starvation.

Chapter 2 presents our initial transcriptomics approach based on macro-arrays obtained from the *Synechocystis* sequencing project. In this chapter we overlaid patterns of gene expression from three distinct stress conditions: high salt stress, nitrogen starvation and phosphorus starvation. This enabled the identification of several general as well as several stress-specific responses. Moreover, based on the results of this chapter, we selected several hypothetical open-reading frames (ORFs) for targeted gene mutagenesis and showed that ORFs *ssr2016* and *slr1208* play a role in Photosystem-I driven cyclic electron flow (Yeremenko *et al.*, 2005).

To investigate the response of *Synechocystis* to changing environmental conditions in more detail our research group designed an oligonucleotide DNA microarray (Eisenhut *et al.*, 2007; Tuominen *et al.*, 2008; Schriek *et al.*, 2008; Hackenberg *et al*., 2009 and 2012; Aguirre von Wobeser *et al.*, 2011). **Chapter 3** describes methods applied for the data analysis of microarray experiments in chapters 4 and 6 of this thesis.

Chapter 4 describes a pilot experiment on nitrogen starvation for 12 hours in batch cultures of *Synechocystis*. It investigates reliability of the designed microarray platform and the applied method for data analysis.

In **Chapter 5** we have applied our microarray platform to the large-scale investigation of dynamic changes in whole-genome expression of *Synechocystis* during the transition between nitrogen and light-limited growth in continuous culture (Aguirre von Wobeser *et al.*, 2011).

Chapter 6 of this thesis describes our detailed investigation of dynamic changes in whole-genome expression of *Synechocystis* during nitrogen starvation for 96 hours and subsequent recovery for 12 hours in batch cultures. In this chapter, we overlay transcriptomics with physiological data on photosynthetic performance of nitrogen-starved cultures and discuss the involved metabolic pathways (Krasikov *et al.*, 2012).

Finally, **Chapter 7** concludes this thesis with a general discussion and shows how the data support further understanding of the genetic and physiological adaptation of cyanobacteria to nitrogen limitation.

References

Aguirre von Wobeser E, Ibelings BW, Bok J, Krasikov V, Huisman J, Matthijs HCP (2011) Concerted changes in gene expression and cell physiology of the cyanobacterium *Synechocystis* sp. strain PCC 6803 during transitions between nitrogen and light-limited growth. *Plant Physiol* **155**: 1445-1457

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

Aichi M, Takatani N, Omata T (2001) Role of NtcB in activation of the nitrate assimilation genes in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *J Bacteriol* **183:** 5840–5847

Allakhverdiev SI, Nishiyama Y, Miyairi S, Yamamoto H, Inagaki N, Kanesaki Y, Murata N (2002) Salt stress inhibits the repair of photodamaged photosystem II by suppressing the transcription and translation of psbA genes in *Synechocystis*. *Plant Physiol* **130**: 1443-1453

Allen MM, Smith AJ (1969) Nitrogen chlorosis in blue-green algae. *Arch Microbiol* **69:** 114-120

Allewalt JP, Bateson MM, Revsbech NP, Slack K, Ward DM (2006) Effect of temperature and light on growth of and photosynthesis by *Synechococcus* isolates typical of those predominating in the Octopus Spring microbial mat community of Yellowstone National Park. *Appl Environ Microbiol* **72:** 544-550

Anderson L, Seilhamer J (1997) A comparison of selected mRNA and protein abundances in human liver. *Electrophoresis* **18:** 533-537

Asadulghani, Nitta K, Kaneko Y, Kojima K, Fukuzawa H, Kosaka H, Nakamoto H (2004) Comparative analysis of the hspA mutant and wild-type *Synechocystis* sp. strain PCC 6803 under salt stress: evaluation of the role of hspA in salt-stress management. *Arch Microbiol* **182**: 487-497

Badger MR, Hanson D, Price GD (2002) Evolution and diversity of CO_2-concentrating mechanisms in cyanobacteria. *Funct Plant Biol* **29:** 161–173

Baier K, Nicklisch S, Grunder C, Reinecke J, Lockau W (2001) Expression of two *nblA*-homologous genes is required for phycobilisome degradation in nitrogen-starved *Synechocystis* sp. PCC 6803. *FEMS Microbiol Lett* **195**: 35-39

Barnard A, Wolfe A, 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

Battchikova N, Vainonen JP, Vorontsova N, Keränen M, Carmel D, Aro EM (2010) Dynamic changes in the proteome of *Synechocystis* 6803 in response to CO_2 limitation revealed by quantitative proteomics. *J Proteome Res* **9**: 5896-5912

Bird C, 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 Enviro. Microbiol* **69**: 7009–7018

Blankenship RE (1992) Origin and early evolution of photosynthesis. *Photosynth Res* **33:** 91-111

Blot N, Mella-Flores D, Six C, Le Corguillé G, Boutte C, Peyrat A, Monnier A, Ratin M, Gourvil P, Campbell DA, Garczarek L (2011) Light history influences the response of the marine cyanobacterium *Synechococcus* sp. WH7803 to oxidative stress. *Plant Physiol* **156**: 1934-1954

Burillo S, Luque I, Fuentes I, Contreras A (2004) Interactions between the nitrogen signal transduction protein P_{II} and N-acetyl glutamate kinase in organisms that perform oxygenic photosynthesis. *J Bacteriol* **186:** 3346–3354

Choi JS, Kim DS, Lee J, Kim SJ, Kim SI, Kim YH, Hong J, Yoo JS., Suh KH, Park YM (2000) Proteome analysis of light-induced proteins in *Synechocystis* sp. PCC 6803: identification of proteins separated by 2D-PAGE using N-terminal sequencing and MALDI-TOF MS. *Mol Cells* **10**: 705-711

Cohen Y, Jørgensen BB, Revsbech NP, Poplawski R (1986) Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. *Appl Environ Microbiol* **51**: 398-407

Dolganov N, Grossman AR (1999) A polypeptide with similarity to phycocyanin α-subunit phycocyanobilin lyase involved in degradation of phycobilisomes. *J Bacteriol* **181:** 610-617

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, Hess WR (2003) Genome sequence of the cyanobacterium *Prochlorococcus marinus* SS120, a nearly minimal oxyphototrophic genome. *Proc Natl Acad Sci USA* **100**: 10020-10025

Ehira S, Ohmori M, Sato N (2005) Identification of low-temperature-regulated ORFs in the cyanobacterium *Anabaena sp.* strain PCC 7120: distinguishing the effects of low temperature from the effects of photosystem II excitation pressure. *Plant Cell Physiol* **46**: 1237-1245

Ehira S, Ohmori M (2006) NrrA, a nitrogen-responsive response regulator facilitates heterocyst development in the cyanobacterium *Anabaena* sp. strain PCC 7120. *Mol Microbiol* **59**: 1692-1703

Eisenhut M, Aguirre von Wobeser E, Jonas L, Schubert H, Ibelings BW, Bauwe H, Matthijs HCP, Hagemann M (2007) Long-term response toward inorganic carbon limitation in wild type and glycolate turnover mutants of the cyanobacterium *Synechocystis* sp. strain PCC 6803. *Plant Physiol* **144**: 1946-1959

Elmorjani K, Herdman M (1987) Metabolic control of phycocyanin degradation in the cyanobacterium *Synechocystis* PCC 6803: a glucose effect. *J Gen Microbiol* **133:** 1685-1694

Espinosa J, Forchhammer K, Burillo S, Contreras A (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

Espinosa J, Forchhammer K, Contreras A (2007) Role of the *Synechococcus* PCC 7942 nitrogen regulator protein PipX in NtcA-controlled processes. *Microbiol* **53**: 711–718

Espinosa J, Castells MA, Laichoubi KB, Contreras A (2009) Mutations at pipX suppress lethality of PII-deficient mutants of *Synechococcus elongatus* PCC 7942. *J Bacteriol* **191**: 4863–4869

Fisher SH (1999) Regulation of nitrogen metabolism in *Bacillus subtilis*: vive la difference! *Mol Microbiol* **32**: 223–232

Flaherty BL, van Nieuwerburgh F, Head SR, Golden JW (2011) Directional RNA deep sequencing sheds new light on the transcriptional response of *Anabaena* sp. strain PCC 7120 to combined-nitrogen deprivation. *BMC Genomics* 12: 332

Forchhammer K (2004) Global carbon/nitrogen control by PII signal transduction in cyanobacteria: from signals to targets. *FEMS Microbiol Rev* **28**: 319–333

Foulds IJ, Carr NG (1977) A proteolytic enzyme degrading phycocyanin in the cyanobacterium *Anabaena cylindrica*. *FEMS Microbiol Lett* **2:** 117-119

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

Frias JE, Herrero A, 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

Fujimori T, Higuchi M, Sato H, Aiba H, Muramatsu M, Hihara Y, Sonoike K (2005) The mutant of *sll*1961, which encodes a putative transcriptional regulator, has a defect in regulation of photosystem stoichiometry in the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Physiol* **139**: 408-416

Fujisawa T, Narikawa R, Okamoto S, Ehira S, Yoshimura H, Suzuki I, Masuda T, Mochimaru M, Takaichi S, Awai K, Sekine M, Horikawa H, Yashiro I, Omata S, Takarada H, Katano Y, Kosugi H, Tanikawa S, Ohmori K, Sato N, Ikeuchi M, Fujita N, Ohmori M (2010) Genomic structure of an economically important cyanobacterium, *Arthrospira (Spirulina) platensis* NIES-39. *DNA Res* **17**: 85-103

Fulda S, Huang F, Nilsson F, Hagemann M, Norling B (2000) Proteomics of *Synechocystis* sp. strain PCC 6803: identification of periplasmic proteins in cells grown at low and high salt concentrations. *Eur J Biochem* **267:** 5900-5907

Fulda S, Mikkat S, Huang F, Huckauf J, Marin K, Norling B, Hagemann M (2006) Proteome analysis of salt stress response in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *Proteomics* **6**: 2733-2745.

Gan CS, Reardon KF, Wright PC (2005) Comparison of protein and peptide prefractionation methods for the shotgun proteomic analysis of *Synechocystis* sp. PCC 6803. *Proteomics* **5**: 2468-2478

Gill RT, Katsoulakis E, Schmitt W, Taroncher-Oldenburg G, Misra J, Stephanopoulos G (2002) Genome-wide dynamic transcriptional profiling of the light-to-dark transition in *Synechocystis* sp. strain PCC 6803. *J Bacteriol* **184**: 3671-3681

Gygi SP, Rochon Y, Franza BR, Aebersold R (1999) Correlation between protein and mRNA abundance in yeast. *Mol Cell Biol* **19:** 1720-1730

Hackenberg C, Engelhardt A, Matthijs HCP, Wittink F, Bauwe H, Kaplan A, Hagemann M (2009) Photorespiratory 2-phosphoglycolate metabolism and photoreduction of O_2 cooperate in high-light acclimation of *Synechocystis* sp. strain PCC 6803. *Planta* **230:** 625-637

Hackenberg C, Huege J, Engelhardt A, Wittink F, Laue M, Matthijs HCP, Kopka J, Bauwe H, Hagemann M (2012) Low-carbon acclimation in carboxysome-less and photorespiratory mutants of the cyanobacterium *Synechocystis* sp. strain PCC 6803. *Microbiology* **158**: 398-413

Hart SE, Schlarb-Ridley BG, Bendall DS, Howe CJ (2005) Terminal oxidases of cyanobacteria. *Biochem Soc Trans* **33**: 832-835

Haverkamp THA, Schouten D, Doeleman M, Wollenzien U, Huisman J, Stal LJ (2009) Colorful microdiversity of *Synechococcus* strains (picocyanobacteria) isolated from the Baltic Sea. *ISME Journal* **3**: 397-408

Heinrich A, Maheswaran M, Ruppert U, Forchhammer K (2004) The *Synechococcus elongatus* PII signal transduction protein controls arginine synthesis by complex formation with N-acetyl-L-glutamate kinase. *Mol Microbiol* **52:** 1303–1314

Herranen M, Battchikova N, Zhang P, Graf A, Sirpiö S, Paakkarinen V, Aro EM (2004) Towards functional proteomics of membrane protein complexes in *Synechocystis* sp. PCC 6803. *Plant Physiol* **134**: 470-481

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

Higo A, Katoh H, Ohmori K, Ikeuchi M, Ohmori M (2006) The role of a gene cluster for trehalose metabolism in dehydration tolerance of the filamentous cyanobacterium *Anabaena* sp. PCC 7120. *Microbiology* **152**: 979-987

Hihara Y, Kamei A, Kanehisa M, Kaplan A, Ikeuchi M (2001) DNA microarray analysis of cyanobacterial gene expression during acclimation to high light. *Plant Cell* **13**: 793-806

Hihara Y, Sonoike K, Kanehisa M, Ikeuchi M (2003) DNA microarray analysis of redox-responsive genes in the genome of the cyanobacterium *Synechocystis* sp. strain PCC 6803. *J Bacteriol* **185**: 1719-1725

Hihara Y, Muramatsu M, Nakamura K, Sonoike K (2004) A cyanobacterial gene encoding an ortholog of Pirin is induced under stress conditions. *FEBS Lett* **574**: 101-105

Hirasawa M, Rubio LM, Griffin JL, Flores E, Herrero A, Li J, Kim SK, Hurley JK, Tollin G, Knaff DB (2004) Complex formation between ferredoxin and *Synechococcus* ferredoxin: nitrate oxidoreductase. *Biochim Biophys Acta* **1068:** 155–162

Huang F, Parmryd I, Nilsson F, Persson AL, Pakrasi HB, Andersson B, Norling B (2002a) Proteomics of *Synechocystis* sp. strain PCC 6803: identification of plasma membrane proteins. *Mol Cell Proteomics* **1:** 956-966

Huang F, Hedman E, Funk C, Kieselbach T, Schröder WP, Norling B (2004) Isolation of outer membrane of *Synechocystis* sp. PCC 6803 and its proteomic characterization. *Mol Cell Proteomics* **3**: 586-595

Huang L, McCluskey MP, Ni H, LaRossa RA (2002b) Global gene expression profiles of the cyanobacterium *Synechocystis* sp. strain PCC 6803 in response to irradiation with UV-B and white light. *J Bacteriol* **184**: 6845-6858

Hubschmann T, Yamamoto H, Gieler T, Murata N, Borner T (2005) Red and far-red light alter the transcript profile in the cyanobacterium *Synechocystis* sp. PCC 6803: impact of cyanobacterial phytochromes. *FEBS Lett* **579**: 1613-1618

Ihlenfeldt MJ, Gibson J (1975) Phosphate utilization and alkaline phosphatase activity in *Anacystis nidulans* (*Synechococcus*). *Arch Microbiol* **102:** 23-28

Imashimizu M, Yoshimura H, Katoh H, Ehira S, Ohmori M (2005) NaCl enhances cellular cAMP and upregulates genes related to heterocyst development in the cyanobacterium, *Anabaena* sp. strain PCC 7120. *FEMS Microbiol Lett* **252**: 97-103

Inaba M, Suzuki I, Szalontai B, Kanesaki Y, Los DA, Hayashi H, Murata N (2003) Gene-engineered rigidification of membrane lipids enhances the cold inducibility of gene expression in *Synechocystis*. *J Biol Chem* **278**: 12191-12198

Jakoby M, Nolden L, Meier-Wagner J, Kramer R, Burkovski A (2000) AmtR, a global repressor in the nitrogen regulation system of *Corynebacterium glutamicum*. *Mol Microbiol* **37**: 964–977

Jensen TE, Rachlin JW (1984) Effect of varying sulphur deficiency on structural components of a cyanobacterium *Synechococcus leopoliensis*: a morphometric study. *Cytobios* **41:** 35-46

Jepson BJN, Anderson LJ, Rubio LM, Taylor CJ, Butler CS, Flores E, Herrero A, Butt JN, 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

Kahlon S, Beeri K, Ohkawa H, Hihara Y, Murik O, Suzuki I, Ogawa T, Kaplan A (2006) A putative sensor kinase, Hik31, is involved in the response of *Synechocystis* sp. strain PCC 6803 to the presence of glucose. *Microbiology* **152**: 647-655

Kaneko T, Sato S, Kotani H, Tanaka A, Asamizu E, Nakamura Y, Miyajima N, Hirosawa M, Sugiura M, Sasamoto S, Kimura T, Hosouchi T, Matsuno A, Muraki A, Nakazaki N, Naruo K, Okumura S, Shimpo S, Takeuchi C, Wada T, Watanabe A, Yamada M, Yasuda M, Tabata S (1996) Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. *DNA Res* **3**: 185-209

Kaneko T, Nakamura Y, Wolk CP, Kuritz T, Sasamoto S, Watanabe A, Iriguchi M, Ishikawa A, Kawashima K, Kimura T, Kishida Y, Kohara M, Matsumoto M, Matsuno A, Muraki A, Nakazaki N, Shimpo S, Sugimoto M, Takazawa M, Yamada M, Yasuda M, Tabata S (2001) Complete genomic sequence of the filamentous nitrogen-fixing cyanobacterium *Anabaena* sp. strain PCC 7120. *DNA Res* **8**: 205-213

Kaneko T, Nakajima N, Okamoto S, Suzuki I, Tanabe Y, Tamaoki M, Nakamura Y, Kasai F, Watanabe A, Kawashima K, Kishida Y, Ono A, Shimizu Y, Takahashi C, Minami C, Fujishiro T, Kohara M, Katoh M, Nakazaki N, Nakayama S, Yamada M, Tabata S, Watanabe MM (2007) Complete genomic structure of the bloom-forming toxic cyanobacterium *Microcystis aeruginosa* NIES-843. *DNA Res* **14**: 247-256

Kanesaki Y, Suzuki I, Allakhverdiev SI, Mikami K, Murata N (2002) Salt stress and hyperosmotic stress regulate the expression of different sets of genes in *Synechocystis* sp. PCC 6803. *Biochem Biophys Res Commun* **290**: 339-348

Kanesaki Y, Yamamoto H, Paithoonrangsarid K, Shoumskaya M, Suzuki I, Hayashi H, Murata N (2007) Histidine kinases play important roles in the perception and signal transduction of hydrogen peroxide in the cyanobacterium, *Synechocystis* sp. PCC 6803. *Plant J* **49**: 313-324

Karradt A, Sobanski J, Mattow J, Lockau W, Baier K (2008) NblA, a key protein of phycobilisome degradation, interacts with ClpC, a HSP100 chaperone partner of a cyanobacterial Clp protease. *J Biol Chem* **283**: 32394-32403

Katoh H, Asthana RK, Ohmori M (2004) Gene expression in the cyanobacterium *Anabaena* sp. PCC 7120 under desiccation. *Microb Ecol* **47**: 164-174

Kloft N, Forchhammer K (2005) Signal transduction protein PII phosphatase PphA is required for light-dependent control of nitrate utilization in *Synechocystis* sp. strain PCC 6803. *J Bacteriol* **187:** 6683–6690

Kobayashi M, Rodriguez R, Lara C, 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

Kobayashi M, Ishizuka T, Katayama M, Kanehisa M, Bhattacharyya-Pakrasi M, Pakrasi HB, Ikeuchi M (2004) Response to oxidative stress involves a novel peroxiredoxin gene in the unicellular cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Cell Physiol* **45**: 290-299

Kobayashi M, Takatani N, Tanigawa M, Omata T (2005) Posttranslational regulation of nitrate assimilation in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *J Bacteriol* **187:** 498–506

Koropatkin NM, Pakrasi HB, Smith TJ (2006) Atomic structure of a nitrate-binding protein crucial for photosynthetic productivity. *Proc Natl Acad Sci USA* **103**: 9820-9825

Krasikov V, Aguirre von Wobeser E, Dekker HL, Huisman J, Matthijs HCP (2012) Time-series resolution of gradual nitrogen starvation and its impact on photosynthesis in the cyanobacterium *Synechocystis* PCC 6803. *Physiol Plant* **145**: 426-439

Kucho K, Tsuchiya Y, Okumoto Y, Harada M, Yamada M, Ishiura M (2004) Construction of unmodified oligonucleotide-based microarrays in the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1: screening of the candidates for circadianly expressed genes. *Genes Genet Syst* **79**: 319-329

Li H, Singh AK, McIntyre LM, Sherman LA (2004) Differential gene expression in response to hydrogen peroxide and the putative PerR regulon of *Synechocystis* sp. strain PCC 6803. *J Bacteriol* **186**: 3331-3345

Liang P, Pardee AB (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* **257:** 967-971

Liang P, Pardee AB (1995) Recent advances in differential display. *Curr Opin Immunol* **7:** 274-280

Lindell D, Jaffe JD, Coleman ML, Futschik ME, Axmann IM, Rector T, Kettler G, Sullivan MB, Steen R, Hess WR, Church GM, Chisholm SW (2007) Genome-wide expression dynamics of a marine virus and host reveal features of co-evolution. *Nature* **449**: 83-86

Luque I, Flores E, 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, Herrero A (1994) Molecular mechanism for the operation of nitrogen control in cyanobacteria. *Biochim Biophys Acta* **1184**: 296–298

Maeda S, 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, Kawaguchi Y, Ohe T, 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

Maeda S, 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

Marin K, Suzuki I, Yamaguchi K, Ribbeck K, Yamamoto H, Kanesaki Y, Hagemann M, Murata N (2003) Identification of histidine kinases that act as sensors in the perception of salt stress in *Synechocystis* sp. PCC 6803. *Proc Natl Acad Sci USA* **100**: 9061-9066

Marin K, Kanesaki Y, Los DA, Murata N, Suzuki I, Hagemann M (2004) Gene expression profiling reflects physiological processes in salt acclimation of *Synechocystis* sp. strain PCC 6803. *Plant Physiol* **136**: 3290-3300

Martiny AC, Kathuria S, Berube PM (2009) Widespread metabolic potential for nitrite and nitrate assimilation among *Prochlorococcus* ecotypes. *Proc Natl Acad Sci USA* **106**: 10787-10792

Marzluf GA (1997) Genetic regulation of nitrogen metabolism in the fungi. *Microbiol Mol Biol Rev* **61**: 17–32

Matthijs HCP, Ludérus EME, Scholts MJC, Kraayenhof R (1984) Energy metabolism in the cyanobacterium *Plectonema boryanum*: Oxidative phosphorylation and respiratory pathways. *Biochim Biophys Acta* **766**: 38-44

Merrick MJ, Edwards RA (1995) Nitrogen control in bacteria. *Microbiol Rev* **59**: 604–622

Mikami K, Kanesaki Y, Suzuki I, Murata N (2002) The histidine kinase Hik33 perceives osmotic stress and cold stress in *Synechocystis* sp PCC 6803. *Mol Microbiol* **46**: 905-915

Miller LS, Holt SC (1977) Effect of carbon dioxide on pigment and membrane content in *Synechococcus lividus*. *Arch Microbiol* **115:** 185-198

Montesinos ML, Muro-Pastor AM, Herrero A, Flores E (1998) Ammonium/methylammonium permeases of a cyanobacterium: identification and analysis of three nitrogen-regulated amt genes in *Synechocystis* sp. PCC 6803. *J Biol Chem* **273**: 31463–31470

Mulkidjanian AY, Koonin EV, Makarova KS, Mekhedov SL, Sorokin A, Wolf YI, Dufresne A, Partensky F, Burd H, Kaznadzey D, Haselkorn R, Galperin MY (2006) The cyanobacterial genome core and the origin of photosynthesis. *Proc Natl Acad Sci USA* **103**: 13126-13131

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

Nakamura Y, Kaneko T, Sato S, Ikeuchi M, Katoh H, Sasamoto S, Watanabe A, Iriguchi M, Kawashima K, Kimura T, Kishida Y, Kiyokawa C, Kohara M, Matsumoto M, Matsuno A, Nakazaki N, Shinpo S, Sugimoto M, Takeuchi C, Yamada M, Tabata S (2002) Complete genome structure of the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1. *DNA Res* **9:** 123-130

Nakamura Y, Kaneko T, Sato S, Mimuro M, Miyashita H, Tsuchiya T, Sasamoto S, Watanabe A, Kawashima K, Kishida Y, Kiyokawa C, Kohara M, Matsumoto M, Matsuno A, Nakazaki N, Shimpo S, Takeuchi C, Yamada M, Tabata S (2003) Complete genome structure of *Gloeobacter violaceus* PCC 7421, a cyanobacterium that lacks thylakoids. *DNA Res* **10**: 137-145

Ohashi Y, Shi W, Takatani N, Aichi M, Maeda S, Watanabe S, Yoshikawa H, Omata T (2011) Regulation of nitrate assimilation in cyanobacteria. *J Exp Bot* **62**: 1411-1424

Ohmori M, Ikeuchi M, Sato N, Wolk P, Kaneko T, Ogawa T, Kanehisa M, Goto S, Kawashima S, Okamoto S, Yoshimura H, Katoh H, Fujisawa T, Ehira S, Kamei A, Yoshihara S, Narikawa R, Tabata S (2001) Characterization of genes encoding multi-domain proteins in the genome of the filamentous nitrogen-fixing cyanobacterium *Anabaena* sp. strain PCC 7120. *DNA Res* **8**: 271-284

Omata T, Andriesse X, Hirano A (1993) Identification and characterization of a gene cluster involved in nitrate transport in the cyanobacterium *Synechococcus* sp. PCC 7942. *Mol Gen Genet* **236**: 193–202

Omata T (1995) Structure, function and regulation of the nitrate transport system of the cyanobacterium *Synechococcus* sp. PCC 7942. *Plant Cell Physiol* **36:** 207–213

Osanai T, Kanesaki Y, Nakano T, Takahashi H, Asayama M, Shirai M, Kanehisa M, Suzuki I, Murata N, Tanaka K (2005a) Positive regulation of sugar catabolic pathways in the cyanobacterium *Synechocystis* sp. PCC 6803 by the group 2 sigma factor sigE. *J Biol Chem* **280**: 30653-30659

Osanai T, Sato S, Tabata S, Tanaka K (2005b) Identification of PamA as a P_{II}-binding membrane protein important in nitrogen-related and sugar-catabolic gene expression in *Synechocystis* sp. PCC 6803. *J Biol Chem* **280**: 34684-34690

Osanai T, Imamura S, Asayama M, Shirai M, Suzuki I, Murata N, Tanaka K (2006) Nitrogen induction of sugar catabolic gene expression in *Synechocystis* sp. PCC 6803. *DNA Res* **13**: 185-195

Ow SY, Wright PC (2009) Current trends in high throughput proteomics in cyanobacteria. *FEBS Lett* **583**: 1744-1752

Paithoonrangsarid K, Shoumskaya MA, Kanesaki Y, Satoh S, Tabata S, Los DA, Zinchenko VV, Hayashi H, Tanticharoen M, Suzuki I, Murata N (2004) Five histidine kinases perceive osmotic stress and regulate distinct sets of genes in *Synechocystis*. *J Biol Chem* **279**: 53078-53086

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, Waterbury J (2003) The genome of a motile marine *Synechococcus*. *Nature* **424**: 1037-1042

Palenik B, Ren Q, Dupont CL, Myers GS, Heidelberg JF, Badger JH, Madupu R, Nelson WC, Brinkac LM, Dodson RJ, Durkin AS, Daugherty SC, Sullivan SA, Khouri H, Mohamoud Y, Halpin R, Paulsen IT (2006) Genome sequence of *Synechococcus* CC9311: insights into adaptation to a coastal environment. *Proc Natl Acad Sci USA* **103**: 13555-13559

Partensky F, Hess WR, Vaulot D (1999) *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. *Microbiol Mol Biol Rev* **63**: 106-127

Postier BL, Wang HL, Singh A, Impson L, Andrews HL, Klahn J, Li H, Risinger G, Pesta D, Deyholos M, Galbraith DW, Sherman LA, Burnap RL (2003) The construction and use of bacterial DNA microarrays based on an optimized two-stage PCR strategy. *BMC Genomics* **4**: 23

Prakash JS, Krishna PS, Sirisha K, Kanesaki Y, Suzuki I, Shivaji S, Murata N (2010) An RNA helicase, CrhR, regulates the low-temperature-inducible expression of heat-shock genes groES, groEL1 and groEL2 in *Synechocystis* sp. PCC 6803. *Microbiology* **156**: 442-451

Quintero MJ, Muro-Pastor AM, Herrero A, Flores E (2000) Arginine catabolism in the cyanobacterium *Synechocystis* sp. strain PCC 6803 involves the urea cycle and arginase pathway. *J Bacteriol* **182**: 1008–1015

Quintero MJ, Montesinos ML, Herrero A, Flores E (2001) Identification of genes encoding amino acid permeases by inactivation of selected ORFs from the *Synechocystis* genomic sequence. *Genome Res* **11**: 2034–2040

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, Chisholm SW (2003) Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. *Nature* **424**: 1042-1047

Ross PL, Huang YN, Marchese JN, Williamson B, Parker K, Hattan S, Khainovski N, Pillai S, Dey S, Daniels S, Purkayastha S, Juhasz P, Martin S, Bartlet-Jones M, He F, Jacobson A, Rappin DJ (2004) Multiplexed protein quantitation in *Saccharomyces cerevisae* using amine-reactive isobaric tagging reagents. *Mol Cell Proteomics* **3**: 1154-1169

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

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

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

Sato N, Ohmori M, Ikeuchi M, Tashiro K, Wolk CP, Kaneko T, Okada K, Tsuzuki M, Ehira S, Katoh H, Okamoto S, Yoshimura H, Fujisawa T, Kamei A, Yoshihara S, Narikawa R, Hamano T, Tabata S, Kuhara S (2004) Use of segment-based microarray in the analysis of global gene expression in response to various environmental stresses in the cyanobacterium *Anabaena* sp. PCC 7120. *J Gen Appl Microbiol* **50**: 1-8

Sauer J, Schreiber U, Schmid R, Volker U, Forchhammer K (2001) Nitrogen starvation-induced chlorosis in *Synechococcus* PCC 7942: low-level photosynthesis as a mechanism of long-term survival. *Plant Physiol* **126**: 233–243

Sazuka T, Yamaguchi M, Ohara O (1999) Cyano2Dbase updated: linkage of 234 protein spots to corresponding genes through N-terminal microsequencing. *Electrophoresis* **20:** 2160-2171

Scanlan DJ, Ostrowski M, Mazard S, Dufresne A, Garczarek L, Hess WR, Post AF, Hagemann M, Paulsen I, Partensky F (2009) Ecological genomics of marine picocyanobacteria. *Microbiol Mol Biol Rev* **73:** 249–299

Schena M, Shalon D, Davis RW, Brown PO (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* **270:** 467-470

Schmidt A, Erdle I, Kost HP (1982) Changes of c-phycocyanin in *Synechococcus* 6301 in relation to growth on various sulfur compounds. *Z Naturforsch* **37:** 870-876

Schopf J. W. (1993) Microfossils of the Early Archean Apex chert: new evidence of the antiquity of life. *Science* **30**: 640-646.

Schopf JW (2006) Fossil evidence of Archaean life. *Philos Trans R Soc Lond B Biol Sci* **361**: 869-885

Schriek S, Aguirre-von-Wobeser E, Nodop A, Becker A, Ibelings BW, Bok J, Staiger D, Matthijs HCP, Pistorius EK, Michel KP (2008) Transcript profiling indicates that the absence of PsbO affects the coordination of C and N metabolism in *Synechocystis* sp PCC 6803. *Physiol Plant* **133**: 525-543

Schwarz R, Grossman AR (1998) A response regulator of cyanobacteria integrates diverse environmental signals and is critical for survival under extreme conditions. *Proc Natl Acad Sci USA* **95:** 11008-11013

Schwarz R, Forchhammer K (2005) Acclimation of unicellular cyanobacteria to macronutrient deficiency: emergence of a complex network of cellular responses. *Microbiology* **151**: 2503-2514

Shcolnick S, Summerfield TC, Reytman L, Sherman LA, Keren N (2009) The mechanism of iron homeostasis in the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 and its relationship to oxidative stress. *Plant Physiol* **150**: 2045-2056

Sherman DM, Sherman LA.(1983) Effect of iron deficiency and iron restoration on ultrastructure of *Anacystis nidulans*. *J Bacteriol* **156:** 393-401

Shestakov SV, Khyen NT (1970) Evidence for genetic transformation in blue-green alga *Anacystis nidulans*. *Mol Gen Genet* **107**: 372-375

Shoumskaya MA, Paithoonrangsarid K, Kanesaki Y, Los DA, Zinchenko VV, Tanticharoen M, Suzuki I, Murata N (2005) Identical Hik-Rre systems are involved in perception and transduction of salt signals and hyperosmotic signals but regulate the expression of individual genes to different extents in *Synechocystis*. *J Biol Chem* **280**: 21531-21538

Shui J, Saunders E, Needleman R, Nappi M, Cooper J, Hall L, Kehoe D, Stowe-Evans E (2009) Light-dependent and light-independent protochlorophyllide oxidoreductases in the chromatically adapting cyanobacterium *Fremyella diplosiphon* UTEX 481. *Plant Cell Physiol* **50**: 1507-1521

Singh AK, McIntyre LM, Sherman LA (2003) Microarray analysis of the genome-wide response to iron deficiency and iron reconstitution in the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Physiol* **132**: 1825-1839

Singh AK, Li H, Sherman LA (2004) Microarray analysis and redox control of gene expression in the cyanobacterium *Synechocystis* sp. PCC 6803. *Physiol Plant* **120**: 27-35

Singh AK, Li H, Bono L, Sherman LA (2005) Novel adaptive responses revealed by transcription profiling of a *Synechocystis* sp. PCC 6803 delta-isiA mutant in the presence and absence of hydrogen peroxide. *Photosynth Res* **84**: 65-70

Singh AK, Summerfield TC, Li H, Sherman LA (2006) The heat shock response in the cyanobacterium *Synechocystis* sp. strain PCC 6803 and regulation of gene expression by HrcA and SigB. *Arch Microbiol* **186**: 273-286

Stal LJ, Moezelaar R (1997) Fermentation in cyanobacteria. *FEMS Microbiol Rev* **21**: 179-211

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

Steglich C, Futschik M, Rector T, Steen R, Chisholm SW (2006) Genome-wide analysis of light sensing in *Prochlorococcus*. *J Bacteriol* **188**: 7796-7806

Sterner RW, Elser, JJ (2002) *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton Univ. Press, Princeton

Stöckel J, Welsh EA, Liberton M, Kunnvakkam R, Aurora R, Pakrasi HB (2008) Global transcriptomic analysis of *Cyanothece* 51142 reveals robust diurnal oscillation of central metabolic processes. *Proc Natl Acad Sci USA* **105**: 6156-6161

Stomp M, Huisman J, De Jongh F, Veraart AJ, Gerla D, Rijkeboer M, Ibelings BW, Wollenzien UI, Stal LJ (2004) Adaptive divergence in pigment composition promotes phytoplankton biodiversity. *Nature* **432**: 104-107

Stomp M, van Dijk MA, van Overzee HMJ, Wortel M, Sigon C, Egas M, Hoogveld H, Gons HJ, Huisman J (2008) The timescale of phenotypic plasticity and its impact on competition in fluctuating environments. *Am Nat* **172**: E169-E185

Stowe-Evans EL, Ford J, Kehoe DM (2004) Genomic DNA microarray analysis: identification of new genes regulated by light color in the cyanobacterium *Fremyella diplosiphon*. *J Bacteriol* **186**: 4338-4349

Straub C, Quillardet P, Vergalli J, Tandeau de Marsac N, Humbert JF (2011) A day in the life of *Microcystis aeruginosa* strain PCC 7806 as revealed by a transcriptomic analysis. *PLoS One* **6**: e16208

Sugita C, Ogata K, Shikata M, Jikuya H, Takano J, Furumichi M, Kanehisa M, Omata T, Sugiura M, Sugita M (2007) Complete nucleotide sequence of the freshwater unicellular cyanobacterium *Synechococcus elongatus* PCC 6301 chromosome: gene content and organization. *Photosynth Res* **93**: 55-67

Summerfield TC, Sherman LA (2008) Global transcriptional response of the alkali-tolerant cyanobacterium *Synechocystis* sp. strain PCC 6803 to a pH 10 environment. *Appl Environ Microbiol* **74**: 5276-5284

Summerfield TC, Nagarajan S, Sherman LA (2011) Gene expression under low-oxygen conditions in the cyanobacterium *Synechocystis* sp. PCC 6803 demonstrates Hik31-dependent and –independent responses. *Microbiology* **157**: 301-312

Suzuki I, Horie N, Sugiyama T, Omata T (1995) 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

Suzuki I, Kanesaki Y, Mikami K, Kanehisa M, Murata N (2001) Cold-regulated genes under control of the cold sensor Hik33 in *Synechocystis*. *Mol Microbiol* **40**: 235-244

Suzuki I, Kanesaki Y, Hayashi H, Hall JJ, Simon WJ, Slabas AR, Murata N (2005) The histidine kinase Hik34 is involved in thermotolerance by regulating the expression of heat shock genes in *Synechocystis*. *Plant Physiol* **138**: 1409-1421

Suzuki I, Simon WJ, Slabas AR (2006) The heat shock response of *Synechocystis* sp. PCC 6803 analysed by transcriptomics and proteomics. *J Exp Bot* **57**: 1573-1578

Suzuki S, Ferjani A, Suzuki I, Murata N (2004) The SphS-SphR two component system is the exclusive sensor for the induction of gene expression in response to phosphate limitation in S*ynechocystis*. *J Biol Chem* **279**: 13234-13240

Thompson AW, Huang K, Saito MA, Chisholm SW (2011) Transcriptome response of high- and low-light-adapted *Prochlorococcus* strains to changing iron availability. *ISME J* **5**: 1580-1594

Toepel J, Welsh E, Summerfield TC, Pakrasi HB, Sherman LA (2008) Differential transcriptional analysis of the cyanobacterium *Cyanothece* sp. strain ATCC51142 during light-dark and continuous-light growth. *J Bacteriol* **190**: 3904-3913

Toepel J, McDermott JE, Summerfield TC, Sherman LA (2009) Transcriptional analysis of the unicellular, diazotrophic cyanobacterium C*yanothece* sp. ATCC 51142 grown under short day/night cycles. *J Phycol* **45**: 610–620

Tu CJ, Shrager J, Burnap RL, Postier BL, Grossman AR (2004) Consequences of a deletion in dspA on transcript accumulation in *Synechocystis* sp. strain PCC 6803. *J Bacteriol* **186**: 3889-3902

Tuominen I, Pollari M, Aguirre von Wobeser E, Tyystjärvi E, Ibelings BW, Matthijs HCP, Tyystjärvi T (2008) Sigma factor SigC is required for heat acclimation of the cyanobacterium *Synechocystis* sp. strain PCC 6803. *FEBS Lett* **582**: 346-350

Vazquez-Bermudez MF, Herrero A, Flores E (2000) Uptake of 2-oxoglutarate in *Synechococcus* strains transformed with the *Escherichia coli* kgtP gene. *J Bacteriol* **182:** 211–215

Vazquez-Bermudez MF, Paz-Yepes J, Herrero A, Flores E (2002) The NtcA-activated amt1 gene encodes a permease required for uptake of low concentrations of ammonium in the cyanobacterium *Synechococcus* sp. PCC 7942. *Microbiology* **148**: 861–869

Velculescu VE, Zhang L, Vogelstein B, Kinzler KW (1995) Serial analysis of gene expression. *Science* **270:** 484-487

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* **13**: 87-115

Wang Y, Sun J, Chitnis PR (2000) Proteomic study of the peripheral proteins from thylakoid membranes of the cyanobacterium *Synechocystis* sp. PCC 6803. *Electrophoresis* **21:** 1746-1754

Welsh EA, Liberton M, Stoeckel J, Loh T, Elvitigala T, Wang C, Wollam A, Fulton RS, Clifton SW, Jacobs JM, Aurora R, Ghosh BK, Sherman LA, Smith RD, Wilson RK, Pakrasi HB (2008) The genome of *Cyanothece* 51142, a unicellular diazotrophic cyanobacterium important in the marine nitrogen cycle. *Proc Natl Acad Sci USA* **105**: 15094-15099

Wood NB, Haselkorn R (1980) Control of phycobiliprotein proteolysis and heterocyst differentiation in *Anabaena*. *J Bacteriol* **141:** 1375-1385

Yamaguchi K, Suzuki I, Yamamoto H, Lyukevich A, Bodrova I, Los DA, Piven I, Zinchenko V, Kanehisa M, Murata N (2002) A two-component Mn^{2+}-sensing system negatively regulates expression of the mntCAB operon in *Synechocystis*. *Plant Cell* **14**: 2901-2913

Yamanaka G, Glazer AN, Williams RC (1978) Cyanobacterial phycobilisomes: characterization of the phycobilisomes of *Synechococcus* sp. 6301. *J Biol Chem* **253:** 8303-8310.

Yeremenko N, Jeanjean R, Prommeenate P, Krasikov V, Nixon PJ, Vermaas WF, Havaux M, Matthijs HCP (2005) Open reading frame ssr2016 is required for antimycin A-sensitive photosystem I-driven cyclic electron flow in the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Cell Physiol* **46**: 1433-1436

Yoshimura H, Yanagisawa S, Kanehisa M, Ohmori M (2002) Screening for the target gene of cyanobacterial cAMP receptor protein SYCRP1. *Mol Microbiol* **43**: 843-853

Zhang LF, Yang HM, Cui SX, Hu J, Wang J, Kuang TY, Norling B, Huang F (2009) Proteomic analysis of plasma membranes of cyanobacterium *Synechocystis* sp. strain PCC 6803 in response to high pH stress. *J Proteome Res* **8**: 2892-2902

Zhang S, Bryant DA (2011) The tricarboxylic acid cycle in cyanobacteria. *Science* **334**: 1551-1553

Zinser ER, Lindell D, Johnson ZI, Futschik ME, Steglich C, Coleman ML, Wright MA, Rector T, Steen R, McNulty N, Thompson LR, Chisholm SW (2009) Choreography of the transcriptome, photophysiology, and cell cycle of a minimal photoautotroph, *Prochlorococcus*. *PLoS One* **4**: e5135