Welcome

Welcome at the 10th European Workshop on the Molecular Biology of Cyanobacteria!

The organizer team from Biological Research Center Jibou, are pleased to meet you in the beautiful city of Cluj Napoca in the heart of Transylvania, the North-Western province of Romania. The venue of this meeting is Grand Hotel Italia Cluj Napoca, which offers the best accomodating conditions for the workshop and is a landmark of the hotel industry in Cluj.

Biological Research Center Jibou, a small research center and Botanical Garden, located approximately 70 km North-East of Cluj-Napoca, in Salaj county, is honored to organize, for the first time in Romania, the 10th edition of this successful European Workshop. Every three years, cyanobacteriologists from all over the world meet together to exchange their knowledge in this exciting conference series. The 10th edition promises to be very exciting with a high number of very interesting oral presentations and posters.

With the support of the local staff, the best conference facilities and the beautiful city of Cluj-Napoca we hope that this meeting will be a very memorable experience from scientific and social point of view.

Chair of the Organizing Committee
Dr. Cosmin Sicora
Director of Biological Research Center Jibou

Committees

International Scientific Committee

- **Prof. Dr. Annegret Wilde**
  Institute of Biology III, University Freiburg, 79104 Freiburg, Germany
- **Prof. Dr. Conrad Mullineaux**
  School of Biological & Chemical Science, Queen Mary, University of London, Mile End Rd, London E1 4NS, UK.
- **Prof. Dr. Enrique Flores**
  Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas and Universidad de Sevilla, Spain,
- **Prof. Dr. Eva-Mari Aro**
  Molecular Plant Biology, University of Turku, Turku, Finland
- **Prof. Dr. Lucas J. Stal**
  NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry and Utrecht University
- **Prof. Dr. Masahiko Ikeuchi**
  Department of Life Sciences (Biology), Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan
- **Prof. Dr. Min Chen**
  School of life and environmental Sciences, University of Sydney, NSW 2006, Australia
- **Prof. Dr. Paula Tamagnini**
  Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal;
- **Prof. Dr. Wim Vermaas**
  School of Life Sciences and Center for Bioenergy and Photosynthesis, Arizona State University, Tempe, AZ 85287-4501, USA

Local Organizing Committee

Biological Research Centre Jibou, Romania

- Dr. Cosmin Sicora
- Dr. Oana Sicora
- Eng. Lia Mladin
- Dr. Mara Naghi
- Dr. Tünde-Éva Jakó
- Ms. Iulia Chis
- Ms. Ildikó Fehér
- Mr. Cristian Sebeni-Grec

Contact: 10ewmbc@ccbjb.ro Phone: (+40) 0732 002 251

Fax: (+40) 0260 644 95 Honepage: www. ccbjb.ro/10EWMBC/

Useful information

Information

Cluj-Napoca is one of the largest cities in Romania in the heart of Transylvania. Is considered among the most beautiful romanian cities and is connected to major european transit hubs by direct flights. For more information about Cluj-Napoca, please, check www.visitclujnapoca.ro/en/.
Cluj Napoca can be reached via plane the "Avram Iancu" International Airport (IATA code: CLJ) offers good connections with many european cities and other romanian cities. For more details check the airport's website: www.airportcluj.ro.
Theere are good national and international connections by train, for more detailed trains schedule, please check on www.infofer.ro/en/.
Ig you want to explore the city by public transportation - check the city public transport link:www.ctpcj.ro.
in Cluj-Napoca there are plenty of taxi and also it is availabele the UBER. A trip from the airport to the conference venue cost about 8 euros.

The 10th European Workshop on the Molecular biology of Cyanobacteria will be held at the Grand Hotel Italia, Cluj Napoca Romania.
Grand Hotel Italia, Cluj Napoca http://grandhotelitaliacluj.ro/

Wheather

The average maximum and minimum temperature on Cluj Napoca in August are 25 ºC and 14 ºC.

Lectures

The duration of each keynote lectures should be 30 minutes (including discussions).
The duration of each oral presentation should be 15 minutes (including discussions).
We recommend you to save your PowerPoint presentation using PPT(X) format instead of PPS. Please, keep in mind that organizers cannot guarantee the quality of Macintosh-based presentations; so check in advance (3 hours before your session starts) their Windows compatibility. Note that the presentation system used during the congress also supports the PDF presentations.
There are no parallel sections.
We recommend providing your presentation on the USB stick, external hard or Virtual cloud (One drive, Dropbox, Google drive…) and have it loaded on the PC of the auditorium during the breaks.

Posters

Posters (A0 portrait) will be exhibited on the Poster Area, where you will find fixing material (stickers, pins etc.). Each poster will have an ordinal number which indicates the position of the poster. The posters should be put up on Sunday afternoon or Monday morning and should be removed after the last lecture on Wednesday. The posters can be viewd all times during the workshop.

Badges

The badge repseresnt your ticket to the workshoip and meals, please wear at all times!

Sunday 20 August

16:30-21:00 Arrival and registration of participants
19:00-21:00 Welcome Cocktail

Monday 21 August

Hour	Time	Event name	Name of the lecturer and title of the presentation
8:30	10 min	Opening remarks	**Cosmin Sicora**, director of Biological Research Center Jibou, Romania
8:40	10 min	Official message	
8:50	35 min	Keynote lecture	**Lucas Stal**, Royal Netherlands Institute for Sea Research (NIOZ), Yerseke and University of Amsterdam, Netherlands "Gregarious Cyanobacteria"
9:25	35 min	Keynote lecture	**Susan S. Golden**, Division of Biological Sciences, UC San Diego, USA "A day in the life of a cyanobacterium: integrating temporal and environmental information"
10:00	30 min	Coffee break	
Session 1- Evolution and Phylogeny Chairs: Cosmin Sicora (BRC Jibou, Romania) and Josef Komenda (Institute of Microbiology, Czech Republic)			
10:30	30 min	Invited lecture	**Jesus A. Ochoa de Alda**, Universidad de Extremadura, Caceres, Spain "Sources of inconsistencies in the phylogenomic approach to the evolution of cyanobacteria and plastids exemplified with aminoacyl tRNA synthetases and FtsZ"
11:00	20 min	Contributed lecture	**Patricia Sanchez Baracaldo,** United Kingdom "The early evolution of the chloroplast lineage"
11:20	20 min	Contributed lecture	**Sarit Avrani,** Israel, "Convergent evolution toward an improved growth rate and a reduced resistance range in *Prochlorococcus* strains resistant to phage"

Hour	Time	Event name	Name of the lecturer and title of the presentation
11:40	20 min	Contributed lecture	**Filipe Branco dos Santos,** Netherlands**, "**Aligning microbial fitness with engineered photoautotrophic product formation"
12:00	1 hour	Lunch	
Session 2-Molecular Ecology Chairs: Lucas Stal (NIOZ and University of Amsterdam, Netherlands) and Enrique Flores (IBVF, University of Sevilla, Spain)			
13:00	30 min	Invited lecture	**Wolfgang Hess***,* University of Freiburg, Germany "Specific sets of regulatory RNAs and interacting proteins as drivers of cyanobacterial stress acclimation"
13:30	20 min	Contributed lecture	**Gen Enomoto**, Japan, **"**Cyanobacteriochrome-mediated blue/ green light signaling is a population density-sensing system under photosynthesis-driving red light"
13:50	20 min	Contributed lecture	**Silvia Cretoiu**, Netherlands, "The microbiome of *Microcystis* aggregates – the unseen driver of water blooms in the Danube Delta"
14:10	30 min	Invited lecture	**Elke Dittmann**, University of Postdam, Germany "Chemical mediators facilitating multilateral interactions in symbiotic strains of the genus *Nostoc*"
14:40	20 min	Contributed lecture	**Rakefet Schwarz**, Israel, "Self-suppression of biofilms in cyanobacteria: role of type II/type IV secretion systems"
15:00	2 ½ hours	Coffee break & Poster Session	
17:30	20 min	Contributed lecture	**Muñoz-Marin Maria del Carmen**, USA and Spain, "Unicellular cyanobacterial symbiosis facilitates aerobic nitrogen fixation"

Hour	Time	Event name	Name of the lecturer and title of the presentation
17:50	20 min	Contributed lecture	**Sophia Zborowsky**, Israel, "Resistance strategies differ against generalist and specialist cyanophages"
Session 3- Gene Regulation Chairs: Wim Vermaas (Arizona State University, USA) and Min Chen (University of Sydney, Australia)			
18:10	30 min	Invited lecture	**Annegret Wilde**, University of Freiburg, Germany, "Detection of light direction by cyanobacterial cells"
18:40	20 min	Contributed lecture	**Christin Koebler**, Germany, "Mechanism and function of non-standard circadian clock systems in cyanobacteria"
19:00	2 hours	Dinner	

Tuesday 22 August

8:30	30 min	Invited lecture	**Martin Hagemann**, University of Rostock, Germany, "Acclimation to limiting CO2 amounts: photorespiration, inorganic carbon sensing and synthetic biology approaches"
9:00	20 min	Contributed lecture	**Yuzenkova Yulia**, United Kingdom, "RNA polymerase active centre compensates for the absence of the essential transcription factors in Cyanobacteria"
9:20	20 min	Contributed lecture	**Matthias Futschik**, Portugal and United Kingdom, "Identification of the direct regulon of NtcA during early acclimation to nitrogen starvation in the cyanobacterium *Synechocystis* sp. PCC 6803"
9:40	20 min	Contributed lecture	**James Golden**, USA, "Broad-host-range genetic tools for cyanobacteria and heterologous expression of natural products"
10:00	20 min	Contributed lecture	**Asuncion Contreras**, Spain, "Partner swapping of cyanobacterial nitrogen regulators PII and PipX during diurnal cycles"

Hour	Time	Event name	Name of the lecturer and title of the presentation
10:20	30 min	Coffee break	
Session 4- Cellular Differentiation and Cell Biology Chairs: Annegret Wilde (University of Freiburg, Germany), Conrad Mullineaux (Queen Mary University of London, UK) and Julian Eaton-Rye (University of Otago, New Zealand)			
10:50	30 min	Invited lecture	**Antonia Herrero**, CSIC (Consejo Superior de Investigaciones Científicas), Spain, "Cell division in the filamentous, pluricellular cyanobacterium *Anabaena*"
11:20	1 h 40 min	Poster Session	
13:00	1 hour	Lunch	
14:00	4 hours	Social Programme	
19:00	2 hours	Dinner	

Wednesday 23 August

Hour	Time	Event name	Name of the lecturer and title of the presentation
8:30	30 min	Invited lecture	**Iris Maldener**, University of Tubingen, Germany, "Overcoming the septal barrier: how communication structures are formed in the septum of filamentous cyanobacteria"
9:00	20 min	Contributed lecture	**Gregor Weiss**, Switzerland, "A novel contractile injection system in cyanobacteria"
9:20	20 min	Contributed lecture	**Dirk Schneider**, Germany, "Janus-faced IM30 ring is involved in thylakoid membrane fusion in cyanobacteria"
9:40	20 min	Contributed lecture	**Paulo Oliveira**, Portugal, "At the crossroads of cyanobacterial secretion mechanisms: multidrug efflux meets protein modification and export"
10:00	20 min	Contributed lecture	**Julia Walter**, Finland, "Discovery of the novel calcium-binding protein CSE in the filamentous cyanobacterium *Anabaena* sp. PCC 7120"

Hour	Time	Event name	Name of the lecturer and title of the presentation
10:20	30 min	Coffee break	
10:50	20 min	Contributed lecture	**David Lea-Smith**, United Kingdom, "Spatial mapping of a cyanobacterial proteome reveals distinct subcellular compartment organisation and dynamic metabolic pathways"
11:10	20 min	Contributed lecture	**Karl Forchhammer**, Germany, "The awakening of a dormant cyanobacterium reveals a cryptic program for maintenance of viability"
11:30	20 min	Contributed lecture	**Shukla Mahendra**, Czech Republic, "Remodelling of a cyanobacterial chlorophyll-synthase complex by high-light inducible proteins"
11:50	20 min	Contributed lecture	**Sara Pereira**, Portugal, "Production of extracellular polymeric substances (EPS) in *Synechocystis* PCC 6803: the role of Wzc and Wzb"
Session 5- Bioenergetics and Photosynthesis **Chairs: Paula Tamagnini (University of Port, Portugal), Wolfgang Hess (University of Freiburg, Germany) and Debbie Lindell (Technion Institute, Israel)**			
12:10	30 min	Invited lecture	**Yagut Allahverdiyeva-Rinne**, Turku University, Finland "Alternative-electron transport pathways in cyanobacteria"
12:40	20 min	Contributed lecture	**Tina Summerfield**, New Zealand, "The *psbA* gene family in heterocystous cyanobacteria"
13:00	20 min	Contributed lecture	**Wim Vermaas**, USA, "What happens if the photosystem I/photosystem II (PSI/PSII) stoichiometry changes?"
13:20	10 min	Buffer time	
13:30	1 hour	Lunch	
14:30	20 min	Contributed lecture	**Dennis J. Nurnberg**, United Kingdom, "Chlorophyll *f* photosynthesis in the cyanobacterium *Chroococcidiopsis thermalis*"

Hour	Time	Event name	Name of the lecturer and title of the presentation
14:50	30 min	Invited lecture	**Josef Komenda**, Institute of Microbiology, Czech Republic "The PSII repair cycle in cyanobacteria: news and views"
15:20	20 min	Contributed lecture	**Min Chen**, Australia, "Switchable photosynthetic apparatus in chlorophyll *f* producing cyanobacterium"
15:40	20 min	Contributed lecture	**Claudia Hackenberg**, Germany, "Hexameric CBS-CP12 and thioredoxin form a redox-regulon for CO2 fixation in bloom-forming cyanobacteria"
16:00	30 min	Coffee break	
16:30	20 min	Contributed lecture	**Luning Liu**, United Kingdom, "Probing the organisation, self-assembly and nanomechanics of bacterial microcompartments"
16:50	20 min	Contributed lecture	**Enrique Flores,** Spain, "The arginine catabolism pathways of *Anabaena* sp. PCC 7120"
17:10	20 min	Contributed lecture	**Weimin Ma**, China, "Novel NDH-1L-CpcG2-Phycobilisome-Photosystem I supercomplex is important for efficient cyclic electron transport in cyanobacteria"
17:30	20 min	Contributed lecture	**Arthur Guljamow**, Germany, "Impact of microcystin on RubisCO and the carbon concentrating mechanism of *Microcystis aeruginosa*"
17:50	20 min	Contributed lecture	**Peter Lindblad**, Sweden, "Design and construction of cyanobacteria for direct solar fuels production"
18:10	20 min	Contributed lecture	**Amanda Cockshutt**, Canada, "Strain specific differences in capacity to repair photosystem II in picocyanobacteria"
18:30	20 min	Contributed lecture	**Julian Eaton-Rye**, New Zealand, "Targeted mutation of D2 amino acids residues associated with bicarbonate binding and the bicarbonate-dependent protonation of plastoquinone b"

Hour	Time	Event name	Name of the lecturer and title of the presentation
18:50	20 min	Contributed lecture	**Yvonne Zilliges**, Germany, “Identification of key protonation sites and paths in photosynthetic water oxidation via site-directed mutagenesis of cyanobacterial photosystem II”
19:10	5 min	Closing remarks	
19:15	3 hours	Gala dinner	

Thursday 24 August

Departure

We thank our Sponsors:

- Federation of European Microbiological Societies
- International Society of Photosynthesis Research
- Photon Systems Instruments
- Heinz Walz
- Filara Biomed
- Dialab Solutions
- Elta 90 Medical Research
- CellDeg

Have a great conference in Cluj Napoca!

Social program

Welcome cocktail
Date and time: 20.08.2017, 19:00 - 21:00
Place: Grand Hotel Italia

All participants will be invited to this evening cocktail, after arriving at the hotel.

City tour of Cluj-Napoca
Date and time: 22.08.2017 between 14:00 - 19:00
Place: Cluj Napoca

The tour is an invitation to discover the story of the "hearth" of Transylvania, offering you the opportunity to explore the fascinating architectural and cultural history, including well-known touristic attractions. Throughout the stories and legends which bring the city to life, we will recreate the medieval atmosphere of the citadel.
Website: http://www.visitclujnapoca.ro/en/

Visit of Turda Salt Mine.
Date:22.08.2017 between 14:00 - 19:00
Place: Turda

Turda salt mine is one of the oldest saltmine, whichrepresents a veritable history of salt minig. The Buissnes insider website ranked it on their list between the top ten "coolest underground places in the world". You can visit different compartments of the mine: the Iosif Mine, the octagonal room hosts a winch called "crivac", Rudolf mine and Gizela mine. The submerged wonderland of mine is at 400 feet down, where you will find theme park, bowling alley and underground lake. If these arguments don't convince you to visit Turda Salt Mine please access the following website
Website: http://salinaturda.eu/

Gala Dinner
Date: 23.08.2017 between 19:00-21:00
Place: Grand Hotel Italia

INDEX OF CONTENT

KEYNOTE LECTURES **1**

ORAL PRESENTATIONS **5**

- SESSION 1: EVOLUTION AND PHYLOGENY 5
- SESSION 2: MOLECULAR ECOLOGY 13
- SESSION 3: GENE REGULATION 27
- SESSION 4: CELLULAR DIFFERENTIATION AND CELL BIOLOGY 41
- SESSION 5: BIOENERGETICS 61

POSTERS **91**

- SESSION 1: EVOLUTION AND PHYLOGENY 91
- SESSION 2: MOLECULAR ECOLOGY 97
- SESSION 3: GENE REGULATION 125
- SESSION 4: CELLULAR DIFFERENTIATION AND CELL BIOLOGY 163
- SESSION 5: BIOENERGETICS 213

AUTHOR INDEX **267**

LIST OF PARTICIPANTS **272**

Keynote Lectures

Gregarious Cyanobacteria

Lucas J. Stal

NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry and Utrecht University
and University of Amsterdam, IBED Department of Aquatic Microbiology,
POBox 94248, 1090 GE Amsterdam, The Netherlands

Aggregation is common among cyanobacteria and it offers many advantages as compared to a free-living lifestyle. Cyanobacteria have evolved a plethora of different morphologies with cells varying 2 orders of magnitude in size, ranging from single cells to filamentous, multicellular forms. Each of these types may form larger, more or less structured, aggregates. It has been hypothesized that small unicellular forms may be more vulnerable to grazing and that aggregation is an inducible defense against it. However, there are alternative explanations. For instance, phytoplankton that possess gas vesicles benefit from the larger size of aggregates because they float much faster to the water surface after being mixed deep into the water column. Aggregation may be induced by the exudation of sticky extracellular polymeric substances (eps), which can be the result of a metabolic overflow caused by nutrient limitation. Such sticky eps also allow cyanobacteria to attach to a surface and form a biofilm or microbial mat. These aggregates are home to a complex microbiome that effectively recycle elements and exhibit the behavior of a living entity.

A day in the life of a cyanobacterium: integrating temporal and environmental information

Susan S. Golden

Center for Circadian Biology & Division of Biological Sciences, University of California – San Diego, La Jolla, CA 92093 USA

✉ *sgolden@ucsd.edu*

Cells of diverse organisms, from cyanobacteria to humans, execute temporal physiological programs that are driven by circadian oscillators. The circadian clock of the cyanobacterium *Synechococcus elongatus* regulates global patterns of gene expression, the timing of cell division, and metabolism. We use *S. elongatus* as a model to understand how a cell keeps track of time, executes activities according to a temporal program, and synchronizes the internal clock with the external solar cycle. The components of the circadian oscillator are known (proteins KaiA, KaiB, and KaiC), their structures have been solved, and the rhythm in phosphorylation of KaiC can be reconstituted *in vitro*. One oscillator component, KaiB, undergoes a metamorphosis to a new protein fold – a rare event that is key to the slow progression of the circadian cycle. Furthermore, fold-switched KaiB initiates the "night" phase of the oscillator, and connects the oscillator to the downstream components that broadcast time to the cell. Although the oscillator can keep time *in vitro*, the situation *in vivo* is more complicated, with the clock components undergoing temporally regulated changes in intracellular localization. We are also investigating the metabolic consequences for *S. elongatus* when it is grown with or without an intact clock, in continuous light or in a diel cycle, to determine how circadian timing contributes to fitness. We found that wild-type cells turn off nighttime metabolic pathways before dawn, enabling a clock-dependent switch from primary metabolism to the synthesis of more complex molecules early in the day.

Oral presentations

Session 1: Evolution and Phylogeny

Sources of inconsistencies in the phylogenomic approach to the evolution of cyanobacteria and plastids exemplified with aminoacyl-tRNA synthetases and FtsZ.

Jesús A. G. Ochoa de Alda[1, ✉], Rocío Esteban[1], Jose Maria Marcos-Merino[1] Antonia Herrero[2] and Ignacio Luque[2,]

[1]Department of Science and Mathematics Education, University of Extremadura, Avd. Universidad s/n, 10003-Cáceres, Spain.

[2] Instituto de Bioquímica Vegetal y Fotosíntesis CSIC and Universidad de Sevilla, Avda Américo Vespucio N 49 E-41092 Sevilla, Spain

✉ *ochoadealda@unex.es*

Cyanobacteria likely predate to about 3,700-million years ago. Since then, they have adapted to the changing environmental conditions of Earth through evolution. The reconstruction of Cyanobacteria phylogeny is a way to decipher sources of molecular innovation and complexity, and the order of events leading to diversification of these prokaryotes as well as the origin of eukaryotic phototrophs.
To obtain accurate and well-supported phylogenies, the reconstruction of deep-level relationships within Cyanobacteria and plastids requires the concatenation of multigene datasets able to provide a congruent signal. However, this phylogenomic approach often produces highly incongruent findings, yet statistically highly supported, despite the use of considerable sequence data.
A comparison of the most sophisticated approaches to reconstruct Cyanobacteria and plastid phylogeny evidenced important methodological differences and sources of incongruence derived from i) genome annotations, ii) number of species, iii) selection of protein or DNA sequences that are resistant to HGT and possess both strong evolutionary signals and a common phylogeny iv) alignment and selection of reliable positions least subject to saturation and v) selection of the most realistic model of sequence evolution.
In cyanobacteria, genes coding for elements of the translational machinery, such as aminoacyl-tRNA synthetases, constitute good examples of a misconceived evolutionary stability since they show a complex evolutionary pathway. We used this group of proteins, together with FtsZ protein, to exemplify sources of incongruence that can affect phylogenomic reconstructions.
Acknowledgements: Work was supported by grants BFU2016-77097-P from Agencia Estatal de Investigación, Spain, cofinanced by the European Regional Development Fund (EU).

The early evolution of the chloroplast lineage

Patricia Sánchez-Baracaldo

School of Geographical Sciences, University of Bristol, Bristol BS8 1SS, UK.

✉*p.sanchez-baracaldo@bristol.ac.uk*

The early evolutionary history of the chloroplast lineage remains an open question. It is widely accepted that the endosymbiosis which established the chloroplast lineage in eukaryotes can be traced back to a single event in which a cyanobacterium was incorporated into a protistan host. It is still unclear, however, which cyanobacteria are most closely related to the chloroplast, when the plastid lineage diverged from its cyanobacterial sisters, and in what habitats this endosymbiotic event occurred. Here, I present phylogenomic and molecular clock analyses, including 119 taxa from cyanobacterial and chloroplast genomes with the aim of estimating the age for the primary endosymbiotic event. Phylogenomic analyses support the hypothesis that the chloroplast lineage diverged from its closet relative, *Gloeomargarita*, a basal cyanobacterial lineage, ~2.1 billion years ago (Bya). The analyses suggest that the Archaeplastida, consisting of glaucophytes, red algae, green algae and land plants, share a common ancestor that lived ~1.9 Bya. Whereas crown group Rhodophyta evolved in the Mesoproterozoic Era (1600-1000 million years ago, Mya), crown group Chlorophyta and Streptophyta began to radiate early in the Neoproterozoic (1000–542 Mya). Stochastic mapping analyses indicate that the first endosymbiotic event occurred in low salinity environments. Both red and green algae colonized marine environments early in their histories, with prasinophyte green phytoplankton diversifying 850-650 Mya.

Convergent evolution toward an improved growth rate and a reduced resistance range in *Prochlorococcus* strains resistant to phage

Sarit Avrani and Debbie Lindell

Prochlorococcus is an abundant marine cyanobacterium that grows rapidly in the environment and contributes significantly to global primary production. This cyanobacterium coexists with many cyanophages in the oceans, likely aided by resistance to numerous co-occurring phages. Spontaneous resistance occurs frequently in *Prochlorococcus* and is often accompanied by a pleiotropic fitness cost manifested as either a reduced growth rate or enhanced infection by other phages. Here, we assessed the fate of a number of phage-resistant *Prochlorococcus* strains, focusing on those with a high fitness cost. We found that phage-resistant strains continued evolving toward an improved growth rate and a narrower resistance range, resulting in lineages with phenotypes intermediate between those of ancestral susceptible wild-type and initial resistant substrains. Changes in growth rate and resistance range often occurred in independent events, leading to a decoupling of the selection pressures acting on these phenotypes. These changes were largely the result of additional, compensatory mutations in noncore genes located in genomic islands, although genetic reversions were also observed. Additionally, a mutator strain was identified. The similarity of the evolutionary pathway followed by multiple independent resistant cultures and clones suggests they undergo a predictable evolutionary pathway. This process serves to increase both genetic diversity and infection permutations in *Prochlorococcus* populations, further augmenting the complexity of the interaction network between *Prochlorococcus* and its phages in nature. Last, our findings provide an explanation for the apparent paradox of a multitude of resistant *Prochlorococcus* cells in nature that are growing close to their maximal intrinsic growth rates.

Aligning microbial fitness with engineered photoautotrophic product formation

Filipe Branco dos Santos

Molecular Microbial Physiology Group, Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands

✉*f.brancodossantos@uva.nl*

Human societies are mostly fuelled by the oxidation of fossilized deposits leading to CO_2 release. We face the global challenge of reversing this alarming trend. Processes using photosynthetic microorganisms to convert CO_2 into compounds of interest, fuelled by sunlight, are very pertinent in this context. Over the last decade, there has been a 'cyanobacterial bloom' in the (scientific) literature of proof-of-principle studies, showcasing the applicability of this approach to different products. Unfortunately, the majority has yet to blossom into large-scale sustainable processes. We have recently demonstrated that as carbon is increasingly deviated during growth toward product formation, there is an increased fixation rate in the population of spontaneous mutants harboring an impaired production pathway. This apparent instability of engineered strains poses enormous challenges in the scale-up of processes using green cell factories. A way to counter this is by aligning microbial fitness with engineered photoautotrophic product formation. Here, we present a novel method to Find Reactions Usable In Tapping Side-products (FRUITS), that (i) lists all the metabolites in any genome-scale metabolic model that can be formed in a growth-coupled manner; (ii) identifies the gene deletions that are required for such; and (iii) computes the maximal biomass formation and minimum product formation rates. We apply this method to *Synechocystis* PCC6803 under photoautotrophic conditions and identify 9 products of biotechnological interest that can be engineered in a stable fashion. Furthermore, we experimentally implement this approach and report the first photoautotrophic growth-coupled producers, along with evidence supporting their remarkable stability.

Session 2: Molecular Ecology

Specific sets of regulatory RNAs and interacting proteins as drivers of cyanobacterial stress acclimation

Wolfgang R. Hess

Albert-Ludwigs-University Freiburg, Institute for Biology 3, Schänzlestrasse 1, 79104 Freiburg, Germany

✉*wolfgang.hess@biologie.uni-freiburg.de*

Regulatory small RNAs (sRNAs) play versatile roles in bacteria in the coordination of gene expression during various physiological processes, especially during stress acclimation. Indeed, hundreds of potentially regulatory sRNAs have been identified in model strains and in environmentally relevant cyanobacteria such as *Synechocystis* sp. PCC 6803, *Anabaena* sp. PCC 7120, *Trichodesmium erythraeum* IMS101, *Nodularia spumigena* CCY9414, multiple *Synechococcus* and *Prochlorococcus* isolates.
Recent work in *Synechocystis* sp. PCC 6803 on the molecular mechanisms these sRNAs are involved in have provided surprising insights. The only 68 nt long sRNA IsaR1 ("Iron stress activated RNA 1") impacts the photosynthetic apparatus and the machinery making several of its most important structures in three fundamentally different ways. First, IsaR1 directly inhibits the expression of multiple photosynthetic proteins. Secondly, IsaR1 interferes with several steps of the biochemical pathway leading to chlorophyll, which is not needed at the same quantity when iron is becoming scarce. Thirdly, IsaR1 antagonizes the expression of proteins for iron-sulfur clusters, a particularly important cofactor in photosynthetic electron transport. However, IsaR1 functions not only in the acclimation response to low iron but possesses also integrating functions, connecting to the acclimation to high salt and other physiological features. The 70 nt sRNA NsiR4 ("Nitrogen stress inducible RNA 4") is under NtcA control and affects the assimilation of nitrogen by targeting the translation of glutamine synthetase inactivating factor IF7, which acts at the level of enzyme activity regulation. However, these sRNAs do not function in a vacuum but depend and interact with protein factors in multiple ways. One such example was provided by the 131 nt long sRNA PsrR1 ("Photosynthesis regulatory RNA1"). The expression of PsrR1 is under RpaB control and becomes stimulated when cells are transferred to high light. PsrR1 targets several mRNAs for photosynthetic proteins, among them *psaL* encoding the PSI reaction center protein subunit XI. Upon PsrR1:*psaL* base-pairing, endoribonuclease E is recruited that cleaves the 5'UTR and first two codons off the remaining mRNA sequence, rendering it untranslatable and destabilizing it. Extending on the long list of RNase E functions, we found its involvement in the maturation of certain transcripts from the native CRISPR system in *Synechocystis* sp. PCC 6803. The sequestration of RNase E by the CRISPR apparatus may constitute a pool of RNase molecules that becomes activated only in extreme stress situations when the entire transcriptome needs to be remodeled in a short time.

Cyanobacteriochrome-mediated blue/ green light signaling is a population density-sensing system under photosynthesis-driving red light

Gen Enomoto[1], Masahiko Ikeuchi[2]

Department of Life Sciences (Biology), Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan

Department of Life Sciences (Biology), Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan

✉[1] *genenomoto@bio.c.u-tokyo.ac.jp, mikeuchi@bio.c.u-tokyo.ac.jp*

Cyanobacteriochromes (CBCRs) are cyanobacteria-specific photoreceptors distantly related to more widespread phytochromes. CBCRs show diverse spectral properties typified by the prevailing blue/green light-absorbing type, while phytochromes preserve red/far-red light absorbing properties. We previously reported that the three blue/green-type CBCRs (SesA/B/C) cooperatively regulate *Thermosynechococcus* cell aggregation in a blue light-specific manner (Enomoto *et al.* 2015 *PNAS*). However, it is unknown where the light-color specific signaling occurs in nature.
Here, we assess the physiological and evolutionary implications of the CBCR-mediated blue/green light signaling. We found that cell aggregation was strongly dependent on population density under mixture of blue/green light; cell aggregation of dense culture was greatly weakened even under high blue/green ratio. As cyanobacterial cells show low transmittance ratio of blue/green light, the shade of other cells should be green light-rich environment. Cell aggregation was unaffected by population density under only blue or green light, indicating that light-independent population density sensing system does not contribute. These results suggest that SesA/B/C estimate cell population density by sensing blue/green light ratio to regulate cell aggregation in a natural habitat. Cell aggregation is reversible and dispersed by green light signal. We also found that the dispersion was impaired when the background red light irradiation was missing or 3-(3,4-dichlorophenyl)-1,1-dimethylurea was added. These results indicate that the dispersion requires red light-driven photosynthesis. Cyanobacteria may have developed blue/green CBCRs as a shade detector in an upper layer of microbial mats where sufficient photosynthesis-driving red light is still available while primitive phytochromes may be effective in a darker deep area.

The microbiome of *Microcystis* aggregates – the unseen driver of water blooms in the Danube Delta

Mariana Silvia Cretoiu[1], Mirela Moldoveanu[2], Lucas J. Stal[1,3]

[1] *Department of Freshwater and Marine Ecology, University of Amsterdam, Amsterdam, The Netherlands*

[2] *Department of Ecology, Taxonomy and Nature Conservation, Institute of Biology, Romanian Academy of Science, Bucharest, Romania*

[3] *Department of Marine Microbiology and Biogeochemistry, NIOZ Royal Netherlands Institute for Sea Research and University of Utrecht, Texel, The Netherlands*

Microcystis is a unicellular cyanobacterium that forms extensive blooms in freshwater lakes. Hypertrophic conditions and the hydrological regime in aquatic ecosystems foster toxic blooms. However, *Microcystis* blooms in the Danube Delta are non-toxic even though the strains possess the genetic potential for toxicity. In order to understand this phenomenon, we investigated these blooms by revealing the genomic treats of the cyanobacterial consortia. The DNA of the surface blooms from three lakes in the Danube Delta (Lakes Erenciuc, Puiu, and Rosu) was shotgun deep-sequenced and the data analyzed through digital normalization, assembly, annotation and genome recruitment. We explored the core microbiome and other features responsible for processing genetic and environmental cues. The microbiome consisted of *Cyanobacteria*, *Proteobacteria* and *Actinobacteria*. It contained a high abundance of protein-coding genes of the unicellular cyanobacteria – *Cyanobacterium*, *Cyanothece*, *Microcystis*, *Stanieria* and *Synechocystis*. Novel draft genomes of *Cyanothece* and *Synechocystis* were obtained with a higher coverage than *Microcystis*. Predicted proteins of *Cyanothece* outnumbered those of other cyanobacteria, whereas repetitive genomic regions were mostly assigned to *Synechocystis*. Consistent SNPs patterns were observed in hydrogenases, N6-adenine-specific DNA methylases and methyltransferases of *Cyanothece*. Various integrases, transposases, twitching motility protein PilT, and cyanophage genome fragments hint to an efficient co-existence and resilience of the consortium members. The results offer multiple cues to support the conclusion that the aggregates of *Microcystis* offer the physicochemical and structural environment to support other microorganisms. Associated unicellular cyanobacteria may improve consortium fitness by the mobility of enzymatic traits such as hydrolytic cleavage capacity between different species.

Chemical mediators facilitating multilateral interactions in symbiotic strains of the genus *Nostoc*

Anton Liaimer[1], Arthur Guljamow[2], Marco Kreische[2], Daniel Dehm[2], Keishi Ishida[3], Eric Helfrich[3], Christian Herweck[3] and Elke Dittmann[2, ✉]

[1] Molecular Environments Group, Department of Arctic and Marine Biology, Faculty of Biosciences, fisheries and Economics, University of Tromsø, 9037 Tromsø, Norway

[2] University of Potsdam, Institute for Biochemistry and Biology, Karl-Liebknecht-Str. 24/25, 14476 Potsdam-Golm, Germany

[3] Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Beutenbergstr. 11a, 07745 Jena, Germany

✉editt@uni-potsdam.de

Terrestrial symbiotic cyanobacteria of the genus *Nostoc* are of enormous environmental importance, providing fixed nitrogen to a diverse range of hosts as well as playing a central role in soil formation and fertility. Strains of this genus have revealed a high potential for the production of bioactive compounds, exhibiting cytotoxic, antifungal and antibacterial activities.

While the genomic potential for secondary metabolite production in this group of bacteria is high, the compounds itself remain mostly cryptic. I will present our recent insights into the role of natural products for cellular differentiation and biotic interactions of *Nostoc sp.* and discuss possibilities how this knowledge can be used to develop novel genomic mining strategies to discover and characterize new chemical mediators in this underexplored group of bacteria. We have used fluorescence reporter strains, RNAseq studies, MALDI-Imaging and agar diffusion assays to dissect the role of individual compounds and observed a gradual impact and a major governing role for the nonribosomal peptide nostopeptolide in strain ATCC29133. Our data further support the existence of a quorum-sensing like mechanism in *Nostoc* sp. and uncover a pronounced cross-talk network between secondary metabolites. I will discuss how far intraspecific and cross-kingdom interactions are expected to shape the *Nostoc* community inside and outside of symbiotic plant partners and the overarching impact of chemical diversity.

Self-suppression of biofilms in cyanobacteria: role of type II/type IV secretion systems

Rakefet Schwarz[1, ✉], Elad Nagar[1], Rami Parnasa[1], Yevgeni Yegorov[1], Ryan Simkovsky[2], Susan S. Golden[2] and Eleonora Sendersky[1]

[1]*The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel*

[2]*Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093, USA*

✉ *Rakefet.Schwarz@biu.ac.il*

✉ *e-mails of co-authors: elad@danyel.co.il; rami.parnasa@gmail.com, jenyay85@gmail.com; rsimkovsky@ucsd.edu; sgolden@ucsd.edu; ES@rakefetlab.com*

Information on the molecular mechanisms underlying biofilm formation in cyanobacteria is scarce in spite of their environmental prevalence and the economic loss associated with these microbial assemblages. We recently demonstrated that the planktonic nature of the cyanobacterium *Synechococcus elongatus* is a result of a self-inhibition mechanism, which depends on the deposition of a factor to the extracellular milieu. This inhibitory substance governs expression of small secreted proteins that enable biofilm development. Inactivation of a gene encoding a homolog of the ATPase subunit of type II protein secretion or type IV pilus assembly systems impairs the inhibitory process and results in biofilm formation (1-3). We recently identified additional components of the biofilm inhibitory mechanism, including the RNA-chaperone homolog, Hfq, and a highly conserved protein, thus far defined as "hypothetical". Inactivation of either one of the genes encoding these proteins, results in aberrant protein secretion and absence of pili, suggesting involvement of the Hfq-homolog and the "hypothetical protein" in modulation of type II/type IV complexes. Localization of these modulators to the photosynthetic membranes implies a new mode of regulation of type II/ Type IV systems in cyanobacterial cells.

Schatz D et al. (2013) Environ Microbiol, 15(6), 1786-1794.
Nagar E, Schwarz R (2014) Environ Microbiol. doi: 10.1111/1462-2920.12583.
Parnasa R et al. (2016) Sci. Rep. 6:32209

Unicellular cyanobacterial symbiosis facilitates aerobic nitrogen fixation

Maria del Carmen Muñoz-Marin[1, 2, ✉], Irina N. Shilova [1], Shi T [1, 3] Hanna Farnelid [1, 4] and Jonathan P. Zehr [1]

[1] *Ocean Sciences Department, University of California, Santa Cruz, CA, USA.*

[2] *Departamento de Bioquímica y Biología Molecular, Universidad de Córdoba, Córdoba, Spain.*

[3] *State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361102, China.*

[4] *Centre for Ecology and Evolution in Microbial Model Systems (EEMiS), Linnaeus University, Kalmar, Sweden.*

✉b32mumam@uco.es

Biological nitrogen (N_2) fixation, the reduction of inert atmospheric N_2, is a major source of fixed nitrogen for natural and agricultural ecosystems, and is common in photosynthetic cyanobacteria and in symbioses between bacteria and higher plants. The uncultivated symbiosis between a marine alga and a N_2-fixing cyanobacterium (UCYN-A) appears to fix N_2 aerobically in the light, which is unusual in unicellular N_2-fixing phototrophs. It is unclear how this unicellular symbiosis functions since N_2 fixation is an energetically expensive process that is also inhibited by the oxygen evolved in oxygenic photosynthesis. By analyzing gene transcription in natural, uncultivated populations, we found that the symbiosis has shifted diel transcription patterns to facilitate aerobic N_2 fixation during the day. The results show that even simple unicellular symbioses can lead to adaptations that facilitate coupling N_2 fixation directly to the energy obtained from photosynthesis in the light.

Acknowledgements. J.P.Z was supported by grants from the Simons Collaboration on Ocean Processes and Ecology (SCOPE, grant 329108), the Simons Foundation (grant 545171), and Gordon and Betty Moore Foundation (grant 493.01). M.M.M. was supported by a Marie Curie International Outgoing Fellowship within the 7th European Community Framework Programme. T.S. was supported by National Natural Science Foundation of China (NSFC) (41676092) and National Key Research and Development Program of China (2016YFA0601202). H.F. was supported by the Swedish Research Council VR 637-2013-7502.

Resistance strategies differ against generalist and specialist cyanophages

Sophia Zborowsky and Debbie Lindell

Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel

Cyanobacteria of the genera *Synechococcus* and *Prochloroccocus* and the viruses that infect them are abundant in the marine environment. Coexistence between them is thought to be facilitated by the presence of both resistant and susceptible cells. While extracellular resistance by alterations in receptors is established, known intracellular defense mechanisms are lacking in these cyanobacteria. In order to investigate whether intracellular resistance exists in *Prochlorococcus* and *Synechococcus* we sought to identify infections that are halted inside the cell. We found that generalist T4-like cyanophages tend to adsorb to resistant hosts more often than specialist phages. We next tested DNA expression and replication of generalist and specialists phages inside resistant strains. This revealed that viral RNA of generalists and specialists was expressed. In addition, we often observed replication of viral genome in these infections. In one of these infections of a generalist phage we tested for protein synthesis and found that while translation occurs in the resistant strain, multiple morphogenesis proteins are lacking, noticeably among them are proteins responsible for DNA recognition and packaging into capsids. These data indicate that resistance to broad-host-range phages is often conferred inside the cell and may be due to novel intracellular mechanisms of defense.

Session 3: Gene Regulation

Detection of light direction by cyanobacterial cells

Annegret Wilde[1], Annik Jakob[1], Nils Schuergers[1], Conrad Mullineaux[2]

[1]*Institute of Biology III, University of Freiburg, Germany*

[2]*School of Biological and Chemical Sciences, Queen Mary University of London*

The cyanobacterium *Synechocystis* 6803 exhibits flagellar-independent twitching motility using type IV pili. Phototaxis is a mechanism that allows cyanobacteria to respond to fluctuations in the quality and quantity of illumination by moving either towards or away from a light source. Regulation of phototactic motility involves many different gene products, including various photoreceptors, second messengers and the RNA chaperone Hfq. Previously, we showed that individual *Synechocystis* 6803 cells do not respond to a spatiotemporal gradient in light intensity, but rather they directly and accurately sense the position of a light source. Single cells of *Synechocystis* 6803 focus the light from a unidirectional light source in a sharp focal point on the distal side of the cell. We were able to demonstrate that this focusing effect correlates with phototactic movement. However, the signal transduction pathway regulating the motility apparatus in a polar light-dependent manner remains elusive. We propose a model where the strong focal point induces a local inhibition of the motility apparatus at the shadow-side surface of the cell. At least three operons which gene products show similarity to known classical chemotaxis regulators are involved in the regulation of phototaxis. Using fluorescence microscopy we determined the localization of CheY-like and PATAN-domain response regulators as well as type IV pili in the cell. We show that the expression level of these response regulators can regulate direction of movement and that ethylene and the second messenger c-di-GMP modulate this response.

Mechanism and function of non-standard circadian clock systems in cyanobacteria

Christin Koebler[1, ✉], Anja Doerrich[2], Anika Wiegard[3], Annegret Wilde[1]

[1]*Molecular Genetics, Institute of Biology III, University of Freiburg, Germany;*
[2]*Institute of Microbiology and Molecular Biology, Justus-Liebig-University Giessen, Germany;*
[3]*Synthetic Microbiology, Heinrich-Heine-University Duesseldorf, Germany*
✉ *christin.koebler@biologie.uni-freiburg.de*

Through the rotation of the earth, all organisms are subjected to daily environmental changes. To facilitate their adaption, many organisms generate an internal rhythm with a period length of around 24 hours, referred to as circadian rhythm. Within cyanobacteria *Synechococcus elongatus* sp. PCC 7942 (hereafter *Synechococcus*) functions as a model organism for the circadian clock. Its clock consists of three core proteins: KaiA, KaiB and KaiC. Phosphorylation and dephosphorylation of KaiC maintains the timing mechanism. The circadian system is rather well understood in *Synechococcus*, but can be quite diverse in other cyanobacteria. Some species lack *kai* genes, whereas others acquired additional *kai* homologs. *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) for example, contains two additional homologs of the *kaiB* and *kaiC* genes. However, it is suggested that they form other time keeping mechanisms, but their function is still unknown. Using deletion mutants for each additional *kai* gene we want to elucidate their respective phenotype and uncover their function. Additionally, we want to explore a putative cross talk between the non-canonical Kai homologs via protein interaction studies. Furthermore, the core oscillator of *Synechocystis* seems to employ a different output signaling pathway than *Synechococcus* and until now, most of this pathway remains unsolved. We aim to identify new components of the output signaling pathway, screening for impaired growth of oscillator deficient strains under dark-light cycles. Finally, we want to determine interaction partners and their position within the regulatory network of the circadian clock.

Acclimation to limiting CO_2 amounts: photorespiration, inorganic carbon sensing and synthetic biology approaches.

Martin Hagemann

University Rostock, Dept. Plant Physiology, A.-Einstein-Str. 3, D-18059 Rostock, Germany;

✉ *martin.hagemann@uni-rostock.de*

Cyanobacterial evolved an efficient inorganic carbon metabolism (CCM) that allows Rubisco to work under saturating CO_2-conditions. Nevertheless, photorespiratory metabolism remained essential for cyanobacteria under ambient air conditions to remove toxic byproducts of the Rubisco oxygenase reaction. The acclimation to low CO_2 (LC) conditions involves the coordinated up-regulation of many genes mostly encoding for components of the CCM and but also many other proteins. These expression changes are accompanied by characteristic shifts in metabolite pools; among them many photorespiratory intermediates were transiently accumulated. The transcript and metabolic patterns of wild-type cells were compared to mutants defective in the CCM to support the role of metabolic signals such as phosphoglycolate as potential signal molecules. In contrast to *in vitro* data, increased amounts of photorespiratory intermediates did not lead the expected transcriptional changes in CCM-related genes. Hence, other signals might be involved in the sensing of LC conditions. The potential role of the newly discovered PII-like protein SbtB as LC-sensor among cyanobacteria will be discussed. Moreover, photorespiration is an energy-demanding process that is also leading to the loss of carbon Therefore, attempts were initiated to generate artificial photorespiratory pathways, which are supposed to work without CO_2 loss or can even fix additional CO_2. Cyanobacteria such as *Synechocystis* sp. PCC 6803 provide ideal chassis to test such pathways in a living photosynthetic cell.

RNA polymerase active centre compensates for the absence of the essential transcription factors in Cyanobacteria

Amber Riaz-Bradley and Yulia Yuzenkova

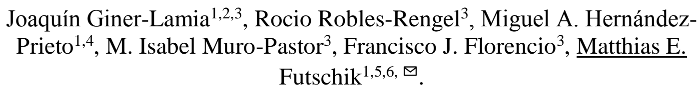
Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne, NE2 4AX, UK

Transcription – copying genetic information into RNA form, is the first step of gene expression. While copying, the main enzyme of transcription, RNA polymerase, inevitably makes mistakes, incorporating wrong, non-base paired with genomic DNA, nucleotides into RNA. Misincorporation leads to immediate pause of the RNA polymerase on DNA. To correct mistakes in real time, vast majority of all living organisms encode specific transcription proofreading factors. Role of proofreading factors goes beyond making a correct RNA. In bacterial cell they help to prevent transcriptional "traffic jams" and collisions of RNA polymerases with replisome, caused by paused RNA polymerases.

Remarkably, cyanobacteria do not encode any proofreading factors. How they compensate for their absence? We found two compensatory mechanisms: very efficient intrinsic (factor-independent) proofreading and overall low propensity of cyanobacterial RNA polymerase to pause on DNA. Our results suggest that active site configuration of cyanobacterial RNA polymerase is the same as *E.coli* one, but its dynamic is different. By having less flexible "hinges" of the molecule, cyanobacterial enzyme tends to clasp DNA more tightly, compared to the *E.coli* RNA polymerase. This closed conformation results in fast and highly processive enzyme. Additionally, transcription and replication might be temporally or spatially separated in cyanobacteria, further relaxing the need for proofreading factors.

Identification of the direct regulon of NtcA during early acclimation to nitrogen starvation in the cyanobacterium *Synechocystis* sp. PCC 6803

Joaquín Giner-Lamia[1,2,3], Rocio Robles-Rengel[3], Miguel A. Hernández-Prieto[1,4], M. Isabel Muro-Pastor[3], Francisco J. Florencio[3], Matthias E. Futschik[1,5,6,] ✉.



[1] *System Biology and Bioinformatics Laboratory, University of Algarve, 8005-139 Faro, Portugal.*

[2] *Laboratory of Intracellular Bacterial Pathogens, Department of Microbial Biotechnology, Centro Nacional de Biotecnología-Consejo Superior de Investigaciones Científicas (CNB-CSIC), Darwin, Madrid , Spain.*

[3] *Instituto de Bioquímica Vegetal y Fotosíntesis. Universidad de Sevilla-CSIC, Av. Américo Vespucio 49, E 41927 Seville Spain.*

[4]*ARC Centre of Excellence for Translational Photosynthesis and School of Biological Sciences, University of Sydney, NSW 2006, Australia.*

[5]*Centre of Marine Sciences, University of Algarve, 8005-139 Faro, Portugal.*

[6]*School of Biomedical & Healthcare Sciences, Plymouth University Peninsula Schools of Medicine and Dentistry, Plymouth, PL6 8BU, UK*

✉ *mfutschik@ualg.pt*

In cyanobacteria, nitrogen homeostasis is maintained by an intricate regulatory network around transcriptional factor NtcA. Although mechanisms controlling NtcA activity appear to be well understood, its regulon remains poorly defined. To determine the NtcA regulon during the early stages of nitrogen starvation for the model cyanobacterium *Synechocystis* sp. PCC6803, we performed chromatin immunoprecipitation, followed by sequencing (ChIP-seq), in parallel with transcriptome analysis (RNA-seq). Through combining these methods, we determined 51 genes activated and 28 repressed directly by NtcA. In addition to genes associated with nitrogen and carbon metabolism, a considerable number of genes without current functional annotation were among direct targets providing a rich reservoir for further studies. The NtcA regulon also included eight non-coding RNAs, of which *ncr0710*, Syr6 and NsiR7 were experimentally validated, and their putative targets were identified. Surprisingly, we found substantial NtcA binding associated with delayed expression changes indicating that NtcA can reside in a poised state controlled by other factors. Indeed, a role of PipX as modulating factor in nitrogen regulation was confirmed for selected NtcA-targets. We suggest that the indicated poised state of NtcA can enable a more differentiated response to nitrogen limitation and can be advantageous in native habitats of *Synechocystis.*

Broad-host-range genetic tools for cyanobacteria and heterologous expression of natural products

Taton, Arnaud, Amy T. Ma, You Chen, Vinayak Agarwal, Young-Saeng Kim, Brian P. Tieu, Bryan Bishe, and James W. Golden✉

Division of Biological Sciences, Univ. of California, San Diego, La Jolla, CA, USA

✉ *jwgolden@ucsd.edu*

Improved genetic tools for cyanobacteria provide new opportunities for scientific research and biotechnology. We have developed a set of broad host range standardized genetic parts and devices for genetic modification and engineering diverse cyanobacterial strains. The parts and devices are carried in a library of donor vectors. These donor vectors contain origins of replication for *E. coli*, origins of transfer for conjugation, origins of replication and neutral sites for various cyanobacteria, antibiotic markers, expression cassettes with different promoters, and reporter cassettes. This library of donor vectors allows the construction of modular shuttle vectors, designed for the component parts to be easily replaced or additional parts to be easily inserted. Different types of vectors can be assembled, including autonomously replicating vectors and integrating vectors for gene knockout and gene expression from the chromosome. Assembled modular vectors allowed a thorough characterization of different genetic parts and devices in several diverse cyanobacterial strains. The system has been used for the characterization of mutant versions of the broad-host-range plasmid RSF1010, theophylline-inducible riboswitches, a set of constitutive promoters with different levels of activity, and vectors based on the *S. elongatus* plasmid pANS. The genetic tool kit has been used for the heterologous expression of several genes, including a 20-kb gene cluster required for the biosynthesis of the polyunsaturated fatty acid EPA, a *hs_bmp7* gene and *hs_bmp7 to hs_bmp12* gene cluster involved in synthesis of polybrominated diphenyl ethers, and the dehydroascorbate reductase (DHAR) gene from *Brassica juncea* (*BrDHAR*).

Partner swapping of cyanobacterial nitrogen regulators PII and PipX during diurnal cycles

Javier Espinosa, Jose I. Labella, Raquel Cantos and Asunción Contreras✉

Departamento de Fisiología, Genética y Microbiología, Universidad de Alicante, Spain,

✉ *contrera@ua.es*

Cyanobacteria require regulatory strategies to adapt their metabolic processes to the challenges imposed by the succession of days and nights. PII, a protein found in all three domains of life as an integrator of signals of the nitrogen and carbon balance as well as of energy signals, functions by protein-protein interactions. In cyanobacteria PII stimulates arginine biosynthesis in complex with N-acetyl-L-glutamate kinase and controls acetyl-CoA levels by binding to acetyl-CoA carboxylase. Unique to cyanobacteria is the existence of PipX, another nitrogen regulator mediating its function by protein-protein interactions. Alternate binding of PipX between PII and the transcriptional regulator NtcA provides a mechanistic link between PII signaling and NtcA-regulated gene expression. PII-PipX complexes can also bind to the transcriptional regulator PlmA, whose regulon remains unknown. Here we demonstrate that PII and PipX proteins display distinct localization patterns during diurnal cycles and that at dark periods they co-localize into the same foci at the periphery and poles of the cells, a process requiring wild type PII and a low ATP/ADP ratio. We also show that PII inhibits nitrate transport in darkness, unless the ATP/ADP ratio is artificially increased. We propose that the energy-driven partner swapping of PII and PipX with their protein targets plays a role in the nocturnal down regulation of nitrogen assimilation and gene expression, and therefore in dormancy of cyanobacterial cells.
Funded by MIMECO-FEDER

Session 4: Cellular Differentiation and Cell Biology

Cell division in the filamentous, pluricellular cyanobacterium *Anabaena*

Antonia Herrero[1], Ana Valladares[1], Sergio Camargo[1], Laura Corrales[1], Silvia Picossi[1], Jesús Ochoa de Alda[2], Enrique Flores and Ignacio Luque[1]

[1]*Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas and Universidad de Sevilla, Spain,*

[2]*Facultad de Formación del Profesorado, Universidad de Extremadura, Cáceres, Spain.*

✉ *herrero@ibvf.csic.es; avalla@IBVF.csic.es; scamargobernal@gmail.com; corrales@uni-marburg.de; silvia.picossi@ibvf.csic.es; ochoadealda@unex.es; eflores@ibvf.csic.es; ignacio.luque@ibvf.csic.es*

In heterocyst-forming cyanobacteria, the organismic unit is a filament of communicated cells. In the septal regions between neighboring cells, proteinaceous structures called septal junctions provide intercellular binding and molecular exchange functions. These features require specific mechanisms of cell division as compared to those producing separated daughter cells. In most bacteria, cell division is initiated by FtsZ tethering to the cytoplasmic membrane (mediated by membrane-bound proteins) and FtsZ polymerization forming the Z-ring at the future division site. In cyanobacteria of the filamentous-heterocystous clade, FtsZ has a conserved N-terminal sequence preceding the ubiquitous globular core that includes the GTP binding and hydrolysis determinants. Deletion of the N-terminal sequence of FtsZ was not possible in *Anabaena*, and expression of FtsZ variants lacking this part (DN-FtsZ) together with the native protein led to drastic alterations in cell shape and cell size, and the formation of aberrant Z-ring structures that were frequently positioned outside the cell center. In vitro, *Anabaena* FtsZ polymerized forming filaments with a distinct curling shape, in contrast to highly-bundled straight filaments formed by DN-FtsZ. In BACTH analysis, *Anabaena* FtsZ interacted with several divisome proteins including the membrane-bound ZipN. In *Anabaena* derivatives depleted of ZipN the fraction of membrane-bound FtsZ was considerably lower than in the wild type, indicating that ZipN is an FtsZ tether. The septal junction protein SepJ is recruited to the divisome, establishing interactions with many divisome components and influencing the morphology of the resulting septa. Thus, SepJ is a key element during the process of septation in *Anabaena*.

Overcoming the septal barrier: how communication structures are formed in the septum of filamentous cyanobacteria

Iris Maldener✉, Jan Bornikoel, Eva Bok, Katharina Faulhaber, Ann-Katrin Kieninger, Karl Forchhammer

IMIT-Organismic Interactions, University of Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany

✉ iris.maldener@uni-tuebingen.de

Upon nitrogen starvation, *Nostoc* forms nitrogen-fixing heterocysts along the trichome and exhibits an intercellular elaborated communication system. Metabolites and signal molecules cross the septal peptidoglycan (PG) between adjacent cells, presumably via septal junction complexes. Isolated PG of cyanobacterial trichomes is contiguous, preserving the filamentous structure of the trichomes. The septal PG between adjacent cells forms a rigid disc and is perforated by approximately 100 nanopores. This nanopore array is involved in intercellular transfer of small molecules and we suggest that it is necessary for the formation of the septal junctions.

By a functional study of cell wall lytic amidases in two *Nostoc*-strains we showed that AmiC drills the pores in the septal cell wall, creating the nanopore array. The intercellular transfer of small molecules is affected in *amiC*-mutants, showing the importance of the nanopores for the formation of the septal junctions. Putative septal junction protein SepJ is functionally linked to AmiC as shown by mutational and localization studies.

The structure of the catalytic domain of AmiC2 has been resolved in high resolution and enzymatic studies illustrate unique features of this enzyme. In addition, the nanopore-septal junction complex was visualized by electron cryotomography solving the *in situ* architecture at a macromolecular resolution.

In conclusion, our study shows that the septal cell wall of *Nostoc* exhibits unique features with regularly arranged nanopores and septal junctions. It represents a dynamic structure, which influences vital processes in the complex life style of multicellular cyanobacteria.

A novel contractile injection system in cyanobacteria

Gregor L. Weiss✉, Hannah Minas, Martin Pilhofer

Institute of Molecular Biology & Biophysics, Eidgenössische Technische Hochschule Zürich, CH-8093 Zürich, Switzerland

✉ *gregor.weiss@mol.biol.ethz.ch*

Contractile injection systems are important mediators of bacterial cell-cell interactions, delivering effectors by a bacteriophage tail-like structure (Leiman et al.). One example is the Type 6 Secretion System (T6SS), acting as an inverted phage tail anchored to the cytoplasmic membrane. We recently reported the first *in situ* architecture of a novel T6SS termed subtype IV (T6SSiv) (Böck et al.). Evolutionary sequence analyses predicted that contractile secretion systems are much more widespread than previously thought.
Here we investigated the presence of the T6SS*iv*-like gene cluster in diverse bacteria. We detected highly significant candidates in 17 bacterial phyla including the *Cyanobacteria*. We showed that the system was expressed in two cyanobacterial model organisms by purifying contractile structures from cultures and imaging them. The structures were identified being encoded by the T6SSiv-like gene cluster by mass spectrometry. We then vitrified cells by plunge-freezing, thinned the samples by cryo-focused ion beam milling and imaged the cells by electron cryotomography in order to visualize T6SSiv *in situ* (Weiss et al.). Strikingly, we observed a high abundance of contractile injection systems, which were always associated with the outermost thylakoid membrane (Figure).
To our knowledge, this is the first report of contractile injection systems in *Cyanobacteria*. The high expression level indicates the physiological relevance of the system. We would like to take the possibility to present our results to the *Cyanobacteria* community to mediate discussions in particular regarding a possible function of this intriguing system.

References:
Leiman, P. G., Basler, M., Ramagopal, U. A., Bonanno, J. B., Sauder, J. M., Pukatzki, S., et al. (2009). Type VI secretion apparatus and phage tail-associated protein complexes share a common evolutionary origin. *Proc Natl Acad Sci USA*, 106(11), 4154–4159.
Böck, D., Medeiros, J. M., Tsao, H., Penz, T., Weiss, G. L., Aistleitner, K., Horn, M., Pilhofer, M. (2017). In situ architecture, function, and evolution of a contractile injection system. *Science*, in print.
Weiss, G. L., Medeiros, J. M., & Pilhofer, M. (2017). In Situ Imaging of Bacterial Secretion Systems by Electron Cryotomography. *Methods Mol Biol*, 1615(Chapter 27), 353–375.

A Janus-faced IM30 ring is involved in thylakoid membrane fusion in cyanobacteria

Jennifer Suppes[1], Raoul Hennig[1], Benedikt Junglas[1], Michael Saur[2], Jürgen Markl[2] and Dirk Schneider[1]

[1]*Institute of Pharmacy & Biochemistry,*
[2]*Institute of Zoology, Johannes Gutenberg University Mainz, Germany*

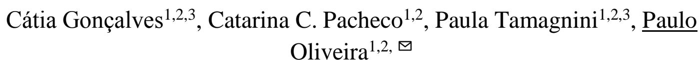
Thylakoid membranes are unique cellular membranes in cyanobacteria and chloroplasts. However, despite their apparent importance, remarkably little is known as to the biogenesis, dynamics and maintenance of thylakoids. The “inner membrane-associated protein of 30 kDa” (IM30), also known as the “vesicle inducing protein in plastids 1” (Vipp1), was known to be involved in the biogenesis of the thylakoid membrane system, although the exact physiological function of IM30 has remained mystic for a long time. We now have identified the IM30 protein of the cyanobacterium *Synechocystis* sp. PCC 6803 as being a thylakoid membrane fusion protein, which turns out to be a key player for thylakoid membrane formation, dynamics and maintenance in chloroplasts and cyanobacteria. IM30 monomers pre-assemble into stable tetrameric building blocks, which further align to form oligomeric ring structures, and differently sized IM30 rings bind to membranes. However, ring formation is not crucial for, but even counteracts interaction of IM30 with negatively charged membrane surfaces, albeit membrane fusion appears to depend on IM30 ring formation.

In summary, based on our analyses we presume that IM30 establishes contacts between internal membranes and promotes membrane fusion to enable regulated exchange of proteins and/or lipids in cyanobacteria and chloroplasts.

References:

Hennig, R., *et al.* (2015), *Nat. Commun*. 6, 7018
Heidrich J. *et al.* (2016) *JBC* 291, 14954ff
Hennig R. *et al.* (2017) *BBA* 1858, 126ff
Heidrich, J., *et al.* (2017) *BBA* 1859, 537ff

At the crossroads of cyanobacterial secretion mechanisms: multidrug efflux meets protein modification and export



Cátia Gonçalves[1,2,3], Catarina C. Pacheco[1,2], Paula Tamagnini[1,2,3], Paulo Oliveira[1,2,] ✉

[1] *i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal*

[2] *IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal*

[3] *Faculdade de Ciências, Departamento de Biologia, Universidade do Porto, Porto, Portugal.*

✉*paulo.oliveira@ibmc.up.pt*

✉ *catia.goncalves@ibmc.up.pt, cclopes@ibmc.up.pt, pmtamagn@ibmc.up.pt*

TolC-mediated secretion fulfills a number of important physiological roles, ranging from drug efflux to protein export. Following up on previous work in which a TolC-homologue was identified in the unicellular cyanobacterium *Synechocystis* sp. PCC6803 (1), here we report on the identification of inner membrane (IM) and periplasmic adaptor protein (PAP) components involved in TolC-dependent secretory mechanisms. The genome of *Synechocystis* sp. PCC6803 was investigated, resulting in the identification of 11 candidate genes putatively involved in TolC-dependent secretion mechanisms. Gene-inactivation of each of the candidates followed by a comprehensive phenotypic characterization allowed to link specific protein components to particular functional roles in the secretion of defined compounds. Interestingly, we present experimental evidence showing that secretion of proteins via the classical type I secretion system in *Synechocystis* sp. PCC6803 is dependent on the function of more than a single PAP. Furthermore, mutants inactivated in specific IM or PAP components present phenotypic impairments that were not detected in the *tolC*-deletion mutant, namely on the glycosylation of pilin subunits. To the best of our knowledge, these observations represent the first evidence that TolC-functional partners may play additional and TolC-independent roles, highlighting how intricate and functionally dependent secretory mechanisms are in cyanobacteria. Finally, we show that the hyper-vesiculating phenotype presented by the TolC minus mutant does not result solely from impairment in secretion capacity, but may also be dependent on cell envelope stress management.

1. Oliveira, P., *et al.* 2016. The versatile TolC-like Slr1270 in the cyanobacterium *Synechocystis* sp. PCC6803. *Environ. Microbiol.* 18:486-502.

Discovery of the novel calcium-binding protein CSE in the filamentous cyanobacterium *Anabaena* sp. PCC 7120

Julia Walter[1], Ute C. Vothknecht[2], Eva-Mari Aro[1], Peter J. Gollan[1]

[1] *Molecular Plant Biology, University of Turku, Turku, Finland*

[2] *IZMB – Plant Cell Biology, University of Bonn, Bonn, Germany*

✉ *julia.walter@utu.fi, vothknecht@uni-bonn.de, evaaro@utu.fi, peter.gollan@utu.fi*

Filamentous cyanobacteria represent optimal model organisms for studies investigating multicellularity. Under nitrogen-limited conditions, some species, such as *Anabaena* and *Nostoc*, are capable of differentiating photosynthetic vegetative cells into specialised cells, called heterocysts, which are responsible for fixing atmospheric nitrogen. During heterocyst differentiation, a calcium signal and cyanobacterial calcium-binding protein (CcbP) are involved in this highly regulated process in *Anabaena* 7120. However, other calcium-binding proteins in cyanobacteria have not been discovered, and calcium signalling in cyanobacteria, in general, is poorly understood.

Here, we describe a novel calcium-binding protein in *Anabaena* 7120. Calcium Sensor EF-hand (CSE) is a small soluble protein consisting of only two EF-hand domains that bind calcium *in vitro*. CSE is highly conserved in filamentous cyanobacteria species. A mutant strain lacking CSE (Δ*cse*) displayed a striking short-filament phenotype, with more than 90 % of filaments containing 1-5 cells per filament. Bright-field and electron micrographs showed the absence of heterocysts from the short filaments, and the presence of partially differentiated cells. Transcriptomics analysis in nitrogen-replete conditions revealed down-regulation of genes involved in heterocyst differentiation/function, and upregulation of genes involved in inhibition of heterocyst development. These results suggest an impairment of heterocyst formation in Δ*cse*. Furthermore, immediate down-regulation of *cse* expression in *Anabaena* WT and changed expression patterns of heterocyst differentiation genes in Δ*cse* upon nitrogen step-down indicate that CSE is involved in the early heterocyst differentiation process by sensing and/or controlling calcium signalling. Calcium was shown to fine-tune the carbon and nitrogen balance intracellularly, and thus might regulate heterocyst differentiation too.

Spatial mapping of a cyanobacterial proteome reveals distinct subcellular compartment organisation and dynamic metabolic pathways

Laura L. Baers[1], David J. Lea-Smith[1,δ], Laurent. Gatto, Lisa M. Breckels, Michael J. Deery, Tim J. Stevens[2], Christopher J. Howe[1], Kathryn S. Lilley[1,3,*].

[1] *Department of Biochemistry, Hopkins Building, Downing Site, University of Cambridge, CB2 1QW, United Kingdom*

[2] *MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, United Kingdom*

[3] *Cambridge Systems Biology Centre, and Cambridge Centre for Proteomics*

[δ] *Current Address: School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK*

Cyanobacteria are complex prokaryotes, incorporating a Gram-negative cell wall and internal thylakoid membranes. However, localisation of proteins within cyanobacterial cells is poorly understood. Using subcellular fractionation and quantitative proteomics we report the most extensive subcellular map of the proteome of a cyanobacterial cell, identifying ~67 % of *Synechocystis* sp. PCC 6803 proteins, ~1000 more than previous studies. 1,711 proteins were assigned to six specific subcellular regions. Proteins involved in energy generation localised to thylakoid membranes whereas transporters and regulatory proteins predominantly reside in the plasma membrane. The majority of metabolic enzymes are soluble although numerous biosynthetic pathways terminate in membranes. Ribosomal proteins and enzymes synthesising the storage compound polyhydroxybuyrate localise to distinct regions of the cell. Moreover, heterogeneity within membrane and cytoplasmic regions is observed, indicating further cellular complexity. Cyanobacteria thylakoid membrane protein localisation is conserved in *Arabidopsis thaliana* chloroplasts, suggesting similar proteome organisation in higher photosynthetic organisms. The dynamic organisation of a cyanobacterial cell we reveal will aid our understanding of these environmentally and biotechnological important organisms.

The awakening of a dormant cyanobacterium reveals a cryptic program for maintenance of viability

Karl Forchhammer

Interfakultäres Institut für Mikrobiologie und Infektionsmedizin der University Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany

✉*karl.forchhammer@uni-tuebingen.de*

When the unicellular cyanobacterium *Synechocystis* PCC 6803 is subjected to nitrogen deprivation, the cells become chlorotic and enter a dormant state that enables survival for prolonged periods of time. Upon re-addition of nitrate, almost all cells start a highly organized, genetically determined resuscitation program. In an initial preparatory phase, the cells restore the machinery for protein synthesis. During that period, they switch to a heterotrophic mode of metabolism: despite illumination, they turned off residual photosynthesis and turned on glycogen catabolism, generating energy by respiration. After having reset the basic functions (after about 16 hours), the cells start to re-build the photosynthetic machinery and gradually switch on photosynthesis. Only after having re-established full photosynthetic capacity (after about 48 hours), the cell-cycle arrest is released and cells divide again. The entire process is manifested at the transcriptomic and proteomic level. The transcriptome revealed a major contribution of non-coding RNA and many genes that are highly expressed under dormancy encode proteins of unknown functions. The resuscitation experiments revealed functions for several of these so-far unknown proteins. In addition, proteomic analysis showed abundant protein phosphorylation during prolonged starvation and it appears that the phosphorylation state controls protein stability.

Reference:

Klotz et al. (2016). Awakening of a Dormant Cyanobacterium from Nitrogen Chlorosis Reveals a Genetically Determined Program. Current Biology *26*, 2862-2872.

Remodelling of a cyanobacterial chlorophyll-synthase complex by high-light inducible proteins

Mahendra K. Shukla[1,2], Philip J. Jackson[3], Lena Moravcová[1], Amanda A. Brindley[3], C. Neil Hunter[3], Roman Sobotka[1,2]

[1] *Institute of Microbiology, Academy of Sciences, 37981 Treboň, Czech Republic*

[2] *Faculty of Sciences, University of South Bohemia, 370 05 České Budejovice, Czech Republic*

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

Cyanobacteria contain a family of one-helix high-light-inducible proteins (Hlips), which are widely accepted as ancestors of plant light harvesting antennae. Hlips are known to bind chlorophyll and carotenoids and to play an essential role in the photoprotection and in chlorophyll synthesis/recycling; however the mechanism is not explained1. The cyanobacterium *Synechocystis* 6803 contains four Hlips (HliA-D) and while the HliD appears to be constantly present in the cell, other Hlips are detectable only after exposure to stress conditions. The HliD has been found previously in a pigmented complex with chlorophyll synthase (ChlG) and putative alcohol dehydrogenase Ycf39 and the formation of this complex was shown to be important for the proper functioning of chlorophyll biosynthesis2. Here, we demonstrated that the stress-induced HliC binds tightly to HliD and the resulting HliC-HliD pairs monomerize ChlG and prevent Ycf39 to be bound to the ChlG complex. Moreover, we observed that under low-stress the ChlG is attached to either trimeric or monomeric Photosystem I but the HliD/C-ChlG complex interacts also with an enigmatic low-abundant form of Photosystem I designated as PSI[1]*. The HliC-driven structural changes appear to be particularly important during cold stress as the *Synechocystis* strain lacking HliC is poorly viable at 20°C due to inhibited chlorophyll biosynthesis and the loss of functional Photosystem II. We suggest that Hlips work like a safe glue/anti-glue, promoting formation of 'stress-ready' protein complexes (biogenesis centres) that integrate terminal steps of chlorophyll biosynthesis and the biogenesis of Photosystem II.

References

1. Komenda, J. and Sobotka, R. Biochim Biophys Acta, 2016, 1857, 288-95.
2. Chidgey, J. W. *et al.*, Plant Cell, 2014, 26, 1267-79

Production of extracellular polymeric substances (EPS) in *Synechocystis* PCC 6803: the role of Wzc and Wzb

Sara B. Pereira[1,2], Marina Santos[1,2,3], José P. Leite[1,2], Carina Eisfeld[1,2], Carlos Flores[1,2,3], Rita Mota[1,2,4], Federico Rossi[5], Roberto de Philippis[5], Luís Gales[1,2,3], João Cabral[1,2]and Paula Tamagnini[1,2,4]

[1]. *i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal;*

[2]. *IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal;*

[3]. *ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal;*

[4]. *Faculdade de Ciências, Departamento de Biologia, Universidade do Porto, Porto, Portugal.*

[5] *Department of Agrifood Production and Environmental Sciences, University of Florence, Florence, Italy.*

Many cyanobacteria produce extracellular polymeric substances (EPS), mainly composed of heteropolysaccharides, with unique characteristics that make them suitable for biotechnological and biomedical applications [1]. However, their biosynthetic pathway(s) remain poorly understood, limiting the manipulation/optimization. A phylum-wide analysis led to the identification of genes/proteins putatively involved in the assembly and export of EPS and revealed that cyanobacterial EPS production may not strictly follow one of the pathways previously characterized for other bacteria [2]. Using the model cyanobacterium *Synechocystis* sp. PCC 6803, mutants on EPS-related genes are being generated to assess the specific role of the encoded proteins, namely Δ*wzc* (Δ*sll0923*), Δ*wzb* (Δ*slr0328*), Δ*wzc*Δ*wzb* (Δ*sll0923*Δ*slr0328*) and wzc_{Trunc} (encoding a protein lacking the C-terminal Y-rich region where autophosphorylation/dephosphorylation by Wzb is expected to occur). These mutants produced different amounts and qualities of CPS and/or RPS compared to the wild type. The results emphasize the role of Wzc in CPS and RPS production [3] and suggest a phosphoregulatory interaction between Wzc and Wzb. To address this issue, these proteins were overexpressed, purified and used *in vitro* assays. The phosphatase activity of Wzb was confirmed [4] and its structure resolved. In addition, Wzc was shown to have ATPase and autokinase activities. Most importantly, Wzb is able to dephosphorylate Wzc *in vitro*. Currently, these studies are being extended to *in vivo* conditions.

[1] Leite et al. 2017 Macromol Biosci 17:1600206 [2] Pereira et al. 2015 Sci Rep 5:14835 [3] Jittawuttipoka et al. 2013 PLoS ONE 8:e55564 [4] Mukhopadhyay & Kennelly 2011 J Biochem 149: 551-562.

Session 5: Bioenergetics

Alternative electron transport pathways in cyanobacteria

Y. Allahverdiyeva✉, M. Ermakova, A. Santana-Sanchez, L. Bersanini, Eva-Mari Aro

Molecular Plant Biology, Department of Biochemistry, University of Turku, FI-20014 Turku, Finland.
✉ allahve@utu.fi

Aquatic photosynthetic microorganisms experience strong fluctuations in environmental conditions including nutrient availability, light quality and light quantity. In order to avoid large-scale ROS damage to photosynthetic machinery, various auxiliary electron transport pathways have evolved. In this respect, flavodiiron proteins (FDPs, Flv) play an important role as strong safety valves. In *Synechocystis* sp. PCC 6803, 4 different genes encode Flv1-to-Flv4 proteins, these function as hetero-oligomers in Mehler-like reactions reducing O_2 to water down-stream of PSI. Growth of cyanobacteria under rapidly fluctuating light intensities is enabled by Flv1/Flv3 acting on a timescale of seconds, whilst Flv2/Flv4 function in a pH dependent manner over a relatively longer time scale. Interestingly, the Flv3 homo-oligomer also plays a role in the photo-protection of cyanobacteria. However, it is not involved in O_2 photoreduction. The exact photo-protective mechanism of Flv3 is unclear. More precise study of different *flv4-sll0218-flv2* operon mutants demonstrated that the majority of observed PSII phenotypes were due to the absence of Sll0218, which contributes to PSII repair and stability. In addition to FDPs, thylakoid localized terminal oxidases (Cox and Cyd) play important roles in photo-protection, but operate more slowly than FDPs and on a limited scale. The impact of FDPs on the photoproduction of hydrogen in *Anabaena* sp. PCC7120 will also be discussed.

The *psba* gene family in heterocystous cyanobacteria

Kevin Sheridan and Tina C. Summerfield

Department of Botany, University of Otago, PO Box 56, Dunedin, New Zealand

✉ *Tina.Summerfield@otago.ac.nz, Kevin.Sheridan@otago.ac.nz*

The *psbA* gene encodes D1, a core protein of Photosystem II. The D1 protein is damaged during light-induced inactivation of Photosystem II, consequently sustained activity requires rapid degradation of photodamaged protein balanced by rapid synthesis of new copies of D1. In cyanobacteria, the response to a variety of stress conditions includes altered transcription of different *psbA* genes and in some cases exchanging D1 isoforms in the PS II reaction centre. Many cyanobacteria contain *psbA* gene families with 2–8 copies, typically encoding 2–4 different D1 proteins. The heterocystous cyanobacteria are monophyletic [Howard-Azzeh M, Shamseer L, Schellhorn HE, Gupta RS. 2014. Phylogenetic analysis and molecular signatures defining a monophyletic clade of heterocystous cyanobacteria and identifying its closest relatives. Photosynthesis Research 122:171-185]. We have used available genome sequences to examine the distribution of the *psbA* gene families in these cyanobacteria.

What happens if the photosystem I/photosystem II (PSI/PSII) stoichiometry changes?

Vicki Moore and Wim Vermaas✉

School of Life Sciences and Center for Bioenergy and Photosynthesis, Arizona State University, Tempe, AZ 85287-4501, USA,

✉ *wim@asu.edu*

Some cyanobacteria have much more PSI than PSII: for example, *Synechocystis* sp. PCC 6803 maintains a PSI/PSII ratio of 2-5. More PSI offers the potential for abundant cyclic electron flow around PSI to generate sufficient ATP as *Synechocystis* cells have low respiratory activity. To test the hypothesis that a high PSI/PSII ratio correlates with increased cyclic electron flow, we generated photoautotrophic mutants with 2-to-4-fold less PSI using combinatorial mutagenesis. Low-PSI strains may have less cyclic and more linear photosynthetic electron flow, resulting in an increased NADPH/ATP ratio. These strains were tested for growth and, after introduction of appropriate additional mutations, for their ability to produce free fatty acids, which is an NADPH-intensive process. We found that strains with less PSI: (a) retained their PSII levels, resulting in unaltered oxygen evolution per cell at saturating light intensity; (b) grew more slowly at lower light intensity (50 mmol photons/m^2/s) but grew about as well as the wild type at higher intensities, in line with a higher light intensity required for saturation of photosynthetic electron transport; and (c) produced more free fatty acids. The results indicate that: (a) in wild type there is an overabundance of PSI for linear electron flow; (b) low-PSI strains do best at higher light intensity and may have an evolutionary advantage; and (c) increased fatty acid production and a concomitantly increased NADPH level is evidence for more linear photosynthetic electron transfer. These results illustrate that the NADPH/ATP supply ratio in cyanobacteria can be modulated via the PSI/PSII ratio.

Chlorophyll *f* photosynthesis in the cyanobacterium *Chroococcidiopsis thermalis*

Dennis J. Nürnberg[1], Andrea Fantuzzi[1], Stefano Santabarbara[2], Alison Telfer[1], Pierre Joliot[3], Farbrice Rappaport[3], Alain Boussac[4], and A. William Rutherford[1]

[1]*Department of Life Sciences, Imperial College, London, United Kingdom*

[2]*Istituto di Biofisica, Consiglio Nazionale delle Ricerche, Milano, Italy*

[3]*Institut de Biologie Physico-Chimique, CNRS -Univ P et M Curie, Paris, France*

[4]*iBiTec-S, CNRS UMR 8221, CEA Saclay, Gif-sur-Yvette, France*

Oxygenic photosynthesis uses chlorophyll-*a*to convert solar energy into the chemical energy that drives the biosphere. Chlorophyll-*a* absorbs visible light up to ~700nm, the so-called “red limit”. Some cyanobacteria extend this limit by using modified chlorophylls. Chlorophyll-*f*, is the longest wavelength and most recently discovered chlorophyll but its role in light-harvesting and photochemistry remains unknown. When the cyanobacterium *Chroococcidiopsis thermalis* is grown in far-red/near IR light it forms around 10 % chlorophyll *f* in addition to chlorophyll *a*. Here we investigate the effect of far-red light on the remodelling of photosystem PSI andPSII, and the phycobilisome complexes using various spectroscopic, microscopical and biophysical methods. Our results show evidence for (i) densely packed thylakoid membranes with phycobilisome complexes strongly reduced in quantity and size, (ii) highly oligomerised photosynthetic complexes of PSI, and (ii) chlorophyll *f* involved in charge separationin both photosystems, PSI and PSII. These findings have important implications for the understanding the bioenergeticsof the oxygenic photosystems and the red-limit.

Photosystem II repair cycle in cyanobacteria: news and views

Josef Komenda[1], Vendula Krynická[1], Martina Bečková[1], Shengxi Shao[2], Jianfeng Yu[2], James W. Murray[2] and Peter Nixon[2]

[1]Institute of Microbiology, Center Algatech, 37981 Třeboň, Czech Republic

[2] Department of Life Sciences, Imperial College London, London, SW7 2AZ, UK

✉ *Email: komenda@alga.cz, krynicka@alga.cz, beckova@alga.cz, s.shao11@imperial.ac.uk, j.yu@imperial.ac.uk, j.w.murray@imperial.ac.uk, p.nixon@imperial.ac.uk*

The Photosystem II complex (PSII) represents the key component of the oxygenic photosynthetic apparatus which is responsible for water oxidation. It is a multi-subunit protein complex consisting of large subunits D2, D1, CP47 and CP43, a number of small polypeptides and many cofactors. The complex is very vulnerable to the light-induced damage and the most important protective mechanism counterbalancing this damage is so called repair cycle, which is based on the selective replacement of the central PSII protein subunit D1. Cyanobacterial homologues of the bacterial protease FtsH play a crucial role in this replacement. Namely, the FtsH2/FtsH3 heterohexamer processively degrades the D1 protein from its N-terminus during the repair and also maintains low level of unassembled PSII proteins and assembly intermediate complexes. FtsH2/FtsH3 directly interacts with the Psb29 protein which seems to stabilize this heterocomplex. The degradation of the D1 (and D2) protein is not necessarily triggered by photodamage while the main factor is its accessibility to the protease. The mutant expressing the CP43-D1 fusion instead of individual CP43 and D1 protein copies shows impaired repair and the fusion protein is degraded slower in comparison with the D1 protein in the control strain. This documents an importance of the evolutionary splitting of the ancient 11 helix photosynthetic reaction center proteins into 6 plus 5 helix couple for high effectivity of the PSII repair.

Switchable photosynthetic apparatus in chlorophyll *f* producing cyanobacterium

Min Chen

School of life and environmental Sciences, University of Sydney, NSW 2006, Australia

Discovery of chlorophyll *f*, the most red-shifted chlorophyll currently known, challenged the established view that long wavelength light (>700nm) does not contain sufficient energy to power oxygenic photosynthesis (Chen *et al.* 2010). The red-shifted spectral qualities of chlorophyll *f* make it a good candidate for further research into the ways of improving the efficiency of photosynthesis, by effectively expanding the photosyntheticcally active region from 700 nm to 760 nm (Chen and Blankenship 2011). The cyanobacterium *Halomicronema hongdechloris* was the first organism identified to produce chlorophyll *f* under infar-red light conditions (Chen *et al.* 2012). Recently, we reported a new phycobilisome that absorbs red and far-red light, having the red-shifted absorption peaks of 712 and 730 nm along with the production of chlorophyll *f* (Li *et al.* 2016). Under white light condition (control conditions), *H. hongdechloris* uses chlorophyll *a*-binding photosystems and has phycobilisomes as its major light-harvesting protein complexes. However, *H. hongdechloris* will produce chlorophyll *f* and red-shifted phycobilisomes when they are grown under far-red light conditions. Here, we isolated chlorophyll *f*-binding protein complexes using sucrose gradient ultracentrifugation and characterised the function of isolated chlorophyll-binding protein complexes by pigment and spectral analysis. The interaction between chlorophyll *f*-binding protein complexes and the red-shifted phycobilisomes has been characterised. The pigment composition analysis revealed that chlorophyll *a* is the main chlorophyll in the isolated PSI enriched fraction although there are less than 5% chlorophyll *f* detected in the fraction. Further spectral analysis confirmed that chlorophyll *f* only functions as accessory chlorophylls in the isolated PSI fraction. Interestingly, chlorophyll *f* is mainly presented in the isolated light-harvesting complexes and PSII enriched fractions. Using fluorescence spectral analysis, the new energy transfer pathway is proposed, which is different from the photosynthetic energy pathways in the cells grown under white light. The role of chlorophyll *f* in the chlorophyll-binding light-harvesting complexes of *H. hongdechloris* and its relationship to the phycobiliprotein complexes will be discussed.

References

Chen, M., M. Schliep, R. D. Willows, Z. L. Cai, B. A. Neilan and H. Scheer (2010). "A Red-Shifted Chlorophyll." *Science* 329: 1318-1319.

Chen, M. and R. E. Blankenship (2011). "Expanding the solar spectrum used by photosynthesis." *Trends in Plant Science* 16(8): 427-431.

Chen, M., Y. Li, D. Birch and R. D. Willows (2012). "A cyanobacterium that contains chlorophyll f - a red-absorbing photopigment." *FEBS Letters* 586(19): 3249-3254.

Li, Y., Y. Lin, C. J. Garvey, D. Birch, R. W. Corkery, P. C. Loughlin, H. Scheer, R. D. Willows and M. Chen (2016). "Characterization of red-shifted phycobilisomes isolated from the chlorophyll fcontaining cyanobacterium Halomicronema hongdechloris." *Biochimica Et Biophysica ActaBioenergetics* 1857(1): 107-114.

Hexameric CBS-CP12 and thioredoxin form a redox-regulon for CO_2 fixation in bloom-forming cyanobacteria

Claudia Hackenberg[1,✉], Johanna Hakanpää[1], Fei Cai[2,3], Svetlana Antonyuk[4], Caroline Eigner[1], Sven Meissner[5], Cheryl A. Kerfeld[2,3,6], Elke Dittmann[5], Victor S. Lamzin[1]

[1]European Molecular Biology Laboratory (EMBL) Hamburg Outstation, c/o DESY, Hamburg, Germany;

[2]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA;

[3]Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA;

[4]Institute of Integrative Biology, Faculty of Health and Life Sciences, University of Liverpool, UK

[5]Department of Microbiology, Institute for Biochemistry and Biology, University of Potsdam, Potsdam-Golm, Germany;

[6]MSU-DOE Plant Research Laboratory and Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA.

✉hackenbergclaudia@gmail.com

CBS-CP12 proteins are unique fusion-proteins of unknown structure and function, consisting of i) CBS-domains (cystathionine ß-synthase), important for the regulation of various proteins in all kingdoms of life, always occurring in pairs and generally assembling in homodimers; and ii) CP12, a well-known regulator of the Calvin cycle enzymes glyceraldehyde-3-P dehydrogenase (GAPDH) and phosphoribulokinase (PRK). In contrast to CBS-domains, structural information o CP12 is scarce, due to its intrinsic disorder. We have systematically analysed the structure, function and occurrence of a CBS-CP12 protein from the cyanobacterium *Microcystis aeruginosa* PCC 7806.
We present the first characterisation of the N-terminal part of a CP12 protein and the unprecedented discovery of a hexameric CBS-domain proteins. We further reveal the critical role of the CP12-domain for hexamerisation of CBS-CP12, binding of AMP and regulation of thioredoxin (TrxA). Transcription analysis demonstrates the light-induced co-regulation of CBS-CP12 and TrxA, both encoded in a conserved gene cluster that is prevalent in bloom-forming cyanobacteria. Our findings establish a previously unknown role of the hexameric
CBS-CP12 within the TrxA-mediated redox-regulation of photosynthesis, carbon fixation and oxidative stress response in cyanobacteria exposed to harsh environmental conditions, and further suggest a conservation of the hexameric organisation of CBS-CP12.

Probing the organisation, self-assembly and nanomechanics of bacterial microcompartments

Matthew Faulkner[1], Jorge Rodriguez-Ramos[1], Yaqi Sun[1], Fang Huang[1], Luning Liu[1, ✉]

[1]*Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, United Kingdom*

✉*Luning Liu, luning.liu@liverpool.ac.uk.*

✉ *M.Faulkner@liverpool.ac.uk; jrramos@liverpool.ac.uk; Yaqi.Sun@liverpool.ac.uk; fang.huang@liverpool.ac.uk*

Bacterial microcompartments (BMCs) are proteinaceous organelles widespread among bacterial phyla. They compartmentalise enzymes within a selectively permeable shell and play important roles in CO_2 fixation, pathogenesis, and microbial ecology. The carboxysomes of cyanobacteria were the first BMCs identified and serve as the central CO_2-fixing machinery. Carboxysomes are composed of an icosahedral proteinaceous shell encapsulating the key carbon fixation enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), in the interior. Due to the modularity nature of BMCs, there is growing interest in installing BMCs into higher plants, with the aim of enhancing photosynthetic efficiency. Our studies aim to unravel how the bacterial self-assembling machinery is formed and regulated to maintain its function. Using live-cell confocal fluorescence imaging, we explored the biosynthesis and subcellular positioning of β-carboxysomes in *Synechococcus elongatus* PCC7942 and demonstrated that carboxysome biosynthesis is accelerated by increasing light intensity and the subcellular localisation of carboxysomes correlates with the redox state of photosynthetic electron transport chain (Plant Physiol, 2016, 171: 530-541). Using atomic force microscopy and electron microscopy, we characterised the three-dimensional organisation and intrinsic physical mechanics of native β-carboxysomes from *Synechococcus* 7942 (Nanoscale, 2017, DOI:10.1039/C7NR02524F), and visualised for the first time the assembly dynamics of BMC shell proteins to form shell facets (Nano Letters, 2016, 16: 1590-1595). Advanced knowledge of BMC assembly and regulation is instrumental for design and synthetic engineering of new molecular scaffolds and biological nanoreactors in heterologous organisms, e.g. crop plants, to supercharge photosynthetic CO_2 fixation and biomass productivity.

! Please, see the Enrique Flores's abstract at the Poster Section No 60 (page 209)

Tetrameric photosystem I widespread in cyanobacteria: implications in physiology and evolution

Barry D. Bruce[1, ✉], Meng Li[2,3,†], Alexandra Calteau[3], Dmitry A. Semchonok[5], Thomas A. Witt[2], Jonathan T. Nguyen[2], Nathalie Sassoon[6], Egbert J. Boekema[5], Julian Whitelegge[7], Muriel Gugger[5],

[1]*Biochemistry & Cellular and Molecular Biology Dept., & Bredesen Center for Interdisciplinary Research and Graduate Education &*[7]*Microbiology Dept., University of Tennessee, Knoxville, TN, USA.*

[2]*Biochemistry & Cellular and Molecular Biology Dept., University of Tennessee, Knoxville, TN, USA;*

[3]*Bredesen Center for Interdisciplinary Research and Graduate Education, University of Tennessee, Knoxville, TN, USA;*

[4]*Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA), Genoscope & CNRS, UMR 8030; Laboratoire d'Analyse Bioinformatique en Génomique et Métabolisme, Evry cedex, FRA;*

[5]*Electron Microscopy Dept., Groningen Biomolecular Sciences & Biotechnology Institute, University of Groningen, Groningen, NLD;*

[6]*Collection of Cyanobacteria, Institut Pasteur, Paris, FRA;*

[7]*Pasarow Mass Spectrometry Laboratory, Geffen School of Medicine, University of California, Los Angeles, CA*

✉ *bbruce@utk.edu*

Photosystem I (PSI) forms trimeric complexes in most characterized cyanobacteria, yet in plants/algae PSI is monomeric. Recently reports on the tetrameric PSI raised questions and speculations about its occurrence, formation mechanism as well as physiological and evolutionary significance. In this study, by examining PSI in 61 cyanobacteria, we show that tetrameric PSI, correlating with a unique *psaL* gene, is widespread in the heterocyst-forming cyanobacteria and their close relatives. Physiological studies on these cyanobacteria revealed that the formation of tetrameric PSI is favored under high light, with increased relative PSI tetramer abundance, stability, and carotenoids content. These carotenoids include some novel PSI cofactors: myxoxanthophyll, canthaxanthan and echinenone, which putatively play photoprotective roles for PSI. Together this work suggests that tetrameric PSI is an early adaptation to high light and supports the hypothesis of tetrameric PSI being the evolutionary intermediate in the transition from cyanobacterial trimeric PSI to monomeric PSI in plants/algae.

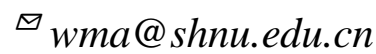
Novel NDH-1L-CpcG2-Phycobilisome-Photosystem I supercomplex is important for efficient cyclic electron transport in cyanobacteria

Fudan Gao[1], Jiaohong Zhao[1], Liping Chen[1], Natalia Battchikova[2], Zhaoxing Ran[1], Eva-Mari Aro[2], Teruo Ogawa[3], Weimin Ma[1],✉

[1]*Department of Biology, Shanghai Normal University, Guilin Road 100, Shanghai 200234, China;*

[2]*Department of Biochemistry, Molecular Plant Biology, University of Turku, FI-20520 Turku, Finland;*

[3]*Bioscience Center, Nagoya University, Chikusa, Nagoya 464-8601, Japan.*



✉ *wma@shnu.edu.cn*

Two mutants isolated from a tagging library of *Synechocystis* sp. strain PCC 6803 were sensitive to high light and had a tag in *sll1471* encoding CpcG2, a linker protein for photosystem I (PSI)-specific antenna. Both mutants demonstrated strongly impaired NDH-1-dependent cyclic electron transport (NDH-CET). Blue-native PAGE followed by immunoblotting and mass spectrometry analyses of the wild-type and a mutant containing CpcG2 fused with YFP-His6 indicated the presence of a novel NDH-1L-CpcG2-PSI supercomplex, which was absent in the *cpcG2* deletion mutant, the PSI-less mutant and several other strains deficient in NDH-1L and/or NDH-1M. Co-immunoprecipitation and pull-down analyses on CpcG2-YFP-His6, using antibody against GFP and Ni-column chromatography, confirmed the association of CpcG2 with the supercomplex. Conversely, the use of antibodies against NdhH or NdhK after blue-native PAGE and in co-immunoprecipitation experiments verified the necessity of CpcG2 in stabilizing the supercomplex. Further, deletion of CpcG2 destabilized NDH-1L as well as its degradation product NDH-1M and significantly decreased the number of functional PSI centers, being consistent with involvement of CpcG2 in NDH-CET. The CpcG2 deletion had, however, no effect on respiration. We thus propose that formation of an NDH-1L-CpcG2-PSI supercomplex in cyanobacteria facilitates PSI cyclic electron transport via NDH-1L. Recently, our results further reveal that formation of the supercomplex is essential for acclimation of cyanobacterial cells to green light environment.

Impact of microcystin on RubisCO and the carbon concentrating mechanism of *Microcystis aeruginosa*

Arthur Guljamow[1], Sven Meissner[1], Tino Barchewitz[1], Alexander Erban[2], Joachim Kopka[2], Martin Hagemann[3] and Elke Dittmann[1]

[1] *guljamow@uni-potsdam.de, smeiss@uni-potsdam.de, barchewitz@uni-potsdam.de, editt@uni-potsdam.de University Potsdam, Department of Microbiology, Karl-Liebknecht-Straße 24-25, 14476 Potsdam, Germany*
[2] *erban@mpimp-golm.mpg.de, kopka@mpimp-golm.mpg.de, Max Planck Institute of Molecular Plant Physiology, Applied Metabolome Analysis, Am Mühlenberg 1, 14476 Potsdam, Germany*
[3] *martin.hagemann@uni-rostock.de, University of Rostock, Plant Physiology, Albert-Einstein-Str. 3, 18059 Rostock, Germany*

The bloom-forming cyanobacterium *Microcystis aeruginosa* has garnered world-wide attention because of its production of microcystin, a hepatotoxin that poses a serious threat to livestock and drinking water. While the biosynthesis of microcystin is well understood, its biological function remains largely unresolved. An elementary function for microcystin is frequently discussed, as it is known that it binds to a number of proteins of the carbon metabolism, the most prominent being RubisCO. Our evidence shows that microcystin binding is stimulated at elevated pH levels which are triggered by high light and, consequently, accelerated photosynthesis. In metabolic analyses we found a much faster accumulation of RubisCO products in the wild type as compared to a microcystin-free mutant. This points to an influence of microcystin on RubisCO activity, a finding that we could confirm with *in vitro* activity measurements. Additionally, microcystin seems to influence the distribution of RubisCO between the cytosol and the carboxysome, the main site of carbon fixation. In the presence of microcystin we detected a rapid accumulation of glycolate, a product of the oxygenase reaction of cytosolic RubisCO. This is surprising, as it suggests a substantial rerouting of carbon into a pathway that is commonly perceived as wasteful. However, we found a concomitant increase of metabolites such as malate, which could be the result of a yet unidentified pathway for the detoxification of oxygenase products. We conclude that microcystin plays a significant role in the regulation of carbon fixation and metabolization, especially under conditions of high light and fluctuating CO_2 levels.

Design and construction of cyanobacteria for direct solar fuels production

Peter Lindblad

Department of Chemistry – Ångstrom, Uppsala University, Sweden
✉ *Peter.Lindblad@kemi.uu.se*

Introduction. There is an urgent need to develop sustainable solutions to convert solar energy into energy carriers used in the society. In addition to solar cells generating electricity, there are several options to generate solar fuels. Native and engineered cyanobacteria have been as model systems to examine, demonstrate, and develop photobiological hydrogen production. Moreover, custom designed and engineered cyanobacteria have been shown to be able to produce carbon-based solar fuels as well as other chemicals and hydrocarbons.

Results. We have developed a molecular toolbox for advanced genetic engineering, using a synthetic biology approach, to custom-design, engineer and construct cyanobacteria to produce a desired product (1). I will present the status of our work to engineer cyanobacteria with a focus on controlled transcription and translation of selected introduced capacities. This will be followed by examples of different approaches to engineer cyanobacteria to produce solar fuels and chemicals (hydrogen and alcohols, 2 - 4) and to grow faster with increased carbon fixation (5 - 7).

References

1. Camsund D, Lindblad P (2014) *Front Bioeng Biotechn* 2: 40
2. Nyberg M, Heidorn T, Lindblad P (2015) *J Biotechn* 215: 35-43
3. Raleiras P, Khanna N, Miranda HH, Mészáros L, Krassen H, Battchikova N, Aro E-M, Magnuson A, Lindblad P, Styring S (2016) *Energy & Environ Sci* 9: 581-594
4. Khanna N, Esmieu C, Meszaros LS, Lindblad P, Berggren G (2017) *Energy & Environ Sci* 10: 1463-1567
5. Liang F, Lindblad P (2016) *Metabol Eng* 38: 56-64
6. Liang F, Lindblad P (2017) *Metabol Eng Com* 4: 29-36
7. Durall C, Rukminasari N, Lindblad P (2016) *Algal Res* 16: 275-281

Strain specific differences in capacity to repair photosystem II in picocyanobacteria

Cole Murphy, Erin Bonisteel, Brooke Turner, Kui Xu, Jenna-Rose Melanson, Douglas Campbell and Amanda Cockshutt

Department of Chemistry & Biochemistry, Mount Allison University, Sackville NB E4L 1G8 CANADA

✉ *Cole Murphy (cdmurphy@mta.ca), Erin Bonisteel (embonisteel@mta.ca), Brooke Turner (beturner@mta.ca), Kui Xu (kxu@mta.ca), Jenna-Rose Melanson (jmelanson2@mta.ca), Douglas Campbell (dcampbell@mta.ca) and Amanda Cockshutt (acockshutt@mta.ca)*

Picocyanobacteria are major primary producers in the oligotrophic regions of the oceans. We previously reported the differential allocation of protein resources in different strains of picocyanobacteria, with certain strains allocating relatively more nitrogen and iron towards the cytochrome b6f complex, and showing differing Photosystem I per Photosystem II ratios. The overall rate of Photosystem II repair after inactivation relies on either the rate of removal of the core protein subunit of Photosystem II (PsbA), or upon the rate of synthesis of new PsbA and concomitant reassembly of the active complex. We measured the total cellular contents of FtsH, the protease thought to be responsible for the removal PsbA from Photosystem II, and the PsbA and PsbD subunits of Photosystem II. We correlate this catalytic capacity for PsbA removal with the measured rate constants for the removal of PsbA and for the repair of Phosotosystem II. We demonstrate that some strains allocate less protein resource to FtsH, and that this lower capacity to remove damaged PsbA lowers the rate at which Photosystem II can be repaired. We also show that the FtsH content relative to PsbA depends on the growth light, with relatively more allocation to FtsH when picocyanobacterial cells are grown at higher irradiance. *Prochlorococcus* strains MED 4 and MIT 9313 differ significantly in their capacity to induce expression of *ftsh* transcripts in response to a shift to higher light levels.

Targeted mutation of D2 amino acids residues associated with bicarbonate binding and the bicarbonate-dependent protonation of plastoquinone b

Julian J. Eaton-Rye✉ and Victor Zhong

Department of Biochemistry, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand;

✉*julian.eaton-rye@otago.ac.nz*

The bicarbonate co-factor of the quinone-Fe-acceptor complex of Photosystem II (PSII) is a bidendate ligand to the non-heme iron. It has been hypothesized that the protonation of the secondary plastoquinone electron acceptor, Q_B, proceeds first through D1-His252 and D1-Ser264 to give $Q_B^-(H^+)$ followed by the second proton (resulting in Q_BH_2) being delivered via the D1-His272 and D1-His215 ligands of the non-heme iron. The pathway for the second proton is hypothesized to include two waters: W675A and W1138A in PDB 3ARC. W1138A has hydrogen bonds to both bicarbonate and W675A, and protons are suggested to pass via this route to D1-His272 and D1-His215: furthermore, our targeted mutations in the D1 protein support this interpretation. The W675A water is also hydrogen-bonded to D2-Thr243: additionally, D2-Glu242 interacts with D1-Glu244 that is also hydrogen-bonded to W675A and D2-Lys264 is hydrogen-bonded to D2-Glu242, potentially stabilizing the hydrogen-bond network around W675A. We have introduced mutations at D2-Glu242, D2-Thr243 and D2-Lys264: in these mutants, which assemble near wild-type levels of PSII, Q_A to Q_B electron transfer is substantially slowed while oxygen evolution is depressed by more than 50% but rescued by addition of bicarbonate. Moreover, D2-Tyr244 is hydrogen-bonded to bicarbonate and targeting this residue also disrupts Q_A to Q_B electron transfer. Our results indicate: (1) D2 plays an equal role to D1 in supporting the putative pathway of protons from the cytosol to bicarbonate; and (2) support the hypothesis that bicarbonate is involved in protonation of $Q_B^{2-}(H^+)$ to Q_BH_2 before Q_BH_2 is released to the plastoquinone/plastoquinol pool.

Identification of key protonation sites and paths in photosynthetic water oxidation via site-directed mutagenesis of cyanobacterial photosystem II

Yvonne Zilliges, Zhiyong Liang, Matthias Schönborn, Philipp Simon and Holger Dau

Freie Universität Berlin, Arnimallee 14, 14195 Berlin

Sequential absorption of minimally four photons by the chlorophylls of the photosystem II (PSII) protein complex is required to drive one turnover of the water-oxidation cycle. In this process, the spatio-temporal orchestration of proton removal from the protein-bound Mn_4Ca complex is functionally crucial. However, these protonation dynamics as well as the structural basis for proton release are only insufficiently understood.
The tight coupling of local protonation dynamics and long-distance proton relocations is most likely mediated by the hydrogen-bonded clusters formed by charged amino acids of the PSII proteins and water molecules (~1300 water molecules per PSII monomer). To understand the protonation dynamics at the atomic level, various tools for monitoring proton relocations as well as site-directed mutagenesis of crucial amino acids involved in the hydrogen-bonded network will be employed. Major focus of the present study are the selection of potentially crucial amino acids and eligible amino acid substitutions (i), as well as different mutagenesis approaches for genetic modification of different photosystem-II core proteins and variants (isoforms) in (i) strict photoautotrophic and (ii) facultatively photoheterotrophic cyanobacteria such as *Thermosynechococcus elongatus* and *Synechococcus* sp. PCC 7002, respectively.

Posters

Session 1: Evolution and Phylogeny

1. Thermoacclimation of the membrane lipidome in marine *Synechococcus* cyanobacteria.

Solène Breton[1], Justine Pittera[1], Juliette Jouhet[2], Laurence Garczarek[1], Frédéric Partensky[1], Éric Maréchal[2], Ngoc An Nguyen[1], Hugo Doré1, Morgane Ratin[1], and Christophe Six[1]

[1] Sorbonne Universités, Université Pierre and Marie Curie (Paris 06) and Centre National de la Recherche Scientifique, UMR 7144, Marine Phototrophic Prokaryotes group, Station Biologique, Place Georges Teissier, CS 90074, 29688 Roscoff cedex, France.

[2]Laboratoire de Physiologie Cellulaire et Végétale, UMR 5168 Centre National de la Recherche Scientifique-Commissariat à l'Energie Atomique-Université Grenoble Alpes, Institut de Recherche en Sciences et Technologies pour le Vivant, Commissariat à l'Energie Atomique Grenoble, 38054 Grenoble cedex 9, France.

Phylogeography studies on *Synechococcus* have shown that the natural communities are dominated by 4 genetically distinct lineages showing ecophysiological adaptations to different thermal niches (thermotypes). These specialization processes raise questions about the underlying adaptive mechanisms. Among them, membrane lipid metabolism represents a large part of the global physiology of these organisms, since their membranes are notably the site of photosynthesis, a process indispensable to growth. Membrane fluidity, a crucial parameter for the membrane functioning, is directly impacted by the ambient temperature and first results showed that a model *Synechococcus* strain carries out unusual mechanisms tomodify the membrane composition to regulate fluidity. We hypothesize that membrane regulation capacities have evolved differently in the distinct *Synechococcus* thermotypes to adapt to different thermal niches. This study discusses the results of a comparative physiology study, with the aim to highlight differences in lipid metabolism among different cyanobacterial strains representative of different thermotypes.

2. Evolution of chaperonin gene duplication in stigonematalean cyanobacteria

Julia Weissenbach[1], Judith Ilhan[1], David Bogumil[2], Nils Hülter[1], Karina Stucken[1], and Tal Dagan[1]

[1]*Institute of General Microbiology, Christian-Albrechts University of Kiel, Am Botanischen Garten 11, Kiel, Germany*

[2]*Present address: The Department of Life Sciences & The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva,* Israel

Chaperonins promote protein folding and are known to play a role in the maintenance of cellular stability under various conditions including heat, UV radiation, salinity, and light stress. In most eubacteria (70 %) the GroES/GroEL chaperonin is encoded by a single-copy bicistronic operon, whereas in cyanobacteria up to three groES/ groEL paralogs have been documented. Here, we study the evolution and functional diversification of *groES/groEL* paralogs in the heterocystous multi-seriate filament forming cyanobacterium Chlorogloeopsis *fritschii* PCC6912 which encodes two *groEL/groES* operons (*groELS1*, *groELS1.2*) and a monocistronic *groEL* gene (*groEL2*). A phylogenetic reconstruction reveals that the *groEL2* duplication is as ancient as cyanobacteria, whereas the *groESL1.2* duplication occurred at the ancestor of heterocystous cyanobacteria. A comparison of the groEL paralogs transcription levels under different growth conditions shows that they have adapted distinct transcriptional regulation. Furthermore, protein–protein interaction assays suggest that paralogs encoded in the two operons assemble into hybrid complexes. The monocistronic encoded GroEL2 is not forming oligomers nor does it interact with any oft he two co-chaperonins. Interaction between GroES1.2 and GroEL1.2 could not be documented, suggesting that the *groESL1.2* operon does not encode a functional chaperonin complex. Functional complementation experiments in *Escherichia coli* show that only GroES1/GroEL1 and GroES1/GroEL1.2 can substitute the native operon. In summary, the evolutionary consequences of chaperonin duplication in cyanobacteria include the retention of *groESL1* as a housekeeping gene, subfunctionalization of *groESL1.2* and neofunctionalization of the monocistronic *groEL2* paralog.

3. Molecular detection of toxin producing and water-blooms forming cyanobacteria in bathing water

Hana Némová, Lucia Chomová

[1]*Department of materialization of living conditions factors-National Reference Centre for Hydrobiology, Public Health Authority of the Slovak Republic, Bratislava,* ✉*hana.nemova@uvzsr.sk*

[2]*Department of materialization of living conditions factors-National Reference Centre for Hydrobiology, Public Health Authority of the Slovak Republic, Bratislava Slovakia* ✉*lucia.chomova@uvzsr.sk*

The presence of cyanobacterial bloom in bathing water can cause potential health hazards. In this study, we aimed at the identification of cyanobacteria coming from bathing water and cultured in our laboratory. The 16S ribosomal RNA genes and adjacent internal transcribed spacer (ITS) regions were amplified by PCR from 1 mixed cyanobacteria sample and 3 samples of pure cultures. PCR product of mixed sample was cloned into plasmids followed by sequencing reactions. Total of 6 clones were identified by similarity search (BLASTN) to the *Cyanobium* sp. Sequence comparison was also used to determining sample of the pure culture to the level *Pseudanabaena* sp. Some sequences obtained from PCR analyses of the last 2 cyanobacteria, identified like *Microcystis aeruginosa* and *Microcystis ichthyoblabe* by microscopy, showed 100% similarity with different strains of *Microcystis*, which means that *Microcystis* strains examined here may be only morphospecies of *Microcystis* strains.

Session 2: Molecular Ecology

4. Overview of genetic diversity of culturable Brazilian cyanobacteria

Marli Fátima Fiore[1], Ana Paula Dini Andreote[1] Danillo Oliveira Alvarenga [1,2], Renata Beatriz Cruz[1], Bruno Costa Evangelista de Souza[1], Janaina Rigonato[1]

[1]Center for Nuclear Energy in Agriculture, University of São Paulo (USP), Piracicaba, SP, Brazil;

[2]College of Agricultural and Veterinary Science, São Paulo State University (UNESP), Jaboticabal, SP, Brazil

✉ *fiore@cena.usp.br, apdini@cena.usp.br, danillo.alvarenga@gmail.com, rbcruz@cena.usp.br), Evangelista de Souza brunoc.uefs@gmail.com, jana_rigonato@yahoo.com.br*

The tropical and subtropical climates of Brazil and its highly heterogeneous biomes (Amazon, Atlantic Forest, Pantanal, Caatinga, Cerrado and Pampa) favor the colonization and diversity of cyanobacteria, and during several decades these microorganisms were mostly been accessed only by floristic surveys. In 2001 our laboratory initiated studies on genetic diversity of cultured cyanobacteria. Currently, CENA cyanobacterial culture collection includes 402 Brazilian cyanobacterial strains characterized morphologically and phylogenetically. Representatives of the majority of Brazilian biomes from different habitats (planktonic, benthic, epiphytic, epilithic, aerophitic, subaerophytic, etc.) were retrieved. Morphological identification and phylogenetic analyses based on the 16S rRNA sequences showed that the strains belong to the orders Synechococcales, Oscillatoriales, Chroococcales, Pleurocapsales, Chroococcidiopsidales and Nostocales. A total of 11 new genera and one new species were discovered and described so far. This broad distribution and novelty makes the CENA collection particularly interesting for genomic studies. To this end, the sequencing of the genome of eight selected strains was completed and some more are currently underway. The whole genome sequencing approach allowed identify biosynthetic pathways of the Brazilian strains involved in production of bioactive compounds, such as microcystins, saxitoxins, nodularins, aeruginosins, anabaenopeptins, spumigins, hassallidins, namalides, scytonemins, mycosporines and geosmins. Currently, ~4,200 cyanobacterial nucleotide sequences generated by our laboratory can be found in the public database GenBank of the NCBI. In summary, the CENA collection is a biological resource biobank to conserve and document the biodiversity of Brazilian cyanobacteria, as well as a repository for discovery of novel bioactive compounds.

5. Cyanophage-encoded lipid-desaturases: oceanic distribution, diversity and function

Sheila Roitman[1], Ellen Hornung[2], José Flores-Uribe[1], Itai Sharon[3], Ivo Feussner[2,4], and Oded Béjà[1*]

[1]*Faculty of Biology, Technion - Israel Institute of Technology, Haifa, Israel;*

[2]*University of Göttingen, Albrecht-von-Haller-Institute for Plant Sciences, Department of Plant Biochemistry, Göttingen, Germany;*

[3]*Migal Galilee Research Institute, Kiryat Shmona, 11016, Israel. Tel Hai College, Upper Galilee 12210, Israel;*

[4]*University of Göttingen, Göttingen Center for Molecular Biosciences (GZMB), Department of Plant Biochemistry, Göttingen, Germany.*

Cyanobacteria are among the most abundant photosynthetic organisms in the oceans; viruses infecting cyanobacteria (cyanophages) can alter cyanobacterial populations, and therefore affect the local food web and global biochemical cycles. These phages carry auxiliary metabolic genes (AMGs), which rewire various metabolic pathways in the infected host cell, resulting in increased phage fitness. Coping with stress resulting from photodamage appears to be a central necessity of cyanophages, yet the overall mechanism is poorly understood. In order to expand our knowledge regarding cyanophages metabolic capabilities we performed a metagenomic search and found a novel, widespread cyanophage AMG, encoding a fatty acid desaturase (FAD), found in two genotypes with distinct geographical distribution. FADs are capable of modulating the fluidity of the host's membrane, a fundamental stress response in living cells. We show that both viral fatty acid desaturases (vFADs) families are Δ9 lipid desaturases, catalyzing the desaturation at carbon 9 in C16 fatty acid chains. In addition, we present the first fatty acid profiling for marine cyanobacteria, which suggests a unique desaturation pathway of medium to long chain fatty acids no longer than C16, in accordance to the vFADs activity. Our findings suggest that cyanophages fiddle with the infected host's cell, leading to increased photoprotection and potentially enhancing viral-encoded photosynthetic proteins, resulting in a new viral metabolic network.

6. Genetic and chemical investigation of MIB and geosmin biosynthesis in brazilian cyanobacterial strains

Fabiane Dörr[1], Danillo Oliveira Alvarenga[2], Rafael Vincentini Popin[3] , Alessandro de Mello Varani[2], Marli de Fátima Fiore[3] , Ernani Pinto[1]

[1] Faculty of Pharmaceutical Sciences, University of São Paulo (USP), São Paulo-SP, Brazil

[2] School of Agricultural and Veterinarian Sciences, São Paulo State University (UNESP), Jaboticabal-SP, Brazil

[3] Center for Nuclear Energy in Agriculture, University of São Paulo (USP), Piracicaba-SP, Brazil

✉ *fabidorr@usp.br, danillo.alvarenga@gmail.com, Popin[3] rvpopin@gmail.com, amvarani@fcav.unesp.br, fiore@cena.usp.br, ernani@usp.br*

Cyanobacteria are considered the most common source of the taste-and-odor (T&O) compounds geosmin and 2-methylisoborneol (MIB), which impart unpalatable earthy and musty flavors to drinking water. Although MIB and geosmin are relatively nontoxic, their identification and quantification are essential for water utilities because consumers generally rely on the taste as the primary indicator of safety. Genomic and chemical analyses were performed for MIB and geosmin production assessment in 11 Brazilian cyanobacterial strains. Previously assembled genomes were submitted to the antiSMASH server for secondary metabolite gene cluster prediction, while manual search and curation was carried out with the Artemis genome browser and BLAST with reference geosmin and MIB sequences. MIB and geosmin production was confirmed by targeted analysis using headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC/MS). Although geosmin synthetase genes were identified in four of the tested strains, a total of six geosmin producers were chemically detected. The inconsistency between the results of two unicellular strains might be due to an insufficient sequencing. Since assemblies produced draft, unfinished genomes, it is possible that terpene synthase genes are retrieved with further sequencing. Thus, additional analyses will be done to confirm and untangle this contradiction. MIB analysis were negative for all strains tested in both genetic and GC/MS assessments. Therefore, the use of both genetic and chemical approaches might be useful in providing reliable data on T&O compounds production, since knowledge of the microorganisms responsible for their biosynthesis is important for early warning detection and prediction of impending water quality impairment.

7. Decreased NtcA responsiveness to 2-oxoglutarate in prochlorococcus in adaptation to more stable environments

María Agustina Domínguez-Martín[1], Antonio López-Lozano[1], Rafael Clavería-Gimeno[2], Adrián Velázquez-Campoy[2], Gerald Seidel[3], Andreas Burkovski[3], Jesús Díez[1], José Manuel García-Fernández[1]

[1] *Dpto. Bioquímica y Biología Molecular, Campus de Excelencia Agroalimentaria CEIA3, Universidad de Córdoba, Córdoba. Spain.*

[2] *Institute of Biocomputation and Physics of Complex Systems (BIFI), Joint Units BIFI-IQFR-CSIC and GBsC-BIFI-CSIC, Universidad de Zaragoza, Zaragoza. Spain*

[3] *Lehrstuhl für Mikrobiologie, Department Biologie, Friedrich-Alexander-Universität, Erlangen-Nürnberg, Staudtstrasse 5, 91058 Erlangen. Germany*

Previous studies showed differences in the regulatory response to C/N balance in *Prochlorococcus* with respect to other cyanobacteria, but no information was available about its causes, or the ecological advantages conferred to thrive in oligotrophic environments. We addressed the changes in key enzymes (glutamine synthetase, isocitrate dehydrogenase) and genes (*glnA, icd, glsF, ntcA, glnB, pipX*) involved in C/N metabolism and its regulation, in three model *Prochlorococcus* strains: MED4, SS120 and MIT9313. We observed a remarkable level of diversity in their response to different conditions, especially to the concentration of the key metabolite 2-oxoglutarate, used to sense the C/N balance by cyanobacteria. Besides, we studied the binding between the global nitrogen regulator (NtcA) and the promoter of the *glnA* gene in the same *Prochlorococcus* strains, and its dependence on the 2-oxoglutarate concentration, by using isothermal titration calorimetry, surface plasmon resonance and electrophoretic mobility shift. Our results show a reduction in the responsiveness of NtcA to 2-oxoglutarate in the MED4 and SS120 strains. This suggests a trend to streamline the regulation of C/N metabolism in late-branching *Prochlorococcus* strains (MED4 and SS120), in adaptation to the rather stable conditions found in the oligotrophic ocean gyres where this microorganism is most abundant.

Funding:
Grants BFU2016-76227-P & BFU2016-78232-P (MINECO, Spain), P12-BIO-2141 (Junta de Andalucía), Programa Propio de Investigación (Universidad de Córdoba) and Fondo de Investigaciones Sanitarias (PI15/00663).

8. Mechanism of radiation resistance in *Arthrospira* sp. PCC 8005

A.Yadav[1], N. Leys[1], A. Cuypers[2], A. Misztak[3], M. Waleron[3], K. Waleron[4], and P. J. Janssen[1]

[1]*Molecular and Cellular Biology, Belgian Nuclear Research Centre (SCK•CEN), Boeretang 200, B-2400 Mol, Belgium (ayadav@sckcen.be),*

[2]*University of Hasselt, Centre for Environmental Sciences, Universiteit Hasselt, Agoralaan Gebouw D, B-3590 Diepenbeek, Belgium (ann.cuypers@uhasselt.be),*

[3]*Dept. of Biotechnology, University of Gdańsk, ul. Abrahama 58, 80-307 Gdańsk, Poland,*

[4]*Pharmaceutical Microbiology, Medical University of Gdańsk, ul. Gen. Hallera 107, 80-416 Gdańsk, Poland.*

Background

The cyanobacterium *Arthrospira* is globally used as feed- and foodstock owing to its high nutritional value. At SCK-CEN, we study *Arthrospira* sp. strain PCC 8005 as a principal organism and edible end product of MELiSSA, a life support system developed by ESA. Particular caveats to study this highly versatile organism are its assay-interfering auto-fluorescence and the lack of a genetic system.

Objectives

The objective of this study is to understand the genetic and biochemical pathways involved in the resistance of strain PCC 8005 to high doses of ionizing radiation (IR)

Methods

To study the effect of radiation on growth and morphology and to test whether extreme IR-resistance is a general trait in Arthrospira, different strains were exposed to increasing doses of gamma radiation (up to 5 kGy) and analyzed for culture-based growth recovery, morphological changes, and cellular and molecular effects (by TEM microscopy and LC-MS).

Conclusions

Arthrospira strains and species from different origins display a variable sensitivity towards IR. Even the two morphotypes of *Arthrospira* sp. strain PCC 8005 (straight versus helical) show, in terms of growth recovery, distinct sensitivities towards IR. Once all data are analyzed, LC-MS, TEM, and biochemical analyses should provide a detailed insight in the cellular responses of *Arthrospira* sp. PCC 8005 towards IR

Acknowledgments: The authors are grateful for funds obtained via the SCK•CEN Doctoral Studies Grant to AY, and a FWO-Flanders / Polisch Academy of Sciences Scientific Exchange Grant to AM, MW, KW, and PJ.

9. Analysis of biomass composition of *Arthrospira* strains under various conditions.

Agnieszka Misztak[1], Malgorzata Waleron[1], Magda Furmaniak[2], Krzysztof Waleron[2]

[1] *Department of Plant Protection and Biotechnology, Intercollegiate Faculty of Biotechnology UG-MUG, Abrahama 58, 80-307 Gdańsk, agnieszka.misztak@biotech.ug.edu.pl, malgorzata.waleron@biotech.ug.edu.pl*

[2] *Chair&Department of Pharmaceutical Microbiology,Medical University of Gdańsk, Al. Gen. J. Hallera 107, 80-416 Gdańsk, krzysztof.waleron@gumed.edu.pl, magda.michalak@gumed.edu.pl*

Cyanobacteria from the genus *Arthrospira* are interesting study subject with vast biotechnological potential. Their basic trait, large content of sugars and proteins makes them an atractive source of food and feed. *Arthrospira* biomass is also rich in vitamins, microelements and fatty acids (fa) such as γ-Linolenic acid (GLA) which makes them a valued supplement of varied diet.
Arthrospira is an organism characterized by cylindrical, multicellular trichoms that are either free floating or mat-creating. In nature it can be found in alkaline and saline water bodies localized in tropical and subtropical climate. The reported salinity of the water in which *Arthrospira* was found varied from 0,1 – 300 g/L of total salt. This difference in growing condition can heavily influence *Arthrospira* biomass composition. Still there are no literature data available concerning changes of nutritional values in biomass.
In our work we investigated the survivability of *Arthrospira* strains in media containing different amounts of salts (0 - 300g/L). We also analyzed changes in protein levels and fa composition of biomass grown in a/m media (25-100 g/L of salt) and in 21 °C, 28 °C and 35 °C. Additionally we investigated the distribution of fa in lipids fractions.
The tested strain survived the salinity of medium up to 200g/L of total salt. Optimal growth of biomass was observed in 28 °C. Analysis revealed similar changes in ratio of identified fatty acids in all temperatures. With growing salinity the levels of GLA and palmitoleic acids were decreasing in favor of palmitic and cis-vaccenic acids.

10. Cyanobacterial strains with an unusual fatty acid composition

Maria A. Sinetova[1,✉], Kenzhegul Bolatkhan[2], Aizhan A. Usserbaeva[2], Bolatkhan K. Zayadan[2], Roman A. Sidorov[3], Kirill S. Mironov[3], Elena V. Kupriyanova[3], Alexandra G. Markelova[3], Alexander Y. Starikov[3], Dmitry A. Los[3]

[1]Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, 127276 Moscow, Russia

Kenzhegul Bolatkhan (kenge83@mail.ru), Aizhan A. Usserbaeva (aizhan.userbaeva@gmail.com), Bolatkhan K. Zayadan (zbolatkhan@gmail.com)

[2]Department of Biotechnology, Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University,

Almaty, Kazakhstan

Roman A. Sidorov (roman.sidorov@mail.ru), Kirill S. Mironov (ksmironov@gmail.com), Elena V. Kupriyanova (ivlaanov@mail.ru), Alexandra G. Markelova (fialma@yandex.ru), Alexander Y. Starikov (starikovay1393@gmail.com), Dmitry A. Los (losda@ippras.ru)

[3] Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya Street 35, 127276 Moscow, Russia

✉maria.sinetova@mail.ru

According to fatty acid composition, all cyanobacteria have been divided into four groups. We have studied four cyanobacterial strains with fatty acid composition which was found to be not typical for any of these groups. Two strains of genus *Cyanobacterium*, IPPAS B-1200 and IPPAS B-1201, have myristic (14:0) and myristoleic (14:1Δ9) acids at high amounts (35-45 % of all FAs), while palmitic (16:0) and palmitoleic (16:1Δ9) acids make 50-55% and C18 fatty acids are present in negligibly low amounts. Interesting, that despite having similar fatty acid content strains differs ecologically: strain B-1200 was isolated from warm salt lake, while strain B-1201 inhabits fresh cold water. Another two strains, both thermotolerant from warm fresh water, *Desertifilum* sp. IPPAS B-1220 and *Gloeocapsa* sp. IPPAS B-1203, are characterized by significant amount (28–40%) of hexadecadienoic acid (16:2 Δ7,10). Possible role of an unusual fatty acid composition of these strains in adaptation to their environments is discussed. Analysis of B-1200 and B-1220 draft genomic sequences allowed suggesting the possible schemes for fatty acid biosynthesis in these strains.

This work was supported by a grant from the Russian Science Foundation [no. 14-14-00904] to MAS, and by a grant from the Ministry of Education and Science of the Republic of Kazakhstan to BKZ [no. 1582/GF4].

11. Construction of a cyanobacterium synthesizing 10-methyl stearic acid

Shuntaro Machida[1], Iwane Suzuki[2]

[1] *Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan. s1430345@u.tsukuba.ac.jp*

[2] *Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan. iwanes6803@gmail.com*

Methylesters of fatty acids from microalgae attract attention as a biodiesel. However, most of the fatty acids in microalgae are C16-18 saturated and polyunsaturated fatty acids. The saturated fatty acids are stable against oxidation under the atmosphere, but they are solidified at the ambient temperature because their melting points are high. Whilst the melting points of the polyunsaturated fatty acids are low and they are liquid at the temperature. However, C=C double bonds in the carbon skeleton are unstable due to oxidation by the atmospheric oxygen. The branched-chain fatty acid in which certain bacteria compose has low melting-points and stable to the oxidation because it is saturated. In this study, we succeeded in characterizing the synthetic pathway of the branched-chain fatty acid, 10-methyl stearic acid, in *Mycobacterium chlorophenolicum* and introduced the synthetic pathway for the branched-chain fatty acid in a cyanobacterium, *Synechocystis* sp. PCC 6803. In addition, we examined the effect of synthesis of 10-methyl stearic acid in *Synechocystis* on the growth and the photosynthetic activity. The transformants synthesizing 10-methyl stearic acid demonstrated slower growth rate than the parental strains at 34˚C, although the photosynthetic activity of these cells did not show significant difference. The amount of 10-methyl stearic acid is approximately 2 % of the total fatty acids. Even in such small amount 10-methyl stearic acid in the membrane lipids may have a negative effect on the cell growth. Or expression of the synthetic enzymes may suppress the growth except the function of photosynthesis.

12. Global phylogeography of marine cyanobacterium *Lyngbya majuscula* and *Moorea producens* reveal distinct genetic structure among oceanic regions

Emily Curren and[1,2,✉], Sandric Chee Yew Leong[2]

[1]Department of Biological Sciences, National University of Singapore, 21 Lower Kent ridge road, Singapore 117543.

✉e0013223@u.nus.edu

[2]Tropical Marine Science Institute, National University of Singapore, 18 Kent Ridge road, 119227.

Cyanobacteria are important oxygenic organisms that are major contributors to primary production levels in the marine environment. They are long assumed to have a widespread global distribution. However, few comparative studies of closely related populations across geographic boundaries have been conducted. We examined the phylogeography of the filamentous cyanobacterium *Lyngbya majuscula* and its tropical counterpart *Moorea producens.* The spatial distribution of both species was studied based on 1042 loci of the 16S rRNA gene using new data from the tropics in combination with data from previous studies to form a dataset that encompasses three regions (America, Asia and Oceania). There was distinct clustering as demonstrated by the neighbour network phylogenetic tree. Principal coordinate analysis and isolation by distance also showed significant genetic structure between the three populations. Temperate and tropical clustering of these individuals produced significant differences between the two groups. These results are fundamental to the global phylogeographic study of *L. majuscula* and *M. producens* and can suggest that dispersal barriers could have been present to prevent interregional dispersion of *L. majuscula* and *M. producens.*

13. Effects of glucose uptake in the marine cyanobacteria *Prochlorococus* and *Synechococcus*

José Ángel Moreno-Cabezuelo, Antonio López-Lozano, María Agustina Domínguez-Martín, Jesús Díez and José Manuel García-Fernández

Dpto. Bioquímica y Biología Molecular, Campus de Excelencia Agroalimentaria CEIA3, Universidad de Córdoba, Córdoba, Spain.

Prochlorococcus and *Synechococcus* are the most abundant cyanobacteria in the ocean and therefore are among the main players in the global carbon cycle, showing different distributions: *Prochlorococcus* dominates the temperate intertropical oligotrophic oceans, while *Synechococcus* is more abundant close to the coast and in cold waters.
We have previously shown that *Prochlorococcus* can take up glucose, increasing the expression of genes that participate in its metabolization. Subsequently we characterized the Pro1404/ *glcH* gene (annotated as *melB* in cyanobacterial genomes), which encodes a biphasic glucose transporter in *Prochlorococcus* sp. SS120. Besides, glucose transport was detected in natural populations of *Prochlorococcus* in the Atlantic.
Since this gene is present in all *Prochlorococcus* and *Synechococcus* strains, we decided to perform comparative experiments addressing the glucose uptake capabilities in several representative strains of both genus, subjected to different concentrations of glucose and also to light vs darkness. Our results suggest that *glcH* expression changes depending on the adaptation of the cyanobacterial strain, indicating this process has been subjected to selective pressures in the evolution of these organisms.
On the other hand, the implication of several amino acids in the glucose uptake kinetics is being studied. For this, some specific residues conserved in this transporter in *Prochlorococcus* SS120 have been identified. They have been mutated in constructions later expressed in *Synechococcus* sp. PCC 7942. Studies on the effects of these mutations on the transport kinetics are underway.

Funding:
Grants BFU2016-76227-P (MINECO, Spain), P12-BIO-2141 (Junta de Andalucía) & Programa Propio de Investigación (Universidad de Córdoba).

14. Anatoxin-a(s) biosynthetic pathway in a *Sphaerospermopsis torques-reginae* strain

Stella Thomaz Lima[1], Danillo Oliveira Alvarenga[2], Felipe Augusto Dörr [3], Augusto Etchegaray[4], Fernanda Cristina Rodrigues Paiva[5], Marcio Vinícius Bertacine Dias[5], Olivier Ploux[6], Alessandro de Mello Varani[2], Ernani Pinto[3], Marli Fátima Fiore[1]

[1]*Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba-SP, Brazil;*

[2]*College of Agricultural and Veterinary Sciences, São Paulo State University, Jaboticabal-SP, Brazil;*

[3]*School of Pharmaceutical Sciences, University of São Paulo, São Paulo-SP, Brazil;*

[4]*Chemistry College, Pontifical Catholic University of Campinas, Campinas-SP, Brazil;*

[5]*Department of Microbiology, Institute of Biomedical Science, University of São Paulo, São Paulo-SP, Brazil;*

[6]*Laboratoire Interdisciplinaire des Énergies de Demain, Université Paris Diderot, France.*

✉ *stella@cena.usp.br, danillo.alvarenga@gmail.com, fadorr@usp.br, aejr07@gmail.com, fcrpaiva@hotmail.com, mvbdias@usp.br, olivier.ploux@chimie-paristech.fr, amvarani@gmail.com, ernani@usp.br, fiore@cena.usp.br*

Sphaerospermopsis torques-reginae ITEP-024 is a cyanobacterial strain known to produce the neurotoxic organophosphate anatoxin-a(s). This cyanotoxin is a potent and irreversible inhibitor of acetylcholinesterase, an enzyme that acts on the peripheral nervous system of mammals. The structure of anatoxin-a(s) has been elucidated and some of its biosynthetic pathway steps have been proposed. However, there is currently no information about the specific enzymes and genes involved on its biosynthesis. In this study, the genome of the anatoxin-a(s)-producing Brazilian strain *S. torque-reginae* ITEP-024 was sequenced and assembled and genes potentially involved in the biosynthesis of anatoxin-a(s) were annotated. Total genomic DNA was extracted from ITEP-024 and a genomic library was constructed for sequencing in the MiSeq platform (Illumina). The genome was assembled using the software SPAdes 3.5.0 and Platanus 1.2.1. Gene prediction and annotation was performed using Prokka 1.9 and Artemis 16.0.0, while comparative conserved domains of proteins were carried out using BLAST from the National Center for Biotechnology Information (NCBI). The proposed anatoxin-a(s) gene cluster encodes nine biosynthetic enzymes, including two pyridoxal phosphate dependent aminotransferases, one arginine beta-hydroxylase/enduracididine beta-hydroxylase, two methyltransferases, one kinase, and a few other enzymes. The clustering of genes encoding these enzymes suggests a unique conserved pathway to synthesize anatoxin-a(s). The hypothetic anatoxin-a(s) pathway that we propose differs from other cyanotoxins since it does not involve NRPS or PKS enzymes. The *S. torques-reginae* ITEP-024 genome is the first from an anatoxin-a(s) producing cyanobacterium.

15. The unseen face of cyanobacteria from Danube Delta lakes

Maria Iasmina Moza [1,2,3,✉], Ciprian Valentin Mihali [4], Carmen Postolache [1]

[1]*Faculty of Biology, University of Bucharest, Romania,*
✉carmen_postolache83@yahoo.com

[2]*Institute of Biology, Romanian Academy of Science, Bucharest, Romania*

[3]*Faculty of Science, "Lucian Blaga" University, Applied Ecology Research Centre, Pediatric Respiratory Medicine Research Centre, no.5-7, Ion Raţiu Street, 550012, Sibiu, Romania, email: iasmina_moza@yahoo.com*

[4]*International Centre of Electronic Microscopy "George Emil Palade", Life Science Institute, "Vasile Goldis" Western University of Arad, Arad, Romania, mihaliciprian@uvvg.ro*

Is well known that cyanobacteria (CB) represent a very important part of phytoplankton, including also species with toxic potential. This study aimed to describe the ultramorphology of few CB a to expose specific colonies features and to establish any relationship between their appearance and their "bad food quality" status for zooplankton. Therefore, we analyzed a series of pure cultures using a method for: (1) *in situ* isolation of living CB on solid BG11 medium, (2) transfer the isolated cultures into the liquid medium and (3) microscopy techniques like optical microscopy (OM), environmental scanning electron microscopy (ESEM) and elemental analyses (EDX) to study their colonies morphology. Interestingly, some of the CB colonies shows unusual deposits that we called "cyano flowers". The structures were well represented and they appeared as exudates at the surface of the colonies. Those structures were formed by calcium and magnesium and their role still remain unknown. However, the most interesting was that those features prevail in CB sampled from lakes with strong waves and high grazers abundance, so this could be a defense strategy. The ESEM technique permit a closer examination of CB using living samples without damaging their natural aspect. In this way, amazing CB features can be expose and chemical described and novel aspects of the bloom can be reconstructed and the ecological relevance of CB to grazers could be elucidate. Also, the first cyanoteque with species from Danube Delta was initiated.

This work was supported by the Swiss Enlargement Contribution, project IZERZ0 – 142165, "CyanoArchive".

16. Evaluating the microbial community shift in brackish water *Arthrospira* cultures

Edina Szekeres[1, 2,] Cristian Coman[1], Andreea Baricz[1, 2,] Cecilia Chiriac[1, 2,] Adriana Hegedűs[1,] Nicolae Dragoş[1,]

[1] *NIRDBS, Institute of Biological Research, Cluj Napoca, Romania*

[2] *Babeş-Bolyai University, Cluj Napoca, Romania*

Institute of Biological Research, Cluj-Napoca, Romania

✉ edina.szekeres@icbcluj.roş *Cristian Coman:* cristian.coman@icbcluj.roş *Andreea Baricz:* andreea.baricz@icbcluj.ro; *Cecilia Chiriac:* cecilia.chiriac@icbcluj.ro; *Adriana Hegedűs:* addenne@yahoo.com; *Nicolae Dragoş: dragos.nicolae@ubbcluj.ro*

The filamentous cyanobacterium Arthrospira, due to high nutritional value and health benefits, has been widely used as a dietary supplement in modern health-food industry. The majority of the Arthrospira biomass used in human nutrition is produced in outdoor systems, although this approach is limited due to high costs involved especially in temperate climate conditions.

An empirical growth medium (EGM) was formulated by incorporating selected nutrients of the standard Zarrouk's medium in natural brackish water that can ensure an optimal growth of Arthrospira. The present study serves as a "first step" to assess possible bacterial contamination and non-target microorganism variation in Arthrospira EGM. Thus, we applied NGS, optical and electron microscopy examinations to assess cyanobacterial and total bacterial occurrence in parallel experimental series of different combinations of Z medium, EGM, one of the Arthrospira strains, and homogenized mat. Arthrospira fusiformis was dominant among Cyanobacteria, with highest abundance in the mat-autochthonous Arthrospira combinations. Although competitor growth should be limited by high salt concentrations, multiple cyanobacterial genera were detected in all experimental series. Actinobacteria prevailed in the brackish water from the alkaline basin, while the homogenized mat also contained higher Firmicutes abundance. In the experimental series the balance shifted, majority of the bacterial OTUs observed being members of five phyla: Proteobacteria>Bacteroidetes>Planctomicetes>Actinobacteria>Firmicutes. Some OTUs were identified as possible pathogens, but their abundance was suppressed in experimental cultures and disappeared altogether from the ones with the autochthonous strain. Although the dominant cyanobacterium in EGM was Arthrospira, several series contained other cyanobacteria and bacterial contaminants, which shows the need of further adjustments of EGM before it can be considered for large scale cultivation of Arthrospira.

Acknowledgements: This work was supported by a grant from the Romanian Ministry of National Education, project PN 16 19 BIODIVERS. Edina Szekeres wishes to acknowledge the receipt of FEMS Young Scientist Meeting Grant.

17. Temperature-induced expression of fatty acids desaturase genes in *Artrhrospira platensis*

Bivol Cezara[1], Becze Anca[2], Cepoi Liliana[3], Rudi Ludmila[3], Chiriac Tatiana[3], Simedru Dorina[2]

[1] *Institute of Microbiology and Biotechnology, Moldova*

[2] *Research Institute for Analitical Instrumentation, Romania*

[3] *Institute of Microbiology and Biotechnology, Moldova*

✉ *bivolcezara@yahoo.com, anca.naghiu@icia.ro, liliana.cepoi@imb.asm.md, rudiludmila@gmail.com, chiriac_tv@yahoo.com, dorina.simedru@icia.ro*

Arthrospira platensis (spirulina) is widely used in human food due to its valuable biochemical content. The biomass contains proteins (up to 70 %), sulphated polysaccharides, vitamins and polyunsaturated fatty acids, among which the most valuable is gamma-linolenic acid (GLA). Spirulina contains up to 1,5 % GLA of the dry biomass or up to 30 % of the total fatty acids. In the cyanobacterial cells polyunsaturated fatty acids, including GLA, are responsible for their survival under low temperature condition. For the human health GLA is important as precursor in prostaglandins biosynthesis. The polyunsaturated fatty acids production in spirulina is achieved by desaturation process, in which the double bonds are introduced into fatty acids molecules by desaturase enzymes encoded by the desatuase genes – *desA, desC* and *desD.*

To observe the influence of low temperatures on the expression of the desaturase genes in *Arthrospira platensis* CNM-CB-11, the culture was exposed for 3 and for 72 hours at 20°C (the optimal temperature is 35 ºC). The short-term exposure to low temperature does not alter the expression of desaturase genes. In condition of long-term exposure of spirulina to low temperature the expression of *desC* was also not affected. At the same time, the expression of *desA* and *desD* gene has increased. These genes are responsible for introducing the double bonds at position Δ12 of oleic acid and Δ6 of linoleic acid respectively. Thus, desaturation of fatty acids under low temperature conditions is a slow process.

Session 3: Gene Regulation

18. Compatible solute breakdown during salt acclimation in *Synechocystis* sp. PCC6803

Friedrich Kirsch[1], Nadin Pade[1], Stephan Klähn[2], Wolfgang R. Hess[2] and Martin Hagemann[1]

[1]Plant Physiology Department, University of Rostock, D-18059 Rostock, Germany

[2]Genetics & Experimental Bioinformatics, Institute of Biology III, University of Freiburg, Germany

The model cyanobacterium *Synechocystis* sp. strain PCC 6803 responds to increasing salinity with the accumulation of the compatible solutes glucosylglycerol (GG) and sucrose. The synthesis of both compounds has been well studied at the biochemical and genetic level during the last years.

Less attention has been paid to physiological mechanisms allowing the acclimation toward decreasing salinity. Since the high amounts of cellular compatible solutes represent a major carbon pool it is likely that there exist processes for internal recycling of these substances. Genome searches revealed candidates that potentially encode for proteins involved in breakdown of GG or sucrose.

A mutant strain of *Synechocystis* showing a deletion in the *slr*1670 gene, encoding a putative glycosyl hydrolase, is unable to adjust the internal GG pool according to decreasing salt concentrations or after trehalose addition. Recombinant Slr1670 protein degraded GG into glucose and glycerol *in vitro*. The experimental data provide evidence that Slr1670 shows specific GG-degrading activity and represents an overseen part of the cyanobacterial salt acclimation.

The secondary compatible solute sucrose can be degraded by an A/N-invertase in *Synechocystis*. This enzyme is encoded by the gene *sll*0626. The deletion of *sll*0626 leads to increased sucrose contents in the resulting mutant strain, which was then combined with other genetic manipulations to trigger sucrose over-accumulation. Since sucrose became a promising candidate in cyanobacteria-based organic carbon production, the knockout of invertase represents a useful tool to increase product yield.

19. Regulation of motility and phototaxis in a model cyanobacterium

Thomas Wallner[1], Veronika Angerer[1], Karsten Voigt[1], Stephan Klähn[2], Annegret Wilde[1]

[1]Molecular Genetics, Institute of Biology III, University of Freiburg, Freiburg, Germany

[2]Genetics and Experimental Bioinformatics, Institute of Biology III, University of Freiburg, Freiburg, Germany

Many cyanobacteria are able to move actively using different motility machineries thus enabling the cells to make decisions about lifestyle and to actively search for suitable environmental conditions for life. Although light-controlled movement of cyanobacteria was observed and has been described for a long time the basic biophysical and molecular mechanisms are poorly understood and investigated. The model cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) shows light-induced motility on surfaces using type IV pili depending on the quantity and quality of light. The movement towards blue light is inhibited by the second messenger c-di-GMP generated via the unique photoreceptor Cph2 [1].
To understand how altered c-di-GMP levels control motility from a mechanistic point of view and what other processes this molecule regulates, we performed analyses at the level of c-di-GMP concentration and gene expression. The transcriptomes of the wild type and the Δ*cph2* mutant were compared using microarray analysis. Several genes known to be involved in phototaxis were differentially expressed in the Δ*cph2* mutant grown under blue-light, but not under green light. However the functions of many of these gene products are not known and their role within phototaxis remains unclear. Furthermore, several of these Cph2-controlled genes are also known to be regulated by cAMP related signalling pathways implying a cross talk of the second messengers.
Our results point to a highly complex regulatory network for the motility of *Synechocystis* including transcription factors, photoreceptors, several second messengers and sRNAs.

References:
[1] Savakis, P. *et al.* (2012). "Light-induced alteration of c-di-GMP level controls motility of *Synechocystis* sp. PCC 6803." Mol Microbiol **85**(2): 239-251

20. Micro-optical properties of *Synechocystis* sp. PCC 6803

Annik Jakob[1,2], Nils Schürgers[3], Conrad Mullineaux[4], Annegret Wilde[2]

[1]*Spemann Graduate School of Biology and Medicine Freiburg, Germany;*

[2]*Albert-Ludwigs-Universität Freiburg, Germany,*

[3]*École Polytechnique Fédérale de Lausanne, Switzerland,*

[4]*Queen Mary University of London, England*

Phototaxis is a mechanism that allows cyanobacteria to respond to fluctuations in the quality and quantity of illumination by moving either towards or away from a light source. While phototactic behavior is exhibited by many prokaryotes using rotating flagella to generate thrust, the model organism *Synechocystis* sp. PCC6803 uses retractile type IV pili (T4P) to move across moist surfaces in a jerky motion referred to as twitching motility.
Previously, it was assumed that single bacterial cells are not capable of directly detecting the direction of a light source due to their size of a few micrometers only. In contrast, latest research results of our group revealed that single cells of *Synechocystis* sp. PCC6803 focus the light from a unidirectional illuminant in a sharp focal point on the distal side. We were able to demonstrate that this focusing effect correlates with a directional phototactic movement. However, the signal transduction pathway regulating the motility apparatus in a polar light-dependent manner remains elusive. We propose a model where the strong focal point induces a local inhibition of the motility apparatus at the shaded side of the cell.
At least 3 operons encode signaling systems that are possibly involved in the regulation of phototaxis and show similarity to known classical chemotaxis regulators. Using fluorescence microscopy we determined the localization of CheY-like and PATAN-domain response regulators and phototaxis photoreceptors. We show that the expression of these response regulators can regulate direction of movement.

21. Identification and characterization of novel promoter sequences in *Synechocystis* 6803

Patricia Caicedo Burbano and Filipe Branco dos Santos

Molecular Microbial Physiology Group, Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands

Microorganisms are widely used in biotechnology applications to produce several added value products. *Synechocystis* sp. PCC 6803, a model microorganism of cyanobacteria, has been repeatedly investigated as an alternative for sustainable biotechnological production of many different compounds. The use of microorganisms as cell factories frequently requires extensive molecular manipulation. However, in contrast to E. Coli, the synthetic biology toolbox available for *Synechocystis* is still somewhat limited. The ability to tune gene expression is critical for any redesign of cellular machinery; thus, the identification and/or engineering of constitutive and inducible promoter sequences that span large dynamic ranges are necessary steps to further develop synthetic biology in cyanobacteria (Ramey et al 2015). Since currently limited knowledge is available directly comparing the performance of various native and foreign promoters in cyanobacteria, a systematic investigation on behaviors of promoters is important (Wang et al 2012). In this work, screening systems are being constructed in *Synechocystis* sp. PCC 6803 to identify and characterize promoter sequences in *Synechocystis* that can be used in cyanobacteria genetic engineering.

References

Ramey C. J, Barón Sola A., Aucoin H. R., Boyle N. R. (2015) Genome Engineering in Cyanobacteria: Where we are and where we need to go. ACS Synthetic Biology, 4, 1186- 1196.
Doi: 10.1021/acsynbio.5b00043
Wang B, Wang J, Zhang W, Meldrum D, (2012). Application of synthetic biology in cyanobacteria and algae. Frontiers in Microbiology. Volume 3. Article 344. Doi: 10.3389/fmcib.2012.00344.

22. The iron-stress activated RNA 1 (IsaR1) coordinates osmotic acclimation and iron starvation responses in the cyanobacterium *Synechocystis* sp. PCC 6803

Stephan Klähn[1], Henriette Rübsam[1], Friedrich Kirsch[2], Martin Hagemann[2], Wolfgang R. Hess[1]

[1]*Genetics & Experimental Bioinformatics, Faculty of Biology, University of Freiburg, D-79104 Freiburg, Germany*

[2]*Plant Physiology department, Institute of Biological Sciences, University of Rostock, D-18059 Rostock, Germany*

Iron is crucial for many biological processes. However, iron is a major limiting factor for bacterial and phytoplankton growth in most environments. Thus bacterial iron homeostasis is tightly regulated, mainly by the transcriptional regulator Fur as well as on the posttranscriptional level by small, regulatory RNAs (sRNAs). For instance, in cyanobacteria the iron-stress activated RNA 1 (IsaR1) was shown to be a critically important riboregulator affecting the acclimation of the photosynthetic apparatus to iron starvation [1]. Unexpectedly, upon IsaR1 overexpression in the model strain *Synechocystis* sp. PCC 6803 a decreased abundance of the mRNA encoding glucosylglycerol phosphate synthase (GgpS) was observed. GgpS is the key enzyme for the essential accumulation of the compatible solute glucosylglycerol (GG) at high salinities. Using a heterologous reporter system, the direct interaction between IsaR1 and the 5'UTR of *ggpS* was verified. In *Synechocystis*, this interaction drastically reduced the *de novo* synthesis of the GgpS enzyme hence lowering the initial glucosylglycerol synthesis rate upon salt shock. The data indicate that beyond its main regulatory function in photosynthesis, IsaR1 also interferes with the salt acclimation process in *Synechocystis* in a specific way. IsaR1 is involved in the integration of the responses to different environmental perturbations, slowing down the osmotic adaptation process in cells suffering from parallel iron starvation. This integrating function provides a paradigm for other sRNA regulators in bacteria.

[1] Georg J, Kostova G, Vuorijoki L, Schön V, Kadowaki T, Huokko T, Baumgartner D, Müller M, Klähn S, Allahverdiyeva Y, Hihara Y, Futschik ME, Aro EM, Hess WR. (2017) Acclimation of oxygenic photosynthesis to iron starvation is controlled by the sRNA IsaR1. *Current Biology*, in press, DOI: 10.1016/j.cub.2017.04.010.

23. RNA polymerase and transcription regulation in cyanobacteria

Juha Kurkela, Satu Koskinen, Kaisa Hakkila, and Taina Tyystjärvi✉

Department of Biochemistry, University of Turku, FI-20014 Turku, Finland
✉*taityy@utu.fi*

During transcription, a DNA-dependent RNA polymerase (RNAP) synthetizes an RNA copy of the template DNA, initiating and terminating synthesis at the desired genomic locations at correct time. The RNAP core of cyanobacteria has a unique six-subunit composition ($\alpha_2\beta\beta'\gamma\omega$) and for transcription initiation, RNAP core recruits one of the σ factors to form a transcription initiation competent RNAP holoenzyme. Our research focuses on the specific roles of different σ subunits and to the small ω subunit of the RNAP core in the model cyanobacterium *Synechocystis* sp. PCC 6803. The group 2 σ factors that structurally resemble the essential primary σ factor SigA are essential for acclimation to stress conditions although a ΔsigBCDE strain without any group 2 σ factors grows well in optimum conditions. The RNAP core recruits more primary σ factor in ΔsigBCDE than in the control strain in standard conditions. Efficient recruitment of SigA leads to over production of transcriptional and translational machineries, but not photosynthetic complexes, in ΔsigBCDE. Extra RNAP is transcriptionally active while extra ribosome subunits do not form translationally active ribosomes. The ω subunit is non-essential in *Synechocystis* in standard growth conditions but the acclimation capacity of the ω subunit deficient cells to environmental changes is low. Our results suggest that the ω subunit plays a regulatory role in *Synechocystis* as transcription of only a subset of highly expressed genes, not all, is down-regulated in the ω–less strain. This regulation is at least partially due to effects on recruitment of σ factors.

24. Metabolic engineering of mannitol production in *Synechocystis* sp. PCC6803

Wenyang Wu and Filipe Branco dos Santos

Molecular Microbial Physiology Group, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands

Mannitol has been widely used in the food and pharmaceutical industries as a key ingredient used mostly for energy storage, osmoregulation and pathogen defense. Nowadays, commercial production of mannitol is mainly carried out by chemical hydrogenation of fructose, a process that has the main drawback of being costly. Using microbial biosynthesis as a method to produce mannitol has been taken previously considered. To obtain a *Synechocystis* derivative that is able to directly convert CO_2 into mannitol, two genes involved in its biosynthesis pathway need to be heterologously expressed from another organisms into *Synechocystis* sp. PCC6803. The first gene, coding for mannitol-1-phosphatase dehydrogenase (M1PDH), converts fructose-6-phosphatase (F6P) into mannitol-1-Phosphate (M1P). Then M1P is dephosphorylated into mannitol by another that encodes for mannitol-1-phosphatase. In this study, we have selected both genes from different organisms and express them under the control of different constitutive or inducible promoters to make mannitol production at a high level. Further optimization is being considered by transforming the existing mannitol-producing strains with, a mannitol transporter that increases the permeability of the cells and enable further optimization of mannitol production.

25. The alternative sigma factor F (Slr1564) and the production of EPS in *Synechocystis* PCC 6803

Carlos Flores[1,2,3,] Marina Santos[1,2,] Rita Mota[1,2,4], Paulo Oliveira[1,2,] Sara B. Pereira[1,2,] and Paula Tamagnini [1,2,4]

[1]. i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal;

[2]. IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal;

[3]. ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal;

[4]. Faculdade de Ciências, Departamento de Biologia, Universidade do Porto, Porto, Portugal.

Many cyanobacteria produce extracellular polymeric substances (EPS), mainly composed of heteropolysaccharides that can remain associated to the cell or be released into the surrounding environment (RPS). Their particular characteristics, such as the presence of two different uronic acids, the high number of different monosaccharides and the high content in sulphate groups make them very attractive for biotechnological applications (1, 2). Despite the increasing interest on cyanobacterial EPS, the knowledge on the regulatory factors involved in EPS production and export is still limited (3). In this work, we show that the group 3 alternative sigma factor F (SigF, Slr1564) is involved in EPS production in *Synechocystis* PCC 6803. The results obtained with a *Synechocystis* knockout mutant ΔsigF (4) indicate that, although growth is significantly impaired (≈50 %), the production of EPS (mainly RPS) strongly increases compared to the wild-type. The ΔsigF mutant also shows a decrease in carotenoid content and a consequent susceptibility to oxidative damage. Furthermore, differences in ultrastructure and protein secretion corroborate previous findings that reported alterations in cell surface and absence of phototactic motility in ΔsigF mutants (5). Overall, these results provide new insights about the role of SigF on *Synechocystis* physiology, highlighting its importance for environmental adaptation.

(1) Mota, R et al. 2016. Appl Microbiol Biotechnol 1-11. (2) Leite, JP et al.

26. Design of an artificial heterocyst specific promoter for biofuel production in *Nostoc punctiforme*

Xin Li

Uppsala University / Dept. of Chemistry – Ångström Laboratory

With the ability to capture solar irradiation and fix atmospheric CO2, photosynthetic organisms, especially cyanobacteria, are becoming attractive green cell factories for producing renewable biofuels, such as bio-hydrogen.
Nostoc punctiforme ATCC 29133 is a filamentous nitrogen-fixing cyanobacterium, which under nitrogen-depleted conditions forms a special cell type along its filaments, the heterocyst, to fix atmospheric nitrogen. Hydrogenase, the key enzymes for bio-hydrogen production, is oxygen sensitive, which is a technical challenge in our research of using photosynthetic cyanobacteria for hydrogen production. However heterocysts provide a micro-aerobic environment, which makes them native cell factories allowing active hydrogen production.
Recently a specific motif conferring heterocyst specific transcription was identified by a transcriptional regulation study with promoter-GFP reporter constructs. The "DIF motif", TCCGGA, located at -35 region with respect to the transcriptional start site, has been identified to be associated with heterocyst-specific transcription within the promoter of one *dps* gene in Nostoc.
Synthetic biology tools can help to engineer new functions in cyanobacteria to expand the production ability. The "BioBricks" developed for cyanobacteria is still limited. Since currently the knowledge concerning promoters for biotechnological applications in cyanobacteria is very scarce and in filamentous the situation is even worse, a systematic investigation on behaviours of various native promoters in cyanobacteria would be important.
Based on conservation of the "DIF motif" in 220 heterocyst specific promoters, a fully artificial heterocyst specific promoter was designed for expression of oxygen sensitive proteins of importance for heterocyst based bio-hydrogen production. Potential applications and further development of this synthetic promoter will be presented.

27. Could stpks supplement existing signalling pathways in *Synechocystis* sp. PCC 6803

Anna A Zorina

Laboratory of Cellular Regulation, Timiryazev Institute of Plant Physiology, Moscow 127276, Russia

✉*zorina.anna@yahoo.com*

Plethora of different signalling routes can be explored to transmit information about the same stressful event. These pathways function either complementary and/or independently from each other activating identical or different sets of genes. In our work we studied if serine/threonine protein kinases (STPKs) together with two-component systems and DNA supercoiling could also be involved in perception and cold stress signal transduction. We tested 12 mutant strains using as target in PCR after reverse transcription known cold stress responsive genes. Surprisingly none of the kinases with transmembrane domains seemed to participate. Group of STPKs (enzymes SpkB, SpkD, SpkE, and SpkG) was determined as potentially involved in regulating such genes as *ndhD2*, *crhR*, and *desB*. Further, experiments focused on SpkE as its transcription profile of the cold stress response genes was similar to that of the Hik33 (sensor histidine kinase that perceives decrease in environmental temperature). We studied possible differences in polypeptide profile and phosphorylation of individual proteins. The soluble protein profiles were rather complex still some differences were detectable even in normal conditions. Also, the mutation of SpkE abolished phosphorylation of a group of high-molecular weight proteins. Tested enzymatic activity of expressed SpkE protein in the phosphorylation reaction *in vitro* showed no autophosphorylation but kinase activity toward such exogenous substrate as histone H1. Thus, SpkE can be an additional member in the regulatory pathway of the response *Synechocystis* cells on cold stress.

28. Introduction of phosphate deficiency-induced lysis system to *Synechocystis* sp. PCC 6803 for efficient collection of cellular products

Ryo Asada[1], Yoshihiro Shiraiwa[2], Iwane Suzuki[2]

[1]*Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan (ryo.spring@gmail.com)*

[2] *Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan (shiraiwa.yoshihir.ft@u.tsukuba.ac.jp, iwanes6803@biol.tsukuba.ac.jp)*

Production of useful substances using microalgae has attracted much attention. However, problems regarding its practical use are still remaining. For example, to recover products accumulated in the cell, it is necessary to destroy the cell wall for extraction which requires large energy costs. We introduced a lytic system induced by phosphate deficiency in the model cyanobacterium *Synechocytosis* sp. PCC 6803. This system consists of promoter of the *phoA* gene for alkaline phosphatase, induced by the phosphate deficiency, and holin and endolysin, which are cell wall degrading lytic enzymes. The objective of this study is to induce the expression of the lytic enzymes under phosphate deficiency to release cell contents.

The obtained lysis mutant (*lic*), wild-type (WT), negative control (Δ*phoA*) strain were grown in phosphate-deficient BG-11 medium. Approximately 80% of the cells of the *lic* strain died after 24 h whereas WT and Δ*phoA* strains only showed approximately 7% death. In addition, pigments derived from the cells were significantly increased in the supernatant of culture of the *lic* strain.

From a practical point of view, it is possible to recover highly concentrated products by concentrating *lic* cells to completely consume the phosphate remaining in the medium and achieve lysis. The *lic* strain cells grown under phosphate sufficient medium were concentrated 20 times and further cultured for 24 hours. Then, cell lysis was observed. Using this system, it is possible to control the cell growth (substance production) and lysis by the amount of phosphate supplied in the media.

29. Determination of symbiotic inducing signals from the host moss to the cyanobiont

Eric Pederson

Department of Ecology, Environment and Plant Sciences, Stockholm University, Sweden

The ability of nitrogen fixing cyanobacteria to form a symbiosis with other organisms not only allows for both organisms to benefit but also increase the amount of nitrogen in the ecosystem. For example, the symbiosis between certain feather mosses and cyanobacteria is essential in the nitrogen poor boreal forests found in many places around the northern hemisphere. The boreal forest is one of the largest terrestrial C storage on Earth and have a tremendous effect on the C and N global cycling, where the productivity is largely dependent on the cyanobacterial nitrogen input. Previously our research focused on the cyanobacterial partner where genes and proteins are presently being identified due to the presence of the host. However, to get a deeper understanding of the interaction between the symbiosis of cyanobacteria and mosses, the signalling from the host must be ascertained. Thus, my project involves the investigation of genes and gene families, which are potentially involved in the symbiosis between mosses and cyanobacteria from the host moss. To address this question I have isolated an axenic line of the feather-moss *Pleurozium schreberi*, which is able to form a symbiosis with several species of cyanobacteria such as *Nostoc*. After several colonization studies with different *Nostoc* strains and mutants (*Nostoc punctiforme ATCC 29133*), it is clear that colonization can be disrupted. However, even though I can show that disruptions of certain genes results in the inability to properly colonize the host moss further research into the gene regulation is still required.

30. PacR is a global transcriptional regulator of photosynthesis-related genes in *Anabaena*

Silvia Picossi[1], Anita Santana[2], Enrique Flores[1], Yagut Allahverdiyeva-Rinne[2] and Antonia Herrero[1].

[1]Instituto de Bioquimica Vegetal y Fotosintesis. CSIC-Universidad de Sevilla. Avda. Americo Vespucio 49. 41092 Sevilla. Spain.

[2]University of Turku. Department of Biochemistry. Molecular Plant Biology. Biocity 6th floor. Turku 20520, Finland

✉ *silvia.picossi@ibvf.csic.es, anita.i.santanasanchez@utu.fi, eflores@ibvf.csic.es, allahve@utu.fi, herrero@ibvf.csic.es*

The LysR-type transcriptional regulator All3953 of *Anabena* sp. PCC 7120 has been identified as a regulator of genes involved in the assimilation of inorganic carbon, including the *rbcL* operon encoding ribulose bisphosphate carboxylase[1]. A mutant lacking the *all3953* gene was unable to activate the expression of *rbcL* under low carbon conditions. In addition, it grew poorly, formed short filaments and was sensitive to high light. Interestingly, the *all3953* mutant formed heterocysts when grown in the presence of combined nitrogen. We used ChIP-Seq to identify All3953-bound DNA regions 3 h after transfer of filaments from a high carbon to a low carbon regime. Putative targets included, besides *rbcL*, genes encoding elements of the carbon concentrating mechanism, genes putatively involved in the photorespiratory pathway, genes encoding photosystem and electron transport-related elements, and factors putatively involved in protection against oxidative damage. Interestingly, at least two genes involved in heterocyst differentiation were also identified as putative targets of PacR, namely *patS* and *hetN*. A transcriptional analysis of several of the putative target genes was performed, which confirmed their dependence on All3953. Besides, the growth of the mutant under different light and carbon regimes was analysed, as well as other photosynthetic parameters. We conclude that PacR globally regulates transcriptional responses to the inorganic carbon supply and illumination conditions in the context of a phototrophic metabolism.

1. Picossi, S., E. Flores, and A. Herrero, The LysR-type transcription factor PacR is a global regulator of photosynthetic carbon assimilation in Anabaena. Environ Microbiol, 2015. **17**(9): p. 3341-3351.

31. Development of synthetic biology strategies and validation of genetic elements for engineering *Synechocystis* sp PCC 6803

Pekka Patrikainen, Csaba Nagy, Eva-Mari Aro, Pauli Kallio

Molecular Plant Biology, Department of Biochemistry, University of Turku, Turku, Finland

✉ *ptpatr@utu.fi, csaist@utu.fi, evaaro@utu.fi, pataka@utu.fi*

Because of the native ability of cyanobacteria to utilize solar energy for direct capture of CO_2, they are attractive research targets for generating future biotechnological platforms for the production of environmentally friendly carbon-based chemicals. Our research has focused on developing a set of carefully validated genetic elements which would enable more efficient and predictable expression of individual enzymes, and precise control over entire heterologous biosynthetic pathways in *Synechocystis* sp. PCC 6803. To achieve these goals, we have collected and characterized a set of compatible promoters and ribosome binding sites, which can be used to efficiently tune the transcription and translation, respectively, over a broad dynamic range. We have also evaluated a set of novel homologous integration sites in *Synechocystis* which expand the possibilities for over-expression of specific genes, without disturbing the native metabolism. By applying a modified BioBrick approach to assemble the over-expression constructs, the genetic components in our library can be pieced together in any desired combination, thus providing flexibility and higher throughput in the preparative phase, and more precise control over individual genes in the pathways. These tools have been used for the alternative production pathways for sucrose, ethanol, and ethylene, which are used as quantitative markers for monitoring the effects of different metabolic modifications in the cell. Besides improving the efficiency of the systems, the tools developed in this project can be used to construct more complex biosynthetic entities, thus expanding the scope of chemicals which can be produced in *Synechocystis*.

32. Altering carbon and reductant flux for photosynthetic butanol production in *Synechocystis* using CRISPRI

Kiyan Shabestary, Josefine Anfelt, Lun Yao, Michael Jahn and Paul Hudson

School of Biotechnology, KTH - Royal Institute of Technology, Science for Life Laboratory, Stockholm, Sweden

We used a genome-scale model to predict genetic interventions to improve productivities of various products (butanol, ethanol, lactate) in *Synechocystis*. The model predicted suppression of competing electron sinks (including NDH complexes) in all cases. We further used the CRISPRi system to knock-down the target genes accordingly. However, only lactate showed a 5-fold increase in titers upon ndhB knock-down. We hypothesized that, unlike lactate, carbon supply is the limiting factor in butanol and ethanol production. We further identified citrate synthase (gltA) as a potential valve to redirect carbon flux from the TCA cycle and thus biomass formation to butanol production. By partially or completely shuting down gltA, improved butanol productivities and titers were obtained. In addition, growth could be controlled by repression of gltA and could be a promising target to regulate cell density and improve light availability in cultures.

33. Engineering cyanobacteria into biosolar cell factories for green commodities

Ruth Perez Gallego [1], Klaas J. Hellingwerf [1,2] and Aniek D. van der Woude[1,✉]

1 Photanol BV, Amsterdam, the Netherlands

2 Molecular Microbial Physiology, Swammerdam Institute of Life Sciences, University of Amsterdam, the Netherlands

✉ *aniek.vanderwoude@photanol.com*

The ability of cyanobacteria to capture (sun)light and CO_2 to grow can be exploited for the production of chemical commodities by genetic engineering. The resulting biosolar cell factories present an opportunity to fight global concerns over rising atmospheric CO_2 levels and (future) uncertain/fluctuating availability of sugar and oil as raw material. Moreover, direct conversion of CO_2 into chemical commodities by cyanobacteria is expected to be more efficient than first fixing CO_2 into sugar by plants, followed by a specific sugar fermentation process.

At Photanol, we have developed cyanobacterial cell factories for a large number of compounds, such as biofuels, sweeteners and organic acids. Next to metabolic engineering of cyanobacteria, we also develop an upscaling process to explore industrial feasibility. Within the framework of the FP7 program DEMA (Direct Ethanol From Microalgae) we contributed to optimization of *Synechocystis* as a cell factory for the production of ethanol; this included strategies focused on investigating the best growth conditions as well as genetic engineering to increase the ethanol productivity of strains.

34. The primary transcriptome of a fast-growing *Synechococcus elongatus* utex 2973

Xiaoming Tan[1, 2], Shengwei Hou[2], Xuefeng Lu[1,*], Wolfgang R. Hess[2,*]

[1] *Key Laboratory of Biofuels, Shandong Provincial Key Laboratory of Synthetic Biology, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Beijing, China*

[2] *Genetics and Experimental Bioinformatics, Faculty of Biology, University of Freiburg, Freiburg im Breisgau, Germany*

✉ *tanxm@qibebt.ac.cn, housw2010@gmail.com, lvxf@qibebt.ac.cn, wolfgang.hess@biologie.unifreiburg.de*

In recent years, cyanobacteria have shown their promising potential for the production of biofuels and bio-chemicals, which will be beneficial for sustainable development and carbon footprint reduction. *Synechococcus elongatus* UTEX 2973 is a newly characterized cyanobacterial strain with the fastest measured growth rate reported up to now and good tolerances to high temperature and illumination. With these notable characters, this strain was proved as an ideal host for photosynthetic production of sugars. However, although there are some reports on its genomic, proteomic and metabolic properties, as well as the application of novel genome editing tools, there is no transcriptomic information available for this strain. Moreover, insight is lacking so far into why this strain could rapidly accumulate biomass and carbohydrates under the high-light and high-temperature conditions. Here, we analyzed and compared the transcriptome profiles of *S. elongatus* UTEX 2973 grown under normal, high-light, high-temperature and dark culture conditions by the differential RNA-seq technique. We present a genome-wide map of the 3491 transcription start sites (TSS) active in *S. elongatus* UTEX 2973 and identified some differentially expressed small regulatory RNAs, which might play important roles in stress acclimation. The comprehensive information for all transcripts will be helpful for further research on stress acclimation and metabolic engineering of this species.

35. *In silico* predictions and experimental verification of small RNAs from bacterial metatranscriptome datasets

Lott, S.C.[1], Voigt K.[1] and Steglich C.[1]

[1]*Institute of Biology III, University of Freiburg, Germany;*

Bacterial small (s)RNAs are important components of regulatory circuits in the global control of gene expression. Their functional roles were studied mainly in model organisms, restricting this work to laboratory strains and conditions. In contrast, the search for RNA regulators within actual microbial communities is still challenging because a suitable pipeline for bioinformatics analysis and experimental verification is lacking. Here, we propose a workflow based on metatranscriptome data from the Red Sea. Samples from 60 m, 100 m and 130 m were collected during summer, when the ecologically important marine cyanobacterium *Prochlorococcus* is one of the predominant species reaching up to 200.000 cells per mL [(1)]. RNAseq data derived from these samples were mapped against the NCBI database using BLAST and *Prochlorococcus*-specific reads were assembled to contigs, which then were assigned to the best matching *Prochlorococcus* strain. Contigs that mapped within intergenic regions of the reference genome were analyzed with GLASSgo [(2)] – a new search tool for sRNA homologues – and classified as potential sRNAs based on the Z-score of RNAz, experimental verification by northern blots and target predictions using CopraRNA. For the conserved sRNA Yfr22, that is present in *Prochlorococcus* and *Synechococcus*, we validated the top predicted target in a GFP-based reporter system.

[1]Steglich, C.et al. (2015) Dataset for metatranscriptome analysis of *Prochlorococcus*-rich marine picoplankton communities in the Gulf of Aqaba, Red Sea. Mar. Genomics *19*, 5–7.

[2]Lott, S.C. et al. (2017). GLASSgo - Automated and reliable detection of sRNA homologs from a single input sequence. NAR, under revision.

36. Peculiarities of cyanobacterial transcription

Amber Riaz-Bradley, Yulia Yuzenkova

Yuzenkova Lab, The Centre for Bacterial Cell Biology, Level 3, Newcastle University, UK

✉ *A.Riaz-Bradley1@ncl.ac.uk, Yulia.Yuzenkova@ncl.ac.uk*

RNA polymerase (RNAP) is the major protein responsible for carrying out transcription, and is modulated through transient interaction with secondary channel binding factors (SCBFs) in nearly all bacterial species. The highly conserved GreA is the most prevalent of this family of binding factors and has important roles in maintaining the fidelity and continuity of the transcription process through providing increased hydrolytic activity. The function of GreA is important not only for the production of correct RNA and therefore protein, but also for the removal of stalled RNAP molecules which can cause queuing of RNAP molecules and collisions with the replication machinery.

Cyanobacteria are one of the very few species to have no identified SCBFs and it is unknown how RNAP is modulated in their absence and how their loss is compensated for. This study compared natively purified RNAP from the model species Synechocystis *sp* 6803, *Synechococcus elongatus* 7942 and E. *coli* to report an extremely fast rate of intrinsic RNA hydrolysis for the GreA-absent cyanobacterial enzymes.

Identification of the potential residues responsible for this increased cleavage involved sites within the integral trigger loop and bridge helix regions of RNAP which work together at the active site. Mutations in these regions affect their flexibility and interaction and are known to alter RNAP activity. Substitution of the E. *coli* RNAP residues in these areas with the respective S. *sp* residues highlighted the importance of having two particular substitutions – F773V and G1136Q – for dramatically increased cleavage activity and pause resistance.

Session 4: Cellular Differentiation and Cell Biology

37. Structural insights into the CcmR-controlled transcriptional regulation of carbon/nitrogen metabolism homeostasis in *Synechocystis*

Yong-Liang Jiang, Xue-Ping Wang, Hui Sun, Dong-Dong Cao, Wang Cheng, Cheng-Cai Zhang✉, Yuxing Chen✉, and Cong-Zhao Zhou✉

School of Life Sciences, University of Science and Technology of China

Institute of Hydrobiology, Chinese Academy of Science

✉ *cczhang@ihb.ac.cn, cyxing@ustc.edu.cn, zcz@ustc.edu.cn*

Cyanobacteria are capable of acclimating and growing under a wide range of ambient CO_2 concentrations, which is conducted by the CO*2* concentrating mechanism (CCM). Two LysR-type transcription regulators (LTTRs), CcmR and CmpR, and one AbrB family of transcriptional regulator CyAbrB are involved in the regulation of CCM. As aglobal regulator of the CCM, CcmR acts as repressor of several CCM related genes. Despite of extensive studies of the LTTR activators, little is known about the regulatory mechanism of LTTR repressors, due to the absence of the full-length structures. Here we found that 2-phosphoglycolate (2-PG) binds to CcmR regulatory domain with a Kd value of 0.43 mM, which indicates that 2-PG might be an inducer of CcmR. To find the structural insights into 2-PG regulated CcmR transcription, we solved the full-length structure of CcmR and the complex structure of the CcmR regulatory domain with 2-PG. The full-length structure revealed that CcmR has a tetrameric arrangement assembled via two distinct dimerization interfaces. Binding of 2-PG induces a significant conformational change of the CcmR regulatory domain, which facilitates themovement of the DNA-binding domains. Consequently, the CcmR tetramer alleviates the DNA-binding ability by relocation of the DNA-binding sites, which subsequently relieves the repressive effect of CcmR and induces the transcription of target genes. Moreover, the DNA-binding affinity of CcmR is significantly augmented in the presence of 2-oxoglutarate, which is a key metabolite bridging the nitrogen and carbon metabolic pathways. Thus we propose that CcmR might be a transcription factor that balance the homeostasis of the carbon/nitrogenmetabolism.

Key Words: transcriptional regulation, crystal structure, CO_2 concentrating mechanism, nitrogen metabolism, cyanobacteria.

38. Novel insights into the role of Glnb (P_{II}) regulating carbon metabolism

Jörg Scholl[1, ✉], Waldemar Hauf, Karl Forchhammer[1]

Institute for Organismic Interactions, University of Tübingen, auf der Morgenstelle 28
✉*joerg.scholl@uni-tuebingen.de*

The family of the highly conserved P_{II} signal transduction proteins is commonly known to regulate the nitrogen metabolism at several functional levels. P_{II} proteins sense the metabolic state of the cell by competitive binding of ATP and ADP, with the ATP-bound state additionally binding to 2-oxoglutarate (2-OG) (1). In *E. coli*, where the first P_{II} protein GlnB was identified, its main function is the regulation of glutamine synthetase. A P_{II} homolog in the cyanobacterium *Synechococcus elongatus* PCC 7942 was later found to regulate arginine biosynthesis by activating N-acetyl-L-glutamate kinase (NAGK), which catalyzes the arginine-feedback-inhibited step in the pathway. Furthermore, *Synechococcus* P_{II} indirectly controls the global nitrogen control factor NtcA by binding to its co-activator PipX. The P_{II}-PipX interaction is highly sensitive towards the ATP/ADP ratio and antagonized by 2-OG. By contrast, interaction with NAGK is mainly responding to 2-OG fluctuations (2). More recent studies show that beside its role in regulating processes related to the nitrogen metabolism, there is a close link to carbon metabolism, particularly in the regulation of fatty acid biosynthesis. As shown in *Synechocystis* strain PCC 6803, by interaction with biotin carboxyl carrier protein (BCCP), P_{II} proteins control the cellular acetyl-CoA levels (3). In our search for additional P_{II} interaction partners, we identified an interaction between P_{II} and phosphoenolpyruvate carboxylase (PEPC), which directly links P_{II} to the TCA cycle. P_{II} and PEPC were found to interact *in vitro* and PEPC activity is drastically reduced in a P_{II} mutant strain of PCC 6803.

References

1. Forchhammer, K. and Lüddecke, J. (2016). Sensory properties of the P_{II} signalling protein family. *FEBS J, 283: 425–437. doi:10.1111/febs.13584*
2. Lüddecke J. and Forchhammer K. (2015). Energy Sensing versus 2-Oxoglutarate Dependent ATPase Switch in the Control of *Synechococcus* P_{II} Interaction with Its Targets NAGK and PipX. *PLoS ONE 10(8): e0137114. https://doi.org/10.1371/journal.pone.0137114*
3. Hauf, W., Schmid, K., Gerhardt, E. C. M., Huergo, L. F., & Forchhammer, K. (2016). Interaction of the Nitrogen Regulatory Protein GlnB (PII) with Biotin Carboxyl Carrier Protein (BCCP) Controls Acetyl-CoA Levels in the Cyanobacterium *Synechocystis sp.* PCC 6803. *Frontiers in Microbiology, 7, 1700. http://doi.org/10.3389/fmicb.2016.01700*

39. Structure of *Synechocystis sp.* PCC 6803 biofilms

Fabian Conradi

PhD student with Conrad Mullineaux

Queen Mary, University of London

Synechocystis sp. PCC 6803 is a widely used model cyanobacterium. It is commonly used in a planktonic context, but does form biofilms in different circumstances. *Synechocystis* has adaptations for this mode of existence, such as movement by twitching motility using type IV pili. Type IV pili require surface attachment and thus are not suited to the planktonic state.

My project aims to investigate the impact of directional light on the arrangement of cells in *Synechocystis* biofilms. Recent work by Schürgers *et al.* showed that *Synechocystis* act as miniature eyeballs, lensing light to a spot in the back of the cell as part of their ability to sense light and perform phototaxis. Light lensing is likely to have an important effect in a biofilm context. Even though light attenuation through individual *Synechocystis* is low, attenuation through layers of cells in a biofilm is strong, so the formation of substructures within *Synechocystis* biofilms would convey a substantial competitive advantage that increases with biofilm thickness.

We are currently growing *Synechocystis* biofilms in sterile flow cells and generating 3D images by confocal laser scanning microscopy, allowing us to look at the arrangement of cells within the biofilm. We are visualising the cells using chlorophyll fluorescence. Initial results under different growth conditions have been obtained. We are currently working to generate mutants containing different fluorescent proteins as well as mutations in their phototaxis and/or motility apparatus in order to investigate the impact of motility on the arrangement of cells in the biofilm.

40. Determination of 2-oxoglutarate variations in the filamentous cyanobacterium *Anabaena* using a specific biosensor based on fluorescence resonance energy transfer

Hailin Chen, Chen-Cai Zhang, Amel Latifi and Christophe S. Bernard✉

Aix-Marseille Université – CNRS
Institut de Microbiologie de la Méditerranée
LCB – Marseille – France
✉*cbernard@imm.cnrs.fr*

Determination of 2-oxoglutarate 2-oxoglutarate (2-OG), an intermediate of the Krebs cycle is not only a central metabolite, but also a signalling molecule in both prokaryotes and eukaryotes. In the nitrogen-fixing filamentous Anabaena the accumulation of 2-OG within cells constitutes the signal of nitrogen starvation resulting in the differentiation, at a semi regular pattern, of vegetative cells in heterocysts specialized in N2 fixation. How the first step of signalling correlates with cell fate determination remains completely unknown. As the cellular levels of 2-OG vary rapidly in response to environmental changes an easy approach is essential for the measurement of 2-OG. We present a biosensor based on the 2-oxoglutarate-dependent dissociation of the PII–PipX complex using the FRET technology. We demonstrate that, in vitro, the FRET signal is negatively affected, in a specific and concentration-dependent manner, by the presence of 2-OG. This 2-oxoglutarate biosensor responds specifically and rapidly to a large range of 2-OG levels and is highly robust under different conditions in vitro conditions as well as in bacterial cell extracts. This biosensor paves the way for probing the dynamics of 2-OG in various organisms. Using cell extracts, we could follow 2-OG levels during nitrogen starvation and determine the role of the 2-OG oxoglutarate decarboxylase in heterocyst differentiation.

41. A highly conserved Ferredoxin with unique function

Michael Schorsch[1], Marion Eisenhut[2] and Guy T. Hanke[1]

[1] School of Biological and Chemical Sciences, Queen Mary University of London, E1 4NS

[2]Department of Botany, Faculty of Molecular Biology, University of Düsseldorf, Germany

✉*m.schorsch@qmul.ac.uk*

Ferredoxins (Fds) are present in all photosynthetic organisms and function in distribution of electrons derived from the photosynthetic electron transport chain (PET) to many enzymes. FdC2 proteins are among the most intriguing conserved groups of Fds, having no known function, but strong homology from prokaryotic cyanobacteria throughout higher plants. These are named for their unique, highly conserved C-terminal extensions. Preliminary work showed unique properties of FdC2 in higher plants. In contrast to photosynthetically active Fds, FdC2 is impaired in receiving electrons from Photosystem I or FNR. In addition, initial data gave evidence that FdC2 may play a role in metal homeostasis. Furthermore, knock-out of the FdC2 gene appears lethal in *Synecchocystis* PCC6803. Therefore we developed a strategy to generate inducible knock-down of FdC2 and perturbed function through C-terminal truncation in PCC6803 to further test the impact of protein loss in these two systems. Screening of cyanobacterial growth on media with variable metal contents indicates FdC2 may be critical in responding to altered Fe and Cu concentrations.

42. Critical roles of Dps proteins in cellular fitness of heterocyst forming cyanobacteria

Karin Stensjö

Department of Chemistry – Ångström Laboratory, Microbial Chemistry, Uppsala University, Box 523, SE 751 20 Uppsala, Sweden,

✉*karin.stensjo@kemi.uu.se*

Maintenance of cellular homeostasis, including the ability to control the level of free iron and reactive oxygen species, are critical for biological fitness, and thus for efficient bio-production of fuels and other valuable metabolites. We study cellular acclimation strategies in the N_2-fixing, heterocyst-forming, cyanobacterium *Nostoc punctiforme*, as a strategy to explore and provide metabolic engineering tools to enhance the potential of cyanobacteria for biotechnological applications. We have recognized the Dps proteins as being of importance for the ability of cyanobacteria to acclimate to stressful conditions (1,2,3). The specificity and function of the Dps in cyanobacteria are poorly understood. The Dps proteins are wide-spread in cyanobacteria and *dps* genes are represented in almost all bacterial genomes. *N. punctiforme* comprise a superior number of Dps proteins as compared to other bacteria. Using a multidimensional strategy: in-vivo characterization by gene-inactivation, overexpression of proteins, cell localization studies as well as in-vitro biochemical and biophysical characterizations of isolated proteins. We aim to resolve the functions of the Dps proteins in *N. punctiforme*. Here we present individual biochemical properties of each of the five Dps proteins, and the importance of specific Dps proteins in iron regulation, hydrogen peroxide tolerance, and high light tolerance. The results advise the importance of Dps proteins in heterocyst metabolism and consequently in bio production.

[1] Ekman M. et al. (2014) Environ Microbiol 16: 829-44.
[2] Howe Christoph (2016) Licentiate Thesis, Uppsala University.
[3] Moparthi V. et al. (2016) Biochim Biophys Acta (BBA) - Bioenergetics, 1857: 1766-1776.

43. An interacting partner of FtsZ couples cell development to cell cycle control in *Anabaena* 7120

Cheng-Cai Zhang

Institute of Hydrobiology, Chinese Academy of Science

FtsZ is a tubulin-like GTPase that initiates cell division in almost all bacteria, and it constitutes a target of regulation for cytokinesis in response to stress or developmental cue. In the filamentous multicellular cyanobacterium *Anabaena* PCC 7120, 5-10 % of the cells along each filament differentiate into heterocysts, specialized in N_2-fixation, and heterocysts and vegetative cells are mutually dependent on each other for filament growth through the exchange of nitrogen or carbon resources. Heterocysts are terminally differentiated, furthermore, inhibition of cell division blocks heterocyst development. These observations suggest the existence of a coupling mechanism between heterocyst development and cell cycle. Here we report the identification of *patD* whose expression is activated in developing cells by the transcriptional factor NtcA. The inactivation of *patD* increases heterocyst frequency whereas its overexpression decreases heterocyst frequency and inhibits cell division. Increasing the expression level of *ftsZ* mimics the phenotypes of *patD* inactivation, while production of *ftsZ* inhibitors such as SulA, or Cdv3 gave similar phenotypes as the overexpression of *patD*. The effect of PatD is mediated through direct interaction with FtsZ by controlling its polymerization activity. We conclude that PatD is a new FtsZ-interacting factor that antagonizes the function of FtsZ during heterocyst development and participates in the optimization of heterocyst frequency. Thus, the PatD-FtsZ interaction provides a molecular mechanism for the coupling between cell division control and heterocyst pattern formation.

A few publications in relation to the topics

Zhao, M.-X., Jiang, Y.-L., He, Y.-X., Chen, Y.-F., Teng, Y.-B., Chen, Y., Zhang, C.-C.*, and Zhou, C.-Z.* (2010) Structural basis for the allosteric control of the global nitrogen-regulating transcription factor NtcA by the nitrogen starvation signal 2-oxoglutarate. Proc. Natl. Aacd. Sci. USA. 107 :12487-12492.
Latifi, A., Ruiz, M. and Zhang, C.-C*. (2009) Oxidative stress in cyanobacteria. FEMS Microbiol. Rev., 33:258-278.
Zhang, C.-C*., Laurent, S., Sakr, S., Peng, L. & Bédu, S. (2006) Heterocyst differentiation and pattern formation in cyanobacteria: a chorus of signals. Mol. Microbiol. 59:367-375.
Laurent, S., Chen, H., Bédu, S., Ziarelli, F., Peng, L. & Zhang, C.-C.* (2005) Nonmetabolizable analogue of 2-oxoglutarate elicits heterocyst differentiation

44. CRISPR-interference as a tool to improve fatty alcohol production and study membrane homeostasis in cyanobacteria

Ivana Cengic[1], Danuta Kaczmarzyk[2] Lun Yao[2], Elton P. Hudson[2]

[1] icengic@kth.se; School of Biotechnology, KTH - Royal Institute of Technology, Science for Life Laboratory, Stockholm, Sweden

[2] danutak@kth.se, lunyao@kth.se, huds@kth.se;

School of Biotechnology, KTH - Royal Institute of Technology, Science for Life Laboratory, Stockholm, Sweden

** These authors contributed equally*

.

Fatty alcohol production in *Synechocystis* sp. PCC 6803 was introduced by heterologous expression of a fatty acyl reductase. Our previously established inducible CRISPRi tool in cyanobacteria was used to improve fatty alcohol production by repression of pathways competing for fatty acyl-ACP substrates. This included the alkane production pathway and four acyltransferases involved in membrane lipid synthesis. Simultaneous repression of six genes resulted in an 80% increase in fatty alcohol production. The effect was isolated and found to be solely due to repression of *slr1510*, encoding the PlsX phosphate acyltransferase responsible for initiation of phosphatidic acid synthesis. This enzyme has not been characterized in *Synechocystis* previously, but the inability to construct a full knockout indicates that it is essential for viability. Strains where PlsX was repressed, either alone or in combination with the alkane pathway, had a higher content of 18:3(9, 12, 15) acyl chains in their membrane lipids. The desaturase *desB* responsible for the specific desaturation of the ω3 position was found to be upregulated, this is in line with the observed change in lipid composition. This phenotype, commonly observed for cells experiencing cold stress, indicate that the presence of fatty alcohols and the CRISPRi introduced perturbations in membrane synthesis altered the membrane fluidity and invoked a cellular response that aims at restoring the cells membrane homeostasis.

45. Cell-type specific heterocyst spacing pattern in the branching cyanobacterium *Mastigocladus laminosus* (Stigonematales)

Laura A. Antonaru, A. William Rutherford, and Dennis J. Nürnberg

Department of Life Sciences, Imperial College, London, United Kingdom

✉ *laura.antonaru12@imperial.ac.uk, a.rutherford@imperial.ac.uk, d.nurnberg@imperial.ac.uk.*

Cell differentiation is one of the marks of multicellular organisms. Heterocysts are terminally specialized cells in cyanobacteria that can fix atmospheric nitrogen. The development of their spacing pattern, a classic study in developmental biology, has been thoroughly investigated in the model organism *Anabaena sp*. PCC 7120.

This submission focuses on the more complex, branching cyanobacterium *Mastigocladus laminosus* (Stigonematales, Section V). Contrary to what has been previously published, a heterocyst spacing pattern is present in *M. laminosus* but it varies with the age of the culture and the morphology of the cells. Heterocysts in young, narrow trichomes were more widely spaced (~ 14.8 cells) than those in old, wide trichomes (~ 9.4 cells).

Biochemical and transgenic experiments reveal that the heterocyst spacing pattern is affected by morphogen PatS. Addition of the pentapeptide RGSGR (PatS-5) to the growth medium and overexpression of *patS* from *Anabaena sp.* PCC 7120 in *M. laminosus* resulted in the inability to differentiate heterocysts under nitrogen deprivation. Bioinformatics investigations highlighted that putative PatS sequences within cyanobacteria are highly diverse, and fall into two main clades. Both are present in most Section V cyanobacteria.

Despite its more complex, branching phenotype, *M. laminosus* appears to use a PatS-based pathway for heterocyst differentiation which is not too dissimilar from *Anabaena*. These findings highlight how additional variables may add layers of complexity to a relatively simple biological pattern.

46. Does the Glycine Cleavage System form a defined multiprotein complex in *Synechocystis* sp. PCC 6803?

Maria Wittmiß, Martin Hagemann and Hermann Bauwe

University of Rostock, Institute for Biological Science, Plant Physiology, A.-Einstein-Str. 3, 18059 Rostock, Germany

The Glycine Cleavage System (GCS) is ubiquitous distributed and performs essential functions in all eukaryotes and most prokaryotes. Malfunction of GCS usually results in strong phenotypes. One exception to this rule are cyanobacteria, which are able to circumvent the loss of a functional GCS. The GCS itself, together with the Serine Hydroxymethyltransferase (SHMT), catalyzes the glycine-to-serine conversion, which is part of the photorespiration and the C1 metabolism. GCS consists of three enzymatically active proteins: the P-Protein (Slr0293, dimeric 214 kDa), the L-Protein (Slr1096, dimeric 100 kDa) and the T-Protein (Sll0171, monomeric 41 kDa). Additionally, the H-Protein takes part in the GCS reaction, but without direct enzyme activity. The H-Protein (Slr0879, monomeric 14 kDa) with its attached lipoyl cofactor acts as a carrier between the other proteins.

For the GCS in pea leaf mitochondria, a stable complex formation has been suggested [1]. Under high protein concentrations (>0.25 mg/ml) the GCS complex could be reformed *in vitro*, but no intact GCS complex has ever been purified, because of its high instability.

By using recombinant and enzymatically active GCS proteins from *Synechocystis* sp. PCC 6803, we want to investigate a possible GCS complex formation in cyanobacteria. Furthermore, due to their homology to the plant proteins, we might also obtain further insights into the plant GCS complex.

Our *in vitro* experiments using membrane filtration techniques suggested that the four GCS subunits indeed can form a defined multiprotein complex, with a different stoichiometry than suggested for plants. These results could be verified via further investigations with Size Exclusion Chromatography and the Pull down method. To proof the GCS complex formation *in vivo* further experiments are in progress, such as the purification of tagged GCS complex from cyanobacterial cells. The different GCS complex preparations will be explored regarding possible changes of the GCS enzyme activity. These new information on structure-function relation in the ubiquitous GCS complex will shed new light on its regulation to maintain cellular metabolism.

[1] Oliver DJ, Neuburger M, Bourguignon J and Douce R (1990) Interaction between the Component Enzymes of the Glycine Decarboxylase Multienzyme Complex. *Plant Physiology* 94: 833-839

47. Intercellular communication for motility control in hormogonia of *Nostoc punctiforme*

Conrad W. Mullineaux[1, ✉], Deyana A. Ivanova[1], Benjamin J. Dorgan[1], Josef Lehner[2], Iris Maldener[2]

[1] *School of Biological & Chemical Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, U.K.*

[2] *IMIT Organismic Interactions, Eberhard-Karls-Universität Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany*

✉ *c.mullineaux@qmul.ac.uk*

The filamentous cyanobacterium *Nostoc punctiforme* can generate specialised motile filaments called hormogonia, which glide on surfaces due to the co-ordinated action of modified Type IV pili located close to each cell junction. Hormogonia occasionally reverse direction even when moving in apparently homogeneous environments, indicating that a motility decision can be rapidly communicated throughout the filament. To investigate the communication mechanism, we looked at the effects of a *sepJ* null mutation. SepJ is a protein located at the cell junctions that has been shown in *Anabaena* PCC7120 to be important for the intercellular exchange of metabolites and signals. It is a putative component of septal junction complexes: channels that allow smaller hydrophilic molecules to diffuse from cytoplasm to cytoplasm. We used Fluorescence Recovery after Photobleaching measurements to show that the intercellular exchange of calcein, a 623 Da fluorescent tracer, is strongly impaired in vegetative filaments of the *Nostoc sepJ* mutant. Although the mutant can still form motile hormogonia, their movement shows different characteristics from the wild-type. Wild-type hormogonia typically show smooth motility and occasional rapid motility reversals, complete in a median time of 8 s. By contrast, *sepJ* mutant hormogonia show frequent "stalling" and motility reversals are slower, requiring a median time of 18 s ($p < 10^{-6}$). We conclude that the co-ordination of hormogonium motility requires the intercellular diffusion of a messenger molecule or ion through the septal junctions. The septal junctions are crucial for rapid information exchange, in addition to exchange of metabolites and cellular differentiation signals.

48. Physiological studies on akinete differentiation and germination of filamentous cyanobacteria

Rebeca Perez[1] and Iris Maldener[2]

[1, 2] Department of Microbiology/Organismic Interactions, University of Tuebingen, Auf der Morgenstelle 28, 72076 Tuebingen, Germany.

+49 (7071) 29-78847

✉ *[1]rebecazperez@gmail.com; [2]iris.maldener@uni-tuebingen.de*

Cyanobacteria of the order *Nostocales* are multicellular organisms that grow as filaments of hundreds of cells, some of which can differentiate to carry out specialized tasks. Such differentiated cells include nitrogen-fixing heterocysts, motile hormogonia and resting cells called akinetes. The akinetes represent a survival strategy under unfavourable environmental conditions. In contrast to the profound knowledge on heterocyst differentiation, much less is known about akinete differentiation and germination processes (1, 2). Here, we investigate the morphological and physiological changes that take place during both processes in two model species, *Anabaena variabilis* ATCC 29413 and *Nostoc punctiforme* ATCC 29133. The metabolic and morphologic changes were analysed by measurement of O_2 photosynthesis and respiration rates, by chemical determination of reserve materials, and various microscopic techniques. During akinete development the storage compounds cyanophycin, glycogen and lipid droplets transiently increased, and the photosynthesis and respiration activities decreased (1). Both strains showed an asynchronous germination of akinetes confirmed by time-laps microscopy. Moreover, an unusual fast cell division and heterocyst differentiation were detected during germination in *A. variabilis*. In the end of the germination, the photosynthesis and respiration rates were similar to a vegetative cells culture. Finally, a mutant in a cell wall related protein was involved in akinete development and germination. In summary, this study provides the first detailed characterization on akinete formation and germination of two important cyanobacterial species, revealing clear differences in the metabolic and morphological adaptation mechanisms. These results paved the way for further genetic and functional studies in both species.

Perez R, Forchhammer K, Salerno G, Maldener I. (2016) Clear differences in metabolic and morphological adaptations of akinetes of two Nostocales living in different habitats. Microbiology. **162**:214-23.

Maldener et al. (2014) Cell differentiation in filamentous cyanobacteria. In: Flores E. & Herrero A. (eds) The Cell Biology of Cyanobacteria. Caister Academic Press, Norfolk, UK. pp. 263-292

49. The missing cyanobacterial carbon sensor; sensory properties of a novel cell signaling PII-like protein (SbtB): functional and structural insights

Khaled Selim[1,2, ✉], Marcus Hartmann[2], Martin Hagemann[3] and Karl Forchhammer[1]

[1] *IMIT / Organismic Interactions Department, Tübingen University, Auf der Morgenstelle 28, 72076 Tübingen, Germany.*

[2] *Protein Evolution Department, Max Planck Institute for Developmental Biology, Tübingen, Germany.*

[3] *Institute of Biological Sciences, Plant Physiology Department, Rostock University, Rostock, Germany.*

✉ *khaled.selim@uni-tuebingen.de*

PII proteins are widely distributed signaling proteins in nature, and found in all domains of life. Canonical PII proteins bind to different effector molecules (ATP, ADP, 2-OG), the binding induces conformational changes, and enables the PII protein to bind to different targets to modulate different cellular functions. In general, PII proteins are involved in nitrogen metabolism, sensing the cellular energy state with ATP/ADP binding, and sensing C/N state of the cell. All cyanobacteria contain GlnB gene encoding PII homologues. A close examination of available cyanobacteria genomes on CyanoBase revealed further genes with similarity to *glnB* but lacking PII signature sequences, we termed the putative gene products ‘’PII-like proteins’’. Thus, it is tempting to speculate that PII-like proteins are involved in regulation of different cellular activities, which differ markedly from classical PII proteins [1-3]. In this project, we focused on a potential PII-like protein named SbtB, which is located in one operon next to sodium dependent bicarbonate transporter (SbtA). To figure out *in-vivo* cellular function of SbtB protein, we created SbtB knockout mutants in deferent backgrounds to characterize cellular function of SbtB protein in carbon metabolism. The recombinant protein was analyzed by ITC for binding of different effectors molecules to determine the sensory properties of SbtB protein in comparison to central effector metabolites of classical PII proteins (like, 2-OG). The dynamics of subcellular localization was performed with GFP fusion to SbtB, and using specific antibody. For structural functional analysis, we solved various crystal structures of SbtB protein.

References:

1. Sant'Anna FH, Trentini DB, de Souto WS, Cecagno R, da Silva SC, Schrank, IS (2009) The PII superfamily revised: a novel group and evolutionary insights. Journal of Molecular Evolution, 68(4), 322-336.

2. Chellamuthu VR, Alva V, Forchhammer K (2013) From cyanobacteria to plants: conservation of PII functions during plastid evolution. Planta, 237(2), 451-62.

3. Forchhammer K, Jan Lüddecke J (2016) Sensory properties of the PII signalling protein family. FEBS Journal, 283(3):425-37.

50. CYDIV, a protein of the filamentous cyanobacteria with a critical role in the cell division

Derly Andrade[1], Miguel Vicente[2], Mónica Vásquez[1]

[1]Molecular Genetic and Microbiology Department, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago, Chile,

[2]*Microbial Biotechnology, CSIC National Biotechnology Centre, Madrid, Spain*

✉ *Derly Andrade, derlyan11@gmail.com; Miguel Vicente, mvicente@cnb.csic.es; Mónica Vásquez, mvasquez@bio.puc.cl;*

In this study, our goal was to determine the role of the CyDiv protein of the filamentous cyanobacteria *Anabaena* sp. PCC7120 in divisome assembly. We analyzed phenotypic variations at different transcription levels controlled by a copper-inducible promoter and determined their effect on morphology by TEM and SEM. In addition, the association of CyDiv with other proteins involved in cell division was probed by BIFc (Bimolecular fluorescence complementation). The secondary structure of the periplasmatic region of the protein was determined by circular dichroism.

Our results from controlled transcription, cellular localization and protein interaction suggest that CyDiv is essential for cell division in this filamentous cyanobacterium. Alteration of *cyDiv* transcription in *Anabaena* sp. PCC7120 led to effects in the Z-ring positioning and to cell wall damage triggering cell lysis. CyDiv interaction with elements of the divisome, was confirmed by BIFc analysis. Immunofluorescence microscopy revealed that CyDiv and FtsQ had a similar subcellular localization; these proteins are localized from the pole to the midcell depending on the stages of cell division; and they also co-localized with the Z-ring.

Biochemical characterization of CyDiv determined that the periplasmic region adopts an alpha-helix structure, a feature similar to the *E. coli* FtsB protein.

In summary, CyDiv is essential for cell division in filamentous Cyanobacteria, it is likely the homologue of FtsB and it interacts directly with other cyanobacterial divisome proteins. The absence of CyDiv affects the Z-ring position, produces cell wall damage and finally causes cell death.

Disclosure of Interest: None Declared
Keywords: Cell division, cyanobacteria, Morphology

51. Isolation and quantitation of ribosomes by immunodetection from *Prochlorococcus* and marine *Synechococcus*

Jenna-Rose Melanson[1] and Dr. Amanda Cockshutt[1] ✉

[1]*Department of Chemistry & Biochemistry, Mount Allison University, Sackville, NB E4L 1E2 CANADA*

✉ *jmelanson2@mta.ca, acockshutt@mta.ca*

Prochlorococcus and *Synechococcus* are numerically abundant picocyanobacteria that contribute significantly to primary production in oligotrophic waters. Quantifying cellular components that limit growth rates are used to create models for understanding how these organisms will adjust to changing environments. Methods to quantify biochemical ratios of cellular components such as carbon (C), nitrogen (N) and phosphorous (P) in order to understand the C:N:P ratio have been designed. At present, there are no existing protein quantification methods for cyanobacterial ribosomes, which are thought to limit cell growth due to their high production cost. Here we report a novel method for the quantification of ribosomes in *Prochlorococcus* marinus MED4, MIT9313 and *Synechococcus* sp. WH8102. We have quantified number of ribosomes per cell from cultures harvested at varying light levels using quantitative immunoblotting. We also show the effect of growth rates on ribosome concentration in picocyanobacterial cells.

52. Testing *Synechocystis* thylakoids as stores for recombinant membrane proteins

Mariano Battistuzzi[1], Mattia Niero[1], Anna Segalla[1], Francesca Zito[2], Bruno Miroux[2] and Elisabetta Bergantino[1]

[1] *Department of Biology, University of Padova, Viale G.Colombo 3, 35121 Padova, Italy*

[2] *Institut de Biologie Physico-Chimique, UMR 7099, CNRS/Univerité Paris Diderot, Rue Pierre et Marie Curie 13, 75005 Paris, France*

Membrane proteins (MPs) characterization at the 3D level is by far lagging behind that of soluble proteins, essentially because of the difficulties in purifying them from native cells or tissue and also, alternatively, in over-producing them as recombinant products. A recent survey (Hattab et al., 2014) has shown that the T7 RNA polymerase-based expression system and the *E.coli* BL21(DE3) derivative strains C41 and C43, characterized by the proliferation of intracellular membranes upon overexpression of MPs (Miroux and Walker, 1996; Arechaga et al., 2000), account for more than 60 % of MP structures obtained after heterologous production. In these strains but also in other hosts, overproduction of lipids and development of a large network of internal membranes have proven to be extremely useful and productive in buffering the toxicity of MP recombinant expression.
We have recently improved a *Synechocystis* strain that represents the cyanobacterial counterpart of the *E.coli* strain BL21-C43, being similarly based on the induction of the T7 RNA polymerase and naturally possessing a wide internal membrane system, the thylakoids. We have planned to overexpress in it a palette of MPs including the *E.coli* ATPase b subunit to test its impact on membrane proliferation (as found by Miroux and Walker in 1996), bacteriorhodopsin (BR) as model protein and a mammalian mitochondrial uncoupling protein (UCP).

Arechaga & al (2000), FEBS Lett 482, 215-219.
Hattab & al (2014), in: Membrane Proteins Production for Structural Analysis. Mus-Veteau (Ed.), Springer-USA. Chapter 4, 87-106.
Miroux & Walker (1996), J Mol Biol 260, 289-298.

53. Mutational analysis of a gene affecting septal cell wall modifications and cell-cell communication in the filamentous cyanobacterium *Anabaena* sp. Pcc 7120

Andreas Jan Bornikoel, Karl Forchhammer and Iris Maldener

IMIT/Organismic Interactions, University of Tübingen, Tübingen, Germany

Cell separation in *E. coli* is stimulated by the reversible relief of amidase auto-inhibition through the LytM-domain proteins NlpD and EnvC [1]. Filamentous cyanobacteria like *N. punctiforme* ATCC 29133 and *Anabaena* sp. PCC 7120 do not split into single cells after cell division. Instead they grow in filaments of several hundred cells that are connected by the continuous outer membrane and the common peptidoglycan (PG) layer. The septal PG is perforated by an array of nanopores and it has been demonstrated that periplasmic amidases are involved in the formation of these structures and influence cell-cell communication and cell differentiation [2, 3]. However, the spatial and temporal regulation of the specific amidase activity to modulate the septal PG is unknown. To identify regulatory factors involved in nanopore array formation and to investigate, whether LytM proteins are involved in this process, we started the functional characterization of orthologous genes in *Anabaena*. Here we describe the phenotype of a mutant with an insertion in a gene, which influences diazotrophic growth, nanopore array formation and cell-cell communication.

Yang *et al.* (2012) Mol. Microbiol. **85**: 768-781

Berendt, S. *et al.* (2012). Cell wall amidase AmiC1 is required for cellular communication and heterocyst development in the cyanobacterium *Anabaena* PCC 7120 but not for filament integrity. *J. Bacteriol.*, *194*(19), 5218-5227.

Lehner, J. *et al.* (2013). Prokaryotic multicellularity: a nanopore array for bacterial cell communication. *FASEB J., 27*(6), 2293-2300.

54. Use of ribosome profiling in cyanobacteria to reveal gene regulatory mechanisms in response to environmental changes

Jan Karlsen, Quentin Thomas[1]

[1]*Royal Institute of Technology, Proteomics and Nanobiotechnology, Stockholm, Sweden*

✉ *jan.karlsen@scilifelab.se, quentin.thomas@scilifelab.se*

Metabolic engineering and cultivation of cyanobacteria is in many cases troublesome due to unexpected cellular responses to gene modifications or changes in the growth environment. My research aims to improve the knowledge of how changes in the growth environment affect gene regulation at multiple levels of the central dogma. By combining data from transcriptomic, translatomic and proteomic high-throughput measurements, sampled at different growth conditions, valuable information can be acquired regarding the gene-regulatory landscape. While transcriptomes and proteomes of cyanobacteria has been analyzed before, a method for measuring the translational activity of genes in a massively parallel fashion has not yet been shown. Therefore, I have adapted a ribosome profiling protocol to work for cyanobacteria, which allows relative quantification of the number of ribosomes translating individual genes. For example, by comparing the change in transcript abundance with the change in ribosome occupancy between different sampling conditions, it is possible to deduce the occurrence of transcriptional and/or translational regulation. Furthermore, we use an mRNA seq assay that enriches the 5'-most part of the transcript for sequencing. This enables the detection and quantification of asRNA and mRNA transcript isoforms. Cyanobacteria has been shown to have extensive asRNA expression and we are interested how these regulatory RNA and transcript isoforms affect the translational activity of the gene.

55. Signal transduction for negative phototaxis in *Synechocystis* PCC6803

Ruiqian Zhou[1], Annegret Wilde[2] and Conrad Mullineaux [1]

[1]*School of Biological & Chemical Science, Queen Mary, University of London, Mile End Rd, London E1 4NS, UK.*

[2]*Institute for Biology III, Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany*

Synechocystis PCC6803 is able to sense light directions and glide on surface in order to find optimal light conditions. They use Type IV pilus (T4P) for motility controlled by various light sensing systems that respond to different wavelengths and intensites of light. Under UV-A (315-400nm), photoreceptor UirS receives light signal and phosphorylates UirR which regulates the gene expression of response regulator LsiR. To investigate the role of UirS in directional motility, we investigated the distribution of UirS at the cell membrane by confocal microscopy. If UirS is a directional photoreceptor, it must be evenly distributed at the cell periphery. We constructed a UirS knock-out mutant and complemented it with UirS-eYFP expressed from a neutral site under the control of an artificial promoter. A strain expressing UirS-eYFP from the native locus was also constructed. UirS function in each mutant was tested by motility assays. To check the mechanism of LsiR involvement in T4P activation, we expressed LsiR-eYFP in an LsiR knock-out background. Affinity pull-downs will be used to purify components which interact with LsiR. Mass spectrometry will be utilised for protein analysis. Simultaneously, we will compare distribution of LsiR in wild-type *Synechocystis* with a PilB1 null mutant. PilB1 is T4P motor to drive pilus extension. If LsiR directly interacts with PilB1 the distribution of LsiR-eYFP would be loss of PilB1. The distribution will be investigated by confocal microscopy.

56. FtsZ of filamentous, heterocyst-forming cyanobacteria has a conserved N-terminal domain required for normal FtsZ polymerization

Ana Valladares, Sergio Camargo, Silvia Picossi, Laura Corrales and Antonia Herrero

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

In prokaryotes, cell division is normally achieved by binary fission, a process in which the key player FtsZ, the protein that iniciates cell division, is essential. In cyanobacteria, much remains unknown about several aspects of cell division, including the role of various components involved in the division process. Sequence comparisons have revealed that the N-terminal region that precedes the conserved globular domain of FtsZ is highly conserved in the clade of filamentous cyanobacteria that are able to undergone cell differentiation events, including the model strain *Anabaena* sp. PCC 7120. An *Anabaena* derivative (CSL110) expressing an FtsZ version lacking the N-terminal region (ΔN-FtsZ) together with the native FtsZ shows notorious alterations in cell size and morphology. This strain expresses ΔN-FtsZ from the native *ftsZ* promoter and FtsZ from a conditionally regulated promoter. Under restrictive conditions for FtsZ expression cells progressively elongate and enlarge, reflecting restricted cell division. Under these conditions, aberrant Z-structures were detected. In bacterial 2-hybrid assays FtsZ interacts with itself, and ΔN-FtsZ interacts with itself and with FtsZ. However, in vivo ΔN-FtsZ forms aberrant Z-structures when expressed together with FtsZ. *Anabaena* FtsZ polymerizes in vitro forming filaments that show a distinct curling shape, whereas ΔN-FtsZ form thick bundles of straight filaments. Thus, the N-terminal sequence of *Anabaena* FtsZ appears to contribute to a distinct FtsZ polymerization mode that is important for cell division and division plane location through the filament.

57. Acclimation of *Synechocystis* to surface

Sabrina Oeser✉, Thomas Wallner, Annegret Wilde

Molecular Genetics, Institute of Biology III, University of Freiburg, Freiburg, Germany

✉*sabrina.oeser@biologie.uni-freiburg.de*

Many cyanobacteria are able to move on surfaces, make decisions about their lifestyle, and search actively for suitable environments. The most important environmental factor for photosynthetic organisms is light. Nevertheless, the biophysical and molecular mechanisms of light-controlled movement remain poorly understood. The model cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) shows light-induced motility across surfaces using type IV pili. Microarray analysis of *Synechocystis* cells grown either on agar plate or in liquid medium revealed altered transcription of minor pilin genes.

The functions of these minor pilins are unknown in *Synechocystis* so far. They are less abundant than the major pilin PilA1, the monomeric subunit of the pilus fibre, and have a common N-terminal sequence. In *Pseudomonas aeruginosa* a role of the minor pilins in pilus formation and function is proposed. For *Synechocystis* we observe distinct phenotypes of minor pilin mutants under different light environments. Interestingly, the expression of the minor pilins genes is also regulated by the cellular c-di-GMP level.

Our results suggest an important role of the minor pilins in the acclimation to surface growth and during phototaxis.

Additionally, genes potentially involved in EPS production are differentially expressed in liquid and solid culture. These data also hint at a role of EPS in surface acclimation and biofilm formation.

58. Genomic mining of plant-induced secondary metabolites in symbiotic cyanobacteria

Daniel Dehm[1], Anton Liaimer,[2] Eric Helfrich,[3] Katharina Graupner[4], María García-Altares Pérez[4], Christian Hertweck[4], Elke Dittmann[1]

[1]dehm@uni-potsdam.de, elke.dittmann@uni-potsdam.de; University of Potsdam, Institute of Biochemistry and Biology, Department of Microbiology, Potsdam, Germany

[2]anton.liaimer@uit.no; University of Tromsø, Faculty of Biosciences, Fisheries and Economics, Molecular Environments Group, Department of Arctic and Marine Biology, Tromsø, Norway

[3]ehelfrich@ethz.ch; Eidgenössische Technische Hochschule (ETH) Zurich, Institute of Microbiology, Department of Biology, Zürich, Switzerland

[4]christian.hertweck@leibniz-hki.de; Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany

Cyanobacteria such as *Nostoc punctiforme* are often found symbiotically associated with plants including *Gunnera* species and bryophytes like *Blasia pusilla* or *Anthoceros sp*. (Meeks, 1998).

Besides the great variety of symbiotic partners *N. p*unctiforme has one of the most complex life cycles and can differentiate up to four different cell types (Meeks and Elhai, 2002). This complexity is partly reflected by the huge amount of structurally diverse secondary metabolites that are encoded by the cyanobacterium.

While bioinformatic investigations of known secondary metabolite producers often reveal a huge potential secondary metabolome the actual products are often not detectable in the investigated strain. Those orphan gene clusters are silent and only induced under very specific conditions, such as the interaction with symbiotic partners as it was already described for other natural product producers like fungi. MALDI-Imaging analysis first time revealed the production of new types of compounds while *N. punctiforme* is in its symbiotic state. In some cases, the addition of cultivation supernatant was sufficient to induce the production of otherwise undetectable compounds (Liaimer, 2015).

The presented project aims at identifying secondary metabolite clusters that get induced upon exposure of *N. punctiforme* to varying cultivation conditions e.g. the presence of a N-starved host plant exudate in the media or growth in vicinity to a different *Nostoc* species. The ultimate aim is to reveal the structure and biological activity of those so far cryptic compounds. Therefore, a set of CFP reporter mutants was produced each reporting for another orphan secondary metabolite cluster detected by antiSMASH. Here we will present timeline experiments utilizing CFP reporter mutants that revealed a clear heterogeneity of expression levels within the *N. punctiforme* colony. Furthermore, we conducted new MALDI-Imaging studies that confirmed the production of compounds that are only present when *N. punctiforme* is either grown in the vicinity of another *Nostoc* species or in presence of N-starved host plant exudate.

59. Functional interactions between specific glucoside transporters and the SepJ septal protein in *Anabaena*

Mercedes Nieves-Morión[1], Conrad W. Mullineaux[2] and Enrique Flores[1]

[1]Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC and Universidad de Sevilla, Seville, Spain, and [2]School of Biological and Chemical Sciences, Queen Mary University of London, London, United Kingdom

✉ *mercedes.nieves@ibvf.csic.es; c.mullineaux@qmul.ac.uk; eflores@ibvf.csic.es*

The cells in the filaments of heterocyst-forming cyanobacteria exchange regulators and nutrients through septal junctions, which traverse the septal peptidoglycan through perforations known as nanopores. Intercellular molecular exchange can be traced in filamentous cyanobacteria with fluorescent markers including calcein (622 Da), 5-carboxyfluorescein (376 Da) and the sucrose analog esculin (340 Da), which are transferred between cells by simple diffusion [1]. The SepJ, FraC and FraD proteins are located at the intercellular septa and are putative components of the septal junctions, since mutants of *Anabaena* sp. PCC 7120 lacking these proteins make about 15% of the normal number of nanopores and are impaired in the intercellular transfer of the fluorescent markers [2]. We have recently identified *Anabaena* genes encoding proteins involved in glucoside transport including HepP (a MFS permease), GlsC and GlsD (nucleotide-binding domains of ABC transporters), GlsP and GlsQ (permeases of ABC transporters) and GlsR (a periplasmic substrate-binding protein). *Anabaena hepP*, *glsC*, *glsP* or *glsQ* mutants are impaired in the intercellular transfer of fluorescent markers, mainly calcein, suggesting a functional relation of these proteins and the septal junctions. Whereas GlsC appears to be necessary for localization of SepJ at the intercellular septa [3], HepP, GlsP and GlsQ may influence SepJ activity by means of protein-protein interactions, which were observed by BACTH analysis. Complex interactions between septal junctions and other transporters appear to occur at the intercellular septa.

[1] Nieves-Morión et al., mBio 8:e01756-16 (2017); [2] Nürnberg et al., mBio 6:e02109-14 (2015); [3] Nieves-Morión et al., J. Bacteriol. 7:e00876-16 (2017).

60. The arginine catabolism pathways of *Anabaena* sp. PCC 7120

Mireia Burnat[1], Bin Li[2], Silvia Picossi[1], Antonia Herrero[1], Anthony J. Michael[2] and Enrique Flores[1]

[1]Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC and Universidad de Sevilla, Sevilla, Spain, and [2]Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA.

mireia.burnat@ibvf.csic.es; bin.li@utsouthwestern.edu; silvia.picossi@ibvf.csic.es; Anthony.Michael@UTSouthwestern.edu; herrero@ibvf.csic.es; eflores@ibvf.csic.es

Arginine provided in the external medium or derived from β-aspartyl-arginine transferred from heterocysts to vegetative cells can be used as a nitrogen source by *Anabaena* sp. PCC 7120 [1]. Filaments of *Anabaena* supplemented with [^{14}C] arginine generated labelled proline and glutamate as major products. Inactivation of an *Anabaena* gene encoding a protein of the guanidine-modifying superfamily, AgrE, abolished the production of proline and glutamate not only from [^{14}C] arginine but also from [^{14}C] ornithine, suggesting that AgrE generates proline from arginine using ornithine as an intermediate, and inactivation of the *putA* gene abolished production of labelled glutamate from [^{14}C]proline. Thus, AgrE-PutA constitutes a major arginine catabolism pathway in *Anabaena*. In the *agrE* mutant supplemented with [^{14}C] arginine, labelled agmatine could be detected indicating the operation of arginine decarboxylase. Inactivation of *speA* (arginine decarboxylase), *speB* (agmatinase [2]) and a putative homospermidine synthase abolished production of *sym*-homospermidine, which is the polyamine characteristically found in heterocyst-forming cyanobacteria [3]. All arginine decarboxylase pathway mutants were severely impaired in diazotrophic growth, implying a requirement of homospermidine for heterocyst function. An *agrE speA* double mutant did not catabolize [^{14}C] arginine. GFP fusions showed that during diazotrophic growth AgrE, SpeA and SpeB concentrate in vegetative cells, whereas the putative homospermidine synthase is present in both vegetative cells and heterocysts. Hence, arginine catabolism in *Anabaena* proceeds via two pathways and is largely compartmentalized taking place mainly in vegetative cells.

[1] Burnat et al. (2014) PNAS 111:3823-3828; [2] Burnat & Flores (2014) MicrobiologyOpen 3:777-792; [3] Hamana et al. (2016) Microb Resour Syst 32:179-186.

61. Genomic and genotypic characterization of *Cylindrospermopsis raciborskii*

Vinicius A. C. Abreu[1], Rafael V. Popin1, Danillo O. Alvarenga[1,2,] Patricia D. C. Schaker[1,] Caroline Hoff-Risseti[1], Alessandro M. Varani[2], Marli F. Fiore[1]

[1]*University of São Paulo (USP), Center for Nuclear Energy in Agriculture, Piracicaba, São Paulo, Brazil*

[2]*São Paulo State University (UNESP), School of Agricultural and Veterinarian Sciences, Jaboticabal, São Paulo, Brazil*

✉ *vini.abreu@gmail.com; rvpopin@gmail.com; danillo.alvarenga@gmail.com; patischaker@gmail.com; carolhoff2006@gmail.com; amvarani@gmail.com; fiore@cena.usp.br*

Cylindrospermopsis raciborskii is a freshwater cyanobacterial with increasing bloom reports worldwide likely to factors related to climate change. In addition to the bloom effect on ecosystems, the majority are capable of synthetizing toxic secondary metabolites, thus causing public health issues. To overcome the harmful effects of C. raciborskii blooms it is important to advance the knowledge of diversity and evolutionary processes. An efficent approach to exploring this diversity and understanding the evolution of C. raciborskii is to use comparative genomics. We report two draft genomes of C. raciborskii (CENA302 and CENA303) from Brazilian isolates of different origins and explore their molecular diversity, phylogeny, and evolutionary diversification by comparing their genomes with sequences from other strains available in public databases. The results obtained comparing eight C. raciborskii genomes revealed a set of conserved core genes and a variable set of accessory genes, such as those involved in the biosynthesis of natural products, heterocyte formation and nitrogen fixation. Gene cluster arrangements related to the biosynthesis of the antifungal cyclic glycosylated lipopeptide hassallidin was identified in four genomes, including the non-nitrogen fixer strain CENA303. Shift in toxic gene clusters according to geographic origins was observed as well as lack of nitrogen fixation and heterocyte glycolipid gene clusters in some strains. Analyses have shown with high support values that the species C. raciborskii is monophyletic. This comparative genomics study allowed a species-wide view of the biological diversity of C. raciborskii and in some cases linked genome differences to phenotype.

Keywords: Cyanobacteria, Cylindrospermopsis, pan-genome, cyanotoxins, nitrogen fixation

Session 5: Bioenergetics

62. Ndhv is a subunit of NADPH dehydrogenase essential for cyclic electron transport in *Synechocystis* sp. Strain PCC 6803

Fudan Gao, Jiaohong Zhao, Xiaozhuo Wang, Shen Qin, Lanzhen Wei ✉, Weimin Ma

Department of Biology, Shanghai Normal University, Guilin Road 100, Shanghai 200234, China

✉*weilz@shnu.edu.cn*

Two mutants sensitive to heat stress for growth and impaired in NADPH dehydrogenase (NDH-1)-dependent cyclic electron transport around photosystem I (NDH-CET) were isolated from the cyanobacterium *Synechocystis* sp. strain PCC 6803 transformed with a transposon-bearing library. Both mutants had a tag in the same *sll0272* gene, encoding a protein highly homologous to NdhV identified in Arabidopsis. Deletion of *sll0272* gene (*ndhV*) did not influence the assembly of NDH-1 complexes and the activities of CO_2 uptake and respiration but reduced the activity of NDH-CET. NdhV interacted with NdhS, a ferredoxin-binding subunit of cyanobacterial NDH-1 complex. Deletion of NdhS completely abolished NdhV but deletion of NdhV had no effect on the amount of NdhS. Reduction of NDH-CET activity was more significant in Δ*ndhS* than in Δ*ndhV*. We therefore propose that NdhV cooperates with NdhS to accept electrons from reduced ferredoxin.

Reference

[1] Gao F, Zhao J, Wang X, Qin S, Wei L, Ma W (2016) NdhV is a subunit of NADPH dehydrogenase essential for cyclic electron transport in *Synechocystis* sp. strain PCC 6803. Plant Physiol 170(2): 752–760
[2] Battchikova N, Wei L, Du L, Bersanini L, Aro EM, Ma W (2011) Identification of a novel Ssl0352 protein (NdhS), essential for efficient operation of cyclic electron transport around photosystem I, in NADPH:plastoquinone oxidoreductase (NDH-1) complexes of *Synechocystis* sp. PCC 6803. J Biol Chem 286(42): 36992–37001

63. Glycogen catabolism is essential for resuscitation of cyanobacteria from nitrogen starvation

Sofía Doello[1], Alexander Klotz[1], Kirstin Gutekunst[2], Karl Forchhammer[1]

[1] *Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), University of Tübingen, Tübingen, Germany*

[2] *Department of Biology, Botanical Institute, Christian-Albrechts-University, Kiel, Germany*

Glycogen synthesis is essential for cyanobacteria to cope with stress and maintain viability during longer periods of darkness. During nitrogen starvation, *Synechocystis* sp. PCC 6803 reduces photosynthesis to a low level and produces reserve biopolymers like glycogen and polyhydroxybutyrate. This adaptation allows it to remain viable for a long period of time (Sauer et al. 2001). Mutants deficient in glycogen synthesis are not able to adapt to nitrogen depletion (Gründel et al. 2012).
We studied the role of glycogen in the resuscitation of *Synechocystis* from nitrogen starvation, which is a genetically determined program (Klotz et al. 2016). During the first phase of this process, cells switch off the residual photosynthesis and consume glycogen despite illumination. Transcriptome analysis revealed that, instead of being induced, the expression of genes required for glycogen degradation was slightly repressed, suggesting that cells prepare for degrading the polymer already during its synthesis. In phase two cells switch to photosynthetic metabolism, but continue to degrade glycogen. In order to understand the role of glycogen and its mobilization during resuscitation, mutants deficient in the glycogen phosphorylases, which degrade glycogen to glucose-1-P, were created and analyzed. These mutants are unable to degrade glycogen and cannot recover from nitrogen starvation. Further mutants in key glucose catabolic enzymes revealed a major contribution of the oxidative pentose phosphate and Entner-Doudoroff pathways, while the Embden-Meyerhof-Parnas pathway seemed to be of less importance. Our results demonstrate that glycogen plays a major role during stress adaptation and stress relief in cyanobacteria.

References

Gründel, M., Scheunemann, R., Lockau, W. & Zilliges, Y. Impaired glycogen synthesis causes metabolic overflow reactions and affects stress responses in the cyanobacterium Synechocystis sp. PCC 6803. *Microbiol.* **158,** 3032–3043 (2012).
Klotz, A. *et al.* Awakening of a Dormant Cyanobacterium from Nitrogen Chlorosis Reveals a Genetically Determined Program. *Curr. Biol.* **26,** 2862–2872 (2016).
Sauer, J., Schreiber, U., Schmid, R., Völker, U. & Forchhammer, K. Nitrogen starvation-induced chlorosis in Synechococcus PCC 7942. Low-level photosynthesis as a mechanism of long-term survival. *Plant Physiol.* **126,** 233–243 (2001).

64. Dominant role of isozymes in metabolic regulation of cyanobacteria

Jiri Jablonsky

Institute of Complex Systems, FFPW, University of South Bohemia, Cenakva, Czech Republic

We have extended the traditional kinetic model, based on metabolic data from one environmental condition, to multi-level kinetic model which requires metabolic and transcriptomic data from various conditions. This model can be extended by fluxomic, proteomic and other data. We have analysed various isozymes from *Synechococcus* sp. PCC 7942 and *Synechocystis* sp. PCC 6803, particularly phosphoglycolate phosphatases, phosphoglycerate mutases andglyceraldehyde-3-phosphate dehydrogenases. The results were surprising, showing that i) glyceraldehyde-3-phosphate dehydrogenases regulate hexoses homeostasis instead of their substrates, ii) it is beneficial having 3 phosphoglycerate mutases and iii) the impact of phosphoglycolate phosphatases is intransient states and not in steady states. Lastly, we have focused on the possible role of group of isozymes and showed that higher number of different isozymes in *Synechocystis* 6803 improves homeostatic stability of 3PGA by 275 % against changes in gene expression, compared to *Synechococcus* sp. PCC 7942. These and other data imply that the biochemical control dominates over transcriptional regulation in *Synechocystis* 6803 to acclimate central carbon metabolism in the environment of variable inorganic carbon availability without extra cost carried by large changes in the proteome.

65. Rubredoxin A associated with Photosystem II assembly intermediates controls chlorophyll biosynthesis

Eva Kiss, Pascual Aznar Guillem, Roman Sobotka, Josef Komenda

Institute of Microbiology, Center Algatech, Opatovický mlýn, 37981 Třeboň, Czech Republic

✉ *kiss@alga.cz, pascual@alga.cz, sobotka@alga.cz, komenda@alga.cz,*

Rubredoxins are the simplest Fe-S proteins designed to perform robust one electron transfer processes. Soluble rubredoxins are found in a wide variety of bacteria and archaea; however, the membrane-anchored rubredoxin (RubA) is specific for oxygenic photosynthesis. Previous studies using different cyanobacterial models are inconsistent, showing either Photosystem II (PSII) or Photosystem I (PSI) malfunctioning in the absence of RubA. We identified the protein in PSII assembly subcomplexes isolated from *Synechocystis* and ranging from the D1 module up to the PSII core complex lacking CP43 (RC47). Moreover, the Flag-tagged RubA protein expressed in *Synechocystis* was also purified as a part of RC47 and additionally associated with a monomeric PSII core complex. The data suggest that RubA binds to the newly synthesized D1 subunit and remains bound to it until the formation of the monomeric PSII core complex. The *Synechocystis* RubA deletion mutant accumulated low levels of both photosystems; nonetheless the assembled photosystems were fully intact. The chlorophyll biosynthetic pathway was however strongly inhibited, most probably due to restricted synthesis of aminolevulinic acid. Interestingly, the inhibition of ferrochelatase activity, which elevates chlorophyll formation, restored the photosystem levels and accelerated autotrophic growth of the mutant. The potential regulatory function of RubA in the coordination of photosystem assembly and chlorophyll biosynthesis is discussed.

66. Designing microbial photoelectrosynthesis using *Synechocystis* sp. PCC6803 as a host

Pasi Paananen[1]✉, Pauli Kallio[1], Jussi Jäntti[2], Eva-Mari Aro[1]

[1]*Molecular Plant Biology, Department of Biochemistry, University of Turku, 20014 Turun Yliopisto, Finland*

[2]*VTT Technical Research Centre of Finland, 02044 Espoo, Finland*

✉ *pjpaan@utu.fi*

The discovery of electrotrophs, microorganisms that can directly accept electrons from electrodes, has led to the emergence of a research field called microbial electrosynthesis (MES). This offers a potential strategy for capturing electrical energy in the form of carbon-carbon bonds as MES organisms are capable of transporting electrons through their outer membrane. The most studied system is the modular Mtr respiratory pathway of *Shewanella oneidensis* MR-1. The primary pathway consists of a conduit of multiheme c-type cytochromes MtrA and MtrC, a non-heme outer membrane β-barrel MtrB, and a cytoplasmic membrane associated quinol oxidase CymA which is capable of transferring reducing equivalents into the quinone pool.

The ultimate objective here is to engineer a hybrid photo-electro-organism, which is capable of accepting electrons in the form of electric current and directing them further to the carbon fixation reactions. This could provide a biological chassis for new approaches in the production of renewable biofuels, commodity chemicals and high-value products. The strategy is to introduce the heterologous *S. oneidensis* MR-1 electron capture/relay system into photosynthetic cyanobacterium *Synechocystis* sp. PCC6803. In practice, different combinations of selected Mtr pathway components and their homologs have been assembled into multi-gene operons using different synthetic biology approaches and molecular biology tools. The most promising operons have been transferred into *Synechocystis* compatible expression vectors, and transformed into the organism for functional analysis *in vivo*. Different customized photoelectrobioreactor systems have been developed, and are currently being evaluated and optimized to allow the characterization and functional comparison of the engineered strain.

67. Adaptation and regulation of carotenogenesis in cyanobacteria to protect oxygeneic photosynthesis

Gerhard Sandmann

Biosynthesis Group, Molecular Biosciences, Goethe University Frankfurt, Frankfurt, Germany

✉ *sandmann@bio.uni-frankfurt.de*

Cyanobacteria have to cope with high-light and oxygen stress. For them, carotenoids are essential to protect photosynthesis. These pigments transfer excess excitation energy from chlorophyll and quench singlet oxygen. During evolution, different carotenoid structures were acquired by different species. By genetic modification of the carotenoid pathway in *Synechococcus* or deletions in *Synechocystis,* it could be demonstrated that canthaxanthin exhibits best in situ protection of photosynthesis followed by zeaxanthin. Protection is invasive for carotenoids. Therefore, a high demand under high-light stress is compensated by accelerated synthesis regardless of the type of carotenoid. Enhanced carotenoid synthesis under high-light conditions was quantitated together with enhanced photo degradation. Higher synthesis was caused by transcriptional up-regulation of the overall pathway and a fast conversion of carotenoid intermediates to the protective end-product. In Synechococcus and *Nostoc* 7120, regulation of zeaxanthin or canthaxanthin synthesis proceeded via binding of the transcription factor NtcA to the promoter regions of the carotenogenic genes. In *Nostoc*, the binding was absolutely dependent on the presence of reductants and oxo-glutarate and negatively influenced by magnesium ions.

Schäfer L, Sandmann M, Woitsch S, Sandmann G (2006) Coordinate up-regulation of carotenoid biosynthesis as a response to light stress in *Synechococcus*. Plant Cell Envir 26, 1349-1356.

Schöpf L, Mautz J, Sandmann G (2013) Multiple ketolases involved in light regulation of canthaxanthin biosynthesis in *Nostoc punctiforme* PCC 73102. Planta 237, 1279-1285.

Sandmann G, Mautz J, Breitenbach J (2016) Control of light-dependent keto carotenoid biosynthesis in *Nostoc* 7120 by the transcription factor NtcA. Z Naturforsch C 71:303-311.

68. *Anabaena* sp. PCC 7120 mutant deficient in the vegetative-specific FLV3A protein produces H_2 under oxic conditions

Anita Santana-Sánchez[1], Maria Ermakova[1*], Melin He[1], Yagut Allahverdiyeva[1]

[1] *Department of Biochemistry, Molecular Plant Biology, University of Turku, FI-20014 Turku, Finland*

[*] *Current address: Research School of Biology, College of Medicine, Biology and Environment, Australian National University, Canberra ACT 2601, Australia.*
Co-authors: Maria.Ermakova@anu.edu.au (Maria Ermakova), meilin.he@utu.fi (Meilin He), allahve@utu.fi (Yagut Allahverdiyeva)

Flavodiiron proteins (Flvs) are involved in protection of photosynthesis and N_2-fixation machinery in cyanobacteria. In the unicellular non-N_2-fixing cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*), the Flv1/Flv3 heterodimer catalyzes a light-dependent reduction of O_2 to water, also called the Mehler-like reaction (Helman et al. 2003). Additionally, the Flv3 homo-oligomer in *Synechocystis* is involved in alternative electron pathway(s) where the terminal acceptor is not O_2 (Mustila et al. 2016). A filamentous heterocystous N_2-fixing cyanobacterium *Anabaena* sp. PCC 7120 (hereafter *Anabaena*) contains two copies of genes encoding Flv1 and Flv3 proteins: Flv1A and Flv3A are expressed in vegetative cells, whereas Flv1B and Flv3B are localised in heterocysts (Ermakova et al. 2013). The Flv1A and Flv3A proteins of *Anabaena* functionally match to Flv1 and Flv3 from *Synechocystis* and protect PSI from rapid changes in light intensity (Allahverdiyeva et al. 2013).
In contrast to the *Anabaena* wild-type (WT) and Δ*flv1A* mutant, the filaments lacking vegetative-cell specific Flv3A protein (Δ*flv3A*), demonstrated stable H_2 photoproduction under oxic conditions. Importantly, H_2 production yield of Δ*flv3A* was only slightly lower than Δ*hupL,* lacking large subunit of uptake hydrogenase, and the double mutant Δ*flv3A/*Δ*hupL.*
The Δ*flv1A* and WT cells demonstrated similar H_2-uptake capacity, while Δ*flv3A* showed significantly lower H_2 uptake rate, suggesting a strong impairment of H_2 recycling pathways in this mutant. This finding explains the stable production of H_2 in Δ*flv3A*, although the regulatory mechanisms behind this phenomenon is still unclear

69. Enhanced recovery from photosystem I photoinhibition by the assembly factor.

Mai Watanabe[1], Hiroaki Yoshino[2], Masako Matsumura[2], Yukiko Okuda[2], Masahiko Ikeuchi[1]

[1] *Department of Life Sciences (Biology), Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan. Japan Science and Technology Agency (JST), CREST.*

[2] *Department of Life Sciences (Biology), Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan.,*

✉ *maimai@bio.c.u-tokyo.ac.jp, uchiage.senkouhanabi@gmail.com, matsumura-masako367@g.ecc.u-tokyo.ac.jp, y_okuda@bio.c.u-tokyo.ac.jp, mikeuchi@bio.c.u-tokyo.ac.jp*

Photosystem I (PSI) is generally tolerant and protected from excess light by various mechanisms. However, PSI photoinhibition takes place under specific conditions and damaged PSI cannot be recovered efficiently. We focused on the assembly factors to investigate their roles for PSI damage. Here, we evaluated the PSI assembly factor Ycf3. We constructed a phylloquinone knockout mutant (Δ*menD*) as a model system of PSI photoinhibition. Δ*menD* is highly sensitive to photoinhibition and is unable to grow under normal light. Overexpression of the *ycf3* in the Δ*menD* ("Δ*menD/ycf3ox*") alleviated the growth retardation under normal light. In Δ*menD*, PSI-to-PSII ratio was markedly reduced compared with wild type (WT), even though cells were maintained under low light. Overexpression of *ycf3* recovered the PSI-to-PSII ratio of the Δ*menD* to the WT level. After treatment with high light irradiation, the growth recovery of Δ*menD* was slow under low light conditions, whereas that of Δ*menD/ycf3ox* was fast like WT. These results demonstrated that overexpression of the assembly factor is effective in the recovery of PSI photoinhibition. We measured P700 re-reduction rate which reflects PSI photoinhibition at the acceptor side. Even under low light condition, PSI of Δ*menD/ycf3ox* was cleary affected and PSI of Δ*menD* was more severly affected. It is not clear whether *ycf3* protects from photoinhibition or enhances the recovery process. For this purpose, we are currently measuring the re-reduction rate after high light treatment. We will discuss the role of *ycf3* overexpression in the PSI photoinhibition and its recovery process.

70. A new confocal microscopy method to study thylakoid membrane microdomains structure and properties.

Grzegorz Konert[1], Adela Strašková[1], Myriam Canonico[1], Gabor Steinbach[1,2], Radek Kaňa[1]

[1] *Institute of Microbiology, ALGATECH, Třeboň, Czech Republic*

[2] *Biological Research Centre, Szeged, Hungary*

✉ *konert@alga.cz, straskova@alga.cz, canonico@alga.cz, steinbach.gabor@gmail.com, kana@alga.cz*

Cyanobacteria are an excellent model organism to study structure and function of photosynthetic machinery. Photosystem I (PSI) and Photosystem II (PSII) are essential pigment-proteins complexes of light photosynthetic reactions. Both photosystems are capable of binding other pigment protein, Phycobilisomes (PBS), a large extra-membrane complex allowing utilisation of wavelengths not accessible by chlorophyll. Distribution of PBS between PSI and PSII within thylakoid membrane is an important structural way of coping with environmental conditions.
We have developed new methods of 3D confocal microscopy and image processing to study and quantified PSI, PSII and PBS distribution on single cell of cyanobacteria (*Synechocystis sp.* PC 6803). Single cell sectioning and image analysis allowed us to quantify co-localisation of each separate pigment-protein. Further investigation allowed us to develop a new photosynthetic parameter defined as protein-arrangement factor ("PA-factor") that can characterise organisation of photosynthetic machinery in particular single cells. The PA-factor represents a single number obtained by allocating pixels from images to corresponding CIE colour space area and analysing their distribution. It allows precise and a relatively simple investigation of physiological status of cells; its variability between cyanobacteria has been tested in different physiological conditions.

71. Light-driven whole-cell biocatalysis with recombinant cyanobacteria

Marc M. Nowaczyk[1, ✉] and Robert Kourist[2]

1Plant Biochemistry, Ruhr University Bochum, Bochum, Germany,
✉marc.m.nowaczyk@rub.de

2Molecular Biotechnology, TU Graz, Austria

Oxidoreductases, highly selective enzymes that catalyze oxidative redox reactions undermild conditions, have been established for the environmental friendly production of pharmaceuticals and chemicals. However, one limitation for the use of oxidoreductases is the stoichiometric requirement for organic co-substrates like glucose as electron donors in heterotrophic production systems like *Escherichia coli.* The use of water as electron donor would avoid the demand for co-substrates and the generation of unwanted waste products. To test the feasibility of photosynthetic biocatalyst production, two enantioselective model enzymes were expressed in *Synechocystis* sp. PCC 6803, cell extracts were used for biotransformations and synthesis of products was proven [1]. Subsequently, a NADPH-dependent enoate reductase was expressed in *Synechocystis* and whole-cells were applied for light-driven asymmetric C=C reductions. The efficiency of the reaction was comparable to typical whole-cell biotransformations with E. coli. Under optimized conditions, a solution of 100 mg prochiral 2-methylmaleimide was reduced to optically pure 2-methylsuccinimide (99 % ee, 80 % yield of isolated product). Moreover, volumetric productivities of up to 10 mm h-1 and product titers of up to 2 g L-1 were obtained [2].

[1] Bartsch M, Gassmeyer SK, Köninger K, Igarashi K, Liauw P, Dyczmons-Nowaczyk N, Miyamoto K, Nowaczyk MM, Kourist R (2015) Photosynthetic production of enantioselective biocatalysts Microb Cell Fact 14:53

[2] Köninger K, Gómez Baraibar Á, Mügge C, Paul CE, Hollmann F, Nowaczyk MM, Kourist R (2016) Recombinant Cyanobacteria for the Asymmetric Reduction of C=C Bonds Fueled by the Biocatalytic Oxidation of Water. Angew Chem Int Ed 55, 5582–5585

72. Engineering synthetic CO_2-fixing bypass in *Synechocystis* sp. PCC 6803.

Shanshan Song ✉, Eva-Maria Brouwer, Martin Hagemann

Rostock University, Plant Physiology Department, Einsteinstr. 3, 18059 Rostock, Germany,

✉ *shanshan.song2@uni-rostock.de*

Carbon dioxide is incorporated into organic compounds via the Calvin-Benson cycle in all oxygenic phototrophs [1]. Photosynthetic carbon fixation rate depends on the carboxylating enzyme RubisCO, which not strictly discriminates between CO_2 and O_2, leading to photorespiration and loss of assimilated CO_2. With the advent of synthetic biology, it is now feasible to design, synthesize, and introduce artificial biochemical pathways *in vivo* [2]. Here we aim to introduce a synthetic CO_2 fixation bypass supporting RubisCO. The Glycine-Formate Pathway starts with the reduction of CO_2 to formic acid by optimized formate dehydrogenase (FDH). Subsequently, 10-formyl-THF ligase (FTL) converts formate into 10-formyl-THF, which is finally incorporated into the existing photorespiratory metabolism via glycine to serine conversion. External sodium formate incubation improved the growth and resulted in higher content of serine and lower content of glycine in the FTL-expressing strain of *Synechocystis* sp. PCC 6803. Glycine supplementation increased the formate resistance to higher levels than wild type. The growth improvement is consistent with changes in the soluble amino acid levels. These data suggest that the downstream reactions of the glycine-formate pathway are already functioning in *Synechocystis*. These findings represent a good starting point for expressing our synthetic CO_2 fixation pathway.

Reference:

1. Bar-Even, Arren, et al. "Design and analysis of synthetic carbon fixation pathways." *Proceedings of the National Academy of Sciences* 107.19 (2010): 8889-8894.
2. Shih, Patrick M., et al. "Introduction of a synthetic CO_2-fixing photorespiratory bypass into a cyanobacterium." *Journal of Biological Chemistry* 289.14 (2014): 9493-9500.

73. The protein kinase SpkG phosphorylates the ferredoxin-like protein Slr0148 in *Synechocystis* 6803

Battchikova Natalia[1] ✉, Angeleri Martina[1], Zorina Anna[2] and Aro Eva-Mari[1]

[1] *Molecular Plant Biology, Department of Biochemistry, University of Turku, FI-20014 Turku, Finland;*

[2] *Institute of Plant Physiology, Botanicheskaya Street 35, 127276 Moscow, Russia*

✉ *natbat@utu.fi*

Phosphorylation on S/T/Y residues has been shown in cyanobacterial proteins involved in various cellular functions [1-5] including those related to the light capture, photosynthesis and electron transfer. However, a network of the S/T/Y phosphorylation-dependent signaling and regulation based on activities of protein kinases and phosphatases remains largely unknown in these organisms. We investigated protein kinase mutants of *Synechocystis* 6803 [6] with the mass spectrometry-based targeted approach using the SRM assays designed for phosphopeptides detected in photosynthesis-related proteins [5]. Results showed that phosphorylation of two peptides of the ferredoxin-like protein Slr0148 is strongly decreased in the SpkG mutant suggesting a link between phospho-Slr0148 and SpkG which are encoded in the same gene cluster. While no significant changes in phenotype was observed between WT and the mutant, the SpkG deletion caused a large increase in amounts of some phosphopeptides which belong to ApcA, PsaA, PsbA and OCP implying that post-translational regulation based on protein phosphorylation is involved in optimizing the performance of photosynthesis.

Financial support: Academy of Finland Centre of Excellence Project number 307335

1. Piven et al., J. Biol. Chem. 2005, 22, 21667-21672
2. Yang et al., J. Prot. Res. 2013, 4, 1909-1923
3. Chen et al., Plant Cell Physiol. 2015, 10, 1997-2013
4. Spät et al., Front. Microbiol. 2016, 6, 248
5. Angeleri et al., J. Prot. Res. 2016, 15, 4638−4652
6. Zorina et al, DNA Res. 2011, 18, 137–151

74. Synthetic pathway for carbon fixation in *Synechocystis* sp. PCC 6803.

Eva-Maria Brouwer ✉, Shanshan Song, Martin Hagemann

University Rostock, Department Plant Physiology, Einsteinstr. 3, 18059 Rostock, Germany,

✉ *eva-maria.brouwer@uni-rostock.de*

With the increase reliance on renewable energy sources and increased anthropogenic CO_2 emissions the idea rises to use photosynthetic organisms for biotechnological production of various organic compounds. Photosynthetic carbon assimilation in cyanobacteria as well as plants depends on the activity of RubisCO, an enzyme with relatively low activity and affinity toward the substrate CO_2. Here we want to implement an additional CO_2 fixing pathway to end up with a higher net carbon fixing rate. In this synthetic pathway CO_2 is fixed as formate and subsequently incorporated in carbon metabolism. Key enzymes are the *formate dehydrogenase* reducing CO_2 to formate and the *formate tetrahydrofolate ligase*. Strains expressing heterologous enzymes for formate synthesis and assimilation are currently used to achieve an efficient formate assimilation pathway working in parallel to the photosynthetic CO_2 assimilation. The additional fixed carbon can be used to produce organic compounds without harming growth and therefore to achieve higher yields.

75. Role of Type 2 NAD(P)H dehydrogenase NdbC in redox regulation of carbon allocation in *Synechocystis*

Tuomas Huokko, Dorota Muth-Pawlak, Natalia Battchikova, Yagut Allahverdiyeva, Eva-Mari Aro

NAD(P)H dehydrogenases comprise type 1 (NDH-1) and type 2 (NDH-2s) enzymes. Even though the NDH-1 complex is a well characterized protein complex in the thylakoid membrane of *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) the exact roles of different NDH2s remain poorly understood. To elucidate this question we studied the function of NdbC, one of the three NDH-2s in *Synechocystis*, by constructing a deletion mutant (Δ*ndbC*) for corresponding protein and submitting the mutant to physiological and biochemical characterisation as well as to comprehensive proteomics analysis. We demonstrate that the deletion of NdbC, localized to the plasma membrane, affects several metabolic pathways in *Synechocystis* in autotrophic growth conditions without prominent effects on photosynthesis. Foremost, the deletion of NdbC leads, directly or indirectly, to compromised sugar catabolism, to glycogen accumulation and to distorted cell division. Deficiencies in several sugar catabolic routes were supported by severe retardation of growth of the Δ*ndbC* mutant under light activated heterotrophic growth conditions but not under mixotrophy. Thus NdbC has a significant function in regulating carbon allocation between storage and the biosynthesis pathways. In addition, the deletion of NdbC increases the amount of cyclic electron transfer, possibly via the NDH-1_2 complex, and decreases the expression of several transporters in ambient CO_2 growth condition.

76. Sustained hydrogen production from a [FeFe] hydrogenase heterologously expressed in the diazotrophic cyanobacterium *Nostoc* PCC 7120

Luisana Avilan, Véronique Risoul, Christophe Sébastien Bernard, Baptiste Roumezi, Arlette Kpebe, Myriam Brugna, Amel Latifi

In addition to being a key metabolic in several biological communities, Hydrogen has gained huge interest as a good candidate for an environmentally friendly fuel. Conversion of solar energy into H_2 constitutes a highly attractive strategy for the bioproduction of renewable and clean energy and the use of photosynthetic organisms, is tested worldwide for such strategy. A decade of research in this field has demonstrated that several problems must be resolved before photosynthetic organisms can be efficiently used of H_2 production. Hydrogenases such as that found in anaerobic bacteria such *Clostridium*, are very efficient for H_2 production, and thus could be heterologously expressed in photosynthetic organisms for efficient production of H_2. However, to reach such a goal, a critical problem to be solved is that such hydrogenases are highly sensitive to O_2, thus biochemical incompatible with oxygen-evolving photosynthesis. To circumvent this limit, we have produced the [FeFe] hydrogenase HydA from *Clostridium acetobutylicum* in the heterocysts of *Nostoc* PCC 7120, thus making possible the otherwise incompatible biochemical reactions (H_2 production and O2-evolving photosynthesis) to happen in the same organism. Another part of our work focused on getting a broad knowledge of the genetic capacity of Cyanobacteria to produce hydrogenases (H_2ases). In this purpose, we conducted a phylum wide analysis of the distribution of the genes encoding these enzymes in 130 cyanobacterial genomes. This analysis highlighted the surprising presence in 5 cyanobacterial genomes of homologues to oxygen tolerant H_2ases; which might open new perspectives in the exploitation of photosynthesis for H_2 production.

77. Instead of functioning in O_2 photoreduction, Flv3 homo-oligomer mediates another electron transport pathway *in vivo* in *Synechocystis*

Henna Mustila[1,✉], Pasi Paananen[1], Natalia Battchikova[1], Anita Santana-Sánchez[1], Dorota Muth-Pawlak[1], Martin Hagemann[2], Eva-Mari Aro[1] and Yagut Allahverdiyeva[1]

[1]*Molecular Plant Biology, Department of Biochemistry, University of Turku, Turku, Finland*

[2]*Institut Biowissenschaften, Pflanzenphysiologie, Universität Rostock, Rostock, Germany*

✉*hmsile@utu.fi*

The cyanobacterial flavodiiron proteins Flv1 and Flv3 mediate O_2 photoreduction to water without accumulating reactive oxygen species. The Flv1 and Flv3 proteins safeguard photosystem I, and are thus crucial for the survival of cyanobacteria (*Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120) under fluctuating light, a characteristic light condition in aquatic environments. Under constant light conditions, including high light intensities, Flv1 and Flv3 are dispensable, and they may be compensated with other protective mechanisms. The Flv1/Flv3-dependent Mehler-like reaction occurs transiently during the low- to high-light transition, when the low background light is frequently interrupted with short high light pulses.

Flv1 and Flv3 can also form homo-oligomers *in vivo* in *Synechocystis*. However, in contrast to *in vitro* assay with recombinant Flv3, the Flv3 homo-oligomer in *Synechocystis* do not perform O_2 photoreduction. Either Flv1 or Flv3 was expressed in excess in a *Synechocystis* mutant lacking Flv3 or Flv1, respectively. Due to the function of Flv1 or Flv3 homooligomer, the growth phenotype of the strains was improved compared to inactivation mutants. The overexpression strains of expressing exclusively Flv1 or Flv3 demonstrated a recovery of CO_2 uptake and net photosynthesis, yet neither of the strains was capable of performing light-induced O_2 uptake. Therefore, the terminal acceptor of Flv1 and Flv3 homo-oligomers is not O_2, and the components of this electron transport pathway remain to be identified. Considering the small quantity of Flv1, it is probable that mostly Flv3 homo-oligomers are functional in WT cells.

78. Analysis of engineered *Synechocystis* PCC 6803 cells containing additional copies of phosphoenolpyruvate carboxylase

Claudia Durall[1, ✉], Pia Lindberg[1] and Peter Lindblad[1]

[1] *Chemistry Ångström, Uppsala Univeristy, Uppsala, Sweden*

✉ *claudia.durall@kemi.uu.se*

It is possible to genetically engineer cyanobacteria to produce a variety of biofuels [1-3]. However, the amount of the selected product is low but increasing carbon fixation can increment the production of carbon based substances in photosynthetic organisms. In addition to RuBisCO, Phosphoenolpyruvate carboxylase (PEPc) may also fix carbon in cyanobacteria. I have designed three different engineered strains by overexpressing the native *pepc* in the cyanobacterium *Synechocystis* PCC 6803, with one (WT+PEPc) or two (WT+ 2xPEPc) additional copies of *pepc*, and with additional copies of *pepc, ppsa* and *mdh* (WT+PPSA+ PEPc+ MDH). However, since the additional copy of *pepc* is identical to the native, single recombination with the native *pepc* has occurred in all engineered cells. SDS-PAGE/Immunoblot demonstrated that more PEPc protein is present in the engineered cells compared to in wild type. Interestingly, the WT+ 2xPEPc engineered cells grow faster than the control strain in low light (2-3 $\mu E \cdot m^{-2} \cdot sec^{-1}$) and it has shown a higher *in vitro* PEPc activity [4].
According to our experience, the heterelogous expression has shown better results in terms of protein level and activity. Thus, the *pepc* from *Synechococcus* PCC 7002 and 7942 was introduced in the cyanobacterium *Synechocystis* PCC 6803 either in the chromosome or in a self replicative vector. Interestingly, when a single copy of *pepc* from either *Synechococcus* strain was introduced into the chromosome, the *in vitro* PEPc activity was similar than the WT+2xPEPc. Currently, the PEPc protein level and the growth of the engineered strains is being examined.

References:

[1] S.A. Angermayr, K.J. Hellingwerf, P. Lindblad, M.J. Teixeira de Mattos, Energy biotechnology with cyanobacteria, Curr Opin Biotechnol 20 (3) (2009) 257-263.
[2] P. Lindblad, P. Lindberg, P. Oliveira, K. Stensjö, T. Heidorn, Design, engineering, and construction of photosynthetic microbial cell factories for renewable solar fuel production, Ambio 41 (2) (2012) 163–168.
[3] H. Li, J.C. Liao, Biological conversion of carbon dioxide to photosynthetic fuels and electrofuels, Energy Environ Sci 6 (2013) 2892-2898.
[4] Durall, Rukminasari and Lindblad (2015). Enhanced growth at low light intensity in the cyanobacterium *Synechocystis* PCC 6803 by overexpressing phosphoenolpyruvate carboxylase. Manuscript accepted in Algal Research.

79. Estimation of cyclic photosynthetic electron flow in the cyanobacterium *SynechocystiS SP.* PCC6803

Stefania Viola[1], Benjamin Bailleul[2], Francis-André Wollman[3]

Laboratoire de Physiologie Membranaire et Moléculaire du Chloroplaste-UMR7141, IBPC, CNRS-Université Pierre et Marie Curie (UPMC-Paris VI), Paris, France

✉ *[1]stefania.viola@ibpc.fr, [2]bailleul@ibpc.fr, [3]wollman@ibpc.fr*

In cyanobacteria, both the photosynthetic and the respiratory electron transfer pathways are located in the thylakoid membranes. The two pathways also share some of the chain components, thus giving rise to a complex interplay between the alternative electron transfer routes. This makes the estimation of cyclic electron flow even more challenging than in chloroplasts. The most classical methods to estimate it (comparing PSII and PSI quantum yields) are difficult to apply in cyanobacteria because of changes in the functional antenna size of the two photosystems (state transitions/spillover) and because of a strong limitation of the electron flow on the acceptor side of PSI. We are generating various mutants in which we expect those limitations to be overcome, and developing new methods to investigate the relative contribution of the electron transfer pathways in the model organism *Synechocystis sp.* PCC6803. We also try to estimate how the relative importance of the different pathways affects the redox state of the intersystem components of the chain and, in turn, the distribution of excitation energy between the two photosystems.

80. A Baeyer-Villiger monooxygenase from *Cyanidioschyzon merolae* prolongs growth in *Synechocystis* sp. PCC6803.

Mattia Niero; Elisabetta Bergantino

Department of Biology, Università degli Studi di Padova, Padova, Italy

✉ *mattia.niero@unipd.it, elisabetta.bergantino@unipd.it*

Baeyer-Villiger monooxygenases (BVMOs) are enzymes that catalyse the insertion of an oxygen atom into a carbon-carbon bond. These enzymes represent a promising tool in the field of organic chemistry to get rapid access to enantiomerically pure esters or lactones.

In order to test the possibility to use cyanobacteria as photosynthetic microbial vector for the production of recombinant BVMOs, we introduced into *Synechocystis* sp. PCC6803 chromosome the coding sequence of the BVMO from the red algae *Cyanidioschyzon merolae.* To evaluate possible undesired side effects of the genomic manipulations worked out, we compared the growth of the recombinant strains to that of the wild-type. Unexpectedly, we observed that the strain expressing the BVMO presents a prolonged growth and a delayed stationary phase compared to the wild-type. Our preliminary data suggest an active role of the recombinant enzyme in the metabolism of *Synechocystis*, leading to an increased biomass accumulation. In relation with the data reported by Zhou et al.[1] in a recent work, we are heading our research to find a possible link between the observed phenotype and an increased NADPH consumption caused by the BVMO activity.

We speculate that the BVMO expressed by the recombinant strain would oxidize an unknown substrate at the expense of molecular oxygen and NADPH, unbalancing the redox state and promoting a prolonged growth. Moreover, since the biocatalityc characterization of this Bayer-Villiger monooxygenase revealed a substrate preference towards long aliphatic ketones [2], we are going to investigate on secondary metabolites from the fatty acid pathway, as possible *in vivo* substrates for the heterologous enzyme.

Zhou, Jie, et al. "Introducing extra NADPH consumption ability significantly increases the photosynthetic efficiency and biomass production of cyanobacteria." *Metabolic engineering* 38 (2016): 217-227.

Beneventi, Elisa, et al. "Discovery of Baeyer–Villiger monooxygenases from photosynthetic eukaryotes." *Journal of Molecular Catalysis B: Enzymatic* 98 (2013): 145-154.

81. Thermodynamic and kinetic evaluation of pathways in cyanobacteria

Markus Janasch

The photoautotrophic metabolism of cyanobacteria is of ecological and potentially industrial importance. In addition to being primary producers of organic carbon, engineered cyanobacteria have been used as producers of non-native compounds. To facilitate future metabolic engineering efforts in this host, we have developed tools to enumerate and rank production pathways of a particular compound while considering the constraints imposed by the cyanobacterial metabolic network.

We first explored the thermodynamic landscape of the metabolism of *Synechocystis* by combining metabolomic and fluxomic data with a metabolic model. The "expansion potential," *i.e.* which types of compounds can be produced by taking advantage of natural driving forces, was assessed and compared the heterotroph *E. coli*. We next used available systems biology data to create and constrain a kinetic model of the cyanobacterial Calvin cycle, the main carbon entry point in most photoautotrophs. This model is used to estimate the kinetic parameters of all Calvin cycle enzymes by sampling the thermodynamically feasible metabolite and parameter space.

Our preliminary analysis identified potential regulatory steps and metabolite levels that may lead to non-stable operation.

82. Modules of co-occurrence in the cyanobacterial pan-genome

Christian Beck, Henning Knoop and Ralf Steuer ✉

Humboldt-Universität zu Berlin, Institut für Biologie, FachInstitut für Theoretische Biologie (ITB), Invalidenstr. 43, 10115 Berlin, Deutschland

✉ *ralf.steuer@hu-berlin.de*

The increasing availability of fully sequenced cyanobacterial genomes opens unprecedented opportunities to investigate the manifold adaptations and functional relationships that determine the genetic content of individual bacterial species. Here, we use comparative genome analysis to investigate the cyanobacterial pan-genome based on 77 strains whose complete genome sequence is available. Our focus is the co-occurrence of likely ortholog genes, denoted as CLOGs. We conjecture that co-occurrence CLOGs is indicative of functional relationships between the respective genes. Going beyond the analysis of pair-wise co-occurrences, we introduce a novel network approach to identify modules of co-occurring ortholog genes. Our results demonstrate that these modules exhibit a high degree of functional coherence and reveal known as well as previously unknown functional relationships. We argue that the high functional coherence observed for the extracted modules is a consequence of the similar-yet-diverse nature of the cyanobacterial phylum.

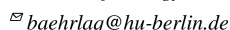
83. Exploring the potential of ultrahigh-density cultivation of cyanobacteria for the production of cyanophycin

Lars Bähr [1,2] ✉, Luca Lippi [3], Arne Wüstenberg [2], Annegret Wilde [3], Ralf Steuer [1]

[1] *Institute for Biology, Humboldt-University of Berlin, 10115 Berlin, Germany*

[2] *CellDEG GmbH, 13357 Berlin, Germany*

[3] *Institute of Biology III, University Freiburg, 79104 Freiburg, Germany*


✉ *baehrlaq@hu-berlin.de*

Cyanophycin is a non-ribosomaly synthesized biopolymer and can be found in many cyanobacteria and several heterotrophic bacteria. Accumulation of cyanophycin is triggered by high cellular arginine levels and is regulated by the interaction between the central regulator PII and the enzyme N-acetylglutamate kinase (NAGK). Modifying the central regulator PII ca n lead to a decoupling of amino acid synthesis and has resulted in strains with high abundance of cyanophycin (1).
As yet, however, the overall productivity of cyanobacterial cultures is limited due to the low cell densities presently obtainable in pilot scale photobioreactors. Here, we explore the use of a novel technology for ultrahigh-density (HD) cultivation of cyanphycin production strains: the recently developed and patented technology (2) is based on a two-tier vessel in which cyanobacterial growth is facilitated by a membrane mediated CO_2 supply. These novel cultivators enable rapid growth up to ultrahigh cell densities with biomass yields about 10 times higher than those reached with conventional bubble-stream techniques.
We demonstrate that HD cultivators are suitable for the cultivation of cyanophycin production strains and report a cyanophycin productivity per culture volume and day that is up to 5 times higher than previously reported. Cyanophycin yield per cell is similar to highest reported abundance of cyanophycin so far, while cultivation time to reach final cell densities is significantly reduced. Our novel approach may overcome some of the challenges with respect to the use of phototrophic bacteria for biotechnological applications.

1. Watzer B, Engelbrecht A, Hauf W, Stahl M, Maldener I, Forchhammer K. (2015) Metabolic pathway
engineering using the central signal processor PII. Microb Cell Fact. 14:192.
2. Bähr, L., Wüstenberg, A. & Ehwald, R. (2016) Two-tier vessel for photoautotrophic high-density cultures. J
Appl Phycol 28: 783. doi:10.1007/s10811-015-0614-5

84. A minimal model of phototrophic growth for cyanobacterial proteome allocation

Marjan Faizi [1], Tomáš Zavřel [2], Cristina Loureiro [3], Jan Červený [2], Ralf Steuer [1]

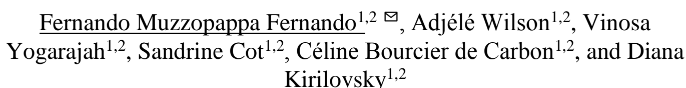
[1] *Humboldt-Universität zu Berlin, Institut für Biologie, Fachinstitut für Theoretische Biologie*
[2] *Department of Adaptive Biotechnologies, Global Change Research Institute CAS, Brno, CzechRepublic*
[3] *Department of Applied Physis, Polytechnic University of Valencia, Valencia, Spain*

Cyanobacterial metabolism must continuously adapt to changing environmental conditions to ensure best possible growth and simultaneously meet cellular demands for energy requirements, synthesize metabolic precursors, and provide sufficient storage compounds for periods of darkness, while avoiding photodamage. Our aim is to understand the underlying mechanisms behind these adaptive properties of cellular metabolism. In particular, we are interested in the relationship between proteome allocation to different cellular processes and the resulting growth rate under different environmental conditions.

Previous computational models that focused on large-scale metabolism have made quantitative predictions on cyanobacterial growth. Such models, however, do not provide details about the intrinsic mechanisms that limit phototrophic growth. To tackle this question, we developed a minimal coarse-grained model of proteome allocation for cyanobacterial growth, based on existing growth models for heterotrophic bacteria.

The model uses ordinary differential equations to describe the dynamics of proteome allocation for different light and CO_2 conditions. The model predicts the relationships that govern optimal resource allocation of phototrophic growth and is able to reproduce cyanobacterial growth laws based on minimal assumptions about key parameters. The model is backed by experimental analysis of cyanobacterial growth under different environmental conditions.

85. The paralogs to the C-terminal domain of the cyanobacterial orange carotenoid protein.


Fernando Muzzopappa Fernando[1,2], Adjélé Wilson[1,2], Vinosa Yogarajah[1,2], Sandrine Cot[1,2], Céline Bourcier de Carbon[1,2], and Diana Kirilovsky[1,2]

[1] *Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Sud, Université Paris-Saclay, 91198 Gif sur Yvette, France*

[2] *Institut Fréderic Joliot, Commissariat à l'Energie Atomique (CEA), 91191 Gif-sur-Yvette, France.*

fernando.muzzopappa@cea.fr

The photoactive Orange Carotenoid Protein photoprotects cyanobacteria cells by quenching singlet oxygen and excess excitation energy. Its N-terminal domain (NTD-OCP) is the active part of the protein and the C-terminal domain (CTD-OCP) regulates the activity. Recently, it was described the characteristics of a family of soluble carotenoids (HCPs), paralogs of the NTD-OCP. Bioinformatics studies also revealed the existence of genes coding for homologs of the CTD (CTDH). Here, we show the first characterization of these genes. This family of proteins contains two subgroups with distinct characteristics (CTDH1 and CTDH2). *Termosynechococcus elongatus* (Clade 1) and *Anabaena* PCC7120 (Clade 2) CTDHS were further characterized. The CTDHs form homodimers stabilized by the binding of a carotenoid molecule that is shared by both monomers. They are very good singlet oxygen quenchers. *T. elongatus* CTDH is able to transfer the carotenoid to *T elongatus* HCP while *Anabaena* CTDH form a disulfide bond between monomers (C103-C103) that avoids carotenoid transfer. When this cysteine is reduced or mutated to a Phe, *Anabaena* CTDH is able to transfer the carotenoid molecule not only to the *Anabaena* HCP but also to *T elongatus* apo-HCP and apo-OCP. The apo-OCPs and CTDHs are able to take the carotenoid directly from membranes with high efficiency while HCPs are almost unable. Results will be presented suggesting that one principal role of CTDHs is as donors of carotenoid molecules to HCPs.

86. Characterization of an intriguing substance from the growth medium of *Synechocystis* sp. PCC6803

István Zoltán Vass, Péter B. Kós, Ateeq Ur Rehman, Imre Vass

Laboratory of Molecular Stress and Photobiology, Institute of Plant Biology, Biological Research Centre, Szeged, Hungary

✉ *vass.istvan_zoltan@brc.mta.hu, kos.peter@brc.mta.hu, rehman.ateeq_ur@brc.mta.hu, vass.imre@brc.mta.hu*

The cyanobacterium *Synechocystis* sp. PCC6803 is a key model organism in photosynthetic research. For laboratory purposes cells are cultured until they reach an exponential growth phase, when they are harvested for experiments. It has been a long-time observation that the medium in which the cells grow might affect the outcome of experiments and consequently data are more consistent and reliable if the growth medium is replaced with fresh medium. Our inquiries revealed that a yellow substance accumulates in the growth medium that has the capacity to produce singlet oxygen. In this study we elaborate on the characteristics of the yellow substance. We provide details of the major factors that affect its production as well as details on its molecular structure, chemical properties and biological effects.

87. D1' Induced Changes in PSII Electron Transport in Cyanobacterium *Synechococcus* sp. PCC 7002

Iuliana-Cristina Chis✉, Ciprian Chis, Oana Sicora, Tünde Éva Jakó, Cristina Copaci, Mladin Lia, Mara Naghi and Cosmin Ionel Sicora

Ministry of National Education, Biological Research Center Jibou, Romania
✉ *simeoni_iulia@yahoo.com*

The structure of the photosynthetic machinery in cyanobacteria is highly conserved, as well as in green algae and higher plants. The core proteins of photosystem II (PSII), D1 and D2, bind all the redox-active components involved in electron transfer of PSII. D1 protein is one of the main sites of damage by a variety of environmental factors, requiring its replacement, whereas most of the other PSII subunits remain ordinarily undamaged. The D1 protein family from cyanobacteria contains members with different functionality as an adaptation to different environmental conditions. There are members of the protein family involved in adaptation to high-light conditions, others to UV-B stress and more recently were discovered members induced in low oxygen or micro-aerobic conditions hinting about a role these D1 form play in cellular adaptation to above mentioned conditions.
In this study we used a *Synechococcus* sp. PCC 7002 mutant that has an inactive *psbA* gene, encoding D1' isoform in comparison with the wild type, in order to better understand the role of this D1 protein isoform under a range of environmental factors (UVB, high-light, micro-aerobic conditions). The standard growth conditions for this strain were: light irradiance of 50 µE x m^{-2} x s^{-1}, and 38°C. During the high-light experiments *Synechococcus* sp. PCC 7002 shows a change in the decay of the fluorescence curve, not seen previously in other species. In our experiments we try to understand the nature and origin of these changes in PSII function in this cyanobacterium in an effort to gain more insight into the mechanisms of cyanobacterial photosynthetic electron transport.

88. Cooperative effect of carotenoids and lipid desaturation on temperature and high-light stress resistance of *Synechocystis* mutants

Tomas Zakar[1], Eva Herman, Sindhujaa Vajravel, Laszlo Kovacs, Mihaly Kis, Tunde N. Toth, Zoltan Gombos, Hajnalka Laczko-Dobos

Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, H-6701 Szeged, Hungary

✉ *tamas.zakar@gmail.com*, *hermaneva91@yahoo.com;, vajravel.sindhujaa@brc.mta.hu; kovacs.laszlo@brc.mta.hu; kis.mihaly@brc.mta.hu; tunde.toth.biol@gmail.com; gombos.zoltan@brc.mta.hu; laczkodobos.hajnalka@brc.mta.hu*

Xanthophylls are the main photoprotective agents, can assist in protection against light stress, and are crucial in the recovery from photoinhibition. Polyunsaturated lipids are important components of photosynthetic membranes. Xanthophyll- and polyunsaturated lipid-deficient ROAD mutant of *Synechocystis sp.* PCC6803 (Synechocystis) was generated in order to study the little-known cooperative effects of lipids and carotenoids. Electron microscopic investigations confirmed that in the absence of xanthophylls the S-layer of the cellular envelope is missing. In wild-type cells, as well as the xanthophyll-less (RO), polyunsaturated lipid-less (AD), and the newly constructed ROAD mutants the lipid and Car compositions were determined by MS and HPLC, respectively. We found that, relative to the WT, the lipid composition of the mutants was remodeled and the Car content changed accordingly. In the mutants the ratio of non-bilayer-forming (NBL) to bilayer-forming (BL) lipids was found considerably lower. Xanthophyll to β-carotene ratio increased in the AD mutant. In vitro and in vivo methods demonstrated that saturated, monounsaturated lipids and xanthophylls may stabilize the trimerization of Photosystem I. Fluorescence induction and oxygen-evolving activity measurements revealed increased light sensitivity of RO cells compared to those of the WT. ROAD showed a robust increase in light susceptibility and reduced recovery capability, especially at moderate low (ML) and moderate high (MH) temperatures, indicating a cooperative effect of xanthophylls and polyunsaturated lipids. We suggest that both lipid unsaturation and xanthophylls are required for providing the proper structure and functioning of the membrane environment that protects against light and temperature stress.

Author index

Adela Strašková 229
Adjélé Wilson 259
Adrián Velázquez-Campoy 103
Adriana Hegedűs 121
Agnieszka Misztak 105, 107
Aizhan A. Usserbaeva 109
Alain Boussac 67
Alessandro de Mello Varani 101, 117, 211
Alexander Erban 81
Alexander Klotz 215
Alexander Y. Starikov 109
Alexandra Calteau 77
Alexandra G. Markelova 109
Alison Telfer 67
Amanda A. Brindley 57
Amanda Cockshutt 85, 191
Amber Riaz-Bradley 33, 161
Amel Latifi 169, 241,
Amy T. Ma 37
Ana Paula
Ana Paula Dini Andreote
Ana Valladares 41, 201, 211
Andrea Fantuzzi 67
Andreas Burkovski 103
Andreas Jan Bornikoel 49, 195,
Andreea Baricz 121
Angeleri Martina 235
Aniek D. van der Woude _155
Anika Wiegard 29
Anita Santana-Sánchez 61, 149, 225,243
Anja Doerrich 29
Anna A Zorina 143
Anna Segalla 193
Annegret Wilde 27, 29, 127, 129, 199, 203, 255
Annik Jakob 27,129
Ann-Katrin Kieninger 49
Anthony J. Michael 209
Anton Liaimer 19, 205,
Antonia Herrero 5, 41, 149, 201, 209,211
Antonio López-Lozano 103, 115
Arlette Kpebe 241
Arne Wüstenberg 255
Arthur Guljamow 19,81
Asunción Contreras 39
Ateeq Ur Rehman 261
Augusto Etchegaray 117
Baptiste Roumezi 241
Barry D. Bruce 77
Battchikova Natalia 235
Becze Anca 123
Benedikt Junglas 47
Benjamin Bailleul 247
Benjamin J. Dorgan 183
Bersanini L. 61
Bin Li 209
Bivol Cezara 123
Bolatkhan K. Zayadan 109
Brian P. Tieu 37
Brooke Turner 85
Bruno Costa Evangelista de Souza 97
Bruno Miroux 193
Bryan Bishe 37
C. Neil Hunter 57
Carina Eisfeld 59
Carlos Flores 59, 139
Carmen Postolache 119
Caroline Eigner 73
Caroline Hoff-Risseti 211
Catarina C. Pacheco 49
Cátia Gonçalves 49
Cecilia Chiria 121
Céline Bourcier de Carbon 259
Cepoi Liliana 123
Chen-Cai Zhang 163, 169, 175
Cheryl A. Kerfeld 73
Chiriac Tatiana 123
Christian Beck 253
Christian Hertweck 19, 205
Christin Koebler 29
Christophe Sébastien Bernard 169, 241
Christophe Six 91
Christopher J. Howe 53
Ciprian Chis 263
Ciprian Valentin Mihali 119
Claudia Durall 245
Claudia Hackenberg 73
Cole Murphy 85
Cong-Zhao Zhou 163

Conrad W. Mullineaux 129, 183, 207
Cosmin Ionel Sicora 263
Cristian Coman 121
Cristina Copaci 263
Cristina Loureiro 257
Csaba Nagy 151
Cuypers A. 105
Daniel Dehm 19, 205,
Danillo Oliveira Alvarenga 97, 117, 101, 211
Danuta Kaczmarzyk 177
David Bogumil 93
David J. Lea-Smith 53
Debbie Lindell 9, 25
Dennis J. Nürnberg 67, 179
Derly Andrade 189
Deyana A. Ivanova 183
Diana Kirilovsky 259
Dirk Schneider 47
Dmitry A. Los 109
Dmitry A. Semchonok 77
Dong-Dong Cao 163
Dorota Muth-Pawlak 239, 243
Douglas Campbell 85
Edina Szekeres 121
Egbert J. Boekema 77
Elad Nagar 21
Elena V. Kupriyanova 109
Eleonora Sendersky 21
Elisabetta Bergantino 193, 249
Elke Dittmann 19,73, 81, 205,
Ellen Hornung 99
Elton P. Hudson 177
Emily Curren 113
Enrique Flores 41, 149, 207, 209,
Eric Helfrich 19, 205
Éric Maréchal 91, 205
Eric Pederson 147
Erin Bonisteel 85
Ernani Pinto 101, 117
Eva Bok 49
Eva Herman 265
Eva Kiss 219
Eva-Mari Aro 51, 61, 79, 151, 221, 235, 239, 243
Eva-Maria Brouwer 233, 237
Fabian Conradi 167
Fabiane Dörr 101
Fang Huang 75
Farbrice Rappaport 67
Federico Rossi 59
Fei Cai 73
Felipe Augusto Dörr 117
Fernanda Cristina Rodrigues Paiva 117
Fernando Muzzopappa Fernando
Filipe Branco dos Santos 11, 131, 137,
Francesca Zito 193
Francis-André Wollman 247
Francisco J. Florencio 35
Frédéric Partensky 91
Friedrich Kirsch 125, 133,
Fudan Gao 79, 213
Gabor Steinbach 229
Gen Enomoto 15
Gerald Seidel 103
Gerhard Sandmann 223
Gregor L. Weiss 45
Grzegorz Konert 229
Guy T. Hanke 171
Hailin Chen 169
Hajnalka Laczko-Dobos 265
Hana Némová 95
Hanna Farnelid 23
Hannah Minas 45
Henna Mustila 243
Henning Knoop 253
Henriette Rübsam 133
Hermann Bauwe 181,
Hiroaki Yoshino 227
Holger Dau 89
Hugo Doré 91
Hui Sun 163.
Ignacio Luque 5, 41
Imre Vass 261
Irina N. Shilova 23
Iris Maldener 41, 183, 185, 195
István Zoltán Vass 261
Itai Sharon 99
Iuliana-cristina Chis 263
Ivana Cengic 77
Ivo Feussner 99
Iwane Suzuki 11, 145
James W. Golden 37
James W. Murray 69
Jan Červený 257
Jan Karlsen 197

Janaina Rigonato 97
Janssen P. J. 105
Javier Espinosa 39
Jenna-Rose Melanson 85, 191
Jennifer Suppes 47
Jesús A. G. Ochoa de Alda 5, 41
Jesús Díez 103, 115
Jianfeng Yu 69
Jiaohong Zhao 85, 213
Jiri Jablonsky 217
Joachim Kopka 81
João Cabraland 59
Joaquín Giner-Lamia 35
Johanna Hakanpää 73
Jonathan P. Zehr 23
Jonathan T. Nguyen 77
Jörg Scholl 165
Jorge Rodriguez-Ramos 75
José Ángel Moreno-Cabezuelo 115
José Flores-Uribe 99
Jose I. Labella 39
José Manuel García-Fernández 103, 115
Jose Maria Marcos-Merino 5
José P. Leite 59
Josef Komenda 69, 219
Josef Lehner 183
Josefine Anfelt 153
Judith Ilhan 93
Juha Kurkela 135
Julia Walter 51
Julia Weissenbach 93
Julian J. Eaton-Rye 87
Julian Whitelegge 77
Juliette Jouhet 91
Jürgen Markl 47
Jussi Jäntti 221
Justine Pittera 91
Kaisa Hakkila 135
Karin Stensjö 173
Karina Stucken 93
Karl Forchhammer 49, 55, 165, 187, 195,
Karsten Voigt 127
Katharina Faulhaber 49
Katharina Graupner 205
Kathryn S. Lilley 53
Keishi Ishida 19
Kenzhegul Bolatkhan 109
Kevin Sheridan 63
Khaled Selim 187
Kirill S. Mironov 109
Kirstin Gutekunst 215
Kiyan Shabestary 153
Klaas J. Hellingwerf 155
Krzysztof Waleron 105, 107
Kui Xu 85
Lanzhen Wei 213
Lars Bähr 255
Laszlo Kovacs 265
Laura A. Antonaru 179
Laura Corrales 41, 201, 211
Laura L. Baers 53
Laurence Garczarek 91
Laurent. Gatto 53
Lena Moravcová 57
Leys N. 105
Liping Chen 79
Lisa M. Breckels 53
Lott S.C. 159
Luca Lippi 255
Lucas J. Stal 1, 17
Lucia Chomová 95
Luís Gales 59
Luisana Avilan 241
Lun Yao 153, 177
Luning Liu 75
M. Isabel Muro-Pastor 35
M. Waleron 105
Magda Furmaniak 107
Mahendra K. Shukla 57
Mai Watanabe 227
Malgorzata Waleron 107
Mara Naghi 263
Marc M. Nowaczyk 231
Marcio Vinícius Bertacine Dias 117
Marco Kreische 19
Marcus Hartmann 187
Maria A. Sinetova 109
María Agustina Domínguez-Martín 103, 115
Maria del Carmen Muñoz-Marin
Maria Ermakova 23, 61, 225
Maria Garcia -Alteras Perez 205
Maria Iasmina Moza 119
Maria Wittmiß 181
Mariana Silvia Cretoiu 17
Mariano Battistuzzi 193

Marina Santos 59, 139
Marion Eisenhut 171
Marjan Faizi 257
Markus Janasch 251
Marli de Fátima Fiore 97, 101, 117, 211
Martin Hagemann 31, 181, 187, 233, 237
Martin Pilhofer 45
Martina Bečková 69
Masahiko Ikeuchi 15, 227
Masako Matsumura 227
Matthew Faulkner 75
Matthias E. Futschik. 35
Matthias Schönborn 89
Mattia Niero 193, 249
Meilin He 225
Meng Li 77
Mercedes Nieves-Morión 207
Michael J. Deery 53
Michael Jahn 153
Michael Saur 47
Michael Schorsch 171
Miguel A. Hernández-Prieto 35
Miguel Vicente 189
Mihaly Kis 265
Min Chen 71
Mireia Burnat 209
Mirela Moldoveanu 17
Mladin Lia..263
Mónica Vásquez 189
Morgane Ratin 91
Muriel Gugger 77
Myriam Brugna 241
Myriam Canonico 229
Nadin Pade 125
Natalia Battchikova 79, 239, 243
Nathalie Sassoon 77
Ngoc An Nguyen 91
Nicolae Dragoș 121
Niels-Ulrik Frigaard 79
Nils Hülter 93, 129
Nils Schuergers 27
Oana Sicora 263
Oded Béjà 99
Olivier Ploux 117
Pascual Aznar Guillem 219
Pasi Paananen 221, 243
Patricia Caicedo Burbano 131
Patricia D. C. Schaker 211
Patricia Sánchez-Baracaldo 7
Paul Hudson 153
Paula Tamagnini 49, 59, 139
Pauli Kallio 151, 221
Paulo Oliveira 49, 139
Pekka Patrikainen 151
Péter B. Kós 261
Peter J. Gollan 51
Peter Lindblad 83, 245
Peter Nixon 69
Philip J. Jackson 57
Philipp Simon 89
Pia Lindberg 245
Pierre Joliot 67
Quentin Thomas 197
Radek Kaňa 229
Rafael Clavería-Gimeno 103
Rafael V. Popin 211
Rafael Vincentini Popin 101
Rakefet Schwarz 21
Ralf Steuer 253, 255, 257
Rami Parnasa 21
Raoul Hennig 47
Raquel Cantos 39
Rebeca Perez 185
Renata Beatriz Cruz 97
Rita Mota 59, 139
Robert Kourist 231
Roberto de Philippis 59
Rocío Esteban 5
Rocio Robles-Rengel 35
Roman A. Sidorov 109,
Roman Sobotka 57
Rudi Ludmila 123
Ruiqian Zhou 199
Ruth Perez Gallego 155
Ryan Simkovsky 21
Ryo Asada 145
Sabrina Oeser 203
Sandric Chee Yew Leong 113
Sandrine Cot 259
Sara B. Pereira 59, 139
Sarit Avrani 9
Satu Koskinen 135
Sergio Camargo 41, 201, 211
Shanshan Song 233, 237
Sheila Roitman 99
Shen Qin 213

Shengwei Hou 157
Shengxi Shao 69
Shi T 23
Shuntaro Machida 111
Silvia Picossi 41, 149, 201, 209, 211
Simedru Dorina 123
Sindhujaa Vajravel 265
Sofía Doello 215
Solène Breton 91
Sophia Zborowsky 25
Stefania Viola 247
Stefano Santabarbara 67
Steglich C. 159
Stella Thomaz Lima 117
Stephan Klähn 125, 127, 133
Susan S. Golden 3, 21
Sven Meissner 73, 81
Svetlana Antonyuk 73
Taina Tyystjärvi 135
Tal Dagan 93
Taton Arnaud 37
Teruo Ogawa 79
Thomas A. Witt 77
Thomas Wallner 127, 203
Tim J. Stevens 53
Tina C. Summerfield 63
Tino Barchewitz 81
Tomas Zakar 265
Tomáš Zavřel 257
Tunde N. Toth 265
Tünde-Éva Jakó 263
Tuomas Huokko 239
Ute C. Vothknecht 51
Vendula Krynická 69
Veronika Angerer 127
Véronique Risoul 241
Vicki Moore 65
Victor S. Lamzin 73
Victor Zhong 87
Vinayak Agarwal 37
Vinicius A. C. Abreu 211
Vinosa Yogarajah 259
Voigt K. 159
Waldemar Hauf 165
Wang Cheng 163
Weimin Ma 79, 213
Wenyang Wu 137
William Rutherford 67, 179
Wim Vermaas 65
Wolfgang R. Hess 13,125, 133, 157
Xiaoming Tan 157
Xiaozhuo Wang 213
Xin Li 141
Xuefeng Lu 157
Xue-Ping Wang 163
Yadav Anu 105
Yagut Allahverdiyeva-Rinne 61, 149, 225, 239, 243
Yaqi Sun 75
Yevgeni Yegorov 21
Yong-Liang Jiang 163
Yoshihiro Shiraiwa 145
You Chen 37
Young-Saeng Kim 37
Yukiko Okuda 227
Yulia Yuzenkova 33, 161
Yuxing Chen 163
Yvonne Zilliges 89
Zhaoxing Ran 79
Zhiyong Liang 89
Zoltan Gombos 265
Zorina Anna 143, 225, 235

List of participants

Name	Contact
Agnieszka Misztak	agnieszka.misztak@biotech.ug.edu.pl
Amanda Cockshutt	acockshutt@mta.ca
Amel Latifi	latifi@imm.cnrs.fr
Andreras Jan Bornikoel	jan.bornikoel@gmail.com
Aniek van der Woude	aniek.vanderwoude@photanol.com
Anita Isabel Santana Sanchez	aisasa@utu.fi
Anna Valladeres-Ruiz	avalla@ibvf.csic.es
Anna Zorina	zorina.anna@yahoo.com
Annegret Wilde	annegret.wilde@biologie.uni-freiburg.de
Annik Jakob	annik.jakob@sgbm.uni-freiburg.de
Antonaru Laura Alexandra	laura_antonaru@yahoo.com
Antonia Herrero	herrero@ibvf.csic.es
Anu Yadav	ayadav@sckcen.be
Arthur Guljamow	guljamow@uni-potsdam.de
Asuncion Contreras	contrera@ua.es
Bähr Lars	baehrlaq@hu-berlin.de
Barry Bruce	bbruce@utk.edu
Bivol Cezara	bivolcezara@yahoo.com
Breton Solene	Solene.breton@sb-roscoff.fr
Brouwer Eva-Maria	eva-maria.brouwer@uni-rostock.de
Carlos Eduardo Flores	carlos.flores@ibmc.up.pt
Christophe Bernard	christophe.bernard1@free.fr
Claudia Hackenberg	hackenberg@embl-hamburg.de
Claudia Steglich	claudia.steglich@biologie.uni-freiburg.de
Cong-Zhao Zhou	zcz@ustc.edu.cn
Conrad Mullineaux	c.mullineaux@qmul.ac.uk
Conradi Fabian	f.d.conradi@qmul.ac.uk
Cristina Iacob	m.s.cretoiu@uva.nl
Daniel Dehm	dehm@uni-potsdam.de
David Lea-Smith	djl63@cam.ac.uk

Debbie Lindel	dlindell@tx.technion.ac.il
Dennis J. Nurnberg	d.nurnberg@imperial.ac.uk
Dirk Schneider	dirk.schneider@uni-mainz.de
Durall de la Fuente Claudia	claudia.durall@kemi.uu.se
Elisabetta Bergantino	elisabetta.bergantino@unipd.it
Elke Dittmann	editt@uni-potsdam.de
Emily Curren	200132223@u.nus.edu
Enrique Flores	eflores@ibvf.csic.es
Eric Pederson	eric.pederson@su.se
Eva Kiss	kiss@alga.cz
Fabiane Dorr	fabidorr@usp.br
Fernando Muzzopappa	fernando.muzzopappa@cea.fr
Filipe Branco dos Santos	f.brancodossantos@uva.nl
Friedrich Kirsch	friedrich.kirsch@uni-rostock.de
Gen Enomoto	genenomoto@bio.c.u-tokyo.ac.jp
Gerhard Sandmann	sandmann@bio.uni-frankfurt.de
Gregor Weiss	gregor.weiss@mol.biol.ethz.ch
Grzegorz Konert	konert@alga.cz
Guy Hanke	g.hanke@qmul.ac.uk
Hana Némová	hana.nemova@uvzsr.sk
Huokko Tuomas Taisto Tapani	ttthuo@utu.fi
Iris Maldener	iris.maldener@uni-tuebingen.de
Cristina Copaci	cri441@netscape.net
Ivana Cengic	icengic@kth.se
Jablonsky Jiri	jiri.jablonsky@gmail.com
James Golden	jwgolden@ucsd.edu
Janasch Markus	markus.janasch@scilifelab.se
Jenna-Rose Melanson	jmelanson2@mta.ca
Jesus A. Ochoa de Alda	ochoadealda@unex.es
José Ángel Moreno-Cabezuelo	bb2mocaj@uco.es
Jose Manuel Garcia Fernandez	jmgarcia@uco.es
Josef Komenda	komenda@alga.cz
Julia Weissenbach	Jweissenbach@ifam.uni-kiel.de

Julia Walter	julwal@utu.fi
Julian Eaton-Rye	julian.eaton-rye@otago.ac.nz
Karin Stensjö	karin.stensjo@kemi.uu.se
Karl Forchhammer	karl.forchhammer@uni-tuebingen.de
Karlsen Jan	jan.karlsen@scilifelab.se
Klahn Stephan	stephan.klaehn@biologie.uni-freiburg.de
Koebler Christin	christin.koebler@biologie.uni-freiburg.de
Lanzhen Wei	weilz@shnu.edu.cn
Lucas Stal	lucas.stal@nioz.nl
Lucia Chomová	lucia.chomova@uvzsr.sk
Luning Liu	luning.liu@liverpool.ac.uk
Mai Watanabe	maimai@bio.c.u-tokyo.ac.jp
Marc Novaczyk	marc.m.nowaczyk@rub.de
Maria Sinetova	maria.sinetova@mail.ru
Maria Iasmina Moza	iasmina_moza@yahoo.com
Marjan Faizi	marjan.faizi@hu-berlin.de
Marli Fiore Fatima	fiore@cena.usp.br
Martin Hagemann	martin.hagemann@uni-rostock.de
Matthias Futschik	matthias.futschik@plymouth.ac.uk
Mattia Niero	mattia.niero@unipd.it
Min Chen	min.chen@sydney.edu.au
Mónica Vásquez	mvasquez@bio.puc.cl
Muñoz-Marin Maria del Carmen	b32mumam@uco.es
Mustila Henna	hmsile@utu.fi
Natalia Battchikova	natbat@utu.fi
Pasi Paananen	pjpaan@utu.fi
Patricia Caicedo	s.p.caicedoburbano@uva.nl
Patricia Sanchez Baracaldo	p.sanchez-baracaldo@bristol.ac.uk
Patrikainen Pekka	pekka.patrikainen@utu.fi
Paula Tamagnini	pmtamagn@ibmc.up.pt
Paulo Oliveira	Paulo.Oliveira@ibmc.up.pt
Perez Rebeca	rebecaperez@gmail.com
Peter Lindblad	Peter.Lindblad@kemi.uu.se

Rakefet Schwarz	Rakefet.Schwarz@biu.ac.il
Riaz-Bradley Amber	a.riaz-bradley1@ncl.ac.uk
Ruiqian Zhou	ruiqian.zhou@qmul.ac.uk
Ryo Asada	ryo.spring@gmail.com
Sabrina Oeser	sabrina.oeser@biologie.uni-freiburg.de
Sara Pereira	sarap@ibmc.up.pt
Sarit Avrani	sarit.avrani@gmail.com
Scholl Jorg	joerg.scholl@uni-tuebingen.de
Schorsch Michael	m.scorsch@qmail.ac.uk
Selim Khaled	khaled.selim@uni-tuebingen.de
Shabestary Kiyan	kiyan@kth.se
Shanshan Song	shanshan.song2@uni-rostock.de
Sheila Roitman	roitmans@campus.technion.ac.il
Shukla Mahendra	shukla@alga.cz
Shuntaro Machida	s1430345@u.tsukuba.ac.jp
Silvia Cretoiu	cretoiums@gmail.com
Silvia Picossi-Goni	silvia.picossi@ibvf.csic.es
Sofia Doello Roman	sofia.doello-roman@uni-tuebingen.de
Sophia Zborowsky	sophiaz@campus.technion.ac.il
Stefania Viola	stefania.viola@ibpc.fr
Stella Thomaz Lima	stella@cena.usp.br
Steuer Ralf	ralf.steuer@hu-berlin.de
Sudip Samanta	sudip.k.samanta@ril.com
Susan Golden	susangolden@me.com
Szekeres Edina	edina.szekeres@icbcluj.ro
Taina Tyystarvi	taityy@utu.fi
Thomas Wallner	Thomas.Wallner@biologie.uni-freiburg.de
Tina Summerfield	tina.summerfield@otago.ac.nz
Tomas Zakar	tamas.zakar@gmail.com
Vass Istvan Zoltan	vass.istvan_zoltan@brc.mta.hu
Vinicius Augusto de Abreu	vini.abreu@gmail.com
Weimin Ma	wma@shnu.edu.cn
Wenyang Wu	W.Wu@uva.nl

Wim Vermaas	wim@asu.edu
Wittmiss Maria	maria.wittmiss@uni-rostock.de
Wolfgang Hess	wolfgang.hess@biologie.uni-freiburg.de
Xiaoming Tan	tanxm@qibebt.ac.cn
Xin Li	xin.li@kemi.uu.se
Yagut Allahverdiyeva	allahve@utu.fi
Yuxing Chen	cyxing@ustc.edu.cn
Yuzenkova Yulia	y.yuzenkova@ncl.ac.uk
Yvonne Zilliges	yvonne.zilliges@fu-berlin.de
Zenkin Nikolay	n.zenkin@ncl.ac.uk
Zhang Cheng-Cai	cczhang@ihb.ac.cn