

7th European Workshop on Molecular Biology of Cyanobacteria

**August 31 – September 4, 2008
České Budějovice, Czech Republic**



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Book of Abstracts

arranged and edited by:

Josef Komenda

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Abbreviations

IOP	invited oral presentation; it may not exceed 25+5 min for discussion
SOP	selected oral presentation; it may not exceed 15+5 min for discussion
POS	poster presentation; posters are on display throughout the conference; the official viewing session for odd posters on Tuesday, September 2, from 13:30 till 15:00 and for even posters on Wednesday, September 3, from 13:30 till 15:00
ICS	interactive computer session on CYANOBIKE in the computer room on Tuesday, September 2, from 17:50

Program

Sunday, August 31

16:00 – 21:00 **Registration and welcome beer**

Monday, September 1

9:00 Workshop opening by Josef Komenda and Martin Tichý

*Structural and cell biology of cyanobacteria
(Chaired by Ch. Kerfeld and D. Adams)*

9:10 **Ziv Reich (Weizmann Inst. Rehovot, Israel):** Connectivity and permeability of cyanobacterial thylakoids

9:40 **David Adams (Univ. Leeds, UK):** Cyanobacterial cell wall ultrastructure and gliding motility

10:10 **Coffee break**

10:40 **Matthias Broser (Technical Univ. Berlin, Germany):**
Quinones, lipids and channels: new insights into cyanobacterial photosystem II

11:00 **Dirk Schneider (Univ. Freiburg, Germany):** Structural organization of cyanobacterial membranes

11:20 **David Kaftan (Univ. České Budějovice, Czech Republic):**
Genetically engineered cyanobacteria with high acclimation potential over a broad temperature range

11:40 **Dennis Dienst (Humboldt Univ. Berlin, Germany):**
Phototactic motility of *Synechocystis* PCC 6803: requirement of the RNA chaperone Hfq

12:15 **Lunch**

*Proteins and their complexes: structure, function and biogenesis
(chaired by Peter J. Nixon and G. Schmetterer)*

13:30 **Eva-Mari Aro (Univ. Turku, Finland):** Thylakoid electron transfer routes induced in *Synechocystis* 6803 under low CO₂ conditions

14:00 **Antonia Herrero (Univ. Sevilla-CSIC, Spain):** Proteins involved in transcription activation and intercellular interactions in heterocyst differentiation and function

14:30	<i>Coffee break</i>
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14:50	Friedrich Ossenbühl (Univ. Ulm, Germany) Integration of photosynthetic proteins into the thylakoid membrane
15:10	Ghada Ajlani (CNRS Saclay, France): Some cyanobacteria have two isoforms of the ferredoxin:NADP oxidoreductase
15:30	Roman Sobotka (Inst. Microbiology Třeboň, Czech Rep): The role of ferrochelatase in the regulation of the tetrapyrrole biosynthesis pathway in <i>Synechocystis</i> 6803
15:50	Anne Karradt (Humboldt Univ. Berlin, Germany): NblA, the key protein of phycobilisome degradation, interacts with the Hsp100 chaperone partner of a Clp protease
16:10	Julian J. Eaton-Rye (Univ. Otago, N. Zealand): Directed mutagenesis of the transmembrane domain of the PsbL subunit of photosystem II in <i>Synechocystis</i> sp. PCC 6803
16:30	Ildikò Szabò (Univ. Padova, Italy): A new potassium channel in cyanobacteria

18:00	<i>Excursion to the Budvar Brewery(only for a limited number of participants registered in advance)</i>
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Tuesday, September 2

*Regulation of gene expression
(chaired by P. Lindblad and H. Matthijs)*

9:00	Susan Golden (Texas A&M Univ., USA): A day in the life of a cyanobacterium
9:30	Kan Tanaka (Chiba University, Japan): Light-dependent transcriptional regulation in cyanobacteria

10:00	<i>Coffee break</i>
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10:30	George W. Owttrim (Univ. Alberta, Canada): Omic analysis of a cyanobacterial RNA helicase
10:50	Tina C. Summerfield (Univ. Otago, N. Zealand): Anaerobic induction of a gene cluster containing psbA1 and petC2 in <i>Synechocystis</i> sp. strain PCC 6803

11:10	Annegret Wilde (Univ. Giessen, Germany): Evolution and function of circadian clock proteins from <i>Prochlorococcus</i> MED4
11:30	T. Tyystjärvi (Univ. Turku, Finland): Characterization of single, double and triple inactivation strains reveals new physiological roles for group 2 sigma factors in the cyanobacterium <i>Synechocystis</i> sp. PCC 6803
11:50	Paulo Oliveira, (Uppsala Univ., Sweden): The effects of LexA over-expression on the transcription of the <i>hox</i> genes in the cyanobacterium <i>Anabaena</i> sp. strain PCC 7120
12:15	Lunch
13:30	Poster Session (odd poster numbers) <i>Cell Differentiation</i> (chaired by Birgitta Bergmann and Enrique Flores)
15:00	Cheng-Cai Zhang (Univ. Marseille, France): A newly uncovered missing link between NtcA and HetR for the initiation of heterocyst differentiation
15:30	James Golden (Texas A&M Univ., USA): Heterocyst development and pattern formation in <i>Anabaena</i> PCC 7120
16:00	Coffee break
16:30	Birgitta Bergman (Univ. Stockholm, Sweden): Complex differentiation events are required to maintain the cyanobacterial – <i>Azolla</i> symbiosis
16:50	Assaf Sukenik (Limnol. Lab. Migdal, Israel): The akinete expressed transporter (Aet) gene can be used a specific akinete marker
17:10	Gustaf Sandh (Univ. Stockholm, Sweden): The regulation of diazocyte differentiation and the role of flavodoxins in <i>Trichodesmium</i>
17:30	Danny Ionescu (Hebrew Univ. Jerusalem, Izrael): Small non-coding RNAs are involved in the response to nitrogen depletion in the filamentous cyanobacterium <i>Anabaena</i> PCC 7120
17:50	Arnaud Taton (Univ. Richmond, USA): CyanoBIKE Interactive Session - an opportunity to learn how to use an on-line resource to exploit genomics and massive experimental data sets

Wednesday, September 3

*Molecular aspects of taxonomy, ecophysiology and evolution
(chaired by Wolfgang Hess and Krzysztof Waleron)*

9:00 **Frederic Partensky (CNRS Roscoff, France):** The role of lateral gene transfer in niche adaptation of marine Synechococcus

9:30 **Debbie Lindell (Technion, Israel):** Cyanobacteria-cyanophage interactions: impacts on genome evolution and genome expression

10:00 **Coffee break**

10:30 **Muriel Gugger (Inst. Pasteur Paris, France):** 16S rDNA phylogeny of the Pasteur Culture Collection of cyanobacteria (PCC)

10:50 **Claudia Steglich (Univ. Freiburg, Germany):** A systematic microarray screen for ncRNAs in Prochlorococcus

11:10 **Yehonatan Bar-Yosef (Hebrew Univ. Jerusalem, Israel.):** "Novel Enslavement" in phytoplankton communities – the biological role of Cyldrospermopsin

11:30 **John Copley (Univ. San Francisco, USA):** The gene, tcpA, required for photoregulation of phycobilisome abundance and for heterotrophic growth in Fremyella diplosiphon, is a useful phylogenetic marker specific for the phylum, Cyanobacteria

11:50 **Ignacio Luque (Univ. Sevilla-CSIC, Spain):** Intraphylum diversity and complex evolution of cyanobacterial aminoacyl-tRNA synthetases

12:15 **Lunch**

13:30 2nd Poster Session (even poster numbers)

*Nutrients, metabolism and biotechnology
(chaired by A. Kaplan and T. Börner)*

15:00 **Martin Welker (Tech. Univ. Berlin, Germany):** Diversity of cyanobacterial oligopeptides – from genes to structures

15:30 **Nigel Robinson (Univ. Newcastle, UK):** Allocating the correct metals to proteins in the periplasm of Synechocystis PCC 6803

16:00 **Coffee break**

16:30	Thomas Börner (Humboldt Univ. Berlin, Germany): Microcystins affect surface properties of Microcystis cells
16:50	David Fewer (Univ. Helsinki, Finland): The biosynthetic genes and novel cyclic peptides anacyclins in strains of the genus Anabaena
17:10	Jose Manuel García-Fernández (Univ. Cordoba, Spain): Glucose uptake and utilization by Prochlorococcus strains
17:30	Paula Tamagnini (Univ. Porto, Portugal): From cyanobacterial hydrogenases to BioModularH2
17: 50	Concluding remarks, announcement of the next workshop venue and official closing of the workshop
19:00	<i>Conference Dinner</i>

Thursday, September 4

8: 00 -14:00	<i>Excursion to the town and castle Český Krumlov (only for a limited number of participants registered in advance)</i>
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Abstracts

STRUCTURAL AND CELL BIOLOGY OF CYANOBACTERIA

Cyanobacterial thylakoid membrane networks: permeability, connectivity, and (dis-) similarity to higher-plant networks

Reinat Nevo¹, Dana Charuvi^{1,2}, Eyal Shimon³, Silvia G. Chuartzman¹, Ophir Rav-Hon¹, Rakefet Schwarz⁴, Aaron Kaplan⁵, Itzhak Ohad⁵, Vlad Brumfeld⁶, Ziv Reich¹

¹*Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel*

²*The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, Rehovot 76100, Israel*

³*Electron Microscopy Unit, Weizmann Institute of Science, Rehovot 76100, Israel*

⁴*Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel*

⁵*The Institute of Life Sciences and Avron-Even-Ari Minerva Center for Photosynthesis Research, The Hebrew University of Jerusalem, Jerusalem 91014, Israel*

⁶*Department of Plant Sciences, Weizmann Institute of Science, Rehovot 76100, Israel*

ziv.reich@weizmann.ac.il

Cyanobacterial thylakoid membrane networks are the most ancient membrane networks that support oxygenic photosynthesis known. As such, they provide a valuable window to glimpse into the fundamental designs employed by evolution in the construction of lamellar systems. I will begin my talk by addressing the basic requisites and constraints imposed on thylakoid membranes by their function as well as by the general homeostasis of their hosting cells or organelles. I will then describe how these requisites and constraints are met in cyanobacteria, as revealed by electron microscope tomography studies. This will be followed by a comparison with the most evolved thylakoid networks existing, those of higher plants, and by a short commentary on what it takes to form and remodel thylakoid membranes.

References:

- [1] Chuartzman, SG, Nevo R et al (2008) *Plant Cell*. **20**: 1029-1039
- [2] Nevo R, Charuvi D et al (2007) *EMBO J* **26**: 1467-1473
- [3] Ohad I, Nevo R et al (2005) *Photochem Photobiol Sci* **4**: 977-982
- [4] Shimon E, Rav-Hon O et al (2005) *Plant Cell* **17**: 2580-2586

Cyanobacterial cell wall ultrastructure and gliding motility

Dave G. Adams¹, Toby Tatsuyama-Kurk², Daniel Whalley¹, Simon Connell², Neil Thomson²

¹Faculty of Biological Sciences and ²School of Physics and Astronomy, University of Leeds, United Kingdom

d.g.adams@leeds.ac.uk

Filamentous cyanobacteria of the genus *Oscillatoria* are motile by a process known as gliding, which requires attachment to a surface. There are two main theories to explain the mechanism of gliding. The first proposes that power is derived from the extrusion of slime from junctional pores that encircle each cell septum. According to the second, fibrils beneath the outer membrane generate waves on the cell surface and it is the progress of these waves that pushes the filament forwards. We have been using Atomic Force Microscopy (AFM) and Field Emission Gun Scanning Electron Microscopy (FEGSEM) to study the ultrastructure of *Oscillatoria* cell walls and cell surfaces, to identify structures that may be associated with motility. Using these techniques we have identified an array of parallel corrugations on the surface of filaments of *Oscillatoria* sp. strain A2 [1]. These corrugations are a consequence of the presence of an array of parallel, 35 nm diameter fibrils beneath the outer membrane [2]. Purification of these fibrils has enabled us to confirm that they consist of protein, which is probably glycosylated, possibly explaining their extreme insolubility. We are now developing AFM imaging of fully hydrated, immobilised *Oscillatoria* filaments to visualise the surface of live cells and to detect surface waves, should they exist. The fibrillar array is much less easily visualised in the very large cyanobacterium *Oscillatoria princeps*, which has filaments 30-40 µm in diameter. This cyanobacterium has an extremely thick peptidoglycan layer which is penetrated by large pores that bring the cytoplasmic and outer membranes into close proximity. We have recently visualised some unique structural aspects of these pores. This talk will illustrate how the use of FEGSEM and AFM can reveal much about the ultrastructure of the *Oscillatoria* cell wall and cell surface, and will consider what the consequences are for theories about gliding motility.

References:

- [1] Read N, Connell S, Adams DG (2007) J Bacteriology **189**: 7361-7366
- [2] Adams DG, Ashworth D, Nelmes B (1999) J Bacteriology **181**: 884-892

Quinones, lipids and channels: new insights into cyanobacterial photosystem II

Albert Guskov¹, Matthias Broser², Jan Kern², Azat Gabdulkhakov¹, Athina Zouni²,
Wolfram Saenger¹

¹*Institut für Chemie und Biochemie/Kristallographie, Freie Universität Berlin, Takustr. 6, D-14195 Berlin, Germany*

²*Institut für Chemie/Max Volmer Laboratorium für Biophysikalische Chemie, Technische Universität Berlin, Strasse des 17. Juni 135, D-10623 Berlin, Germany*

matthias.broser@tu-berlin.de

Photosystem II (PSII) is a large protein-cofactor complex that is located in the photosynthetic thylakoid membrane of plants, green algae and cyanobacteria. It acts as light-driven water:plastoquinone oxidoreductase leading to the production of atmospheric oxygen. In this contribution the current structural model of the crystallized homo-dimeric PSII from *Thermosynechococcus elongatus* at 2.9 Å resolution [1] will be described with a special focus on the structural and functional role of lipids and possible mechanisms for plastoquinol/plastoquinone exchange between PSII and the thylakoid membrane. Furthermore an overview of the model – that contains now all 20 protein subunits of each monomer characterized by mass spectrometry as well as the complete side chains of all 35 chlorophyll *a*, 12 carotenoid molecules, 25 integral lipids and a novel plastoquinone Q_C - will be given. Our data show that the pattern of post-translational modifications of 12 small subunits is dependent on the orientation of the protein within the membrane. Lipids could have several functions in PSII: they mediate most of the monomer-monomer contacts indicating their importance for dimer formation/dissociation necessary for the exchange of photodamaged D1. In addition lipids could support diffusion of formed dioxygen to the cytoplasmic side of PSII and thereby help to prevent oxidative damage to the reaction centre. This role is supported by the putative oxygen positions obtained from a Xe derivative. The location of the novel plastoquinone Q_C combined with a second plastoquinone transfer channel suggests mechanisms for rapid plastoquinol/plastoquinone exchange. Finally a short overview of possible water, oxygen and proton channel connecting the catalytic site of water oxidation, the unique Mn₄Ca cluster, with the lumen will be presented.

References:

[1] Guskov A et al (2008) submitted

Structural organization of cyanobacterial membranes

Dirk Schneider¹, Eva Fuhrmann¹, Peter Graumann²

¹*Departement of Biochemistry and Molecular Biology*

²*Faculty of Biology, Albert-Ludwigs-University Freiburg, Germany*

Dirk.Schneider@biochemie.uni-freiburg.de

In contrast to other bacteria, cyanobacteria contain two internal membrane systems. Besides the outer and inner (cytoplasmic) membrane, cyanobacteria contain internal thylakoid membranes, which carry the active photosynthetic machinery. It is, however, still unclear how these thylakoid membranes evolve and if they are an independent membrane system or if they are invaginations of the cytoplasmic membrane. We have used different fluorescent dyes to study the organization of thylakoid membranes in living cyanobacterial cells. Our results strongly indicate that the cytoplasmic and thylakoid membrane are not directly connected in the cyanobacterium *Synechocystis* PCC 6803 but rather represent separated entities. Furthermore, after continuous supply of a fluorescent dye, which stains the cyanobacterial cytoplasmic, but not the thylakoid membrane, we observed formation of fluorescent structures in cyanobacterial cells. These structures may represent membrane vesicles involved in a transport system between the two inner membrane systems in cyanobacteria. The biogenesis of cyanobacterial thylakoid membranes is, however, still a mystery, although some recent data indicate an involvement of the Vipp1 protein in thylakoid membrane biogenesis.

Using fluorescent DNA stains we also studied the organization of the *Synechocystis* chromosome and we observed that the DNA is stacked in the centre of the cell that is devoid of thylakoid membranes. In a striking contrast to e.g. *Bacillus subtilis*, which possesses an active chromosome segregation machinery, the DNA amount differed considerably between two *Synechocystis* daughter cells soon after cell division. These observations may indicate that chromosomes are segregated more randomly and in a passive fashion in cyanobacteria.

Reference:

Schneider D et al (2007) BMC Cell Biol **8**: 39

Genetically engineered cyanobacteria with high acclimation potential over a broad temperature range

Jorge Dinamarca^{1,2}, Oksana Shlyk-Kerner¹, David Kaftan³, Avigdor Scherz¹

¹*Plant Sciences Department, The Weizmann Institute of Science, Rehovot, Israel*

²*Universidad de La Frontera, Temuco, Chile*

³*Institute of Physical Biology, University of South Bohemia in České Budějovice and Institute of Systems Biology and Ecology ASCR v.v.i., Nové Hrad, Czech Republic*

kaftan@ufb.jcu.cz

Vital growth of wild photosynthetic mesophiles is confined to a narrow range of temperatures between 20–30 °C defined by the interplay between thermal stability and functional flexibility of the enzymes located at the opposite ends of the photosynthetic reactions chain: the Rubisco complex and, for the most part, the photosystem II (PS II) that operates as a light driven water/plastoquinone oxidoreductase in thylakoid membranes of plant and algal chloroplasts and in bacteria performing oxygenic photosynthesis. Predicted global climate change by a few degrees may therefore lower oxygen evolution, carbon dioxide fixation and biomass production by mesophiles, shaking-up ecosystems worldwide and poses a threat to both food and sustainable energy resources. Key factor in this predicted catastrophe is the impairment of the PS II activity at elevated temperatures because of the accelerated PSII degradation that is not matched by its repair. Here we show that certain mutations at the D1-212¹ and D1-209 sites are capable of shifting the maximal photosynthetic rates to higher temperature in *Synechocystis* sp. PCC 6803. The sites are a part of a GXXXG like sequence motif located above the point where D helices of the D1 and D2 proteins cross each other in close to a C2 symmetry. The mutations markedly decrease the degradation rate of the D1 protein subunit at elevated temperature yet even more importantly, the mutated cells become thermotolerant and exhibit efficient oxygen evolution and electron transfer above the physiological temperature range without compromising the activity at the lower temperature range. Perpetuation of the Rubisco and PSI complexes activities suggest that such mutations may serve applications for biomass production or even provide a first step for adaptations of eco-systems at elevated and fluctuating temperatures.

References:

[1] Shlyk-Kerner O, Samish I et al (2006) Nature **442**: 827-830

Phototactic motility of *Synechocystis* PCC 6803: requirement of the RNA chaperone Hfq

Dennis Dienst¹, Ulf Dühring¹, Hans-Joachim Mollenkopf², Jochen Golecki³, Jens Georg³, Björn Voß³, Wolfgang R. Hess³, Annegret Wilde⁴

¹Humboldt-University Berlin, Institute of Biology, Chausseestr. 117, 10115 Berlin, Germany

²Max Planck Institute for Infection Biology, Charitéplatz 1, 10117 Berlin, Germany

³Faculty of Biology, Experimental Bioinformatics and Genetics, University of Freiburg, Germany

⁴Institute of Micro- and Molecular Biology, University of Giessen, Germany

dennis.dienst@gmx.de

Hfq is a versatilely acting, ubiquitous bacterial RNA chaperone that manages multiple regulatory processes particularly with respect to cellular stress responses. One of its best studied molecular functions is the support of post-transcriptional regulation events mediated by small non-coding RNAs (ncRNAs). Orthologues of Hfq were detected in a variety of cyanobacteria [1]. But while there are several reports on the presence and functions of ncRNAs in cyanobacteria [2,3,4,5], the functional importance of Hfq within this context has not been addressed thus far. We generated an insertion mutant regarding the *hfq* homologue in a motile strain of *Synechocystis* PCC 6803 and observed a non-phototactic behaviour that was due to the loss of type IV pili on the cell surface, as demonstrated by electron microscopy. Piliation and motility were re-established by introduction of the wild-type gene on an autonomously replicating plasmid. Microarray analyses revealed a small set of genes with drastically reduced transcript levels in the knock-out mutant over the wild-type cells. Among the most strongly affected genes, there were members of two operons that had previously been shown to be involved in motility, controlled by the cAMP receptor protein SYCRP1 [6]. In addition we suggest an involvement of Hfq within specific stress response regulons in *Synechocystis*, since transcript levels of the chaperonine encoding genes *groES* and *groEL* as well as of *hspA* likewise appeared dramatically reduced in the *hfq* mutant. Further expression analyses applying a tiled array, which covers one-third of the *Synechocystis* genome, detected a handful of candidates for *trans*- encoded ncRNAs, whose abundances were drastically affected by the *hfq* knock-out. Moreover, we have engineered a *Synechocystis* strain expressing a FLAG-tagged version of Hfq. This protein is able to complement the non-motile phenotype of Δhfq mutants and should be instrumental in the identification of Hfq-associated RNAs by co-immunoprecipitation in combination with microarray analysis. Functional characterization of candidate ncRNAs is in progress and will provide further insight into the riboregulatory potential within cyanobacteria.

References:

- [1] Valentin-Hansen P, Eriksen M et al (2004) Mol Microbiol **51**: 1525-1533
- [2] Axmann IM, Kensche P et al (2005) Genome Biol **6**: 1-16
- [3] Nakamura T, Naito K et al (2007) Plant Cell Physiol **48**: 1309-1318
- [4] Dühring U, Axmann IM et al (2006) Proc Natl Acad Sci USA **103**: 7054-7058
- [5] Hernandez JA, Muro-Pastor AM (2005) J Mol Biol **355**: 325-334
- [6] Yoshimura H, Yanagisawa S et al (2002) Mol Microbiol **43**: 843-53

Femtosecond laser processing of *Anabaena* sp. PCC7120 cells

Juan B. Arellano¹, Javier R. Vázquez de Aldana², Cruz Méndez², Sergio González-Pérez¹, Pablo Moreno² and Luis Roso²

¹*Departamento de Estrés Abiótico, Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA-CSIC), Salamanca, Spain*

²*Servicio Láser, Universidad de Salamanca, Salamanca, Spain*

juan.arellano@irnasa.csic.es

Filamentous cyanobacteria develop heterocysts in response to deprivation for combined nitrogen under aerobic conditions. The most prominent structural change in heterocysts is the biosynthesis of an envelope that restricts gas permeability, providing an appropriate microoxic environment for N₂ fixation inside. The additional thickness of the differentiated cells, when compared to vegetative cells, makes filamentous cyanobacteria an attractive biological system to investigate cellular response against femtosecond laser processing. By irradiating the cyanobacterial filaments with 120-fs, 795-nm, 1-kHz pulses focused through a 100× microscope objective with a numerical aperture of 0.85, we have determined that the pulse energy threshold for an apparent disruption of the cell wall of vegetative cells is 13±4 nJ/pulse. A further increase in the pulse energy to 43±13 nJ causes the complete removal of vegetative cells. In contrast, the pulse energy threshold has to be augmented about three-fold for heterocyst envelope disruption or two-fold for complete removal of heterocysts. We propose that the singular cross linked structure of the glycolipid multilayer of the envelope, required to restrict gas permeability, accounts for the remarked difference in the ablation energy threshold between vegetative cells and heterocysts.

The *prqRA* operon coding for TetR-like regulator and MATE family efflux pump is involved in adaptive response to salt stress in *Synechocystis* sp. PCC 6803

Inessa Kirik¹, Thuy T. Do¹, Martin Tichý², Michael Babykin³

¹*Department of Genetics, Moscow State University, Moscow, Russia*

²*Laboratory of photosynthesis, Institute of Microbiology of the Academy of Sciences of the Czech Republic, Trebon, Czech Republic*

³*International Biotechnological Centre, Moscow State University, Moscow, Russia*

babykin@mail.ru

The *prqRA* operon encoding TetR-like regulator (the operon repressor) and MATE (Multi Antimicrobial Extrusion) family exporter contributes to methyl viologen resistance in *Synechocystis* sp. PCC 6803. However, methyl viologen does not serve as the operon inducer and resistance to it can develop through the *prqA* derepression caused by mutation in *prqR*. DNA microarray analysis has recently revealed that the *prqR* gene is induced under salt stress; however, no *prqA* induction was shown, probably, due to strain variability. To elucidate the role of the *prqRA* operon in salt acclimation, the wild-type strain (wtM; motile Moscow variant) and mutants with the *prqA* gene inactive (PqA) or derepressed (PqR) were studied under various salt stresses. In the wtM cells, a moderate salt treatment (0.7 M NaCl) actually induced strong elevation of the transcript levels of both *prqR* and *prqA* genes. In comparison with wtM strain, PqR mutant possessed an increased cell tolerance to a lethal salt treatment (2 M NaCl for 6 h), while the PqA mutant had reduced ability to develop tolerance to a lethal salt treatment after a moderate salt pre-treatment (0.5 M NaCl for 2 h). Chlorophyll *a* and phycobiliproteins undergoing efficient decomposition under lethal salt stress in all strains (2 M NaCl for 72 h), nevertheless, persisted at higher level in the PqA mutant. A moderate salt pre-treatment followed by lethal salt stress induced noticeable accumulation of the photosynthetic pigments: up to a normal content in PqR mutant but to a half normal content in the wtM and PqA strains. Interestingly, among the closest PrqA homologues in *Arabidopsis thaliana* are the known salt-stress induced MATE proteins; moreover, a rate of their homology to PrqA, in general, directly correlates with a level of salt-stress induction of genes encoding them. The results give evidence that the *prqRA* operon is involved in salt stress response of *Synechocystis* cells and that the MATE-type PrqA transporter contributes to control of pigment content during salt acclimation.

Carotenoid assisted assembly and functions of the photosynthetic reaction centers in *Synechocystis* sp. PCC 6803

Ozge Bozkurt¹, Josef Komenda², Shigeru Itoh³, Masayuki Komura³, Krisztián Cser¹, Imre Vass¹, Przemyslaw Malec⁴, Zoltán Gombos¹, Mihály Kis¹

¹*Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary*

²*Laboratory of Photosynthesis, Institute of Microbiology, Trebon, Czech Republic*

³*Department of Physics, Graduate School of Science, Nagoya University, Nagoya, Japan*

⁴*Faculty of Biochemistry, Biophysics and Biotechnology; Jagiellonian University, Krakow, Poland*

ozgebozkurtozge@hotmail.com

Carotenoids are indispensable components in all photosynthetic organisms and have various protective functions. They are part of the light-harvesting complexes and can prevent the organisms against the deleterious effects of light. Furthermore, they are constituents of PSI, PSII and cytochrome b_6f pigment protein complexes, where they have structural and/or functional roles. In order to get better understanding of these roles, we created a mutant strain of the cyanobacterium *Synechocystis* sp. PCC6803 defected in carotenoid biosynthesis and consequently having no carotenoid at all. We found that the mutant is not viable in the light but can grow in the dark. In these cells photosynthetic electron transport is inhibited, however remarkable PSI activity occurred. The efficiency of energy transfer and charge separation in the PSI complex was comparable to that in the wild type. We further demonstrated that there is no detectable decrease in the amounts of PsaA and PsaB the reaction center proteins of PSI. Apparently, the trimer to monomer ratio of the PSI RCs decreased. The energy transfer from phycobilisomes to PSII is inhibited and PSII activity was not detectable. Small amounts of D1 and D2 but even not a trace of CP43 and CP47 proteins could be detected by immunoblot analysis. Two dimensional gel analysis revealed the presence of monomer cytochrome b_6f complex. The majority of D1 protein was present in an incompletely processed form. In summary our results demonstrated that in cells lacking carotenoids PSI and cytochrome b_6f can be assembled and functional while PSII has an obligate structural and functional requirement for carotenoids.

Acclimation from photoautotrophic to photomixotrophic growth – how does it work?

Maya Haimovich¹, Shira Kahlon¹, Yukako Hihara², Judy Lieman-Hurwitz¹, Aaron Kaplan¹

¹*Plant and Environmental Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel*

²*Department of Biochemistry and Molecular Biology, Faculty of Science, Saitama University, Urawa, Saitama 338-8570, Japan*

Maya.haimovich@mail.huji.ac.il

The cyanobacterium *Synechocystis* sp. PCC 6803 grows photoautotrophically under light conditions and survives in the dark by catabolizing storage carbohydrates such as glycogen. A glucose-tolerant (GT) mutant of *Synechocystis* that was isolated can grow photomixotrophically under light conditions when provided with glucose as well as heterotrophically in the dark if provided with a daily pulses of white light. Little is known about the regulation of the switching from photoautotrophically growth to photomixotrophically growth.

An histidine kinase, Hik31, was found to be involve at glucose sensing. Inactivation of *hik31*, resulted in inactivation of glucokinase at the post transcriptional level. Glucokinase mediate the conversion of glucose to G6P in the oxidative pentose pathway and its inactivation causes the GT strain to be unable to grow on glucose.

PmgA an homolog to an anti σ factor, is essential for the acclimation of *Synechocystis* to changing light intensity, but the growth of its mutant, *pmgA*, was found to be impaired under photomixotrophic conditions. This phenotype could be rescued by inactivation of *ndhF3* (involved in CO₂ uptake) in the background of the *pmgA* mutant. The mechanism involved is being examined.

Broadening growth temperature in cyanobacteria by directed mutagenesis opens new avenues to intelligent design of tolerant crops

Jorge Dinamarca^{1,2}, Oksana Shlyk-Kerner¹, David Kaftan³, Avigdor Scherz¹

¹*Plant Sciences Department, The Weizmann Institute of Science, Rehovot, Israel*

²*Universidad de La Frontera, Temuco, Chile*

³*Institute of Physical Biology, University of South Bohemia in České Budějovice and Institute of Systems Biology and Ecology ASCR v.v.i., Nové Hrad, Czech Republic*

kaftan@ufb.jcu.cz

Global temperature changes are predicted to increase in frequency in a future climate and have raised concerns that ecosystems relying on biomass production of photosynthetic cyanobacteria and algae may come seriously out of equilibrium. These organisms stand at the base of the food chain, comprise the main source of atmospheric oxygen and sink for the biosphere carbon dioxide and may be prospective in future biotechnological production of minerals, proteins, lipids and bioactive compounds. Although the expected changes in global temperatures are only in the order of several degrees, they are predicted to strongly affect phenotype and biochemical reaction rates including the energy and matter exchange with the environment in the photosynthetic organisms, even in the tropical arena, where thermo-tolerant species have prospered up until now. The response of PSII RC plays a key role in the destructive effect of elevated temperature on the photosynthetic activity and overall vitality of mesophilic organisms. Here we succeeded at broadening the range of photosynthetic activity in mesophiles by increasing the D1 thermostability and PSII functionality beyond the higher temperature limit of the physiological range while maintaining the functional flexibility at the low temperature of that range. These demands appear contradictory to natural evolution and pose a major challenge in acclimation and adaptation of photosynthetic activities of mesophilic organisms to a broad temperature range. Fortunately, the residues' sequence in the D1 protein subunit and their 3-D organization within the PSII RC complex, allow for increasing the protein thermostability at elevated temperatures while maintaining functional flexibility at the lower temperature range. Two of these residues, D1-212 and 209 are located within a GXXXG-like motif at the D1/D2 protein interface and seem to have a dual role: 1. By controlling the size of cavities in the PSII RC core, adjust the complex localized flexibility and thereby the activation entropy of the Q_A to Q_B ET rate constant, to the prevailing temperature¹; 2. By introducing inter-subunits hydrogen bonds and packing interactions with residues in the D2 subunits enhance the complex thermal stability.

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Characterization of cyanobacterial plastoglobule-like structures through overexpression of fibrillin proteins

Vicki Moore, Wim Vermaas

*Centre for Bioenergy & Photosynthesis and School of Life Sciences, Arizona State University
Tempe, AZ 85287-4501*

vlmoore@asu.edu

Synthesis of thylakoid membranes in cyanobacteria remains a mystery. In higher plants structures known as plastoglobules, or lipid bodies, are sites of storage for metabolic products such as lipids, carotenoids, and other compounds associated with thylakoid membrane genesis. While plastoglobules have been well-characterized in many higher plants, they have yet to be thoroughly investigate among cyanobacteria, which appear to also contain plastoglobule-like structures near thylakoid membranes. The cyanobacterium *Synechocystis* sp. PCC 6803 contains two genes for fibrillin proteins, *slr1568* and *slr1024*, which appear associated with plastoglobules, based on strong sequence similarity to known plastoglobule-associated proteins. We aim to determine metabolic and structural effects of increased expression of increased expression of one or both of these genes by inserting them into the *Synechocystis* genome under the control of a strong promoter. We anticipate increased numbers of plastoglobule-like structures with overexpression of the genes for these proteins, as well as an increase in yield of metabolic products stored within these structures.

Thylakoid features of a filamentous cyanobacterium grown under different light and temperature conditions

Katia Sciuto¹, Isabella Moro¹, Nicoletta La Rocca¹, Gabriele Marcolongo², Alessia Primon¹, Nicoletta Rascio¹, Carlo Andreoli¹

¹*Department of Biology, University of Padova (Italy)*

²*Department of Chemical Sciences of Padova (Italy)*

katia.sciuto@unipd.it

Biological membranes have a central role in cells, being seats of several metabolic activities. Cyanobacteria are prokaryotic organisms with a well developed membrane system, the thylakoids, where photosynthesis takes place. These membranes are composed by four major glycerolipids: monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), sulphoquinovosyl diacylglycerol (SQDG) and phosphatidylglycerol (PG). The maintenance of an efficient photosynthetic apparatus is strictly connected with membrane fluidity and this, in turn, varies with the unsaturation degree of the fatty acids bound to the glycerol moiety. Environmental conditions, such as temperature and light, can influence membrane fluidity, implying changes in fatty acid composition. Desaturases are enzymes widespread among living organisms, which insert double bonds between two carbons of the fatty acid acyl chains. In cyanobacteria, at least four types of acyl-lipid desaturases have been found, each able to insert the double bond in a different position. This report regards the thylakoid features of a filamentous cyanobacterium, isolated from the Euganean thermal district (Padova, Italy) and grown in laboratory, under different lights and temperatures. Three desaturases genes were sequenced (*desA*, *desB*, *desC*) in this strain. Fatty acid composition and other thylakoid features were differently affected by light and temperature.

Growth of cyanobacteria in conditions simulating the high Fe^{2+} content of ancient seawater

Susanne Rantamäki, Esa Tyystjärvi

Department of Biology, University of Turku, FI-20014 Turku, Finland

esatyy@utu.fi

Banded iron formations (BIFs) are sedimentary rock minerals consisting of iron-rich and iron-poor layers. BIFs were formed 3.8-1.8 billion years ago, and today they are the important forms of iron ore. BIFs contain iron oxides, and it has been speculated that BIFs were formed when cyanobacteria oxidized the soluble Fe^{2+} of ancient seawater, and the insoluble oxides sedimented. Leakage of oxygen to the atmosphere began when most of the iron of seawater had sedimented. This hypothesis can be correct only if cyanobacteria are able to thrive in anaerobic seawater with high Fe^{2+} content and if sedimentation of Fe^{3+} really occurs in such conditions. We measured the growth rates of several strains of the filamentous, nitrogen-fixing species *Nodularia spumigena* and two strains of the single-celled, non-nitrogen-fixing species *Microcystis* in the presence of high concentrations of iron under aerobic and anaerobic conditions. In aerobic conditions, iron was present as Fe^{3+} and in anaerobic conditions, iron was present as Fe^{2+} . High CO_2 content was maintained in the anaerobic growth chamber. The standard growth medium contained 2.8 mg iron/L, and concentrations of 30-60 mg/L Fe^{3+} strongly suppressed the growth of the tested strains in anaerobic conditions. Nitrogen-fixing species were less tolerant to high Fe^{3+} . Under anaerobic conditions, all cyanobacteria grew well and tolerated up to 170 mg/L of Fe^{2+} , depending on the strain. Most importantly, iron precipitate was formed when *Microcystis* cells were grown in anaerobic conditions in the presence of 170 mg/L of Fe^{2+} . The result confirms the hypothesis that cyanobacterial photosynthesis may have caused the massive oxidation and sedimentation of oceanic iron during the Precambrian era.

The gas vesicle gene cluster in *Arthrospira* strains PCC 8005 and PCC 7345

Magdalena Miklaszewska¹, Malgorzata Waleron¹, Annick Wilmotte², Krzysztof Waleron^{1,2}

¹Intercollegiate Faculty of Biotechnology Univ. of Gdansk & Medical Univ. of Gdansk Poland

²CIP, Institute of Chemistry, University of Liege, Belgium

waleron@biotech.ug.gda.pl

The genus *Arthrospira* includes filamentous cyanobacteria with trichomes forming an open helix and containing gas vacuoles. Nutritional properties of *Arthrospira* have been known for hundred years. The cells of *Arthrospira* contain eight essential and twelve non-essential amino acids, as well as lipids, carbohydrates, minerals, vitamins and carotenoids. It has immunomodulative, antioxidant, antiviral and anticancerogenic properties. *Arthrospira* is also used for heavy metal and inorganic nutrients removal from wastewater.

The strain *Arthrospira* PCC 8005 has been chosen to be a part of the life-sustaining system MELISSA (Micro Ecological Life Support System Alternative). This closed-loop bioreactor aims to recover O₂, water and edible biomass from waste, CO₂ and minerals using light as the major energy source during long-term space missions.

The gas vacuoles, whose main function is to provide buoyancy, occur in prokaryotes from aquatic habitats i.e. cyanobacteria. They are made up of stacks of cylindrical gas vesicles. There are two main gv proteins: GvpA - a small hydrophobic protein whose amino acid sequence is highly conserved and GvpC - a larger hydrophilic protein with less conserved amino acid sequence. The *gvpC* genes in different cyanobacterial species vary in size, number of copies and arrangement. Furthermore in cyanobacteria – 8 other genes involved in the gv formation have been identified: *gvpN*, *gvpJ*, *gvpX*, *gvpK*, *gvpF*, *gvpG*, *gvpV* and *gvpW*.

The aim of our study is to investigate the sequence and the structure of the *gvp* operon and to investigate the diversity of *gvp* genes in *Arthrospira* strains PCC 8005 and PCC 7345.

Our analysis has revealed that the gene order in the operon of both strains is: *gvpA-gvpC1-gvpA-gvpC2-gvpA-gvpC3-gvpN-gvpJ*. The sequences of three 72 aa ORFs encoding GvpA are identical and share also 100% identity with GvpA of *Arthrospira maxima* OUCCLXC ABU55390 and 97% with GvpA *Planktothrix rubescens* AJ132354.

In the case of the ORFs of GvpC there are two different gene variants. Two genes *gvpC1* and *gvpC2* encode small protein of 151 amino acids while the *gvpC3* consist of one ORF encoding a 284 amino acid sequence. The 151 aa sequences of GvpC1 and GvpC2 are identical with the first 151 aa of GvpC3. The GvpC1, GvpC2, GvpC3 proteins contain contiguous 33-aa repeats previously reported in *Planktothrix* strains.

The comparison of the *gvp* gene clusters sequences from strains PCC 7345 and PCC 8005 revealed 23 polymorphic positions which do not influence the aa sequence (17 of them are localized in intergenic regions).

PROTEINS AND THEIR COMPLEXES: STRUCTURE, FUNCTION AND BIOGENESIS

Thylakoid electron transfer routes induced in *Synechocystis* 6803 under low CO₂ conditions

Eva-Mari Aro, Pengpeng Zhang, Yagut Allahverdiyeva, Julia Vainonen and Natalia Battchikova

Laboratory of Plant Physiology and Molecular Biology, University of Turku, Finland

evaaro@utu.fi

Growth of *Synechocystis* 6803 cells under air level or high (3 %) CO₂ conditions strongly modify the electron transfer routes in the thylakoid membrane. At low CO₂, complex carbon concentrating mechanisms (CCM) are induced that function to increase the intracellular CO₂ in order to overcome the low affinity of the carbon-fixing enzyme, Rubisco.

The proteomic approach based on 2-D blue-native/SDS PAGE and iTRAQ technology have been crucial in identifying the low-CO₂-induced proteins and protein complexes. Among them, the NDH-1MS complex, a specific form of the NAD(P)H-quinone oxidoreductase accumulates in significant amounts in thylakoids of low-CO₂ grown cells. The subunit composition of the various forms of the NDH-1 complex and their involvement in CCM, cyclic electron flow around Photosystem I and respiratory pathways will be discussed.

Up-regulation of A-type flavoproteins (FLV 1-4) is another distinct feature of the low-CO₂-induced proteome of *Synechocystis* 6803. Real-time RT-PCR revealed the strongest up-regulation of the *flv2* and *flv4* genes upon shift of *Synechocystis* cells from high to low CO₂, and this induction occurred the faster the higher the light intensity was. On protein level, the FLV2,3, and 4 proteins were strongly induced, and the FLV2 and FLV4 proteins were co-regulated and possibly form a heterodimer, which is associated with the thylakoid membrane. Experiments with the knock-out mutants of the flavoproteins revealed the importance of FLV2 and FLV4 in the accumulation photosystem II complexes in the thylakoid membrane during growth under low CO₂ conditions. Indeed the PSII/PSI ratio of the thylakoid membrane was related to the amount of the FLV2 and FLV4 proteins. Moreover, the short term susceptibility of PSII to high light was considerably enhanced in the absence of FLV2 or FLV4.

We conclude that the A-type flavoproteins, particularly FLV2 and FLV4, participate in electron flow that protects the PSII centers against photodamage under conditions of excess excitation of the photosynthetic apparatus.

Proteins involved in transcription activation and intercellular interactions in heterocyst differentiation and function

Antonia Herrero, Ana Valladares, Vicente Mariscal, Enrique Flores

Instituto de Bioquímica Vegetal y Fotosíntesis, Centro de Investigaciones Científicas Isla de la Cartuja, Americo Vespucio 49, E-41092 Sevilla, Spain

herrero@ibvf.csic.es

NtcA, a transcriptional regulator of the cyanobacteria, is required for the triggering of heterocyst differentiation and for activation of the expression of multiple genes that are involved in later stages of the differentiation process. Expression of the *ntcA* gene is itself activated upon combined nitrogen deprivation in all cells of the filament and, specially, in spatially patterned cells that have entered the differentiation process. To study the mechanism of transcription activation by NtcA, we have set up in vitro systems that include the purified RNA polymerase of *Anabaena* sp. PCC 7120, reconstituted from its individual subunits expressed in *E. coli*, and have studied the influence of NtcA and the signaling effector 2-oxoglutarate (2-OG) in different stages of the process of transcription initiation at promoters of the *hetC*, *devB* and *nrrA* genes involved in heterocyst differentiation. 2-OG has a moderate effect on NtcA binding to these regulated promoters, and NtcA and 2-OG have a moderate effect on RNAP recruitment. However, both NtcA and 2-OG are stringently required for open complex formation and hence, transcript production at the three investigated promoters. Thus, 2-OG appears to play a crucial role at activation of gene expression during development. For both heterocyst differentiation and performance of the mature heterocyst, chemical intercellular interactions take place resulting in exchange of regulatory molecules and nutrients between the cells in the filament. Noteworthy, the PatS peptide synthesized in proheterocysts negatively influences the differentiation of neighboring cells, whereas sugars are transferred from vegetative cells to heterocysts to serve as carbon skeletons for the incorporation of the fixed nitrogen, and amino acids are transferred, as fixed nitrogen vehicles, from heterocysts to vegetative cells. Two possible paths for intercellular traffic have been investigated. The GFP protein has been produced specifically in proheterocysts and targeted to the periplasm, and it has been shown to move to the periplasm surrounding the neighboring cells, indicating that this compartment is functionally continuous along the filament. Thus, the filament's periplasm is a shared compartment through which certain molecules could be made available to cells other than those in which they are produced. Also, calcein introduced into the cellular cytoplasm can move between neighboring cells likely through inter-cytoplasmic connections that would allow the transference of certain other molecules. This pathway is dependent on the protein SepJ, which is localized to the septum between vegetative cells as well as between vegetative cells and heterocysts and appears to serve both as a transport conduit and as a cohesive element required for maintenance of filament integrity.

Integration of photosynthetic proteins into the thylakoid membrane

M. Sikorski¹, S. Piekenhayn¹, D. Schünemann², F. Ossenbühl¹

¹*Molecular Botany, Albert-Einstein-Allee 11, 89069 Ulm*

²*Department of General and Molecular Botany, Universitätsstr. 150, 44780 Bochum*

friedrich.ossenbuehl@uni-ulm.de

During photosynthesis photosystem (PS) II and in particular the reaction center protein D1 is damaged by reactive oxygen species. Due to this photoinhibition, D1 has a high turn-over rate. This includes the removal and degradation of the inactivated D1 and its functional replacement by a newly synthesized, integrated and folded D1. The protein SynOxa1p (Slr1471p) has recently been shown to be required for integration, folding and assembly of D1 into thylakoid membranes. Co-immunoprecipitation (CoIP) suggests an interaction of D1 with SynOxa1p. To identify and characterize a possible direct interaction of D1 with SynOxa1p in more detail we initiated respective experiments with the yeast split-ubiquitin-system. Assays with this system indeed identified a direct interaction of D1 with SynOxa1p. To locate the interaction site(s) we generated D1-fragments covering various regions of the active D1 protein. The data obtained suggest two putative interaction sites within the N-terminal half of D1.

Since the results of the split-ubiquitin-system are derived in a heterologous system (yeast) we set out to verify the data by analyzing the interaction of D1 with SynOxa1p in *Synechocystis*. We therefore isolated polysomes and found that SynOxa1p co-fractionates with polysomes. CoIPs of radioactively labeled polysomes of *Synechocystis* with antibodies against D1 demonstrated an interaction of SynOxa1p already with nascent chains of D1. In summary we conclude that D1 integration into thylakoid membranes occurs co-translationally with SynOxa1p as essential, membrane integral chaperone.

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Some cyanobacteria have two isoforms of the ferredoxin:NADP oxidoreductase

Ghada Ajlani

IBITECS, CNRS, CEA Saclay, F-91191 Gif-sur-Yvette, France

gajlani@cea.fr

Ferredoxin-NADP oxidoreductases (FNR) catalyze the exchange of reducing equivalents between ferredoxin and NADP(H). In cyanobacteria and photosynthetic plastids the main role of the FNR is to provide NADPH for CO₂ assimilation. In non-photosynthetic plastids a genetically distinct FNR isoform is postulated to function in the opposite direction (1). It has been suggested, for both cyanobacteria and plastids, that FNR could participate in respiration and in cyclic electron transfer as an NADPH dehydrogenase --or oxidase (2,3). Despite the similarity of cyanobacterial and plastid FNR, the unique *petH* gene in most phycobilisome (PBS)-containing cyanobacteria encodes a \approx 46 kDa FNR that contains an N-terminal domain, similar to PBS-linker polypeptides (6). FNR purified from several cyanobacteria, *Spirulina* sp. (5), *Anabaena cylindrica* (6) and *Synechocystis* sp. strain PCC6803 (7), was found to have a molecular mass of \approx 35 kDa, this result was attributed to proteolytic degradation of the 46-kDa FNR (3, 4).

We showed that a smaller FNR isoform (FNR_S) was produced by a second translation-initiation site located within the orf from which FNR_L (46 kDa) is translated. Furthermore, FNR_S (34 kDa) accumulates under conditions where NADPH accumulates, this seems to occur only in cyanobacteria capable of heterotrophy (8). We hypothesized that FNR_L attachment to the PBS might provide a way for the FNR to optimize electron flow for CO₂ fixation (NADPH production). Mutants of *Synechocystis* sp. PCC6803, lacking FNR_L (containing only FNR_S), have slower photoautotrophic growth, which supports our hypothesis. FNR_S accumulation, during heterotrophic growth and nitrogen starvation, in the wild type, plus the impairment of heterotrophic-growth in a mutant lacking FNR_S (containing only FNR_L), suggest that the attachment to PBS is a hindrance for the FNR activity (NADPH consumption) required under these conditions.

I will present the latest experiments we performed in order to understand the function of each of the isoforms.

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The role of ferrochelatase in the regulation of the tetrapyrrole biosynthesis pathway in *Synechocystis* 6803

Roman Sobotka^{1,2}, Neil Hunter³, Annegret Wilde⁴, Jiří Patera¹, Josef Komenda^{1,2}, Martin Tichý^{1,2}

¹*Institute of Microbiology, Department of Autotrophic Microorganisms, Trebon, Czech Republic*

²*Institute of Physical Biology, University of South Bohemia, Nove Hradky, Czech Republic*

³*Department of Molecular Biology and Biotechnology, University of Sheffield, Firth Court, Sheffield S10 2TN, United Kingdom*

⁴*Justus-Liebig-Universität Giessen, Institut für Mikro- und Molekularbiologie, Heinrich-Buff-Ring 26 39392 Giessen*

sobotka@alga.cz

Heme and chlorophyll (Chl) share a common biosynthetic pathway up to the branch point where magnesium chelatase and ferrochelatase (FeCH) insert either magnesium for Chl biosynthesis or iron for heme biosynthesis. Because heme and chlorophyll are being synthesized in various amounts according to growth or light conditions, special regulatory mechanism(s) must evolve in photosynthetic organisms to control heme/Chl formation. Such mechanism is still elusive, however, it is possible that the FeCH plays a key regulatory role since: **a)** this enzyme lies at the point of heme/Chl branching, **b)** heme, product of the FeCH, is expected to inhibit synthesis of aminolevulinic acid, an early precursor in the pathway, **c)** cyanobacterial and plant FeCHs contain a membrane domain with a highly conserved binding motif for Chl (Cab domain).

Using *Synechocystis* mutants with regulatable FeCH expression, we were first to demonstrate that the FeCH activity modulates the chlorophyll availability in the cell (see poster of Patera et al., for details). To query significance of the Cab domain for the FeCH function, we prepared *Synechocystis* strain, which contains a truncated FeCH lacking this domain. This mutation dramatically reduced FeCH *in vivo* activity (< 5%). Interestingly, aminolevulinic acid synthesis was found to be highly upregulated in this strain implying a direct role for heme in the regulation of the tetrapyrrole pathway. Analysis of the recombinant full-length and truncated FeCHs demonstrated that the Cab domain is critical for FeCH activity and it is strictly required for dimerization of this enzyme. To verify data obtained using recombinant proteins, we purified the full-length and truncated FeCH directly from *Synechocystis* using 3xFLAG tag. Also in this case, the Flag-FeCH was purified as an active dimer, whereas the truncated 3xFLAG-FeCH was purified only as a monomeric protein possessing only traces (~ 2%) of activity. We propose that the dimerization of the enzyme via Cab domain could be a mechanism controlling FeCH activity in the cell. Another mechanism might be based on the redox signalling as we have demonstrated that the *Synechocystis* FeCH can form a disulphide bridge. Our data about *Synechocystis* mutants, where cystein(s) in FeCH were replaced by serine(s), will be discussed.

NbIA, the key protein of phycobilisome degradation, interacts with the Hsp100 chaperone partner of a Clp protease

Anne Karradt, Johanna Sobanski, Wolfgang Lockau, Kerstin Baier

Institute of Biology, Plant Biochemistry, Humboldt-University Berlin, Chausseestrasse 117, D-10115 Berlin, Germany

anne.karradt@gmx.de

Cyanobacterial light-harvesting complexes, the phycobilisomes, are proteolytically degraded when the organisms are starved for combined nitrogen, a process referred to as chlorosis or bleaching. Inactivation of gene *nbIA* leads to a non-bleaching phenotype in non-diazotrophic cyanobacteria like *Synechococcus elongatus* PCC 7942 or *Synechocystis* sp. PCC 6803. In diazotrophic, filamentous cyanobacteria such as *Anabaena* strains, the non-bleaching phenotype is most obvious in heterocysts, N₂ fixing cells that are usually devoid of phycobiliproteins. Homologs of the *nbIA* gene are found in all phycobilisome-containing organisms including red algae. Phycobilisome degradation depends on the strongly increased expression of the *nbIA* gene (Collier & Grossman (1994) EMBO J 13, 1039-1047).

We have previously shown that NbIA interacts with phycobilisomes *via* a conserved motif on its C-terminal helix, and proposed a detailed structural model for this interaction (Bienert *et al.* (2006) JBC 281, 5216-5223). In order to understand the degradation process more precisely, we now searched for additional interaction partners of NbIA. Using pulldown assays with GST-tagged NbIA, an Hsp100/chaperone partner of Clp proteases, ClpC, was identified. A refined analysis showed that ClpC interacts with a conserved motif on the N-terminal helix of NbIA. These results strongly suggest that NbIA acts as an adaptor protein that links phycobiliproteins and ClpC/ClpP complex, thereby initiating the phycobilisome degradation process.

Directed mutagenesis of the transmembrane domain of the PsbL subunit of photosystem II in *Synechocystis* sp. PCC 6803

Hao Luo, Julian J. Eaton-Rye

Department of Biochemistry, University of Otago, Dunedin, New Zealand

julian.eaton-rye@stonebow.otago.ac.nz

The PsbL protein is one of three low-molecular-weight subunits identified at the monomer-monomer interface of photosystem II (PSII) [1,2]. We have employed site-directed mutagenesis to investigate the role of PsbL in *Synechocystis* sp. PCC 6803 cells. Truncation of the C-terminus by deleting the last four residues (Tyr-Phe-Phe-Asn) prevented association of PsbL with the CP43-less monomeric sub-complex and therefore blocked PSII assembly resulting in an obligate photoheterotrophic strain. Replacement of these residues with Ala created four photoautotrophic mutants. The Y36A, F37A, F38A and N39A strains had levels of PSII proteins similar to those of wild type; however, the F37A and F38A strains were readily photodamaged and PSII dimer formation was impaired in F38A cells. In addition, each of these strains had elevated levels of the CP43-less inactive monomeric complex. Mutations targeting a putative hydrogen bond between Arg-16 and sulfoquinovosyldiacylglycerol resulted in mutants that were also highly susceptible to photodamage. Similarly mutations targeting a conserved Tyr residue (Tyr-20) also destabilized PSII under high light and suggest that Tyr-20-lipid interactions or interactions of Tyr-20 with PsbT influence the ability of PSII to recover from photodamage.

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A new potassium channel in cyanobacteria

Manuela Zanetti, Nicoletta La Rocca, Enrico Teardo, Giorgio Mario Giacometti, Elisabetta Bergantino, Ildikò Szabò

Department of Biology, University of Padova, Viale G. Colombo 3, Padova, 35121 Italy

ildi@civ.bio.unipd.it

We recently identified a new putative potassium channel (SynK) in the genome of the cyanobacterium *Synechocystis* sp. PCC6803. SynK was cloned, expressed in Chinese Hamster Ovary cells in fusion with GFP, and demonstrated to function as a potassium selective channel by patch clamping SynK-GFP expressing cells. Western blotting experiments, performed with anti-GFP antibody and an antibody raised against recombinant SynK, revealed expression of the channel protein in CHO cells with correct molecular weight. The same antibody was used to determine location of SynK in intact *Synechocystis* cells by immunogold electron microscopy and to follow its expression under various growth conditions. In order to study its physiological role, a SynK-deficient heteroplasmic *Synechocystis* mutant, which is unable to grow in normal illumination conditions, was obtained.

Possible functions of cyanobacterial c_6 -like cytochromes

Wojciech Bialek¹, Andrzej Szczepaniak¹, Toivo Kallas²

¹Department of Biophysics, Faculty of Biotechnology, University of Wrocław, Poland

²Department of Biology & Microbiology, University of Wisconsin-Oshkosh, Oshkosh, WI 54901, USA

wojciech.bialek@ibmb.uni.wroc.pl

A discovery of two genes *petJ1* and *petJ2* in the cyanobacterium *Synechococcus* sp. PCC 7002 (1) encoding cytochrome c_6 and cytochrome c_6 -like proteins, respectively, raised the question about a possible function of the new cytochrome. Our further phylogenetic analysis of plant, algal and cyanobacterial genomes resulted in a discovery of two novel clusters of previously uncharacterized, cyanobacterial c_6 -like cytochromes. We recently characterised for the first time some properties of a member of the c_6 -like group of cytochromes and revealed its lowered midpoint potential of +148 mV, basic pI of 9.89 and very distinct distribution of surface potential when compared with cytochrome c_6 (2). This preliminary characteristic allows to present possible functions of cytochrome c_6 -like proteins: 1) an electron donor to cyanobacterial terminal oxidases CoxI, CoxII and/or ARTO; 2) an involvement in redox or oxygen sensing related to heterocyst differentiation; 3) a direct electron acceptor from PetC3; 4) a possible electron acceptor from thiosulfate or sulfide during anoxygenic photosynthesis or, analogously to cytochrome c_{555} and flavocytochrome of *Chlorobium thiosulfatophilum*, cytochrome c_6 -like may form a complex with sulfide-quinone reductase; 5) an electron acceptor for hydrogenases in heterocysts and for nonheterocystous, nitrogen-fixing cyanobacteria in anaerobic environments.

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Probing the role of the Band 7 protein superfamily in the cyanobacterium *Synechocystis* sp. PCC 6803

Marko Boehm¹, Jon Nield², Peter Nixon¹

¹Division of Biology, Faculty of Natural Sciences, Imperial College London, South Kensington Campus, London SW7 2AZ, United Kingdom

²School of Biological Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, United Kingdom

marko.boehm03@imperial.ac.uk

The Band 7 protein superfamily is found throughout nature and features the SPFH domain (Stomatin, Prohibitin, Flotillin and HflK/C) as a common motif [1]. Despite intensive research, little is known about the functions of its member proteins. In *S. cerevisiae* mitochondria, prohibitin homologues form large, hetero-multimeric complexes with a FtsH protease and are implicated in the assembly of membrane protein complexes [2]. In the cyanobacterium *Synechocystis* sp. PCC 6803 the FtsH homologue (*slr0228*) found has been shown to be important for the repair of the photosystem two (PSII) complex and the protection of the cell from the damaging effects of light [3,4]. We tested the hypothesis that the Band 7 proteins identified in the *Synechocystis* sp. PCC 6803 genome might interact with FtsH (Slr0228) and play an important physiological role in photoprotection, membrane protein assembly and PSII repair. Bioinformatic analyses revealed the presence of five Band 7 proteins in *Synechocystis* sp. PCC 6803, designated here as prohibitin (Phb) 1-5, that are only distantly related to other known prohibitin homologues and among each other. Immunoblotting experiments using monospecific polyclonal antisera, generated against *E. coli*-overexpressed protein, confirmed that Phb1 (*slr1006*), Phb2 (*slr1768*), Phb3 (*slr1128*) and Phb4 (*slr0815*) were membrane-anchored proteins that form large protein complexes. Membrane fractionation experiments revealed that Phb1 was present in the cytoplasmic and thylakoid membranes, whereas Phb2 and Phb3 were found mainly in the cytoplasmic membrane. Analyses using blue-native (BN) gel electrophoresis of specific single Band 7 gene inactivation mutants combined with immunoprecipitation experiments suggested that the complexes were homooligomeric. In the case of Phb3, single-particle electron microscopy experiments indicated that it forms a ring-like complex with a diameter of approximately 160 nm consisting of at least ten subunits. Co-immunoprecipitation experiments additionally revealed that Phb1 interacted with FtsH (*slr0228*) at low level, and possibly a subunit of the NDH-1 respiratory complex. Mutants lacking Phb2 seemed to be affected in phototaxis. A triple (Δ Phb1-3) and a quadruple (Δ Phb1-4) mutant were found not to be drastically impaired in their ability to repair PSII or to assemble photosynthetic and respiratory complexes. Thus, even though no physiological link between the Band 7 proteins of *Synechocystis* sp. PCC 6803 and the hypothesised role in photoinhibition could be established, our experiments represent the first detailed biochemical and mutational examination of Band 7 proteins in a cyanobacterium or any photosynthetic organism.

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Abolition of *in vitro* *Synechocystis* magnesium chelatase activity by the *gun5* mutation and reversal by Gun4

Paul A. Davison, Daniel P. Canniffe, Neil C. Hunter

mbp06dpc@sheffield.ac.uk

The (genome *uncoupled*) mutants in *Arabidopsis thaliana* show a phenotype in which signalling between the nucleus and chloroplast is disrupted. *Gun4* and *gun5* also exhibit pale phenotypes indicating a disruption in chlorophyll biosynthesis. The *gun5-1* and *cch* *Arabidopsis* mutants carry single point mutations in the H subunit of the magnesium chelatase enzyme, which catalyses the first committed step of chlorophyll biosynthesis, namely the conversion of protoporphyrin to Mg protoporphyrin IX. The *gun5-1/cch* mutations were introduced into the gene encoding *Synechocystis* H subunit and the mutant genes were overexpressed in *E. coli* and the recombinant proteins purified. Neither H-*gun5* or H-*cch* show any Mg-chelatase activity despite tryptophan fluorescence quenching studies which show no significant change in their ability to bind either substrate or product. In addition, the formation of the 3 subunit (H, I, D) magnesium chelatase complex is not affected by the presence of either H-*gun5* or H-*cch*. Remarkably, the addition of Gun4 restores Mg-chelatase activity to assays containing H-*gun5* or H-*cch*, which is consistent with the pale, but not white, phenotype of the *A. thaliana* *Gun5* mutants.

Role and properties of NAD(P)H:quinone oxidoreductase encoded by *drgA* gene of the cyanobacterium *Synechocystis* sp. PCC 6803

Irina Elanskaya¹, Victoria Toporova², Vera Grivennikova¹, Elena Muronets¹, Lira Davletshina¹, Eugeny Lukashev¹, Kirill Timofeev¹

¹Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia

²Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russia

ivelanskaya@mail.ru

Soluble NAD(P)H:quinone-oxidoreductase (NQR) encoded by *drgA* gene of the cyanobacterium *Synechocystis* sp. PCC 6803 is responsible for the cell resistance to nitroaromatic and quinone-type inhibitors. The EPR spectroscopy method was used to analyze the kinetics of Photosystem I reaction center (P700) re-reduction after its photooxidation with white light in *Synechocystis* sp. PCC 6803 intact cells. Lower rate of P700⁺ re-reduction in the absence of Photosystem II activity and lower rate of P700⁺ re-reduction by electrons derived from exogenous glucose were observed in DrgA-deficient mutant in comparison to wild type cells. Comparison of the rates of P700⁺ re-reduction in *Synechocystis* sp. PCC 6803 mutants deficient in individual dehydrogenases indicate that in the absence of Photosystem II activity, NDH-1, DrgA, and succinate dehydrogenase are important for electron transfer to plastoquinone pool in thylakoid membrane of cyanobacteria. The results obtained may indicate that NQR encoded by *drgA* gene, as well as NDH-1, participates in regulation of electron transport from NADPH to plastoquinone pool in thylakoid membranes of cyanobacteria.

The expression of *drgA* gene was analyzed by means of Northern blot hybridization and RT-PCR technique. Two transcripts, which gave a positive hybridization signal with *drgA* probe, were indicated in photoautotrophically grown cells. One of them (0.6 kb) corresponds in size to mRNA read from the *drgA* gene; another transcript (1.3 kb), to mRNA transcribed from two genes: *drgA* and *slr1718* located upstream of *drgA* and having homology with genes of the *comB* family. The expression of genes *drgA* and *slr1718* was repressed during cell incubation in the dark, but the addition of glucose led to a drastically enhanced expression both in the dark and after illumination of cells. Menadione or nitrophenolic herbicide dinoseb did not induce the expression of *drgA* or *slr1718*. The results obtained suggest that the expression of these genes might be regulated by the NADPH content in the cytoplasm of the cyanobacterium cell.

Coding sequences for *drgA* and *slr1718* were amplified by PCR. Several types of constructs for direct constitutive and inducible intracellular expression in *E.coli* of DrgA and Slr1718 proteins were designed and investigated. In order to facilitate protein purification we have spliced the 3'-ends of *drgA* and *slr1718* with oligohistidine tag coding sequences. The recombinant proteins were purified by IMAC-chromatography method. The enzyme activity of DrgA was tested. The purified DrgA-12His protein exhibited high quinone reductase and nitroreductase activity. The rate of re-reduction of photooxidized Photosystem I reaction center in isolated cyanobacterial thylakoid membranes was increased after addition of DrgA-12His protein and NADPH. Thus, DrgA protein may participate in electron transfer from NADPH to plastoquinone pool in thylakoid membranes of the cyanobacterium *Synechocystis* sp. PCC 6803.

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Towards the understanding of the role of pilin-like proteins in *Synechocystis* 6803

Markéta Foldynová^{1,2}, Josef Komenda^{1,3}, Martin Tichý^{1,3}, Roman Sobotka^{1,3}

¹*Department of Autotrophic Microorganisms, Institute of Microbiology, Trebon, Czech Republic*

²*Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic*

³*Institute of Physical Biology, University of South Bohemia, Nove Hradky, Czech Republic*

meta@email.cz

Pilins belong to a class of fibrous proteins that are found in pilus structures on the surface of many gram-negative bacteria. These structures are responsible for the cell adhesion, motility and participate in the general secretory pathway. Interestingly, pilin-like proteins PilA1 and PilA2 were described to be implicated in chlorophyll metabolism in the cyanobacterium *Synechocystis* 6803 [1]. To investigate the function of pilin-like proteins in this organism, a double mutant lacking *pilA1* and *pilA2* genes was prepared. Analysis of this *pilA1/A2* strain did not revealed any significant phenotype when it was grown aerobically. Interestingly, *pilA1/A2* grown under microaerobic conditions (bubbled with the mixture of 3% CO₂, 97% N₂) formed one large aggregate. Similar, less pronounced aggregation was observed when the mutant was grown aerobically in the presence of glucose. In order to identify PilA1 interacting proteins, PilA1 possessing StrepII tag on its C-terminus was expressed in both wild-type and *pilA1/A2* backgrounds. The PilA1-StrepII protein was localized in the membrane fraction and its presence partly reverted the *pilA1/A2* aggregation phenotype. Only two bands were apparent on the SDS-PAGE of the PilA1-Strep protein purified under native conditions, both representing the PilA1-Strep. The faster migrating band disappeared when the sample was reduced by DTT, which usually indicates the presence of a disulphide bridge. Indeed, there are two cysteines in the PilA1 structure. The results suggest an important role of PilA1 protein in a process of cell aggregation. As there are other homologous pilin-like proteins in *Synechocystis*, also containing conserved cysteines, that could compensate for the absence of PilA1, we speculate that the formation of the putative disulphide bridge in pilin-like proteins is important for regulation of the cell aggregation.

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Evolution of the D1 encoding genes in marine *Synechococcus* spp.

Laurence Garczarek¹, Nicolas Blot¹, Alexis Dufresne¹, Douglas A. Campbell², Amanda M. Cockshutt², Ludovic Joubin¹, Anne Peyrat¹, Christophe Six²

¹Station Biologique, UMR 7144 CNRS et Université Pierre et Marie Curie, BP74, place Georges Tessier, 29682 Roscoff cedex, France

²Photosynthetic Molecular Ecophysiology, Biology Department, Mount Allison University, 63B York Street, Sackville, New Brunswick, Canada

garczarek@sb-roscoff.fr

The D1 polypeptide is a key protein of the core of the PSII, and is the primary target for photoinactivation which must be countered by a repair cycle to replace photodamaged D1 proteins [1]. While in eukaryotes D1 is usually encoded by a single chloroplastic *psbA* gene, a multigene *psbA* family is present in most cyanobacterial genomes encoding multiple isoforms of D1 proteins [2, 3, 4]. Examination of eleven marine *Synechococcus* genomic sequences identified up to six *psbA* copies per genome, with always a single gene encoding D1:1. The extremely low level of sequence divergence among the D1:2 coding genes and their independent clustering within all marine *Synechococcus* indicates a concerted evolution of these genes within a genome. This strongly suggests a homogenization of these sequences by gene conversion. This mechanism is associated with the occurrence of numerous translocations of orthologs adjacent to D1:2 encoding genes, indicating recurrent recombination events between these genes. Statistical tests and recombination analyses showed that these recombination events occurred both between genes encoding both isoforms and between marine *Synechococcus* strains co-occurring in a same niche. However, it seems that the need to maintain the functional specificity of the D1:1 isoform prevents gene conversion between D1:1 and D1:2 genes. Thus, both genes encoding D1 isoforms seem to be submitted to two distinct evolutionary processes: while all *psbA* gene copies follow a concerted evolution through intragenomic recombinations and lateral transfers from other strains and possibly viruses, D1:2 encoding genes seem to undergo an additional homogenisation through gene conversion. In any case, functional and structural constraints of D1 protein isoforms appear to be the driving force of the co-evolution of *psbA* genes.

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***In situ* expression of genes involved in carbon concentrating mechanisms in hot spring cyanobacteria**

Sheila I Jensen^{1,2}, Anne-Soisig Steunou², Devaki Bhaya², Arthur R Grossman², Michael Kühl¹

¹*Marine Biological Laboratory, Department of Biology, University of Copenhagen, Copenhagen, Denmark*

²*Department of Plant Biology, The Carnegie Institution, Stanford University, Stanford, CA, USA*

sijensen@bio.ku.dk

The photosynthetic microbial mat in the effluent channel of an alkaline hot spring (Mushroom Spring) in Yellowstone National Park experiences extreme diel fluctuations in physicochemical parameters. During the day, photosynthesis causes the oxygen concentration within the mat to rise to highly supersaturating levels, and the intense photosynthetic activity of the cyanobacteria causes a pH >9.5 in the euphotic zone of the mat. During the night, the mat rapidly becomes anoxic, and intense respiration, reoxidation of reduced solutes and fermentation acidifies the mat to pH ~7.5. High temperature (55-70 °C) itself imposes a constraint on the availability of Ci for photosynthesis because of the low solubility of CO₂ at elevated temperatures; this is exacerbated by the relatively high O₂-concentration and high pH in the mat during the day. Therefore, it is likely important for the cyanobacteria in the mat to develop the capacity to accumulate Ci over the course of the day through the induction of genes associated with the carbon concentrating mechanism (CCM). The genomes of two *Synechococcus* isolates from the mat have revealed the presence of two CO₂ hydration systems, two putative HCO₃⁻ transporters and a β-type carboxysome [1] that are likely associated with the CCM. In this study we have performed experiments with axenic *Synechococcus* isolates in the laboratory and are attempting analogous experiments *in situ* to elucidate the function and diel regulation of putative CCM genes.

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The spectral characteristic of the fluorescence induction in cyanobacterium PCC 7942 – role of state transition

Radek Kaňa^{1,2}, Ondřej Prášil^{1,2}, Ondřej Komárek², Govindjee³, George C. Papageorgiou⁴

¹*Inst. of Microbiology, Academy of Sciences Czech Republic, Třeboň, Czech Republic*

²*Institute of Physical Biology, University of South Bohemia, Nové Hrad, Czech Republic*

³*Departments of Biochemistry and Plant Biology, University of Illinois, Urbana, USA*

⁴*National Center for Scientific Research Demokritos, Institute of Biology, Athens, Greece*

kana@alga.cz

We have studied spectral properties of fluorescence induction in the freshwater cyanobacterium *Synechococcus* sp. (PCC 7942). The thylakoid membranes of this prokaryote house embedded Chl *a* core antenna system of photosystem I (PSI) and photosystem II (PSII) and externally attached phycobilisomes (PBS). PBS are assembled from the C-phycocyanin and allophycocyanin that emit fluorescence at different wavelengths in comparison to the both photosystems. We have used newly adapted system suitable for the separate detection of all these fluorescence bands kinetically. We have explored the fluorescence emissions kinetics of PBS and PSI (PSII) during 300s-long photosynthetic induction on continual orange (622 nm) and blue light (460 nm). During irradiation, the fluorescence emission spectra have shown increase in the PBS emission (at 652nm) together with the fluorescence emission of photosystem II and photosystem I (observed at 682 nm and 715 + 740nm respectively). However, deconvolution of spectra into the particular fluorescence bands has shown variability only in the F682 (PSII) and F740 (red chlorophylls of PSI) bands. Kinetically, the F682 and F740 band peaked 100s after light on-set that resembled M-peak usually observed in cyanobacterium. We have attributed this peak to the state II-to-state I transition caused by PBS movement that can be observed in dark-adapted cyanobacteria [1]. The constant intensity of PBS emission peak (F652) during this PBS movement has indicated no reduction in the efficiency of energy transport from PBS to PSI (or to PSII) that could be explained by a very fast PBS movement between photosystems [2].

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The Psb28 protein is involved in the biogenesis of chlorophyll-binding proteins CP47 and PsaA/PsaB in the cyanobacterium *Synechocystis* sp. PCC 6803

Josef Komenda, Marika Dobáková, Roman Sobotka, Martin Tichý

Laboratory of Photosynthesis, Institute of Microbiology Třeboň and Institute of Physical Biology, University of South Bohemia, Nové Hradky, Czech Republic

komenda@alga.cz

The role of the Psb28 protein in the structure and function of the Photosystem II complex (PSII) has been studied in the cyanobacterium *Synechocystis* sp. PCC 6803. The protein was detected in the membrane fraction of wild-type cells as an unassembled protein or bound to the PSII core complex lacking the CP43 antenna (RC47). The association of Psb28 with RC47 was further confirmed by preferential isolation of RC47 from the strain containing a His-tagged derivative of Psb28 using nickel chromatography. However, this affinity-purified PSII preparation also contained small amounts of the monomeric PSII core complex and unassembled CP47 bound to Psb28-His indicating a structural relationship between Psb28 and CP47. This relationship was further supported by a substantial decrease in the level of unassembled CP47 in the *psbC* and *psbEFLJ* deletion mutants after the additional inactivation of the *psb28* gene. A *psb28* single deletion mutant grew autotrophically but slower than wild type although the absence of Psb28 did not affect the functional properties of PSII. The cells of the mutant exhibited slightly accelerated turnover of the D1 protein, faster PSII repair and a decrease in the cellular content of Photosystem I. The radioactive labeling of mutant cells showed limitation in the synthesis of CP47 and the PSI chlorophyll-binding subunits PsaA/PsaB. All *psb28* null mutants accumulated an increased amount of Mg-protoporphyrin IX-methylester and released large quantities of protoporphyrin IX into the medium. Overall our results indicate importance of Psb28 for chlorophyll incorporation during synthesis of CP47 and PsaA/PsaB.

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The purification of His-tagged Scp proteins from *Synechocystis* sp. PCC 6803 using Ni-NTA chromatography

Jana Kopečná^{1, 2}, Martin Tichý^{1, 3}, Roman Sobotka^{1, 3}

¹*Department of Autotrophic Microorganisms, Institute of Microbiology of the Academy of Sciences of the Czech Republic, Trebon, Czech Republic*

²*Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic*

³*Institute of Physical Biology, University of South Bohemia, Nove Hradky, Czech Republic*

kopecna@alga.cz

The genome of the cyanobacterium *Synechocystis* sp. PCC 6803 contains four genes coding for small Cab-like proteins (ScpB–ScpE). Scp proteins contain a single membrane-spanning region highly similar to two transmembrane regions of light harvesting complex II with conserved chlorophyll-binding motif. We have shown that Scp proteins are associated with photosystem (PS) II. However, the function of SCPs is still unclear. To investigate whether Scp proteins are constituents of multisubunit membrane complex and to determine their potential ability to bind photosynthetic pigments, His-tagged proteins ScpB and ScpD were over-expressed in wild type and also in PSI-less background. His-tagged proteins were purified under native conditions using nickel affinity chromatography, in both cases yielding yellow-green eluates. Analysis of the ScpBHis eluate using SDS-PAGE and immunodetection revealed that ScpE and ScpC/D proteins were co-purified with ScpB. This result suggests formation of a specific Scp complex in *Synechocystis* cells. Analyses of the ScpDHis eluate as well as determination of specifically bound pigments are in progress.

Simple purification and characterization of PSI complex from the *Synechocystis* sp. PCC6803 strains expressing His-tagged subunits

Hisako Kubota¹, Isamu Sakurai¹, Naoki Mizusawa¹, Pengpeng Zhang², Eva-Mari Aro², Hajime Wada¹

¹*Department of Life Sciences, University of Tokyo, Tokyo, Japan*

²*Plant Physiology and Molecular Biology, Department of Biology, University of Turku, Turku, Finland*

hkubota@bio.c.u-tokyo.ac.jp

We generated *Synechocystis* sp. PCC6803 strains, F-His and J-His, which express His-tagged PsaF and PsaJ subunits, respectively, for simple and rapid purification of PSI complex. In the generated strains, the C-terminus of PsaF and the N-terminus of PsaJ were tagged with 6 histidine residues. Growth profile of F-His and J-His cells under photoautotrophic growth condition was similar to that of wild-type cells. No difference in photosynthetic activity was also observed among wild-type, F-His and J-His cells. PSI complexes could be simply purified from the F-His and J-His cells by solubilization of thylakoid membranes with dodecylmaltoside followed by a Ni²⁺ affinity column chromatography. The purified PSI complexes were separated to monomer and trimer by ultracentrifugation with glycerol density gradient. PSI activity of monomer and trimer prepared from F-His and J-His cells was similar to that of PSI trimer prepared from wild-type cells that was purified only by ultracentrifugation with glycerol density gradient. Blue-Native PAGE and SDS-PAGE analyses of the purified PSI complexes from F-His and J-His cells indicated the difference of subunit composition between monomer and trimer, and the existence of two distinct monomers having different subunit composition. Trimer from F-His and J-His cells contained PsaK1 and PsaK2, but monomer contained only PsaK1. One monomer from both cells contained PsaL, whereas the other monomer did not contain PsaL subunit. We also analyzed the lipid composition of trimers from F-His and J-His cells and found that they contain about 6 lipid molecules (2 molecules of monogalactosyldiacylglycerol, 1 molecule of digalactosyldiacylglycerol, 1 molecule of sufoquinovosyldiacylglycerol, and 2 molecules of phosphatidylglycerol) per reaction center.

Electronic excitation migration in photosystem II supercomplex of cyanobacterium

Juha Linnanto, Jouko Korppi-Tommola

Physical Chemistry Laboratory, P.O.Box 35, FIN-40014 University of Jyväskylä, Finland

linnanto@jyu.fi

Excitation energy transfer in a photosystem II supercomplex of cyanobacterium has been thoroughly studied using quantum chemical calculations and an exciton theory. Studied photosystem II supercomplex model structure was based on experimental X-ray structures of photosystem II (PSII) of the cyanobacterium *Thermosynechococcus elongates* and different cyanobacterial light harvesting apparatus consisting of phycobilisomes (PBSs) [1-5]. Because the PSII supercomplex contains a huge number of atoms, modelling of excitation energy transfer from the outmost pigment all the way to the reaction center (RC) by utilising available structural information and theoretical methods is a major task.

Excitation energy transfer in the light harvesting antenna complex II (LHCII) of green plants has been successfully modelled [6] and the calculated excitation energy transfer rates are in good agreement with experiments. Calculations have shown that excitation of the blue side of the Q_y band of LHCII results in energy transfer from chlorophyll *b*'s of the luminal side to chlorophyll *a*'s located primarily on the outer edge of one of the monomers of the stromal side of LHCII [6]. Such localisation of excitation on the terminal chlorophylls allows excitation energy "to flow" efficiently from the LHCII antenna towards to the RC of the PSII complex. It will be demonstrated that PSII supercomplex of cyanobacterium has spatially and energetically donor and acceptor states that allow efficient energy migration from PBSs via several antenna complexes of PSII supercomplex to the RC. It will be shown that there is some freedom for lateral and vertical positioning of the PBS protein complexes with respect to PSII complex in the photosynthetic membrane without significant loss in efficiency of excitation energy transfer.

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***In vivo* regulation of ferrochelatase expression modulates chlorophyll accumulation in *Synechocystis* PCC 6803**

Jiří Patera¹, Roman Sobotka^{2,3}, Martin Tichý^{2,3}

¹*Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic*

²*Institute of Microbiology, Department of Autotrophic Microorganisms, Trebon, Czech Republic*

³*Institute of Physical Biology, University of South Bohemia, Nove Hradky, Czech Republic*

patera@alga.cz

Chlorophyll (Chl) and heme share common biosynthetic pathway branched at the point, where magnesium chelatase and ferrochelatase (FeCH) insert either magnesium for Chl biosynthesis or ferrous iron for heme biosynthesis. The mechanism that drives the distribution of the precursor between both branches is unknown, however our previous data imply that that FeCH can play a key role in this process.

To elucidate the significance of the FeCH activity in the regulation of Chl/heme biosynthesis, we have prepared two *Synechocystis* mutant strains where FeCH is expressed under the *nirA* promoter and its expression is controlled by ammonia.

Both constructs differ in the presence or absence of the Shine-Dalgarno sequence (SD). In both strains the wild type copy of the gene coding for FeCH was deleted. Using these constructs we are able to control the accumulation of the FeCH from 300 – 400% of the wild type to the level at which the cells are no longer viable (<5%). Expression levels in strains with and without SD overlap at around 40 % wild type level.

Our data obtained using these strains confirm the important role of the FeCH activity in the regulation of the Chl biosynthesis. High FeCH activity resulted in Chl deficiency with Chl level in cells reaching only 60 % of the wild type. In contrast its decrease to about 25% led to substantially increased Chl accumulation (120 – 130% of the wild type), whereas accumulation of phycobiliproteins was strongly impaired.

Oxygen evolution in genotypes of *Prochlorococcus marinus*

Ondřej Prášil¹, Radek Kaňa¹, Eva Kotabová¹, Daniella Mella², Frédéric Partensky², Laurence Garczarek²

¹Laboratory of Photosynthesis, Institute of Microbiology AVCR in Trebon and University of South Bohemia, Ceske Budejovice, Czech Republic

²Marine Photosynthetic Prokaryotes Team, Station Biologique de Roscoff, CNRS & University Paris 6, France

prasil@alga.cz

Most *Prochlorococcus marinus* strains sequenced to date, with the sole exception of MIT9313 lack two photosystem II (PSII) genes which encode structural proteins *psbU* and *psbV* associated with the oxygen evolving center of PSII. Since the deletion of *psbU* (encoding a 12 kD protein) and *psbV* (encoding cytochrome c550) in the freshwater cyanobacterium *Synechocystis* PCC6803 leads to significant defects in PS II function (slower photoautotrophic growth rate, decreased oxygen evolution activity and decreased structural stability of the water-splitting system), we analysed the oxygen evolution efficiency in several *P. marinus* strains. Our results indicate significantly lower capacity of oxygen evolution in strains that lack *psbU* and *psbV* (strains SS120 and PCC9511) compared to the fully competent strain MIT9313 and reference model organisms (*Chlorella vulgaris*). In addition, we found that strains lacking *psbU* and *psbV* are also less tolerant to stresses known to affect the OEC. Our results help to explain the observations why in the field *Prochlorococcus* populations can be found in oxygen minimum zones of the Arabian Sea and the Eastern Tropical North Pacific off Mexico. Thus, even though *Prochlorococcus* represents about half of the chlorophyll biomass in the intertropical, oligotrophic zone of the world ocean, our results imply that its global oxygen production would be much lower than expected.

Carotenoid-triggered energy dissipation in phycobilisomes diverts excitation energy from reaction centers of both photosystems

Marina G. Rakhimberdieva¹, Irina V. Elanskaya², Navassard V. Karapetyan¹

¹*A.N. Bakh Institute of Biochemistry, Russian Academy of Sciences, Moscow, Russia*

²*Department of Genetics, Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia*

rakhimberd@inbi.ras.ru

We have investigated the molecular mechanism of blue-light induced quenching [1] in *Synechocystis* sp. PCC 6803 – the wild type, a PSII-less and a PSI-less strain – using a combination of fluorescence spectroscopy, PAM-fluorometry and P700 measurements. Both room temperature and 77 K fluorescence emission spectra revealed that the site of quenching includes all allophycocyanin (APC) forms in the core of the phycobilisome (PBS) – both the APC and the terminal emitters, whereas chlorophyll (Chl) is not involved in the quenching mechanism. There is no blue-light-induced quenching of Chl excitation in PSII-less cells, where fluorescence originates from a fraction of uncoupled PBSs [2], whereas the other PBSs are energetically coupled to PSI. However, we have seen in the PSI-less strain that at room temperature blue light effectively quenches Chl excitation in PSII. This confirms our theory [3] that Chl excitation could be quenched via an APC quenching mechanism if the acceptor side of PSII is fully reduced, i.e. PSII reaction centers are closed. The light-saturation curve of fluorescence rise under PBS absorbed flashes (620 nm) demonstrates a 30% decrease in excitation energy transfer from PBS to PSII centers in the quenched state. Thus the quenching is indeed responsible for the down regulation of photosynthesis – the carotene-triggered process results in so efficient energy dissipation that it can actually compete with the energy transfer from PBS to PSII with an open reaction center. The light-saturation curve of P700 photooxidation in PSII-less strain also demonstrates a 30% decrease in 620 nm excitation energy transfer to the PSI reaction centers, which accompanies blue light induced fluorescence quenching. At the same time, there is no change in Chl absorbed 730 nm excitation energy transfer to the PSI reaction center. These facts indicate that energy transfer from PBS to PSI is diminished in the quenched samples. The comparable decrease of the excitation pressure on both reaction centers suggests that energy is transferred from PBS to PSI in the PSII-less strain at about the same efficiency as to PSII in the PSI-less strain. It seems that all PBSs attached to the thylakoid membrane may be subject to carotene-triggered dissipation of absorbed energy without any difference between “uncoupled” PBSs and PBSs coupled to PSI or PSII with open or closed centers.

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The concept of the ARTO: Alternate Respiratory Terminal Oxidases in cyanobacteria

Georg Schmetterer, Bernhard Wurzing, Ronald Stebegg, Geraldine Graser
Institute of Physical Chemistry, University of Vienna, Vienna, Austria

georg.schmetterer@univie.ac.at

All cyanobacteria contain (at least) one or more (up to 10!) respiratory electron transport chains. The key enzymes of these respiratory chains are the Respiratory Terminal Oxidases (RTOs), which - in cyanobacteria - invariably catalyze the reduction of dioxygen to water. Cyanobacterial RTOs belong to at least three different families: 1) the homologs of mitochondrial iron-copper cytochrome *c* oxidases, 2) the homologs of *Escherichia coli* cytochrome *bd* quinol oxidase and 3) the homologs of the plastidic terminal oxidase (Ptox). The total genomic sequences of about 40 cyanobacterial strains are now available and it has become clear that no single type of RTO is present in all cyanobacteria. The iron-copper RTOs in cyanobacteria fall into (at least) three different categories: a) the classical cytochrome *c* oxidases (Cox) that are well known from mitochondria and certain heterotrophic bacteria such as *Paracoccus denitrificans*, b) homologs of enzymes called cytochrome *cbb3* that have been characterized e.g. in some purple bacteria and c) a group of enzymes closely related to Cox but clearly different in sequence and function that appears to have no relatives in non-cyanobacteria, which we have called Alternate Respiratory Terminal Oxidases (ARTOs). The name ARTO was originally coined by us to describe an RTO that was discovered by sequencing *Synechocystis* sp. PCC6803. Although it displayed high sequence similarity with genuine cytochrome *c* oxidases of the mitochondrial type, it quickly became clear that the ARTO had a different function. At least in strain PCC6803 the ARTO has no measurable *in vitro* cytochrome *c* oxidase activity, although we have evidence that cytochrome *c* (encoded by the *petJ* gene) probably is a component of the electron transport chain ending in ARTO. In other strains, such as *Nostoc* sp. PCC7120 and *Anabaena variabilis* ATCC29413 there is more than one ARTO locus in the chromosome. Especially the latter strain allowed us to define the difference between a genuine Cox and an ARTO by simply analyzing the sequence of the corresponding gene loci: in cyanobacteria: both enzyme seem to have invariably three subunits (exactly those subunits of the mitochondrial Cox that are encoded by the mitochondrial DNA in most mitochondria), but a very characteristic WAHHMF sequence in subunit I (the major catalytic subunit) is changed to WVHHMF (or a very similar sequence) in all ARTOs. This characteristic change apparently makes ARTOs unable to function as efficient bioenergetic enzymes and therefore ARTOs are unable to support heterotrophic growth. This has been conclusively shown in both *Synechocystis* sp. PCC6803 and in *Anabaena variabilis* ATCC29413. Therefore, the ARTOs have one or more different functions, which will be discussed based on the available evidence. For all Coxes and almost all ARTOs the corresponding three genes are located in a single locus that contains the genes for subunits II, I, and III, in that order. For *Anabaena variabilis* ATCC29413 we have shown that this locus constitutes a single operon (*coxBAC*). In contrast, a unique situation exists in *Synechocystis* sp. PCC6803: the genes for the ARTO subunits are not present in a single locus, with the gene for the putative subunit II far removed from the other two adjacent genes. Recent results on the ARTO of *Synechocystis* sp. PCC6803 will also be presented.

Phenomenon of State 1- State 2 transitions in cyanobacteria after 40 years of the study. Participation of photosystem II, photosystem I and phycobilisomes

Igor Stadnichuk¹, Evgenii Lukashev²

¹A.N. Bakh Institute of Biochemistry Russian Academy of Sciences, Moscow

²Moscow Lomonosov State University, Biological Department, Moscow

stadnichuk@mail.ru

Light quality and quantity are key factors determining the efficiency of photosynthesis. The phenomenon of short-term adaptation to unbalanced light absorption by photosystem I (PSI) and photosystem II (PSII) in phycobilisome (PBS)-containing algae [1] and higher plants [2] was first described nearly 40 years ago. It is a way to adjust optimal rate of electron flow between the photosystems under conditions when light is limiting for growth. In higher plants, it is based on the partial redistribution of phosphorylated light-harvesting chlorophyll *a/b*-protein between PSI and PSII. Contrary, the cause of state transitions in cyanobacteria is still in debate. Three main models have been proposed. In the spillover model, the excitation energy under State 2 conditions is transferred from PSII to PSI core chlorophylls. The partial dissociation of PBS from PSII and its reassociation without docking to PSI is the main event of the detachment model. In the mobile model, the PBS could reversibly migrate from the surface of PSII to PSI in the plane of the thylakoid membrane [3]. The described docking of PBS to the trimers of PSI [4] and the newly found mechanism of OCP-induced PBS quenching [5, 6] complicate the description of all the three models. We have used the photosynthetic action spectra and steady state fluorescence emission and fluorescence excitation spectra measurements in the presence of fluorescence standards to study the state transitions in the unicellular cyanobacterium *Synechocystis* sp. 6803. The detectable changes of energy migration from PBS to the PSI and PSII in the *Synechocystis* sp. 6803 during the transition from State I to State 2 and vice versa were not registered. The identified constant level of fluorescence emission of PSI during State 1-State 2 transitions and simultaneous increase of fluorescence quantum yield of PSII in State 1 allows to propose that spillover is also an unlikely mechanism of this phenomenon. We conclude that the increase of fluorescence emission from PSII in State 1 regulated by the redox status of the plastoquinone pool is the main event of this phenomenon. The changes of PBS emission are secondary comparing with the fluorescence changes of chlorophyll belonged to PSII while the emission of PSI stays unchangeable at all. It could be possible that none of the exposed three models of state transitions would be correct after additional investigations.

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Crystal structure of a mutated RbcX protein from *Thermosynechococcus elongatus*

Mirosław Tarnawski¹, Szymon Krzywda³, Mariusz Jaskolski^{2,3} and Andrzej Szczepaniak¹

¹Department of Biophysics, Faculty of Biotechnology, University of Wrocław, Przybyszewskiego 63/77, 51-148 Wrocław, Poland

²Center for Biocrystallographic Research, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 60-704 Poznań, Poland

³Department of Crystallography, Faculty of Chemistry, A. Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland

mirekt@ibmb.uni.wroc.pl

RuBisCO is a key enzyme responsible for atmospheric carbon dioxide fixation during the photosynthetic process. In land plants, green algae and cyanobacteria RuBisCO is composed of eight large (RbcL) and eight small (RbcS) subunits. In some cyanobacteria, the RuBisCO operon contains three genes, *rbcL*, *rbcX* and *rbcS*. It has been suggested that the RbcX protein could be an assembly chaperone for RuBisCO. The latest studies of RbcX from *Synechococcus* sp. PCC7002 have confirmed that cyanobacterial RbcX is a RuBisCO chaperone, it assists RuBisCO folding and assembly of L₈ core complex by binding to the C-terminus of partially unfolded RbcL subunits before they assemble with RbcS into the native L₈S₈ complex [1]. Recently, the crystal structure of RbcX from *Synechocystis* sp. PCC6803, has been reported [2]. Here, we report the crystal structure of RbcX from the thermophilic cyanobacterium *Thermosynechococcus elongatus* BP-1. To obtain this new RbcX structure it was necessary to mutate unusual Cys103 residue to alanine or arginine in order to prevent cysteine oxidation mediated aggregation. Four different crystal forms were obtained. Crystal form described here, containing two molecules per asymmetric unit, belongs to the orthorhombic space group *P*212121, with unit-cell dimensions *a* = 45.96, *b* = 67.86, *c* = 94.81 Å. The structure was determined at 1.96 Å resolution and solved by molecular replacement. The overall fold was found to be similar to that of previously reported RbcX structures.

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A novel ATP-binding cassette transporter involved in paraquat (methyl viologen) resistance in the cyanobacterium *Synechocystis* sp. PCC 6803

Jana Prosecká¹, Artem Orlov², Yuri Fantin², Michael Babykin³, Martin Tichý^{1,4}

¹*Institute of Microbiology, Department of Autotrophic Microorganisms, Trebon, Czech Republic*

²*Department of Genetics, Moscow State University, Moscow, Russia*

³*International Biotechnological Centre, Moscow State University, Moscow, Russia*

⁴*Institute of Physical Biology, University of South Bohemia, Nove Hrad, Czech Republic*

tichym@alga.cz

Paraquat (methyl viologen) is a charged quaternary ammonium compound that generates reactive oxygen species under aerobic growth conditions. The compound is one of the most widely used herbicides in the world. In an extensive screening for spontaneous paraquat-resistant mutants in *Synechocystis* sp. PCC 6803, mutations of two classes were identified to be responsible for the resistant phenotype. The point mutations of the first class localized into the transcriptional regulator gene *prqR* (*slr0895*) resulted in derepression of the *prqA* gene (*slr0896*) coding for a protein homologous to multidrug efflux pumps of the MATE family [1]. The mutations of the second class were localized into the *slr1174* gene coding for a protein related to a permease subunit of ABC-2 type transporters (DUF990 protein family). Sequencing of three independent resistant mutants revealed point mutations leading to substitution of the same amino acid R115 (R115G, R115L and R115C) in the Slr1174 protein. Insertion inactivation of *slr1174* resulted in increased cell sensitivity to paraquat suggesting that putative Slr1174 permease is involved in paraquat export. In bioinformatic search for ATPase component forming a functional ABC transporter with Slr1174, it was realized that gene coding for such ATPase component is in immediate neighborhood of the respective *slr1174* homologues in genomes of various bacteria. Moreover, third gene coding for another DUF990 family protein was usually present in the putative operon. No such operon was found in the genome of *Synechocystis* sp. PCC 6803; however, there was another gene encoding the DUF990 family protein Slr0610 and ATPase protein Slr1901, both with significant homology to presumed ABC-2 transporter components. To confirm existence of the heteromeric Slr1174/ Slr0610/ Slr1901 ABC transporter in *Synechocystis* sp. PCC 6803, we constructed the *slr0610* and *slr1901* interruption mutants in the paraquat resistant *slr1174* point mutant background. As would be expected for paraquat exporter, inactivation of any of its putative subunits led to the loss of paraquat resistance. We conclude that three-component Slr1174/ Slr0610/ Slr1901 ABC-2 type transporter is involved in paraquat efflux in *Synechocystis* sp. PCC 6803. This is the first report of a function for the DUF990 family protein.

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A-type flavoproteins Flv2 and Flv4 are crucial for sustenance of photosystem II in *Synechocystis* sp. PCC 6803

Pengpeng Zhang, Yagut Allahverdiyeva, Eva-Mari Aro

Department of Biology, Plant Physiology and Molecular Biology, University of Turku, FI-20014, Turku, Finland

pzhang@utu.fi

Cyanobacteria contain several genes encoding putative A-type flavoproteins, but the specific roles of these proteins are still obscure. To address this, various flavoprotein inactivation mutants of *Synechocystis* sp. PCC 6803 were investigated. It is shown that the expression of Flv2, Flv3 and Flv4 proteins is negligible at 3% CO₂ and is largely enhanced by CO₂ limiting conditions. Moreover, the induction rate of the *flv2* and *flv4* genes under low CO₂ is strongly dependent on light intensity. A specific decline in PSII centers was observed in the $\Delta flv2$ and $\Delta flv4$ strains grown at low CO₂, particularly under saturating light the $\Delta flv2$ and $\Delta flv4$ mutants were unable to acclimate. On the contrary, the $\Delta flv1$ and $\Delta flv3$ strains had higher amounts of the Flv2 and Flv4 proteins than WT and also higher amounts of PSII centers under steady state growth at air level of CO₂. When the cells were subjected to short-term high light, the $\Delta flv2$ and $\Delta flv4$ mutants appeared to be more susceptible to photoinhibition and incapable of inducing a fast cyclic electron flow around PSI as compared with WT, $\Delta flv1$ and $\Delta flv3$. These data suggest that the flavoproteins in cyanobacteria have an important function in photoprotection of PSII when the cells are acclimated to low CO₂ environments but these proteins are dispensable at high CO₂. The mechanism of PSII photoprotection by Flv2 and Flv4 proteins may be related to a route of cyclic electron flow around PSI which is induced under low CO₂ but impaired in the $\Delta flv2$ and $\Delta flv4$ mutants.

REGULATION OF GENE EXPRESSION

Integrating the circadian clock into the life of the cyanobacterial cell

Susan S. Golden

*Center for Research on Biological Clocks, Department of Biology, Texas A&M University,
College Station, Texas 77843*

sgolden@tamu.edu

Cyanobacteria, like diverse eukaryotes, possess circadian clocks that allow cells to coordinate physiological processes with the predictable daily patterns of environmental fluctuations on Earth. An autonomous oscillator comprised of three distinctive proteins, KaiA, KaiB, and KaiC, underpins the circadian clock of the cyanobacterial model organism *Synechococcus elongatus* PCC 7942. Our work applies genetic, genomic, biochemical, and structural approaches, integrated with cell biology, to achieve a comprehensive understanding of how the *S. elongatus* cell harnesses this oscillator to control cellular activities. The emerging picture is one of a clock that is set daily by sampling the cellular redox environment as a proxy for light and which uses protein-protein interactions and subcellular localization to coordinate gene expression, cell division, and chromosome dynamics. A functional genomics project has produced inactivation alleles of nearly all *S. elongatus* loci. Gene expression assays of the 700 mutants tested so far suggest that about 10% of loci affect circadian period or phasing, and that many genes which are essential for viability are connected to the clock.

Light-dependent transcriptional regulation in cyanobacteria

Kan Tanaka*, Asako Seki, Mitsumasa Hanaoka

Institute of Molecular & Cellular Biosciences, the University of Tokyo, Japan

**present address: Graduate School of Horticulture, Chiba University, Japan*

kntanaka@iam.u-tokyo.ac.jp

Changing gene expression dependent on light environment is especially important for survival for photosynthetic organisms as cyanobacteria. Excess energy influx into two photosystems results in damages of various cellular machineries, and thus in case of medial-to-high light shift, cyanobacteria are known to transcriptionally induce a series of high light (HL)-inducible (*hli*) genes for the acclimation. Recently, we analyzed the transcriptional regulation of two HL-inducible genes, *hliA* and *rpoD3*, in *Synechococcus elongatus* PCC 7942 to understand the underlying mechanism for the light signal transduction. *RpoD3* is an RNA polymerase group 2 sigma factor that is involved in the survival under the high light conditions. In this study, we mapped the transcriptional start site of *rpoD3*, and found HRE1 sequences, previously proposed HL-responsive element involved in the NblS-RpaB two-component regulatory system [1], in the promoter region as in *hliA*. Using a recombinant RpaB protein, we demonstrated that RpaB directly binds to HRE1 in vitro, and in vivo overexpression of a truncated-variant of RpaB suggested that RpaB negatively regulates HL-inducible genes under the normal light condition and the derepression under HL results in the transcriptional activation [2]. This model was demonstrated using chromatin-immunoprecipitation (ChIP) analysis, which is a versatile technique for analysis of in vivo protein-DNA interactions [3]. Involvement of sigma factor regulation in other light changing regimes will be also discussed in the presentation.

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Omic analysis of a cyanobacterial RNA helicase

Lige Wu, Laura Patterson-Fortin, George W. Owtttrim

Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E9

g.owttrim@ualberta.ca

Rearrangement of RNA secondary structure by RNA helicases is a crucial component controlling the post-transcriptional regulation of gene expression. While numerous RNA helicases have been associated with general RNA metabolic processes, including mRNA splicing and turnover, only a single precise target RNA has been identified. We have initiated an omic analysis to identify RNA and protein targets of the cyanobacterial RNA helicase, *crhR*. *crhR* expression is regulated by the redox status of the electron transport chain and as a result, is significantly enhanced at low temperature. Results will be presented from co-IP and microarray analysis of WT and *crhR* mutants to identify RNA targets for CrhR. These results will be correlated with proteomic analysis designed to identify not only proteins whose abundance is altered by *crhR* mutation, but also protein partners that interact with CrhR. The combined observations will provide insight into the physiological role(s) that CrhR performs in cyanobacteria.

Anaerobic induction of a gene cluster containing *psbA1* and *petC2* in *Synechocystis* sp. strain PCC 6803

Tina C. Summerfield^{1,2}, Jörg Toepel¹, Louis A. Sherman¹

¹*Department of Biological Sciences, Purdue University, West Lafayette, IN, USA*

²*Department of Biochemistry, University of Otago, Dunedin, New Zealand*

tina.summerfield@stonebow.otago.ac.nz

Many cyanobacteria contain multiple copies of the *psbA* gene that encodes D1, a reaction centre protein of photosystem II (PSII). Following photodamage, the repair cycle of PSII includes replacement of the D1 protein with a newly translated copy. It has been demonstrated that alternate copies of *psbA* are involved in acclimation to different stresses including: high light, blue light, low temperature, and UV-B. In a number of cyanobacterial strains, additional copies of *psbA* are transcribed at trace levels and are not induced under stress conditions, e.g., the *psbA1* gene in the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803. We show that *Synechocystis psbA1* is induced strongly under anaerobic conditions to represent at least 20% of the *psbA* transcripts. Using a strain lacking the constitutively expressed *psbA2* gene and stress induced *psbA3* gene, we have shown the *psbA1* transcripts are translated and incorporated into PSII centres that are capable of evolving oxygen. After a ten-hour incubation under anoxic conditions, the photoheterotrophically grown $\Delta\text{PsbA2}:\Delta\text{PsbA3}$ strain was able to evolve oxygen at almost 90% the rate of wild type. However, the $\Delta\text{PsbA2}:\Delta\text{PsbA3}$ strain did not exhibit photoautrophic growth in anoxic conditions. In addition, wild type *Synechocystis* cultures became bleached following prolonged exposure to anaerobic conditions. This is consistent with microarray results indicating that under these conditions there is down-regulation of genes involved in photosynthesis (including transcripts encoding ATP synthase) and translation (including most of the genes encoding ribosomal proteins). We have identified the anaerobic induction of specific *psbA* genes in two other cyanobacteria, the unicellular diazotroph *Cyanothece* sp. strain ATCC 51142 and the filamentous diazotroph *Anabaena* sp. strain PCC 7120. However, we did not observe anaerobic induction of any of the five *psbA* genes in the deeply divergent cyanobacterium *Gloeobacter violaceus* sp. strain PCC 7421. In both *Synechocystis* and *Cyanothece*, the induced *psbA* gene is in a cluster containing a *petC2* gene encoding an alternative Rieske iron-sulphur protein. We suggest that this gene cluster, encoding important proteins for two of the major photosynthetic complexes, may represent adaptation to changing O₂ levels for which both marine and freshwater cyanobacteria must be prepared.

Evolution and function of circadian clock proteins from *Prochlorococcus* MED4

Annegret Wilde¹, Ulf Dühring³, Luiza Seeliger³, Anne Arnold², Ilka M. Axmann²

¹*Institute of Microbiology and Molecular Biology, Justus-Liebig University Giessen, Germany*

²*Institute for Theoretical Biology, Humboldt-University Berlin, Germany*

³*Institute of Biology, Humboldt-University Berlin, Germany*

Annegret.Wilde@mikro.bio.uni-giessen.de

Organisms coordinate biological activities into daily cycles using an internal circadian clock. The simplest clock has been described for cyanobacteria where only three proteins, KaiA, KaiB and KaiC, are sufficient to generate 24-hour oscillations. KaiC, the principal protein of this biochemical oscillator, undergoes rhythmic autophosphorylation and dephosphorylation modulated by KaiA and KaiB. Marine cyanobacteria of the genus *Prochlorococcus* lost the *kaiA* gene by genome reduction. Our experiments indicate that the two remaining clock proteins of *Prochlorococcus* MED4, KaiB and KaiC, are functional. In contrast to the *Synechococcus elongatus* PCC 7942 system, KaiC from *Prochlorococcus* MED4 shows substantial autophosphorylation without KaiA. KaiB does not promote dephosphorylation of KaiC from *Prochlorococcus* MED4. However, in the *in vitro* clock system of *Synechococcus elongatus* PCC 7942 *Prochlorococcus* KaiB is able to replace the *Synechococcus* protein. We also observed ATPase activity of KaiC from *Prochlorococcus*, again pointing out that this protein is functional. Although we did not observe circadian oscillations using the two-protein system of *Prochlorococcus* MED4 in a test tube, a minimized but functional clock is likely for the living *Prochlorococcus* cell. Describing cell cycle and expression of selected clock and non-clock genes in synchronized cultures of the closely related *Prochlorococcus* PCC9511, the idea has been presented that a core oscillatory mechanism and output apparatus is functional in *Prochlorococcus*, but working in an hourglass-like fashion rather than as a self-sustained oscillator [1]. These findings are fully compatible with the biochemical properties we have observed here. Thus, the mechanism driving circadian oscillations in *Prochlorococcus* is obviously distinct from the standard cyanobacterial clock model. Future studies may clarify if it requires transcription/translation or other processes.

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Characterization of single, double and triple inactivation strains reveals new physiological roles for group 2 sigma factors in the cyanobacterium *Synechocystis* sp. PCC 6803

**Taina Tyystjärvi¹, Maija Pollari¹, Liisa Gunnelius¹, Ilona Tuominen¹, Virpi Ruotsalainen¹,
Tiina Salminen², Esa Tyystjärvi¹**

¹*Department of Biology, University of Turku, FI-20014 Turku, Finland*

²*Department of Biochemistry and Pharmacy, Åbo Academi University, FI-20520 Turku, Finland*

taityy@utu.fi

The initiation of transcription, mediated by the RNA polymerase holoenzyme, is an important determinant of gene regulation in eubacteria. The σ factor of the RNA polymerase holoenzyme is responsible for the recognition of a promoter sequence. In the cyanobacterium *Synechocystis* sp. PCC 6803 the primary σ factor, SigA, is essential for cell viability. The SigB, SigC, SigD and SigE factors show significant sequence similarity with the SigA factor but are nonessential. According to the three dimensional models, the overall structures of group 1 and 2 σ factors are similar, the SigB and SigD factors being the most similar ones [1]. We have constructed a complete set of group 2 σ factor single, double [1] and triple inactivation strains. All mutants grew well under standard growth conditions (BG11 medium buffered with 20 mM Hepes pH 7.5, continuous light 40 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, 32 °C) but differences were observed in stress conditions. Both ΔsigB and ΔsigC strains are sensitive to heat stress [2, 3]. These two σ factors regulate different sets of genes at high temperature, which makes the double mutant ΔsigBC extremely sensitive to heat stress. All strains lacking the SigD factor are sensitive to bright light. The transition from lag-phase to exponential growth is slow in the ΔsigBD strain. Furthermore, all group 2 σ factors were found to be involved in acclimation to salt- or sorbitol-induced osmotic stresses.

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The effects of LexA over-expression on the transcription of the *hox* genes in the cyanobacterium *Anabaena* sp. strain PCC 7120

Paulo Oliveira, Xiaohui Zhang, Johannes Sjöholm, Daniel Kamsund, Peter Lindblad
Department of Photochemistry and Molecular Science, Uppsala University, Uppsala, Sweden

paulo.oliveira@fotomol.uu.se

The bidirectional [NiFe]-hydrogenase in the cyanobacterium *Anabaena* sp. strain PCC 7120 is composed of five different subunits, encoded by the *hoxEFUYH* genes. These genes are found on the genome as two clusters, separated by approximately 8.8 kb. In agreement with the genomic arrangement, their transcription was investigated and demonstrated to be carried out as two, possibly three, operons [1]. In the same study, the transcription factor LexA was shown to interact with the promoter regions of both *hox* operons [1]. Interestingly, in *Synechocystis* sp. PCC 6803, LexA has been shown to bind to two different regions of the *hox* promoter [2, 3], and further suggested to regulate the *hox* genes expression [3]. In addition, Domain *et al.* [4] showed that LexA in *Synechocystis* sp. PCC 6803 is not involved in the classical SOS-response system, implicated when DNA damage occurs, but instead is *e.g.* critical to the survival of the cells facing inorganic carbon starvation. In the present work, we describe an *Anabaena* sp. PCC 7120 strain capable of over-expressing LexA upon induction with nitrate. This expression was confirmed both on the RNA and protein levels. The role of LexA on the *hox* genes transcription, as well as on genes putatively involved in the SOS response system, was monitored by Northern blotting and qRT-PCR, and will be further discussed.

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Transcription of the extended *hyp*-operon in *Nostoc* sp. strain PCC 7120

Åsa Agervald, Karin Stensjö, Marie Holmqvist, Peter Lindblad

Department of Photochemistry and Molecular Science, The Ångström Laboratories, Uppsala University, Box 523, SE-751 20 Uppsala, Sweden

Asa.Agervald@fotomol.uu.se

The maturation of hydrogenases into active enzymes is a complex process. A correctly assembled active site requires the involvement of at least seven proteins, encoded by *hypABCDEF* and a hydrogenase specific protease, encoded either by *hupW* or *hoxW* [1, 2]. While the maturation process of the hydrogenase large subunit is extensively studied, the maturation and assembly of the small subunit is still unclear. The small subunit of most [NiFe]-hydrogenases contains three iron-sulphur clusters which transport the electrons in and out from the active site [1].

The N₂-fixing cyanobacterium *Nostoc* sp. strain PCC 7120 may contain both an uptake and a bidirectional hydrogenase, encoded by the structural genes *hupSL* and *hoxEFUYH*, respectively. The gene cluster *asr0689-alr0693*, positioned in the extended *hyp*-operon, was shown to be transcribed together with the *hyp*-genes. These ORFs are present in cyanobacterial strain containing only an uptake hydrogenase, or both an uptake and a bidirectional hydrogenase, but are absent in strains harbouring only a bidirectional hydrogenase. In N₂-fixing cyanobacteria the ORFs are located between the *hupSL* and the *hyp*-genes as in *Nostoc* sp. strain PCC 7120 [3]. All ORFs in the extended *hyp*-operon upstream the *hyp*-genes are annotated as encoding unknown proteins, with the exception of Alr0692 which is identified as a NifU-like protein. Two of the ORFs, alr0691 and alr0692, contain functional domains resembling *hupH* and *hupG* which are proved to be involved in the maturation process of the small subunit of the hydrogenase in *Rhizobium leguminosarum* bv. Viciae st. UPM791 and HoxQ and HoxO respectively in *Ralstonia eutropha* [4,5].

Based on the finding that *asr0689-alr0693* are transcribed together with the *hyp*-genes, the existence of highly conserved homologue regions in N₂-fixing cyanobacteria positioned between the structural genes of the uptake hydrogenase and the maturation genes for the hydrogenase active site it is tempting to suggest that the upstream genes of the *hyp*-operon are involved in the assembly and maturation process of the cyanobacterial uptake hydrogenase small subunit [3].

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Exploitation of sequenced cyanobacteria to unravel the DNA repair systems in the desiccation-, radiation-tolerant cyanobacterium *Chroococcidiopsis*

Daniela Billi¹, Patrizia Ghelardini^{2,1}

¹Dipartimento di Biologia, Università di Roma "Tor Vergata", Rome, Italy

²Istituto di Biologia e Patologia Molecolari del CNR, Rome, Italy

billi@uniroma2.it

Cyanobacteria of the genus *Chroococcidiopsis* constantly appear in the most extreme and dry habitats on Earth, including the McMurdo Dry Valleys (Antarctica) and the Atacama Desert (Chile),- considered the terrestrial Martian analogues [1] -, where they colonize the last refuges for life inside porous rocks or at the stone-soil interfaces. In order to withstand lethal damages induced by entry into, and exit from, the fully desiccated state, desert strains of *Chroococcidiopsis* must have evolved mechanisms to protect and/or repair their cellular structures [2]. Hints on the existence in these cyanobacteria of an interplay between mechanisms to avoid (or limit) DNA damage and repair it, arise from the fact that: i) they mend genome fragmentation induced by ionizing radiation [3]; ii) desiccation-survivors protect their genome from complete fragmentation; and iii) the survival of dried cells to simulated Martian UV flux is greater than that of *Bacillus subtilis* spores [4]. Ongoing researches aim to unravel the DNA repair systems in *Chroococcidiopsis* sp. 029, hence, in order to overcome impairments due to the lack of its genome sequence, two approaches were developed, which take advantage of sequenced cyanobacterial genomes. The first one aims to the screening of a prey genomic library of *Chroococcidiopsis* by using DNA repair baits obtained from *Synechocystis* PCC 6803. The rationale relies on the homo/heterodimerization occurring between DNA repair proteins and validation tests were carried out by performing the two hybrid assay in *Escherichia coli*. This method assesses the interaction between two proteins by following their ability to form a functional repressor, finally resulting in a beta-galactosidase activity reduction [5]. Results provided proper baits to identify MMR genes in *Chroococcidiopsis*, but, for the first time, also allowed an *in vivo* characterization of the MMR pathway of *Synechocystis* PCC 6803. The second approach aims to identify fragments of DNA repair genes in *Chroococcidiopsis* by using evolutionary PCR. The alignment of MMR, nucleotide excision repair (NER) and SOS proteins of sequenced cyanobacteria allowed the design of degenerative primers which amplified *mutS1*, *UvrB* and *recA* fragments from *Chroococcidiopsis*. Finally, to localize DNA repair factories in *Chroococcidiopsis*, a GFP-tagging system was developed and experimental conditions were set up by tagging MutL, MutS1 and RecA of *Synechocystis* PCC 6803. Results of the *in vivo* imaging analysis further corroborated the characterization on the MMR pathway of *Synechocystis* PCC 6803.

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Response of *Synechococcus* sp. WH7803 to light and oxidative stress

Nicolas Blot, Christophe Boutte, Frédéric Partensky, Laurence Garczarek

Station Biologique, UMR 7144 CNRS & University Paris VI, 29682 Roscoff, France

blot@sb-roscoff.fr

A whole-genome microarray has been developed to assess the transcriptomic response of the marine cyanobacterium *Synechococcus* sp. WH7803 to various stresses. We acclimated cultures this strain to different growth irradiances then exposed them to either high light only, high light plus ultraviolet radiations [1], hydrogen peroxide or methyl viologen-induced oxidative stress. The transcriptomic profiles showed similar features, including the repression of a number of photosynthesis genes expression, the induction of *hli* genes (encoding high light-inducible proteins) and the up- or down-regulation of other stress response genes. Differences were nevertheless observed in processes like the iron and nitrogen uptakes or the phosphate transport. These data have been complemented by measurements of physiological responses (quantum yield, antioxidant activities). Comparative analyses allowed us to point out a common response to these various stresses.

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Antisense RNAs, another level of gene regulation in cyanobacteria?

Jens Georg¹, Ingeborg Scholz¹, Jan Mitschke¹, B. Voss¹, Dennis Dienst², Annegret Wilde², Wolfgang R. Hess¹

¹*Faculty of Biology, University Freiburg, Freiburg, Germany*

²*Institute of Biology, Humboldt-University Berlin, Germany*

jens.georg@biologie.uni-freiburg.de

So far only very few examples for chromosomally encoded antisense RNAs (asRNAs) in bacteria are published. We used a terminator prediction based bioinformatic approach combined with a 105K tiling array, spanning one third of the *Synechocystis* PCC6803 genome, to identify the global asRNA distribution in the cyanobacterium.

To date, 43 new asRNAs have been verified by 5'RACE and Northern Blot. Extrapolated to the whole genome there are 300 to 900 asRNAs, corresponding in number to 10 to 30% of all genes. That is a multiple of all known noncoding or small RNAs in the well investigated *E.coli*. The sizes of detected asRNAs range from 60 up to 800 nucleotides.

Expression levels vary from barely detectable to levels comparable with strongly accumulating mRNAs. Also the mRNA/ asRNA ratios are very variable. In most cases the asRNA is lower or equally expressed as its mRNA, but sometimes asRNA expression levels are significantly higher.

These findings suggest for these asRNAs different modes of action and different grades of importance. It seems possible that transcriptional noise can gain a vital function during evolution. At least the previously published asRNA *isrR* has an important role in iron stress response.

The occurrence of antisense RNAs is not limited to special genomic regions or gene functions. For example photosynthesis genes, protein kinase genes, a ribosomal protein gene, RNA metabolism genes, a DNA repair gene and general housekeeping genes, are affected.

Altogether the current knowledge of asRNAs is only the tip of the iceberg, they probably have an important function in the fine tuning of gene regulation.

Small noncoding RNAs in the marine cyanobacterium *Synechococcus* WH7803

Gregor Gierga, Björn Voß, Wolfgang R. Hess

Faculty of Biology, Experimental Bioinformatics and Genetics, University of Freiburg, Germany

gregor.gierga@biologie.uni-freiburg.de

Small noncoding RNAs in prokaryotes are known for a couple of years. Nowadays it has been well established that sRNAs are involved in a variety of regulatory processes in the cell.

Our goal is to identify the sRNAs in a widely distributed and ecologically important marine unicellular cyanobacterium, *Synechococcus* WH7803.

Based on the semiconductor technology introduced by the company CombiMatrix we designed a tiling microarray targeting exclusively the intergenic "empty" regions of the genome of *Synechococcus* WH7803 using 12000 35-mer probes with an overlap of approximately 11 bases. We worked with two different types of microarrays, one type uses recently developed electrasense technology with biotin labeled RNA, the other classically Cy5- fluorescence-labeled RNA.

The results of the microarray experiments were compared with the outcomes of a comparative analysis to predicted sRNA genes and other RNA elements using genomes of 4 other marine *Synechococcus* evolutionarily closely related to *Synechococcus* WH7803. Electrasense data showed 600 probes with a signal clearly above threshold. Compared with the computationally predicted sRNAs, where we found 89 IGRs with a probability value for ncRNAs >0.5, 48 of them had corresponding signals on the array.

The joined results helped to identify a number of new sRNAs and candidate sRNA genes which have been verified and analyzed further by Northern Blot and RACE experiments. Since the expression of many regulatory RNAs is coupled to the process they help regulate, we explored the differential expression of sRNAs as a function of different ecologically relevant stresses, like cold stress, phosphate and iron starvation. Compared to the standard growth conditions we discovered sRNAs differentially expressed under cold stress e. g. an sRNA about 80 nucleotides long that becomes downregulated under cold stress and shows good similarity to the cyanobacterial functional RNA family Yfr2-5, as well as putative sRNAs upregulated under cold stress.

Three-dimensional modeling of RNA polymerase and group 1 and 2 σ factors from *Synechocystis* sp. PCC 6803

Liisa Gunnelius¹, Tiina Salminen², Taina Tyystjärvi¹

¹Department of Biology, University of Turku, FI-20014 Turku, Finland

²Department of Biochemistry and Pharmacy, Åbo Akademi University, FI-20520 Turku, Finland

liisa.gunnelius@utu.fi

Bacteria have only one RNA polymerase (RNAP), which is responsible for all transcription. This RNAP consists of a catalytic core and a sigma (σ) factor, which is needed for promoter-specific initiation of transcription. All bacteria have one group 1 σ factor, which is essential for growth. Many bacteria even have additional σ factors, of which the group 2 σ factors share extensive sequence similarity with group 1 σ factors. However, group 2 σ factors are nonessential and activated in various stress conditions. The core of RNAP is composed of two α subunits and β , β' , and ω subunits. In cyanobacteria, the β' subunit is split in two and the amino-terminal part is called the γ subunit. The cyanobacterial β' subunit, which corresponds to the carboxy-terminal part of β' of other bacteria, harbors a large insertion. In this study, structural models of RNAP with group 1 and 2 σ factors from *Synechocystis* sp. PCC 6803 were constructed using the crystal structure of *Thermus thermophilus* RNAP at 2.8 Å resolution (PDB code 2A6E [1]) as a template. Approximately half of the amino acids were identical between the template and *Synechocystis*. However, the identity of the ω subunits remained below 20%, and the ω subunit was excluded from the models. The sequences were aligned and the models were built and optimized with automated modeling software based on the alignments [2]. Ramachandran plots showed that approximately 90% of amino acid residues were in the most favoured regions, with less than 1% of residues in disallowed regions, which meets the criteria for good quality models. The RMSD values between the template and models were around 1.6 Å, which is satisfactory. The model of the holoenzyme showed that the splitting of the β' subunit does not affect the overall structure much. Furthermore, the cyanobacterial insert in the β' subunit is located on the surface of RNAP, where it is easily accommodated. The models of individual σ factors were used to compare group 1 and 2 σ factors. The σ factors have very similar structures, all being almost entirely α -helical, but there is a non-conserved domain of variable length. This non-conserved domain does not seem to be in direct contact with DNA, but might play a regulatory role. The structural similarity of some σ factors gives some explanation to why the simultaneous inactivation of certain σ factors makes *Synechocystis* more susceptible to certain stresses than the inactivation of either σ factor alone.

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Finding the targets of an ultraconserved sequence element in the non-coding RNA Yfr1

Stephanie Hein, Wolfgang R. Hess, Claudia Steglich

Faculty of Biology, University Freiburg, Freiburg, Germany

stephanie.hein@jupiter.uni-freiburg.de

Non-coding RNAs (ncRNA) are important key players in regulation of gene expression. Yfr1 is an abundant ncRNA (size between 54 and 69 nt) with ubiquitous appearance in cyanobacteria except for two low light-adapted strains of *Prochlorococcus*. A feature of Yfr1 is an ultraconserved undecanucleotide (5'-ACUCCUCACAC-3') within an unpaired stretch of sequence flanked by two stem-loop elements [1]. Putative interaction partners for this conserved Yfr1 motif have been predicted in various cyanobacteria based on computational analysis.

Data obtained in a GFP expression based interaction screen in *E. coli* [2] revealed that Yfr1 has a strong impact on the translation of at least one of the predicted targets. These results suggest that Yfr1 acts as a repressor of translation.

Functional assignments have not been performed for the vast majority of cyanobacterial ncRNAs yet. The used screening method is an appropriate way for rapid investigation of RNA-RNA interactions in vivo. The use of *E. coli* as a heterologous host appears particularly advantageous since interference of the foreign ncRNA with the host gene expression machinery can be excluded. We anticipate this approach to be a helpful tool for the functional analysis of ncRNAs in cyanobacteria.

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Photoactive holocomplex assembly of cyanobacteriochrome TePixJ

Takami Ishizuka¹, Ayumi Kamiya², Rei Narikawa¹, Takayuki Kohchi³, Katsuhiko Inomata², Masahiko Ikeuchi¹

¹Department of Life Sciences (Biology), The University of Tokyo, Tokyo, Japan

²Division of Material Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Japan

³Graduate School of Biostudies, Kyoto University, Kyoto, Japan

takami-i@bio.c.u-tokyo.ac.jp

Cyanobacteria harbor many putative GAF-containing photoreceptors that may bind a linear tetrapyrrole as a chromophore (cyanobacteriochrome) in addition to typical phytochromes. One of them, TePixJ of *Thermosynechococcus elongatus* BP-1 is essential for phototaxis. Previously, we reported novel properties of the GAF domain of TePixJ (denoted TePixJ_GAF) that was expressed in *Synechocystis* PCC 6803. Purified TePixJ_GAF showed reversible photoconversion between the 433nm (Pb form) and 531nm-absorbing forms (Pg form). Molecular mass of the chromophore was identical to phycocyanobilin (PCB) but was slightly different in spectral properties [1]. Moreover, we carefully compared TePixJ_GAF with PCB-bearing *Synechocystis* phytochrome Cph1 after denaturation with acidic urea. The spectral properties of TePixJ chromophore were clearly different from those of Cph1 PCB, but were very similar to those of phycoviolobilin (PVB), an isomer of PCB [2]. Here, we report reconstitution of TePixJ_GAF: *in vitro* reconstitution of TePixJ_GAF apoprotein with synthetic PCB and *in vivo* co-expression of TePixJ_GAF apoprotein and PCB in *E. coli* cells. In the early phase of *in vitro* reconstitution, free PCB was covalently incorporated with concomitant accumulation of photoconvertible Pb-like blue-absorbing form. Longer incubation resulted in slow isomerization to PVB. The holocomplex from *in vivo* co-expression included PCB and PVB as chromophore. When this holocomplex was further incubated, additional isomerization from PCB to PVB was detected. These results suggest an assembly intermediate form of photoactive Pb-like PCB-TePixJ_GAF holocomplex and subsequent isomerization from PCB to PVB. Thus, we can conclude that the GAF domain of TePixJ is sufficient for both lyase, isomerase and photoconversion activities. We also discuss the mechanism of the photoconversion between Pb and Pg form.

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Characterization of the amino-terminal region of SphS, a histidine kinase sensing phosphate availability, in *Synechocystis* sp. PCC6803

Satoshi Kimura, Yoshihiro Shiraiwa, Iwane Suzuki

Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki, 305-8572 Japan

s0730467@ipe.tsukuba.ac.jp

In cyanobacteria, a two-component system consisting of a histidine kinase, SphS, and a response regulator, SphR, is involved in the transcriptional activation of the phosphate (Pi)-acquisition system, which includes high-affinity Pi-transporters, alkaline phosphatase (AP), and extracellular nuclease, under Pi-deficient conditions [1, 2]. In addition, SphU also might be involved in the signal sensing since the mutation of SphU constitutively activates the Pi-acquisition system [3]. However, molecular mechanisms for sensing the signals are unknown. According to the analogy with other histidine kinases, the amino-terminal region of SphS might function to sense the signal of Pi availability and it contains a putative transmembrane helix and a presumable PAS domain, suggesting that these domains might play an important role for perception of the signal.

In order to analyze the function of the amino-terminal region of SphS, we constructed mutants, which express genetically modified SphS from its original promoter, and determined effects of the modification on SphS activities by measuring the cellular AP activity. Deletion of the transmembrane helix resulted in loss of the AP activity under the Pi-deficient or Pi-replete conditions. However, when the transmembrane region was substituted with that of Ni-sensing histidine kinase, NrsS [4], the chimeric SphS regulated AP induction as the native SphS does. These results suggested that the transmembrane helix of SphS did not play any special roles on perception of the signal, but it might be crucial to localize the protein on suitable membrane or to activate the kinase domain after stimulation through the other signal-input domain. On the other hand, deletion of the PAS domain resulted in the constitutive induction of AP activity regardless of the Pi-availability, as observed in the mutant of SphU [3]. To characterize the PAS domain more in detail, four amino acid residues conserved in the PAS domain were substituted with alanine. Among the constructed mutants, a mutant (R121A) constitutively expressed the AP activity, suggesting that the arginine residue is particularly important to the function of PAS. Our observations indicated that the presence of any kind of transmembrane helix at the amino-terminal is critical for the activity of SphS and the PAS domain is involved in the signal perception of Pi-deficiency perhaps via an association with SphU.

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Characterization of *ftsZ* in cyanobacteria

Fernando A. Lopes Pinto, Cecilia Blikstad, Mikael Strömmer, Paulo Oliveira, Peter Lindblad

Uppsala University, Department of Photochemistry and Molecular Science, Uppsala, Sweden

fernando.lopespinto@fotomol.uu.se

The transcription of *ftsZ* has been thoroughly investigated in *Escherichia coli* and *Bacillus subtilis*, but for cyanobacteria only a limited amount of information is available.

FtsZ plays a central role in cell division. The transcription of *ftsZ*, including the characterization of the transcript and its regulation, should provide vital information to better understand cyanobacterial cell division. A higher level knowledge of this process could in the future allow for biotechnological applications, like more cost effective hydrogen production.

As a result of our ongoing research, we will introduce novel data for the transcription of *ftsZ* in three cyanobacterial strains: *Synechocystis* sp. PCC 6803, *Nostoc punctiforme* PCC 73102 and *Anabaena* sp. PCC 7120. This data will include Northern, RT-PCR, 5' and 3'RACE results characterizing the transcripts including the genes(s) encoding for FtsZ. We will also present results from his-tag pull-down using FtsZ.

Tools for improved RT and RACE experiments

Fernando A. Lopes Pinto, Håkan Svensson, Jesper Svedberg, Peter Lindblad

Uppsala University, Department of Photochemistry and Molecular Science, Uppsala, Sweden

fernando.lopespinto@fotomol.uu.se

In order to better understand different aspects of metabolism it is important to study the underlying transcriptional profile. RT-PCR is an extremely sensitive tool used extensively for the detection of even very low copy mRNA transcripts.

Unfortunately, a number of obstacles can interfere with this tool especially during the reverse transcription stage: i) DNA contamination of RNA samples, ii) GC rich regions in mRNA, iii) strong mRNA secondary structures, or even iv) the overall experimental design.

In order to avoid the above described obstacles we have: 1) developed bioinformatics tools, 2) designed specific oligos for use with cyanobacteria, 3) adopted and modified more effective RT/RACE methodologies and 4) altered RT buffer chemistry. We will describe the different strategies developed and show the improvements over the standard used protocols.

Redox Regulation of the Ser/Thr Kinase SpkB in the cyanobacterium *Synechocystis* sp. PCC 6803

Alejandro Mata-Cabana, Francisco J. Florencio, Marika Lindahl

Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC – Universidad de Sevilla, Seville, Spain

matacabana@ibvf.csic.es

Thioredoxins are enzymes capable of reducing disulfide bonds of others proteins and, thus, regulate their activity. In order to search for new redox-regulated processes in cyanobacteria we have previously identified potential thioredoxin targets by means of proteomic approaches involving 2-DE SDS-PAGE under non-reducing/reducing conditions [1]. However, there are some proteins of notoriously low abundance, e.g. protein kinases and phosphatases, which cannot be detected by this method. *Synechocystis* sp. PCC 6803 harbours seven genes encoding a subfamily (Pkn2) of eukaryotic-type Ser/Thr kinases. These genes are *slr1575* (SpkA), *slr1697* (SpkB), *slr0599* (SpkC), *slr0776* (SpkD), *slr1443* (SpkE), *slr1225* (SpkF) and *slr0152* (SpkG) [2]. Two of them, SpkB and SpkF, present two Cys motives with two cysteines separated by two or three amino acids [2], which makes them attractive candidates for a possible redox regulation. Initially, we performed experiments to test the influence of reagents that affect the cysteine thiol redox state on the pattern of protein phosphorylation in *Synechocystis*. To this end, we used radioactive labelling by [γ - 32 P]ATP *in vitro* and autoradiography to visualise stable protein phosphorylation and found that low concentrations of Cu $^{2+}$, which promotes disulphide formation, and oxidised glutathione change the phosphorylation patterns quite dramatically. The phosphorylation of a 90 kDa protein, that disappeared specifically upon treatment with μ M concentrations of Cu $^{2+}$, could be recovered following reduction by thioredoxin, raising the possibility that the kinase responsible for this activity might be regulated by thioredoxin. Using knockout mutants of the seven kinases we compared the protein phosphorylation patterns between the wild type and mutant strains. Indeed, the *spkB* mutant displayed a pattern similar to the wild type strain treated with copper and, notably, the phosphorylation of the 90 kDa protein did not occur. Western blot using specific antibodies raised against phosphorylated threonine residues confirmed this result. By cloning and expressing the *spkB* gene in *E. coli* for *in vitro* assays using histones and casein as artificial substrates, we could confirm a redox regulation of its kinase activity. The truncated versions of the kinase lacking one or two of the Cys motives were not active. Currently, we are intending to identify the target protein(s) of the SpkB kinase by means of IEF/SDS-PAGE 2-DE and native-denaturing 2-DE combined with western blot as well as radioactive labelling autoradiography. We are also constructing site-directed mutants for each of the cysteines of the two Cys motives and will test their *in vitro* activities in order to propose a model for its redox regulation.

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Towards genome-wide promoter identification in the model cyanobacteria *Synechocystis* PCC 6803

Jan Mitschke, J. Bantscheff, B. Voss, Claudia Steglich, Wolfgang R. Hess

Faculty of Biology, University Freiburg, Freiburg, Germany

jan.mitschke@biologie.uni-freiburg.de

Transcription, the process whereby RNA copies are made from sections of the DNA genome, is directed by promoter regions. These define the transcription start site (TSS), and also the set of cellular conditions under which the promoter is active and therefore the respective gene is expressed. It appears to be frequent for prokaryotic genes to have several different TSS, which may be active under different conditions. Transcription has been investigated in several bacteria, with *Escherichia coli* being by far the best studied example. A consensus sequence has been established, and is defined by two hexamers, the -10 element and the -35 element. So even while the basic principles of transcription are known, promoters are complex and sequence conservation between families is unsteady. Therefore promoters have proven hard to detect *in silico*, based on the conserved sequence-motifs of other species alone.

Synechocystis PCC 6803 was one of the first prokaryotes and the first cyanobacterium whose genome was completely sequenced (1996) and has developed since then into an important model organism. Hence, the lack of a systematic study of TSS has become a major bottleneck for molecular genetic studies and systems biology.

Since cyanobacteria are clearly distinct from *Escherichia coli* the use of the determined consensus sequences for the -10 and -35 elements from *E. coli* was of no use here.

Due to the mentioned reason we experimentally determined a set of 32 promoter regions in *Synechocystis* PCC 6803 with 5'RACE. This set was the basement for our species specific analysis of the transcriptional start sites in *Synechocystis* PCC 6803. With the experimental dataset a position-specific scoring matrix (PSSM) was calculated. This PSSM enabled us to make a genome-wide scan for putative TSS in intergenic regions. Several 1000s of putative -10 Elements were predicted. Other structures like the -35 element are also under estimation, but are more difficult to classify. The computational algorithm was designed, to be easily adjustable for analysis of other species, and will be made available as a web tool soon.

Petri net modeling of transcriptional dynamics for sugar catabolic genes

Takashi Osanai^{1,2}, Miyuki Azuma², Kan Tanaka², Masahiko Ikeuchi¹

¹*Department of Life Sciences, The University of Tokyo, Tokyo, Japan.*

²*Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan.*

cosanai@mail.ecc.u-tokyo.ac.jp

Recent development of systems biology requires modeling and simulation of biological pathways. Among several architectures, Petri net is one of graphical and mathematical representations for modeling distributing systems. Petri net consists of two types of nodes (places and transitions) and arcs which connect between places and transitions. For the modeling of biological processes based on hybrid functional Petri net, a software "Cell Illustrator" was generated by Dr. Miyano's group (The Univ. Tokyo) and succeeded in modeling biological pathways such as metabolic pathways and gene regulatory networks. Using Cell Illustrator, we here construct a model of transcriptional regulation of sugar catabolic genes in unicellular cyanobacteria. In *Synechocystis* sp. PCC 6803, the mRNA levels of genes for sugar catabolism (especially the oxidative pentose phosphate pathway) were increased by light-to-dark transition depending on a sigma factor SigE [1]. In addition, we recently found that ChlH, an H-subunit of Mg-chelatase for chlorophyll biosynthesis, interacts with SigE and represses its transcriptional activity [2]. Immunoprecipitation analysis revealed that SigE and ChlH interact with each other under light conditions and dissociate under dark conditions. Using these data, we constructed Petri net model of these transcriptional regulations and performed simulation, revealing dynamics of proteins for transcriptional regulation and decoupling of the transcript and protein levels of sugar catabolic genes in *Synechocystis*.

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Group 2 sigma factors SigB and SigD participate in light acclimation in *Synechocystis* sp. PCC 6803

Maija Pollari, Virpi Ruotsalainen, Esa Tyystjärvi, Taina Tyystjärvi

Plant Physiology and Molecular Biology, Department of Biology, University of Turku, 20014 Turku, Finland

maija.pollari@utu.fi

Bacteria acclimate to changes in environmental conditions by changing the gene expression pattern. In eubacteria the control of gene expression occurs mainly at the level of transcription initiation. The RNA polymerase holoenzyme is composed of a catalytically active core and a sigma factor, which recognizes promoter sequences. The switching of one sigma factor to another is a major determinant in changing the gene expression pattern. The cyanobacterium *Synechocystis* sp. PCC 6803 has nine sigma factors. The primary (group 1) sigma factor SigA is essential for cell viability, while group 2 sigma factors SigB, SigC, SigD and SigE are required for specific stress responses. Group 3 is composed of the alternative sigma factors SigF, SigG, SigH and SigI.

Light is a key environmental factor affecting the growth of cyanobacteria. We investigated the roles of the group 2 sigma factors SigB and SigD in acclimation to different light conditions. Under standard light (40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) conditions the inactivation strains ΔSigB , ΔSigD and ΔSigBD grew as well as the control strain. Moderate light (80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) promoted growth in all strains but the ΔSigBD strain was unable to take full advantage of the greater light availability. The effect was similar but less pronounced in the ΔSigD single inactivation strain. SigB and SigD factors are structurally the most similar pair of the *Synechocystis* group 2 sigma factors and may have partial redundancy in their physiological roles [1].

Although light is essential for photosynthesis it also causes photoinhibition. Photoinhibition occurs when the rate of light-induced damage to photosystem II exceeds the rate of repair. Photoinhibition was induced by illuminating the cells with 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. In the ΔSigBD strain the PSII repair cycle did not function as efficiently as in the control strain and the cells were more sensitive to photoinhibition than control cells. Labelling with radioactive methionine showed a lower overall translation activity in the ΔSigBD strain during high light illumination. Western blot analysis revealed that the amount of the D1 reaction centre protein decreased slightly in the ΔSigBD strain during high light illumination while its amount remained constant in the control strain. Next we investigated the expression of the *psbA2* and *psbA3* genes encoding the D1 protein and found that the ΔsigBD strain is unable to enhance the expression of these genes as well as the control strain upon exposure to 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

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Characterization of a tRNA encoding gene cluster in plasmid δ of *Anabaena* (*Nostoc*) sp. PCC7120

Leonor Puerto-Galán, Agustín Vioque

Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla-CSIC, Sevilla, Spain

leonor@ibvf.csic.es

In cyanobacteria, tRNA genes are generally scattered in the genome and are transcribed as individual precursors, with the exception of tRNA^{Tyr} and tRNA^{Thr} that are cotranscribed in a single precursor containing both tRNAs in most strains. The number of tRNA genes oscillates between 37 and 45, and they do not code for the 3'-CCA sequence in almost any case. The CCA sequence is added posttranscriptionally by CCA adding enzymes. In *Anabaena* 7120 there are 49 tRNA genes in the chromosome that in theory would be enough to decode all sense codons. In addition, there is a tRNA gene cluster encoding 19 closely linked tRNA genes in plasmid δ . *Nostoc punctiforme* carries in its chromosome also a cluster of 23 tRNA genes, unrelated to the cluster present in *Anabaena*. In contrast to the general rule, several of the genes present in both clusters do encode the CCA sequence. We have undertaken the characterization of the expression, processing and function of the tRNAs encoded in plasmid δ of *Anabaena* 7120. tRNA genes encoded in plasmid δ are transcribed and correctly processed, as determined by northern blot, primer extension and RT-PCR. *In vitro* transcribed RNA precursors containing several tRNAs are correctly processed by purified RNase P holoenzyme as well as by the RNase P ribozyme. What is more, northern blots in acidic conditions indicate that tRNAs encoded in plasmid δ are aminoacylated *in vivo*. In addition to the 19 well-predicted tRNAs, the cluster contains a possible tRNA^{Glu}(UUC) and a sequence that resembles an aberrant tRNA^{Ser}(GCU) (Int1). Int1 accumulates to high levels, similar to tRNAs, is generated by processing of the flanking tRNAs, and by further processing at the 5' end at a RNase P predicted site, which has been confirmed *in vitro*. Rifampicin experiments indicate that processed Int1 is stable, with a half-life similar to tRNAs. Int1 contains the determinants for recognition by serine tRNA synthetase and it seems to be aminoacylated *in vivo*. In contrast the possible tRNA^{Glu}(UUC) is not aminoacylated. Our results indicate that the tRNA genes present in plasmid δ , spite their redundancy, are expressed and functional. Int1 might be a *bona fide* tRNA^{Ser} used in protein translation, or, taking in account its aberrant structure, it might be involved in another unknown function.

Activity of antisense RNA promoters in *Synechocystis* PCC 6803

Verena Schön, S. Ude, Jens Georg, Wolfgang R. Hess

Faculty of Biology, University Freiburg, Germany

verena.schoen@biologie.uni-freiburg.de

Small regulatory non-coding RNAs control gene expression in a plethora of processes, e.g. stress responses. These non-coding RNAs can be classified into two groups: trans-encoded RNAs and antisense RNAs (asRNA). About the latter there are only few examples published. However, the regulatory role of chromosomally encoded asRNAs in Cyanobacteria may have been underestimated.

Recently, we found several novel asRNAs in *Synechocystis* sp. PCC6803. The 5' ends of these asRNAs have been mapped by 5' RACE and information on these RNAs has been collected by Northern Blot analysis.

To analyse the function and the way of regulation of the asRNAs, we determined the activity of asRNA promoters under different stresses. For this purpose promoter fusions with the reporter *luxAB* were introduced by homologous recombination into *Synechocystis* and activity was tested by luciferase assays. The investigated asRNA are complementary to the RNA and DNA polymerase genes *rpoB* and *dnaX*, the DNA repair gene *uvrA* and the housekeeping gene *accA*. Fusions with promoters of well-characterized protein-coding genes: *isiA*, *psbA* and a novel trans-acting non-coding RNA, 44f, served as control. For negative control we cloned promoter fragments in the reverse orientation.

Promotor activity of the different probes varied from barely detectable to levels comparable with strongly accumulating mRNAs. Several fusions responded to stresses caused by high light, entry into stationary phase or iron limitation. The asRNA to *uvrA* was the only one responding to UV stress.

Contrary to our expectation the promoter of *isiA* (iron-stress-induced protein) showed an even further increased activity during recovery from iron limitation, in addition to its already enhanced activity under iron deficiency. Promoters of the trans-acting RNA 44f and of the asRNA *isrR* turned out to exhibit a very similar pattern of promoter activity after release from iron limitation.

Yfr1 – a small regulatory RNA in *Synechocystis* sp. PCC 6803

Thomas Wallner¹, Dennis Dienst², Annegret Wilde¹

¹Institute for Microbiology and Molecular Biology, Justus-Liebig-University Giessen, Germany

²Institute of Biology, Humboldt-University Berlin, Germany

Thomas.Wallner@mikro.bio.uni-giessen.de

From a comprehensive dataset of computationally predicted and experimentally confirmed small, noncoding RNAs in *Synechocystis* sp. PCC 6803, Yfr1 has been selected for functional investigations on the physiological and molecular level. Here we report on this potentially *trans*-acting ncRNA, which is a highly abundant ncRNA between 54 and 69 nt in size present in most cyanobacteria except for two *Prochlorococcus* strains adapted to extreme low light conditions [1]. The predicted secondary structure displays two stem-loop elements enclosing an unpaired sequence of 16-20 nucleotides containing the ultra conserved undecanucleotide 5'-ACUCCUCACAC-3' [2]. In *Synechococcus elongatus* PCC 6301, the *sbtA* mRNA, encoding a sodium-dependent bicarbonate transporter, was suggested as a putative target of Yfr1 [3]. Since our attempts to create a *yfr1* null mutant in *Synechocystis* were not successful, we presume an essential role for Yfr1 in this cyanobacterium. The Yfr1 RNA is highly expressed in *Synechocystis*, and its cellular amounts do not depend on the presence of the RNA chaperone Hfq. A genetic approach to enhance the abundance of Yfr1 succeeded to only a limited extent. The knock-down *yfr1*-mutant shows considerable growth defects, particularly in regard to high light conditions, whereas investigations of the mutant overexpressing Yfr1 revealed considerable growth alterations under low light conditions. This suggests a possible function of Yfr1 as a regulatory element involved in the adaptation to various light conditions. Since a complete chromosomal segregation of the *yfr1* mutant failed even under photoheterotrophic growth conditions, we actually propose a fundamental role of Yfr1 in general housekeeping functions in *Synechocystis*. This idea is further supported by expression analyses of Yr1 under various applied stress conditions, as its abundance did not appear to be differentially regulated. However, a specific function of Yfr1 during the stationary growth phase of *Synechocystis* is currently under examination and may provide deeper insights into riboregulatory adaptation mechanisms of cyanobacteria

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CELL DIFFERENTIATION

A newly uncovered missing link between *ntcA* and *hetR* for the initiation of heterocyst differentiation

Cheng-Cai Zhang^{1,2}, Jichan Jang¹, and Lei Shi²

¹Laboratoire de Chimie Bactérienne, Aix-Marseille Université, Marseille, France

²Huazhong Agricultural University, Wuhan, China

cczhang@ibsm.cnrs-mrs.fr

In response to deprivation of combined nitrogen, the filamentous cyanobacterium *Anabaena* sp. strain PCC 7120 can form a particular cell type, a heterocyst, in which N₂ fixation takes place [1]. The initiation of heterocyst differentiation depends on the mutual regulation between *ntcA* and *hetR*. The control of the *hetR* expression by NtcA is partly mediated by *nrrA*, but other factors must be involved in this regulation because heterocyst differentiation is only delayed in the *nrrA* mutant. *Anabaena* PCC 7120 has two closely related PP2C-type protein phosphatases, here called PrpJ1 (previously PrpJ) and PrpJ2. We have reported that PrpJ1 was involved in heterocyst maturation since the *prpJ1* mutant arrested heterocyst differentiation at a stage of proheterocyst, and lacked a major heterocyst-specific glycolipid [2]. We will present evidence to show that both PrpJ1 and PrpJ2 have a Mn²⁺-dependent phosphatases activity in vitro. We further demonstrate that whereas *prpJ2* is dispensable for cell growth under different conditions tested, a double mutant with both *prpJ1* and *prpJ2* disrupted did not initiate heterocyst differentiation. Ectopic expression of *hetR* in the double mutant could rescue the failure of the initiation of heterocyst development, but these heterocysts were not mature like those of the *prpJ1* single mutant. The expression of *prpJ2* was enhanced during heterocyst development and this upregulation was directly under the control of NtcA. The upregulation of both *ntcA* and *hetR* was affected in the double mutant. We propose that PrpJ1 and PrpJ2 are required for the mutual regulation of *ntcA* and *hetR*, and thus control the initiation of heterocyst differentiation.

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Heterocyst development and pattern formation in *Anabaena* PCC 7120

M. Ramona Aldea, Rodrigo A. Mella-Herrera, James W. Golden

Department of Biology, Texas A&M University, College Station, Texas, USA

jgolden@tamu.edu

The regulatory mechanisms that govern heterocyst development in *Anabaena* sp. strain PCC 7120 have been continuously refined over the last two decades. In this work, we show that three of the sigma factor genes present in the *Anabaena* sp. strain PCC 7120 genome are developmentally regulated. Time-lapse microscopy of *gfp* reporter strains indicated that expression of *sigC*, *sigG*, and *sigE* is upregulated specifically in differentiating cells at 4 h, 9 h, and 16 h, respectively, after induction of heterocyst development. We proposed that the sigma factors encoded by these genes are involved in regulation of heterocyst genes whose expression is relatively coincident with that of *sigC*, *sigG*, or *sigE*. Indeed, inactivation of the *sigC* gene caused delayed and reduced expression of genes required for the early stages of heterocyst development, and caused delayed development. Inactivation of the *sigE* gene caused a considerable drop in expression of *nifH*, a late gene required for nitrogen fixation. We also provide evidence that c-di-GMP, a novel bacterial second messenger, is involved in regulating heterocyst development. The all2874 gene encodes a bona fide diguanylate cyclase, which synthesizes c-di-GMP, and the gene's inactivation resulted in a decreased tendency to form heterocysts; this phenotype was exacerbated by high light intensity. We hypothesize that the putative operon all2875-all2874 senses and relays information about light conditions and this information is integrated into the decision to form heterocysts. Finally, we identified the all0187 gene, which is up regulated in heterocysts at 9 h, a time when cells that have initiated differentiation commit to complete the process. In nitrogen-free medium, all0187 mutant filaments formed abnormally long heterocysts and were unable to grow diazotrophically. Septum formation between heterocysts and their flanking vegetative cells was incomplete, leaving one or both poles of the heterocysts more opened and potentially more permeable to oxygen. Despite having nitrogenase activity, the all0187 mutant was unable to grow diazotrophically. We hypothesize that the diazotrophic growth defect is caused by the inability of the heterocysts to transport fixed nitrogen to the neighboring vegetative cells.

Complex differentiation events are required to maintain the cyanobacterial-*Azolla* symbiosis

Birgitta Bergman¹, John Larsson¹, Theoden Vigil-Stenman¹, Karolina Bauer, Liang Ran¹, Wei Wen Zheng²

¹Department of Botany, Stockholm University, SE-10691 Stockholm, Sweden

²Biotechnology Center, Fujian Academy of Agricultural Sciences, Fuzhou, China, 350003

bergmanb@botan.su.se

In spite of their abundance and importance, only a limited number of cyanobacteria form stable nitrogen-fixing symbioses with diverse eukaryotes, such as plants [1]. For instance, in the small free-floating water-fern *Azolla*, a nitrogen-fixing filamentous cyanobacterium (cyanobiont) inhabits, extracellularly, leaf cavities found in the pigmented dorsal leaves throughout the plant body [2]. The symbiosis shows a pronounced intimacy between the partners and is the only perpetual N₂-fixing symbiosis, i.e. the cyanobiont is vertically transmitted (in the sporocarps) between plant generations; and the cyanobiont seems incapable of independent growth. The cyanobiont in *Azolla* undergoes several cell developmental processes during colonization of new cavities and during the reproduction of the symbiosis, including differentiation of hormogonia (motile filaments), heterocysts (nitrogen-fixing entities) and akinetes (spores). Proteomic profiles [3] and other data (see posters from my lab at Stockholm University) demonstrate that complex developmental and adaptive mechanisms of the cyanobacterium are prerequisites, e.g. including metabolic adjustments and endoreduplication of their DNA. This points to a highly coordinated co-evolution between the symbiotic partners, possibly dictated by the plant or the plant environment offered. Potentially this intimate interaction is on its way to generate (the first) nitrogen-fixing plant. The genome of the cyanobiont is currently being sequenced (DOE/JGI, USA).

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The akinete expressed transporter (*Aet*) gene can be used a specific akinete marker

Assaf Sukenik¹, Ruth N. Kaplan-Levy¹, Michael Kube², Nechama Malinsky-Rushansky¹, Yehudit Viner-Mozzini¹, Richard Reinhardt², Ora Hadas¹

¹Israel Oceanographic and Limnological Research, The Yigal Allon Kinneret Limnological laboratory, P. O. Box 447, Migdal, 14950 Israel

²Max Planck Institute for Molecular Genetics, Ihnestrasse 64-73, D-14195 Berlin, Germany

assaf@ocean.org.il

Vegetative cells of many filamentous cyanobacteria from the order Nostocales and Stigonematales can differentiate into encysted spores called akinetes. Akinetes provide the capacity for germination after long-term exposure to stresses such as cold, desiccation, and phosphate limitation. Matured akinetes differ from vegetative cells physiologically and functionally. They have a thicker cell wall; they accumulate large amounts of nucleic acids, store nitrogen in the form of cyanophycin they lose most of their photosynthetic capabilities and maintain residual metabolic activity. In order to facilitate genetic analysis of akinete differentiation in *Anabaena variabilis* a specific gene marker for developing or mature akinetes (*AvaK*) was identified [1]. The homologue of *AvaK*, *AoaK* was cloned and characterized from the toxic cyanobacterium *Aphanizomenon ovalisporum* isolated from the freshwater Lake Kinneret (Sea of Galilee, Israel). The *AoaK* gene was found to be primarily expressed in akinetes, with transcript levels enhanced as the number of akinetes increased and they reached maturity. We have recently identified and characterized an additional specific akinete marker gene *Aet*, from *A. ovalisporum*. *Aet* - akinete expressed transporter gene was differentially expressed in isolated mature akinetes as previously described for *Nostoc punctiforme* [2]. While the functional role of *AoaK* remained unknown the *Aet* contains an ATP-binding cassette (ABC)-type transporter, homologous to multidrug transporters that export hydrophobic compounds and components of lipopolysaccharides. Based in its deduced primary structure, *Aet* is similar to *MsbA* from *Escherichia coli* which is responsible for the transport of lipopolysaccharides to the outer membrane. Thus the cellular location of the protein encoded by the akinete gene-marker *Aet* could be in the akinete envelope layers.

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The regulation of diazocyte differentiation and the role of flavodoxins in *Trichodesmium*

Gustaf Sandh¹, Senjie Lin², Edward J. Carpenter³, Liang Ran¹, Birgitta Bergman¹

¹Department of Botany, Stockholm University, SE-10691 Stockholm, Sweden

²Department of Marine Sciences, University of Connecticut, Groton, USA

³Romberg Tiburon Center, San Francisco State University, Tiburon, USA

sandh@botan.su.se

The marine filamentous diazotrophic cyanobacterial genus *Trichodesmium* is an important contributor of fixed C and N in tropical and sub-tropical oceans. Although limited by the availability of iron in many of their natural environments a decrease in flavodoxin levels was apparent in iron deprived cultures of *Trichodesmium* IMS101. This goes against the current notion that flavodoxin in many phototrophic organisms, including some cyanobacteria, replaces ferredoxin under iron limiting conditions. In addition, two highly diverse flavodoxin genes were found and the transcription of the two *fld* forms reacted differently depending on nitrogen source, indicating a functional differentiation between the two forms.

Although being non-heterocystous, *Trichodesmium* spp. are able to fix nitrogen during the day. A striking feature related to this phenomenon is its nitrogen-fixing strategy with nitrogenase being localized in series of cells, diazocytes, within the filaments [1, 2]. Several specific phenotypic features have been attributed to the diazocytes, such as the exclusive localization of the nitrogenase enzyme, higher cytochrome oxidase levels and considerably fewer intracellular storage granules and gas vacuoles [1, 2, 3]. Diazocytes frequencies varied during a diurnal cycle, with highest frequencies during the day in cultures of *Trichodesmium*. These diurnal changes may be a reflection of the diurnal variations in cell division detected as indicated by data obtained following gene expression and cell specific division patterns (in vegetative cells and diazocytes) and variations in diazocyte abundance over a diurnal cycle. Moreover, our data implicated that the heterocyst regulator protein HetR may be involved in regulating diazocyte differentiation, as it co-expressed with the cell division gene *ftsZ*, and the expression of these genes was in turn followed by an increase in diazocyte abundance.

Ongoing efforts to better understand development and function of diazocytes include the production and use of an antibody against *Trichodesmium* HetR to in detail follow its localization in time and space; the use of comparative proteomics during induction and repression of diazocyte differentiation and cell type specific analysis using Laser Capture Microdissection, followed by liquid chromatography-tandem mass spectrometry, to reveal the diazocyte proteome.

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Small Non-Coding RNAs Are Involved in the Response to Nitrogen Depletion in the Filamentous Cyanobacterium *Anabaena* PCC 7120

Danny Ionescu^{1,2}, Bjoern Voss³, Aharon Oren², Wolfgang R. Hess³

¹The School of Marine Sciences, The Ruppin Academic Center, Michmoret, Israel.

²The Hebrew University of Jerusalem, Dept. of Plant and Environmental Sciences, Jerusalem, Israel.

³University of Freiburg, Faculty of Biology, Experimental Bioinformatics, Freiburg, Germany,

danny.ionescu@mail.huji.ac.il

Non-coding RNAs (ncRNAs) have been shown to be part of regulatory mechanisms in all three domains of life. Due to lack of data and lack of conservation, identification of these molecules can be very difficult. We have scanned the genome of the filamentous cyanobacterium *Anabaena* PCC 7120 for potential ncRNAs by predicting Rho-Independent transcriptional terminators within intergenic regions and by a comparative genomics approach. As the process of nitrogen fixation in heterocystous cyanobacteria requires numerous regulatory actions, we have focused on ncRNAs potentially involved in this process. For this purpose we have analyzed the expression of ncRNAs encoded in the vicinity of the different nitrogenase and heterocyst differentiation genes. Using 5' RACE (Rapid Amplification of cDNA Ends) and northern analysis we show the expression of several ncRNAs. Among these, one RNA molecule was significantly over-expressed in the absence of combined nitrogen sources and repressed upon its addition, the reason why we suggest this ncRNA tentatively to be called NsiR1 for nitrogen stress induced RNA 1. This ~60 nt ncRNA is part of a larger construct consisting of seven 133 nt repeats upstream of *hetF*. Similar sequence patterns are found at the same location in the genomes of *Anabaena variabilis*, *Nostoc punctiforme* and *Nodularia spumigena*, including some highly conserved regions. In the case of *Anabaena* PCC 7120, six of these repeats are further arranged in 3 larger repeats of 266 nt. The expression of these 266 nt repeats decreases upon nitrogen depletion, while the expression of NsiR1 increases. This suggests that the larger sequences may serve as precursors from which the final 60 nt small ncRNA is derived when needed. This is the first report of a bacterial ncRNA to be processed depending on nitrogen availability. Two point mutated strains, one in the *hetR* gene and one in the *ntcA* gene were tested for the expression pattern of NsiR1. A weak increase in expression was found in the *ntcA* mutant, whereas no expression at all was found in the *hetR* mutant. This suggests NsiR1 to belong to the HetR regulon. Since HetR is the major factor in the cell fate decision process leading to heterocyst differentiation, NsiR1 may be a novel component in this process. The role of this small ncRNA in the response to nitrogen depletion in *Anabaena* PCC 7120 is still unknown but its occurrence in several different species, the complexity of its arrangement and expression pattern suggest it may have an important function.

The thermophilic cyanobacteria of Zerka Ma'in, Jordan - diversity and nitrogen fixation

Danny Ionescu^{1,2}, Aharon Oren², Orly Levitan³, Muna Hindiyeh⁴, Hanan Malkawi⁵, Ilana Berman-Frank³

¹*The School of Marine Sciences, The Ruppin Academic Center, Michmoret, Israel*

²*The Hebrew University of Jerusalem, Dept. of Plant and Environmental Sciences, Jerusalem, Israel*

³*Bar Ilan University, Mina and Everard Goodman Faculty of Life Sciences, Ramat Gan, Israel*

⁴*Jordan University of Science and Technology, Irbid, Jordan*

⁵*Yarmouk University, Department of Biological Sciences, Irbid, Jordan*

danny.ionescu@mail.huji.ac.il

Thermal springs with waters up to 74 °C (the upper limit of photosynthesis) are often densely inhabited by cyanobacteria. We examined the cyanobacterial diversity of a series of thermal springs located in the mountains on the eastern shore of the Dead Sea. The Zerka Ma'in springs are characterized by temperatures between 51-63 °C, low sulfide concentrations, and very low concentrations of combined nitrogen. The cyanobacterial diversity of the springs has been assessed microscopically, as well as by culture-dependent and culture-independent 16S rRNA-based phylogenetic analysis including sequencing and denaturing gradient gel electrophoresis. The latter showed a greater diversity than previously estimated. One cultured *Mastigocladus*-like isolate from these springs acclimated to temperatures up to 53 °C and fixed atmospheric nitrogen with an optimum at 45 °C. Nitrogen fixation by this heterocystous cyanobacterium is preceded by a morphological change. Following nitrogen depletion, the rate of nitrogen fixation peaked and decreased to zero in a cycle of 6 hours that was not affected by natural or artificially induced light/dark periods. Quantitative PCR analysis showed a similar pattern in the expression of the genes for dinitrogenase reductase, *nifH*, and the circadian clock protein, *kaiC*. In addition we have noticed that the general cyanobacterial small non-coding RNA YFR1 is over-expressed upon initial nitrogen depletion and remains the same throughout the 96 hours experimental procedure. Oxygen concentration measurements performed under constant light conditions over a period of 96 hours in a nitrogen depleted culture showed an initial decrease in dissolved oxygen followed by a constant level. This is in contrast to a constantly increasing concentration in dissolved oxygen in a similarly grown culture in a nitrate containing medium. Our observations in relation to nitrogen fixation in thermophilic filamentous cyanobacteria have not been previously reported and require further investigation.

Dormancy in cyanobacteria – the formation and maturation of akinetes

Ruth N. Kaplan-Levy¹, Michael Kube², Nechama Malinsky-Rushansky¹, Yehudit Viner-Mozzini¹, Richard Reinhardt², Ora Hadas¹ and Assaf Sukenik¹

¹Israel Oceanographic and Limnological Research, The Yigal Allon Kinneret Limnological laboratory, P. O. Box 447, Migdal, 14950 Israel

²Max Planck Institute for Molecular Genetics, Ihnestrasse 64-73, D-14195 Berlin, Germany

ruth@ocean.org.il

The toxic cyanobacterium *Aphanizomenon ovalisporum* (Nostocales) invaded the freshwater Lake Kinneret (Sea of Galilee, Israel), in 1994. Under stress conditions, this filamentous cyanobacterium produces dormant cells, akinetes which survive harsh conditions, reside in the lake's sediments and germinate when favorable conditions reoccur. In this study we investigated cellular processes that occur during the differentiation, formation and maturation of akinetes. Under laboratory conditions, akinete formation in *A. ovalisporum* is triggered by depletion of potassium [1]. Following this physiological induction, akinetes differentiate from vegetative cells in an unsynchronized manner going through several developmental stages to reach filament-free matured forms. Matured akinetes differ from vegetative cells physiologically and functionally. They have a thicker cell wall; they accumulate large amounts of nucleic acids, store nitrogen in the form of cyanophycin and they lose most of their photosynthetic capabilities. However, protein and spectra analyses showed that both photosystems (PSI and PSII) existed in mature akinetes whereas the phycobilisome antennae proteins were degraded [1] by NblA (Non-Bleaching A, Phycobilisome degradation protein). This process is supported by increasing expression levels of *NblA* transcript during akinetes development, with a maximum level reached in mature, filament-free, isolated akinetes. Genes that are associated with cell protection from reactive oxygen species were found to be upregulated during akinete differentiation and maturation. The expression of superoxide dismutase (*SOD*) and glutathione reductase (*GRX*) increased during the akinetes differentiation process. In both cases, transcript levels varied in a similar manner as recorded for *NblA*, with a maximum level measured in mature, isolated akinetes. The ecological success of akinetes is measured by their capacity to germinate when environmental conditions improve. Migration of sunken akinetes from the deep dark sediment water interface to the photic zone is assured by the development of gas vesicles. We postulate that the potency of this process is maintained during akinete formation and in matured akinetes by the constitutive expression of *GvpF* (Gas Vesicle Protein F), a gene involved in gas vesicle organization and biogenesis.

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Developmental adaptations in the cyanobacterial symbiont of *Azolla filiculoides* (a water-fern)

John Larsson, Theoden Vigil-Stenman, Birgitta Bergman

Department of Botany, Stockholm University, SE-10691 Stockholm, Sweden

John.Larsson@botan.su.se

The cyanobacterial symbiont (cyanobiont) residing in *Azolla filiculoides* leaf cavities was isolated from the apex to mature and senescing plant parts and examined by light- and fluorescence microscopy (Zeiss Axiovert 200). Cyanobiont cell differentiation, cell division, cell size, and DNA content was measured along the host developmental gradient in hormogonia, vegetative cells and heterocysts. The fluorochrome DAPI was used to stain DNA of cyanobiont cells and measurements of fluorescence intensities were made on digital images using the AxioVision digital imaging software.

As expected, the frequency of heterocysts increased rapidly, from 0% (in hormogonia) to around 25%, along the developmental gradient of the host main stem. Cyanobiont cell division frequency was highest at the apex where 16% of the vegetative cells were dividing, while it declined to 2% within 10 leaves from the apex (the whole plant represents 30-40 leaves). The vegetative cell area increased by 68% from the apex to the basal leaf cavities, and was primarily due to an increase in cell length. Estimates of vegetative cell volume showed an increase by 55% and the DNA content increased by 42% along the same gradient. In contrast, the heterocyst cell size and DNA content showed no obvious trend along the host developmental gradient, most likely reflecting the terminally differentiated state of this cell type [1]. Cell differentiation and cell division rates were highest in the very early stages of the host developmental gradient. The increase in vegetative cell size and DNA content together with a decrease in cell division points to endoreduplication in vegetative cells of the *Azolla* cyanobiont. This could be imposed by the plant host on vegetative cell mitosis and cytokinesis (M phase) in order to prevent the cyanobiont from outgrowing the plant while allowing genome replication (S phase) and cell elongation to occur. Also, polyploidy is known to stimulate differentiation and metabolic activities. A eukaryotic control on the bacterial cell cycle has recently been proposed for the *Rhizobium*-legume symbiosis [2]. High molecular weight genomic DNA was recently isolated from purified *Azolla* cyanobiont and is currently being sequenced (DOE/JGI, USA). The genomic sequence will help to shed light on the complex phenotypic changes, and underpinning regulatory mechanisms, typical for the cyanobiont in this highly specific symbiotic environment.

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The Azolla cyanobiont: an efficient nitrogen-fixing 'plastid' to be?

Liang Ran, Theoden Vigil-Stenman, Karolina Bauer, John Larsson, Birgitta Bergman

Department of Botany, Stockholm University, SE-10691 Stockholm, Sweden

liang@botan.su.se

The symbiosis between cyanobacteria and the small (~5 cm) water-fern Azolla, has attracted considerable attention due to its nitrogen fixing capacity (biofertilizer) as well as to the unique behavior of the cyanobiont 'inoculum' which is transferred to the next plant generation through the plant reproductive organ, the sporocarp [1]. It is apparent that the development of the cyanobionts, harbored extracellularly in Azolla leaf cavities, is highly adapted to their role as providers of fixed nitrogen to the host plant. This is reflected in a high heterocyst frequency, and in the large quantities of the nitrogen fixing enzyme nitrogenase [2].

A light dependent nitrogenase activity (acetylene reduction-GC assay) was found in diurnal assays of the Azolla filiculoides plants, used as our symbiotic model systems, with about 10% of the highest nitrogenase activity occurring in the dark. This stresses the importance of the cyanobiont as the main nitrogen-fixer in spite of the presence of other bacteria in the Azolla leaf cavities. The Azolla plants were also separated into sections representing different plant developmental stages, and the cyanobionts isolated. The presence and relative levels of several cyanobacterial proteins (e.g. NifH, FtsZ, HetR) were estimated using SDS-PAGE and western blotting. The most dramatic changes in cyanobacterial protein expression levels occurred at the plant apex, which was also reflected in morphological developments. For instance, the nitrogenase activity increased along the plant gradient (<20th leaf cavity) but leveled off in older parts. The nitrogenase enzyme levels also increased with plant age, and were quantitatively abundant in older parts. In contrast, the highest specific nitrogenase activity (expressed per nitrogenase protein level) occurred at the plant apex (within the first 1mm of the stem) with very low levels of nitrogenase. This suggests that the most optimal nitrogenase activity conditions are offered at the plant apex, the potentially most nitrogen demanding (rapidly growing) plant part. Preliminary 2-D gel electrophoresis-based western blotting revealed two copies of the NifH protein; with the second copy possibly representing yet another nitrogen-fixing bacterial symbiont(s) present in the symbiosis. The molecular identification of the NifH copies using LC-MS/MS, and qRT-PCR analyses coupled to sequencing are in progress. The ongoing sequencing of the genome (DOE/JGI) of the Azolla filiculoides cyanobiont will no doubt help resolve these and other important questions related to this symbiosis.

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TolC like outer membrane protein HgdD (Alr2887) is necessary for heterocyst envelope formation in *Anabaena* sp. PCC 7120

Iris Maldener¹, Fadi Al Dehni², Peter Staron¹

¹*Microbiology/Organismic Interactions, EK University, Tübingen, Germany*

²*Plant Physiology & Cell Biology, University Regensburg; present: Department of Physiology, University Regensburg, Germany*

iris.maldener@uni-tuebingen.de

To protect nitrogenase from external oxygen, heterocysts possess a special envelope comprising an external layer of polysaccharides and an innermost layer of glycolipids (HGL). The HGL layer is deposited on top of the outer membrane, after the polysaccharide layer has been formed. Synthesis of the HGLs starts early in heterocyst differentiation, around 3 h. However the laminated layer is not visible before 14 hours. The polysaccharide layer appears as early as 6 hours, and is a prerequisite for HGL deposition. Proteins involved in export and assembly of HGLs outside the outer membrane have to be made or activated during the differentiation process. The previously identified DevBCA ABC-type transporter, which is expressed after 6 hours in developing heterocysts fits well in this time scale [1,2]. It is known from mutant analysis that DevBCA is necessary for deposition of the heterocyst specific glycolipids. The mutants show synthesis of HGLs but no deposition. Therefore the *devBCA* mutants are not able to grow without a source of bound nitrogen. Sequence analysis of the subunits of DevBCA shows highest similarity to protein exporter of gram-bacteria, like the haemolysin exporter of *E. coli* [1]. It was suggested that DevBCA is the exporter of the glycolipids or of a protein involved in assembly of the layer.

If this DevBCA substrate is supposed to traverse the outer membrane, the adaptor protein DevB has to interact with an TolC like outer membrane channel. By transposon mutagenesis, this putative "missing link" of the DevBCA exporter complex has been found, which showed the same phenotype as the mutants lacking *devA*, *devB* or *devC*. The transposon had inserted into gene *alr2887*, whose deduced amino acid sequence and topology shows homology to TolC [3]. The gene is expressed in cells grown on NO₃ and expression transiently increases during the first 9 hours after nitrogen withdrawal [3,4]. A mutant lacking the transcription factor NtcA does not show the elevated expression. Expression of *alr2887* stays high in a mutant in regulatory protein HetR. In contrast transcription of the *devBCA* operon is NtcA and HetR dependent [2]. Site-directed mutagenesis of *alr2887* and complementation of the mutant confirmed the initial phenotype. Ultrastructure of the mutants shows that the HGL layer of heterocysts is not present, however the HGLs can be detected in lipid extracts of the filaments and isolated heterocysts [4]. This is exactly the phenotype described earlier for *devA* mutants. Therefore we named the gene *hgdD*, for heterocyst glycolipid deposition gene D (nomenclature of C.P. Wolk). Currently we investigate the co-localization of the subunits of the entire export machinery and the interaction of the subunits in vitro and in vivo.

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Two conduits for cellular communication in the filamentous cyanobacterium *Anabaena* sp. PCC 7120

Vicente Mariscal, Victoria Merino-Puerto, Rafael Pernil, Conrad Mullineaux*, Antonia Herrero, Enrique Flores

Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas and Universidad de Sevilla, Sevilla, Spain

**School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London, UK*

vicente.mariscal@ibvf.csic.es

The filamentous, heterocyst-forming cyanobacterium *Anabaena* sp. PCC 7120 is a simple multicellular prokaryote. In the absence of combined nitrogen, two different cell types that are mutually dependent are present in the filament: the vegetative cells that supply carbon compounds to the heterocysts and the heterocysts that supply combined nitrogen to the vegetative cells. We investigate the conduits that might be involved in the transference of metabolites and regulatory compounds between heterocysts and vegetative cells. Two possible ways can be considered: 1) the continuous periplasmic space that surrounds the cells in the filament [1] and 2) septal channels that might allow direct transference of small molecules. In order to know if the periplasm of *Anabaena* is functionally continuous, we constructed different altered versions of the gene encoding the green fluorescent protein (GFP) placed under the promoter of the *patS* gene, which is active specifically in developing proheterocysts of *Anabaena* sp. PCC 7120. When the GFP was anchored to the cytoplasmic membrane, it was observed in the periphery of the producing proheterocysts but not in adjacent vegetative cells. This result shows that there is no cytoplasmic membrane continuity between the different cells of the same filament. However, when the GFP was exported to the periplasm of the proheterocysts through the twin-arginine translocation system it was observed also in the periphery of the neighboring vegetative cells. This result shows that the GFP protein can move along the filament through the periplasm, which is functionally continuous and therefore provides a conduit that can be used for chemical communication between cells [2]. On the other hand, we have recently shown that calcein (a small, charged molecule) can be directly exchanged between *Anabaena* cells [3]. We have characterized SepJ as a candidate for a septal channel-forming protein involved in calcein transfer. SepJ is a multidomain protein whose N-terminal region is predicted to be periplasmic and might participate in cell-to-cell anchoring, and whose C-terminal domain resembles export permeases. Using a GFP translational fusion to the carboxyl terminus of SepJ, we have found that during cell division, this protein is localized in a ring whose position is similar to that of a Z ring. In mature heterocysts and vegetative cells, the protein is localized in the cytoplasmic membrane at the intercellular septa [4]. Mutation of *sepJ* produces filament fragmentation and a Fix⁻ phenotype, and additionally impedes calcein transference between cells [3, 4]. We have recently characterized two more genes, *fraC* (originally identified by Bauer et al. [5]) and *fraD*, whose mutation produces filament fragmentation. Mutants of these genes do not localize the SepJ protein at the cell poles and are impaired in calcein transfer. These results suggest that the calcein-transferring channel might be a multiprotein complex.

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Reversible inactivation of cyanobacterial GSI. Mutational analysis of the inactivating factors (IFs)/GSI interaction

Carla V. Galmozzi, Lorena Saelices, Javier Florencio, M. Isabel Muro-Pastor
Instituto de Bioquímica Vegetal y Fotosíntesis CSIC-US, Sevilla, Spain

imuro@ibvf.csic.es

Ammonium assimilation through the glutamine synthetase and glutamate synthase (GS-GOGAT) pathway is tightly regulated in cyanobacteria as in many other organisms. GS is the target of different regulatory mechanisms at transcriptional and posttranscriptional levels, depending on carbon and nitrogen supply. We have studied in detail GS regulation in the cyanobacterium *Synechocystis* sp. PCC 6803 which contains two different types of GS, GSI and GSIII. The reversible mechanism responsible for GSI posttranscriptional regulation implicates the action of two small proteins, named IF7 and IF17 (IFs), that inhibit GSI activity by direct protein-protein interaction [1]. The process of GS reactivation involves IFs degradation. The involvement of some soluble metalloproteases in IF7 degradation has been demonstrated and the crucial role of the target protein GSI for *in vivo* IFs stability has been established [2]. The inactivating factors are highly basic homologous proteins. In order to characterize the interaction between GSI and IFs, we tried to identify the amino acid residues responsible for this interaction. For this purpose, we undertook a site-directed mutagenesis study on IF7 and IF17 residues conserved between these two proteins and other cyanobacterial homologous proteins. We found that three conserved arginine residues from IF17 (R90, R103 and R110) are critical for the ability of IF17 to inactivate GSI either *in vitro* or *in vivo*. Mutations that change one of these arginine residues by a glutamic acid residue severely affect IF17 function. Taking into account that IFs homologous are present in several cyanobacterial strains, we investigated the conservation of the IFs-mediated GS regulatory mechanism in the filamentous cyanobacterium *Anabaena* sp. PCC 7120. We found that the system is conserved in this filamentous cyanobacterium.

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Transcriptional regulation of the *ntcA* promoter during heterocyst differentiation in *Anabaena* sp. PCC 7120

Elvira Olmedo-Verd, Ana Valladares, Antonia Herrero, Enrique Flores and Alicia Muro-Pastor

Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Avda. Américo Vespucio 49, E-41092, Seville, Spain.

eoimedo@ibvf.csic.es

Heterocyst differentiation in *Anabaena* sp. PCC 7120, which takes place from vegetative cells under nitrogen deprivation conditions, depends on both the global N-control transcription factor NtcA and the cell-differentiation regulatory protein HetR, with the induction of both genes being mutually dependent. NtcA also directly activates the expression of genes whose products are required during heterocysts maturation or for the function of mature heterocysts. *ntcA* is an autoregulatory gene that is transcribed from a complex promoter region that includes two sites for NtcA binding, a constitutive promoter (P_2) and two promoters that are induced upon N step-down (P_1 and P_3). With the aim of analyzing the regulation of the expression of *ntcA* during heterocyst differentiation, we constructed strains that bear a translational fusion of the complete or modified versions (including deletions and point mutations) of the *ntcA* promoter region to *gfp*. Induction of *ntcA-gfp* takes place at especially high levels in the developing heterocyst suggesting that high levels of NtcA protein are present in cells that are undergoing differentiation. Induction of transcription of *ntcA* in developing cells takes place from promoters P_1+P_3 . Induction of expression of P_1 depends on NtcA binding to a site centered at -103.5 from the translational start site. Additionally, there are two cis-acting elements, a positive one that includes the sequences upstream from P_3 and a negative one (sequence from -182 to -143) that regulates the expression from P_1 in proheterocysts. Our results are consistent with the notion that transcription of complex promoters that are expressed both in vegetative cells and heterocysts, such as that of the *ntcA* gene, might take place *via* different transcriptional settings in different cell types.

Chemical probes to study the signaling role of 2-oxoglutaric acid, a key Krebs cycle intermediate in cyanobacterium

Xinjun Liu^{1,2}, Han Chen², Sophie Laurent³, Sylvie Bedu³, Fabio Ziarelli⁴, Cheng-Cai Zhang³, Ling Peng^{1,2}

¹CNRS UMR 6114, Département de chimie, 163 avenue de Luminy, 13288 Marseille, France

²College of Chemistry and Molecular Sciences, Wuhan University, P. R. China

³LCB CNRS UPR 9043, Marseille, France

⁴Spectropole, Universités Aix-Marseille I et III, Marseille, France

ling.peng@univmed.fr

2-Oxoglutaric acid (2-OG), a key Krebs cycle intermediate, provides carbon skeleton for nitrogen assimilation [1]. It has been proposed as a signal to coordinate C/N metabolisms in plants and bacteria, and recently as a ligand for G-protein coupled receptors [2] as well as a substrate for a fat mass and obesity associated protein (Fto) [3], linking metabolism to blood pressure and obesity. Most of these studies have been carried out *in vitro*, and the signalling function of 2-OG is difficult to establish *in vivo* because it is rapidly metabolised. Recently, we have synthesized a series of nonmetabolizable analogues of 2-OG (Scheme 1), which can be traced *in vivo* by High-Resolution Magic Angle Spinning (HRMAS) NMR. These approaches, combined with techniques of genetics and molecular biology enabled us to obtain the first *in vivo* evidence showing that the level of 2-OG constitutes the signal of nitrogen starvation in the cyanobacterium *Anabaena* PCC 7120, leading to the differentiation of heterocyst for nitrogen fixation [4,5]. To better understand the molecular mechanism in response to nitrogen starvation, and the coordination of the metabolic networks according to the physiology and growth conditions, we propose to develop various chemical and photolabeling probes to identify 2-OG receptors and study the interaction between 2-OG and its putative receptors as well as their modulation of the metabolic networks by photolabelling, NMR and biochemical approaches. Structure/activity relationship study of these probes may also provide useful instruction to develop therapeutic means to treat 2-OG related problem of blood pressure and obesity.

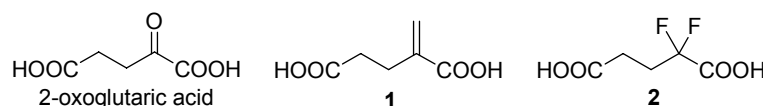
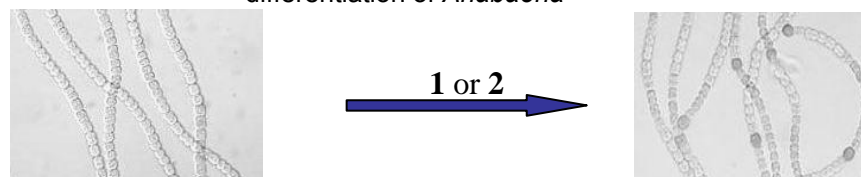


Figure 1: The non-metabolizable analogues of 2-oxoglutaric acid, **1** and **2**, trigger cell differentiation of *Anabaena*



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Identification of akinete-expressed genes by microarray and GFP-reporter analysis

Peter Holmquist, Elsie Campbell, Jack Meeks, Michael Summers

Department of Biology, California State University Northridge, Northridge, California, USA

michael.l.summers@csun.edu

Akinetes are spore-like cells that differentiate from vegetative cells of certain filamentous cyanobacteria. This cell type is resistant to desiccation and cold that kills undifferentiated vegetative cells. Akinetes are visually larger and more granular than vegetative cells, and first appear midway between heterocysts in *N. punctiforme*, with subsequent differentiation of adjacent cells over time [1]. In culture containing combined nitrogen, akinetes form randomly throughout the filament. Akinetes form in old cultures, and in response to low light and phosphate limitation, but are also synchronously induced in a *zwf* mutant lacking the first enzyme of the oxidative pentose phosphate pathway when placed in the dark with exogenous fructose [2]. The *zwf* akinetes were resistant to desiccation, cold, and lysozyme digestion by day 6, with partial resistance developing by day 3 following akinete induction. A whole genome DNA microarray was used to assess gene expression in the *zwf* strain each day for 6 days relative to initial expression. Thirty-seven up-regulated genes were targeted for further study. These included genes encoding proteins with similarity to the following: 18 genes containing 2-component regulatory system components, 9 DNA-binding regulatory proteins, 2 sigma factors, 2 anti-sigma factors (one adjacent to one of the sigma factors), 1 phage-shock transcriptional regulatory protein, 1 putative ribosomal inhibitor protein, 1 heat-shock protein, 1 transporter and 2 proteins of unknown function. To date all 37 genes have 1) transcriptional start sites mapped using RACE, 2) un-mutated promoter-bearing PCR fragment cloned into the GFP transcriptional reporter plasmid pSUN119 [3] confirmed by sequencing, and 3) reporter strains made via electroporation into *Nostoc punctiforme*. Akinetes have been induced in 15 of the 37 reporter strains, and 12 of these have been documented to show induction in akinetes relative to neighboring vegetative cells of the filament. Together these results validate use of the *zwf* model system for identification of akinete-related gene expression.

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Identification of two types of glutaminase in cyanobacteria

Jie Zhou^{*}, Junxia Zhou

Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

**present address: Center of Bio-energy and Industrial Biotechnology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China*

jiezhouw@im.ac.cn

Glutaminase is widely distributed among microorganisms and mammals with important physiological functions. Little is known regarding the biochemical properties and functions of the deamidating enzyme glutaminase in cyanobacteria. In this study the putative glutaminase genes *slr2079*, *all2934* and *all4774* were cloned from *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120, respectively. These three genes were overexpressed in *Escherichia coli* as histidine-tagged fusion protein, respectively. Glutaminase activity assay and kinetic analysis of recombinant protein Slr2079, All2934 and All4774 demonstrated that these proteins all possessed glutaminase activity using glutamine as sole substrate. But the enzyme characterization was different among these recombinant proteins, especially All2934. The optimal pH of Slr2079 and All4774 was basic, while the optimal pH of All2934 was acidic. Slr2079 and All4774 glutaminase activity had the same optimum temperature of 37-40°C, whereas All2934 was found to be optimally activity at temperature of 20°C. Among the three proteins All2934 had the lowest K_m value and the highest V_{max} . Most interestingly, All2934 was phosphate-activated glutaminase, while Slr2079 and All4774 were phosphate-independent. These data indicate that there are two types of glutaminase in cyanobacteria. One is phosphate-independent glutaminase, Slr2079 and All4774, possessing weak enzyme activity; another is phosphate-activated glutaminase, All2934, possessing strong glutaminase activity. As Slr2079 is from non-nitrogen fixing cyanobacterium *Synechocystis* sp. PCC 6803, while All4774 and All2934 are from nitrogen fixing cyanobacterium *Anabaena* sp. PCC 7120, the two types of glutaminase, phosphate-independent glutaminase (Slr2079, All4774) and phosphate-activated glutaminase (All2934) may play different roles in vegetative cells and heterocyst, respectively, in cyanobacteria.

MOLECULAR ASPECTS OF TAXONOMY, ECOPHYSIOLOGY AND EVOLUTION

The role of lateral gene transfer in niche adaptation of marine *Synechococcus*

Alexis Dufresne¹, Martin Ostrowski², David J. Scanlan², Laurence Garczarek¹, Wolfgang R. Hess³, **Frédéric Partensky**³

¹UMR 7144 CNRS and Université Paris 6, Station Biologique, BP 74, 29682 Roscoff, France

²Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK

³University of Freiburg, Faculty of Biology, D-79104 Freiburg, Germany

partensky@sb-roscoff.fr

Synechococcus is a ubiquitous marine picocyanobacterium occurring in the upper layer of most estuarine, coastal or offshore waters over a large range of latitudes. In order to better understand the genetic basis of niche adaptation in this ecologically important genus, we compared the genomes of 11 marine *Synechococcus* isolates, representing 10 distinct lineages and the whole variety of pigment types existing in this group. The core genome of marine *Synechococcus* is composed of 1,572 gene families, which represent 52-67% of the total genome. Genome size, which ranges from 2.22 to ~2.86 Mbp, is strongly correlated with the cumulative lengths of 'islands', which are regions of the genome only composed of accessory genes, including many unique genes. One of these islands encompasses many genes encoding phycobilisome rods, i.e. the most variable part of these light-harvesting complexes. Therefore, this island, which is involved in adaptation to changes in light quality, has seemingly been transferred between members of different *Synechococcus* lineages. This hypothesis is consistent with the fact that phylogenies based on phycocyanin and phycoerythrin notably differ from core genome phylogenies but match the *Synechococcus* pigment types. We also observed that two euryhaline strains which have similar pigmentation and physiologies, have an unusually high number of genes in common, given their phylogenetic distance and many of these genes are also located in islands. Based on these findings, we propose that local niche occupancy is facilitated by lateral gene transfers, a process in which genomic islands clearly play a key role as a repository for transferred genes.

Cyanobacteria-cyanophage interactions: Impacts on genome evolution and genome expression

Debbie Lindell^{1,2}, Maureen L. Coleman², Jacob D. Jaffe³, Matthias E. Futschik⁴, Trent Rector³, Gazalah Sabehi¹, Claudia Steglich^{2,5}, Matthew B. Sullivan², Zackary I. Johnson², Jessica Lee², Erik R. Zinser², Robert Steen³, Wolfgang Hess⁵, George M. Church³, Sallie W. Chisholm²

¹Faculty of Biology, Technion – Israel Institute of Technology, Israel

²Civil Env. Eng. MIT, USA

³Department of Genetics, Harvard Medical School, USA

⁴Institute for Theoretical Biology, Humboldt University, Germany

⁵Faculty of Biology, University of Freiburg, Germany

dlindell@tx.technion.ac.il

Viruses (phages) are an important component of ocean ecosystems, influencing population dynamics, diversity and evolution of their hosts. Here we present evidence for the impact of host-phage interactions on genome evolution and genome expression during infection. Photosynthesis (*psbA* and *psbD*) and high-light inducible stress response (*hli*) genes are present in the genomes of many phages that infect marine cyanobacteria [1,2,3,4]. Phylogenetic analyses show that these phage genes are of cyanobacterial origin and were transferred from host to phage multiple times [4]. The *hli* genes in *Prochlorococcus* phages cluster with multicopy types found exclusively in *Prochlorococcus*, suggesting that phage have mediated the expansion of the *hli* gene family by transferring these genes back to their hosts after a period of evolution in the phage [2]. These horizontally acquired *psbA* and *hli* genes are expressed from within the recipient phage and host genomes [5,6,7] suggesting they may be functional. Using the cyanobacterium *Prochlorococcus* MED4 and the T7-like podovirus P-SSP7 as a model system we investigated whole-genome expression of both host and phage during infection [7]. Phage genome expression progressed from left to right of the genetic map with time after infection (for the most part) with three distinct expression clusters being discerned. Genes thought to be involved in host take-over were expressed first, followed by phage genome replication genes, with phage particle formation genes being expressed last. The DNA replication expression cluster included four 'bacterial-like' genes – *psbA*, *hli*, *nrd* and *talC* – which we hypothesize are required for the acquisition of energy and deoxynucleotides for phage replication. Transcript levels of the vast majority of host genes, including *psbA* and most *hli* genes, declined during infection. Conversely, transcription of 44 host genes, including 3 ncRNAs, was induced with 2 distinct expression profiles being discerned. Upregulated genes included host stress response (including 5 *hli* genes), RNA degradation and protein turnover genes. Intriguingly, many of these up-regulated host genes are located in hypervariable regions thought to be laterally transferred by phages. Furthermore homologues of many of these host genes are found in cyanophage genomes. We therefore propose that the expression of these genes during infection may constitute an initial stage in the co-evolutionary process of gene exchange between host and phage.

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16S rDNA phylogeny of the Pasteur Culture Collection of Cyanobacteria (PCC)

Muriel Gugger¹, Alexandra Calteau², Rosmarie Rippka¹, Thérèse Coursin¹, Thierry Laurent¹, Jennifer Tambosco¹, Corinne Cruaud², Frederik Gavory², Jean Weissenbach², Nicole Tandeau de Marsac¹

¹*Unité des Cyanobactéries, Institut Pasteur, Paris, France*

²*Genoscope – Centre National de Séquençage, Evry, France*

mgugger@pasteur.fr

Based on morphological characteristics, members of the cyanobacterial phylum can be organized into five major groups [1]. Subsection I comprises unicellular cyanobacteria reproducing by equal binary fission or budding. Subsection II assembles unicellular or pseudo-filamentous cyanobacteria that multiply by baeocytes formation. Filamentous cyanobacteria that multiply by fragmentation or hormogonia are assignable to subsections III, IV and V. Strains of the latter two subsections all produce heterocysts, and some may produce akinetes.

In public databases, only a limited number of complete or partial 16S rDNA sequences are available for axenic strains, and the global picture of cyanobacterial interrelationships is largely based on non-axenic isolates for which little information may be available, or on environmental samples representative of very specific ecosystems.

To overcome the paucity of sequences representing the genetic diversity of axenic cyanobacteria, the 16S rRNA genes of all 750 strains presently available in the PCC were sequenced in order to establish their phylogenetic relationships. These organisms include 60 major morphotypes from diverse ecosystems and exhibiting widely physiological characteristics. The sequences analysis confirmed the monophyly of the heterocystous cyanobacteria, and the intermixed relationships of the other three subsections. Although many genera defined by morphotypes are polyphyletic, several sustained clusters were recognized that contain PCC reference strains, previously proposed based on mean DNA base composition and phenotypic characters [1, 2, 3]. Moreover, PCC strains hitherto insufficiently characterized can now be assigned to appropriate genetic clusters within the cyanobacterial phylum, providing a more complete reference frame for cyanobacteria from other culture collections or for those only known from environmental sequences. Consequently, the new 16S rDNA sequence database of the largest culture collection of axenic cyanobacteria should help to better define cyanobacterial taxa and to place the phenotypic properties of the organisms into an evolutionary context.

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A systematic microarray screen for ncRNAs in *Prochlorococcus*

Claudia Steglich¹, M. Futschik², Debbie Lindell³, S.W. Chisholm⁴, Wolfgang R. Hess¹

¹University of Freiburg, Faculty of Biology, Freiburg, Germany

²Humboldt University, Institute of Theoretical Biology, Berlin, Germany

³Technion – Israel Institute of Technology, Faculty of Biology, Haifa, Israel

⁴Massachusetts Institute of Technology, Department of Civil and Environmental Engineering, Cambridge, MA, USA

claudia.steglich@biologie.uni-freiburg.de

Small non-coding RNAs (ncRNAs) are functional RNA molecules, mostly without a protein-coding function, that have been found in all domains of life. In bacteria these functional RNA molecules range in size between 50 – 400 nt and frequently play a crucial role in regulatory networks particularly in response to environmental stress. ncRNAs are also known to control plasmid and viral replication, bacterial virulence and quorum sensing, while the function of others has remained unknown. Another class of regulatory RNAs – chromosomally encoded antisense RNAs (asRNAs) - is important for the regulation of mRNA stability. There are no systematic approaches to screen for asRNAs, but RNomics approaches have inadvertently revealed the presence of asRNAs in *Escherichia coli*. These *cis*-encoded asRNAs are transcribed from the opposite strand of the same genomic locus as the target (m)RNA and feature 100 % base complementarity. In contrast, ncRNAs that are mostly located in intergenic regions, act *trans* in a different genomic locus and exhibit only a short and imperfect base complementarity with their target transcripts.

Our analysis of microarray expression data of intergenic regions from *Prochlorococcus* MED4, together with a previous comparative genomics approach revealed the existence of more than 20 ncRNAs. The relative number of ncRNAs in *Prochlorococcus* thus is comparable with those found in enterobacteria like *Escherichia coli*, each with 1 – 2% of the genes coding for ncRNAs. Genome reduction in *Prochlorococcus* has particularly affected the number of genes coding for regulatory proteins, suggesting that regulation of gene expression through ncRNAs plays an important role in *Prochlorococcus*' response to environmental cues. Some of these functional RNAs are likely to be involved in processes such as light stress adaptation, the response to phage infection or nitrogen starvation as inferred from their mode of regulation. Furthermore, the enrichment in ncRNA genes in genomic islands of *Prochlorococcus* suggests that these islands are an important vehicle for the acquisition of ncRNAs.

"Novel enslavement" in phytoplankton communities – the biological role of cylindrospermopsin

Yehonatan Bar-Yosef, Aaron Kaplan

Department of Plant and Environmental Sciences, the Hebrew University of Jerusalem, 91904, Jerusalem, Israel

Yehonatan.baryosef@mail.huji.ac.il

Intensification of toxic cyanobacterial blooms in fresh water lakes, over the last 15 years, is a matter of growing concern due to their impact on the quality of drinking waters. Nutrient availability is an important factor affecting the dynamics of phytoplankton blooms; many organisms respond to inorganic phosphate (Pi) limitation by enhancing external alkaline phosphatase (AP) activity. Here we show that *Aphanizomenon ovalisporum* which produces the hepatotoxin cylindrospermopsin (CYN) recruits other organisms to supply its Pi needs. In isolated cultures, *A. ovalisporum* responds to removal of external Pi by fast induction of the *aoaA* and *aoaC* genes essential for CYN production, *pstS* involved in the high affinity Pi uptake system and *phoA* encoding AP. In contrast, the activity of AP is observed only after 5-7 days during which it consumes the internal Pi. Treatments of the green algae *Chlamydomonas reinhardtii* (and *Chlorella* sp.) with spent media from *A. ovalisporum*, or with the purified toxin CYN, led to induction of genes typically up-regulated upon Pi limitation such as the *ptb2* (high affinity Pi uptake) and the PHO regulon and a significant rise in the extracellular AP activity in these organisms despite the fact that they were not Pi limited. The "enslavement" of other organisms may explain the observed rise in AP activity in the water body despite the high Pi quota within the *A. ovalisporum* cells and reveals the mechanism whereby it can form massive blooms despite limiting Pi conditions. Data presented here may also provide the biological role of CYN production.

The gene, *tcpA*, required for photoregulation of phycobilisome abundance and for heterotrophic growth in *Fremyella diplosiphon*, is a useful phylogenetic marker specific for the phylum, Cyanobacteria

John Cobley¹, Sumerra Khan¹, Hira Ahmad¹, Shaun Bailey²

¹Dept. of Chemistry, USF, 2130, Fulton St, San Francisco, CA 94117 USA

²Carnegie Institute of Washington, Dept. of Plant Biology, 260 Panama St, Stanford CA 94305

cobley@usfca.edu

In *Fremyella diplosiphon* green light induces the synthesis of phycoerthrin (PE) and represses the synthesis of phycocyanin (PC). We have previously identified a gene, *psaR*, which when mutated causes overproduction of phycobilisomes (PBSs) and a decreased production of PE in green light. *psaR* is present in most cyanobacteria which produce PBSs but is notably absent from *Prochlorococcus* and marine *Synechococcus* strains. Expression of *psaR* from a shuttle plasmid in *F. diplosiphon* wild-type resulted a 10-20 fold reduction in the PBS / chl *a* ratio.

Immediately upstream of *psaR* in *F. diplosiphon* is a gene that encodes a 40 aa peptide which we have called TcpA (tetracontapeptide A). Each one of the 39 cyanobacterial genomes in the NCBI database (June 14th 2008) contains *tcpA*. Furthermore, with a single exception (cyanophage P-SSM2; AY939844.1) *tcpA* is confined to the phylum, Cyanobacteria. If *psaR* is present in the genome of a particular cyanobacterial strain, then *tcpA* is invariably found immediately upstream. We have shown by RTPCR that in *F. diplosiphon* *tcpA* and *psaR* are cotranscribed. A strain of *F. diplosiphon* was created with an unmarked in frame deletion internal to *tcpA*. While this strain was capable of chromatic adaptation it could no longer regulate PBS abundance in response to light intensity, showing a PBS / chl *a* ratio similar to that found for the wild-type growing in the middle of its light intensity range. This mutant strain also failed to grow in the dark on glucose but grew photoheterotrophically on glucose in the presence of DCMU. The ability to grow in the dark was regained when a shuttle plasmid expressing *tcpA* was mobilized into the mutant strain.

Wild type and the mutant strain were grown with 50 mM glucose in high light and then transferred to darkness for three days. After a lag of one day the wild-type resumed growth but the mutant did not. PAM fluorescence was used to compare the strains. In the wild-type the photochemical efficiency of PSII was down regulated after three days in the dark, most evidently in low actinic light. Efficiency of PSII in the mutant remained high even after three days in the dark. Maximum photosynthesis rates and subsequent dark respiration rates were measured with an oxygen electrode. The dark respiration rate for the wild-type incubated in the dark for three days (~30 $\mu\text{mole O}_2 \text{ mg chl}^{-1} \text{ h}^{-1}$) was about six fold greater than for the mutant strain similarly treated.

The presence of *tcpA* exclusively and ubiquitously in the Cyanobacteria suggests that this gene might serve as a phylogenetic marker. Alignment of *tcpA* sequences taken from complete cyanobacterial genomes gives a phylogenetic tree that agrees well with the tree created using SSU rRNA sequences. From the global ocean survey (GOS) we have recovered 293 *tcpA* sequences of high quality and have used them to create a snapshot of cyanobacteria in the near-surface marine planktonic niche. The results reveal a large, unexpected clade of *tcpA* sequences, the significance of which will be discussed.

Intraphylum diversity and complex evolution of cyanobacterial aminoacyl-tRNA synthetases

**Ignacio Luque¹, Elvira Olmedo-Verd¹, María Loreto Riera-Alberola¹, Alfonso Andújar¹,
Jesús A. G. Ochoa de Alda²**

¹*Instituto de Bioquímica Vegetal y Fotosíntesis. C.S.I.C. and Universidad de Sevilla, Avda
Américo Vespucio 49, E-41092 Seville, Spain*

²*Departamento de Biología Molecular y Celular. IE Universidad. Campus de Santa Cruz la Real.
C/ Cardenal Zúñiga 12, E-40003 Segovia, Spain*

Ignacio.luque@ibvf.csic.es

Aminoacyl-tRNA synthetases (AARS) are the enzymes that decipher the genetic code, coupling the tRNAs with their cognate amino acids. AARS are a group of proteins with a double specificity for a particular amino acid and for the corresponding isoacceptor tRNAs. These are enzymes of a very ancient origin whose evolution is most probably linked to that of the genetic code. The group of AARS has been partitioned in two classes. Enzymes of the same class have diversified from a common ancestor and have evolved specificity for a particular amino acid. Many phylogenetic studies have focussed on AARS due to their universal distribution, their relation to the evolution of the genetic code, and their central role in translation.

We have carried out a survey of genes encoding AARS in 35 cyanobacterial genomes, and we have observed that the gene complement for AARS differs within the cyanobacterial phylum. For instance, glutamyl-tRNA synthetase (GluRS) is missing in cyanobacteria whereas asparaginyl-tRNA synthetase (AsnRS) is lacking in some genera. A detailed phylogenetic analysis has been carried out for every AARS. We have observed that whereas phylogeny of most AARS is consistent with the evolution of cyanobacterial species indicating vertical inheritance of the corresponding genes, some of them show evidences of a complex evolutionary course, what could be attributed to horizontal gene transfer, gene duplication and/or gene loss events. In addition to sequence data, some of these evolutionary hypotheses are supported by the presence of indels, duplicated genes or pseudogenes. We have also detected the existence of a novel domain recruited in apparently independent evolutionary events by several AARS in particular cyanobacteria. This novel domain is termed CAAD and its function is presently unknown. We are characterizing the activity of wild-type and truncated versions of several AARS in order to identify the putative role of CAAD.

Evidence for the microcystin-producing *Anabaena* in cyanobacterial blooms in Czech reservoirs

Kateřina Bernardová¹, David Fewer², Eliška Zapomělová¹, Kaarina Sivonen², Luděk Bláha³

¹Biology Centre of AS CR, Institute of Hydrobiology, Laboratory of Phytoplankton Ecology, Na Sádkách 7, 370 05 České Budějovice, Czech Republic

²Department of Applied Chemistry and Microbiology, University of Helsinki, Viikinkaari 9, Finland

³Centre for Cyanobacteria and Their Toxins, RECETOX, Kamenice 126/3, 625 00 Brno, Czech Republic

kacabka@volny.cz

Anabaena is a common, morphological diverse component of fresh and brackish water phytoplankton worldwide [1]. Here we report the first direct evidence for the presence of microcystin-producing *Anabaena* in reservoirs in the Czech Republic. We collected bloom material from Lucina reservoir in 2005. LC-MS demonstrated the presence of LR (368,3 µg/g DW) and RR (312,8 µg/g DW). We extracted DNA and PCR amplified the *mcyE* gene directly from the bloom sample containing microcystins and constructed clone libraries. Sequence analysis suggested that the microcystin load in the Lucina reservoir could be attributed to the genus *Anabaena*. We screened a culture collection of Czech *Anabaena* strains for the production of microcystins through a PCR based assay based on the presence of the *mcyE* gene. We identified a microcystin producing *Anabaena* isolated from the Husinec reservoir. Microcystin production was confirmed through LC-MS. Together our results demonstrate the presence of genetically diverse population of microcystin producing *Anabaena* in the Czech Republic. *Anabaena* is a common component of cyanobacterial blooms worldwide and our results suggest that microcystin production in these blooms may be an under reported phenomenon.

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Cytomorphological and molecular studies on eps-producing filamentous cyanobacteria from hypogean monuments

Laura Bruno, Simona Bellezza, Daniela Billi, Patrizia Albertano

Department of Biology, University of Rome "Tor Vergata", Italy

Laura.bruno@uniroma2.it

Hypogean archaeological sites, such as the Roman Catacombs in the Mediterranean area, are mostly characterized by high relative humidity and nearly constant temperature. These sites are usually equipped with lighting systems that enable the visits of tourists but favour at the same time the development of phototropic biofilms on exposed surfaces [1]. The growth of these complex microbial communities causes aesthetic damages due to the discolouration of the valuable lithic faces and biodeterioration of the artworks by mineralisation processes and biotransformation of stone. Filamentous cyanobacteria in association with bacteria frequently form extensive blue-green, brown or grey patinas on limestone, tufa rock, plaster and frescoes [2].

A polyphasic approach was taken to define the phylogenetic position of the filamentous cyanobacteria so far isolated from various catacombs of Rome (Italy), and to further understand their role in stone deterioration. The specimens included six strains of *Leptolyngbya* sp., two of *Scytonema julianum*, one each of *S. ocellatum*, *Fischerella major* and *Fischerella* sp. Cytomorphological features were analysed by light and transmission electron microscopy using cytochemical stains, while genetic diversity was analysed using 16S rRNA and 16-23S ITS sequencing and total genome PCR fingerprinting using primers derived from highly iterated octameric palindrome (HIP1).

Differences were shown in the ultrastructure of the sheath outer layers and chemical composition of the secreted exopolysaccharides which were rich in anionic groups and had a particular monosaccharide content different to those of other prokaryotes. The production of colloidal biogenic slimes rich in negatively charged exopolymers has the potential to increase stone damage by either mechanical stress to mineral structures or by cation adsorption from the underlying substratum.

The results from DNA fingerprinting were significantly correlated with those of the 16S rRNA analyses, and discrimination was allowed at the inter- and intra-specific level. The phylogenetic analysis based on the sequencing of the 16S rRNA gene confirmed the monophyletic lineage of heterocyst-forming cyanobacteria and the heterogeneity of the genus *Leptolyngbya* as previously described [3, 4, 5]. We also identified variable regions and conserved domains within the ITS sequences, that gave a better resolution of the genetic variability among the strains as has been already reported for aquatic strains [6, 7].

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Cyanobacterial diversity in the Transantarctic Mountains (Antarctica)

Rafael Fernandez-Carazo¹, Dominic A. Hodgson², Annick Wilmotte¹

¹*CIP, Institute of Chemistry B6, University of Liège, B-4000 Liège, Belgium*

²*British Antarctic Survey, NERC, Cambridge, UK*

rfernandez@ulg.ac.be

For the first time, the cyanobacterial diversity in biotopes of the Transantarctic Mountains (82°S) was studied by molecular and microscopic approaches. Two perennially ice-covered lakes were sampled: Forlidas Pond in the Dufek Massif (Pensacola Mountains), one of the most southerly freshwater ponds known in Antarctica that contains plant life, and Lundström Lake (Shackleton Mountains). These lakes are ca 400 kms apart, but separated by a chain of mountains. Samples from Forlidas Pond were taken along a gradient going from a saline slush at the bottom of the lake, an aquatic mat in the littoral zone and 'cabbage-like' terrestrial mats in the vicinity. There is a continuity between close-by habitats that share some OTUs (groups of 16S rRNA sequences with more than 97,5% of similarity). The diversity in the centre of the continent appears lower (2 to 5 OTUs per sample) than in coastal lakes (4 to 12 OTUs per sample). A high degree of endemism (50%) was observed among the total of 10 OTUs. In each site, one new OTU was found. The OTUs in Forlidas Pond and Lundström Lake were different, maybe reflecting the fact that Forlidas was more saline (due to different evaporation histories)

Fossil cyanobacterial sequences in Antarctic lake sediments

Rafael Fernandez-Carazo¹, Krzysztof Waleron^{1,2}, Dominic A. Hodgson³, Elie Verleyen⁴, Wim Vyverman⁴, Annick Wilmotte¹

¹*CIP, Institute of Chemistry B6, University of Liège, B-4000 Liège, Belgium*

²*Department of Biotechnology, University of Gdansk & Medical School of Gdansk, 80-822 Gdansk, Poland*

³*British Antarctic Survey, NERC, Cambridge, UK*

⁴*Laboratory of Protistology and Aquatic Ecology, Ghent University, B-9000 Gent, Belgium*

rfernandez@ulg.ac.be

One of the aims of the BELSPO projects LAQUAN and HOLANT is to reconstruct the Holocene diversity of cyanobacteria, which dominate benthic mats in east Antarctic lakes. In the LAQUAN project, we successfully retrieved 16S rRNA sequences (DGGE and clone libraries) and tested a specific cyanobacterial marker (*rpoC1*) in lake sediments from the Larsemann Hills. Our results were partially validated by parallel analyses in 2 separate laboratories. Except for 5 new phylotypes, the fossil sequences were a subset of the modern cyanobacterial diversity in the region. The oldest layer from which cyanobacterial sequences could be retrieved was 9500 years old and characterized by the presence of a unicellular phylotype corresponding to *Synechococcus* sequences, and by a phylotype related to clone Fr252, previously found in Lake Fryxell (McMurdo Dry Valleys, Antarctica).

For the HOLANT project, the cyanobacterial DNA was extracted from two lakes in Beak Island (Antarctic peninsula) and West Ongul Island (East Antarctica). We tested the use of MDA (Multi Displacement Amplification) as an initial step before starting the analyses in order to increase the low concentrations of fossil DNA. Several protocols were tested. Our results suggest that MDA could improve the study of samples containing low amounts of DNA that could not be analyzed by direct PCR.

A molecular approach to investigation of cyanobacterial mats associated to thermomineral drillings from Western plain of Romania

Cristian Coman^{1,2}, Bogdan Drugă^{1,2}, Adriana Bica¹, Lucian Barbu-Tudoran³, Nicolae Dragoș^{1,2}

¹*Babeș-Bolyai University, Cluj-Napoca, Romania*

²*Institute of Biological Research, Cluj-Napoca, Romania*

³*Electron Microscopy Centre, Babeș-Bolyai University, Cluj-Napoca, Romania*

cr.coman@yahoo.com

Defining the diversity and structure of microbial communities based on the quantification of their constituent populations has proved to be quite a challenge in microbial ecology. Selective enrichment cultivation as an approach for the description of naturally occurring microbial communities has severe limitations because the majority of bacteria in nature cannot be cultivated using traditional techniques. The studies based on phenotypic identification of taxa are largely admitted to provide inconclusive information because this method cannot determine genetic variability and, in many cases, it induces misunderstandings because phenotype diversity is not always correlated with genetic diversity. An alternative approach for understanding the composition of natural communities is the one that uses molecular biology techniques and provides a culture-independent analysis of microorganisms. The question that appears is: can molecular techniques be used independently of morphology to characterize microbial communities? To answer this question, we focused on some cyanobacterial mats associated to thermomineral drillings; they are an excellent model for studying the molecular diversity and the colonizing potential of cyanobacteria due to their precise spatial delimitation and homogenous conditions enforced by constant temperature and water chemistry. Our research was mainly based on culture-independent molecular analysis of cyanobacterial ribosomal *rnm* operon (16S RNA and ITS-Internal Transcribed Spacer). PCR amplification of target fragments with cyanobacterial primers from genomic DNA extracted from field samples was followed by: 1. cloning, restriction map analysis and sequencing of selected amplicons; 2. capillary electrophoresis of PCR amplification products (ITS); 3. Denaturing Gradient Gel Electrophoresis (DGGE) banding patterns analysis and sequencing of selected gel DNA fragments. The conserved fragments of 16S rRNA gene and ITS were used for taxa identification and genotype heterogeneity analysis; a phylogenetic analysis was also conducted. The comparison of the sequences we obtained with those stored in public nucleotide databases revealed that the majority of taxa belong to the following genera: *Phormidium*, *Symploca*, *Oscillatoria* and *Microcoleus*. The use of molecular techniques for biodiversity investigation of cyanobacterial mats associated to thermal springs found in Western Plain of Romania has proved to be successful. They provided all the necessary aspects for determining the number of species in the community (using capillary electrophoresis and DGGE), for species delimitation (using restriction map analysis) and species identification (BLAST analysis of the rDNA-ITS sequences).

Preliminary report on a coccoid diazotrophic cyanobacterium from the Euganean Thermal District (Padova, Italy)

Maria Alessia Fuiano, Isabella Moro, Nicoletta La Rocca, Katia Sciuto, Marion Adelheid Wolf, Carlo Andreoli, Nicoletta Rascio

Department of Biology, University of Padova, Padova, Italy

mafuiano@bio.unipd.it

This research deals with a coccoid strain isolated from cyanobacterial populations growing on the muds of the thermal Euganean District (Padova) and cultured in BG11 medium at 30°C, 12 h photoperiod and 40 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. The cells of this euganean strain were solitary, oval to cylindrical (about 5 μm in width and 6-10 μm in length) and surrounded by a thin mucilaginous layer consisting of fibers arranged perpendicularly to the outer membrane. The division occurred by binary transverse fission yielding two equal daughter cells. The thylakoids were numerous, radially arranged, occasionally fasciculate and distributed over the whole cell body. Inside the cytoplasm there were polyhedral carboxysomes, electron-dense granules of cyanophycin, small granules of glycogen between the photosynthetic membranes and large electron-transparent inclusions, probably of polyhydroxybutyrate [1] enclosed by one thylakoid. The Euganean cyanobacterium showed to be a nitrogen-fixing strain since it was able to grow well in BG11₀ medium without NaNO_3 . Moreover, a molecular analysis led to the finding in its genome of the *nifH* gene which code for a subunit of the enzyme nitrogenase. Most of the ultrastructural and metabolic features exhibited by our strain were common to those which characterize members belonging to the genus *Cyanothece* [1, 2]. From a preliminary phylogenetic analysis, carried out by using the sequence of the 16S rDNA gene of the Euganean strain and those of coccoid diazotrophic cyanobacteria available in GenBank, our strain resulted to be sister taxon to both a *Cyanothece* and a *Gleotheca* strain. Research is now in progress to define the precise taxonomic and phylogenetic position of this coccoid nitrogen-fixing cyanobacterium and to establish whether it belongs to a known species already noticed in other thermal environment or it is a new form proper to the Euganean District.

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Benthic Baltic cyanobacteria of the genus *Anabaena* reveal new insights into their molecular biology

Birgit Heyduck-Söller, Ulrich Fischer

Abteilung Marine Mikrobiologie, Fachbereich 2 und Zentrum für Umweltforschung und nachhaltige Technologien (UFT), Leobener Str., 28359 Bremen, Germany

bhs@uni-bremen.de

The identification of most cyanobacterial isolates existing in culture collections has based mainly on morphological features. The culture collection of our department contains more than 100 benthic, unicyanobacterial isolates, which have been isolated mainly from shallow coastal waters of the Baltic Sea. Ten cyanobacterial strains were designated as members of the genus *Anabaena* due to morphological criteria [1-4] and differentiated on the basis of random amplified polymorphic DNA (RAPD)-fingerprints [5] and fatty acid patterns. By combining all sets of data, three clusters (I to III) with dissimilar isolates were obtained.

At present, nucleotide sequences of the small subunit rDNA (16S rRNA gene) are used for phylogenetic analysis of the above mentioned isolates. In the Basic Local Alignment Search Tool (BLAST, NCBI), the isolates of cluster I were about 99.58% identical to *Anabaena* strain BECID19 [6], whereas all isolates of cluster III had a sequence similarity of about 99.81% to *Anabaena* strain XPORK36C [7]. Interestingly, the molecular identification of four out of five isolates belonging to cluster II was not congruent with the given morphological *Anabaena* description. A sequence similarity of about 98.30% supported the belonging of these strains to the genus *Nodularia* more than to the genus *Anabaena*. The remaining isolate of this cluster II was about 99.85% identical to the result obtained for cluster III. To resolve this discrepancy, additional molecular markers, such as the subunit B protein of DNA gyrase (*gyrB*), the internal transcribed spacer (ITS) for the 16S-23S rRNA region, or the intergenic spacer for the phycocyanin operon (PC-IGS), are under investigation.

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The biodiversity of picocyanobacteria in the *in situ* samples and isolates from epilimnion of lakes from Mazurian Lake District, Poland

Anna Dudziec, Katarzyna Jakubiec, Iwona Jasser, Maja Łukomska, Adriana Mazurkiewicz
Microbial Ecology Department, Institute of Microbiology, University of Warsaw, Warsaw, Poland

jasser.iwona@biol.uw.edu.pl

The biodiversity of picocyanobacteria – prokaryotic component of autotrophic picoplankton (APP) from Mazurian Lake District – *in situ* and isolates were studied using one direct sequencing and two denaturing gradient gel electrophoresis (DGGE) protocols. Environmental samples were collected several times between April and October from epilimnion of nine lakes of different trophic status. For the study DGGE analyses involving a 194 bp fragment of the intergenic transcribed spacer (ITS) and a 500 bp fragment of the phycocyanin operon – *cpcBA*-IGS were used. Both protocols showed higher diversity of picocyanobacteria in spring comparing with late summer and autumn samples.

To isolate picocyanobacteria two approaches were applied: a classic plate method and a modified flow cytometry method [1]. For isolation and cultivation of strains three different media were used: BG11, WC, and BG11 modified by Ernst (N and P concentration reduced to one third). WC – a medium with the lowest N and P concentration, turned out to be the most selective one to isolate picocyanobacteria.

From 32 isolates of picocyanobacteria obtained after 9 months 18 were chosen for direct sequencing of the phycocyanin operon – *cpcBA*-IGS fragment on the base of microscopic analyses and PCR (preparing for direct sequencing). From selected strains seven seem to be unique: four PC (phycocyanin – rich) strains and three PE (phycoerythrin – rich) strains. The PC strains originated as follows: two from mesotrophic Lake Majcz, one identical from Lakes Majcz and eutrophic Mikołajskie and finally one from eutrophic Lake Beldany. In case of PE strains all three came from eutrophic lakes: one from Lake Mikołajskie and two from Lake Beldany. The remaining 11 isolates chosen for sequencing were identical with these given above. The phylogenetic analyses revealed that the obtained sequences belong to five different clusters, recognized before [2], however, the PC and PE strains were located in different clusters.

In addition DGGE *cpcBA*-IGS protocol was used for comparison of environmental, *in situ* samples and isolated strains. Furthermore the morphometry and some ecophysiological properties of selected strains as well as advantages and disadvantages of both isolation methods are discussed.

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A new approach to analyze genotypes of colony-forming cyanobacteria from environmental samples

Yannick Lara¹, Christophe Boutte², Anatoly Peretyatko³, Annick Wilmotte¹

¹Cyanobacteria Group, Center of Protein Engineering, University of Liège, Belgium

²Station Biologique de Roscoff, UMR 7144 France

³Plant Science and Nature Management, Department of Biology, Vrije Universiteit Brussel, Belgium

ylara@ulg.ac.be

Several studies have shown the efficiency of sequences as rRNA-ITS, *cpcBA*, *rbclX* and other housekeeping genes to study taxonomy [1, 2, 3], population, community structure of cyanobacteria, or for Multi Locus Sequence Analysis [4].

Recently, the genotypic analysis of single colonies and single filaments directly isolated from the environment has been carried out by other authors. It appears that different genotypes of *Microcystis* are present in one population in one lake. Besides, succession of toxic and non-toxic genotypes may have a critical influence on toxin concentrations during the blooms [5]. Genotypic analysis of colony-forming cyanobacteria requires enough DNA. So far, the genotypes of environmental single colonies of *Microcystis* were characterized on the basis of one or two PCR [6]. As the DNA content of one single colony only allows for a few PCR reactions, we have developed a new approach using Whole Genome Amplification with *Phi29* polymerase to allow for the Multi Locus Sequences Typing analysis of a single colony or filament. For the first time, we were able to amplify and sequence more than one locus of the genome of a single colony of *Microcystis*. In addition, we have obtained the first sequences of *rpoC1*, *rbclX* and rRNA-ITS from a single colony of the genus *Woronichinia* (identified by microscopy). This approach allows to work with a small amount of DNA, and represents a concrete answer to the lack of data on non-cultivable cyanobacteria.

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Ultrastructural and photosynthetic features of a new coccoid cyanobacterium from the Euganean Thermal District (Padova, Italy)

Nicoletta La Rocca, Isabella Moro, Monica Di Bella, Maria Alessia Fuiano, Barbara Baldan, Carlo Andreoli, Nicoletta Rascio

Department of Biology, University of Padova, Padova, Italy.

nicoletta.larocca@unipd.it

A coccoid cyanobacterium, isolated from the thermal waters of Abano Terme (Padova, Italy), was studied through a phylogenetic, ultrastructural and physiological point of view. The phylogenetic analysis led to consider the examined strain as a new species of the *Cyanobacterium* genus, and it was named *Cyanobacterium aponinum* [1]. Analyses carried out on cultures grown at different temperatures showed that *C. aponinum* survived temperatures until 40–45°C. However, in comparison with cells grown at 30°C, those maintained at 40–42°C showed: a decrease of photosynthesis (as O₂ emission) referred to chlorophyll amount, a reduction of D1 protein of the reaction centre of PSII, evidenced by western analysis, and a lowering of the Fv/FM value (as chlorophyll fluorescence emission). All these results suggested an inhibition or a damage of some PSII units. Interestingly, the analysis of fluorescence emission at 77°K showed that the increase of growth temperature from 30 to 40°C in cells exposed to the same light intensity caused a transition of the photosystems from the state 1, with the phycobilisomes linked to the PSII, to the state 2, with the phycobilisomes linked to PSI. The increase of the temperature at 45–46°C led to the death of the cyanobacterium in few days. The death occurred surprisingly through an orderly programme of events. More precisely, a gradual and directed demolition of the photosynthetic pigments and thylakoids occurred from the centre towards the periphery of the cells, evidenced by a disappearance of chlorophyll autofluorescence. Moreover, the DAPI staining showed that the DNA was degraded. At the end, only ghosts of cells remained, which were apparently integral but lacking in thylakoids and in all the other cellular components.

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Harvesting the ocean - Isolation and characterization of novel strains of marine cyanobacteria and evaluation of their biotechnological potential

Remus Mohr^{1,2}, Wolfgang R. Hess¹

¹*Institute for Biology II, University of Freiburg, Freiburg, Germany,*

²*School of Biological Sciences, University of Sydney, Sydney, Australia*

remus.mohr@biologie.uni-freiburg.de

Marine photooxygenic cyanobacteria are a key player for the global carbon cycle and oxygen production. Recent studies show that cyanobacteria are also an important source for biologically active natural products and potential drugs. Most of these molecules belong to the class of non ribosomal peptides (NRP) or polyketides (PK) [1]. NRP, polyketides and NRP/polyketide hybrids are small molecules with a broad spectrum of biological activities (e.g.: antibiotic, antifungal, insecticide, anti-inflammatory, cytostatic) (Tan 2007). They are synthesized by large enzyme complexes known as Non Ribosomal Peptide Synthetases (NRPS) and Polyketide Synthases (PKS). NRPS and PKS genes are arranged in large gene clusters of up to 70kb length. They have a modular structure with reoccurring domains.

Despite their importance marine cyanobacteria are still poorly studied in particular with regard to the actual number and diversity of species in laboratory culture.

This study aims at isolating new strains of cyanobacteria and takes a closer look at the distribution of natural product genes within these new strains. A set of novel marine cyanobacteria were isolated from Heron Island, Great Barrier Reef, Australia (23.44 °S, 151.91 °E) using standard microbiological techniques. The newly obtained laboratory cultures were classified by 16S rDNA sequence comparison, morphology and pigmentation. In order to assess their biotechnological potential NRPS adenylation domains were detected by PCR and subsequently sequenced. Furthermore mass spectrometry was carried out to detect peptides and polyketides biochemically.

25 cyanobacterial isolated strains were used for this study. Based on 16S rDNA sequence data most isolated strains are novel with a maximum of 97% identity to sequences in the database. Phylogenetically they group within sections I-IV of the cyanobacterial kingdom. The majority belongs to section II or III. Morphologically interesting candidates from section IV are marine *Calothrix* spp., a filamentous species with a single basal heterocyst. Also an *Acaryochloris marina* related red light organism was isolated that seems to be using chlorophyll d as a main light harvesting pigment.

Regarding the production of possible bioactive compounds more than half of the novel cyanobacteria contain genes for the production of NRPs. 40% of the tested strains show relevant amounts of potentially new secondary metabolites.

Despite the selective number of cyanobacterial strains compared to larger culture collections we were able to show that there is an enormous potential still lying within our oceans. Isolating the new secondary metabolite and sequencing of the relevant gene clusters will be the challenge for future research.

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Characterization of *Leptolyngbya* sp. ETS-04 isolated from the Euganean thermal muds (Montegrotto Terme, Italy).

Isabella Moro¹, Katia Sciuto¹, Nicoletta Rascio¹, Nicoletta La Rocca¹, Alberto Lalli², Carlo Andreoli¹

¹*Department of Biology, University of Padova (Italy)*

²*Centro Studi Termali "Pietro d'Abano", Abano Terme (Italy)*

isabella.moro@unipd.it

Leptolyngbya are very common cyanobacteria in soils and in periphyton and metaphyton of freshwater and marine biotopes. Several species are known from thermal and mineral springs or from aerophytic rocky sites or walls. Characteristic species grow endogloeically in mucilage and in colonial slime of other algae [1]. During a research on the biodiversity of the cyanobacterial mats covering the muds of the Euganean thermal District, both coccoid and filamentous strains of cyanobacteria were isolated. This work deals with the characterization of one filamentous strain found on muds from this District (Montegrotto Terme, Italy). It was cultured in laboratory in BG11 medium, at 30°C, under a light intensity of 10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with a 12h photoperiod. A polyphasic research was carried out through morphological, ultrastructural, biochemical and molecular analyses. In particular, the molecular analyses were performed using different markers, such as the 16S rDNA gene and the 16S-23S ITS region. Our experimental results, taken as a whole, suggest that the filamentous cyanobacterium analyzed is a new species belonging to the genus *Leptolyngbya*.

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Polyphasic evaluation and phylogenetic analysis of a marine cyanobacterium strain related to thermophilic cyanobacteria

Vitor Ramos^{1,2,3}, Rui Seabra^{1,2}, Arlete Santos^{1,3}, Agostinho Antunes², Vitor Vasconcelos^{2,4}, Paula Tamagnini^{1,3}

¹IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

²CIMAR/CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Porto, Portugal

³Departamento de Botânica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

⁴Departamento de Zoologia & Antropologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

vmramos@ibmc.up.pt

Historically, the classification of cyanobacteria has been based on simple morphological characteristics but in the last decades, modern ecological investigations, electron microscopy and particularly the advent of molecular methods have substantially influenced our knowledge about this group [1]. As a result, the cyanobacteria taxonomy is still being improved as well as our understanding of their diversity. However, the traditional cellular–morphological approach is still necessary, since classification cannot be yet satisfactorily replaced by any other criteria [1]. In this work, by mean of a polyphasic approach, which combines molecular analysis, cytomorphological and ecological evaluation of the taxa, we characterize a strain isolated from a cyanobacterial mat present in the intertidal zone of the rocky beach of Praia da Luz, southern Portugal (LEAN 26). The highest homologies of the 16S rDNA sequence of LEAN 26 are with three cyanobacteria collected from two geographically distant hot springs – two from Zerka Ma'in Thermal Springs in Jordan and one from La Duke Hot Springs in the Grand Yellowstone Area, USA. The four sequences form a distinct cluster from all the other cyanobacteria. One of these thermophilic cyanobacteria, identified as *Chroogloeocystis siderophila* Brown, Mummey et Cooksey [2], shares similar morphological and ultrastructural features with LEAN 26. Following the criteria presented by the authors, our strain should fit into the same genera *Chroogloeocystis*. However, attending to morphology-based identification our strain must be assigned to the genus *Gloeocapsopsis* Geitler ex Komárek. The possible taxonomic misinterpretation and an evolutionary inference of these findings are discussed.

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Genetic diversity of marine *Synechococcus* in the Southwest Atlantic Ocean

Macarena Pérez-Cenci¹, Ricardo I. Silva², Rubén Negri², Gonzalo Caló¹, Graciela Salerno¹

¹CEBB-Centro de Investigaciones Biológicas, FIBA, Vieytes 3103, Mar del Plata, Argentina

²Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Mar del Plata, Argentina

gsalerno@fiba.org.ar

Unicellular cyanobacteria of the genus *Synechococcus* are known to be widespread and abundant throughout the world's oceans and, together with those of the *Prochlorococcus* genus, are very important contributors to marine and global primary production. Several genetic lineages of *Synechococcus* have been defined through phylogenetic analysis of the 16S rRNA gene [1], while more potential lineages have been proposed more recently, based on ITS and the N-regulatory gene *ntcA* [2,3]. The wide oceanic distribution of these microorganisms is mirrored by high physiological and genetic diversity of cultured isolates and natural populations. Recently, a detailed analysis of the distribution of these genetic lineages was reported including the local and ocean basin scale using data from cruises in the Pacific, Atlantic, Indian, and Arctic Oceans [4,5]. However, to date little is known about the picophytoplankton diversity in the South Atlantic Ocean. As part of a study on the biodiversity and population dynamics of the unexplored picophytoplankton of the Argentinean Sea, the aim of this work was to identify and molecularly characterize the components of the prokaryotic picophytoplankton using microscopic and molecular methodologies. Water samples were collected at a permanent coastal station (EPEA, 38°28'S-57°41'W) or in the shelf in the Patagonian region of the Argentinean Sea. Only cyanobacteria of the genus *Synechococcus* were found, according with their ecophysiological characteristics [6]. We determined the genetic diversity after PCR amplification, cloning and sequencing of the 16S rRNA gene (from genomic DNA or environmental DNA) and analysed the phylogenetic relationships of *Synechococcus* either from cultured isolates or from natural populations. In the latest case, 16S rRNA gene clone libraries were constructed from each water sample collected at 5 m depth. The 16S rRNA gene sequences grouped with *Synechococcus* of the clade 1 of the marine subcluster 5.1 [6].

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New classification systems and molecular methods necessitate the reclassification of the filamentous cyanobacterium *Oscillatoria limnetica* strain Flo1 as a species of the genus *Geitlerinema*

Jan Schrübbers, Birgit Heyduck-Söller, Ulrich Fischer

Universität Bremen, Abt. Marine Mikrobiologie, FB 2, Zentrum für Umweltforschung und nachhaltige Technologien (UFT), Leobener Str., D-28359 Bremen

jan.schruebbers@web.de

Until 1979 cyanobacteria were classified under the Botanical Code exclusively according to morphological characteristics. In the last decades, modern approaches including molecular and genetic methods - as well as ultrastructural studies and ecological features - have been taken into account for a taxonomic classification of these photosynthetic prokaryotes and new classification systems were generated.

The filamentous cyanobacterium strain Flo1 was primarily morphologically classified according to GEITLER (1932) as *Oscillatoria limnetica*. By applying modern techniques and methods we could clearly demonstrate that this species had to be reclassified as a species of the genus *Geitlerinema* of the family *Pseudanabaenaceae* within subsection III, *Oscillatoriales* (see CASTENHOLZ et al. 2001 and ANAGNOSTIDIS and KOMAREK 2005).

The molecular analysis of 16S rDNA and gene *gyrB* of strain Flo1 showed 99.56% and 99.7% sequence similarity to *Geitlerinema* PCC 7105. Additionally, the sequences of the internal spacer region (ITS) and intergenic spacer of phycocyanin (IGS) were determined. The *mcyA* gene, part of the microcystin-cluster, was not detected.

Analysis of optimal growth conditions showed that strain Flo1 grows best at light intensities from 1-10 $\mu\text{E m}^{-2} \text{s}^{-1}$ PAR and tolerates salinities from 7-62‰ with an optimum at 15‰. Higher light intensities accompanied a higher production of exopolymeric substances. Strain Flo1 grows with nitrate, nitrite and ammonium as sole nitrogen sources in concentrations from 1-10 mM. Nitrogen fixation was not observed. Strain Flo1 possesses chlorophyll *a*, the carotenoids myxoxanthophyll, zeaxanthin, β -carotene, as well as the phycobiliproteins phycoerythrin, phycocyanin, and allophycocyanin. Complementary chromatic adaptation in changing light qualities from red- to green-light (and vice versa) was not observed. The position and arrangement of thylakoids were determined using ultrathin sections of the cells and showed concentrically arranged thylakoids, peripheral and parallel to the longitudinal cell walls.

Biodiversity of cyanobacteria derived from two sites of Svalbard inhabited by two different species of seabirds: *Alle alle* and *Uria lomvia*

Magdalena Szefel-Markowska, Malgorzata Waleron, Joanna Głowacka, Krzysztof Waleron

Department of Biotechnology, Intercollegiate Faculty of Biotechnology

University of Gdańsk & Medical University of Gdańsk, Kładki 24, 80-822 Gdańsk

waleron@biotech.univ.gda.pl

Cyanobacteria dominate most polar ecosystems and they are critically important for their functioning. Until recently, little was known about microbial diversity. However, during the last years as a result of the application of molecular techniques (especially clone libraries construction) it has been changed.

The study was performed in order to determine and compare the influence of two species of seabirds (inhabiting two locations of Svalbard) on the diversity of the soil cyanobacteria. These two habitats were named by first letter of the name of the bird species which were characteristic for them (U1- *Uria lomvia*, A1-*Alle alle*). The birds introduce biogens to the soil and in that way change the environmental conditions. It is important to underline that these species of birds have different food strategies, as a result, the composition of the cyanobacterial population in their habitats may vary. In addition, the comparison studies of bird transects U1, A1 and control transects U2, A2 (which were topographically similar to proper transects) were performed in order to estimate if the occurrence of these animals influence the cyanobacterial diversity. The second goal was to determine the correlation between cyanobacteria biodiversity and distance from the nests. In both sites of Svalbard (U1, A1), the soil samples were collected from eight regions (situated in various distances from the nests). Both phenotypic and genotypic methods were applied to describe the cyanobacterial isolates. Phenotypic analysis of these isolates covered morphological and biochemical features and their potential ability to nitrogen fixation. Only five cyanobacterial isolates (four different isolates from U1 and only one from A1) were separated under laboratory conditions. It is due to the fact that many species of cyanobacteria cannot be cultured. The genetic characteristic of cyanobacterial isolates was based on the restriction analysis of the sequences of two house-keeping genes: *rpoC1* (unique for cyanobacteria) and *recA*. Restriction analysis of *rpoC1*-PCR products allowed to describe three different RFLP groups from separated isolates. Therefore, in order to study diversity of cyanobacteria it is necessary to use clone libraries technique. The molecular characteristic of cyanobacteria was based on clone libraries construction of the genes: 16S rRNA and *rpoC1*. Two restriction endonucleases (*TasI*, *TaqI*) were applied in order to analyze the clone libraries. The most interesting results were obtained during the analysis of the sample named U1K2 (from region in the shortest distance from the *Uria lomvia* nest). In the case of this sample, only two different isolates were separated. On the contrary, the analysis of the clone libraries of *rpoC1* gene performed for the same sample permitted to describe 23 different genotypes. In this case, the analysis of 16S rRNA gene allowed to distinguish 12 genotypes. These results allow to draw a conclusion that it is necessary to use clone libraries technique in order to perform in depth study of the diversity of cyanobacteria. Primary results of the comparison study of the bird transects and the control one indicated that the presence of birds increases of the biodiversity of cyanobacteria.

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Polyphasic evaluation of planktonic *Anabaena* and *Anabaena*-like cyanobacteria from the Czech Republic, incl. *Sphaerospermum* gen. nov.

Eliška Zapomělová^{1,2}, J. Jezberová¹, P. Hrouzek^{2,3}, E. Soldati⁴, K. Řeháková^{1,5}, D. Hisem^{2,3}, J. Komárková^{1,5}, S. Ventura⁴

¹Biology Centre of the ASCR, Institute of Hydrobiology, České Budějovice, Czech Republic

²University of South Bohemia, Faculty of Sciences, České Budějovice, Czech Republic

³Institute of Microbiology, AS CR, Department of Autotrophic Microorganisms, Opatovický mlýn, Třeboň, Czech Republic

⁴CNR-ISE, Firenze, Italy

⁵Botanical Institute of ASCR, Algological Centre, Třeboň, Czech Republic

eliska.zapomelova@seznam.cz

Planktonic cyanobacteria from the genus *Anabaena* and related morphospecies were studied using a combination of morphological, molecular and biochemical approaches. Both the field morphologies and morphological features under culture conditions were described in 31 strains of 12 morphospecies (*Anabaena compacta*, *An. circinalis*, *An. crassa*, *An. curva*, *An. flos-aquae*, *An. lemmermannii* var. *lemmermannii*, *An. lemmermannii* var. *minor*, *An. mendotae*, *An. reniformis*, *An. sigmaidea*, *An. spiroides*, and *Aphanizomenon aphanizomenoides*). Partial sequences (1203 bp) of the 16S rRNA gene have been obtained for all of those strains and secondary metabolite contents have been determined for 20 selected strains using HPLC-MS analysis. Based on the 16S rRNA gene sequences, *Anabaena circinalis* and *An. crassa* strains appeared together in a joint cluster, suggesting that these morphospecies are hardly distinguishable one from another. Analogous was the situation with *An. mendotae* and *An. sigmaidea*. These results were in a good agreement with morphological observations, since continuous transitions between *An. mendotae* and *An. sigmaidea* or *An. circinalis* and *An. crassa* were observed both in field and under culture conditions. *An. lemmermannii* formed an isolated cluster, indicating that it is a reliable taxon, where the position of the akinetes is the main identification feature. Clear morphological and molecular delimitation of *An. compacta*, which was previously demonstrated by Rajaniemi et al. (2005), was confirmed by the present study. *An. flos-aquae* strains were spread throughout the whole planktonic-*Anabaena* cluster in the phylogenetic tree (16S rRNA). Based on extremely different sequences of the 16S rRNA gene, *An. reniformis* and *Aph. aphanizomenoides* have been re-classified into a new genus *Sphaerospermum*. These strains appeared in a cluster with *Cylindrospermopsis* and *Raphidiopsis*, closely together with *An. kisseleviana* sequences from GenBank. All of those *Anabaena*-like morphospecies were characterized by the shape and position of the akinetes (spherical, from one or both sides adjacent to the heterocytes).

NUTRIENTS, METABOLISM AND BIOTECHNOLOGY

Diversity of cyanobacterial oligopeptides – from genes to structures

Martin Welker^{1,2}

¹FG Umweltmikrobiologie, Technische Universität Berlin, 10587 Berlin, Germany

²AngnosTec GmbH, 14476 Potsdam-Golm, Germany

martin.welker@tu-berlin.de

Cyanobacteria produce a large variety of oligopeptides, many of which have been shown to be toxic or bioactive. The majority of the known peptides are synthesized by nonribosomal peptide synthetase (NRPS), polyketide synthase (PKS), or hybrid NRPS-PKS pathways and a number of gene clusters of several biosynthetic operons from various genera has been sequenced. Other peptide types are synthesized by ribosomal peptide synthesis followed by post-translational modifications by microcin synthesis-like pathways. Among nonribosomal peptides, several classes or types can be defined based on structural peculiarities, with many individual structural variants in each peptide class. For three peptide classes (microcystins, cyanopeptolins, and aeruginosins) biosynthesis gene cluster sequences from multiple strains and species are available offering insight in the genetic base of cyanopeptide structural diversity.

When four aeruginosin synthetase gene clusters (*aerA-P*) from two genera (*Microcystis* and *Planktothrix*) are compared, a high plasticity is evident, especially with respect to genes coding for modifying enzymes. The operons may or may not include genes for sulfotransferases, glycosyltransferases or halogenases. Further, regions coding for the second adenylation domain are very variable, like as a result of recombination events. In cyanopeptolin synthetase gene clusters (*mcnA-F*, *apdA-F*, *ociA-D*), a core of NRPS genes is rather conserved, even in distant genera like *Anabaena*, *Planktothrix*, and *Microcystis*. In particular genes, however, regions of high sequence divergence can be recognized that code for adenylation domains responsible for the incorporation of amino acids at variable positions in the peptide products. Substrate binding pocket signature sequences, the 'nonribosomal code', of some cyanobacterial adenylation domains do not match to amino acid specific sequences of cyanobacteria and other organisms. Considering the peptide products of individual strains, an activation by adenylation of amino acids as different as arginine, lysine, and tyrosine, for example, is required. This likely is achieved by a yet not fully understood relaxed substrate specificity of particular adenylation domains that allows the production of multiple congeners by a single NRPS system.

Conclusively, the variability of peptide abundance and diversity observed in natural cyanobacterial populations and communities is the result of 1. erratic distribution of particular NRPS gene clusters among species and clones, 2. the plasticity of gene clusters, especially with respect to modifying enzymes, and 3. the co-production of multiple congeners by individual strains. Insight in the evolution and plasticity of gene clusters and the biochemistry of cyanobacterial peptide synthesis will help to understand the biological role of these metabolites and their structural diversity that is still unexplained.

Allocating the correct metals to the correct proteins

Nigel Robinson

Institute for Cell and Molecular Biosciences, University of Newcastle University, UK

n.j.robinson@ncl.ac.uk

Left to their own devices, metalloproteins tend to select the wrong metals. It has been estimated that about a third of proteins require metals although the precise proportion varies in different cells and under different environmental conditions. Cyanobacteria have especially high metal demands requiring ten times more iron than *E. coli*, due in part to the substantial requirements for iron sulphur clusters and for heme in the photosystems, a hundred times more manganese than photosynthetic bacteria devoid of the water splitting enzyme, and plastocyanin is one of the few known bacterial proteins that requires copper internal to the plasmamembrane. Whatever the precise proportion, filling each metalloprotein with the correct metal is, literally, elemental to life.

To understand the challenge associated with correctly populating each protein its necessary to be familiar with the Irving-Williams stability series. The affinities of proteins for metals tend to follow universal orders of preference, one of which is the Irving-Williams series which specifies the order (from weakest binding metals to tightest binding metals): magnesium, calcium, manganese, (ferrous) iron, cobalt, nickel, (cupric and cuprous) copper, and zinc ions are also highly competitive. How can a cell simultaneously contain some proteins that require tight binding metals and others that require weak binding ones? Metals at the top of the series must somehow be kept out of the binding sites for those lower down. Metal-sensors, metal-transporters, metal-storage proteins and metallochaperones have crucial roles in allowing cells to overcome this limitation of bioinorganic chemistry. The fidelity of these proteins in discriminating between metals is at the top of a hierarchy of metal-specificity since their actions influence metal occupancy of all other proteins.

To directly explore how a cyanobacterium overcomes the challenge imposed by the Irving Williams series we have recently identified the most abundant copper protein and the most abundant manganese protein in the periplasm of *Synechocystis* PCC 6803. The metals were chosen because they lie at opposing ends of the affinity series. The crystal structures of both proteins unexpectedly revealed identical metal binding ligands. As predicted from the affinity series, the manganese protein binds copper (and also zinc) in preference to manganese. The mechanism by which cell biology acts to ensure that copper and zinc do not aberrantly bind to the manganese protein will be described and its implications highlighted.

Microcystins affect surface properties of *Microcystis* cells

Jan-Christoph Kehr¹, Yvonne Zilliges¹, Nicole Tandeau de Marsac², Thomas Börner¹, Elke Dittmann¹

¹Humboldt-Universität zu Berlin, Institut für Biologie, Molekulare Ökologie und Genetik, Chausseestr. 117, 10115 Berlin, Germany

²Unité des Cyanobactéries (URA-CNRS 2172), Département de Microbiologie, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France

thomas.boerner@rz.hu-berlin.de

Microcystins are the most common cyanobacterial toxins found in freshwater lakes and reservoirs throughout the world. They are frequently produced by the unicellular, colonial cyanobacterium *Microcystis aeruginosa*; however, the role of the peptide for the producing organism is poorly understood. We have compared the proteomes of the microcystin-producing strain *M. aeruginosa* PCC 7806 and of its microcystin-lacking mutants $\Delta mcyB$ and $\Delta mcyH$. We observed drastic differences in the accumulation of several proteins (Mrps – ‘microcystin-related proteins’) some of which were selected for further characterization [1,2,3, and unpubl. data]. MrpC was found to be a surface-exposed protein that showed increased abundance in PCC 7806 mutants deficient in microcystin production compared to the wild type. Mass spectrometric and immunoblot analyses revealed that the protein is posttranslationally glycosylated. Analyses of field samples of *Microcystis* demonstrated a strain-specific occurrence of MrpC possibly associated with distinct *Microcystis* colony types. Another protein differentially expressed in wild type and mutants is a lectin that we designated ‘microvirin’ (MVN) since it shares 33% identity with the potent anti-HIV protein cyanovirin-N from *Nostoc ellipsosporum*. Carbohydrate microarrays were employed to demonstrate the high specificity of the protein for high-mannose structures containing alpha(1-->2) linked mannose residues. Studies on lectin binding and phenotypic characterization of MVN-deficient mutants suggest that MVN is involved in cell-cell recognition and cell-cell attachment of *Microcystis*. Another striking observation was the specific binding of microcystin to certain *Microcystis* proteins including Mrps that were found to accumulate to different levels in wild-type and mutant strains of PCC 7806. We are about to study the consequences of microcystin-binding to selected proteins.

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The biosynthetic genes and novel cyclic peptides anacyclins in strains of the genus *Anabaena*

David Fewer, Niina Leikoski, Jouni Jokela, Leo Rouhiainen, Kaarina Sivonen

Department of Applied Chemistry and Microbiology, University of Helsinki, Viikinkaari 9, Finland

david.fewer@helsinki.fi

Cyanobactins are small cyclic peptides harboring heterocyclized residues and are produced by a diverse selection of cyanobacteria through proteolytic cleavage of precursor proteins [1]. Here we report the discovery of a novel family of cyclic cyanobactins, anacyclins. We identified an 11-kb cyanobactin biosynthetic gene cluster in the complete genome of *Anabaena* sp. 90. The gene cluster encodes two subtilisin-like proteases and a short 49-aa protein with homology to patellamide, microcyclamide, tenuocyclamide and trichamide precursor proteins from other cyanobactin gene clusters. Bioinformatics combined with stable-isotope labeling led to the identification of the cyclic decapeptide anacyclin product of the gene cluster in *Anabaena* sp. 90. We carried out heterologous expression of the entire pathway in *Escherichia coli* and confirmed cleavage site predictions by comparison to a synthetic peptide. The anacyclin biosynthetic gene cluster is common in *Anabaena* strains from a wide selection of habitats and the products contain only unmodified proteinogenic amino acids. The length of the anacyclin peptides varied from seven to fifteen amino acids in different *Anabaena* strains with little sequence conservation. We carried out a PCR-based survey and demonstrated that the cyanobactin biosynthetic pathway is widespread in planktonic cyanobacteria.

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Glucose uptake and utilization by *Prochlorococcus* strains

Guadalupe Gómez-Baena¹, Antonio López-Lozano¹, Jorge Gil-Martínez², Jose Manuel Lucena², Jesús Díez¹, Pedro Candau², **Jose Manuel García-Fernández¹**

¹Departamento de Bioquímica y Biología Molecular, Universidad de Córdoba, Spain

²Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla-CSIC, Spain

bb1gafej@uco.es

The marine cyanobacteria *Prochlorococcus* have been considered photoautotrophic microorganisms, although the utilization of exogenous sugars has never been specifically addressed in them. We studied glucose uptake in several surface- and depth-adapted *Prochlorococcus* strains, as well as the effect of glucose addition on the expression of several glucose-related genes.

Glucose uptake was studied by adding radiolabelled glucose to *Prochlorococcus* cultures, followed by flow cytometry coupled with cell sorting in order to separate *Prochlorococcus* cells from bacterial contaminants. Sorted cells were recovered by filtration and their radioactivity measured. The expression, after glucose addition of several genes (involved in glucose metabolism, and in nitrogen assimilation and its regulation) was determined in the depth-adapted *Prochlorococcus* SS120 strain by semi-quantitative real time RT-PCR, using the *rnpB* gene as internal control.

Our results demonstrate for the first time that all studied *Prochlorococcus* strains take up glucose at significant rates even at concentrations similar to those found in the oceans, and also exclude the possibility of this uptake being carried out by eventual bacterial contaminants, since only *Prochlorococcus* cells were used for radioactivity measurements. Besides, we show that the expression of a number of genes involved in glucose utilization (namely *zwf*, *gnd* and *dld*, encoding glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and lactate dehydrogenase, respectively) is strongly increased upon glucose addition to cultures of the SS120 strain. This fact, taken together with the magnitude of the glucose uptake, clearly indicates the physiological significance of the phenomenon.

Given the significant contribution of *Prochlorococcus* to the global primary production, these findings have strong implications for the understanding of the phytoplankton role in the carbon cycle in nature. Besides, the ability of assimilating carbon molecules could provide additional hints to comprehend the ecological success of *Prochlorococcus*.

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From cyanobacterial hydrogenases to BioModularH₂

Paula Tamagnini^{1,2}

¹IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

²Departamento de Botânica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

pmtamagn@ibmc.up.pt

Cyanobacteria may contain two distinct NiFe-hydrogenases: an uptake enzyme found in N₂-fixing strains, and a bidirectional one present in both non-N₂-fixing and N₂-fixing strains examined. The uptake hydrogenase (encoded by *hupSL*) catalyzes the consumption of the H₂ produced during N₂-fixation, while the bidirectional enzyme (*hoxEFUYH*) probably plays a role in fermentation and/or acts as an electron valve during photosynthesis [1]. The *hupSL* genes constitute a transcriptional unit, and are mainly transcribed under N₂-fixing conditions. The bidirectional hydrogenase consists of a hydrogenase and a diaphorase part, and the corresponding five *hox* genes are not always clustered or cotranscribed. The synthesis/maturation of NiFe-hydrogenases is a highly complex process requiring several core proteins. In cyanobacteria, the genes that are thought to affect hydrogenases pleiotropically (*hyp*), as well as the genes presumably encoding the hydrogenase-specific endopeptidases (*hupW* and *hoxW*) have been identified and characterized. The presence of a single copy of most of the *hyp* genes/*hyp* operon in cyanobacteria suggests that their products might be responsible for the maturation of both hydrogenases in these organisms. Furthermore, NtcA, LexA, and AbrB-like have been implicated in the transcriptional regulation of the uptake and the bidirectional enzyme respectively. Recently, the phylogenetic origin of cyanobacterial and algal hydrogenases was analyzed, and it was proposed that the current distribution in cyanobacteria reflects a differential loss of genes according to their ecological needs or constraints. In addition, some of the possibilities and challenges of cyanobacterial-based H₂ production will be addressed. The BioModularH₂ project (FP6, NEST-2005-Path-SYN, Contract 043340) aims at designing reusable, standardized molecular building blocks that integrated into a “chassis” will produce a new photosynthetic bacterium containing engineered chemical pathways for competitive, clean and sustainable hydrogen production. Due to their simplest nutritional requirements and photosynthetic machinery, cyanobacteria are good candidates to accommodate these devices. Therefore, a photoautotrophic cyanobacterial *chassis* will be constructed, taking into consideration the putative constraints to the insertion and functionality of the above mentioned modules.

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Molecular aspects of microcystin production in the toxic cyanobacterium *Microcystis aeruginosa*

Sven Becker¹, Hans C.P. Matthijs², Ellen van Donk¹

¹Netherlands Institute of Ecology, NIOO-Centre for Limnology, Rijksstraatweg 6, 3631 AC Nieuwersluis, The Netherlands

²Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Nieuwe Achtergracht 127, 1018 WS Amsterdam, The Netherlands

s.becker@nioo.knaw.nl

Among the known mechanisms of induced defence (against grazers) in cyanobacteria, there are increased toxin (microcystin = MC) production (in toxic strains), colony formation, and spine formation in *Cyanobium* sp. (Jezberova & Komarkova, 2007). Jang et al. (2003) have investigated the effect of direct and indirect grazing on toxin production and colony formation in toxic and non-toxic *Microcystis* strains. Their results contribute to a whole array of conflicting studies in this field, and to date the function and/or biological role of MC in aquatic foodwebs has not been clarified. Additionally, only a limited number of molecular studies in this field are available, e.g. by Rantala et al. (2004), who demonstrated by phylogenetic analysis that the *mcy* genes responsible for toxin production in cyanobacteria emerged early in Earth's history (before the metazoan lineage) and have been lost in many cyanobacterial lineages. Hence, it seems unlikely that microcystins evolved as a means in defence against grazing. One of the striking results in a study by Schatz et al. (2007) demonstrates that MC and other peptides released from disrupted *Microcystis* cells can stimulate the McyB (protein) content and MC production in remaining undisrupted cells. To evaluate in our testing system effects found by Jang et al. (2003) and Schatz et al. (2007), we used batch cultures with *Microcystis aeruginosa* strain PCC7806 (toxic wildtype and non-toxic mutant) and *Daphnia magna* as direct and indirect grazer. In our treatments with high and low light conditions, we determined growth (biovolume), survival time of *Daphnia* individuals, relative cellular microcystin content and aggregate formation. Our results show that the presence of disrupted *Microcystis* cells increased the cellular MC content of the undisrupted cells, as does direct grazing. In contrast, an indirect effect of *D. magna* (via medium) on the cellular toxin production was not observed. Furthermore, the non-toxic mutant of strain *Microcystis* PCC7806 is ingested with a higher rate than the toxic wildtype strain, and in contrast to results by Jang et al. (2003), the survival time of *D. magna* is shorter in a toxic culture than in the non-toxic (mutant) culture. Future work will evaluate the (genome-wide) gene expression effects from this study with quantitative reverse-transcription PCR and micro-arrays.

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Studying a putative calcium-dependent potassium channel in *Synechocystis* sp. PCC 6803

Vanessa Checchetto, Manuela Zanetti, Giorgio Mario Giacometti, Ildikò Szabò, Elisabetta Bergantino

Department of Biology, University of Padova, Italy

vanessa.checchetto@unipd.it

Our work is focused on identification and characterization of a potassium channel in the cyanobacterium *Synechocystis* sp. PCC 6803. A bioinformatic screening of *Synechocystis* proteome identified a protein that displays sequence homology to MthK, a calcium-dependent potassium channel from *Methanobacterium thermoautotrophicum* [1]. Like MthK, this protein is predicted to contain two transmembrane regions and the selectivity filter characteristic of potassium channels. Moreover the *Synechocystis* putative channel (SynCaK) shares a significant similarity to a protein of *Arabidopsis thaliana* classified as a calcium-dependent potassium channel (KCO6) and predicted to localized in chloroplast by programs CloroP and TargetP.

Our goal is to clone and express SynCaK, to verify whether it works as a channel and if its function is somehow related to the photosynthetic processes.

In order to evaluate the SynCaK function we aim to produce a fusion protein SynCaK-GFP and to express it in a mammalian cell system, useful for patch-clamp analyses [2,3].

To study the importance of SynCaK in photosynthesis and in physiology of cyanobacteria, we plan to produce deletion and site-specific mutants in *Synechocystis*.

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The study of cyanobacteria with toxic potential belonging to genus *Microcystis* from Transylvania

Bogdan Drugă^{1,2}, Lucian Barbu-Tudoran³, Nicolae Dragoș^{1,2}

¹*Babeș-Bolyai University, Cluj-Napoca, Romania*

²*Institute of Biological Research, Cluj-Napoca, Romania*

³*Electron Microscopy Centre, Babeș-Bolyai University, Cluj-Napoca, Romania*

bogdandruga_ro@yahoo.com

This study aims the morphological and molecular analysis of the *Microcystis* strains from the Collection of Cyanobacteria and Alga Cultures of the Institute of Biological Research from Cluj-Napoca, Romania. We have also intended to study the hepatotoxic potential of these strains by molecular means and to correlate the results with toxicity tests on mice.

The study material of this project was represented by 24 cyanobacterial strains of the genus *Microcystis*. For a complete morphological characterization all the strains have been analyzed both in light and electron microscopy (scanning – SEM and transmission - TEM). In order to manage the quick and precise strain differentiation we have studied the discriminative capacity of the HIP (Highly Iterated Palindromes), STRR (Short Tandemly Repeated Repetitive) and LTRR (Long Tandemly Repeated Repetitive) genomic repeated sequences by their amplification through PCR. For the validation of the phylogenetic position of our strains we have sequenced the 16S rRNA gene and the ITS fragment, and then we have generated a phylogenetic tree employing certain bioinformatic methods. The hepatotoxic potential of the strains was analyzed by the PCR amplification of certain genomic regions considered responsible for the synthesis of the enzymes involved in toxin production. The results achieved by this molecular method were verified through certain toxicity tests that consisted in the intraperitoneal injection of mice with cyanobacterial suspension concentrated by centrifugation.

The strains analysis with the transmission electron microscope allowed us to observe certain pilus-like structures, known as “fimbria”. Their presence was also shown with the scanning electron microscope, in the form of specific bundles that seem to interconnect the cells.

The PCR amplification of the HIP genomic repeated sequences and the migration of the resulted amplicons in agarose gels lead us to the achievement of specific electrophoretic patterns for all the 24 analyzed strains. Based on these patterns, we made a dendrogram (UPGMA algorithm) that grouped all the strains in accordance with the presence / absence of the electrophoretic bands. Thus we concluded that the HIP repeated sequences are enough to differentiate the chroococcacean cyanobacteria, even at the infraspecific level.

The sequencing of the 16S rRNA gene and the 16S rRNA – 23S rRNA ITS fragment followed by the Interrogation of the public databases has been materialized by the construction of a phylogenetic tree which has confirmed the affiliation of our strains to the genus *Microcystis*. Nevertheless, the precise confirmation of the species was difficult in the case of some strains, probably due to certain inaccuracies in the annotation of the sequences from the databases.

The PCR amplification of some of the DNA fragments involved in the hepatotoxic potential has lead to the generation of specific amplicons in 7 of the 24 analyzed strains. These amplicons have further been characterized. The intraperitoneal injection of mice with cyanobacterial suspension has confirmed the presence of hepatotoxins (microcystins) in 5 of the 7 strains.

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***luxCDABE*-marked filamentous cyanobacteria as lights-off bioreporters of metal bioavailability and toxicity in aqueous samples**

Francisca Fernández-Piñas, Ismael Rodea-Palomares, Coral González-García, Francisco Leganés

Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, 28049 Madrid, Spain

francisca.pina@uam.es

We have developed a novel toxicity bioassay for detecting heavy metals in aqueous samples based on the use of a self-luminescent filamentous cyanobacterial bioreporter strain, which is a derivative of the freshwater cyanobacterium *Anabaena* sp. PCC 7120. The strain, denoted as CPB4337, bears in the chromosome a Tn5 derivative with *luxCDABE* from the luminescent terrestrial bacterium *Photorhabdus luminescens* and shows a high constitutive luminescence with no need to add exogenous aldehyde [1]. The toxicity assay that we have developed is based on the inhibition of bioluminescence caused by biologically available heavy metals ("lights-off" bioreporter). The bioassay allowed for acute as well as chronic toxicity testing. To address the question of bioavailability, we evaluated the effect of a series of potential modifying factors on toxicity of selected metals. For the latter experiments, chemical modelling was used to predict metal speciation and link it with toxicity. Toxicity appeared mainly related to the predicted metal free-ion concentration, although Zn-EDTA complexes and certain Hg chloro-complexes might exhibit some toxicity to cyanobacteria. The sensitive response of the cyanobacterial bioreporter highlights its potential use for rapid determination of heavy metal bioavailability and toxicity in environmental samples from different freshwater sources.

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Detection of toxigenic strains of cyanobacteria in hypertrophic lakes of Wielkopolska region

Ilona Gagala¹, Joanna Mankiewicz-Boczek², Mikolaj Kokocinski^{3,4}, Karolina Stefaniak⁴, Maciej Zalewski^{1,2}

¹Department of Applied Ecology, University of Lodz, ul. Banacha 12/16, 90-237 Lodz, Poland

²International Centre for Ecology, Polish Academy of Sciences, Tylna 3, 90-364 Lodz, Poland

³Collegium Polonicum in Slubice, Adam Mickiewicz University, ul. T. Kosciuszki 1, 69-100 Slubice, Poland

⁴Department of Hydrobiology, Adam Mickiewicz University, Marcelinska 4, 60-801 Poznan, Poland

ilona.gagala@hotmail.com

The most common in Polish water bodies are microcystin-producing cyanobacteria. *Planktothrix agardhii* was observed to be the most dominant species in lakes of Wielkopolska region. The genetic studies on cyanobacteria in lakes Bninskie, Bytynskie and Lubosinskie revealed the presence of microcystin synthetase genes, *mcyA* (96% of samples) and *mcyE* (100% of samples), in two-year lasting monitoring. The toxicity of cyanobacteria was determined by PPIA. In all analyzed samples the toxic microcystin variants were detected at concentration ranging between 1.44 µg/L – 26.54 µg/L (2006) and 0.48 µg/L – 27.28 µg/L (2007). The relationship between the quantity of *mcyE* gene, the toxicity of microcystin and biomass of *Planktothrix agardhii* was determined. The strong correlation between biomass of *Planktothrix agardhii* and quantity of *mcyE* gene in Bninskie Lake ($r = 0.74$; $p < 0.05$) and Lubosinskie Lake ($r = -0.63$; $p < 0.05$) was observed. Detection of dominating toxigenic strains of *Planktothrix agardhii* and synthesized microcystin in all investigated samples from yearlong monitoring indicated on a serious problem for studied water ecosystems and human health. Moreover, the increase of cyanobacterial risk in following summer season could be a consequence of toxigenic strains and cyanotoxins occurrence in winter and spring seasons.

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Rapid quantification of microcystin-producing cyanobacteria using real-time PCR

Henna Hautala¹, Erik Jokisalo^{1,2}, Markus Vehniäinen¹, Sonja Nybom³, Jussi Meriluoto³

¹*Dept of Biotechnology, University of Turku, Turku, Finland*

²*VWR International Oy, Espoo, Finland*

³*Dept of Biochemistry and Pharmacy, Åbo Akademi University, Turku, Finland*

henna.hautala@utu.fi

Mass occurrences, or blooms, of toxic cyanobacteria are common worldwide. They create a serious water quality problem in water resources and recreational waters. Several cyanobacterial genera, including *Microcystis*, *Anabaena* and *Planktothrix*, produce hepatotoxic microcystins which are a serious health risk to humans and have caused acute poisoning and death in animals. Because toxic and non-toxic strains of the same cyanobacterial species exist, even in the same bloom, morphological characteristics cannot be used to identify potentially harmful cyanobacteria. In EU, directive 2006/7/EC requires appropriate monitoring and adequate management measurements of bathing waters when a potential of cyanobacterial proliferation in bathing waters occurs.

Microcystins are synthesized non-ribosomally by large enzyme complexes mainly through peptide synthetase and polyketide synthase activities. The presence of microcystin synthetase genes in the genome of a cyanobacterium strain strongly suggests its ability to produce toxins. Thus these genes can be used as genetic determinants which enable the discrimination of non-toxic and potentially toxic strains. We have developed a real-time PCR-based method for quantifying microcystin-producing cyanobacteria of the genera *Microcystis*, *Anabaena* and *Planktothrix* from natural water samples. Our approach is based on amplification of a target sequence located within the microcystin synthetase operon and fluorescently labeled genus-specific oligonucleotide probes. Amplification of the target is detected in real-time by time-resolved fluorometry [1]. The assay has been tested using a total of 29 cyanobacterial strains obtained from the Pasteur Culture Collection (PCC) and Norwegian Institute for Water Research (NIVA). It has also been successfully applied to the analysis of lake water samples. The results have been verified with HPLC, mass spectrometry based methods, commercial ELISA kits and protein phosphatase inhibition assays [2].

PCR-based monitoring of water resources allows detection of very small amounts of potentially toxic cyanobacteria, serving as a basis for an early-warning alarm system. Genus-specific detection provides information about population dynamics and relative abundances of the different cyanobacterial genera. Indirect information about toxin concentrations could also be obtained if the correlation between microcystin synthetase copy numbers and toxin quantities is established. Rapid and reliable, yet simple methods such as real-time PCR will help in controlling the increasing problem of toxic cyanobacterial blooms as well as in understanding the underlying mechanisms of toxin production.

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Introduction of foreign hydrogenases into the cyanobacterium *Nostoc punctiforme*

Thorsten Heidorn, Peter Lindblad

Dept. of Photochemistry and Molecular Science, Uppsala University, Sweden

thorsten.heidorn@fotomol.uu.se

Photosynthetic microorganisms such as green algae and cyanobacteria can be used for the production of hydrogen from sunlight and water. The main obstacle for this method is that, on the one hand, the H₂ producing enzymes (hydrogenases and nitrogenases) are highly sensitive to oxygen, but on the other hand, oxygen is continuously evolved during photosynthesis (PSII). A temporal or spatial separation of photosynthesis and H₂ evolution are possible strategies for overcoming this problem.

In heterocystous cyanobacteria, the oxygen sensitive nitrogenase is only present in special cells, heterocysts, which lack the PSII-activity and are therefore under anaerobic conditions.

The nitrogenase catalyzes nitrogen fixation, where N₂ from the air is converted to ammonia in an energy consuming reaction. H₂ is produced as a by-product, but for the most part it is immediately oxidized by an uptake hydrogenase. To obtain higher yields, the uptake hydrogenase may be knocked out, and a more efficient [Fe] hydrogenase could be used as the H₂ generating enzyme.

Several uptake hydrogenase deficient mutants are available (including one in our own lab [1]) which evolve H₂ as a consequence of nitrogen fixation. Furthermore, the heterologous expression of different hydrogenases in different organisms (*Synechococcus* [2], *E. coli* [3], *Clostridium* [4]) has already been achieved in a few labs.

The objective of this project is to express an active algal [Fe] hydrogenase in the heterocysts of an uptake hydrogenase deficient mutant of *Nostoc punctiforme*. For this purpose the *Chlamydomonas reinhardtii* hydrogenase gene *hydA1* and two accessory genes (*hydEF* and *hydG*) are to be inserted into an expression vector (pSCR202), the vector is to be transformed into an uptake hydrogenase deficient mutant (NHM5) by electroporation, and finally the expression of the active enzyme is to be demonstrated. Results will be presented and discussed.

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RNA-based detection of hepatotoxic cyanobacteria

Hanna Hirvonen, Anne Rantala-Ylinen, Ilona Oksanen, Kaarina Sivonen

Dept. of Applied Chemistry and Microbiology, Univ. of Helsinki, Finland

hanna.hirvonen@helsinki.fi

Toxic cyanobacterial mass occurrences (blooms) are common in Finnish freshwater bodies and in the Baltic Sea. *Microcystis*, *Planktothrix* and *Anabaena* are the main hepatotoxin producers in Finnish lakes [2] and *Nodularia spumigena* in the Baltic Sea [1]. Toxin-producing and -nonproducing cyanobacterial strains often co-occur in blooms, containing producers even from several different genera [1]. Toxin-producing strains cannot be discriminated from nontoxic strains by traditional microscopy [1]. Toxins, microcystins/nodularins, are synthesised by non-ribosomal polyketide and peptide synthetase enzyme complexes encoded by microcystin/nodularin synthetase gene clusters. Based on these genes, hepatotoxic cyanobacterial strains can be detected and identified with molecular methods. The DNA-chip is a tool for the detection and identification of cyanobacterial groups and potential hepatotoxin producers [3]. Toxin producers from the genera *Anabaena*, *Microcystis*, *Planktothrix*, *Nodularia*, *Nostoc* and different cyanobacterial groups can be identified simultaneously with genus-specific probes for *mcyE/ndaF* and 16S rRNA genes, respectively. *mcyE* and *ndaF* genes encode enzymes responsible for synthesizing amino acids Adda and D-Glu, which are crucial for the toxicity of microcystins/nodularins. The aim of this study was to optimize the DNA-chip method further and use RNA as a target to identify not only the potential but also the active microcystin producers. In addition, *mcyE* gene and transcript copy numbers were quantified with *Anabaena*- and *Microcystis* specific TaqMan quantitative real time PCR (qPCR) methods. For this, RNA extraction and cDNA synthesis protocols for cyanobacterial strains and environmental samples were optimized. Chip results with DNA and cDNA extracted from toxic *Anabaena* and *Microcystis* strains were consistent. Both chip and qPCR methods were used to study the community composition and the potential and active microcystin producers in Lake Tuusulanjärvi samples from June to September 2006. According to 16S rRNA probes, *Anabaena*+*Aphanizomenon* and *Microcystis* were detected most frequently in both DNA and cDNA samples. In addition, *Synechococcus* and/or *Woronichinia* were present in all DNA samples. *Microcystis*- and *Anabaena-mcyE* genes and transcripts were detected with both methods in Lake Tuusulanjärvi. Potentially toxic *Microcystis* were present and expressed the *mcyE* genes earlier than *Anabaena*. Results from the chip and qPCR analyses were consistent when considering *Microcystis* but qPCR was more sensitive than the chip to detect *Anabaena-mcyE* genes. The highest *mcyE* gene and transcript copy numbers coincided with the highest microcystin concentrations in August and September samples when both microcystin producers were present and active. Molecular methods could also detect toxin producers in samples, from which microcystins were not measurable with LC/MS. These methods were excellent for monitoring potential and active microcystin producers from environmental samples, to follow toxic bloom development.

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Characterization of the uptake hydrogenase promoter activity in *Nostoc punctiforme* ATCC 29133

Marie Holmqvist, K. Stensjö, Peter Lindblad

Department of Photochemistry and Molecular Science, Angstrom Laboratory, Uppsala University, Box 523, SE - 751 20 Uppsala, Sweden

Marie.Holmqvist@fotomol.uu.se

Cyanobacteria and green algae are the only organisms capable of producing molecular hydrogen directly from solar energy through oxidative photosynthesis. This characteristic makes them interesting from a biotechnological point of view as future hydrogen producers of commercial use. Before that vision can be realised however more needs to be learned about the hydrogen metabolism in these organisms function. Cyanobacteria have three enzymes directly involved in hydrogen metabolism. These enzymes are nitrogenase, uptake hydrogenase and bidirectional hydrogenase [4]. During conditions of nitrogen starvation nitrogenase fix atmospheric nitrogen. Hydrogen is at the same time produced as a by-product. The uptake hydrogenase recaptures and oxidise the hydrogen produced by nitrogenase and recycles the energy, and are directly coupled to nitrogen fixation (4). In many nitrogen fixing filamentous strains the nitrogenase and the uptake hydrogenase are located to cells specialised for nitrogen fixation, so called heterocysts [4]. *Nostoc punctiforme* ATCC 29133 is a heterocystous filamentous nitrogen fixing cyanobacteria [3]. This strain contains one type of nitrogenase and one type of uptake hydrogenase but lacks the bidirectional hydrogenase [5]. The genes encoding the structural parts of the uptake hydrogenase, *hupS* and *hupL*, have been identified [2] and were found to be transcribed as one operon [1]. Furthermore a *tsp* has been found and putative binding sites for regulatory proteins have mapped in the promoter region [1]. To investigate the importance of these putative binding sites a promoter-deletion study was carried out where parts of the *hupSL* promoter were fused to the reporter gene *gfp*, encoding Green Fluorescent Protein or *luxAB*, encoding luciferase. The self-replicative reporter gene vectors were transferred to *N. punctiforme* ATCC 29133 by electroporation, and the in vivo promoter activity was measured. Furthermore, the actual binding of the regulatory protein NtcA to its corresponding putative binding site in the promoter was also investigated.

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An integrating cavity absorption meter as a detector of cyanobacteria in seas and oceans

Ivan Konyukhov

Biological Faculty, Moscow State University, Russia

vanka.kon@gmail.com

Recently, the chlorophyll fluorescence is widely used for rapid estimation of phytoplankton abundance in surface layer of seas and oceans as well as its distribution among different depths. Before the expansion of these methods oenologists characterized abundance of natural waters by the concentration of chlorophyll "a" – the pigment represented in all taxes of algae and cyanobacteria.

In random area, a researcher converts relative fluorescence units to the chlorophyll content in water through local calibration of his fluorometer. During such procedure fluorescence and concentration of chlorophyll (extracted in organic solvent) are determined simultaneously and then compared in a few samples of natural water. In a case of fixed taxonomical distribution of algae the good linear correlation exists between the chlorophyll content and fluorescence. However in a case of dominant species alteration the precision of the measurement of phytoplankton abundance via fluorescence significantly decreases.

We measured depth profiles of chlorophyll fluorescence and chlorophyll concentration in Black Sea. Under the 25-30 m the fluorescence begins to decrease earlier then the chlorophyll does. At these probes of water the presence of cyanobacteria detected by phycoerythrin absorption is obviously. One of the cyanobacteria feature is relatively poor portion of chlorophyll associated with PS II. The main part is aggregated with PS I reaction centers where the excited states are effectively quenched. For the equal quantity of the pigment, cyanobacteria emit up to 10 times less chlorophyll fluorescence then eukaryotic algae do. Therefore, information on appearance of cyanobacteria and their approximated content to the algal community is highly important for the correctly organized fluorometric mapping of phytoplankton distribution.

Low concentration of photosynthetic pigments in natural water restricts the application of standard spectrophotometers to study water samples. Every sample has to be preliminary concentrated. In a contrast, we present the results of spectroscopic analysis of natural phytoplankton by a hand-made integrating cavity absorption meter (ICAM). Due to the multiple internal reflection of measuring beam the effective optical pathway of the device increases to 5 meters. Optical system used has no movable elements; the total weight with the supply source is less then 6 kg. So it is well adapted for operation during sea expeditions. Just 5-10 min required to measure the dissolved organic matter spectrum and the spectrum of suspended particles (with phytoplankton). It is possible to obtain the information on the presence of cyanobacteria when the total chlorophyll content in water is as low as 0,05µg/l. Accordingly, we suppose ICAM to be a good supplementing instrument for studding cyanobacteria in natural conditions.

Calcium signalling in cyanobacteria: a proteomics approach

Francisco Leganés, Alberto Jorge, Francisca Fernández-Piñas

Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid. 28049 Madrid, Spain

francisco.leganes @uam.es

During the course of evolution, cyanobacteria have developed signal transduction systems that enable them to sense and respond to any change in their extracellular or internal milieu. The signalling systems generally include a series of receptors, intracellular messengers, enzymes and transcription factors; amongst intracellular messengers, calcium has arisen as probably the most versatile one in eukaryotes. Although a similar role for this divalent cation in prokaryotes is still elusive, there is increasing interest in and growing evidence for calcium as a regulator in this group of organisms. In any cell system, calcium signalling is initiated by the generation of a specific calcium signal (the so-called "calcium signature") in response to a stimulus. We have constructed a recombinant strain of *Anabaena* sp. strain PCC 7120 that constitutively expresses the Ca^{2+} binding photoprotein apoaequorin [1]. This strain allows continuous and *in vivo* monitoring of intracellular free calcium levels. By using this strain, we have analyzed the calcium signatures that are triggered by a variety of stimuli such as temperature, salt and osmotic shocks or light-to-dark transitions; interestingly, we have also found a specific calcium signal during the process of heterocyst differentiation [2]; recently, we recorded a similar signal under conditions that mimic nitrogen starvation; i.e. addition of 2-oxoglutarate to cells growing with a source of combined nitrogen. All these calcium signals need to be sensed and decoded by a series of protein components before the cell can give an appropriate response. At present, we have initiated a proteomics approach to identify those components that may constitute the Ca^{2+} signalling toolkit in cyanobacteria.

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Production of cyanophycin, a source for the biodegradable polymer polyaspartate, in transgenic plants

Maja Hühns¹, Tina Hausmann¹, Katrin Neumann¹, Karl Ziegler², Friederike Klemke², Wolfgang Lockau², Uwe Kahmann³, Elfriede K. Pistorius³, Inge Broer¹

¹*University of Rostock, Germany*

²*Humboldt University Berlin, Germany*

³*University of Bielefeld, Germany*

wolfgang.lockau@rz.hu-berlin.de

The production, in transgenic plants, of biodegradable polymers that can substitute for petrochemical compounds in commercial products is a challenge for plant biotechnology. The biodegradable polymer polyaspartate can replace synthetic polycarboxylates in many applications. Polyaspartate can be obtained from the cyanobacterial storage compound cyanophycin, a copolymer of L-aspartate and L-arginine. A single enzyme, cyanophycin synthetase CphA1, catalyzes cyanophycin biosynthesis. Potato tubers are particularly suitable for the production of biopolymers since they allow for cost-effective harvest as byproducts of starch extraction. To establish cyanophycin synthesis in plants, three different CphA1 genes were expressed constitutively in tobacco and potato plants. Only one of the three synthetases produced cyanophycin in plants, with up to 0.1 % polymer yield on a dry weight basis. Cyanophycin granules were detected by electron microscopy in various transgenic lines in leaves and for potato also in tubers. However, due to production of the polymer the transgenic tobacco and potato lines exhibited stress symptoms like reduced growth, variegated leaves and early flower induction. In order to circumvent these problems, the functional CphA1 gene was fused to various transit peptide sequences for import into plastids. Cyanophycin contents up to 5% of dry weight were obtained by tuber-specific production in amyloplasts, without phenotypical changes.

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Changes in lipid and fatty acid composition of cyanobacteria induced by additional carbon sources

Nataliya Mykhaylenko, Olena Zolotareva

Department of Membranology and Phytochemistry, M.G. Kholodny Institute of Botany, Kyiv, Ukraine

nf_mykhaylenko@mail.ru

In addition to oxygenic photosynthesis, cyanobacteria are able to assimilate some organic compounds that contribute to carbon and/or energy supply of the cells. The unique feature of cyanobacterial bioenergetics is the occurrence of both photosynthetic and respiratory electron transport chains in the same thylakoid membrane [1]. Lipid composition of cyanobacterial membranes resembles that of thylakoid and inner envelope membranes of plant chloroplasts [2]. They contain three glycolipids: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol, sulfoquinovosyldiacylglycerol, and the sole phospholipid, phosphatidylglycerol (PG). Therefore the protein complexes involved in cyanobacterial respiration are active in lipid environment that differs substantially from that in mitochondria and bacteria. It seems to be of great interest to examine the effects of glucose, a key metabolite of a photosynthetic cell, on cyanobacterial membrane lipid status. It is well known that in higher plants and algae glucose inhibits photosynthesis not only by feedback regulation of enzyme activities, but also on the level of gene expression (e.g. via hexokinase signal pathway) [3]. The effects of supplementation with organic substances, namely glucose, mannose (poorly metabolizable glucose epimer capable of triggering hexokinase-mediated repression of photosynthetic genes) and sodium citrate on growth, lipid and fatty acid composition of filamentous cyanobacteria, *Spirulina platensis* (Nordst.) Geitl. (alkaliphilic water cyanobacterium) and two species of *Nostoc linckia* (Roth) Born. et Flah. in sensu Elenk. (soil cyanobacterium) were studied in the present research. *S. platensis* cells in comparison with *N. linckia* were characterized by higher absolute contents of chlorophyll and all glycerolipid classes, somewhat lower parts of galactolipids but strikingly higher PG proportion. Glucose evoked the active growth of cyanobacteria but chlorophyll content was reduced to some extent. Mannose inhibited cyanobacterial growth as well as chlorophyll synthesis, however PG content was higher than that in respective control samples. MGDG content tended to diminish when organic compounds were added, and PG content in most cases increased appreciably. Fatty acid composition of *S. platensis* lipids differed from that of *N. linckia* by the lower proportions of stearic, oleic and palmitoleic acids together with the higher percent of octadecatrienoic acid. The proportion of unsaturated fatty acids was reduced markedly in dark-grown *N. linckia*. The addition of hexoses resulted in higher relative content of oleic acid in lipids, and the same effect was observed sometimes for cyanobacteria cultivated with sodium citrate. The increased PG content in cyanobacteria grown under organic supplementation suggests that this lipid may be indispensable for proper functioning of certain components of the respiratory electron transport chain, and the regulation of gene expression may be involved.

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Identification of genes putatively involved in the synthesis of cyanobacterial exopolysaccharides

Sara Pereira^{1,2}, Ângela Brito^{1,2}, Andrea Zille¹, Pedro Moradas-Ferreira^{1,3} & Paula Tamagnini^{1,2}

¹IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

²Departamento de Botânica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

³Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal

sarap@ibmc.up.pt

Many cyanobacterial strains possess outside their outer membrane, additional surface structures, mainly of polysaccharidic nature, that are usually referred to as exopolysaccharides (EPSs). Even though EPSs are common in cyanobacteria, their biosynthetic pathways and the factors that regulate these processes are far from being fully understood [1]. However, the mechanisms involved in the synthesis of EPSs seem to be relatively conserved for Gram⁻ and Gram⁺ bacteria [2-5], requiring the combined action of a large number of gene products that can be divided into two distinct groups: the proteins required for the synthesis of sugar nucleotides from which the repeat unit is constructed (probably encoded by housekeeping genes), and proteins encoded by *eps*-specific genes, which are necessary for the assembly of the polymer, and its translocation across the membrane [2]. The *eps*-specific genes are usually clustered and organized in a similar manner in different bacteria. Within these clusters three different regions can be discerned: a central region constituted by the genes encoding the glycosyltransferases, flanked by two other regions comprising the genes encoding enzymes involved in the chain-length control, export, and polymerization [6].

Gloeotheca sp. PCC 6909/ATCC 27152 is a unicellular N₂-fixing cyanobacterium possessing two types of EPSs: a well defined sheath and an outer amorphous layer [6]. Analysis of the protein profiles of *Gloeotheca* wild type and its sheathless mutant*, revealed that the mutant is deficient in dTDP-glucose 4,6-dehydratase, an enzyme involved in the synthesis of rhamnose, one of the sugars present on *Gloeotheca*'s EPSs [6]. In this work, the gene encoding this enzyme – *rfbB* – was partially sequenced for both the wild type and the sheathless mutant, and considerable differences were observed. *In silico* analysis of the available cyanobacterial genomes revealed the presence of several putative *eps*-specific genes that, in other organisms, are involved in the last steps of the synthesis of EPSs. Those genes, namely, *wza*, *wzb*, *wzc* (*wzz*), *wzx* and *wzy* are frequently clustered, often occurring as multiple copies. Using this information, another gene – *wza* – which encodes a protein involved in the export of the polysaccharide, was identified in *Gloeotheca*. At the moment RT-PCR experiments are underway in order to determine if the genes previously identified are constitutively expressed or if its transcription is affected by the growth conditions.

* (obtained by chemical mutagenesis using nitrosoguanidine, by S. Shestakov, Moscow, and kindly provided by L. Stal, NIOO-KNAW, The Netherlands).

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A synthetic biology approach for H₂ photobiological production, using *Synechocystis* sp. PCC 6803 as a cyanobacterial “chassis”

Filipe Pinto^{1,2}, Miguel Lopo¹, Catarina Pacheco^{1,3}, Daniela Ferreira^{1,2}, Pedro Moradas-Ferreira^{1,3}, Paula Tamagnini^{1,2}

¹IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

²Departamento de Botânica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

³Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal

fvpinto@ibmc.up.pt

Molecular hydrogen (H₂) is an environmentally clean energy carrier that can be a valuable alternative to the limited fossil fuel resources of today. The BioModularH₂ project (FP6, NEST-2005-Path-SYN, Contract 043340) aims at designing reusable, standardized molecular building blocks that integrated into a “chassis” will produce a photosynthetic bacterium containing engineered chemical pathways for competitive, clean and sustainable hydrogen production. This engineering approach will provide the next generation of synthetic biology engineers with the toolbox to design complex circuits of high potential industrial applications such as the photo-production or photo-degradation of chemical compounds with a very high level of integration. The potential “chassis” should be well studied organisms with high throughput genomic and proteomic data available, minimalist in terms of the subset of genes that will allow retaining viability, and easy to engineer with the available molecular tools, becoming a versatile platform for multiple purpose applications. Taking these criteria into consideration the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 emerges as a suitable candidate for a photoautotrophic “chassis”. The synthetic biological approach within this project aims at creating an anaerobic environment within the cell for an optimized and highly active hydrogenase, by using an oxygen consuming device, which will be connected to an oxygen sensing device and regulated by artificial circuits. This project will also help to establish a systematic hierarchical engineering methodology (parts, devices and systems) to design artificial bacterial systems using a truly interdisciplinary approach that decouples design from fabrication. The construction of biological molecular parts will be achieved by engineering proteins with new enzymatic activities and molecular recognition patterns, by combining computational and in vitro evolution methodologies. Subsequently, novel devices will be designed (e.g. input/output, regulatory and metabolic) by combining these parts and by using the emerging knowledge from systems biology. The construction of the photoautotrophic cyanobacterial “chassis”, based on a model-driven biotechnology, will take into consideration the putative constraints to the insertion and functionality of the synthetic circuits. In this context, bioinformatics tools are required to predict, design and simulate biochemical pathway interactions and the responses of the biological systems to changes. Concomitantly, the effect of different physiological conditions on *Synechocystis* growth will be assessed to feed and validate the developed theoretical models.

Environmental diagnostics of hepatotoxic cyanobacteria

Anne Rantala-Ylinen¹, David P. Fewer¹, Hanna Hirvonen¹, Ermanno Rizzi², Kerttu Koskenniemi¹, Kaarina Sivonen¹

¹Dept. of Applied Chemistry and Microbiology, Univ. of Helsinki, Finland

²Institute of Biomedical Technologies - INRC, Milan, Italy

anne.rantala@helsinki.fi

Planktonic, potentially hepatotoxic cyanobacterial genera, *Anabaena*, *Microcystis*, *Planktothrix*, and *Nodularia* form frequently mass occurrences (blooms). A bloom can comprise both toxic and nontoxic strains and it can even include several of these genera. However, toxin-producing strains cannot be separated from the non-producing strains by conventional microscopy [1]. Hepatotoxins, microcystins and nodularins, are synthesized non-ribosomally by peptide synthetase polyketide synthase enzyme complexes encoded by the orthologous microcystin/nodularin synthetase gene clusters. These biosynthetic genes have been utilized in developing molecular methods for recognition of toxin-producing strains [1]. Our object was to develop various methods based on the evolutionary stable *mcyE/ndaF* genes [2] to detect and identify hepatotoxin-producing cyanobacteria in environmental samples. *mcyE/ndaF* genes encode enzymes involved in the synthesis of Adda and D-Glu, the constituent amino acids crucial for the toxicity of microcystin and nodularin molecules. In addition, the *mcyE/ndaF* genes are present in the biosynthetic gene clusters of different producer genera and thus allow detection of both microcystin and nodularin producers. With conventional PCR and genus-specific primers we showed that the co-occurrence of potentially microcystin-producing *Anabaena*, *Microcystis*, and *Planktothrix* was common in Finnish lakes [3]. In addition, the proportion of lakes with multiple toxin producers was greater in more eutrophicated lakes [3]. With quantitative real-time PCR (qPCR) methods, we could identify and quantify the main hepatotoxin producers both in lakes [4] and the Baltic Sea [5]. A DGGE analysis of a general *mcyE/ndaF*-PCR product revealed for the first time the widespread occurrence and genetic variation of microcystin-producing *Anabaena* in the Baltic Sea [6]. DNA-chip using genus-specific probes for *mcyE/ndaF* genes of *Anabaena*, *Microcystis*, *Planktothrix*, *Nostoc*, and *Nodularia* was designed to reveal all potential microcystin/nodularin producers in a single analysis making it suitable for high-throughput analysis and monitoring of environmental samples [4]. DNA-chip and *Anabaena*- and *Microcystis*-specific qPCR methods were further optimized to be used with environmental RNA as target. Using RNA instead of DNA allowed for recognition of active transcription of the biosynthetic genes and thus even a more reliable indication of actual microcystin production in the lake water samples.

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The genome of *Anabaena* sp. strain 90, the microcystin producing filamentous cyanobacterium

Leo Rouhiainen¹, Hao Wang¹, David Fewer¹, Arnaud Taton², Zhijie Li³, Bin Liu⁴, Kaarina Sivonen¹

¹University of Helsinki, Department of Applied Chemistry and Microbiology, Viikki Biocenter, Finland

²Center for the Study of Biological Complexity, Virginia Commonwealth University, Richmond, VA, USA

³BGI Life Tech Co., Ltd., Beijing, P.R.China

⁴Beijing Genomics Institute, Beijing, P.R.China

leo.rouhiainen@helsinki.fi

The whole genomic sequence of the nitrogen fixing, hepatotoxic (microcystin producing) *Anabaena* sp. strain 90 was determined. The genome consists of 4.3 Mb circular chromosome and four circular plasmids with the sizes of 820, 80, 56 and 20 kb. The large gene clusters for the biosynthesis of microcystins [1], anabaenopeptilides [2] and anabaenopeptins (the non-ribosomal peptides regarded as secondary metabolites) were located in the chromosome. These gene clusters together with the microcin-like ribosomal cyclic peptide coding gene cluster comprise approximately 5 % of the genome. The number of the proteins found common in *Anabaena* 90 and in other sequenced genomes of cyanobacteria was explored. Specially those, which are found also in the microcystin producing *Microcystis aeruginosa* NIES-843 [3] and in *Nostoc punctiforme* PCC 32102. The presence of numerous repeated sequences in the genome of *Anabaena* 90 is reported here as well as the preliminary annotation of the proteins coded by plasmid genes. Future studies will use the information gained from genomic sequences to uncover the function of the bioactive peptides in cyanobacteria and to understand the role of the producers in their environment.

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Role of sucrose synthase in the conversion of sucrose to polysaccharides in filamentous nitrogen-fixing cyanobacteria

Leonardo Curatti, Laura E. Giarrocco, Andrea C. Cumino, Graciela L. Salerno

CEBB-Centro de Investigaciones Biológicas, FIBA, Vieytes 3103, 7600 Mar del Plata, Argentina

gsalerno@fiba.org.ar

Sucrose (Suc) is synthesized by plants, unicellular algae and cyanobacteria as part of the carbon dioxide assimilation pathway. While Suc metabolism is an essential pathway in plants, its significance in oxygenic photosynthetic bacteria is still not fully understood. Particularly, Suc synthase (SuS, EC 2.4.1.13), which catalyzes a readily reversible reaction of Suc synthesis and cleavage, is widespread in the plant kingdom and in filamentous heterocyst-forming cyanobacteria [1]. In higher plants, SuS has critical functions, and particularly, in Suc to starch and other polysaccharides interconversion [2]. However, knowledge of the relationship between Suc and glycogen metabolism and the function and regulation of SuS in cyanobacteria, remains still fragmentary. The interconnection between glycogen and Suc metabolism in *Anabaena* sp. PCC 7119 N₂-fixing filaments was recently studied. It has been shown that SuS activity is modulated by the nitrogen source at the transcriptional level [3,4]. Different lines of evidence point to a key role of Suc cleavage by SuS, located in the photosynthetic vegetative cells, in the control of carbon flux in the nitrogen-fixing filaments of heterocystous cyanobacteria [3,4]. In the present work we have studied the functional relationship between SuS and the metabolism of polysaccharides in *Anabaena* strains. We show that the nitrogen and carbon sources and light regulate the expression of the SuS encoding gene (*susA*), in a similar way that they regulate the accumulation of polysaccharides. Furthermore, glycogen content in an *Anabaena* sp. mutant strain with an insertion inactivation of *susA* was lower than in the wild-type strain under diazotrophic conditions, while both glycogen and polysaccharides levels were higher in a mutant strain constitutively overexpressing *susA*. We also show that there are soluble and membrane-bound SuS forms in *Anabaena*. Taken together, these results strongly suggest that SuS is involved in the Suc to polysaccharides conversion according to nutritional and environmental signals in filamentous nitrogen-fixing cyanobacteria. These findings support a role of SuS in the cycling of sugar-nucleotides and in the regulation of the flux of carbon between Suc and polysaccharides. Also, this study contributes to an emerging concept that shows that not only the enzymology of Suc and polysaccharides metabolism has been conserved during evolution from cyanobacteria to higher plants, but also that some basic regulatory aspects of carbon assimilation have been conserved as well. Supported by grants of ANPCyT (PICT 2004 N° 21227-53), UNMdP and CONICET (PID 6105).

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Response of the redox system to arsenate and arsenite in the cyanobacterium *Synechocystis* sp. PCC 6803

Ana María Sánchez-Riego, Luís López-Maury, Francisco J. Florencio

Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla-CSIC. Avda. Américo Vespucio 49, 41092 Sevilla

amriego@ibvf.csic.es

Arsenic is one of the most important global environmental pollutants which displays a variety of toxic effects on living organisms resulting in severe disturbances of cellular metabolism. [1] Arsenic-induced toxic effects are related to its high ability of reacting with protein and nonprotein thiol groups resulting in alterations of critical cellular pathways and oxidative stress induction [2] Because of the wide use and distribution of arsenic compounds, arsenic resistance is wide-spread among living organisms. Arsenic resistance in *Synechocystis* sp. PCC 6803 is mediated by an operon that includes an arsenite transporter gene, *arsB*, an *arsH* homologue without a clear function in arsenic resistance and an arsenate reductase gene, *arsC* [1] that uses the glutathione/glutaredoxin system for reduction. There are two additional genes coding for an additional arsenate reductase: *arsI1* and *arsI2*, which are nearly identical to each other.

Glutaredoxins, peroxiredoxins and thioredoxins, are proteins conserved throughout evolution, and play key roles in maintaining the cellular redox balance [3]. The *Synechocystis* genome contains 5 genes encoding peroxiredoxins (Prxs): *1cys prx*, *2cys prx*, *prxII*, *prxQ1* and *prxQ2*; 4 genes encoding thioredoxins (Trxs): *trxA*, *trxB*, *trxQ* and *trxC* and 3 genes encoding glutaredoxins (Grxs): *grxA*, *grxB* and *grxC*.

We have analyzed the expression of the different Prxs, Trxs and Grxs in response to the arsenic forms As^{V} and As^{III} in WT strains and two mutants unable to metabolize arsenate (As^{V}) and arsenite (As^{III}). Our results indicate that arsenite promotes a general activation of the genes involved in the oxidative stress response. In addition we corroborate that GrxA plays a central role in arsenate reduction since all strains lacking GrxA are less tolerant to the presence of As^{5+} in the media, and we concluded that, in general, tioredoxins, glutaredoxin and peroxiredoxin genes respond to arsenic compounds, because of oxidative stress induction.

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Distribution of nonribosomal peptide synthetases in thermotolerant cyanobacteria and partial genomic analysis of *Fischerella* sp. MV11 using 454 sequencing

Ingeborg Scholz¹, Richard Reinhardt², Michael Kube², Karin Finsinger¹, Jürgen Weckesser¹, Wolfgang R. Hess¹

¹University of Freiburg, Faculty of Biology, Schänzlestr. 1, D-79104 Freiburg

²Max-Planck-Institute for Molecular Genetics, Ihnestr. 63, 14195 Berlin, Germany

ingeborg.scholz@biologie.uni-freiburg.de

Many cyanobacteria are known to possess secondary metabolites produced by nonribosomal peptide synthetases (NRPS) and polyketide synthases (PKS). Here, we have analysed the distribution of NRPS and PKS genes in 78 cyanobacterial isolates from geothermal sites and hot springs of Costa Rica, characterized four of these strains in more detail and performed in case of *Fischerella* sp. MV11 a partial genomic analysis to gain insight into the genomic structure of one of the NRPS cluster.

NRPS-A-domains were amplified by using degenerate PCR and subsequent sequencing of randomly selected cloned PCR fragments, revealing that 51,3 % of the tested strains gave positive amplification results regarding NRPS genes. 41 % of the 78 strains were tested positive for PKS domains. The molecular, morphological and physiological characterization of four selected isolates identified one strain as *Westiellopsis* sp. Ar73, and strains RV14, MV9 and MV11 to belong to the genus *Fischerella* (*Mastigocladus*), forming a distinct clade in phylogenetic analyses. Thermotolerance was measured from 25°C to 60°C, uncovering 35°C as the optimum for the *Fischerella* strains, but growing up to 55 °C. In contrast, *Westiellopsis* sp. Ar73 was rather mesophilic as growth was inhibited at 45°C and optimal at 25-35°C.

A fosmid library for *Fischerella* sp. MV11 was constructed and hybridized with cloned NRPS probes. This screening resulted in 120 possible positives out of 1553 fosmids. 40 hybridizing fosmids were selected for 454 sequencing, which yielded a total of 1,256,000 unique nucleotide sequence assembled into 95 contigs. In parallel, the NRPS-positive fosmid # 102 was used for traditional sequence analysis to control the quality and assembly of 454-derived sequence information. The combined information yielded a contig of 41.604 kb, which comprehends 29 genes, including full length NRPS and a putative halogenase gene. This is the first complete NRPS gene cluster from a thermotolerant cyanobacterium.

The detection and identification of potentially toxic cyanobacteria in Polish water

Joanna Głowacka, Małgorzata Waleron, Magdalena Szefel-Markowska, Krzysztof Waleron
Department of Biotechnology, Faculty of Biotechnology University of Gdańsk and Medical University of Gdańsk. Kładki 24, 80-822 Gdańsk, Poland.

waleron@biotech.univ.gda.pl

Blooms of toxic cyanobacteria, which create major threats to animal and human health, aquaculture, tourism and recreation have become an increasing worldwide problem in aquatic habitats. The main goal of this study was the detection and identification of potentially toxic cyanobacteria directly in environmental samples from selected Polish lakes, ponds, rivers, lagoons, Baltic Sea and Gulf of Gdańsk. Samples were collected from 2002 till 2004 from 9 water bodies where mass occurrence of cyanobacteria were visible and from 40 water reservoirs where blooms were not observed. Sampling from 12 water bodies was periodic. The presence of potentially toxic cyanobacteria was confirmed by detection of the microcystin peptide synthetase genes *mcyB* and *mcyE* (toxigenicity markers). Both toxin encoding genes were found in 7 (per 9) water blooms of cyanobacteria (Bnińskie lake, Bytyńskie lake, Trzesiecko lake, pond in Drzewce, pond in Gumnisko, lagoon in Nadarzyce, lagoon in Sulejów). In Klasztorne and Tuchom lake only *mcyB* gene was detected. These blooms were potentially toxic. In case of samples collected from Polish water bodies (where blooms were not observed) *mcyB* and *mcyE* genes were detected in 41 samples delivered from 20 water bodies. In lakes: Klasztorne Duże, Ostrzyckie, Kielno and lagoon of Wistula the presence of potentially toxic cyanobacteria was observed from July to September, in Trzesiecko lake from June till October and in pond in Kowalewo both genes were detected in October and September. We did not detect *mcyB* gene in Mausz lake nor *mcyE* gene in Raduńskie lake. In lakes: Bielsko, Ciemino, Dobre, Dolsko, Jeleń, Kamienickie, Żur, pond in Lębork, pond in Piaszczyzna, lagoon of Zęborzycki and in rivers: Gwda, Łeba, Radunia, Słupia, Tralalka and also in the Gulf of Gdańsk any of the encoding microcystin genes were observed. In order to identify potentially toxic cyanobacteria we used molecular methods: *rpoC1*-PCR-RFLP analysis and clone libraries of *rpoC1* and 16S rDNA genes. As a result, *Microcystis* strains were identified in ponds (Drzewce, Mniewo), lakes (Chodzieskie, Kielno, Klasztorne, Trzesiecko, Tuchom,) and in lagoon of Sulejów and lagoon of Nadarzyce. Cyanobacteria belonging to genera *Planktothrix* were identified in lakes Bnińskie, Bytyńskie, Klasztorne, Mausz, Ostrzyckie, Raduńskie and in a pond in Gumnisko.

The presence of toxin producing strains (*Microcystis*, *Planktothrix*) in environmental samples was dominant during summer (July, August). These results are in agreement with earlier morphological observations. PCR detection of toxin encoding genes is a useful method for identification of the beginning of cyanobacteria bloom and is valuable method for monitoring of the presence toxic cyanobacteria directly in environmental samples. The conducted research supplied new information about the appearance of potentially toxic cyanobacteria in Polish water reservoirs.

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Cyanobacteria – a source of bioactive substances useful for plant protection and biocontrol

Małgorzata Waleron¹, Joanna Luszczynska¹, Magdalena Miklaszewska¹, Ewa Lojkowska¹, Annick Wilmotte², Krzysztof Waleron^{1,2}

¹Intercollegiate Faculty of Biotechnology Univ. of Gdansk & Medical Univ. of Gdansk, Poland

²Institute of Chemistry, Univ. of Liege, Belgium

goniawu@biotech.ug.gda.pl

One of the numerous interesting properties of Cyanobacteria is the production of biologically active compounds. Among them, there are substances inhibiting the growth of other organisms, as well as the enzymes that inactivate the signal molecules involved in the quorum sensing (QS) mechanism. QS is a kind of cell-to-cell communication between microorganisms that is mediated by chemical signals and dependent on the signal molecule concentrations. The most extensively studied QS systems rely on the use of *N*-acylhomoserine lactones (AHLs) signal molecules. AHLs are used by plant pathogenic bacteria to control the production of virulence factors and for plant colonization. Some bacteria produce enzymes that are able to inactivate AHL signals produced by others and hamper QS-mediated processes via a phenomenon known as quorum quenching (QQ). This mechanism seems to be a promising strategy to control bacterial plant diseases.

The aim of the presented work was to assess whether 72 reference and 17 polar cyanobacteria strains have antimicrobial activities against 12 plant pathogenic strains belonging to *Pectobacterium*, *Dickeya* and *Brenneria* genera, and if they produce enzymes which degrade the AHLs.

The antibacterial activity screening revealed seven positive strains, *Chroococcidiopsis* sp. PCC7203, *Pleurocapsa* sp. PCC7516, *Stanieria* sp. PCC7301, *Nostoc punctiforme* PCC73102, *Oscillatoria* sp. PCC7112, *Lyngbya* sp., *Phormidium laminosum*. The broadest spectrum of antimicrobial activity was observed for water extract of *P. laminosum*, which inhibited growth of all tested phytopathogens. In fact, the substances inhibiting the growth of plant pathogenic bacteria were produced by *Brevibacillus brevis* which grows in cyanobacterial sheaths. Interestingly, the antibiotic substance was secreted only when the *B. brevis* was in consortium with the cyanobacterium, phytopathogen and other *Bacillus* species.

All cyanobacterial strains showing antagonistic activity against plant pathogens were checked if they were also toxigenic. None of them possessed the genetic determinants (*mcyB* and *E* genes) responsible for the synthesis of the most common cyanotoxin (microcystin).

The AHL degradation properties were observed in the stationary phase of growth for four cyanobacterial strains, *Anabaena* sp. PCC 7120, *Gloeobacter violaceus* PCC7421, *Nostoc punctiforme* PCC73102, and *Anabaena* PCC7122. The sequence comparison of the *aiiC* gene encoding AHL-acylase from *Anabaena* sp. PCC 7120 with other cyanobacterial genomes available in the databases allowed designing new PCR primers to detect the enzyme responsible for the degradation of AHLs. The presence of a 1500 bp *aiiC* gene fragment was observed for *Anabaena* sp. PCC7120, *G. violaceus* PCC 7421, *N. punctiforme* PCC 73102, *N. punctiforme* Hegewald 1971-108, *Nostoc* sp. PCC7937, *Nostoc* sp. UM3, *A. variabilis* NIVA-CYA19 and Antarctic strain S8. The sequencing of the PCR products confirmed that the new primers could serve for fast screening of cyanobacterial strains producing enzymes which degrade the AHLs.

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AbrB-like protein plays an important role in modulating hydrogenases expression

Xiaohui Zhang, Åsa Agervald, Peter Lindblad

Department of Photochemistry and Molecular Science, Uppsala University, Uppsala, Sweden

xiaohui.zhang@fotomol.uu.se

AbrB-like proteins in many cyanobacteria regulate different metabolic processes, such as production of cylindrospermospin [1], photosynthesis, CCM process. In our group, AbrB-like proteins were found binding to different hydrogenases promoter regions. In *Synechocystis* sp. Strain PCC 6803, AbrB-like protein Sll0359 binds to *hox* promoter and functions as an activator [2]. In this study, an AbrB-like protein Alr0946 was fished out from promoter region of *hyp* (Hyp proteins are involved in maturation of NiFe hydrogenases). Electrophoretic mobility shift assays were conducted to confirm the protein binding to the promoter region and further to investigate its binding site on the DNA. The EMSA results show it indeed binds to *hypC* promoter region and the binding site is at 5' end of promoter region. The binding ability to shorter region is weaker compared with that to the whole promoter region. It implies this protein forms a polymer and its binding causes long DNA bending, while the shorter segments cannot form a stable protein-DNA complex. In order to study regulation function, an overexpression strain of Alr0946 with the control of *nirA* promoter and an empty vector strain were constructed. Quantitative real time PCR results reveal the transcription level of Alr0946 is more than 30 times higher after the induction of nitrate. The expression of *hyp* gene is dramatically inhibited when Alr0946 overexpressed; the expression level of *hypC* gene drops to 10^{-3} of those in the wild type strain and empty vector strain. Bidirectional hydrogenase gene *hoxE* is also down-regulated slightly. Expression of *alr0946* and *alr0947* operon is repressed by overexpression of Alr0946, simultaneously; the expression of this operon is regulated when medium changed from NH_4^+ to NO_3^- . A deletion mutant is under construction, the regulation network of AbrB-like protein in large scale will be investigated by using microarray or proteomics methods, by comparing different mutant strains and wild type strain.

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The regulatory effect of glucose on *Spirulina platensis* Photosystem II activity

Nataliya Mykhaylenko, Olena Zolotareva

Department of Membranology and Phytochemistry, M.G. Kholodny Institute of Botany, Kyiv, Ukraine

e.zolotareva@mail.ru

Low-molecular carbohydrates, especially glucose, not only play the pivotal role in the metabolism of photosynthetic cell, but they can also act as primary messengers in the transduction of regulatory signals to the genome [1]. One of the principal mechanisms involved in glucose signaling is hexokinase-dependent system [2]. Poorly metabolizable glucose analogues capable of triggering hexokinase-mediated photosynthesis repression are widely used for the discrimination between nutrient and signaling functions of glucose. The details of sugar-induced signal transduction are intensely studied in bacteria, yeast, mammals and higher plants, but less attention is paid to cyanobacteria, the organisms capable of both autotrophic and heterotrophic nutrition. Moreover, the fact that both photosynthetic and respiratory electron transport chains in cyanobacteria are active in the same thylakoid membrane and share some components [3] may impart additional versatility to their metabolism, thus enhancing the regulatory role of glucose. The goal of the present research was to characterize Photosystem II (PSII) activity of the cyanobacterium *Spirulina platensis* (Nordst.) Geitl. grown in the presence of glucose, poorly metabolizable glucose analogues (2-deoxyglucose and mannose), or non-carbohydrate carbon source (sodium citrate), by means of chlorophyll fluorescence assay. The following fluorescence parameters were determined [4]: 1) F_V/F_M , the maximal efficiency of PSII photochemistry; 2) F_V'/F_M' , the efficiency of excitation energy capture by open PSII reaction centres; 3) q_P , the photochemical quenching coefficient, which measures the proportion of open PSII reaction centres; 4) Φ_{PSII} , the quantum yield of PSII electron transport; 5) q_N , the nonphotochemical quenching coefficient; 6) NPQ, Stern-Volmer nonphotochemical quenching. At first glucose supplementation evoked the decrease in all photochemical quenching parameters (F_V/F_M , F_V'/F_M' , q_P and Φ_{PSII}), and the rate of the decrease correlated with glucose concentration. The inhibition of photochemical activity relaxed on the third day of glucose addition. If the concentration of glucose in the cultural medium was high (50 mM), on the fourth day all the indices of photochemical quenching began to diminish again. The relief of glucose repression of PSII photochemical processes was light-dependent. In the presence of glucose analogues the decrease in only one photochemical quenching parameter, q_P , was observed. Nonphotochemical fluorescence quenching parameters (q_N and NPQ) of light-grown *S. platensis* were also reduced under sugar treatment. The addition of 5 mM sodium citrate virtually did not affect the photosynthetic activity. Therefore, the multiplicity of glucose effects in the course of inhibition of *S. platensis* PSII activity was demonstrated. The hexokinase-dependent repression mechanism was playing only a partial role and resulting in the impediments to electron withdrawal from PSII reaction centers.

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Accumulation of protective carotenoids in phosphatidylglycerol deficient *Synechocystis* cells

Ildikó Domonkos, Przemysław Malec², Hajnalka Laczko-Dobos¹, Ozge Bozkurt¹, Kazimierz Strzałka², Zoltan Gombos¹

¹*Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary*

²*Department of Plant Physiology and Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland*

domonkos@brc.hu

Phosphatidylglycerol (PG) depletion resulted in a remarkable suppression of photosynthetic oxygen evolving activity in *Synechocystis* PCC6803 cells. Insufficient amount of PG led to similar effect as high-light exposure of the cells. PG depletion is likely to generate deleterious substances like reactive oxygen species and other free radicals. Carotenoids are among the most potential molecules that are able to protect photosynthetic and other energy generating processes against light-induced damage. PG depletion induced an increase in echinenone and myxoxanthophyll content of the cells. High amounts of these carotenoids can protect the structure and function of photosynthetic apparatus. The increased carotenoid content was localized in isolated thylakoid and cytoplasmic membranes and also in a soluble fraction of the cells. The soluble cell fraction contains carotene derivatives which may bind to proteins. These carotene-protein complexes are similar to orange carotenoid protein that is involved in protection against free radicals and reactive oxygen species. Increase in the content of myxoxanthophyll and echinenone suggests that PG depletion regulates the biosynthetic pathway of specific carotenoids.

INTERACTIVE COMPUTER SESSION

On the road with CyanoBIKE: lessons learned addressing problems in cyanobacterial molecular biology

Arnaud Taton¹, Jeff Elhai¹, JP Massar², Bogdan Mihai¹, Peter Seibel³, Jeff Shrager⁴, Mark Slupesky², Mike Travers⁵

¹*Center for the Study of Biological Complexity, Virginia Commonwealth University, Richmond, VA, U.S.A.*

²*Berkeley, CA, U.S.A.*

³*Gigamonkeys Consulting, Oakland, CA, U.S.A.*

⁴*Symbolic Systems Program (consulting), Stanford University, Stanford, CA, U.S.A.*

⁵*CommerceNet, Palo Alto, CA, U.S.A.*

aataton@vcu.edu

It is a rare biologist concerned with cellular and molecular matters that does not make some use of the wealth of mass biological information now available. However, it is unusual for a biologist to have the computational abilities to take full advantage of that information. Most are confined to the services provided by the small set of programs with which they are familiar.

CyanoBIKE (**C**yanobacterial **B**iological **I**ntegrated **K**nowledge and programming **E**nvironment) offers an alternative that can help cyanobacteriologists make more creative use of biological information. It contains all publicly available cyanobacterial genomes and their annotations and incorporates into its language common concepts of molecular biology (e.g. "translation" and "ortholog"). Many bioinformatics functions (e.g. sequence searches, sequence alignment, motif discovery, and phylogenetic tree construction) can be accessed through a common graphical interface, and users can combine genome analysis with microarray data, protein domains, metabolic pathways and other information available from other resources (e.g. GenBank and KEGG). Its graphical interface provides the power and flexibility of a programming language (e.g. to do repetitive tasks or create new tools) but also caters to users who wish to use simple but powerful commands. In brief, CyanoBIKE allows biologists with little computational experience the opportunity to access and analyze biological information in ways suggested by the problem at hand rather than by a limited set of tools. All the capabilities of CyanoBIKE are publicly accessible through the web (<http://biobike.csbc.vcu.edu>).

Over the past 2 years, one of us (AT) has visited approximately 40 cyanobacterial laboratories. We will report on ways that CyanoBIKE has addressed complex computational problems faced in these labs that would normally require the services of a computer programmer. These problems include:

- Comparison of protein domains from large sets of signal transduction proteins
- Determination of the phylogenies of hundreds of proteins in order to predict lateral transfer
- Annotation of thousands of insertion mutations
- Correlation of the presence/absence of regulatory motifs with proteomic, transcriptomic and metabolomic data
- Prediction of operons based on complex criteria.

Addresses of Participants

Dr. David G. Adams
University of Leeds
Biological Sciences
Leeds LS2 9JT United Kingdom
d.g.adams@leeds.ac.uk

Ms. Åsa Agervald
Uppsala University
Photochemistry and Molecular Science
Uppsala SE-751 20 Sweden
asa.agervald@fotomol.uu.se

Dr. Ghada Ajlani
CNRS/CEA IBITECS
Gif-sur-yvette 91191 France
gajlani@cea.fr

Dr. Juan B. Arellano
Instituto de Recursos Naturales y Agrobiología
(IRNASA-CSIC)
Abiotic Stress
Salamanca 37008 Spain
juan.arellano@irnasa.csic.es

Prof. Eva-Mari Aro
University of Turku
Department of Biology
Turku FI-20014 Finland
evaaro@utu.fi

Dr. Michael M. Babykin
Moscow State University
International Biotechnological Centre
Moscow 119992 Russia
babykin@mail.ru

Mr. Yehonatan Bar-Yosef
Hebrew university
Plant & Environment
Jerusalem 91904 Israel
yehonatan.baryosef@mail.huji.ac.il

Dr. Sven Becker
NIOO-KNAW Centre of Limnology
Aquatic Food Webs
Nieuwersluis NL-3631AC Netherlands
s.becker@nioo.knaw.nl

Dr. Elisabetta Bergantino
University of Padova
Department of Biology
Padova I-35131 Italy
elisabetta.bergantino@unipd.it

Prof. Birgitta Bergman
Stockholm University
Department of Botany
Stockholm SE 10691 Sweden
bergmanb@botan.su.se

Ms. Katerina Bernardova
Biology Centre AS CR

Institute of Hydrobiology
České Budějovice 37001 Czech Republic
kacabka@volny.cz

Mr. Wojciech Bialek
University of Wrocław
Biophysics
Wrocław 51-148 Poland
wojciech.bialek@ibmb.uni.wroc.pl

Dr. Daniela Billi
University of Rome
Biology
Rome 133 Italy
billi@uniroma2.it

Dr. Luděk Bláha
Institute of Botany, Academy of Sciences
Centre for Cyanobacteria
Brno CZ62500 Czech Republic
blaha@recetox.muni.cz

Dr. Nicolas Blot
Station Biologique (CNRS, Paris VI)
UMR7144-Adaptation and Diversity in Marine
Environment
Roscoff 29680 France
blot@sb-roscoff.fr

Dr. Marko Boehm
Imperial College London
Life Sciences
London SW72AZ, United Kingdom
darthvad5@hotmail.com

Prof. Thomas Börner
Humboldt University
Biology
Berlin 10115 Germany
thomas.boerner@rz.hu-berlin.de

Ms. Ozge Bozkurt
Hungarian Academy of Science
Biological Research Center
Szeged 6726 Hungary
ozgebozkurt@hotmai.com

Mr. Matthias J. Broser
Technical University Berlin
Institut für Chemie / Max-Volmer-Laboratorium
Berlin 10623 Germany
matthias.broser@tu-berlin.de

Dr. Laura Bruno
University of Rome
Biology
Rome 173 Italy
laura.bruno@uniroma2.it

Mr. Daniel P. Canniffe
University of Sheffield
Molecular Biology & Biotechnology

Sheffield S10 2TN United Kingdom
mbp06dpc@sheffield.ac.uk

Mr. Rafael Fernandez Carazo
CIP Laboratory of Cyanobacteria
Liège 4100 Belgium
rfernandez@ulg.ac.be

Prof. John G. Cobley
University of San Francisco
Chemistry
San Francisco CA 94117 United States
cobley@usfca.edu

Mr. Cristian Coman
Babes-Bolyai University
Experimental Biology
Cluj-Napoca 40 Romania
cr.coman@yahoo.com

Ms. Elisa Corteggiani Carpinelli
University of Padova
Biology
Padova 35131 Italy
elisa.corteggiani@unipd.it

Dr. Paul A. Davison
Sheffield University
Molecular Biology and Biotechnology
Sheffield S10 2TN United Kingdom
p.davison@sheffield.ac.uk

Mr. Dennis Dienst
Humboldt University Berlin
Plant Biochemistry
Berlin 10115 Germany
dennis.dienst@gmx.de

Dr. Ildiko Domonkos
Hungarian Academy of Sciences
Biological Research Center
Szeged 6726 Hungary
domonkos@brc.hu

Mr. Bogdan Druga
Babes-Bolyai University, Cluj-Napoca
Biology and Geology
Cluj-Napoca 400672 Romania
bogdandrugar_o@yahoo.com

Prof. Julian J. Eaton-Rye
University of Otago
Biochemistry
Dunedin 9054 New Zealand
julian.eaton-rye@stonebow.otago.ac.nz

Dr. Irina Elanskaya
Moscow Lomonosov State University
Biology
Moscow 119992 Russia
ivelanskaya@mail.ru

Dr. Francisca Fernandez-Piñas
Universidad Autonoma de Madrid
Biology
Madrid 28049 Spain
francisca.pina@uam.es

Ms. Daniela Ferreira
IBMC CAM
Porto 4150-180 Portugal
daniferr@ibmc.up.pt

Dr. David P. Fewer
University of Helsinki
Applied Chemistry and Microbiology
Helsinki FI-00014 Finland
david.fewer@helsinki.fi

Prof. Enrique Flores
CSIC-Universidad de Sevilla, Instituto de
Bioquímica Vegetal y Fotosíntesis
Sevilla E-41092 Spain
eflores@ibvf.csic.es

Ms. Markéta Foldynová
University of South Bohemia
Faculty of Science
České Budějovice 37005 Czech Republic
meta@email.cz

Dr. Maria Alessia Fuiano
University of Padova
Department of Biology
Padova 35131 Italy
fuiano.mariaalessia@libero.it

Ms. Ilona Gagala
University of Lodz
Applied Ecology
Lodz 90-237 Poland
ilona.gagala@hotmail.com

Dr. Jose Manuel Garcia-Fernandez
Universidad de Cordoba
Bioquímica y Biología Molecular
Cordoba E14071 Spain
bb1gafej@uco.es

Dr. Laurence Garczarek
Station Biologique (CNRS, PARIS VI)
UMR7144-Adaptation and Diversity in Marine
Environment
Roscoff 29680 France
garczare@sb-roscoff.fr

Mr. Jens Georg
Universität Freiburg
Genetik
Freiburg 79104 Germany
jens.georg@biologie.uni-freiburg.de

Dr. Yoram Gerchman
Haifa University-Oranim
Biology
Tivon 36006 Israel
gerchman@research.haifa.ac.il

Mr. Gregor Josef Gierga
University of Freiburg
Baden-Württemberg
Freiburg 79104 Germany
gregor.gierga@biologie.uni-freiburg.de

Ms. Joanna Glowacka

Intercollegiate Faculty of Biotechnology
UG & AMG
Department of Biotechnology
Gdansk 80-822 Poland
glowacka@biotech.univ.gda.pl

Prof. Susan S. Golden

Texas A&M University
Biology College Station
77843-3258 United States
sgolden@tamu.edu

Dr. James W. Golden

Texas A&M University
Biology College Station
77843-3258 United States
jgolden@tamu.edu

Ms. Vera Lúcia Goncalves

ITQB-UNL
Biological Chemistry Division
Oeiras 2780-157 Portugal
verag@itqb.unl.pt

Dr. Simonetta Gribaldo

Pasteur Institute
Ile de France
Paris 75015 France
simo@pasteur.fr

Dr. Muriel Gugger

Institut Pasteur
Cyanobacteries
Paris 75015 France
mgugger@pasteur.fr

Ms. Liisa Gunnelius

University of Turku
Department of Biology
Turku FIN-20014 Finland
liisa.gunnelius@utu.fi

Ms. Maya Haimovich

Hebrew University
Plant
Jerusalem 96629 Israel
maya.haimovich@mail.huji.ac.il

Ms. Henna Anneli Hautala

University of Turku
Dept. of Biotechnology
Turku 20500 Finland
henna.hautala@utu.fi

Dr. Thorsten Heidorn

Uppsala University
FotoMol
Uppsala 75120 Sweden
thorsten.heidorn@fotomol.uu.se

Ms. Stephanie Hein

University of Freiburg
Biology
Freiburg 79104 Germany
stephanie.hein@jupiter.uni-freiburg.de

Prof. Antonia Herrero

Consejo Superior de Investigaciones Científicas
Instituto de Bioquímica Vegetal y Fotosíntesis
Seville 41092 Spain
herrero@ibvf.csic.es

Prof. Wolfgang R. Hess

University of Freiburg
Faculty of Biology
Freiburg D-79104 Germany
wolfgang.hess@biologie.uni-freiburg.de

Dr. Birgit Heyduck-Soller

University of Bremen Marine Microbiology
Bremen 28359 Germany
bhs@uni-bremen.de

Ms. Hanna Leena Hirvonen

University of Helsinki
Department of Applied Chemistry and Microbiology
Helsinki 14 Finland
hanna.hirvonen@helsinki.fi

Ms. Marie Holmqvist

Uppsala University
Photochemistry and Molecular Science
Uppsala SE-75121 Sweden
Marie.Holmqvist@fotomol.uu.se

Ms. Vanessa Checchetto

University of Padova
Biology
Padova 35131 Italy
vanessa checchetto@unipd.it

Mr. Danny Ionescu

Hebrew University of Jerusalem
Plant and Environmental Sciences
Jerusalem 91904 Israel
danny.ionescu@mail.huji.ac.il

Mr. Takami Ishizuka

The University of Tokyo
Department of Life Sciences (Biology)
Tokyo 153-8902 Japan
takami-i@bio.c.u-tokyo.ac.jp

Dr. Iwona Jasser

Warsaw University
Microbial Ecology
Warsaw 02-096 Poland
jasser.iwona@biol.uw.edu.pl

Ms. Sheila I. Jensen

University of Copenhagen
Marine Biological Laboratory
Helsingør 3000 Denmark
sijensen@bio.ku.dk

Dr. David Kaftan

Institute of Systems Biology and Ecology ASCR
Structure and Function of Proteins
Nové Hrády 37333 Czech Republic
kaftan@ufb.jcu.cz

Dr. Radek Kaňa

Institute of Microbiology, Academy of Sciences
Department of Autotrophic Microorganisms
Třeboň 37981 Czech Republic
kana@alga.cz

Ms. Ruth Noemi Kaplan-Levy

IOLR KLL
Migdal 14950 Israel
ruth@ocean.org.il

Ms. Anne Karradt

Humboldt-University Berlin
Plant Biochemistry
Berlin D-10115 Germany
anne.karradt@gmx.de

Prof. Cheryl A. Kerfeld

DOE-JGI/UCB
Plant and Microbial Biology
Walnut Creek 94598 United States
ckerfeld@lbl.gov

Mr. Satoshi Kimura

University of Tsukuba
Graduate School of Life and Environmental
Sciences
Tsukuba 305-8572 Japan
s0730467@ipe.tsukuba.ac.jp

Dr. Josef Komenda

Institute of Microbiology
Autotrophic Microorganisms
Třeboň 37981 Czech Republic
komenda@alga.cz

Mr. Ivan Konyukhov

Moscow State University
Biophysics, Biological Fac.
Moscow 119991 Russia
vanka.kon@gmail.com

Ms. Jana Kopečná

Institute of Microbiology
Laboratory of Photosynthesis
Třeboň 37981 Czech Republic
kopečna@alga.cz

Mr. Markus Krummenacker

SRI International
BRG
Menlo Park 94025 United States
kr@ai.sri.com

Ms. Hisako Kubota

University of Tokyo
Arts and Sciences
Tokyo 153-0041 Japan
hkubota@bio.c.u-tokyo.ac.jp

Dr. Nicoletta La Rocca

University of Padova
Department of Biology
Padova 35131 Italy
nicoletta.larocca@unipd.it

Mr. Yannick J. Lara

University of Liege
CIP
Liege B4000 Belgium
ylara@ulg.ac.be

Mr. John O.A. Larsson

Stockholm University
Department of Botany
Stockholm 10691 Sweden
John.Larsson@botan.su.se

Dr. Francisco Leganes

Universidad Autonoma de Madrid
Biology
Madrid 28049 Spain
francisco.leganes@uam.es

Ms. Limor Waisberg Keren

Weizman Institute
Rehovot 76100 Israel
lpkeren@weizmann.ac.il

Prof. Peter Lindblad

Uppsala University
Dept Photochemistry and Molecular Science
Uppsala SE-751 20 Sweden
Peter.Lindblad@fotomol.uu.se

Dr. Debbie Lindell

Technion
Biology
Haifa 32000 Israel
dlindell@tx.technion.ac.il

Mr. Juha Matti Linnanto

University of Jyväskylä
Chemistry
Jyväskylä FIN-40014 Finland
linnanto@jyu.fi

Mr. Wolfgang Lockau

Humboldt University Berlin
Biology
Berlin D-10115 Germany
wolfgang.lockau@rz.hu-berlin.de

Mr. Fernando Lopes Pinto

Uppsala University
Photochemistry and Molecular Science
Uppsala SE-75120 Sweden
fernando.lopespinto@fotomol.uu.se

Mr. Miguel Pedro Lopo

IBMC
Cellular and Applied Microbiology
Porto 4150-180 Portugal
mlopo@ibmc.up.pt

Dr. Ignacio Luque

CSIC and Universidad de Sevilla
Instituto de Bioquímica Vegetal y Fotosíntesis
Sevilla 41092 Spain
ignacio.luque@ibvf.csic.es

Dr. Iris Maldener
University of Tübingen
Microbiology/Organismic Interactions
Tübingen 72076 Germany
iris.maldener@uni-tuebingen.de

Dr. Vicente Mariscal Romero
CSIC
Instituto de Bioquímica Vegetal y Fotosíntesis
Sevilla 41092 Spain
vicente.mariscal@ibvf.csic.es

Mr. Alejandro Mata Cabana
CSIC - Universidad de Sevilla
IBVF
Sevilla 41092 Spain
matacabana@ibvf.csic.es

Ms. Adriana Mazurkiewicz
Warsaw University
Microbial Ecology
Warsaw 02-096 Poland
ada.mazurkiewicz@biol.uw.edu.pl

Ms. Daniella Mella
Station Biologique (CNRS, Paris VI)
UMR7144-Adaptation and Diversity in Marine
Environnement
Roscoff 29680 France
dmella@sb-roscoff.fr

Mr. Jan Mitschke
University of Freiburg
Experimentelle Bioinformatik
Freiburg 79104 Germany
jan.mitschke@biologie.uni-freiburg.de

Mr. Remus Mohr
University of Freiburg
Institute for Biology III Genetics
Freiburg 79106 Germany
remus.mohr@biologie.uni-freiburg.de

Ms. Vicki L. Moore
Arizona State University
School of Life Sciences
Tempe AZ 85281 United States
vlmoore@asu.edu

Dr. Isabella Moro
University of Padova
Department of Biology
Padova 35131 Italy
isabella.moro@unipd.it

Dr. M. Isabel Muro-Pastor
CSIC-Universidad de Sevilla
IBVF
Sevilla 41092 Spain
imuro@ibvf.csic.es

Ms. Nataliya F. Mykhaylenko
M.G.Kholodny Institute of Botany, Nat. Acad. Sci.
Ukraine
Membranology and Phytochemistry
Kyiv 1601 Ukraine
nf_mykhaylenko@mail.ru

Prof. Peter J. Nixon
Imperial College
Life Sciences
London SW7 2 United Kingdom
p.nixon@imperial.ac.uk

Mr. Paulo Oliveira
Uppsala University
Dept. of Photochemistry and Molecular Science
Uppsala SE 751 20 Sweden
paulo.oliveira@fotomol.uu.se

Dr. Elvira de Olmedo-Verd
CSIC IBVF
Sevilla 41092 Spain
eolmedo@ibvf.csic.es

Dr. Takashi Osanai
The University of Tokyo
Life Sciences (Biology)
Tokyo 153-8902 Japan
cosanai@mail.ecc.u-tokyo.ac.jp

Dr. Friedrich Ossenbühl
University Ulm
Molecular Botany
Ulm 89077 Germany
friedrich.ossenbuehl@uni-ulm.de

Prof. George W. Owtrim
University of Alberta
Biological Sciences
Edmonton Alberta T6G 2E9 Canada
g.owtrim@ualberta.ca

Ms. Catarina C. Pacheco
IBMC
Cellular and Applied Microbiology
Porto 4150-180 Portugal
cclopes@ibmc.up.pt

Dr. Frederic Partensky
Station Biologique (CNRS, PARIS VI)
UMR7144
Roscoff 29682 France
partensk@sb-roscoff.fr

Mr. Jiří Patera
Institute of Microbiology
Laboratory of Photosynthesis
Třeboň 37981 Czech Republic
patera@alga.cz

Dr. Ling Peng
CNRS UPR 3118
Department de Chimie
163 Avenue de Luminy
Marseille 13288 France
ling.peng@univmed.fr

Ms. Sara Bernardes Pereira
IBMC - Institute for Molecular and Cell Biology
Cellular and Applied Microbiology
Porto 4150-180 Portugal
sarap@ibmc.up.pt

Mr. Filipe Pinto
IBMC MCA
Porto 4150-180 Portugal
fvpinto@ibmc.up.pt

Ms. Maija Elina Pollari
University of Turku
Department of Biology
Turku 20014 Finland
maipol@utu.fi

Dr. Ondřej Prášil
Institute of Microbiology
Laboratory of Photosynthesis
Třeboň 37981 Czech Republic
prasil@alga.cz

Ms. Leonor Puerto
Sevilla University
and CSIC Instituto de Bioquímica Vegetal
Sevilla 41092 Spain
leonor@ibvf.csic.es

Dr. Marina G. Rakhimberdieva
A. N/ Bakh Institute of Biochemistry
Russian Academy of Sciences
Laboratory of Chloroplast Biochemistry
Moscow 119071 Russia
rakhimberd@inbi.ras.ru

Mr. Vitor Manuel Capela Ramos
IBMC MCA
Porto 4150-180 Portugal
vmramos@ibmc.up.pt

Dr. Liang Ran
Stockholm University
Botany
Stockholm 10691 Sweden
liang@botan.su.se

Dr. Anne Rantala-Ylinen
University of Helsinki
Applied Chemistry and Microbiology
Helsinki FI-00014 Finland
anne.rantala@helsinki.fi

Prof. Nicoletta Rascio
University of Padova
Department of Biology
Padova 35131 Italy
nicoletta.rascio@unipd.it

Prof. Ziv Reich
Weizmann Institute of Science
Biological Chemistry
Rehovot 76100 Israel
ziv.reich@weizmann.ac.il

Prof. N. J. Robinson
Newcastle University
Cell and Molecular Biosciences
Newcastle NE2 4HH United Kingdom
n.j.robinson@ncl.ac.uk

Dr. Leo A. O. Rouhiainen
University of Helsinki
Dpt. of Applied Chemistry and Microbiology
Helsinki 14 Finland
leo.rouhiainen@helsinki.fi

Dr. Graciela L. Salerno
Fundacion Inv. Biol. Aplic.
Centro Inv. Biologicas
Mar del Plata 7600 Argentina
gsalerno@fiba.org.ar

Mr. Gustaf Sandh
Stockholm University
Department of Botany
Stockholm 106 91 Sweden
sandh@botan.su.se

Dr. Katia Sciuto
University of Padova
Department of Biology
Padova 35131 Italy
katia.sciuto@unipd.it

Dr. Ivan Šetlík
Institute of Microbiology
Czech Academy of Sciences
Autotrophic Microorganisms
Třeboň 37901 Czech Republic
setlik@alga.cz

Dr. Eva Šetliková
Institute of Microbiology
Czech Academy of Sciences
Autotrophic Microorganisms
Třeboň 37901 Czech Republic
setlikova@alga.cz

Ms. Verena Schön
University Freiburg
Department of Biology
Freiburg 79104 Germany
schoen.verena@web.de
Prof. Georg R. Schmetterer
University of Vienna
Institute of Physical Chemistry
Vienna A-1090 Austria
georg.schmetterer@univie.ac.at

Prof. Dirk Schneider
Albert-Ludwigs-University
Biochemistry & Molecular Biology
Freiburg 79104 Germany
dirk.schneider@biochemie.uni-freiburg.de

Ms. Ingeborg Doris Scholz
University of Freiburg
Experimental Bioinformatics
Freiburg im Breisgau 79104 Germany
ingeborg.scholz@biologie.uni-freiburg.de

Mr. Jan Schrübbers
University Bremen
Marine Microbiology
Bremen 28359 Germany
jan.schruebbers@web.de

Ms. Ana María Sánchez-Riego

Universidad de Sevilla – CSIC
IBVF
Seville 41092 Spain
amriego@ibvf.csic.es

Dr. Roman Sobotka

Institute of Microbiology
Laboratory of Photosynthesis
Třeboň 37981 Czech Republic
sobotka@alga.cz

Dr. Igor N. Stadnichuk

A.N.Bakh Institute of Biochemistry
Russian Academy of Sciences
Moscow 119071 Russia
stadnichuk@mail.ru

Dr. Claudia Steglich

University of Freiburg
Biology
Freiburg 79104 Germany
claudia.steglich@biologie.uni-freiburg.de

Ms. Augusta Maria Streza

Babes-Bolyai University
Business
Cluj-Napoca 40 Romania
samy_augu@yahoo.com

Dr. Assaf Sukenik

Israel Oceanographic & Limnological Research
Kinneret Limnological Laboratory
Migdal 14950 Israel
assaf@ocean.org.il

Dr. Tina Summerfield

University of Otago
Department of Biochemistry
Dunedin 9054 New Zealand
tina.summerfield@stonebow.otago.ac.nz

Prof. Michael L. Summers

California State University Northridge
Biology
Northridge 91330-8303 United States
mls42367@csun.edu

Dr. Iwane Suzuki

University of Tsukuba
Graduate School of Life and Environmental
Sciences
Tsukuba 305-8572 Japan
iwanesh6803@biol.tsukuba.ac.jp

Dr. Ildiko Szabo

University of Padova
Biology
Padova 35121 Italy
ildi@civ.bio.unipd.it

Ms. Magdalena Szeffel-Markowska

Intercollegiate Faculty of Biotechnology UG & AMG
Department of Biotechnology
Gdansk 80-822 Poland
mszeffelm@mp.pl

Dr. Paula Tamagnini

IBMC CAM
Porto 4150-180 Portugal
pmtamagn@ibmc.up.pt

Prof. Kan Tanaka

Chiba University
Graduate School of Horticulture
Matsudo 271-8510 Japan
kntanaka@iam.u-tokyo.ac.jp

Mr. Mirosław Tarnawski

University of Wrocław
Biophysics
Wrocław 51-148 Poland
mirekt@ibmb.uni.wroc.pl

Dr. Arnaud Taton

Virginia Commonwealth University
Center for the Study of Biological Complexity
Richmond VA-23284 United States
aataton@vcu.edu

Mr. Scott A. Taylor

NIOO-KNAW Centre of Limnology
Aquatic Food Webs
Nieuwersluis NL-3631AC Netherlands
s.becker@nioo.knaw.nl

Dr. Martin Tichý

Institute of Microbiology
Czech Academy of Sciences
Autotrophic Microorganisms
Třeboň 37901 Czech Republic
tichym@alga.cz

Dr. Esa Tyystjärvi

University of Turku
Department of Biology
Turku FI-20014 Finland
esatyy@utu.fi

Dr. Taina Tyystjärvi

University of Turku
Department of Biology
Turku FI-20014 Finland
taityy@utu.fi

Mr. Theoden C. Vigil-Stenman

Stockholm University
Botany Department
Stockholm 10691 Sweden
vigil@botan.su.se

Ms. Agnieszka Walczak

University of Gdansk
Marine Biology and Ecology
Gdynia 81-378 Poland
aga_walczak@hotmail.com

Dr. Krzysztof Waleron

University of Liege
CIP Center for Protein Engineering
Liege B 4000 Belgium
waleron@biotech.univ.gda.pl

Dr. Malgorzata Waleron
University of Gdansk Department of
Biotechnology
Gdansk 80-822 Poland
goniawu@biotech.ug.gda.pl

Mr. Thomas Wallner
University of Giessen
Microbiology
Giessen 35390 Germany
ThoWallner@gmx.de

Dr. Martin Welker
Technichal University Berlin
Environmental Microbiology
Berlin 10587 Germany
martin.welker@chem.tu-berlin.de

Prof. Annegret Wilde
Justus-Liebig-University Giessen
Institute of Microbiology and Molecular Biology
Giessen 39392 Germany
Annegret.Wilde@mikro.bio.uni-giessen.de

Ms. Ebru Sebnem Yilmaz
Hacettepe University
Biology Department
Ankara 6800 Turkey
esebnem@hacettepe.edu.tr

Ms. Meral Yilmaz
Anadolu University
Biology
Eskisehir 26470 Turkey
meralyilmaz@anadolu.edu.tr

Ms. Eliška Zapomělová

Biology Centre AS CR
Institute of Hydrobiology
Česke Budějovice CZ-37005 Czech Republic
eliska.zapomelova@seznam.cz

Mr. Cheng-Cai Zhang
CNRS LCB-IBSM
Marseille 13402 France
cczhang@ibsm.cnrs-mrs.fr

Dr. Pengpeng Zhang
University to Turku
Department of Biology
Turku 20520 Finland
pzhang@utu.fi

Dr. Xiaohui Zhang
Uppsala University
Department of Photochemistry and Molecular
Science
Uppsala SE-75120 Sweden
xiaohui.zhang@fotomol.uu.se

Dr. Jie Zhou
Institute of Microbiology
Chinese Academy of Sciences
Center of Bio-energy & Industrial Biotechnology
Beijing 100101 China
jiezhouw@im.ac.cn

Dr. Olena K. Zolotareva
M.G.Kholodny Institute of Botany
Nat. Acad. Sci. Ukraine
Membranology and Phytochemistry
Kyiv 1601 Ukraine
e.zolotareva@mail.ru

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Zapomělová E.	103, 120		
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