EWBC 2024

September 3-6 | **Seville, Spain**

12th European Workshop on the **Biology of Cyanobacteria**

BOOK OF ABSTRACTS

CSIC
Consejo Superior de Investigaciones Científicas

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1542

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This meeting is selected by the Federation of European Microbiological Societies (FEMS) to offer grants for event participation for Early Career Scientists.

Federation of European Microbiological Societies

CONGRESS VISUAL IDENTITY:
Bárbara Luque Martínez

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LIST OF PREVIOUS EWMBC/EWBC MEETINGS

1st European Workshop on the Molecular Biology of Cyanobacteria,
Dourdan (France), May 14-16, 1990

2nd European Workshop on the Molecular Biology of Cyanobacteria,
Bristol (UK), April 4-7, 1992

3rd European Workshop on the Molecular Biology of Cyanobacteria,
Seville (Spain), May 11-14, 1995

4th European Workshop on the Molecular Biology of Cyanobacteria,
Berlin (Germany), September 15-17, 1999

5th European Workshop on the Molecular Biology of Cyanobacteria,
Stockholm (Sweden), June 9-12, 2002

6th European Workshop on the Molecular Biology of Cyanobacteria,
Gdansk (Poland), September 25-28, 2005

7th European Workshop on the Molecular Biology of Cyanobacteria,
České Budějovice (Czech Republic), August 31-September 4, 2008

8th European Workshop on the Molecular Biology of Cyanobacteria,
Naantali (Finland), August 28-September 1, 2011

9th European Workshop on the Molecular Biology of Cyanobacteria,
Island of Texel (The Netherlands), September 7-11, 2014

10th European Workshop on the Molecular Biology of Cyanobacteria,
Cluj-Napoca (Romania), August 20-24, 2017

11th European Workshop on the Biology of Cyanobacteria,
Porto (Portugal), September 7-9, 2020 (on-line)

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WELCOME LETTER

The series of meetings “European Workshop on the Molecular Biology of Cyanobacteria” was started in 1990 by a group of scientists led by the late Nicole Tandeau de Marsac “to provide a forum for the promotion of molecular biological techniques (applied) to these organisms”. Although based in Europe, the organizers explicitly wished to attract participation from further afield. The Workshop became well established, taking place from then onwards every two to four years. However, its focus changed from “the promotion of molecular biological techniques” to become a place for the presentation of results and exchange of ideas centered on the molecular biology of cyanobacteria. The Workshop, pushed enthusiastically by Georg Schmetterer, was organized in an informal way until an International Scientific Committee (ISC) was established in 2014. Because molecular ecology studies on cyanobacteria and the awareness of the interest of these organisms for biotechnological applications expanded along the years, the ISC decided in 2017 to change the name of the meeting to a more general “European Workshop on the Biology of Cyanobacteria” (EWBC). In this way, it is intended to make it clear that the Workshop accommodates every interesting aspect of research on cyanobacteria.

The Local Organizing Committee of the 12th EWBC, working in concert with the ISC, has put together a program covering the state of the art of cyanobacterial research. Additionally, we have arranged a workshop in which researchers will have plenty of opportunities to interact with each other. We hope that the cyanobacterial community of researchers will enjoy a fruitful scientific experience and a splendid time in Seville.

Enrique Flores, *on behalf of the Local Organizing Committee.*

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Annegret Wilde . . . . . *University of Freiburg, Germany*

PROGRAM AT A GLANCE

	Tuesday 3	Wednesday 4	Thursday 5	Friday 6
08:30		Welcome and opening lecture	Session 3.1: Physiology, Metabolism and Bioenergetics	Session 4: Ecology and Interactions with the Environment
09:00				
09:30		Session 1: Cell Biology and Development		
10:00				
10:30		Coffe Break	Coffe Break	Coffe Break
11:00		Session 1 (continued): Cell Biology and Development	Session 3.2: Physiology, Metabolism and Bioenergetics	Session 5: Biotechnology and Synthetic Biology
11:30				
12:00				
12:30				
13:00		Lunch (ISC meeting)	Lunch	Lunch
13:30				
14:00				CyanoCyc Tutorial
14:30			Posters & coffee	Posters & coffee
15:00		Session 2: Evolution, Phylogeny and Genomics		
15:30				
16:00				Session 5 (continued): Biotechnology and Synthetic Biology
16:30			Session 3.3: Physiology, Metabolism and Bioenergetics	
17:00	Registration			Closing Lecture and concluding remarks
17:15				
17:30		Posters & refreshments		
18:00				
18:30				
19:00				
19:30				
20:00			Visit to El Alcázar	
20:30	Get-together			Cocktail Dinner

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SCIENTIFIC PROGRAM

Wednesday September 4th

08:30-09:30 **Welcome and Opening Lecture**

Enrique Flores, Alicia M. Muro-Pastor, María F. Fillat, Paula Tamagnini

08:45-09:30 ***Wolfgang R. Hess***: Cyanobacterial Genomics: The blueprint for systematic analyses of a photosynthetic cell.

09:30-13:00 **Session 1: Cell Biology and Development**

Chairs:
Rocío López-Igual, María del Carmen Muñoz-Marín

09:30-10:00 ***Antonia Herrero***: Specific cell division features in *Anabaena*.

10:00-10:30 ***Iris Maldener***: Exploring established and emerging actors in cell-cell communication within *Nostoc*.

10:30-11:00 **Coffee break**

11:00-11:15 ***Amel Latifi***: Deciphering the roles of two critical factors in heterocyst differentiation and nitrogen fixation in the cyanobacterium *Nostoc* PCC 7120.

11:15-11:30 ***Cheng-Cai Zhang***: Heterocyst differentiation, a controlled process of cell division.

11:30-11:45 ***Marcel Dann***: A possible origin of CurT proteins in gram-positive bacterial cell division.

11:45-12:00 ***Conrad W. Mullineaux***: Mechanics and function of Type IV pili in *Synechocystis.*

12:00-12:15 ***Rakefet Schwarz***: Cell specialization and density dependent intercellular communication underly cyanobacterial biofilm development.

12:15-12:30 ***Yulia Yuzenkova***: Unique machinery of cyanobacterial gene expression: RNA polymerase structure, dynamics and circadian regulation.

12:30-12:45 ***Annegret Wilde***: Subcellular RNA localization in cyanobacteria.

12:45-13:00 ***Dirk Schneider***: *In vivo* and *in vitro punctae* formation of the *Synechocystis* IM30 protein is driven by liquid-liquid phase separation.

13:00-15:00 **Lunch (& Steering Committee meeting)**

15:00-17:15 **Session 2: Evolution, Phylogeny and Genomics**

Chairs:
Ignacio Luque, Patricia Sánchez-Baracaldo

15:00-15:30 ***David Lea-Smith***: What drives plant-like growth in filamentous cyanobacteria?

15:30-16:00 ***Ferran García-Pichel***: The mutualism of *Microcoleus vaginatus* with specific N_2-fixing heterotrophs in soils: based on urea exchange and orchestrated by GABA.

16:00-16:15 ***Beatriz Roncero-Ramos***: Hardening *Stenomitos frigidus* ULC029 by applying sequential stress factors.

16:15-16:30 ***Ellen Yeh***: From free-living cyanobacteria to a nitrogen-fixing endosymbiont in a photosynthetic eukaryote.

16:30-16:45 ***Victoria Calatrava***: Host domestication of the cyanobacterial high light-inducible gene family in the amoeba *Paulinella*.

16:45-17:00 ***Fabian Nies***: Natural competence in the cyanobacteria phylum.

17:00-17:15 ***Debbie Lindell***: Cyanophage host-like photosynthesis genes alter the metabolism of their marine *Synechococcus* host.

17:30-19:30: **Posters & Refreshments**

Thursday September 5th

08:30-10:30 **Session 3.1: Physiology, Metabolism and Bioenergetics**

Chairs:
Francisco J. Florencio, Niels-Ulrik Frigaard

08:30-09:00 ***Kirstin Gutekunst***: The delicate regulation of the central carbon metabolism in *Synechocystis* sp. PCC 6803.

09:00-09:15 ***Martin Hagemann***: Cyanobacterial primary carbon metabolism - Regulation and application.

09:15-09:30 ***Karl Forchhammer***: Metabolic control of the glycogen charge in *Synechocystis* PCC 6803.

09:30-09:45 ***Pablo Ortega Martínez***: Glycogen prevents metabolic imbalance and disruption of photosynthetic electron transport from Photosystem II during transition to photomixotrophy in *Synechocystis* sp. PCC 6803.

09:45-10:00 ***Khaled Selim***: Cyclic-di-AMP signaling in cyanobacteria: a new paradigm in controlling cellular homeostasis.

10:10-10:15 ***Arthur Guljamow***: Cyanobacterial toxins as modulators of carbon fixation.

10:15-10:30 ***María Santos-Merino***: Dramatic restructuring of carbon concentrating machinery accompanies energy imbalance and oxidative stress in cyanobacterial mutants of the circadian regulator RpaA.

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10:30-11:00 **Coffee break**

11:00-13:00 **Session 3.2: Physiology, Metabolism and Bioenergetics**

Chairs:
María F. Fillat, Agustín Vioque

11:00-11:30 ***Yukako Hihara***: Physiological and phylogenetic analyses on diversity in the transcription factor LexA and SOS responses in cyanobacteria.

11:30-11:45 ***Irene Olivan-Muro***: Towards a deeper understanding of biofilm formation and its link to stress response in diazotrophic cyanobacterium *Anabaena* sp. PCC 7120.

11:45-12:00 ***Anna Karlsson***: Studying protein regulation in cyanobacteria and chloroplasts through interaction proteomics.

12:00-12:15 ***Asunción Contreras***: Involvement of the ribosome-assembly GTPase EngA in the PipX interaction network and redox signalling.

12:15-12:30 ***Kintake Sonoike***: Phylogenetic profiling analysis of the phycobilisome revealed a gene encoding novel state-transition regulator in *Synechocystis* sp. PCC 6803.

12:30-12:45 ***Robert L. Burnap***: Towards a mechanism of CO_2 uptake by NDH-1 complexes in cyanobacteria.

12:45-13:00 ***Taina Tyystjärvi***: Inorganic carbon levels regulate growth via a novel SigC signalling cascade in cyanobacteria. Exploring the function of alternative D1 proteins in Photosystem II reaction centre complexes in *Synechocystis* sp. PCC 6803.

13:00-14:30 **Lunch**

14:30-16:30 **Posters & Coffee**

16:30-18:00 **Session 3.3: Physiology, Metabolism and Bioenergetics**

Chairs:
María José Huertas, Claudia Steglich

16:30-16:45 ***Frédéric Partensky***: Deciphering the fitness advantage conferred by chromatic acclimation in marine *Synechococcus*.

16:45-17:00 ***Sadanand Gupta***: High-resolution structures of Photosystem I assembly intermediates support the formation of PsaA-PsaB modules binding PsaC during their heterodimerization.

17:00-17:15 ***Tina C. Summerfield***: Exploring the function of alternative D1 proteins in Photosystem II reaction centre complexes in *Synechocystis* sp. PCC 6803.

17:15-17:30 ***Giorgio Perin***: Functional dependence between cell types is essential to maintain photosynthesis homeostasis in *Anabaena* sp. PCC 7120.

17:30-17:45 ***Laura T. Wey***: Extracellular electron transfer in *Synechocystis* sp. PCC 6803 under photomixotrophy.

17:45-18:00 ***Lisa R. Moore***: The CyanoCyc Cyanobacterial Web Portal.

Friday September 6th

8:30-10:30 **Session 4: Ecology and Interactions with the Environment**

Chairs:
Vicente Mariscal, Elke Dittmann

08:30-09:00 ***Pedro N. Leão***: Metagenomics reveals the biosynthetic potential of cyanobacteria-dominated environmental biofilms.

09:00-09:30 ***Laurence Garczarek***: Comparative and environmental genomics of α-cyanobacteria.

09:30-09:45 ***Henk Bolhuis***: Rhythm on the Beach: Cyanobacterial circadian clock controlled rhythmicity in a complex microbial mat community.

09:45-10:00 ***José Manuel García-Fernández***: Mixotrophy in marine picocyanobacteria: glucose uptake in *Prochlorococcus* and *Synechococcus*.

10:00-10:15 ***David A. Russo***: EXCRETE enables deep proteomics of the cyanobacterial extracellular environment

10:15-10:30 ***Rune Höper***: Modeling microbial communities using biochemical resource allocation analysis.

10:30-11:00 **Coffee break**

11:00-13:00 **Session 5: Biotechnology and Synthetic Biology**

Chairs:
Luis López-Maury, Joan García

11:00-11:30 ***Yagut Allahverdiyeva***: Biocatalytic chemical production using photosynthetic cyanobacteria.

11:30-11:45 ***Paula Tamagnini***: Engineering Syn-6803-based chassis for the expression of heterologous enzymes.

11:45-12:00 ***Julie A. Z. Zedler***: Nanofilaments for *de novo* organisation of heterologous enzymatic pathways in cyanobacteria.

12:00-12:15 ***Wei Du***: Efficient multiplex genome editing of the cyanobacterium *Synechocystis* sp. PCC6803 via CRISPR-Cas12a.

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12:15-12:30 ***Elisabetta Bergantino***: More blue than green: engineering *Synechocystis* sp. PCC6803 to produce bio-indigo.

12:30-12:45 ***Joan García:*** Continuous production of bioplastics from cyanobacteria microbiomes.

12:45-13:00 ***Minmin Pan***: Light regulation strategy on a phototrophic microbial community towards enhanced hydrogen and lipid production: meta-proteomics reveals the microbial interactions.

13:00-14:30 **Lunch**

14:00-15:30 **For those interested, partly overlapping with lunch and poster session: CyanoCyc tutorial by Lisa R. Moore.**

14:30-16:00 **Posters & Coffee**

16:00-17:00 **Session 5: Biotechnology and Synthetic Biology (continued)**

16:00-16:30 ***Jeffrey C. Cameron***: Revealing the hidden life (and death) of cyanobacteria using time-lapse microscopy

16:30-16:45 ***Raul Fernández***: Long-term evolution of a fast-growing cyanobacterium

16:45-17:00 ***Elena Carrasquer-Alvarez***: Cyanobacteria on the edge: How do they respond to very high CO_2?

17:00-17:45 **Closing Lecture**

17:00-17:45 ***Cheryl Kerfeld***: Structural insights into cyanobacterial light harvesting and photoprotection.

17:45-18:00 **Concluding remarks**

Enrique Flores, Alicia M. Muro-Pastor, María F. Fillat, Paula Tamagnini

1 OPENING LECTURE

Cyanobacterial Genomics: The blueprint for systematic analyses of a photosynthetic cell

Wolfgang R. Hess

University of Freiburg, Freiburg, Germany

Abstract text

In 1996, the complete sequence of the *Synechocystis* sp. PCC 6803 chromosome was published [1]. At that time, it was the third complete genome ever and the first genome sequence of a photosynthetic organism. Today, thousands of cyanobacterial genome sequences can be accessed, but in my presentation I will focus on the systematic approaches that have been enabled by the genome analysis of *Synechocystis*. Among these approaches are genome-wide expression analyses, at the RNA and protein level, but also studies focusing on particular features of the gene expression machinery, such as mapping all transcription start sites active under different growth conditions. More recent analyses include CRISPR interference screens over all open reading frames and non-coding RNAs to elucidate the condition-specific gene fitness [2], or fractionation experiments targeting the composition of ribonucleoprotein and multisubunit protein complexes. Recent ribosome profiling experiments have led to the identification of novel, relatively small proteins. Some of these previously unknown small proteins play key roles in fundamental physiological processes such as energy production, the assimilation of essential nutrients, the maintenance of a beneficial C/N balance, or in interactions with other species of the respective microbiome [3, 4]. I will present several of these examples and highlight some very recent findings revealing longer genes that have eluded all previous attempts at identification and characterization

References

[1] Kaneko T., et al. (1996) DNA Research doi: 10.1093/dnares/3.3.109.

[2] Miao R., et al. (2023) Plant Cell doi: 10.1093/plcell/koad208.

[3] Kraus A., et al. (2024) Nature Communications doi: 10.1038/s41467-024-46253-4.

[4] Song K., et al. (2022) Current Biology doi: 10.1016/j.cub.2021.10.051

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218 CLOSING LECTURE

Structural Insights into Cyanobacterial Light Harvesting and Photoprotection

Cheryl Kerfeld

MSU and LBNL, Berkeley, United States

Abstract text

We have determined several cryo-EM structures of the light harvesting antenna, the phycobilisome (PBS), from the model cyanobacteria *Synechocystis* PCC 6803. The resolution of our structure has been extended beyond that in our first report in 2022, with the rods resolved at 1.8 Å and the core at 2.1 Å. The structures reveal the presence of a new linker protein, ApcG that we have further characterized. The structures provide new insight into the inherent flexibility of the phycobilisome; three of the structures correspond to three different conformational states, two of these have not been described before. We are able to place these conformers into a physiological context that suggests how different conformations may affect the balance between light-harvesting and the photoprotective state on different times scales. We also determined the structure of a complex between the phycobilisome and the Orange Carotenoid Protein (OCP). This is the PBS in its fully photoprotected state, providing a complete high-resolution structural description showing how four 34kDa OCPs, each with a single carotenoid, are able to quench the 6.2 MDa PBS with its 396 bilin pigments. Structural details and their photochemical, photophysical and physiological implications will be described, with a comparison to the structure of the pentacylindrical PBS.

References

[1] Dominguez-Martin, M-A., Sauer, et al. Structures of a Phycobilisome in the Light-harvesting and Photoprotected States. Nature 609: 835-845, 2022.

[2] Espinoza-Corral, R.D., et al., The phycobilisome linker protein ApcG interacts with photosystem II and regulates energy transfer to photosystem I in Synechocystis sp. PCC 6803. Plant Physiology kiad615, 2023.

[3] Sauer, P.V., Cupellini, L., Sutter, M., et al.. High-resolution cryo-EM structure of a quenched phycobilisome. Science Advances, in press.

Funding

US Department of Energy, Basic Energy Sciences

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Session 1:
Cell Biology and Development

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Specific cell division features in *Anabaena*

Antonia Herrero

CSIC, Seville, Spain

Abstract text

Cyanobacterial strains of the genus *Anabaena* grow as filaments of communicated cells representing a paradigm of pluricellular bacteria. Accordingly, cell division in *Anabaena* produce adjoined connected cells in contrast to the separate daughters resulting in unicellular bacteria. In *Anabaena*, the initial division ring shows distinctive features including a specific domain in FtsZ [1] and essential FtsZ tethers to the cytoplasmic membrane: the cyanobacterial-specific protein ZipN and the Gram-positive protein SepF, the latter also regulating the extent of FtsZ polymerization [2]. In *Anabaena*, the divisome plays an essential role in the septal localization of protein complexes, termed *septal junctions*, that mediate intercellular molecular exchanges through the filament. Unicellular rod-shaped bacterial models feature two main modes of peptidoglycan growth: The divisome-catalyzed divisional growth, which synthesizes the new poles of the daughter cells during cell division, and the elongasome-catalyzed peripheral growth, which elongates the cells during cell growth. In contrast, *Anabaena* presents divisional, peripheral and septal growth, the latter active throughout the cell cycle and likely involved in maintenance of intercellular communication structures [3]. Moreover, in *Anabaena* the divisome and the elongasome interact and are interdependent [3]. Regarding heterocyst differentiation, the irreversibility of the process is related to loss of cell division capacity involving down-regulation of genes encoding essential cell division proteins such as *ftsZ*, *zipN* or *sepF* [2], and induction of specific factors such as PatA or PatD, which interact with ZipN or ZipN and FtsZ, respectively, representing cell-division inhibitors. Thus, multiple mechanisms cooperate to inhibit cell division setting commitment to differentiation.

References

[1] Corrales-Guerrero et al. Frontiers Microbiol. doi.org/10.3389/fmicb.2018.02260

[2] Valladares et al. Microbiological Res. doi.org/10.1016/j.micres.2023.127489

[3] Velázquez-Suárez et al. mBio. doi.org/10.1128/mbio.01165-22.

Funding

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3 INVITED TALK

Exploring established and emerging actors in cell-cell communication within *Nostoc*

Iris Maldener[1], Ann-Katrin Kieninger[1], Ana Janovic[1], Karl Forchhammer[1], Piotr Tokarz[2], Li Yanxun[2], Martin Pilhofer[2], Gregor L. Weiss[2]

[1.] *Interfaculty Institute of Microbiology and Infection Medicine Uni Tübingen, Tübingen, Germany*

[2.] *Department of Biology, Institute of Molecular Biology & Biophysics, Eidgenössische Technische Hochschule Zürich, Zürich, Switzerland*

Abstract text

Filamentous cyanobacteria of *Nostoc* and *Anabaena* species exhibit a sophisticated communication system that enables these multicellular prokaryotes to adapt to diverse environments through the differentiation of highly specialized cell types. In 2008, the Mullineaux and Flores groups published groundbreaking research on cell-cell communication within the filaments, utilizing fluorescent dyes and FRAP (Fluorescence Recovery After Photobleaching) for observation and measurement of intercellular molecule exchange.

A significant milestone was achieved in 2013 with the discovery of the nanopore array, comprising dozens of regularly arranged pores in the septal cell wall between neighboring cells. These pores, drilled by specific amidases, are indispensable for communication and heterocyst formation [1]. Since these discoveries, an array of mutants exhibiting defects in heterocyst formation and diazotrophic growth have undergone thorough investigation regarding their intercellular communication capabilities and nanopore formation.

A breakthrough was the elucidation of the architecture of cell-cell connections, known as septal junctions, which we achieved through cryo-electron tomography. This revealed a tripartite composition of the septal junction complex, with the FraD and SepN proteins identified as essential components [2, 3].

In this presentation, we will provide an overview of both established and newly identified factors involved in cell-cell communication, and their potential functions. Furthermore, we will delve into the intricate architecture of septal junction complexes, offering unprecedented resolution, and propose a model illustrating the gating mechanism in response to stress.

References

[1] Lehner et al. The FASEB Journal. doi.org/10.1096/fj.12-225854

[2] Weiss et al. Cell. doi.org/10.1016/j.cell.2019.05.055

[3] Kieninger et al. Nature Communications. doi.org/10.1038/s41467-022-34946-7

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4 ORAL

Deciphering the roles of two critical factors in heterocyst differentiation and nitrogen fixation in the cyanobacterium *Nostoc* PCC 7120

Raphael Rachedi[1], Véronique Risoul[1], Anais Scholivet[1], Emmanuel Talla[1], Baddredine Douzi[2], Jean-Michel Jault[3], Maryline Foglino[1], **Amel Latifi**[1]

[1.] *CNRS, Marseille, France*

[2.] *INRAE, Nancy, France*

[3.] *CNRS, Lyon, France*

Abstract text

Our research focuses on cell differentiation in *Nostoc* PCC 7120, a filamentous, multicellular cyanobacterium. This process is triggered by a lack of combined nitrogen, transforming about 10% of cells into heterocysts, specialized cells for fixing atmospheric nitrogen. Since heterocyst formation is a terminal differentiation, the process is intricately regulated, following a semi-regular pattern within the filament and depending on many regulatory genes. These genes, primarily identified through genetic studies, have only begun to be understood at the molecular level, with most insights limited to early developmental stages. Our objective is to unravel the roles of key factors in the later stages of differentiation. In this context, we have provided evidence that the protein PatB is the direct transcriptional regulator of the operon for nitrogenase, crucial for nitrogen fixation [1]. Another focus is HetC, a membrane exporter crucial for heterocyst development in our strain. We have analyzed its topology and demonstrated that its peptidase and ATPase functions are necessary for heterocyst formation. Interestingly, in vitro studies revealed that HetC binds to ppGpp within a domain critical for its in vivo activity [2]. Additionally, our examination of heterocyst-related gene conservation across the cyanobacterial phylum prompted an investigation into the potential functional relationship between HetC and PatB [3]. This investigation not only expands our comprehension of the differentiation process but also underscores the complex regulatory networks overseeing patterned developmental behavior, paving the way for future studies aimed at uncovering how the heterocyst achieves its terminal state.

References

[1] Rachedi, R et al., (2023) Molecular Microbiology. doi: 10.1111/mmi.15044

[2] Rachedi, R et al., (2024) Microbiology Spectrum. doi: 10.1128/spectrum

[3] Xu, X et al., (2022) Molecular Genetics Genomics doi: 10.1007/s00438-022-01902-5

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“Agence Nationale pour la Recherche Scientifique” (ANR-21-CE20-0025-01)

5 ORAL

Heterocyst differentiation, a controled process of cell division

Cheng-Cai Zhang

Institute of Hydrobiology, CAS, Wuhan, China

Abstract text

The cyanobacterium *Anabaena* PCC 7120 is able to form heterocysts for nitrogen fixation. Heterocysts are terminally diferentiated cells, unable to divide again after the comittment step. We found that the active process of cell division is required for heterocyst development at the committment step. The protease HetF and its specific substrate PatU3 are involved in coordination of cell division and heterocyst formation. Interestingly, our data also indicate that cell division proteins, such as FtsZ also play a role in the control of heterocyst formation. We'll present our results on the interplay of cell division and differentiation. Based on these data, we propose that the committment step of heterocyst development, during which the differentiation process becomes irrevesible, mainly relies on the control of and the interaction with cell division.

References

(1) Xing W-Y. et al. (2022) A proteolytic pathway coordinates cell division and heterocyst differentiation in the cyanobacterium Anabaena sp. PCC 7120. PNAS 119:e2207963119.

(2) Liu J. et al. (2023) Three-dimensional coordination of cell-division site positioning in a filamentous cyanobacterium. PNAS Nexus 2 :pgac307.

(3) Zeng X. et al. (2023) A c-di-GMP binding effector controls cell size in a cyanobacterium. PNAS, 120 :e2221874120.

Funding

The work has been founded by the National Natural Science Foundation and the Chinese Academy of Sciences

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6 ORAL

A Possible Origin of CurT Proteins in Gram-Positive Bacterial Cell Division

Marcel Dann

Technical University of Darmstadt, Darmstadt, Germany

Abstract text

Thylakoid membranes have undergone severe morphological changes during cyanobacterial evolution and the transition into endosymbiotic plastids. Today, members of the CurT/CURT1-like transmembrane protein family constitute key determinants of thylakoid architecture in both *Synechocystis* sp. PCC 6803 and *Arabidopsis thaliana* chloroplasts [1, 2]. The evolutionary origin of this protein family, however, remains highly elusive [3], with the appearance of "fully formed" CurT-like proteins roughly coinciding with the evolution of thylakoids themselves.

Our microscopic analyses have revealed a number of hitherto undescribed defects is cell division and thylakoid partitioning in *curT*-depleted mutants of *Synechocystis* sp. PCC6803, which may provide the first relevant cue towards the origin of these fascinating proteins. CurT-depleted *Synechocystis* sp. PCC 6803 cells show impaired Z-ring formation, while a filamentous phenotype can be observed in *Synechococcus* sp. PCC 7942 *curT* mutants, both of which suggests a - possibly original - involvement of CurT in cell division. Through phylogenetic analyses we identified a candidate for a distant sister clade to cyanobacterial/plastid CurT/CURT1 within the inherently thylakoid-less *Firmicutes* phylum. As *Firmicutes* and cyanobacteria are closely related phyla with overlapping cell division features, we consider this possible evidence for CurT/CURT1-like proteins having been originally obtained through horizontal gene transfer and stemming from a non-photosynthetic context altogether. Initially aiming at unravelling a tentative secondary function of CurT/CURT1 in cyanobacteria, we may have uncovered an evolutionary origin of these prominent photosynthesis-associated proteins within Gram-positive-like cell division.

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7 ORAL

Mechanics and function of Type IV pili in *Synechocystis*

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Abstract text

Type IV pili are versatile and dynamic protein fibre appendages found in many bacteria. In cyanobacteria, they are crucial for motility, phototaxis, surface adhesion, cell aggregation and transformability. Here we visualise Type IV pili in action in *Synechocystis* sp PCC 6803 using a site-directed mutation that introduces an additional cysteine residue into the major pilin PilA1. This enables fluorescent labelling of the pili without any major loss of function. We use the method to provide new insights into the mechanism of Type IV pilus action and the specific functions of Type IV pili in *Synechocystis*. PilX proteins are minor pilins that are putative components of a pilus tip complex. Mutants lacking any of the three *Synechocystis pilX* homologs produce abnormally long pili that appear unattached to the agar surface. This indicates that PilX homologs are crucial both for pilus tip attachment to substrates, and for triggering the switch from pilus extension to pilus retraction. It implies that pilus retraction in *Synechocystis* is not purely stochastic, but is triggered by pilus tip attachment to a substrate. Thus, pili follow a deterministic cycle of extension, substrate attachment and retraction. Type IV pili are crucial for flocculation in *Synechocystis.* We use PilA1 labelling to visualise the role of Type IV pili in building cell assemblages. We show that flocculation of *Synechocystis* is promoted by exposure to the opportunistic pathogen *Pseudomonas aeruginosa*, and that flocculation acts as a defence mechanism against bacterial predation.

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8 ORAL

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Cell Specialization and Density Dependent Intercellular Communication Underly Cyanobacterial Biofilm Development

Rakefet Schwarz, Eli Zecharia, Shiran Suban, Alona Frenkel, Yevgeni Yegorov, Eleonora Sendersky

Bar-Ilan University, Ramat-Gan, Israel

Abstract text

Cyanobacterial biofilms are prevalent in the environment and are of biotechnological importance; yet the mechanisms underlying formation of these assemblages were overlooked for many years. We revealed a biofilm self-suppression mechanism that operates in *Synechococcus elongatus* PCC7942, which requires the type IV pilus (T4P) assembly apparatus. This complex is involved in deposition of a biofilm inhibitor to the extracellular milieu, which in turn suppresses expression of the EbfG-operon that comprises genes enabling biofilm formation. The RNA-chaperone Hfq and a protein annotated 'hypothetical', which we denote EbsA (essential for biofilm self-suppression A), form a tripartite complex with the PilB ATPase of the T4P complex. This tripartite complex is required for pilus-assembly, DNA competence and for biofilm self-suppression [1]. We recently demonstrated that SigF1 sigma factor of RNA polymerase controls the EbfG-operon through intra- and intercellular pathways [2]. Additionally, analysis of EbfG-operon expression in individual cells revealed that high expression is limited to a subpopulation in the culture. Further analysis of EbfG4 indicated cell surface as well as matrix localization and EbfG1-3 were shown to form amyloids [3]. These data suggest a beneficial 'division of labor' during biofilm formation where only some of the cells allocate resources to produce matrix proteins – 'public goods' that support robust biofilm development by the majority of the cells. Moreover, gradual accumulation of the biofilm inhibitor in aging wild-type cultures imply a quorum sensing mechanism in *S. elongatus* biofilm regulation. Together, these studies provide deep insight into cyanobacterial communal behavior.

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9 ORAL

Unique Machinery of Cyanobacterial Gene Expression: RNA Polymerase Structure, Dynamics and Circadian Regulation

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Abstract text

Cyanobacteria and chloroplasts possess distinctive machinery for transcription, the first step of gene expression. Two key features - the absence of proofreading factors correcting mistakes of transcription, and a huge insertion domain (SI3) in the largest subunit of RNA polymerase, the main enzyme of transcription, set this machinery apart from that of other organisms. Additionally, the circadian clock imposes an extra layer of regulation on transcription in cyanobacteria.

Based on the first structures of *Synechococcus elongatus* transcription complexes, biochemical, NGS and genetics data we discovered that SI3 domain “gates” promoter complex formation by making an arch-like contact with an initiation factor. The arch breaks when RNA polymerase escapes from a promoter, and SI3 becomes highly mobile, swinging with addition of each nucleotide to the RNA. SI3 completely blocks binding of any potential proofreading factors to cyanobacterial RNA polymerase. To compensate, cyanobacteria possess very efficient intrinsic, factor-independent mechanism of transcription proofreading. I will further discuss our ongoing research into the molecular mechanisms behind circadian rhythms of activation and repression of transcription orchestrated by the master circadian regulator RpaA. Our *in vitro* work is supported by exploration of transcription localization in the cell, and its coordination with other major processes, e.g. replication.

Our research provides a framework for understanding the role of the unique features of cyanobacteria and chloroplast RNA polymerases and further insights into the molecular mechanism of transcription in specific environment of photosynthetic organisms and organelles.

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10 ORAL

Subcellular RNA localization in cyanobacteria

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Abstract text

Textbooks traditionally depict transcription and translation in bacterial cells as tightly linked and nearly simultaneous processes. However, recent studies challenge this notion, suggesting that coupling may not be as strict. Emerging evidence indicates that transcription and translation can occur with temporal or spatial separation. RNA molecules in bacteria are found in various parts of the cell and often accumulate where the corresponding protein is required. This phenomenon is particularly common in mRNAs that encode membrane proteins. However, the importance of mRNA enrichment at membranes and its impact on protein insertion remains largely unexplored. Recent collaborative work in our laboratories has revealed that translation-independent targeting of photosynthetic mRNAs to the membrane is an overlooked mechanism [1]. Membrane-specific localization of the respective mRNAs could explain the correct targeting of proteins to either the inner or the thylakoid membrane. Furthermore, we showed that RNA-binding proteins are involved in translation-independent localization of mRNAs to membranes. Global analysis of the subcellular RNA distribution in *Synechocystis* sp. PCC 6803 revealed a non-uniform transcriptome distribution. 20% of the entire transcriptome was selectively enriched in one of the fractions, mostly in the membrane fraction (predominantly transcripts encoding membrane and ribosomal proteins). Treatment with the translation inhibitor puromycin revealed a major contribution of translation to the membrane localization of transcripts, yet some transcripts remained enriched post-treatment, indicating translation-independent targeting. We will discuss different mechanisms that control subcellular RNA localization and the implications of mRNA enrichment for correct protein targeting the localized assembly of membrane protein complexes.

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11 ORAL

In vivo and *in vitro punctae* formation of the *Synechocystis* IM30 protein is driven by liquid-liquid phase separation

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Abstract text

Cyanobacteria and chloroplasts require the inner membrane-associated protein of 30 kDa (IM30), also known as Vipp1, for the biogenesis and maintenance of thylakoid membranes. IM30 has been shown to form large ring structures in solution[1]. Yet, the oligomeric structures of IM30 exhibits a remarkable plasticity, which has also been observed with eukaryotic ESCRT-III proteins, the structural and functional homologs of IM30[2]. In addition to ring structures, we have observed the formation of rod structures and membrane-covering carpets[2]. Upon binding to membranes, IM30 rings disassemble into smaller oligomers[3]. Ring disassembly involves the partial unfolding of the monomers, and about half of the protein is intrinsically disordered when not being part of the homo-oligomeric rings[3]. Driven by liquid-liquid phase separation, partially unfolded IM30 forms condensates in solution under physiologically relevant conditions. While the *in vivo* formation of IM30 *punctae* under stress conditions is well established, the exact nature of these structures as well as the forces guiding *punctae* formation are still unknown. We show that *in vivo* IM30 assemblies have features typical for biomolecular condensates, and *both in vitro* and *in vivo* formation of such condensates requires a structured core. This suggests that the IM30 *punctae* observed *in vivo* under membrane stress conditions are in fact biomolecular condensates. Our new findings of IM30s capability to form condensates *in vivo* and *in vitro* under physiologically relevant conditions expand the landscape of IM30 assembly states and advance our understanding of how IM30 might be involved in thylakoid membrane biogenesis, maintenance and repair.

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12 POSTER

Microfluidic device to follow the developmental program of a multicellular and differentiated cyanobacteria

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Zuli Yeerkenjiang, Mahdi Mansouri, Léon Espinosa, Nicolas Waisbord, Amel Latifi, **Matthieu Bergé**

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Abstract text

Many cyanobacterial strains, including the model strain Nostoc PCC7120, exhibit a multicellular lifestyle, growing as one-dimensional filament containing hundreds of communicating vegetative cells. In this model, in the absence of combined nitrogen, one vegetative cell every ten cells in the filament will differentiate into heterocyst, creating a micro-oxic compartment dedicated to the functioning of oxygen-sensitive enzymes such as nitrogenase. Therefore, Nostoc PCC7120 is the simplest model to understand critical signaling processes related to multicellularity and morphological development. Quantitative single-cell time-lapse microscopy combined with microfluidic devices offers a powerful method for analyzing gene circuit dynamics and heterogeneous cell behavior. However, cyanobacteria present several challenges that have made them difficult to culture in a microfluidic setting. We developed a microfluidic device that allow for the growth *Nostoc PCC7120* under a microscope over several days, enabling real-time observation of the entire differentiation process. Successful identification and tracking of each cell were achieved through segmentation by machine learning for automated microscopy analysis to detect both cell type, specifically vegetative cells and heterocysts. This platform has several advantages over agarose pads and demonstrates great potential for obtaining high quality, single-cell gene expression data in multicellular cyanobacteria in precisely controlled, dynamic environments over long time periods.

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13 POSTER

RNA-binding proteins are involved in the differentiation of heterocysts

Manuel Brenes Álvarez[1], Halie Rae Ropp[1], Dimitrios Papagiannidis[2], Clement Potel[2], Frank Stein[2], Mikhail Savitski[2], Agustín Vioque[3], Alicia M. Muro-Pastor[3], Wolfgang R. Hess[1]

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Abstract text

RNA-binding proteins (RBPs) are central components of gene regulatory networks. The differentiation of heterocysts in filamentous cyanobacteria is one of the few examples of cell differentiation in prokaryotes. Although multiple non-coding transcripts are involved in this process, not a single possibly involved RBP has been identified thus far. We have analyzed the differential fractionation of RNA-protein complexes after RNase treatment in density gradients (R-DeeP/GradR[1]) yielding 333 RNA-dependent proteins in *Nostoc* sp. PCC 7120. In parallel, a bioinformatic prediction of RBPs was performed. We validated *in vivo* the RNA-binding capacity of 6 RBP candidates. Some of these proteins participate in essential physiological aspects, such as photosynthesis (Alr2890), thylakoid biogenesis (Vipp1) or heterocyst differentiation (PrpA, PatU3), but their association with RNA was unknown. The validated RBPs Asl3888 and Alr1700 were not characterized before. Asl3888 co-fractionates with the 30S ribosomal subunit whereas Alr1700 is an RBP with two OB-fold domains that is differentially expressed in heterocysts. Deletion of *alr1700* led to the complete deregulation of the cell differentiation process, with a striking increase in the number of heterocyst-like cells, and was ultimately lethal in the absence of combined nitrogen. The results of this approach provide a comprehensive resource for the functional assignment of RBPs in a photosynthetic bacterium with two cooperating cell types.

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14 POSTER

Second messenger c-di-GMP-mediated cell size control of *Anabaena* PCC 7120

Xiaoli Zeng

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Abstract text

Cell size control is an essential physical trait of bacteria, and how cell size is maintained and regulated remain as important questions in microbiology. c-di-GMP plays key roles in regulating various physiological processes such as biofilm formation, motility, virulence, cell differentiation, etc [1]. *Anabaena* sp. strain PCC 7120 (*Anabaena*) is a filamentous cyanobacterial which houses 16 genes encoding proteins with GGDEF motif (for c-di-GMP synthesis), or EAL/HD-GYP motif (for c-di-GMP hydrolysis), or both [2]. The presence of such a large number of genes related to c-di-GMP signaling suggests that c-di-GMP must play important functions in *Anabaena*. We recently found that the second messenger c-di-GMP is may be an intracellular proxy for cell size control in *Anabaena*. We identified and characterized a highly conserved c-di-GMP receptor in cyanobacteria, and demonstrated that it regulates cell size via interaction with the transcription factor DevH in *Anabaena* [3]. In this study, we proved that c-di-GMP is essential in *Anabaena.* We found a minimum threshold for intracellular c-di-GMP levels that maintain standard cell size and the lowest c-di-GMP level to maintain cell survival. Furthermore, we identified the c-di-GMP levels required to control cell size are mainly maintained by 4 c-di-GMP metabolic enzymes.These findings highlight the importance of proper c-di-GMP level during cell size control in *Anabaena*.

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15 POSTER

Cyanobacterial sigma factor controls biofilm-promoting genes through intra- and intercellular pathways

Eleonora Sendersky, Shiran Suban, Sapir Yemini, Anna Shor, Hiba Waldman Ben-Asher, Orly Yaron, Sarit Lampert, Rakefet Schwarz

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Abstract text

Cyanobacteria frequently constitute integral components of microbial communities known as phototrophic biofilms, which are widespread in various environments and hold significant industrial relevance. Previous studies of the model cyanobacterium *Synechococcus elongatus* PCC 7942 revealed that its planktonic growth habit results from a biofilm-suppression mechanism that depends on an extracellular inhibitor, an observation that opens the door to investigating cyanobacterial intercellular communication. Here, we demonstrate that the RNA polymerase sigma factor SigF1, is required for this biofilm-suppression mechanism and suggest that *sigF1*-inactivation impairs secretion of the biofilm inhibitor. The *S. elongatus* paralog SigF2, however, is not involved in biofilm regulation. Comprehensive transcriptome analyses identified distinct regulons under the control of each of these sigma factors. Additional data indicate that SigF1 regulates biofilm through its involvement in transcriptional induction of genes that include those for the primary pilus subunit: *sigF1* inactivation both prevents pilus assembly and abrogates secretion of the biofilm inhibitor. Consequently, expression is significantly upregulated for the *ebfG*-operon that encodes matrix components and the genes that encode the corresponding secretion system. Thus, this study uncovers a basic regulatory component of cyanobacterial intercellular communication, a field that is in its infancy. Elevated expression of biofilm-promoting genes in a *sigF1* mutant supports an additional layer of regulation by SigF1 that operates via an intracellular mechanism.

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16 POSTER

Regulation of cell division during heterocyst differentiation in *Anabaena* sp. PCC 7120

Ana Valladares Ruiz, Antonia Herrero

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Abstract text

Anabaena sp. PCC 7120 forms filaments in which the cells exchange metabolites and regulatory molecules, thus representing a pluricellular bacterium. Under nitrogen scarcity, the filament can include two cell types: vegetative cells and heterocysts, the latter specialized in the fixation of atmospheric nitrogen. This multicellular organization not only entails specific structural characteristics, but also distinctive features in the process of cell division. *Anabaena* FtsZ presents a specific N-terminal peptide that is essential for cell division and viability and represents a distinct feature of filamentous cyanobacteria capable of heterocyst differentiation. In *Anabaena*, the cyanobacterial-specific protein ZipN represents an essential tether of FtsZ to the cytoplasmic membrane and a main divisome organizer, and SepF, which is also essential, influences the polymerization of FtsZ in a concentration-dependent manner [1]. During heterocyst differentiation the capacity for cell division is lost, establishing commitment to differentiation. Mechanisms involved in inhibition of cell division during differentiation include the early dismantlement of the Z-ring and the late down-regulation of *ftsZ* gene expression in the differentiating cells [2]. A number of proteins have been found to be upregulated in differentiating cells and involved in inhibition of Z-ring polymerization. These include PatA, which is required for the differentiation of intercalary heterocysts, and interacts with ZipN and SepF [2]; and PatD, which interacts with ZipN and FtsZ and interferes with its in vitro polymerization [3]. Additionally, SepF, which as mentioned above is essential for FtsZ polymerization, is downregulated in differentiating heterocysts, thus contributing to their disability for cell division [1].

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17 POSTER

Light intensity regulates the cell shape of *Anabaena* sp. PCC 7120

Cristina Velázquez-Suárez[1], Manuel J. Mallén-Ponce[1], José L. Crespo[1], Dennis J. Nürnberg[2], Antonia Herrero[1], Ignacio Luque[1]

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Abstract text

Bacterial cell morphology is a genetically determined trait that is transmited from one generation to another. However, morphology can be influenced by environmental conditions that may impinge on the size and/or the shape of cells. The structure that confers its particular shape to bacterial cells is the peptidoglycan cell wall. In this work we have investigated the influence of light on the morphology of *Anabaena* sp. PCC 7120, a filamentous cyanobacteria with bacillary cell morphology. We have observed that light intensity provokes changes in cell morphology, as cells become rounder and bigger as light intensity increases. We have analyzed the role of the elongasome, the complex responsible of lateral peptidoglycan synthesis, on the morphological changes observed. Mutants of the elongasome scafolding proteins MreB, C and D show altered morphology in standard culture conditions but under high light (HL) conditions display a morphology similar to that of the wild type, suggesting that the elongasome is not primarily responsible for the HL morphology. Surprisingly, the *mre* mutants showed growth retardation or lethality under HL, despite displaying normal pigment composition and apparently intact acclimation mechanisms to HL. We have investigated the basis for this phenotype and we have observed that *mre* mutant cells show anomalous septation, with division septa not necessarily at midcell defining in some cases compartments that are devoid of DNA. We propose that this anomalous septation may be the basis for the phenotype of *mre* mutants.

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18 POSTER

The role of the MCE (Mammalian Cell Entry) protein in biogenesis of the thylakoid membrane of *Synechococcus elongatus* PCC7942

Xiaowei Dong, Vidya Darbari, Conrad Mullineaux

Queen Mary University of London, London, United Kingdom

Abstract text

Cyanobacteria have a specialised internal membrane called thylakoids to host the photosynthetic machinery. Photosynthesis in thylakoid membranes is affected by the type and integrity of lipids on the membrane. MCE proteins are conserved lipid-binding proteins in double-membrane bacteria and eukaryotic chloroplasts. In *E. coli*, an MCE transport system called Mla has been implicated in phospholipid trafficking and outer membrane integrity. A similar complex TGD has been found in the *Arabidopsis thaliana* chloroplast, which was suggested to transport phosphatidic acid from the endoplasmic reticulum to the chloroplasts to synthesise galactolipids. MCE domain-containing proteins are also found in cyanobacteria, but their locations and functions are unknown.

In *Synechococcus elongatus* PCC 7942, a single protein Syf0351 has a conserved MCE domain. An MCE knockout mutant was generated. The confocal microscopy showed significantly reduced chlorophyll fluorescence compared to the wild type. A loss of the cellular content of pigments was observed in the whole cell absorption spectra. These phenotypes indicate that the MCE knockout might have perturbed the thylakoid organisation. An MCE GFP tagging overexpression mutant was generated to determine the localisation of the MCE protein in *Synechococcus*. Interestingly, the MCE protein was found in both the thylakoid membrane and plasma membrane by membrane fractionation and western blots. A GFP pull-down experiment will be carried out for TM and PM, and proteomic analysis will be used to identify interacting proteins. Lipidomic analysis of the TM and PM will be carried out in the MCE mutant to explore if it engages in lipid transport.

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19 POSTER

Resolving heterocyst differentiation in a multicellular cyanobacterium using scRNAseq

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Abstract text

Nostoc sp. PCC7120, a filamentous model cyanobacterium and diazotroph, differentiates nitrogen-fixing heterocysts in a complex developmental program.

These heterocysts fix nitrogen and exchange the products for combined carbon products with vegetative cells in the filament [1]. This division of labor and intercalary spatial arrangement creates a unique microenvironment for each cell along the filament. Even though the differentiation process has been extensively studied and numerous participating genes have been identified, the induction of differentiation still remains enigmatic [2]. We aim to resolve the differentiation with single-cell transcriptomics and elucidate the early signals underlying this differentiation as well as the characterization of pre-induction transcriptional heterogeneity among vegetative cells. First promising results validate our approach for fragmenting filaments and sorting of single cells by FACS of both the wildtype and an established reporter strain. Arresting transcriptional activity with commercially available RNAlater solution will enable us to resolve the heterocyst developmental program at specific points of time with single-cell resolution.

This approach is complemented by the characterization of select genes of interest previously identified as putative RNA-binding proteins involved in heterocyst differentiation. Transcriptional as well as translational GFP fusions will be analyzed by confocal microscopy and provide insight into promoter activity, protein expression and localization. Furthermore, CLIPseq will be used to assess RNA-binding capabilities.

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20 POSTER

Long-term live cell microscopy for functional studies in cyanobacteria

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Raquel Gutierrez-Lanza[1], Víctor Campa[1], Alfonso Mendaña[1], Marina Domínguez-Quintero[1], Carlos Díaz-Ceballos[1], María Santos-Merino[2], Alicia M. Muro-Pastor[3], Raúl Fernandez-Lopez[1]

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Abstract text

Fluorescence reporters and live cell imaging convey key information on the dynamics of gene expression and protein function in live cells. The slow growth rates of cyanobacterial species, combined with their particular illumination requirements pose specific challenges for the implementation of these techniques. Here we develop standardized protocols that allow the continuous tracking of cyanobacterial microcolonies for >96 h under the microscope. Power meter sensor heads are used to calibrate microscope illumination to specific light intensities, enabling the healthy growth of cells for several days. We track heterocyst formation in *Anabaena* and circadian gene expression in *Synechococcus elongatus* to showcase the versatility of these approaches in deciphering the molecular dynamics of cyanobacteria.

21 POSTER

Control of positive phototaxis by the Tax1 system in *Synechocystis* sp. PCC 6803

Yu Han[1], **Jonas Hammerl**[1,2], Felicitas E. Flemming[1], Nils Schuergers[1], Annegret Wilde[1]

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Abstract text

To seek out optimal photosynthetic conditions cyanobacteria employ type 4 pili (T4P) navigating towards or away from directional light sources in a process termed positive or negative phototaxis, respectively. In *Synechocystis* sp. PCC 6803 the chemotaxis-like Tax1 system mediates a switch in cell polarity that enables positive phototaxis. The system comprises the blue/green photoreceptor PixJ upstream of the histidine kinase PixL and two CheY-like response regulators, PixG and PixH. However, it remains elusive how the Tax1 system regulates polar T4P activation and thereby movement direction on a molecular level. Here, we investigated the phosphotransfer between PixL and its cognate response regulators *in vitro* and examined the localization and function of wild-type and phosphorylation deficient PixG and PixH during phototactic behavior. We found that the C-terminal receiver domain of PixL modulates PixL kinase activity and is necessary for positive phototaxis. Moreover, both response regulators PixG and PixH can be phosphorylated by PixL but exert antagonistic roles during phototaxis. Phosphorylated PixG can interact with the T4P motor ATPase PilB1 and localizes to the leading cell pole under directional light, thus promoting positive phototaxis. On the other hand, PixH regulates PixG phosphorylation and may act as a phosphate sink to inhibit positive phototaxis. Our findings shed light onto the molecular processes underlying positive phototaxis in *Synechocystis* and provide insights into the different roles of PatA-type and CheY-like response regulators in cyanobacterial chemotaxis-like systems. More generally, these findings reveal similarities in the regulation of twitching motility in phototactic and chemotactic bacteria.

Funding

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22 POSTER

The complexome of the model cyanobacterium *Synechocystis* sp. PCC 6803

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Abstract text

Most cellular functions are performed by protein complexes, rather than by individual proteins. The size of these protein complexes can range from a few subunits to a large number of different components. These complex multimeric protein machineries perform and control many different cellular processes. Cryo-slicing blue native mass spectrometry (csBN-MS) combines gel separation of native protein complexes [1] with micro-slicing, high-performance liquid chromatography-tandem mass spectrometry and label-free quantification [2]. High-resolution fractionation profiles obtained by csBN-MS provide information on the abundance and subunit composition of membrane and cytosolic protein complexes. Here, we present the first application of this method to the model cyanobacterium, *Synechocystis* sp. PCC 6803, as well as the preliminary results. CsBN-MS analysis of cyanobacteria grown under different conditions may help elucidate the dynamic processes during the biogenesis of protein complexes and their cellular functions, providing useful information for other researchers.

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Funding

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23 POSTER

Two interconnected circadian oscillators in one cyanobacterium

Anika Wiegard[1], Christin Köbler[2], Nicolas M Schmelling[1], Alice Pawlowski[1], Gopal Pattanayak[3], Philipp Spät[4], Nina M Scheurer[2], Kim N Sebastian[2], Florian P Stirba[1], Lutz C Berwanger[1], Boris Macek[4], Michael J Rust[3], Annegret Wilde[2], Ilka M Axmann[1]

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Abstract text

The cyanobacterial circadian clock is well characterized in *Synechococcus elongatus* PCC 7942 where it is composed of the three proteins KaiA, KaiB and KaiC. Some cyanobacteria – among them *Synechocystis* sp. PCC 6803 - express multiple diverged Kai proteins, which implies that circadian regulation might be more complex in those organisms. We chose *Synechocystis* as a representative to study the function and interplay of diverged clock proteins in an otherwise well characterized model organism. It encodes three KaiB and three KaiC proteins. Based on homology searches, it was believed that all cyanobacteria contain only one copy of KaiA, but we discovered a second KaiA protein (KaiA3) in *Synechocystis* [1]. It is a chimeric protein, which resembles a KaiA domain in the C-terminus, while the N-terminus is similar to NarL type response regulators. After incubation with KaiA3 and KaiB3, KaiC3 displayed low amplitude, temperature compensated ~24h phosphorylation and dephosphorylation cycles in vitro. 24h phosphorylation rhythms were also detected in entrained cultures after release to constant light and were phase-locked with KaiC1 phosphorylation cycles. Circadian rhythms can be simply read out via online monitoring of the backscatter properties of cells [2]. Studies of *kai* gene deletion strains indicated that the KaiA1B1C1 oscillator and KaiA3B3C3 oscillator are both required to maintain stable backscatter oscillations, which implies that two interconnected oscillators are present in *Synechocystis*. We are currently investigating the interplay of these two oscillators.

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24 POSTER

Possible Involvement of CurT Proteins in Cyanobacterial Cell Division

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Abstract text

The CurT (Curvature Thylakoid) protein is a conserved thylakoid protein found in all but the most early-branching cyanobacteria as well as plant chloroplasts where it regulates 3-dimenional thylakoid architecture through its inherent membrane-shaping properties [1, 2]. In *Synechocystis* sp. PCC 6803 the absence of CurT results in disorganized thylakoid structures, absence of thylapses, and impaired photosynthesis [2], but also seemingly leads to asymmetrical cell division. Intriguingly, CurT is co-regulated with the prominent cell division protein SepF [2] and resembles SepF in several respects, including its capability to form dimers and polymers and its ability to tubulate liposomes *in vitro* due to an N-terminal amphipathic alpha helix [3]. We found first indications for CurT acting in cyanobacterial cell division in a manner similar to SepF and thus propose a possible role of CurT in the function and/or formation of the cyanobacterial divisome. Our project entails the analysis of CurT effects on cell division in *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942 and aims at acquiring a full understanding of the functional interactions between CurT and the components of the cell division complex.

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25 POSTER

Organization of the chlorophyll synthase complex in cyanobacteria

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Abstract text

Chlorophyll (Chl) is a crucial cofactor for oxygenic photosynthesis and the final step of Chl biosynthesis is catalyzed by the integral membrane enzyme Chl synthase (ChlG). In cyanobacteria, ChlG forms a stable complex with High light inducible proteins (Hlips; 1). These single-helix proteins are able to bind Chl and carotenoid pigments in a configuration that dissipates exciton energy. In addition, ChlG can be co-isolated with the Ycf39 protein, which is involved in Photosystem II biogenesis (2). It is known that Ycf39 also binds Hlips but how the ChlG-Hlips complex is organized and what role Hlips and Ycf39 play in this complex is still unknown.

We isolated Flag-tagged ChlG from *Synechocystis* mutants lacking Hlips and/or Ycf39 and characterized the resulting pulldowns by biochemical methods, advanced mass spectrometry (QConCAT) and single particle analysis. We found that the ChlG binds a heterodimer of Hlips (HliC and HliD) but only the HliD is in contact with ChlG. An alternative tetrameric complexes containing dimeric HliD, adjacent to two ChlG or to single ChlG and Ycf39, can be present in the cell under low light. We show that Ycf39 binds to the conserved N-terminus of HliD and structural models of all ChlG assemblies will be presented. Furthermore, we investigated the dynamics of the ChlG complexes under stress conditions. Results indicate that the ChlG-bound Hlips photoprotect the enzyme against photodamage by sequestering free Chl molecules. The role of Ycf39 will be discussed.

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26 POSTER

Identification of RNA-binding proteins in marine picocyanobacteria

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Abstract text

Marine unicellular picocyanobacteria, consisting of the two genera *Prochlorococcus* and *Synechococcus*, are the predominant photosynthetic organisms in the oceans. They play a pivotal role as primary producers and are estimated to contribute up to 25% of the total primary production in the oceans. Recent *in vivo* high-throughput proteomic studies have revealed the presence of hundreds of RNA-binding proteins (RBPs) across both eukaryotic and prokaryotic domains. The exact number of RBPs in any given organism remains unknown, but research indicates that approximately 7-15% of the bacterial proteome may comprise RBPs. These studies uncovered a surprisingly large number of proteins that have not been previously associated with RNA metabolism, but also function as RBPs. Canonical RBPs possess a well-defined RNA-binding domain, such as the RNA-recognition motif, the hnRNP k homology domain, the cold shock domain, the Sm or Sm-like domain, the S1 domain and the DEAD (Asp-Glu-Ala-Asp)-box helicase domain. However, until now, many RNA-binding domains remained uncharacterized due to their high heterogeneity, making it difficult or even impossible to identify them through computational approaches.

This study aims to identify picocyanobacterial RBPs, of which only extensively studied RBPs such as ribosomal proteins, RNA polymerase, RNA helicase and ribonucleases are currently known.

Funding

Deutsche Forschungsgemeinschaft

27 POSTER

FtsH1/3 proteolytic complex controls cell division in *Synechocystis* PCC 6803

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Abstract text

Membrane-bound FtsH proteases are widespread in prokaryotes, mitochondria and chloroplasts and play a crucial role in in a number of cellular functions including protein quality control and proteostasis. The genome of the cyanobacterium Synechocystis sp. PCC 6803 encodes four FtsH homologs, designated FtsH1-4, two of which, FtsH1 (slr1390) and FtsH3 (slr1604), are essential for cell viability. They form a hetero-oligomeric complex located in the cytoplasmic membrane. Recent results have investigated the role of the FtsH1/3 complex in the acclimatization of cells to nutrient deprivation. To gain deeper insights into the physiological role of the FtsH1/FtsH3 complex in *Synechocystis*, we analyzed the phenotype of the FtsH1down mutant with conditionally downregulated expression of the FtsH1 protease. The downregulation of FtsH1, which leads to a reduction of the FtsH1/3 complex, resulted in aberrant and significantly enlarged cells compared to the controlled WT strain. Detailed analyzes of ultrastructural features using transmission electron microscopy revealed that thylakoid membranes were disorganized within the cell and more thylakoid sheets protruded beyond the cell. These data indicate that the mutant cells are unable to divide properly. Interestingly, whole-cell proteomic analysis of the FtsH1down mutant revealed an over-accumulation of the Min system, which prevents the formation of the Z-ring. The proteolytic trap assay FtsH1trap with proteolytically inactivated FtsH1 showed proteins of the Min system, but also FtsZ as a possible substrate for the FstH1/3 complex. Overall, the data suggest that FtsH1/3 plays a crucial role in cell division by controlling the level of proteins important for divisome formation.

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28 POSTER

Membraneless organelle formation and associated translatome and protein interactome mediated by the RNA helicase CrhR in *Synechocystis* 6803

Brendan T. Whitman, **George W. Owttrim**

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Abstract text

We utilized immunofluorescent microscopy, O-propargyl-puromycin-ID (OPP-ID) and BioID to investigate abiotic stress effects on the subcellular organization, translatome and interactome of the DEAD-box RNA helicase, CrhR, in *Synechocystis* 6803. Dramatic temporal and spatial alterations are induced at 20°C where liquid-liquid phase separation (LLPS) mediates CrhR coalescence into a single crescent-shaped membraneless organelle exterior to the thylakoid membrane in an RNA-dependent reaction (1). OPP-ID identified potential components of these organelles and CrhR function in them, revealing that the CrhR translatome was intimately associated with translation of a minor proportion of the transcriptome, comprising transcripts primarily associated with photosynthesis and translation. Intriguingly, the data revealed that the primary role performed by CrhR RNA helicase activity involved downregulation of translation. BioID identification of the CrhR-protein interactome revealed a similar photosynthetic-translation specific regulation. Alleviation of the stress conditions repress CrhR induction by proteolysis mediated by a dimerization domain functioning as a thermometer, temperature upshift causing dimer to monomer conversion exposing a degron motif required for degradation (2). The data reveal that CrhR RNA helicase activity regulates translation and thus functionality of the photosynthetic and translational apparatus in a variety of environments.

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Session 2:
Evolution, Phylogeny and Genomics

29 INVITED TALK

What drives plant-like growth in filamentous cyanobacteria?

David John Lea-Smith[1], Ella Considine[2], David Pearce[1], Keanu S Walsham[1], Shylaja Mohandass[2], Phoenix Whenman[2], Andrew Rj Curson[1], Jonathan D Todd[1], Mark A Trimmer[2], Annegret Wilde[3], Conrad W Mullineaux[2]

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Abstract text

The ability of multicellular eukaryotes to simultaneously exploit resources from multiple locations has been key to their environmental success, for example, plants in terrestrial ecosystems. Prokaryotes have been thought to lack the ability to construct large-scale structures capable of extracting and combining resources from spatially separated environments. However, certain filamentous, diazotrophic cyanobacteria which incorporate their cells within a rigid mucilaginous sheath, demonstrate cm-scale colonial growth, developing tuft-like structures above the surface, combined with penetration of filaments into the underlying sediment or rock. Using a novel model system and analysing environmental populations we aim to determine how and why these plant-like structures form and how nutrients are moved between aerial and subsurface filaments. We demonstrate that ~1 cm high tufts comprise tight bundles of sheaths and that subsurface filaments are not bundled but demonstrate a root like pattern, likely providing stability for aerial formation of tufts. We observed rapid motility of filaments between aerial and subsurface sheaths at about 1.4 mm per hour which may drive transport of nutrients. However, downward movement of calcein is far slower than upward movement, suggesting an additional mechanism, likely transpiration, is driving upward transport of water and nutrients. Surprisingly, nitrogen fixation occurs in aerial cells and since this species lack heterocysts, this suggests a novel mechanism of limiting oxygen exposure to nitrogenase containing cells. Omics analysis of the model system and environmental populations are being conducted to determine the exact roles of aerial and subsurface cells and elucidate why these cyanobacteria form complicated large-scale structures.

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30 INVITED TALK

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The mutualism of *Microcoleus vaginatus* with specific N_2-fixing heterotrophs in soils: based on urea exchange and orchestrated by GABA

Ferran Garcia-Pichel[1], Soumyadev Sarkar[1], Ana Mercedes Heredia-Velásquez[1], Finlay Warsop Thomas[1], Ana Maria Giraldo-Silva[2], Corey Nelson[3]

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Abstract text

M. vaginatus, the most abundant terrestrial cyanobacterium on the planet, is the main pioneer in the formation of biological soil crusts of arid lands, which are perennially nitrogen-limited systems[1]. And yet, it cannot fix nitrogen. Its success is dependent on entering a C for N mutualistic exchange with specific diazotrophic heterotrophs (its cyanosphere)[2]. Unlike in other cyanobacterial mutualisms, this involves no specific structures isolating the partners from adventitious soil bacteria. How does this foundational mutualism ensure specificity and avoid feeding C or N to non-mutualists? Based on investigations using representative cultures of the mutualistic partners as well as multi-species assemblages from the field, we found that behavioral motility responses are key to keeping partners in close proximity and that these are mediated by GABA and Glu acting as interspecies signaling compounds and quorum-sensing effectors[3]. Further, we show that urea vehiculates the N transfer from heterotrophs to the cyanobacterium, which operates a rare allophanase-based, high affinity urea utilization pathway. This avoids losses to other soil bacteria that use the common, low-affinity urease systems, by keeping concentrations under their radar.

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31 ORAL

Hardening *Stenomitos frigidus* ULC029 by applying sequential stress factors

Beatriz Roncero-Ramos[1,2], Valentina Savaglia[1,3], Benoit Durieu[1], Isabelle Van De Vreken[4], Aurore Richel[4], Annick Wilmotte[1]

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Abstract text

Soil degradation in warm drylands is increasing due to land-use intensification. To restore these soils, we need to develop technologies that are efficient under the extreme environmental conditions in drylands. One strategy is the inoculation of cyanobacteria to restore soil biocrusts, because these communities provide multiple benefits to the ecosystem. However, after extended laboratory culturing, the cyanobacteria need to be re-adapted to natural conditions to optimize their survival, even if they were shown to be resistant to extreme stress when isolated. Here, we analyzed the genome and ecophysiological response to sequential stresses (osmotic, desiccation and UVR) of an Antarctic cyanobacterium, *Stenomitos frigidus* ULC029. Chlorophyll *a* concentrations show that preculturing ULC029 under moderate osmotic stress improved its survival during an assay of desiccation plus rehydration under UVR. Additionally, its sequential exposure to these stress factors increased the production of exopolysaccharides, carotenoids and scytonemin. Desiccation, but not osmotic stress, increased the concentrations of the osmoprotectants, trehalose and sucrose. However, osmotic stress might induce the production of other osmoprotectants, for which the complete pathways were found in its genome. Here, we confirm that the sequential application of moderate osmotic stress and dehydration, could improve cyanobacterial hardening for soil restoration, by inducing several resistance mechanisms.

Funding

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32 ORAL

From free-living cyanobacteria to a nitrogen-fixing endosymbiont in a photosynthetic eukaryote

Ellen Yeh, Solene Moulin

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Abstract text

Nitrogen is a limiting nutrient for photosynthetic productivity. Nitrogen in the atmosphere must be fixed into ammonia, a reaction performed by some bacteria. Yet nitrogenase, the enzyme that catalyzes biological nitrogen fixation, is exquisitely sensitive to oxygen produced during photosynthesis. How can plants and photosynthetic eukaryotes acquire nitrogen fixation function? We investigate Epithemia diatoms that contain obligate, nitrogen-fixing endosymbionts, or diazoplasts, derived from cyanobacteria. The diazoplast, which has lost photosynthesis, provides fixed nitrogen to the diatom host in exchange for fixed carbon. These algae are rare examples of photosynthetic eukaryotes that have successfully coupled oxygenic photosynthesis with oxygen-sensitive nitrogenase activity. To identify the metabolic changes associated with this endosymbiotic specialization, we compared the Epithemia diazoplast with its close, free-living cyanobacterial relative, Crocosphaera subtropica. Unlike C. subtropica, in which nitrogenase activity is temporally separated from photosynthesis, nitrogenase activity in the diazoplast is continuous through the day (concurrent with host photosynthesis) and night. Host and diazoplast metabolism are tightly coupled to support nitrogenase activity: Inhibition of photosynthesis abolishes daytime nitrogenase activity, while nighttime nitrogenase activity no longer requires cyanobacterial glycogen storage pathways. Instead, import of host-derived carbohydrates supports nitrogenase activity throughout the day-night cycle. Carbohydrate metabolism is streamlined in the diazoplast compared to C. subtropica with retention of the oxidative pentose pathway and oxidative phosphorylation. These pathways may be optimized to support nitrogenase activity, providing energy and consuming oxygen. Our results reveal critical metabolic adaptations associated with the transition from a free-living cyanobacteria to a nitrogen-fixing specialist endosymbiont inside a photosynthetic host cell.

Funding

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33 ORAL

Host Domestication of the Cyanobacterial High Light-Inducible Gene Family in the Amoeba *Paulinella*

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Abstract text

The evolution of photosynthesis in algae and plants relied on a pivotal event of primary endosymbiosis approximately 1.5 Gya, wherein a free-living cyanobacterium was engulfed by a heterotrophic microbe and evolved into a primary plastid. This primary plastid was subsequently disseminated throughout the tree of life via serial endosymbiosis events. The photosynthetic amoeba *Paulinella* represents a unique case in primary plastid evolution [1]. *Paulinella* harbors “chromatophores,” photosynthetic organelles derived from a relatively recent cyanobacterial association (~120 Mya), distinct from the evolution of primary plastids in plants and algae. The recent nature of this endosymbiosis offers a unique opportunity to uncover key elements facilitating the genetic integration between host and endosymbiont [2]. This work focuses on the evolution of the gene family encoding the cyanobacterial High Light-Inducible Proteins (HLIPs). This gene family, crucial for photoprotection in cyanobacteria, underwent transfer to the host nuclear genome and loss in the cyanobacterial endosymbiont. Within the host genome, the endosymbiont-derived *HLI* gene family suffered extensive duplications and rearrangements, acquiring novel regulatory elements and genetic configurations [3]. This profound genetic adaptation likely enabled the cell to withstand the photooxidative stress generated by oxygenic photosynthesis in the nascent organelle. Leveraging a cyanobacterial heterologous system, we are further studying the functionality of host-domesticated *HLI* genes in photoprotection. Our findings offer key insights into the genetic adaptations underpinning metabolic integration in cyanobacteria-derived organelles, illuminating the evolutionary trajectory of photosynthesis in eukaryotes.

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34 ORAL

Natural competence in the cyanobacteria phylum

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Abstract text

The ability to take up DNA from the environment via natural transformation, termed natural competence, relies in gram negative bacteria on the type IV pilus and additional natural competence specific proteins. Within the cyanobacterial phylum the complete set of the respective genes for natural competence is present in the majority of species. However, experimental evidence for DNA uptake via natural transformation in a cyanobacterial strains has been so far only available for few species. Here we present experimental validation of natural transformation in diverse filamentous and unicellular cyanobacteria representing major parts of the phylum. Transformation trials with candidate strains include incubation with a plasmid carrying an antibiotic resistance gene and integration of the plasmid resistance gene into the recipient’s chromosome by homologous recombination. We present our approach and our results for which species transformation was achieved, and for which it was not. Our conclusions are summed up in the form of guidelines outlining the key factors for establishing transformation protocols for non-model cyanobacteria. Our study links genome-based prediction of natural competence with experimentally validated natural transformation in cyanobacterial species and further supports the view that natural competence is widely distributed in cyanobacteria. The evolutionary advantage of natural competence in the phylum, e.g., as means for rapid adaptation or a potential route for DNA repair, remains an open question. The protocols and tools we established for genetic modification of cyanobacteria species open avenues for recombinant work in so far inaccessible lineages.

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35 ORAL

Cyanophage host-like photosynthesis genes alter the metabolism of their marine *Synechococcus* host

Debbie Lindell, Gazalah Sabehi, Omer Nadal, Ran Tahan, Dror Shitrit, Irena Pekarsky, Andrey Rozenberg, Oded Beja

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Abstract text

Cyanobacteria and cyanophages are abundant biological entities that coexist in the ocean. Cyanophages carry many host-like genes in their genomes, the host-encoded copies of which are involved in photosynthesis, carbon metabolism, nutrient acquisition, and other processes. The presence of these genes in cyanophages suggests an intimate link between host physiology and cyanophage replication that has evolved over time. Using our recently developed genetic manipulation system for cyanophages [1], we are investigating the function and impact of photosynthesis-related genes encoded by cyanophages, including the *psbA* and *nblA* genes. Infection with wild-type cyanophages changed the metabolic profile of the *Synechococcus* sp. strain WH8109 host. Infection with the mutant phages further altered the metabolic profile of the infected cell, indicating that these genes have a direct influence on host metabolism. The deletion of some genes resulted in a longer lytic cycle and/or the production of fewer phage progeny, indicating that these genes provide a fitness advantage to the cyanophage. Surprisingly, a fitness advantage was not apparent for all genes, raising questions as to their utility and the environmental conditions under which they are important to cyanophage.

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A multi-probabilistic model approach to improve annotations of Cyanorak, an information system dedicated to the expert curation of alpha-cyanobacteria genomes

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Abstract text

Besides their relevance in evolutionary biology, cyanobacteria are also of great interest in ecology, given their ubiquity and abundance in many ecosystems. Cyanobacterial genomes have multiplied in recent years due to the constant decrease in sequencing costs and Next Generation Sequencing (NGS) advances. The availability of numerous (near-)complete cyanobacteria genomes makes comprehensive comparative genomics studies possible, but such studies require the development of dedicated databases/tools such as Cyanorak (www.sb-roscoff.fr/cyanorak), a web server for annotating, comparing, and visualising alpha-cyanobacteria genomes [1]. Cyanorak currently encompasses sequences from 97 genomes, covering most of the genetic diversity known so far within this cyanobacterial group. We are currently developing a pipeline to semi-automatically update Cyanorak with a selection of the best quality genomes in public databases in order to fill diversity gaps. Functional annotation is an essential step in Cyanorak, which involves assigning each gene/protein to clusters of likely orthologous groups (CLOGs). Despite Cyanorak using several annotation methods based on different approaches (Blast, alignment, probabilistic models, etc.), the functional annotation of many proteins is still missing. Here, we are investigating if CLADE [2], an annotation method based on multi-probabilistic models, could decrease the number of unknown genes in alpha-cyanobacteria genomes. Moreover, we will exploit recent and promising techniques, such as deep neural networks, which have already reached impressive results in protein three-dimensional structure prediction [3]. The newly developed methods could help reduce the number of “unknowns/hypothetical proteins” in this cyanobacterial radiation, which represents almost 40% of the proteins in the Cyanorak database.

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37 POSTER

CRISPR-Cas alternative interaction with DNA target unique to cyanobacteria

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Abstract text

The CRISPR-Cas system is an immune system of bacteria with different biological functions. The system of type V-K exists exclusively in Cyanobacteria and works as a so-called CAST system (**C**RISPR-**as**sociated **t**ransposon). The system exists on a transposon with other cargo genes and three specific transposases that enable the mobile genetic shift, while the CRISPR compound, works as a targeting mechanism for this shift. From comparing the gene maps of these transposons we could see a highly conserved gene order starting at the left insertion element with a CRISPR-array, a tracrRNA, the typical DNA-interaction gene *cas12k* and its regulator-gene. In our previous work [1] we could identify three different types of regulators, the MerR-like CvkR and two distinct Arc-repressors which we named Arc1 and Arc2. These potential regulators were conserved at the same position and seemed to coevolve with the main CRISPR-Cas gene *cas12k*. Through the comparison with Cas12k, we could see that the CAST systems can be separated into distinct phylogenic groups that correspond to their specific regulator. From that, we could identify key features of these subtypes and found that the tracrRNA, an adaptation molecule between crRNA and Cas12k was missing in all systems with an Arc1 repressor. However, when we analyzed the typical region in these systems, we could still detect conserved sequences partially identical to the typical V-K tracrRNAs. From this, we searched for an alternative tracrRNA and were able to prove its existence *in vivo* via northernblot with RNA from *Scytonema sp.* NIES-4073.

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How far can convergent evolution go: the case of *Merismopedia* and *Microcrocis* (Cyanobacteria)

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Abstract text

The genera *Merismopedia* and *Micrococis* were traditionally considered closely related, emphasising common multicellular flat tabular colonies. *Merismopedia*, originally the type of distinct family Merismopediaceae, was recently included in Microcystaceae based on some of the *Merismopedia* morphotypes. However, recently examined authentic material of *Microcrocis geminata* supported the placement of *Microcrocis* in Geminocystaceae along with other *Merismopedia* morphotypes [1]. Further *Merismopedia* taxa were also assigned to Prochlorococcaceae. Therefore, the true lineage of the genus is indistinct and its determination was complicated by the absence of authentic material of the type species *Merismopedia tranquilla*. In the present study, a complex polyphasic approach was employed, including cultivation-independent molecular methods, and detailed morphological observation using light and transmission electron microscopy. Among our numerous specimens, we also collected material from the geographically accurate type locality of *Merismopedia tranquilla*, which can possibly be used to establish the generic epitype. Extensive sequencing of single-colony isolates provided the first molecular data for multiple taxa of both examined genera. The genera were revealed polyphyletic, which indicates frequent convergent evolution, and an unexpected diversity was discovered in Geminocystaceae, where taxa of both colonial morphotypes clustered with entangled relationships. Morphological criteria for the designation of the genera were questioned, and taxonomical changes are expected to follow. The single colony approach together with data cloning revealed the presence of multiple 16S-23S ITS variants and, rarely-described two variants of 16S rRNA for several studied taxa, which can possibly refer to ongoing speciation and may influence our view on current taxonomy of Cyanobacteria.

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Unicellular, Filamentous and Multicellular Cyanobacterial Genome Evolution Through Geological Time

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Abstract text

Cyanobacteria, which are one of the oldest known life forms on Earth, were also the first organisms to perform oxygenic photosynthesis [1]. Throughout the past 3.0 Ga years, cyanobacteria have undergone major evolutionary transitions, including the shift from oceanic benthic to planktonic habitats, as well as the origins of multicellularity, amongst others [2]. These transitions have had profound implications for Earth's biogeochemical cycles and have been critical drivers of biological innovation that shaped the early Earth environment. By studying the evolution of cyanobacterial genomes, we have gained insights into the origin and early diversification of cyanobacteria over geological time. Multicellularity offered considerable advantages for exploring new ecological niches, thus facilitating the diversification of new lineages. Phylogenetic studies have shown that multicellularity evolved early within Cyanobacteria, and there have been several instances where multicellular linages revert to unicellular [3]. With the increasing quality and quantity of genome-wide data, it is now possible to use comparative genomics techniques to gain insights into the origins and evolution of different morphological cyanobacterial taxa. In this study, we implement phylogenomics and evolutionary genomics pipelines to study 205 complete genomes. Our aim was to identify homologous genes that emerged and/or were lost at key times in cyanobacteria evolution, with a specific focus on transitions from multicellular to unicellular events. Our findings highlight processes behind genetic mechanisms during cyanobacterial morphological transitions and emphasise the pivotal role of conserved novel genes and gene families in the process of cyanobacterial morphological diversification.

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The proheterocyte germinating *Kaarinaea lacus* gen. nov., sp. nov. in culture

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Abstract text

Cyanobacteria are ecologically significant but they are also known to produce cyanotoxins. The cyanobacterium *Nostoc* sp. 152 (PCC 9237) isolated from a freshwater bloom sample from Lake Sääksjärvi (Finland) has been shown to synthesize microcystins and nostophycin [1]. Because of its overall morphology, this strain was assigned to the genus *Nostoc*. However, phylogenetic trees reconstructed from 16S rRNA gene sequences in different studies showed that this strain clustered outside the *Nostoc sensu stricto* clade. Therefore, we reinvestigated the taxonomic status of the axenic strain PCC 9237 using phylogenetic, morphological and genomic analyses. Multilocus and 16S rRNA gene phylogenetic trees both confirmed that this strain represents a novel Nostocalean genus for which we propose the name *Kaarinaea lacus* gen. nov., sp. nov.

Remarkably, PCC 9237 showed interesting morphological attributes such as unusual heterocyte patterning leading to the development of heterocytes in series followed by the fragmentation of trichomes at this site. Among the genes involved in heterocyte differentiation and patterning [2], *patS*, *patX*, *patC* and three homologs of *hetP* were not retrieved from its assembled genome. In addition, the germination of proheterocytes was observed occasionally. Furthermore, genomic investigations revealed a single heterocyte glycolipid gene cluster with a reduced *hglB* but having an additional gene encoding TubC-N terminal docking domain. Finally, the presence of a *pks2*-like gene cluster could also interfere with cellular differentiation like *pks2* does [3].

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Multicellular structures and functions are widespread in filamentous cyanobacteria

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Abstract text

Filamentous, heterocyst-forming cyanobacteria rely on intercellular communication for distribution of carbon- and nitrogen-containing metabolites produced in vegetative cells and heterocysts respectively, as well as for intercellular transfer of cell differentiation regulators [1]. Intercellular communication takes place via septal junctions, proteinaceous structures that traverse the septal peptidoglycan through nanopores. Genes involved in regulation of cellular differentiation (e.g., *hetR*, *patU3*, *patZ*) and in the formation of septal structures (e.g., *sepJ*, *sepI*, *hglK*, *fraCDE*) have been identified [1]. Phylogenomic, Bayesian molecular clock and gene-tree-species-tree reconciliation analyses indicate that some genes encoding septal structures (*sepJ*, *sepI*, *fraE*) and cellular differentiation regulators (*hetR*) appeared early in evolution 2.6-2.7 billion years ago (Ga), being generally present in filamentous cyanobacteria including the early-branching *Pseudanabaena* spp. [2]. Later, at the start of the Great Oxygenation Event about 2.5 Ga, other genes involved in cellular differentiation regulation (namely *hetZ, patU3*) and increased septal complexity (*hglK*) appeared. These are widespread in all filamentous cyanobacteria except *Pseudanabaena* spp.. The appearance time for *fraCD*, which are also absent from *Pseudanabaena* spp., is however unclear. Septal peptidoglycan nanopores and intercellular exchange of fluorescent markers were observed in two filamentous Macro- and one Micro-cyanobacteria that were studied. Furthermore, one central septal pore and intercellular transfer of, specifically, 5-carboxyfluorescein were observed in a true *Pseudanabaena* sp. strain. These and other available [3] results suggest that intercellular communication is widespread in filamentous cyanobacteria, albeit with unique characteristics in *Pseudanabaena* spp., contributing to the behavior of the filament as their organismic unit of growth.

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During the reign of dinosaurs, Cyanobacteria triumphed over Antarctica

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Abstract text

Cyanobacteria are the dominant primary producers in polar regions. Despite the extreme environmental conditions, both Antarctica and the Arctic have been colonised by many unrelated groups of mostly filamentous cyanobacteria [1]. These groups span the whole cyanobacterial diversity, and often also contain strains isolated from temperate environments. Even though genomic data is available for some strains of polar cyanobacteria, the vast majority of this diversity has only been assayed using individual markers (e.g., 16S ribosomal RNA or pigments). This has hindered efforts to study the evolution of these strains and their adaptations to cold climates.

To address these questions, we sequenced the genomes of 32 strains of cyanobacteria from cold environments (CCE) from culture collections. We used phylogenomic analyses and a relaxed Bayesian molecular clock approach to date the origin of groups of CCE. We also performed comparative genomics analyses, comparing CCE with strains from temperate regions.

Our analyses revealed that most groups of CCE originated during the Mesozoic (252 – 66 Mya), which is generally regarded as one of the warmest times in Earth's history, although some cold events have been hypothesised. This suggests that Cyanobacteria colonised modern polar environments at a time when conditions were favourable for their growth and survived in these regions due to their overall resilience, rather than to specific adaptations to extreme cold. This is consistent with CCE strains exhibiting optimal growth in the 15-20°C range [2] and lacking specific genomic features that differentiate them from strains in temperate regions [3].

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New insights of the evolutionary diversification of the marine nitrogen-fixing symbiotic cyanobacteria UCYN-A

Cinthya Vieyra Mexicano[1], Giorgio Bianchini[2], Laurence Garczarek[3], Patrick Wincker[4,5,6], Silvia G. Acinas[1], Kendra A. Turk-Kubo[7], Patricia Sánchez Baracaldo[2], Jonathan P. Zehr[7], Francisco M. Cornejo Castillo[1]

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Abstract text

The unicellular cyanobacterium *Candidatus* Atelocyanobacterium thalassa (hereafter, UCYN-A) is an important marine nitrogen fixer that lives in symbiosis with haptophyte algae related to *Braarudosphaera bigelowii*. The UCYN-A phylogenetic group includes at least eight genetically distinct sublineages, yet only representative genomes of the UCYN-A1 and UCYN-A2 sublineages are currently available. Therefore, despite its biogeochemical relevance, comparative genomics on the UCYN-A phylogenetic group has been limited by the poor representation of genomic data, which hinders our understanding of its evolution. Here, using single cell genomics, we obtained new genomic data of two UCYN-A sublineages, UCYN-A3 and UCYN-A4, for which we reconstructed ca. 61 and 99% of their genomes, respectively. We show that the genome content of UCYN-A3 and UCYN-A4 is similar to the previously sequenced sublineages. However, we also detect some distinct genomic features, e.g., the presence of genes for cell shape and glycerol lipid metabolism that were missing in UCYN-A1 and UCYN-A2, respectively. Phylogenomics and molecular clock analyses show that these four UCYN-A sublineages form a monophyletic group where the UCYN-A1 sublineage separated from the other sublineages ca. 121 Million years ago. We also show that the UCYN-A2 sublineage diverged from the UCYN-A3/UCYN-A4 branch ca. 88 Mya, and that UCYN-A3 and UCYN-A4 diverged from each other ca. 44 Mya. We looked for possible causes of evolutionary diversification by analyzing selection pressure that suggested purifying selection for most UCYN-A genes in the four sublineages. Our findings, together with paleo-oceanographic data, provide a better understanding of the diversification context of the UCYN-A symbiosis.

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A novel sRNA Ncr0700 mediates post transcriptional gene regulation for rapid acclimation to multiple abiotic stress in cyanobacteria

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Abstract text

Cyanobacteria are found in diverse niches and are known to survive in extreme environmental conditions. For rapid acclimation under such stress conditions, several non-coding genes are reported to mediate post transcriptional regulation. A novel small non-coding RNA, Ncr0700, is highly upregulated during multiple stress conditions in unicellular cyanobacterium *Synechocystis sp.* PCC 6803. It is transiently upregulated during heat stress but gradually upregulated during darkness while it is downregulated during cold stress. Its strong accumulation under darkness suggests its functional significance in metabolic re-optimization during circadian cycles. *Δncr0700* knockout mutant was generated by replacing the corresponding non-coding gene with chloramphenicol resistant cassette to functionally characterize Ncr0700. *Δncr0700* exhibited retarded growth under darkness, photo mixotrophic heat and nitrogen limiting conditions, implying its importance under these stress conditions. Ncr0700 may rapidly regulate genes that code for enzymes involved in metabolic re-optimization during these stress conditions for acclimation. Whole transcriptome studies suggest Ncr0700 to be a post-transcriptional regulator of manganese transport operon (*mntABC*), bidirectional hydrogenase (*hox)* cluster, pilin biosynthesis genes, sulphate transporters and genes encoding key metabolic enzymes, thereby re-optimizing the carbohydrate metabolism and redox poise, thus helping the cells to acclimatize under multiple abiotic stress conditions. RNA secondary structure analysis and prediction of interacting regions confirm that these are the targets of Ncr0700. Currently, we are working to elucidate the molecular mechanism of regulation of *mnt* and *hox* operon by Ncr0700.

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Photosynthesis and Genome Evolution of Cyanobacteria from Polar Environments

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Abstract text

Cyanobacteria are the only bacteria capable of oxygenic photosynthesis. They are cosmopolitan, but especially successful in extreme habitats such as Polar regions. However, adaptation of photosynthesis to these habitats, where extreme fluctuations in irradiance and temperature are commonplace, remains poorly understood. Cyanobacterial dominance and ability to thrive in these environments suggests a capacity to acclimate photosynthesis to challenging conditions. The mechanics of such adaptations can provide insights into the past and present evolution of such extremophiles, potential applications in biotechnology for transferring these qualities into eukaryotic algae and crops, and a deeper understanding of the biological plasticity allowing the endurance of oxygenic photosynthesis over the long history of our constantly changing planet.

The aim of the project is to characterize in real-time, at a physiological and genomic level, how photosynthesis in cyanobacteria acclimates to harsh environmental conditions using adaptive laboratory evolution experiments. Experiments will grow Polar cyanobacteria in the laboratory to evaluate their fitness and photosynthetic performance under extreme light intensities in combination with changes in temperature. Selected populations of polar strains will be gradually acclimated to benign laboratory conditions to release selective pressures. In parallel, laboratory cyanobacteria will be gradually acclimated to harsh polar-like conditions and their fitness compared to polar isolates. Genomes and transcriptomes will be sequenced every three months, genetic and physiological changes will be correlated with photosynthetic performance.

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46 POSTER

The Collection of Cyanobacteria at the Leibniz Institute DSMZ: Characterisation of Non-Axenic Cyanobacterial Cultures by Metagenomic Approaches

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Abstract text

The Leibniz Institute DSMZ is one of the world's largest collections of microorganisms and cell cultures, currently housing more than 86,500 bioresources [1]. These include about 1000 strains of "Cyanobacteria" (*Cyanophyceae*) - an ancient group of oxygenic phototrophic bacteria with outstanding ecological and evolutionary significance. The cyanobacterial strains in the DSMZ collection originate from various habitats worldwide, including seawater, sediments, drinking water resources, (ice) deserts and soils at anthropogenic influenced sites. While 160 of the cyanobacterial strains have been characterised and quality-controlled - and are available for research and industrial applications via the DSMZ online catalogue - many of the remaining strains have been classified only on the basis of morphological characteristics and their phylogenetic classification is pending. In addition, most of these strains are non-axenic cultures, complicating their characterisation using standard microbiological methods. Our current aim is to comprehensively characterise non-axenic cyanobacterial cultures kept at the DSMZ and to investigate the biodiversity of heterotrophs present in them. Accordingly, we applied metagenomic methods to analyse cyanobacterial cultures, i.e. (I) 16S rRNA gene - ITS amplicon sequencing and (II) metagenomic sequencing with the PacBio Sequel IIe HiFi long-read technology. The applicability of these methods was investigated exemplarily (I) for 32 filamentous *Coleofasciculus* strains from different oceanic sampling sites and (II) for *Oculatella crustaeformantes* DSM 109267*,* a soil crust isolate from Spitsbergen, Norway. The resulting data (I, II) are used for a taxonomic assessment of the cyanobacteria and associated heterotrophs and (II) for the determination of the cyanobacterial metabolic and toxigenic potential.

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The conjugative potential of Cyanobacteria

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Abstract text

Bacterial conjugation is a horizontal gene transfer process between donor and recipient cells. Currently, bacterial conjugation from *Escherichia coli* to *Cyanobacteria* serves as the primary method for genetically modifying these organisms. However, there is limited evidence of conjugation between *Cyanobacteria* [1]. In this study, we conducted a bioinformatic search for the components of the conjugation machinery in RefSeq212 cyanobacterial genomes originated from 17 taxonomic orders. Among the eight mating pair formation (MPF) systems already described in prokaryotes [2], MPF_C is the sole system present in *Cyanobacteria*, both in plasmids and chromosomes. Of the nine relaxase MOB families described in bacteria [3], five are present in this phylum. A total of 276 plasmids potentially transmissible by conjugation, out of which 85 are conjugative, and 57 Integrative and Conjugative Elements (ICEs) were detected. The phylogenetic analysis suggested recent interchange of MPF_C between plasmids and ICEs. A common core of 5 MPF_C components exists across 10 different orders, with highly conserved organization. Nevertheless, slight variations in MPF_C synteny were detected depending on different factors. Most chromosomally-encoded MPF_C tend to lack the Alr7212 and coupling protein components. Additional genes in the vicinity of VirB4, and thus potential MPF_C components, were exclusively found in taxonomic orders *Leptolyngbyales* and *Nostocales*. In the latter, the presence of these genes correlated with specific associated relaxases. Notably, in *Nostocales*, two Alr7209 homologs were detected and exhibited a high degree of coevolution.

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CvkR is a MerR-type transcriptional repressor of class 2 type V-K CRISPR-associated transposase systems

Marcus Ziemann[1], **Viktoria Reimann**[1], Yajing Liang[2,3,4], Yue Shi[2,3,4], Yuman Xie[5,6], Hui Li[5,6], Tao Zhu[5,3,4,6], Xuefeng Lu[5,3,4,6,7], Wolfgang R. Hess[1]

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Abstract text

A core component of the immune system of cyanobacteria is their diverse set of CRISPR-Cas systems. One of the more exotic systems exclusively existing in Cyanobacteria is the type V-K, which is integrated into a Tn7-like transposon, forming a so-called CRISPR-associated transposon (CAST) system. The CRISPR-Cas system works as a targeting system for the mobile shift of the transposon and therefore avoids uncontrolled genetic mobility in the cell. How the activity of these systems is controlled in situ has remained largely unknown. Here we characterize the MerR-type transcriptional regulator Alr3614 that is encoded by one of the CAST (AnCAST) system genes in the genome of the cyanobacterium *Anabaena* sp. PCC 7120 [1]. We identify a number of Alr3614 homologs across cyanobacteria and suggest naming these regulators CvkR for Cas V-K repressors. Alr3614/CvkR is translated from leaderless mRNA and represses the AnCAST core modules *cas12k* and *tnsB* directly, and indirectly the abundance of the tracr-CRISPR RNA. We identify a widely conserved CvkR binding motif 5'-AnnACATnATGTnnT-3'. The crystal structure of CvkR at 1.6 Å resolution also reveals that it comprises distinct dimerization and potential effector-binding domains and that it assembles into a homodimer, representing a discrete structural subfamily of MerR regulators. CvkR repressors are at the core of a widely conserved regulatory mechanism that controls type V-K CAST systems.

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Funding

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CyanoGenes: A Web-Based Tool for Interactive Exploration of the *Synechocystis* sp. PCC 6803 Genome.

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Abstract text

Synechocystis sp. PCC 6803 is a well-studied cyanobacterium and a valuable model organism for research in photosynthesis and biotechnology. However, the lack of a user-friendly web database for exploring its genome has been noted due to long intermittent shutdowns of the Cyanobase portal. To tackle this issue, we have developed CyanoGenes, a user-friendly web server that facilitates interactive exploration of the *Synechocystis* genome.

Although *Synechocystis*' genome was the first cyanobacterial genome to be sequenced, approximately 23% of its protein-coding genes remain unannotated (last Refseq *Synechocystis* genome). To predict the functions of these unannotated proteins, we performed a structural clustering analysis using AlphaFold [1] and FoldSeek [2]. A total of 2002 proteins, which accounts for approximately 60% of the *Synechocystis* proteome, were grouped into 469 clusters, enabling the inference of functional annotations for 247 hypothetical proteins. These results were integrated with information from UniProt, RefSeq, Cyanobase, and EggNOG5, resulting in CyanoGenes reducing unannotated proteins to only 302 (~7.5%).

CyanoGenes not only allows users to search for genes but also to obtain a comprehensive annotation from a list of genes and calculate their functional enrichment profile. Future plans involve expanding CyanoGenes to other cyanobacteria. Overall, CyanoGenes offers an easy-to-use web-based tool for investigating the *Synechocystis* genome that is freely available at https://cyanogenes.com.

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50 POSTER

Introducing BCCM/ULC public cyanobacteria collection: A tool for *ex-situ* conservation of cyanobacterial biodiversity and support of taxonomic and genomic research.

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Abstract text

The BCCM/ULC is a young public collection, embedded in the Belgian Co-ordinated Collections of Microorganisms funded by BelSPO. It currently hosts more than 500 cyanobacterial strains, of which approximately 140 derive from (sub)polar and alpine environments, followed by (sub) tropical and mediterranean biotopes as well as strains of Belgian origin. An ISO 9001 certificate covers the public deposition & distribution of strains, as part of a multi-site certification for the BCCM consortium.

All strains are characterized by a polyphasic approach workflow (i.e. morphological, molecular, and ecological data). Furthermore, the collection includes more than 20 strains that are the reference (or 'type') for newly described genera and species including *Plectolyngbya*, *Shackletoniella*, *Timaviella, Parakomarekiella*, *Petrachloros, Johannesbaptistia, Leptochromothrix, Vermifilum*, *Tigrinifilum*, *Affixifilum, Brasilonema* and *Neolyngbya*. Recently, 30 more strains of rock-inhabiting cyanobacteria from Finland have been deposited to the collection, most of which represent new taxa such as *Pseudanabaena epilithica* and *P. suomiensis.*

Additionally, the collection has developed a genomic infrastructure, the GEN-ERA toolbox (https://github.com/Lcornet/GENERA), highly reproducible and adhering to the concept of FAIR science. This allows for the study of taxonomically interesting morphotypes, such as polar strains of the recently described genus *Laspinema*, through phylogenomic inference and comparative genomics. Genomic analyses of ULC007, ULC065, and ULC129 revealed the presence of BCGs encoding a wide range of natural products, showing promise as potential drug leads as well as metabolites with potential biotechnological and biomedical applications.

Funding

BCCM/ULC is funded by the Belgian Science Policy Office (BelSPO)

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More than just disorder, metabolite diversity of *Microcystis* strains shows tight correspondence to genotype and may contribute to ecotype specificities.

Benjamin Marie[1], Aurore Huré[2], Charlotte Duval[2], Manon Quiquand[2], Sébastien Halary[2], Muriel Gugger[3]

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Abstract text

Microcystis is one of the most common bloom-forming cyanobacteria in freshwater ecosystems worldwide. This species remarkably produces numerous bio-active accessory metabolites, which are believed to be potentially involved with different ecological and/or physiological processes. Their genuine contribution to the evolutive success of *Microcystis* blooms remains undetermined.

To better depict the potential relation between the local genetic diversity of blooming *Microcystis* populations and their respective associated chemical diversity, we conducted a joined genomic and metabolomic analysis of 65 *Microcystis* strains collected from various lakes from France and surrounding countries. Interestingly, both core- and noncore-gene phylogenic analysis place 59 of these European strains in 12 distinct genetic clades of at least 2 genomes, being widely distributed along the whole *Microcystis* phylogeny and presenting specific signatures of accessory metabolite biosynthesis. Overall, the direct chemical analysis of metabolite diversity produced by those strains, under lab culture conditions, reveals the production of stable metabolite corteges, beyond little variations along culturing generations, growth phases and conditions. Indeed, these strains belonging to 12 different genotypes correspond to 13 distinct metabotypes according to an accurate one-metabotype-for-one genotype rule. This observation reveals that *Microcystis* collected from certain environments present a large set of genetic and subsequent corresponding metabotype diversity whereas all strains originating from certain other lakes present a net strain uniformity. These observations suggest that the genetic diversity may be related to specific environmental pressures that may rather favour or constrain chemical and initial genetic diversity locally and the emergence of certain ecotypes.

Funding

This work is supported by ANR MC-Tox (SJ 1108-22).

52 POSTER

In-depth clone collection and culturomic strategies for the investigation of cyanobacteria microdiversity at the French National Museum of Natural History

Sébastien Duperron[1], Charlotte Duval[1], Sébastien Halary[1], Cécile Bernard[1], Benjamin Marie[2]

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Abstract text

Established in the late 1920s, the culture collection of cyanobacteria at the French National Museum of Natural History (MNHN) now comprises over 1100 non-axenic live strains isolated mostly from freshwater ecosystems in France (Hamlaoui et al. 2022). As a research-oriented collection, it contributes to biodiversity, taxonomy, genomics, and bioactive compound research. Notably, the collection contains multiple strains of various genera of bloom-forming cyanobacteria that are of ecological concern, some of which produce cyanotoxins. Novel strategies for strain identification, conservation and accessibility are being implemented to provide an up-to-date resource to the community.

Using a few recent examples, we here describe how developing a specific strategy for the enrichment of various clones from the same taxa and environments provides a new vista of the micro-diversity of cyanobacteria concerning their evolution and ecology. These enlarged perceptions of clonal diversity offer us new opportunities to better characterize the plasticity of cyanobacteria blooming populations in terms of local adaptation, physiology, ecotoxicology, chemical ecology and microbial interaction.

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MNHN provide financial support to the Paris Museul Collection of Cyanobacteria

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A membrane-associated cAMP receptor protein, SyCRP1 senses inorganic carbon and regulates CCM in *Synechocystis* sp PCC 6803

Jogadhenu Syama Sundar Prakash, Suraj K Chauhan

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Abstract text

Although CCM regulatory mechanisms have been well documented, the mechanism by which CO_2 levels are sensed in cyanobacteria is poorly understood. Here we show a unique membrane-associated cAMP Receptor Protein, SyCRP1, senses the inorganic carbon (C_i) levels and regulates the CCM in *Synechocystis*. The Δ*sycrp1* showed a upregulated expression of CCM genes in transcriptome analysis. Δ*sycrp1* grows slower at low-CO_2, and photosynthetic performance of Δ*sycrp1* reduced when compared to wild-type. The decreased photosynthetic performance could be due to fewer carboxysomes in Δ*sycrp1* at low-CO_2, while high-CO_2 acclimated cells bear similar number. Further, we observed that the SyCRP1 is localized on the membrane. We demonstrated that cAMP and CO_2 trigger release of SyCRP1 into cytoplasm, suggesting that CO_2 mediated increase in cAMP caused the dissociation of SyCRP1 from membrane. The SyCRP1-cAMP, binds to upstream of *sbtA* and *ccmK* with high affinity. We identified that it binds to upstream of various genes involved in Ribosome structure, Carbon fixation & metabolism and Metabolic pathways expanding the regulatory role beyond CCM using ChIP-sequencing with 3x-FLAG-SyCRP1^{+} strain. SyCRP1 has an N-terminal extended peptide unique to cyanobacteria may aid in its association to the membrane. SyCRP1 has two binding sites for cAMP. Microscale thermophoresis analysis indicated that SyCRP1 anchor to the membrane through membrane lipids. SyCRP1 as a key-player mediating C_i response *via* CCM gene-regulation. Further, the study provides insights into SyCRP1's role in broader regulatory networks and insights enrich understanding of its mode of action.

Funding

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54 POSTER

Complete genome sequence of *Crocosphaera watsonii* WH8501 reveals specific features of subtropical unicellular diazotroph.

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Abstract text

In open ocean, diazotrophic cyanobacteria are known as important fixers of nitrogen and carbon. *Crocosphaera* is the only one free-living unicellular cyanobacteria genus which habitats in subtropical ocean [1]. So far, incomplete genome data of them is available in public databases. Thus, it has been difficult to assume the molecular mechanisms of photosynthesis or nitrogen fixation. In the current study, we re-sequenced the genome of *Crocosphaera watsonii* WH8501 (hereafter WH8501). Since, we could not have obtained axenic culture of it, we utilized the combination of Nanopore long-read and illumina short-read sequencing technology for genome sequencing. As the result, we found that WH8501 has a total 6.28 Mbp genome composed by 6.1 Mbp chromosome and 3 plasmids. Although this genome size is comparable with the old dataset (GenBank GCA_000167195.1) [2], our new data has more than 300 genes compared with old data. Interestingly about genes related to photosystems, we found 3 copies of *psbA* gene encoding D1 reaction center protein of PSII, in contrast, previous data has only 2 copies of it. Two of the genes have identical amino acid sequences and the last one encodes rogue D1 which is previously reported as an inactive variant for oxygen evolution [3]. Furthermore, our complete genome revealed 1191 of mobile genetic elements (e.g. transposase). This huge number of mobile elements would make WH8501 genome unstable as previously suggested [2]. Thus, our complete genome data provides new insight for subtropical unicellular diazotroph.

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Session 3.1: Physiology, Metabolism, and Bioenergetics

55 INVITED TALK

The delicate regulation of the central carbon metabolism in *Synechocystis* sp. PCC 6803

Kirstin Gutekunst

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Abstract text

The regulation of the central carbon metabolism in cyanobacteria is especially delicate as anabolic and catabolic reactions are not separated in different compartments but operate in parallel in the cytoplasm. The Calvin-Benson-Bassham (CBB) cycle and glycolytic routes are highly intertwined. Especially glycolysis and the oxidative pentose phosphate pathway share reactions with the CBB cycle but operate in opposite directions. The strongest signals to turn the direction of the central carbon metabolism are darkness and light. However, anabolic and catabolic pathways are not sharply separated but merge e.g. in transitions states when glycolytic shunts fine-tune the CBB cycle [1, 2]. The central carbon metabolism is characterized by a great number of isoenzymes which are mostly distinctly regulated via metabolites and display the elegant fine-tuning of photosynthesis, CO_2 fixation and carbohydrate oxidation.

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56 ORAL

Cyanobacterial primary carbon metabolism - Regulation and application

Martin Hagemann

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Abstract text

Cyanobacteria fix inorganic carbon (Ci) via the Calvin-Benson cycle, which is supported by an inorganic carbon-concentrating mechanism (CCM). The activity of the CCM needs to be coordinated with the activity of the Calvin-Benson cycle under fluctuating Ci conditions. The CCM regulation on transcriptional level is quite well understood, however, changes in carbon flux and transport activities seem to be mainly regulated on the posttranscriptional level. During the last years, we investigated how protein phosphorylation or small regulatory proteins contributed to the regulation of the primary carbon metabolism under different Ci conditions. Phosphorylation of metabolic enzymes and transporters is possibly responsible for the fine-tuning of carbon fluxes into different metabolic branches [1]. In addition, regulatory proteins such as PirC [2] and CP12 [3] have been shown to modulate the flux of carbon under varying nitrogen and light conditions. The new insights into the regulation of carbon metabolism will contribute to the rational design of cyanobacterial cells for biotechnological purposes, e.g. the production of bioethanol or bioplastics. Our current knowledge of carbon regulation and its possible application in cyanobacterial biotechnology will be presented and discussed.

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57 ORAL

Metabolic control of the glycogen charge in *Synechocystis* PCC6803

Karl Forchhammer, Kenric Lee, Niels Neumann, Tim Orthwein, Janette Alford, Sofia Doello

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Abstract text

Glycogen in cyanobacteria functions as a central store of energy and carbon, aiding in the management of metabolic fluctuations and maintaining overall metabolic equilibrium through its rapid turnover. This metabolic battery is tightly regulated, with glycogen hub enzymes responding promptly to metabolic cues, including carbon and nitrogen availability, as well as cellular energy status. Our recent research focuses on elucidating the intricate network of effectors and sensory factors governing this process.

At the heart of this network lies 3-phosphoglycerate (3-PGA), the initial product of RubisCO-catalyzed CO_2 fixation. Activation of glycogen synthesis by GlgC converting Glc-1P into ADP-Glc is under strict metabolic control. Strong cooperative activation by 3-PGA is antagonized by inorganic phosphate (Pi), resulting in sharp activation of ADP-Glc formation at critical 3-PGA thresholds. The concentration of 3-PGA responds to CO2 fixation rates and the balance of carbon and nitrogen supply, sensed by the PII signaling protein. Under nitrogen-poor conditions, dissociation of the PII-PirC complex leads to inhibition of cofactor-independent phosphoglycerate mutase by PirC, elevating 3-PGA levels, thus activating GlgC and promoting glycogen formation [1]. Phosphoglycerate mutase (PGM) governs the entry and exit from the glycogen hub by interconverting Glc-6P and Glc-1P. Its activity requires the activator molecule Glc-1, 6-bisphosphate, synthesized by a specialized PGM using Fru-1, 6-BP as a phosphoryl donor [2]. Additionally, long-term inhibition of PGM1 through protein Ser phosphorylation allows for sustained survival during nitrogen starvation [3]

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58 ORAL

Glycogen prevents metabolic imbalance and disruption of photosynthetic electron transport from Photosystem II during transition to photomixotrophy in *Synechocystis* sp. PCC6803

Pablo Ortega Martínez[1,2], Lauri Nikkanen[3], Laura T. Wey[3], Francisco J. Florencio[1,2], Yagut Allahverdiyeva[3], Sandra Diaz-Troya[1,2]

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Abstract text

Cyanobacteria are photosynthetic prokaryotes that store part of their photosynthates as glycogen. Some cyanobacteria can grow photo-autotrophically or photo-mixotrophically by using simultaneously CO_2 and glucose. Despite improving their growth and production with potential use as photo-biorefineries(1), the switch between trophic modes and the role of glycogen, remains poorly understood.

We analyzed the effect of glucose addition on Synechocystis sp. PCC6803 and mutants lacking phosphoglucomutase and ADP-glucose pyrophosphorylase, with reduced or null glycogen synthesis, respectively(2).

Glucose addition enhanced optical density and glycogen reserves in the wild-type, whereas the glycogen-deficient strains experienced growth arrest, a 4-fold accumulation of reactive oxygen species, PSII core proteins loss and cell death within 24 hours. Merely 30 minutes after glucose addition, metabolites in the OPP and PGI shunt and CBB cycle increased 3-fold in the wild-type strain and 9-fold in the mutants (intermediates like Sedoheptulose-7P, Glucose-6P and Erythrose-4P increased 50, 40 and 30-fold, respectively). These alterations in the metabolic landscape affected the photosynthetic performance of the glycogen mutants, as CO_2 acquisition, and O_2 evolution were both impaired. Our results suggest this is due to restriction of electron transfer from PSII that was reversed when an artificial electron acceptor was added.

These findings indicate complex interplay between metabolic and photosynthetic processes. Their crosstalk allows maintenance of source/sink balance in the cyanobacterial cell and enables growth in diverse trophic modes. Thus, our study lays the fundamental groundwork for being able to optimally harness cyanobacteria to produce valuable carbon compounds by tapping into the energy of photosynthesis

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Cyclic-di-AMP signaling in cyanobacteria: a new paradigm in controlling cellular homeostasis

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Abstract text

Because of their photosynthesis-dependent lifestyle, cyanobacteria evolved sophisticated regulatory mechanisms to adapt to oscillating day-night metabolic changes. How they coordinate the metabolic switch between autotrophic and glycogen-catabolic metabolism in light and darkness is poorly understood. Recently, we showed that the second messenger c-di-AMP is implicated in diurnal regulation[1]. To unravel the signaling functions of c-di-AMP, we identified the cyanobacterial c-di-AMP receptor proteins. The carbon-sensor protein SbtB was identified as the major c-di-AMP receptor[1]. We found that c-di-AMP–bound SbtB interacts with the glycogen-branching enzyme GlgB. Accordingly, both c-di-AMP-free (Δ*dacA*) and Δ*sbtB*–deficient mutants displayed impaired glycogen synthesis and nighttime survival[1]. To gain better understanding of cellular processes regulated by SbtB or c-di-AMP, we compared the metabolomic, transcriptomics, and proteomic landscapes of both mutants[2,,3]. While our results indicate that the cellular roles of SbtB is restricted to carbon/glycogen metabolism[1-5], the Δ*dacA* lethality seems a result of dysregulation of multiple metabolic processes[2,3]. These processes include photosynthesis and redox regulation, which lead to elevated levels of intracellular ROS and glutathione[2]. Further, we showed an impact of c-di-AMP on transcription/transitional regulations, ion homeostasis, and especially on central carbon metabolism. Adding to its cellular functions catalog, for first time, we show physiologically that c-di-AMP signaling influences on nitrogen metabolism, implying new cellular roles for c-di-AMP in controlling nitrogen homeostasis[2]. Additionally, we found that the cyanobacterial pilus biogenesis and natural competence are regulated by c-di-AMP and show that the ComFB signaling protein is a novel c-di-AMP-receptor protein[6], widespread in bacterial phyla, and required for DNA uptake.

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Cyanobacterial toxins as modulators of carbon fixation

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Abstract text

The freshwater cyanobacterium *Microcystis* can rapidly grow to dominance by forming “blooms” which pose serious ecological threats but also have adverse effects on water supply for industrial, agricultural and recreational use. The remarkable success of *Microcystis* in the field is attributed to its phenotypic plasticity in reaction to changing environmental conditions. This strategy is exemplified by a unique carbon concentrating mechanism that is rooted in a genetic variability observed among *Microcystis* sub-strains. Another intriguing feature of *Microcystis* is the capacity to produce various secondary metabolites with unknown biological functions. One prominent example, the hepatotoxin microcystin, functions as an extracellular infochemical but also acts intracellularly by binding to proteins of the carbon metabolism, most notably to RubisCO, the main CO_2-fixing enzyme. Microcystin binding is stimulated by high light and coincides with a relocation of RubisCO from carboxysomes to the cytoplasm. Concomitantly, the enzymatic activity of RubisCO depends on microcystin and the light conditions during cultivation, with carboxylation rates and CO_2 affinities increased under low light in the microcystin-free mutant.

We propose that microcystin is part of a fast response system that adjusts carbon fixation to fluctuating growth conditions by modulating, among others, RubisCO activity through the binding of small effector molecules, thus contributing to the metabolic flexibility and the success of *Microcystis* in the field. Employing X-ray crystallography, cryo-electron microscopy and high-resolution mass spectrometry we aim at isolating and identifying these RubisCO effectors.

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Dramatic restructuring of carbon concentrating machinery accompanies energy imbalance and oxidative stress in cyanobacterial mutants of the circadian regulator RpaA

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Abstract text

Photosynthetic organisms need to balance the rate of photosynthesis with the utilization of photosynthetic products by downstream reactions. While such “source/sink” pathways are well-interrogated in plants, analogous regulatory systems are unknown or poorly studied in single-celled algal and cyanobacterial species. We previously identified a connected network of four two-component systems (TCS) with roles as energy/sugar sensors in cyanobacteria, using an engineered strain of *Synechococcus elongatus* PCC 7942 that allows experimental manipulation of carbon status. Among these four candidates, the transcription factor RpaA (regulator of phycobilisome associated A), also known as the master regulator of circadian transcription in cyanobacteria, was identified. Here, we find that deletion of *rpaA* caused imbalances between photosynthetic activities (measured as the apparent quantum yield of PSII (Φ_{II})) and downstream metabolic processes (rate of sucrose export) in *S. elongatus* PCC 7942. In addition, strains of *S. elongatus* PCC 7942 lacking the circadian master regulator RpaA showed structural changes in carboxysomes in response to energetic changes driven by sucrose export or sucrose feeding. Whereas the activation of sucrose export led to a locked high-Rubisco state and irresponsive carboxysome number to increased carbon demand, sucrose feeding prompted the dramatic disassembly of carboxysomes, accompanied by a decrease in the photosynthetic activity and pigment content. An increase in the accumulation of Reactive Oxygen Species (ROS) was concurred with carboxysome breakdown. Our results provide insight into the role of RpaA in responding to carbon energy demand by restructuring the carbon concentration mechanisms and the direct effect of ROS in this reorganization.

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Session 3.2: Physiology, Metabolism, and Bioenergetics

62 INVITED TALK

Physiological and phylogenetic analyses on diversity in the transcription factor LexA and SOS responses in cyanobacteria

Haruka Kubodera[1], Ryoko Hatsukawa[1], Aoi Ando[1], Ayumi Kizawa[2], **Yukako Hihara**[1]

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Abstract text

Although LexA is well-known as the SOS repressor in heterotrophic bacteria, significant functional diversity has been reported in cyanobacterial LexA. Namely, LexA of the filamentous *Anabaena* sp. PCC 7120 is involved in SOS gene expression [1], while that of the unicellular *Synechocystis* sp. PCC 6803 (S.6803) regulates broad cellular functions and lacks self-cleavage residues essential for SOS response [2, 3]. In order to clarify how LexA function and SOS responses are diversified among cyanobacteria, we analyzed UV responses of the above two species, together with an early-branching species *Gloeobacter violaceus* PCC 7421, and *Gloeothece citriformis* PCC 7424 belonging to the same subclade as S.6803 but having LexA with self-cleavage residues. Each species was subjected to UV-B or UV-C treatment by which doubling time increased to 1.5-1.9 times that of the untreated control. Time course analyses up to 180 min revealed that LexA dynamics and induction levels of *lexA* and *recA*, which are generally considered as target genes of LexA, are markedly different among species. Furthermore, RNA-seq analysis before and after 180 min of UV-C treatment revealed diversification in the strategy to cope with UV stresses. Based on the results of these physiological analyses together with phylogenetic analysis, cyanobacterial-specific evolution of LexA and SOS responses will be discussed.

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63 ORAL

Towards a deeper understanding of biofilm formation and its link to stress response in diazotrophic cyanobacterium *Anabaena* sp. PCC7120

Irene Olivan-Muro[1], Alodia Liesa-Delgado[1], Giulia Veronesi[2], Isabelle Michaud-Soret[2], Emma Sevilla[1], María F. Fillat[1]

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Abstract text

Bacterial biofilms are microbial communities that grow attached to a surface, embedded in a complex matrix composed of polymeric compounds such as exopolysaccharides. They are highly resilient and commonly induced in response to stresses, such as desiccation or depredation. Their undesired growth constitutes a threat to the environment and health, but biofilms also display biotechnological potential in fields like bioremediation and biofertilization. Cyanobacterial biofilms and their exopolysaccharides are of particular interest due to their negative charge, allowing positively-charged metal chelation, among other applications.

Model cyanobacterium *Anabaena* sp. PCC7120, typically cultured planktonically, is able to form biofilms. Their observation through scanning electron microscopy shows a distinct morphology compared to planktonic cultures, which do not appear embedded in a visible matrix. To advance in the understanding of phototrophic biofilm formation, we carried out comparative transcriptomic analysis of biofilm and planktonic *Anabaena* cells and observed vast alterations. The expression profile of biofilms partially overlaps with what is observed for drought response and adaptation to nitrogen deficiency, consistent with the relation of biofilms with stress resistance.

Biofilm growth assays under iron deprivation, saline stress, and nitrogen-fixing conditions unveiled that while the biomass obtained was slightly higher for the latter two, the absence of iron practically voided biofilm formation. Lastly, synchrotron radiation-X-ray fluorescence (SR-XRF) nano-imaging allowed us to map metal distribution on sessile and planktonic cultures at single-cell level, displaying differences in concentration of several orders of magnitude that showcase the relevance of metals on biofilm formation and strengthen their potential in heavy metal removal.

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Irene Olivan-Muro is supported by a FPU predoctoral grant.

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64 ORAL

Studying protein regulation in cyanobacteria and chloroplasts through interaction proteomics

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Abstract text

Metabolite-level regulation of enzymes provides rapid adaptability for microbes in response to changes in the environment. The stringent response, triggered by the secondary messenger ppGpp, leads to global regulatory adjustments in bacteria in response to nutrient starvation. The stringent response is conserved in cyanobacteria and plant chloroplasts and exhibits unique ppGpp regulation adapted to phototrophic growth. In this study we shed light on ppGpp interacting proteins through the high-throughput proteomics technique thermal proteome profiling in *A. thaliana* chloroplasts and the two cyanobacteria *Synechocystis sp*. PCC 6803 and *Synechococcus sp*. PCC 7942. Our proteomic screening suggests ppGpp to interact with multiple enzymes in the central metabolism, including transcription, translation, carbon fixation, the citric acid cycle, pyruvate metabolism, glycogen metabolism, and nitrogen regulation. To validate the interactions, we use recombinant expression and *in vitro* assays. Overall, our findings highlight the importance of understanding protein interactions for regulating enzyme activity and suggest a role for this in improving productivity of photosynthetic organisms.

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65 ORAL

Involvement of the ribosome-assembly GTPase EngA in the PipX interaction network and redox signalling

Asunción Contreras, Sirine Bibak, Antonio Llop, Raquel Cantos, Laura Fuertes, Trinidad Mata, Paloma Salinas

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Abstract text

The ribosome-assembly GTPase EngA, with a unique domain structure involving two tandemly repeated G domains (GD1_GD2_KH-like) is essential in all systems studied so far. In *Synechococcus elongatus* PCC7942 EngA also plays a role in acclimatization to environmentally relevant stress such cold or high light conditions, where the EngA levels rise. A sudden increase in light intensity turns EngA into a growth inhibitor, a response involving residue Cys122 of EngA, a cysteine conserved just in the cyanobacteria-chloroplast lineage which is part of the GD1-G4 motif NKCES.

PipX, a small protein exclusive of cyanobacteria that form complexes with the nitrogen regulators NtcA and PII according to the C/N ratio, was first connected to EngA in the Cyanobacterial Linked Genome (CLG), a web tool that generates flexible gene networks based on synteny (https://dfgm.ua.es/es/cyanobacterial-genetics/dclg/index.htm). EngA and PipX interact physically using, respectively, their GD1 and tudor-like domains. Genetic analyses in *Synechococcus elongatus* PCC7942 are consistent with the idea that PipX binds to EngA to slow down growth under stress conditions. This in turn makes EngA a candidate to be involved in the phenomenon of PipX toxicity, an arrest of growth observed whenever the PipX/PII ratio is increased.

We are trying to get additional insights into the regulatory functions and peculiarities of cyanobacterial EngA and its regulatory connections with PipX, paying particular attention to the role of Cys122. We will discuss the latest results, their implications and future research in the context of an updated regulatory model.

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66 ORAL

Phylogenetic Profiling Analysis of the Phycobilisome Revealed a Gene Encoding Novel State-Transition Regulator in *Synechocystis* sp. PCC 6803

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Abstract text

Phycobilisomes play a crucial role in the light-harvesting mechanisms of cyanobacteria, red algae, and glaucophytes, but the molecular mechanism of their regulation is largely unknown. In the cyanobacterium, *Synechocystis* sp. PCC 6803, we identified a gene *slr0244*, as a phycobilisome-related gene using the phylogenetic profiling analysis, a prediction method of gene function based on comparative genomics. To investigate the physiological function of the *slr0244* gene, we performed spectroscopic characterization of the *slr0244* mutants. The *slr0244* mutants did not exhibit normal state transition, a process in which phycobilisomes regulate the distribution of light energy between two photosystems in response to the changes in light conditions. The Slr0244 protein seems to act somewhere at or downstream of the sensing step of the redox state of the plastoquinone pool for the regulation of state transition. We assume that the *slr0244* gene is a novel state-transition regulator that integrates the redox signal of plastoquinone pools with that of the photosystem I-reducing side through the two conserved cysteines. Our study also showed the efficacy of the phylogenetic profiling analysis in predicting the function of cyanobacterial genes that have not been annotated so far.

Funding

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67 ORAL

Towards a mechanism of CO_2 uptake by NDH-1 complexes in cyanobacteria

Robert L Burnap, Zhifen Zhang, Ross M Walker

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Abstract text

Cyanobacteria have evolved a CO_2-concentrating mechanism (CCM), which efficiently supplies CO_2 to the photosynthetic mechanism functioning as a 'supercharger' for CO_2 by concentrating it within the cell, thereby saturating the active sites of the CO_2-fixation enzyme, Rubisco, thereby increasing the efficiency of photosynthesis. This includes specialized Type-1 NDH complexes that couple photosynthetic redox energy to CO_2 hydration forming the bicarbonate that accumulates to high cytoplasmic concentrations required for effective carbon fixation. We used a *Synechococcus* PCC7942 expression system to investigate the vectoral CO_2 hydration reaction by a putative Zn-centered carbonic anhydrase catalyst near the interface between CupB protein and the proton-pumping subunits of the NDH-1 complex. Redox coupling drives the directional CO_2-hydration reaction, allowing the build-up of HCO_3^- in the cytoplasm at the expense of redox energy in the form of Fd_{red}. A homology model has been constructed and most of the targeted conserved residues are in the vicinity of a Zn ion modeled to form the catalytic site of deprotonation and CO_2 hydration. A new model strain, designated DualB/ΔF1, and abundantly expresses only the CO_2 uptake NDH-1_4 complex and no NDH-$1_{1/2}$ nor NDH-1_3. Remarkably, the elimination of the gene for the respiratory/CEF subunit, NdhF1 in DualB/ΔF1, resulted in something unanticipated: the greater abundance of the desired NDH-1_4 complex beyond the levels in the overexpression strain alone (DualB) and the restoration of lost CEF in the ΔF1 strain The results are discussed in the context of a possible mechanism that couples transmembrane proton pumping to CO_2-hydration activity in the cyanobacterial NDH-1 complexes.

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68 ORAL

Inorganic carbon levels regulate growth via a novel SigC signalling cascade in cyanobacteria

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Abstract text

In cyanobacteria, elevated CO_2 concentrations down-regulate the carbon concentrating mechanism and accelerate photosynthesis and growth. Although many molecular mechanisms controlling the carbon concentrating mechanism have already been revealed, less is known how inorganic carbon regulates photosynthesis and growth. Here we report a novel signalling cascade linking CO_2 levels and growth in the model cyanobacterium *Synechocystis* sp. PCC 6803. Deletion of the *rpoZ* gene, which encodes the ω subunit of the RNA polymerase (RNAP), prevented the up-regulation of many photosynthetic and nutrient uptake genes normally induced by high CO_2 leading to low photosynthetic activity of ΔrpoZ cells in high CO_2. In parallel, the decreased expression of peptidoglycan synthesis genes resulted in the lysis of dividing ΔrpoZ cells in high CO_2. The high CO_2-sensitive phenotype of the ΔrpoZ strain was rescued by spontaneously occurring secondary mutations in the *ssr1600* gene. Bioinformatics, 3D structural modelling and biochemical analyses showed that the *ssr1600* gene encodes an anti-σ factor antagonist of the group 2 σ factor SigC and that Slr1861 functions as the cognate anti-SigC factor. Consequently, the observed high CO_2-sensitive phenotype of ΔrpoZ is due to the excess formation of RNAP-SigC holoenzyme complexes. In the suppressor mutants, drastically decreased Ssr1600 levels lowered the amounts of RNAP-SigC holoenzyme complexes to similar levels as in the control strain, leading to an almost normal transcriptome composition and growth in high CO_2. The data reveals that the SigC factor, the anti-SigC factor Slr1861 and the anti-SigC antagonist Ssr1600 form a growth-regulating signalling cascade in cyanobacteria.

Funding

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Session 3.3: Physiology, Metabolism, and Bioenergetics

69 ORAL

Deciphering the fitness advantage conferred by chromatic acclimation in marine *Synechococcus*

Louison Dufour[1], Francesco Mattei[2], Morgane Ratin[1], Anna E. Hickman[3], Stephanie Dutkiewicz[4], David M. Kehoe[5], Laurence Garczarek[1], **Frédéric Partensky**[1]

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Abstract text

Most *Synechococcus* cells thriving in open ocean waters possess phycobilisomes comprising both phycoerythrin-I and -II, which bind the green-light (GL) absorbing chromophore phycoerythrobilin (PEB) and blue-light (BL) absorbing chromophore phycourobilin (PUB) [1]. GL-specialists display a low PUB:PEB ratio (≈0.4), BL-specialists a high ratio (≈1.6), while other strains can dynamically modulate their ratio, a process known as Type-IV chromatic acclimation (CA4). Strikingly, CA4 appeared twice during evolution, once to transform GL-specialists and a second time BL-specialists into CA4-able cells [2]. Although the two resulting pigment types have a similar PUB:PEB ratio in GL (≈0.6) and BL (≈1.6), their ratios differ in intermediate blue-green light, and they display distinct acclimation dynamics after a shift from BL to GL or vice-versa [3]. Competition experiments between a GL-specialist, a BL-specialist and a CA4-able strain showed that while the GL-specialist wins in both low and high GL, the BL-specialist wins in low BL but is outcompeted by the CA4-able strain in high BL [Dufour et al., submitted]. Analyses of *Tara* Oceans metagenomes previously showed that the CA4 process has a global significance, since about 41.5% of *Synechococcus* cells along the transect were potentially capable of CA4 [1]. To further understand the fitness advantage conferred by CA4, we adapted the global ocean model Darwin and showed that BL-specialists are restricted to blue, nutrient-poor open ocean waters and GL-specialists to green, costal and upwelling, nutrient-rich waters, whereas CA4-able cells are more ubiquitous and show highest abundances in intermediate regions between the two specialists [Mattei et al. submitted].

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70 ORAL

High-resolution structures of Photosystem I assembly intermediates support the formation of PsaA-PsaB modules binding PsaC during their heterodimerization

Sadanand Gupta[1,2], Martin Tichy[1], Roman Sobotka[1], Josef Komenda[1], Andreas Naschberger[3]

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Abstract text

Photosystem I (PSI) is the membrane embedded pigment-protein complex performing the photochemical energy conversion during oxygenic photosynthesis in cyanobacteria, algae and plants. While the detailed structural and functional aspects of the mature PSI complex are well established, our understanding of the PSI biogenesis process remains incomplete. Our recent detailed study of the *Synechocystis* mutant lacking the large PsaB subunit revealed the formation of dimeric FLAG-tagged PsaA, which can be considered a prototype of the ancient PSI-like homodimeric reaction centre. Its high-resolution structure showed the presence of chlorophylls (Chl) including chlorophyll a', carotenoids, phylloquinone and also the small subunit PsaK implying its PsaB-independent incorporation. In order to block assembly process at the stage of PsaA/PsaB heterodimer, we also deleted PsaC and isolated the accumulated PSI assembly intermediate. Its detailed cryoEM analysis revealed the lack of all stromal subunits while all transmembrane ones were present with exception of PsaL. The complex binds almost all pigments and electron transport cofactors as the mature PSI including P700 Chls, phylloquinone, and F_X iron-sulfur cluster, which is however, incomplete most probably due to a locally modified conformation of PsaB caused by the absence PsaC. Our findings substantially modify existing PSI assembly models and are in agreement with the modular principle similar to PSII biogenesis. We propose that small membrane subunits can be attached to individual PsaA and PsaB before their heterodimerization, during which PsaC protein needs to be attached in parallel in order to get correct formation of the Fx cluster.

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71 ORAL

Exploring the function of alternative D1 proteins in Photosystem II reaction centre complexes in *Synechocystis* sp. PCC 6803

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Abstract text

The *psbA* gene encodes D1, a core protein of Photosystem II. The D1 protein is damaged during light-induced inactivation of Photosystem II, consequently degradation of photodamaged protein is balanced with synthesis of new copies of D1 in order to sustain activity. In cyanobacteria, the response to specific environmental conditions includes altered transcription of different *psbA* genes and in some cases exchanging D1 isoforms. Phylogenetic analyses identified two new groups of D1, $D1^{FR}$ and $D1^{INT}$ [1]. We investigated the performance of these proteins by introducing the corresponding *psbA* genes into *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis* 6803). The $D1^{FR}$ protein is part of the far-red-light-induced up-regulation of alternative copies of multiple proteins of Photosystem II. We show that in *Synechocystis* 6803, $D1^{FR}$ can be incorporated into Photosystem II centres that evolve oxygen at low rates but cannot support photoautotrophic growth. Modification of the *Synechocystis* 6803 D1 showed that changes in $D1^{FR}$ helix A produced a strain similar to the $D1^{FR}$ strain. Changes in helices B and C impacted Photosystem II function and compensating effects occurred with specific combinations of residue changes in helix C. In contrast, a *Synechocystis* 6803 mutant expressing only $D1^{INT}$ grew photoautotrophically but with increased susceptibility to high light. Consistent with this, the gene encoding $D1^{INT}$ was not part of a high light response in *Nostoc punctiforme* sp. PCC 73102. The $D1^{INT}$ is predominantly found in heterocystous cyanobacteria. Progress on investigating the role of the $D1^{INT}$ isoform will be presented.

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72 ORAL

Functional dependence between cell types is essential to maintain photosynthesis homeostasis in *Anabaena* sp. PCC 7120.

Mattia Storti[1], Massimo Mezzavilla[1], Francesca Arcudi[2], Enrique Flores[3], Tomas Morosinotto[1], **Giorgio Perin**[1]

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Abstract text

In diazotrophic cyanobacteria the assimilation pathways of atmospheric carbon (CO_2) and nitrogen (N_2) are biologically incompatible because C fixation via photosynthesis evolves molecular oxygen which inhibits nitrogenase. Nevertheless, the two processes must be tightly coordinated because N assimilation depends on reduced C skeletons generated by photosynthesis, whilst the latter depends on pigments and proteins synthetized after N assimilation. To overcome the biological constraint whilst enabling functional cooperation, diazotrophic cyanobacteria evolved either a temporal or spatial segregation between the two metabolisms. In this work we focused on the model diazotrophic cyanobacterium *Anabaena* sp. PCC 7120, in which C and N assimilation occur in two distinct cell types (i.e. vegetative cells and heterocysts, respectively VC and HC), and investigated the metabolic cooperation between them. We ran an extensive photosynthetic characterization of cells exposed to systematic variations of metabolic inputs (e.g., light and CO_2) and compared diazotrophic vs. nitrogen replete conditions. We observed that the biological system responds to the availability of metabolic inputs by modulating both composition and activity of photosynthetic components, in particular tuning the contribution of complementary electron pathways. We observed the nitrogen fixation activity of HC is favoured when the photosynthetic activity of VC is larger, underlining a finely tuned metabolic dependence of HC on VC. In a mutant strain affected in the vehiculation of fixed N from HC to VC [1] instead we observed a substantial re-modulation of the photosynthetic response of VC vs the WT strain, indicating the metabolic dependence between cell types is mutualistic.

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73 ORAL

Extracellular electron transfer in *Synechocystis* sp. PCC 6803 under photomixotrophy

Laura T. Wey, Mithila Ray, Tuomas Huokko, Lauri Nikkanen, Yagut Allahverdiyeva

University of Turku, Turku, Finland

Abstract text

A variety of cyanobacteria exhibit a behaviour called ‘exoelectrogenesis’, whereby electrons predominantly derived from photosynthesis are exported outside the cell under illumination.[1] Even in darkness, there exists some level of current generation, attributed to respiration or other metabolic dark reactions.[2] While strategies aimed at boosting exoelectrogenesis for biotechnological purposes have involved the addition of glucose to the electrolyte in electrochemical cells, the long-term implications of these interventions, including the role of acclimatisation as cyanobacteria undergo a metabolic shift to photomixotrophy, has not been explored yet. Moreover, the fundamental bio(photo)electrochemical mechanisms governing exoelectrogenesis, and its interaction with the storage of excess carbon in the form of glycogen, which serves as an intracellular electron sink, remain largely obscure. In this study, we investigated the exoelectrogenesis of *Synechocystis* sp. PCC 6803 cultivated under various trophic conditions using biofilm photoelectrochemistry, as well as photosynthetic activity before and post-harvesting current. Cells grown under photomixotrophy exhibited elevated dark current, consistent with heightened respiration. Peak current output during the first minute of illumination was two orders of magnitude greater in photomixotrophically grown cells compared to photoautotrophically grown cells. However, with prolonged illumination, the current became increasingly cathodic, up to 400-fold more negative. These observations suggest that ‘exoelectrogenesis’ represents a bidirectional process, modulated by cyanobacteria under varying trophic conditions in response to organic carbon availability. By advancing our comprehension of this behaviour in *Synechocystis* sp. PCC 6803, we can rationally harness cyanobacteria for biotechnological applications, including biophotovoltaic devices for sustainable electricity generation and intracellular reactions powered by photosynthesis.

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The CyanoCyc Cyanobacterial Web Portal

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Lisa R Moore[1], Ron Caspi[1], Douglas A Campbell[2], John R Casey[3], Sophie Crevecoeur[4], David J Lea-Smith[5], Bin Long[6], Naaman M Omar[7], Suzanne M Paley[1], Nicolas M Schmelling[8], Alejandro Torrado[9], Jonathan P Zehr[10]

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Abstract text

CyanoCyc.org [1] is a web portal that integrates an exceptionally rich collection of information about cyanobacterial genomes with an extensive suite of bioinformatics tools. It was developed to address the needs of the cyanobacterial research and biotechnology communities. CyanoCyc currently contains 277 annotated cyanobacterial genomes obtained from NCBI’s RefSeq and are supplemented with computational inferences including predicted metabolic pathways, operons, protein complexes, and orthologs; and with data imported from external databases, such as protein features and Gene Ontology (GO) terms imported from UniProt. Five of the genome databases have undergone manual curation, the most recent of which is the *Synechocystis* sp. PCC 6803 substr. Kasuza. More than a dozen cyanobacteria experts contributed to the curation by integrating information from more than 1,765 published articles. As part of the BioCyc collection of genome databases, CyanoCyc has access to all the bioinformatics tools which encompass genome, metabolic pathway and regulatory informatics; omics data analysis; and comparative analyses, including visualizations of multiple genomes aligned at orthologous genes, and comparisons of metabolic networks for multiple organisms. I will provide a separate tutorial on how to use these tools. CyanoCyc is a high-quality, reliable knowledgebase that accelerates scientists’ work by enabling users to quickly find accurate information using its powerful set of search tools, to understand gene function through expert mini-reviews with citations, to acquire information quickly using its interactive visualization tools, and to inform better decision-making for fundamental and applied research.

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Ca^{2+} signaling in filamentous heterocyst forming cyanobacteria

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Abstract text

Free calcium (Ca^{2+}) ions can be highly toxic as they can precipitate phosphate ions, which are important for various metabolic pathways. Therefore, cyanobacteria regulate their intracellular Ca^{2+}-concentration $[Ca^{2+}]_i$ via pumps, channels and calcium binding proteins. In multicellular cyanobacteria, Ca^{2+} signaling also plays an important role in heterocyst differentiation [1]**.** Shortly after nitrogen depletion, a condition that triggers heterocyst differentiation, a transient increase of $[Ca^{2+}]_i$ in pro-heterocysts have been reported [1]. In the filamentous heterocyst forming model organism *Nostoc* sp. PCC 7120 two calcium binding proteins have been identified: CcbP (cyanobacterial calcium binding protein) [1] and CSE (Ca^{2+} Sensor EF-hand) [2]. CcbP is mainly known for its buffer property in capturing free Ca^{2+} ions. CSE binds Ca^{2+} via two characteristic Ca^{2+} sensor EF hand domains [2]. The CSE appears unfolded in the absence of calcium and undergoes a strong conformation change upon Ca^{2+} binding. CSE is strongly downregulated during nitrogen depletion [2,3]. Although there is a strong connection between Ca^{2+} signaling and heterocyst differentiation, the specific functions of both calcium binding proteins, CcbP and CSE, remain elusive. Our aim is to further investigate the role of calcium signaling and calcium homeostasis in multicellular cyanobacteria with respect to heterocyst formation and photosynthesis. Therefore, we investigate the characteristics of knockout or overexpressing mutants of those proteins in *Nostoc* sp. PCC 7120 regarding cell-cell-communication, growth under nitrogen limitation and heterocyst formation.

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76 POSTER

Regulation of the Carbon Flux in *Synechocystis* Using the PGAM-PirC Switch

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Abstract text

For a sustainable bioeconomy, CO_2 neutrality is pivotal. Therefore, *Synechocystis* sp. PCC 6803 is studied concerning the metabolic flux to broaden the bioengineering platform for biotechnological applications. An important control point of the carbon flux is the 2, 3-bisphosphoglycerate-independent phosphoglycerate-mutase (PGAM), which converts the first CO_2 fixation product 3-phosphoglycerate to 2phosphoglycerate. This reaction directs carbon flow towards lower glycolysis for the production of amino acids, fatty acids, biopolymers, such as polyhydroxybutyrate (PHB) and more.

PGAM activity is inhibited through binding of the small protein PirC, which itself is repressed by the PII protein, a signal integrator of carbon and nitrogen [1]. Furthermore, based on the *pirC* mutation, a strain was constructed that can produce up to 80 % PHB per cell dry mass under nitrogen starvation [2].

Our work aims to use this PGAM-PirC key hub to direct the metabolic flux towards lower glycolysis and the production of PHB and other feedstock chemicals. Therefore, different promoters are tested to adjust both PGAM expression and repression by PirC. The level of PGAM, glycogen levels, PHB production and other metabolites will be analyzed to gain insight into how the carbon flow can/will be influenced in the different strains. Preliminary work revealed that levels of glycogen are lower, whereas PHB amounts are higher under nitrogen starvation in a strain with constitutively overproduced PGAM.

Further analysis will show the role PGAM is playing in the metabolism. By tuning the PGAM-PirC switch, a platform will be established to redirect carbon flow for enhanced valuable chemical production.

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New Mechanisms Regulating the CO_2-Concentrating Mechanism in Cyanobacteria

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Abstract text

Cyanobacteria adapted to the present day low availability of inorganic carbon (C_i), i.e., CO_2 and HCO_3^-, by the evolution of the CO_2-concentrating mechanism (CCM) that accumulates bicarbonate inside the cell using different uptake systems. The CCM activity largely avoids the accumulation of toxic photorespiratory metabolites and adapts carbon fixation efficiency to different Ci levels.

In *Synechocystis* sp. PCC 6803 and other cyanobacteria, the SbtA protein represents the major low Ci-induced HCO_3^- transporter, which is not only regulated transcriptionally but also at the protein level via the P_{II}-like regulator SbtB. SbtB itself seems to integrate signals of Ci availability via second messengers as well as redox regulation and most likely phosphorylation. We aim to elucidate the regulatory network around SbtB further.

For this purpose, SbtA with or without SbtB was reintroduced in the multiple knockout mutant Δ5. This mutant does not possess any C_i uptake machinery and requires elevated Ci conditions to grow. Reintroducing SbtA rescues viability at ambient air and permits a detailed analysis of SbtA functions without interference by other Ci uptake systems. Examining this mutant, we are able to analyse the different roles of SbtB and potential additional regulators on SbtA functions and on other targets.

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German Academic Scholarship Foundation

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The second messenger c-di-AMP controls natural competence via ComFB signaling protein

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Abstract text

Natural competence is a conserved mechanism of horizontal gene transfer that permits massive genetic variation and genomic plasticity via uptake of extracellular DNA. Natural competence requires a contractile pilus system and an assemblage of competence-accessory proteins. Here, we provide evidence that the pilus biogenesis and natural competence in cyanobacteria are regulated by the second messenger c-di-AMP. The c-di-AMP-free (*DdacA*) mutant showed significantly lower transformation efficiency than the WT cells. Transcriptome and proteome analysis revealed a strong downregulation of specific genes and proteins involved in pilus biogenesis and DNA uptake in the *DdacA* mutant. These proteins include PilT1, an essential motor required for retraction of type IV pilus. In contrast to WT cells, transmission electron microscopy revealed that *dacA* cells possess only thick pili with hyperpiliation phenotype similar to the non-competent *pilT1* mutant. Furthermore, we show that the competence factor B signaling protein (ComFB) is a novel c-di-AMP-receptor protein, widespread in bacterial phyla. A *DcomFB* mutant was created to test if ComFB is playing a role in natural competence, and like *DdacA* mutant, it showed reduced transformation efficiency. Our findings further support that natural competence depends on c-di-AMP signaling and is controlled by a pathway that involves ComFB as a c-di-AMP receptor.

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Being lazy is not always bad: The effects of c-di-AMP signalling on glutamine toxicity in cyanobacteria

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Abstract text

Cyanobacteria exhibit a distinctive lifestyle as photoautotrophic bacteria, necessitating rapid responses to environmental shifts that jeopardize their carbon/nitrogen (C/N) balance. To maintain homeostasis, cyanobacteria employ cyclic nucleotide second messengers, such as cyclic di-adenosine monophosphate (c-di-AMP). While mechanistic aspects of C-regulation have been elucidated [1], N-regulation remains unexplored. In the search for novel c-di-AMP targets, we identified the glutamine (Gln) transporter BgtAB as a c-di-AMP target. Gln was reported to be toxic for cyanobacteria [2], but its mode of action remained elusive. Surprisingly, the c-di-AMP-free mutant (Δ*dacA*) was able to grow on toxic Gln concentrations and use it as sole N-source, while wildtype (WT) died. We found that Δ*dacA* exhibited impaired Gln uptake compared to WT. Here, targeted metabolomics unveiled an overaccumulation of arginine and its intermediates in WT cells growing on Gln. In contrast, Gln metabolization was severely hampered in Δ*dacA*, highlighting a role for c-di-AMP in controlling N-metabolism. We further show that the high Gln uptake in WT induces *dacA* gene expression and c-di-AMP overproduction, which is known to be toxic for bacteria [3]. Overall, our findings suggest that c-di-AMP positively regulates BgtAB, and the resulting Gln uptake triggers c-di-AMP overproduction, establishing a lethal feedback loop. This work presents the first mechanistic explanation of Gln toxicity in cyanobacteria due to c-di-AMP signalling and showcases the role of c-di-AMP in N-metabolism.

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80 POSTER

Novel Insights into Overflow Metabolism in *Synechocystis*

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Abstract text

Glycogen is a storage compound produced by many bacteria to cope with temporary shortage of nutrients. This is crucial for survival in a fluctuating environment [1]. *Synechocystis* relies on glycogen as carbon and energy storage to survive dark periods and transient nutrient deprivation. During nitrogen starvation, inhibition of glycogen synthesis triggers overflow metabolism, leading to increased pyruvate and 2-oxoglutarate release. Unable to divert excess energy and carbon into glycogen synthesis, carbon flux is directed towards lower glycolysis, and surplus metabolites are excreted [2]. In cyanobacteria, the key control point for regulating carbon flow toward lower glycolysis is the PirC-modulated PGAM reaction. Absence of PirC increases carbon flux towards lower glycolysis, resulting in an increase in intracellular pyruvate and 2-oxoglutarate concentrations during nitrogen starvation [3].

Here we aim to investigate if the absence of PirC also results in overflow metabolism. Additionally, we explore whether concurrent deletion of PirC and glucose-1-phosphate adenylyltransferase (GlgC), the enzyme catalyzing the first step of glycogen synthesis [2], amplifies metabolite excretion.

Surprisingly, the PirC-deficient strain exhibited no overflow metabolism, contrasting previous findings of elevated intracellular pyruvate and 2-oxoglutarate levels. Investigation of the *pirC-glgC*-double-mutant revealed no additive effect. Overflow metabolism could solely be attributed to the deletion of *glgC* and the resulting inability to generate glycogen. A systematic comparison of intracellular and extracellular metabolites confirmed that the exometabolome is not simply a reflection of intracellularly increased metabolites. This indicates that *Synechocystis* uses specific excretion mechanisms instead of simple overflow mechanics to release metabolites into the medium.

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Regulation of cyanobacterial glucose-6-phosphate dehydrogenase by the redox sensor OpcA

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Abstract text

The oxidative pentose phosphate (OPP) pathway is a major carbon catabolic route that generates reducing power and metabolic intermediates essential for biosynthetic processes (1). Additionally, the first two reactions of this pathway form the OPP shunt, replenishing the Calvin-Bassham cycle under specific conditions [2]. Glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the first and rate-limiting reaction of this pathway. In photosynthetic organisms, G6PDH is redox-regulated to allow fine-tuning and to prevent futile cycles while carbon is being fixed [3]. In cyanobacteria, regulation of G6PDH requires the redox protein OpcA, but the underlying molecular mechanisms behind this allosteric activation remain elusive [4]. Here, through enzymatic assays and in vivo interaction analyses, we demonstrate OpcA’s binding to G6PDH under various environmental conditions. However, complex formation enhances G6PDH activity when OpcA is oxidized and inhibits it when OpcA is reduced. Cryogenic electron microscopy reveals that OpcA binds the G6PDH tetramer, inducing conformational changes in its active site. OpcA’s redox sensitivity arises from intramolecular disulfide bridge formation, influencing G6PDH’s allosteric regulation. In vitro assays suggest that the level of G6PDH activation correlates with the number of bound OpcA molecules, facilitating delicate fine-tuning [5]. These findings uncover a novel molecular mechanism governing OPP pathway regulation in cyanobacteria, shedding light on the intricate control of cellular metabolism in photosynthetic organisms.

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Regulatory implications of antisense transcription in *Nostoc* sp. PCC 7120

Alicia M. Muro-Pastor[1], Manuel Brenes-Alvarez[2], Isidro Álvarez-Escribano[1], Belén Suárez-Murillo[1], Sara B. Hernández[1], Agustín Vioque[1]

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Abstract text

Antisense transcription is consistently observed in bacterial transcriptomes, and accumulating evidence suggests regulatory consequences on gene expression. The recent definition of the transcriptome of the model heterocyst-forming cyanobacterium *Nostoc* (*Anabaena*) sp. PCC 7120 [1] reveals that antisense transcription is also prevalent in this organism, in which about 65% of the transcriptional units contain regions in antisense orientation to other transcripts. Overlapping transcripts are commonly produced in the case of adjacent genes that are transcribed in a head-to-head or tail-to-tail disposition, invading the transcriptional space of adjacent genes. This observation raises the possibility that specific physiological conditions regulating one of the overlapping partners may have consequences on the expression of an adjacent gene transcribed in the opposite direction. In addition to overlapping mRNAs, another category of entirely non-coding, bona fide antisense RNAs is also observed. We have analyzed antisense transcription in *Nostoc* with a focus on the identification of nitrogen-regulated antisense transcripts. The observation that transcription of some of these antisense RNAs is under control of the global regulators NtcA or HetR, or even produced exclusively in heterocysts, raises the possibility that they have some function specifically under nitrogen deficiency and/or in this specialized cell type. We have already analyzed the possible physiological implications of some heterocyst-specific antisense RNAs that would contribute to metabolic remodeling in these specialized cells. In addition, several cases of overlapping nitrogen-regulated antisense transcripts are currently under analysis.

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Metalloregulator Zur / FurB of *Anabaena* sp. PCC7120 beyond zinc homeostasis: impact on metal trafficking, biofilm formation and heterocyst development

Irene Olivan-Muro[1], Cristina Sarasa-Buisan[1], Jorge Guío[1], Jesús A. Arenas-Busto[2], Emma Sevilla[1], María F. Fillat[1]

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Abstract text

Zinc is a key element in processes such as gene regulation and redox homeostasis, as well as for the enzymatic activity of numerous proteins. In the diazotrophic filamentous cyanobacterium *Anabaena* sp. PCC7120, the metalloregulator FurB/Zur is the main controller of the uptake and transport of zinc. Through comparative transcriptomics of a *zur* deletion mutant with the parent strain, this work has unveiled links of Zur to the homeostasis of various other metals and an unexpected altered expression of genes related to pathways such as carbohydrate metabolism or heterocyst development. The ability of Zur to bind *in vitro* to the promoter regions of a selection of genes has been studied through electrophoretic mobility shift assays (EMSA), leading to the identification of novel regulatory targets.

A significant number of genes differentially expressed in response to *zur* deletion are related to desiccation response, according to previous works. One mechanism of resistance against this stress is the formation of biofilms, which are microbial communities embedded in a matrix with a relevant polysaccharidic component. This process was found to be affected by the expression level of Zur: a deletion strain yields the least biofilm biomass, while Zur overexpression increases biofilm formation. Furthermore, *zur* deletion leads to a lower heterocyst differentiation frequency under nitrogen deficiency and possible underdevelopment, based on a reduced alcyan blue staining of envelope polysaccharides. We suggest that Zur may regulate the expression of proteins involved in the synthesis and transport of external polysaccharides, thus influencing heterocyst development and biofilm formation.

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CikA as a multifunctional light signal transducer in a cyanobacterium

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Abstract text

The CikA protein in *Synechococcus* sp. PCC 7942 is known for its role in resetting the circadian clock by sensing light indirectly via its pseudo-receiver domain, in response to redox signals from the photosynthetic electron transport chain. Here, we investigated the role of the CikA homolog in phototaxis and regulation of photosynthesis in *Synechocystis* sp. PCC 6803 by constructing a series of mutants, including a *cikA* null mutant, a heterologous *cikA* overexpressor and mutants expressing truncated forms of the protein. Deletion of *cikA* in *Synechocystis* results in loss of directionality in phototaxis assays and a decrease in chlorophyll content and photosystem expression.

Δ*cikA* mutants move randomly in single cell motility assays and display no movement on average in the colony motility assays, indicating that CikA is specifically involved in directional control of motility. PilA1 staining and fluorescence microscopy show normal pilus extension and retraction dynamics in Δ*cikA*, confirming that the basic activity of Type IV pilus system is unaffected. Our localization studies reveal the presence of CikA in both plasma membrane and thylakoid membrane fractions, as well as the cytoplasm. Yeast two hybrid experiments suggest an interaction between CikA and PilB, the Type IV pilus extension motor. These results suggest the possibility of direct interaction between CikA and the Type IV pilus apparatus. CikA may transmit directionality signals to the Type IV pili.

Our studies established that CikA protein is a multifunctional signalling molecule, which we presume to control diverse aspects of physiology in connection with phototaxis and photosynthesis.

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Regulatory networks performed by FUR proteins and NtcA in the cyanobacterium *Anabaena* sp. PCC 7120

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Abstract text

FUR proteins (FurA/Fur, FurB/Zur and FurC/PerR) in *Anabaena* sp. PCC7120 are a family of transcriptional regulators involved in the control of highly important metabolic processes such as the maintaining of metal homeostasis, the regulation of oxidative stress response and the adaptation to nitrogen starvation. Previous RNAseq analyses comparing strains in which FUR proteins were misregulated in the *Anabaena* wild type strain, together with gel-shift assays (EMSA), unveiled a broad panel of genes directly regulated by these proteins. Among them, genes encoding several regulatory proteins also showed altered expression indicating a second level of regulation in the regulon of FUR proteins.

In this work we have identified nearly 30 genes with regulatory functions directly regulated by FUR proteins, including transcriptional regulators, two-component systems, serine-threonine kinases and sigma factors, revealing that FUR paralogues are cornerstones of novel regulatory networks in cyanobacteria. In addition, taking into account the role of FUR proteins in the regulation of nitrogen metabolism, the crosstalk between FUR and NtcA regulatory networks was studied. We have found that several members of these networks are also regulated by the global nitrogen regulator NtcA, suggesting that these networks could be involved in orchestrating responses to nitrogen deficiency. Taking together, these results unveiled the existence of a complex network in *Anabaena* in which regulatory proteins hierarchically below FUR or NtcA proteins can be controlling the expression of several genes and connecting the cyanobacterial responses to different stress situations.

Funding

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Diversity and function of carbonic anhydrases in cyanobacteria

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Abstract text

The inorganic forms of carbon, CO_2 and HCO_3^-, are essential metabolites in all organisms. The abiotic conversion between these two forms is slow and therefore a number of evolutionary unrelated carbonic anhydrases have evolved to catalyze the reaction: $CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$.

Many functions of carbonic anhydrase are well described in both photosynthetic and non-photosynthetic organisms. However, genome sequence analyses reveal that most microbes encode multiple carbonic anhydrases, of which only some have an assigned function.

The predominant types of carbonic anhydrases in cyanobacteria belong to the α, β, and γ classes. The carboxysomes contain one or two β or γ class carbonic anhydrases, but the cellular location and function of most other carbonic anhydrases is not clear. Here we present analyses of the distribution, phylogeny, and functions of carbonic anhydrases in cyanobacteria. Our research interests encompass the function and significance of all cellular and extracellular carbonic anhydrases in cyanobacteria.

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Environmental regulation of PipX complexes in *Synechococcus elongatus* PCC7942

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Abstract text

PipX is a small protein conserved in cyanobacteria that forms alternative complexes with NtcA and PII, both involved in carbon/nitrogen homeostasis, providing a mechanistic link between PII signalling of carbon/nitrogen and energy levels and the NtcA-regulated expression of nitrogen assimilation genes. PipX can also bind to the ribosome-assembly GTPase EngA and, in the presence of PII, to the transcriptional regulator PlmA. Furthermore, genetic and synteny studies suggest the existence of additional PipX targets. However, the regulatory complexity of the PipX interaction network makes it difficult to study the physiological significance and the contribution of environmental factors to the formation of the different types of PipX complexes. These depend on the relative levels of the different partners and of the effector molecules binding to them, which are not always known. In this intricated context, maintaining the intracellular environment as untouched as possible is important to confirm candidate interactions and gaining further insights into already known interactions with PipX. We will discuss our results using the NanoBiT complementation system to analyze regulation of complex formation within the PipX interaction network in *Synechococcus elongatus* PCC7942.

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Establishing a Minimal Endosymbiotic Metabolism in Cyanobacteria

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Abstract text

During primary endosymbiosis, a proto-cyanobacterium was engulfed by a eukaryotic cell and became the precursor of the plastids present in all recent eukaryotic photosynthetic organisms. This symbiosis likely developed via linkage of the endosymbiont's and host's energy metabolisms. To recapitulate key steps in the event of primary endosymbiosis, we test the hypothesis that metabolic connectivity was initiated by the loss of the endosymbiont's ability to store carbon for energy production. Cyanobacteria in which glycogen synthesis is disrupted via deletion of the ADP-glucose pyrophosphorylase (GlgC) are unable to store carbon and energy over extended periods of time. This results in compromised growth during periods of darkness and export of excess carbon in the light. As per the *ménage-à-trois* (MAT) hypothesis, this is a central step in enabling the development of a stable endosymbiotic relationship[1]. By introducing hexose-phosphate transporters and ATP/ADP-antiporters similar to those found in recent plastids into the D*glgC* mutants of the model cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* UTEX 2973, we seek to control the metabolic overflow reactions and enable rescue of the impaired growth during dark cycles by external energy supply. IC-MS measurements and ^{13}C-labelling will be used to illumine metabolic changes during diurnal growth and will thus allow identification of metabolites involved in establishing metabolic connectivity.

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DNA methylation, a regulator of keystone enzyme of chlorophyll biosynthesis in *Synechocystis* sp. PCC 6803

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Abstract text

In bacteria epigenetics regulates DNA repair, cell replication and gene expression by DNA methylation provided by DNA methyltransferases (MTases). *Synechocystis* sp. PCC 6803 harbors at least five functional MTases [1]. The aim of this project is to reveal the purpose and impact of the genomic DNA methylation in cyanobacteria.

Mutants lacking the MTase M.Ssp6803II (*sll0729*) possesses an altered phenotype. The cells are decreased in size, contained less chlorophyll a and are sensitive for UV exposure. However, this phenotype is unstable, after long-term cultivation of the Δ*sll0729* strain single clones displaying wild-type-like phenotype [2]. Whole genome sequencing of this suppressor clones revealed a single nucleotide exchange in the promoter of *slr1790*. This gene encodes the protoporphyrinogen IX oxidase (HemJ), a keystone enzyme of the chlorophyll biosynthesis.

Transcriptome data of the original Δ*sll0729* clones showed significantly reduced amounts of *slr1790* transcripts. HPLC measurements revealed accumulation of phototoxic chlorophyll precursors. To verify that the mutated *slr1790* promoter is responsible for the wild-type like phenotype of the Δ*sll0729* suppressor clones, *slr1790* promoter/Δ*sll0729* double mutants were generated. Physiology and chlorophyll precursor accumulation of these strains support the *slr1790* promoter hypothesis. The native promoter double mutant shows similarly high levels of chlorophyll precursors like the Δ*sll0729* single mutant. The Chlorophyll precursors in the mutated promoter double mutant are on a wild-type-like level.

Induced expression systems shall enable complementation of Δ*sll0729*. In summary, we propose altered expression of *slr1790* as reason for Δ*sll0729* phenotype, suspecting indirect regulation by promoter methylation due to M.Ssp6803II.

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90 POSTER

Hmx1 and Hmx2 enable constitutive manganese uptake in cyanobacteria

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Abstract text

Manganese (Mn) is an essential micronutrient with special importance for photosynthetic organisms due to its function in water oxidation. To avoid critical imbalances, Mn needs to be specifically and timely allocated to the place where it is needed, and sequestered in a safe storage place if accumulating in excess. That is, key to Mn homeostasis is thecontrolled uptake from the environment and appropriate intracellular distribution of the metal. We have identified the Unknown Protein Family (UPF) 0016 as a new group of Mn transporter. The founding member Mnx in the model cyanobacterium *Synechocystis* sp. PCC6803 facilitates Mn transport from the cytoplasm into the thylakoid lumen and is involved in Mn delivery to Photosystem II as well as sequestration of excess Mn [1]. Mnx-type transporters were endosymbiontically conveyed to plants and are here constituting efficient Mn uptake systems at the chloroplast envelope and the thylakoid membrane [2]. Two additional UPF0016 members, Hmx1 and Hmx2, are exclusively encoded in cyanobacterial genomes. They function as the long sought constitutive Mn importer at the plasma membrane. Hmx1/Hmx2 likely coevolved with the internalization of the water oxidation complex [3]. Here, results of the functional and phylogenetic characterization of Hmx1 and Hmx2 will be presented and discussed in terms of their function in Mn homeostasis.

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Antisense regulation of glutamine synthetase in *Nostoc* sp. PCC 7120

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Abstract text

Glutamine synthetase (GS) is a key enzyme for nitrogen assimilation and maintenance of the C/N balance and is subjected to strict regulation in all bacteria. In cyanobacteria, glutamine synthetase expression is under control of the transcription factor NtcA, that operates global nitrogen regulation. Additionally, post-transcriptional regulation of GS is operated by protein-protein interaction with GS inactivating factors (IFs). According to the recent definition of the transcriptome of the model heterocyst-forming cyanobacterium *Nostoc* sp. PCC 7120 [1] the transcriptional units covering the *glnA* gene (encoding GS) and the *gifA* gene (encoding IF7A) constitute one example of two overlapping, strongly regulated transcripts that are produced in antisense orientation. Because the *glnA* and *gifA* transcripts overlap tail-to-tail, the 3' end of the *gifA* transcript extends antisense over the *glnA* transcript. Therefore, we considered the possibility that transcription of this antisense RNA could affect the accumulation and/or translation of the *glnA* mRNA. By increasing the levels of such antisense RNA either in *cis* or in *trans* we could demonstrate that the amount of GS can be modulated by the presence of the antisense RNA. The *gifA* transcript would constitute a dual RNA with two regions, a 5' protein-coding region, encoding IF7A, and a 3' untranslated region acting as an antisense to the *glnA* mRNA. The tail-to-tail disposition of the *glnA* and *gifA* genes observed in many cyanobacterial strains from the Nostocales clade suggests the prevalence of such antisense RNA-mediated regulation of GS in this group of cyanobacteria.

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Funding

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Physiological Responses of the two Cyanobacterial strains *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 11901 under Nitrogen Starvation

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Abstract text

Cyanobacteria are photoautotrophic prokaryotic Gram-negative organisms that exhibit ubiquity across diverse environments. In addition to their ability of fixing atmospheric CO_2, these microorganisms produce various secondary metabolites with pharmaceutical and industrial benefits. In nature, one of the most common growth constraints is limitation of nitrogen supply both in marine and terrestrial ecosystems. Nitrogen regulation in cyanobacteria is mediated by the transcriptional regulator NtcA, in conjunction with its co-activator PipX and by the ubiquitous P_{II} signaling protein. In this study, two unicellular cyanobacterial strains, *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) and the newly isolated, fast growing strain *Synechococcus* sp. PCC 11901 (hereafter *Synechococcus*) are compared based on their response to nitrogen starvation conditions; the process known as “chlorosis”. Under optimal growth conditions, *Synechocystis* displays a doubling time of 12h whereas the fast-growing *Synechococcus* of only 2-3h of doubling time. During chlorosis, in both species the cellular metabolism experiences a reduction in flux, marked by diminished protein synthesis and anabolic processes. Moreover, the photosynthetic apparatus is compromised, evident in the absence of chlorophyll α and phycobiliproteins, resulting in a transition from a blue-green to yellow color in *Synechocystis* and white in *Synechococcus.* Remarkably, *Synechococcus* also displays a shift in the morphology from rod-shaped to spherical. Chlorotic cells accumulate glycogen in a form of carbon and energy source, subsequently utilized during cellular resuscitation. Transcriptomic analysis of chlorotic cells in *Synechocystis* reveals heightened expression of the *nblA* gene, responsible for phycobilisome degradation, and of genes associated with glycogen metabolism.

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The Role of Photosystem I Oligomerisation in Far-Red Photosynthesis

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Abstract text

Some cyanobacteria are capable of using far-red light (FRL; >700 nm) for oxygenic photosynthesis. Using low energy light requires a photoacclimation process during which core proteins of the photosystems are modified, red-shifted chlorophylls, chl *d* and chl *ƒ* are synthesised and the oligomeric state of photosystem I (PSI) changes [1]. However, the impact of the oligomeric state on the functionality of photosynthesis in FRL remains largely unknown.

Here, we show that the FRL variant of the PSI protein PsaL is essential for trimerisation in FRL, but not in white light (WL). We deleted either the WL or FRL variant of *psaL* in *Chroococcidiopsis thermalis* PCC 7203 and characterised the resulting FRL monomeric PSI mutant by native PAGE, growth analysis, HPLC, steady state and time resolved spectroscopy. Our results indicate the loss of one chl *ƒ* molecule, presumably in proximity to the reaction centre, showing that the trimeric PSI and PsaL protein environment contribute to the stabilisation of the f790 chlorophyll [2].

We further describe two new cyanobacterial strains which are unique among all known FRL cyanobacteria by lacking the FRL variants of their PSI core proteins, as well as PsaL [3]. Native PAGE confirms that no trimeric PSI is present in FRL. Moreover, our findings reveal a naturally lower chl *ƒ* content in these strains as well as the absence of the f790 pool.

We conclude that changes in supramolecular protein organisation and amino acid environment can lead to the loss of chl *ƒ* in PSI.

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94 POSTER

Unveiling the CalB regulon in the cyanobacterium *Anabaena* sp. PCC 7120

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Abstract text

Cyanobacterial AbrB regulators (commonly known as cyAbrBs) are members of the AbrB (antibiotic resistance protein B) family of transcriptional regulators. All cyanobacterial genomes contain at least one *cyabrB* gene and, in almost all species, there are frequently two copies, which are classified into two different clades, A and B. The heterocyst forming cyanobacterium *Anabaena* sp. PCC 7120 contains two CyAbrB regulators, known as CalA and CalB. Previous works found several direct targets for CalA involved in oxidative stress response, cell division, hydrogen metabolism or heterocyst differentiation, but targets and roles for CalB were not defined.

In the present work, we have obtained the RNAseq profile of a *calB* deletion strain and we have found that CalB is a repressor that controls the expression of genes involved in carbohydrate metabolism, nitrogen metabolism, heterocyst differentiation and oxidative stress response, among others. Specifically, CalB seems to be involved in the control of the production of fructose-containing oligosaccharides, the synthesis of heterocyst envelopes and the cellular responses to H_2O_2. Overall, our work unravels CalB as an important global regulator able to integrate different cellular processes.

Funding

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95 POSTER

Purification and characterization of two transcriptional regulators of the FurC (PerR) regulatory network in *Anabaena* (*Nostoc*) sp. PCC 7120

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Abstract text

The FUR (Ferric Uptake Regulator) family in *Anabaena* (*Nostoc*) sp. PCC 7120 consists of three paralogs: FurA (Fur), FurB (Zur) and FurC (PerR), which are considered global regulators in this cyanobacterium. Transcriptomic analyses of a *furC*-overexpressing strain compared to the WT *Anabaena* under nitrogen starving conditions unveiled that FurC is directly involved in the modulation of the expression of nitrogen metabolism and heterocyst differentiation genes. Noteworthy, the direct targets of FurC encompass genes of various functional categories, including a group of previously uncharacterized transcriptional regulators [1]. As a result, FurC extends its influence indirectly through other transcriptional regulators or two-component systems, generating a comprehensive transcriptional regulatory network.

In this work, Alr1976 and All0345, two transcriptional regulators belonging to this regulatory network, have been purified and characterized. Bioinformatic studies were performed to gain information about their biochemical and structural properties. A purification protocol was optimized for both regulators, making it possible to determine the conditions required for these regulators to bind to DNA. Since our transcriptomic analysis, as well as previous studies [2], pointed to a possible relationship of Alr1976 and All0345 to nitrogen metabolism, we sought to evaluate their potential interaction with the promoter regions of a selection of genes involved in nitrogen metabolism and heterocyst differentiation. Our results showed that Alr1976 and All0345 bind to the promoter regions of various genes involved in these biological processes, suggesting that these regulators are new players in the complex regulatory network that controls nitrogen metabolism and heterocysts differentiation in cyanobacteria.

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96 POSTER

The bioactive sugar 7dSh and its effect on cyanobacterial carbon metabolism

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Abstract text

We previously described the effects of the bioactive sugar 7-deoxy-sedoheptolose (7dSh) that was isolated from culture supernatant of *Synechococcus elongatus.* 7dSh inhibits the growth of various phototrophic organisms including species of cyanobacteria, as well as the model plant organism *Arabidopsis thaliana.* Untargeted metabolome analysis of 7dSh treated organisms showed a strong accumulation of 7-deoxy-D-arabino-heptulosonic acid 7-phosphate (DHAP) the substrate of the 3-dehydoquinate synthase (DHQS) a key enzyme of the shikimate pathway. This indicates that 7dSh inhibits the shikimate pathway, an assumption that was confirmed through inhibition assays with purified DHQS and 7dSh. These findings explained the herbicidal activity of 7dSh, since the shikimate pathway is essential for bacteria, fungi, and plants. To gain further insights into physiological consequences of 7dSh treatments, additional experiments were undertaken with the non-diazotrophic cyanobacterium *Synechocystis sp. PCC6803. Synechocystis* is well-studied for its ability to perform chlorosis, a survival mechanism under nitrogen starvation. During chlorosis, cells degrade phycobiliproteins, accumulate glycogen and finally loose their photosynthetic apparatus, until after prolonged starvation, they enter a dormant state. Upon addition of a nitrogen source, the cells regreen in a coordinated manner, thereby metabolizing the previously accumulated glycogen. Here, we show that 7dSh inhibits both the chlorosis process as well as impairs dark resuscitation of freshly chlorotic cells, a process that strictly depends on efficient glycogen catabolism. Our current data suggest that 7dSh has an immediate effect on the carbon metabolism by inhibiting glycogen accumulation and consumption, implying an immediate inhibition of carbon metabolism by 7dSh.

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97 POSTER

Conditional PC/Cc6 mutants defective in soluble electron carriers *Synechocystis* sp. PCC 6803 strain

María Del Carmen Pérez Nieto, José M. Ortega, Manuel Hervás, Mercedes Roncel, Ana I. López-Pérez, José A. Navarro, Luis López-Maury

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Abstract text

In cyanobacteria, photosynthesis and respiration share some common elements of the electron transport chain, nonetheless, many aspects of the molecular pathways are still unclear. The photosynthetic electron transport between citb6f and PSI is carried out by PC in copper-containing media and by Cc6 in copper-deficient media. The presence of either of these proteins is essential for the correct functioning of photosynthesis, and a double mutant lacking both is not viable.

Conditional mutants were generated by expressing either PC or Cc6 under the arsenite-inducible promoter, P_{arsB}, in a double KO background in *Synechocystis* sp. PCC 6803. Growth was dependent on arsenite in these conditional strains as well as PC/Cc6 expression. O_2 electrode experiments, PAM analysis, and TL were used to photosynthetically characterized these strains. O_2 evolution was found to be completely dependent on arsenite, consistently with growth . The DUAL-PAM fluorescence analysis revealed non-detectable PSII photochemistry and altered PSI activity, whereas PSI content remained unaffected. Additionally, thermoluminescence assays of PSII revealed non-functional reaction centers in non-induced conditional mutants. Shockingly these mutants were able to survive for weeks after arsenite removal. Further physiological and photosynthetic characterization will be presented.

Funding

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Molybdenum is preferred over tungsten by nitrate reductase of *Synechocystis* sp. strain PCC 6803

Ronald Stebegg, Georg Schmetterer, Annette Rompel

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Abstract text

The nitrate reductase of *Synechococcus elongatus* strain PCC 7942 is a molybdoenzyme that is inhibited by tungsten ions dependent on the outer concentration of the latter. The inhibition of nitrate reductase by tungsten ions can be reversed immediately, when sufficient amounts of molybdenum ions are supplied [1]. In *Synechocystis* sp. strain PCC 6803 nitrate reductase is encoded by the gene *sll1454* (*narB*) [2] and nitrate reductase is inhibited by tungsten ions as well [3]. We investigated, if tungsten ions exhibit any negative effect on the growth rate of *Synechocystis* sp. strain PCC 6803. Therefore, the cells were cultivated in a medium, where molybdenum ions had been equimolarly replaced by either tungsten or vanadium ions. Only tungsten ions inhibited the growth, whereas vanadium ions did not alter the growth rate. When the cells were incubated in the absence of any of these metal ions, they exhibited normal growth as well. The effects of different concentrations of tungsten ions (in the absence of molybdenum ions) on the growth rate of *Synechocystis* sp. strain PCC 6803 were compared, however, no difference in growth rate was observed. If molybdenum ions were coadded in the standard concentration, normal growth occurred regardless of the concentration of tungsten ions. Finally, cultures, whose growth had been inhibited for a month, restored their growth, when high amounts of molybdenum ions (five-fold molarity of the standard concentration) were added to the growth medium.

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Investigating the regulation of the carbon switch in central metabolism of *Synechocystis* sp. PCC 6803

Ravi Shankar Ojha, Lu Shen, Carmen Peraglie, Christopher Bräsen, Bettina Siebers

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Abstract text

Dependent on the light/nutrient conditions *Synechocystis* can grow auto-, hetero-, and mixotrophically for which the reversibility of sugar metabolism particularly the Embden-Meyerhof-Parnas pathway (EMP) is essential. To switch between anabolic and catabolic direction sophisticated regulation is required and also the interplay of e.g. EMP and Calvin cycle for CO_2 fixation must be tightly balanced. The antagonistic PFK and FBPase operate exclusively in the catabolic or anabolic direction of the EMP, respectively, and PFK is the classical control point of glycolysis. Two paralogous copies of both enzymes are present in *Synechocystis*. Furthermore, the reversible phosphoglucose isomerase (PGI) has been discussed to direct fluxes between EMP and CBB under different growth conditions. However, the control points and the function and regulatory capacities of the enzymes are not established in *Synechocystis*.

Both PFKs are ADP dependent, thereby defining a new class of ADP-dependent PFK-A superfamily. PFK-A1 is inhibited by 3-phosphoglycerate (3PG), and PFK2 by ATP. FBPase1 is not regulated by various effectors. The PGI displays a preference for the gluconeogenic direction and is inhibited by erythrose 4-phosphate (E4P).

Our investigations reveal that both PFKs are active under conditions of low energy-charge, facilitating the breakdown of glycogen/D-glucose. The inhibition of PFK-A1 and PFK-A2 by 3PG and ATP, respectively, helps in rapid generation of CO_2 acceptor molecule. The sustained FBPase1 activity suggests its housekeeping function to enable reversibility of the EMP pathway in response to growth conditions. E4P enables PGI function as a valve to balance the non-oxidative pentose phosphate pathway intermediates and glycogen synthesis.

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100 POSTER

Viral Take-over of Host Photosynthetic Electron Transport: Understanding the role of Cyanophage-encoded Plastocyanin

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Abstract text

Cyanobacteria perform oxygenic photosynthesis and can be found in almost every habitat on earth. They are infected by specific viruses, cyanophages. During phage infection, key metabolic pathways of the cyanobacterial host (such as photosynthesis, carbon-, and fatty acid- metabolism) are significantly altered. So-called auxiliary metabolic genes (AMGs) encoded in the genome of the infecting phage are believed to contribute to the takeover of the cyanobacterial cell by maintaining the host metabolism during the infection cycle. AMGs are frequently found in cyanophage genomes.[1],[2] We are investigating the role of a particular AMG, the plastocyanin-like gene *petE* from the cyanophage Syn9. Plastocyanins are small blue copper proteins that transfer electrons from the cytochrome b_6f complex to photosystem I and are commonly found in most cyanobacteria, algae, and plants.[3] To verify the functionality of the phage gene, *Syn9petE* was heterologously expressed in *E. coli*. The purified recombinant protein displayed the blue colour typical for plastocyanins and an absorption maximum at ~600 nm. The protein can shift between different redox states. Furthermore, EPR spectroscopy confirmed its identity as a type I copper-binding protein and a midpoint potential of 290 mV was estimated by redox titration. In parallel, a recombinant Syn9 phage lacking a functional *petE* was generated. Recombinant phage particles were first enriched, subsequently purified and assessed for their efficiency in infection and viral particle production. In summary, our objective is to understand the role of cyanophage-encoded plastocyanin, its importance for phage progeny production and to elucidate how cyanophage proteins interact with host metabolism.

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Investigation of the functional coordination between nitrogen and carbon metabolisms in *Anabaena sp.* PCC 7120 using genetically-encoded fluorescence probes.

Mattia Storti, Anna Santin, Francesco Casturà, Tommaso Storti, **Giorgio Perin**

Department of Biology, University of Padova, Padova, Italy

Abstract text

Diazotrophic cyanobacteria fix both atmospheric carbon (C) and nitrogen (N) into biomass and are thus placed at a strategic intersection between the biogeochemical cycles of these two elements in nature, contributing to their integration into assimilable chemical species, essential to sustain life in the biosphere. Understanding the metabolism of diazotrophic cyanobacteria can therefore contribute to predicting perturbations of natural ecosystems’ functionality, as well as developing robust biotechnological solutions for a sustainable global economy. The contribution of the assimilation pathways of C and N to the metabolic homeostasis of diazotrophic cyanobacteria has been investigated separately so far, even though the two processes must be tightly coordinated to avoid metabolic inefficiencies. In fact, N assimilation depends on reduced C skeletons generated by photosynthesis, whilst the latter depends on pigments and proteins synthetized after N assimilation.

In this work, we used the model diazotrophic cyanobacterium *Anabaena sp.* PCC 7120, in which C and N assimilation occur in two distinct cell types (i.e. vegetative cells and heterocysts, VC and HC respectively), to address the functional coordination between them. We implemented genetically-encoded fluorescence probes to measure the pH, redox status, NADH/NAD^+ ratio and the concentration of ATP and 2-oxoglutarate *in vivo*. We used different promoters to induce cell-specific expression and achieve spatial resolution. The whole set of probes has been used to investigate photosynthesis-dependent changes in the metabolic status of both VC and HC, revealing the metabolic coordination between cell types is essential to maintain homeostasis as a functional of variable metabolic inputs.

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Investigating the molecular mechanism of intracellular amorphous carbonates (iACC) inclusions formation by cyanobacteria

Monis Athar Khan[1], Soufian Ouchane[1], Anne Durand[1], Fériel Skouri-Panet[2], Karim Benzerara[2], Franck Chauvat[1], Corinne Cassier-Chauvat[1]

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Abstract text

Cyanobacteria are photosynthetic organisms involved in biomineralization with the formation of carbonate sedimentary deposits and impact therefore biogeochemical cycles of calcium (Ca) and carbon (C). This biomineralization was thought to occur only extracellularly and not genetically controlled. However, recently several cyanobacterial species that form intracellular amorphous calcium carbonate (iACC) inclusions in thermodynamically unfavourable conditions were isolated from various environments.[1] Using *in-silico* approach, a candidate gene named *ccyA*, present in all iACC-forming cyanobacteria and absent in genome of other cyanobacteria, was identified as a marker of the iACC formation.[2] Furthermore, *ccyA* encodes for an orphan protein (named calcyanin) harbouring a conserved C-terminal domain and a variable N-terminal domain that can be classified into four types. Our goal is to identify the *in vivo* function of the *ccyA* gene by using a genetic approach. We were able to validate Cas12-mediated deletion in iACC forming strain *Cyanothece* sp. PCC 7425 (C7425) and attempt to obtain *ccyA* deletion is ongoing. As caclyanin harbours variable N-ter domain, conjugative expression vectors allowing the constitutive expression of *ccyA* from various iACC forming strains were constructed and conjugated into C7425 and non-iACC forming, *Synechococcus elongatus* PCC 7942 (S7942). We showed that overexpression of *ccyA* in S7942 resulted in Ca hotspots formation suggesting a role of *ccyA* in Ca homeostasis.[2, 3] In C7425, overexpression of its native *ccyA* gene led to better growth of the strain that made more iACC inclusion at low Ca, further analysis should provide more insights on the role of this gene in iACC formation.

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The effect of far-red D1 variants on electron transfer efficiency of Photosystem II in *Synechocystis* sp. PCC 6803

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Abstract text

Photosystem II (PSII) is a large multiprotein membrane complex with D1 (PsbA) as one of the core proteins. D1 binds the essential cofactors such as chlorophylls, quinones and the Mn_4CaO_5 cluster, which is involved in the light-driven oxidation of water. The most commonly found PSII has a so called standard D1 and 35 chlorophyll *a*. Some cyanobacteria use however alternative PSII variants to expand their absorption from the visible into the far-red region of the solar spectrum. One type of these far-red (FR)- PSII variants consists of an FR-specific D1 (FR-D1) and the red-shifted chlorophylls, chl *d* and *f*. The other FR-PSII has a standard D1 that mainly contains chl *d* (chl *d*-D1). However, these two variants have slower oxidation rates and higher energy activation than standard PSII, and overall, the water oxidation in red shifted photosystems remains poorly understood. To further study the far-red D1 variants we introduced them into the non-FR model cyanobacterium *Synechocystis* sp. PCC 6803 and analysed these mutants by various biochemical and biophysical methods. Our results indicate that all the D1 variants were expressed, but restored only part of the PSII activity, as indicated by oxygen evolution and variable fluorescence. The mutants containing the FR-D1 were furthermore unable to grow photoautotrophically, while the ones containing chl *d*- D1 could. This suggests that chl *d*-D1 can incorporate chl *a* and form a functional complex.

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Double blocking of carbon metabolism causes a large increase of Calvin–Benson cycle compounds in cyanobacteria

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Abstract text

Carbon-flow-regulator A (CfrA) adapts carbon flux to nitrogen conditions in non-diazotrophic cyanobacteria. Under nitrogen deficiency, CfrA leads to the storage of excess carbon, which cannot combine with nitrogen, mainly as glycogen [1, 2]. *cfrA* overexpression from the arsenite-inducible, nitrogen-independent P_{arsB} promoter allows analysis of the metabolic effects of CfrA accumulation. Considering that the main consequence of *cfrA* overexpression is glycogen accumulation, we examined carbon distribution in response to *cfrA* expression in *Synechocystis* sp. PCC 6803 strains impaired in synthesizing this polymer. We carried out a comparative phenotypic analysis to evaluate *cfrA* overexpression in the wild-type strain and in a mutant of ADP-glucose pyrophosphorylase (DglgC), which is unable to synthesize glycogen. The accumulation of CfrA in the wild-type background caused a photosynthetic readjustment although growth was not affected. However, in a DglgC strain, growth decreased depending on CfrA accumulation and photosynthesis was severely affected. An elemental analysis of the H, C, and N content of cells revealed that *cfrA* expression in the wild-type caused an increase in the C/N ratio, due to decreased nitrogen assimilation. Metabolomic study indicated that these cells store sucrose and glycosylglycerol, in addition to glycogen. However, cells deficient in glycogen synthesis accumulated large amounts of Calvin-Benson cycle intermediates as *cfrA* was expressed. These cells also showed increased levels of some amino acids, mainly alanine, serine, valine, isoleucine and leucine [3]. The findings suggest that by controlling *cfrA* expression, in different conditions and strains, we could change the distribution of fixed carbon, with potential biotechnological benefits.

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Exploring the influence of atmospheric CO_2 and O_2 levels on use of $\delta^{15}N$ as a biological N_2 fixation proxy.

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Abstract text

Biological N_2 fixation can be traced to the Archean eon, over three billion years ago. The nitrogen isotopic fractionation signal ($\delta^{15}N$) of sedimentary rocks is commonly used to reconstruct the presence of diazotrophic ecosystems in the past. While the $\delta^{15}N$ has been calibrated with analyses of diazotrophs grown under modern environmental conditions; it has not been tested under Archean conditions, when atmospheric pCO_2 was higher and pO_2 was lower than present. Here we explore $\delta^{15}N$ in the laboratory under three different simulated atmospheres with (i) elevated CO_2 and no O_2, (ii) present day CO_2 and O_2 and (iii) elevated CO_2 and current O_2 in marine and freshwater heterocystous cyanobacteria. Additionally, we supplement our data set with a compilation of literature data to search for more generalized dependencies of nitrogen fractionation during BNF across a range of organisms (Archaea, Bacteria, Cyanobacteria) and habitats. Statistical analyses of the expanded data set reveal correlation of fractionation ($\varepsilon = \delta^{15}N\text{biomass} - \delta_{15}NN_2$) during BNF with CO_2 concentrations, toxin production and light. Moreover, the magnitude of $\delta^{15}N$ correlated with species type, as well as C/N ratios and toxin production in heterocystous cyanobacteria, albeit it within a small range (-1.44 ± 0.89). We therefore conclude that $\delta^{15}N$ is likely robust when applied to the Archean; however, we stress that current knowledge is strongly biased towards cyanobacteria. Moreover, the increase in fractionation of $^{15}N/^{14}N$ in toxin producing *Nodularia* and *Nostoc* spp. suggests a heretofore unknown role of toxins in modulating preserved isotopic signals that warrants future investigation

Funding

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PerR (FurC) from *Anabaena* (*Nostoc*) PCC7120: Not limited to peroxide regulation

Cristina Sarasa-Buisan[1,2], Jorge Guio[1], Richard F Helm[3], Mercedes Nieves-Morión[2], Sergio Arévalo[2], Ignacio Luque[2], Emma Sevilla[1], **María F. Fillat**[1]

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Abstract text

Ferric uptake regulator (FUR) proteins function as global regulators in *Anabaena* sp. PCC7120, controlling a multitude of genes involved in various metabolic pathways. The gene *alr0957* encodes the FurC (PerR) paralog, which, in heterotrophic bacteria, is involved in the response to peroxide stress. While *perR* is dispensable in non-diazotrophic, unicellular cyanobacteria, in the nitrogen-fixer *Anabaena* sp. PCC7120, *alr0957* has been revealed as an essential gene. FurC/PerR is involved in the photosynthetic performance of *Anabaena* and plays an important role in the modulation of nitrogen fixation and heterocyst development. Furthermore, genome-wide identification of PerR novel targets enabled us to pinpoint genes involved in the modulation of central carbon metabolism, thereby reinforcing the role of PerR as a central regulator in *Anabaena* [1, 2].

Comparative analysis of the extracellular metabolites and proteins of the *furC*-overexpressing variant with those from the WT *Anabaena* unveiled unexpected roles for this FUR paralogue. While several stress-related proteins accumulated in the exoproteome of the *furC*-overexpressing variant, reduced levels of several export proteins and *amiC* gene products, responsible for nanopore formation were detected. Phenotypically, the *furC*-overexpressing strain presented odd septal nanopore formation and impaired intercellular molecular transfer. Additionally, the release of 1, 6-anhydro-N-acetyl-β-D-muramic acid and its associated disaccharide (β-D-GlcNAc-(1-4)-anhydroMurNAc) unveiled alterations in peptidoglycan breakdown and recycling in *furC*-overexpressing cells [3].

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Thioredoxin A regulates protein synthesis to maintain carbon and nitrogen partitioning in cyanobacteria.

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Abstract text

Thioredoxins (Trxs) are proteins that are essential for regulating enzyme activity in response to environmental changes, especially in photosynthetic organisms. In cyanobacteria, Trxs are crucial for metabolic regulation, but the key redox-regulated processes are unknown. In this study, we analysed a conditional mutant of the essential thioredoxin gene trxA (STXA2) from *Synechocystis* sp. PCC 6803 [1] and observed that depletion of TrxA levels alters cell morphology and induces a dormant-like state. Depletion of TrxA also affects protein synthesis, leading to changes in amino acid, nitrogen and carbon pools, and results in oxidation of the elongation factor EF-Tu. Transcriptomic analysis of low TrxA levels in STXA2 shows a strong transcriptional response. Genes involved in photosynthesis, ATP synthesis and CO_2 fixation are down-regulated, whereas genes involved in respiratory electron transport, carotenoid biosynthesis, amino acid metabolism and protein degradation are up-regulated. These results highlight the role of TrxA as a key regulator of cyanobacterial metabolism, directing the switch from anabolic to maintenance metabolism and regulating carbon and nitrogen partitioning.

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108 POSTER

Reconstitution of the PE-III phycobiliprotein of *Prochlorococcus marinus* SS120 in *Escherichia coli*

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Abstract text

The marine cyanobacterium Prochlorococcus has abandoned the effective light-harvesting phycobilisomes (PBS) and instead relies on divinyl-chlorophyll-antennae to efficiently harvest blue light. However, Prochlorococcus has kept small amounts of a remnant of the PBS as a single phycobiliprotein, phycoerythrin III (PE-III). This PE-III of low-light adapted Prochlorococcus strains is composed of an α- and β-subunit (SU) and likely carries covalently attached phycoerythrobilin (PEB) and phycourobilin (PUB) chromophores. Genes required for the assembly of PE-III are encoded in a ~10 kb gene cluster, encoding the SUs and five putative phycobiliprotein lyases for the proper stereochemical attachment of chromophores to apo-PE-III. Apart from the cluster, genes are found encoding biosynthetic enzymes for PEB and phycocyanobilin (PCB).

The function of lyases and their role in the assembly of apo-PE-III were investigated by a heterologous E. coli expression system. While the lyase CpeT is proposed to attach a PEB chromophore to cysteine residue Cys163 on the β-SU, the function of the CpeS lyase was already confirmed, ligating (3Z)-PEB to Cys82 on the β-SU CpeB. Furthermore, we identified MpeX as the first isomerase lyase in Prochlorococcus, attaching PUB at Cys51/60 in CpeB. Interestingly, MpeX shows only activity in the presence of ‘helper-lyase’ CpeZ and a prior attachment of PEB to Cys82 by CpeS. Similarly, the α-SU CpeA will be reconstituted. Our ultimate goal is to reconstitute the whole PE-III in E. coli and subsequently in Synechocystis sp. PCC6803. Overall, this project aims to understand the assembly and function of unusual phycobiliproteins in low-light-adapted Prochlorococcus strains.

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Monitoring intracellular ATP levels in cyanobacteria in single cells

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Abstract text

In cyanobacteria, ATP levels regulate a variety of key physiological processes, such as the progression of the circadian cycle. ATP is an unstable molecule prone to fast degradation, which complicates obtaining accurate measurements of its intracellular concentrations. Here, we develop a single-molecule ratiometric ATP sensor, based on the epsilon subunit of the ATP synthase from Bacillus. This biosensor provides a quantitative estimate of intracellular ATP levels by measuring the fluorescence of a circularly permuted GFP molecule. We validate the performance of the biosensor in E.coli and introduce it into the genome of the cyanobacterium S.elongatus, obtaining a strain in which intracellular ATP levels can be tracked using live-cell microscopy. By measuring these levels in different illumination conditions, we demonstrate the interrelation between ATP levels and light intensity in cyanobacteria.

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Light acclimation in CA1 and CA3 cyanobacteria *Synechocystis* sp. PCC 6803 and *Nostoc* sp. CCAP 1453/38 and implications for productivity

Tomáš Zavrel[1], Tobias Pfennig[2,3], Anna Matuszynska[2,3], Jan Cervený[1], Gábor Bernát[4]

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Abstract text

Cyanobacteria play a key role in primary production in both oceans and fresh waters and hold great potential for sustainable production of a large number of commodities. During their life, cyanobacteria cells need to acclimate to a multitude of challenges, including shifts in intensity and quality of incident light. Despite our increasing understanding of metabolic regulation under various light regimes, detailed insight into fitness advantages and limitations under shifting light quality has been missing. Here, we will describe photoacclimation strategies in *Nostoc* sp. CCAP 1453/38 and *Synechocystis* sp. PCC 6803, as representatives of chromatic acclimator (CA) type 1 and type 3. Rods of phycobilisomes of the CA1 strain *Synechocystis* contain only phycocyanin whereas rods of CA3 strain *Nostoc* contain both phycocyanin and phycoerythrin. Cultivation of both strains under narrow-band cultivation LEDs revealed clear growth advantage of *Nostoc* in the blue-green part of the light spectrum that corresponds with phycoerythrin absorption. The faster growth was allowed by more efficient linear electron flow (LEF) between PSII and PSI. In addition to LEF, many other parameters show specific dependencies on light quality in *Nostoc*, compared to *Synechocystis*. These include cycling and respiratory electron flows, cellular composition and pigment distribution within cells. These trends will be described in detail. In addition, using a wavelength-dependent mathematical model of photosynthesis [1], a prediction of wavelength optimality for various metabolic pathways (such as Calvin-Benson-Basham Cycle) and products (isoprene, ethylene) will be provided and discussed.

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Functional characterization by CRISPR mutagenesis of marine *Synechococcus* genes involved in Type IV-B chromatic acclimation.

Louison Dufour[1], **Morgane Ratin**[1], Laurence Garczarek[1], Théophile Grébert[1], Wendy Schluchter[2], David Kehoe[3], Frédéric Partensky[1]

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Abstract text

The phycobiliprotein and phycobilin composition of marine *Synechococcus* phycobilisomes is highly diverse, reflecting the wide variety of spectral niches colonized by this ubiquitous microorganism [1]. The most sophisticated *Synechococcus* pigment type is cells capable to dynamically modify their light absorption properties to match the ambient color: blue, green or blue-green. This process called 'Type IV chromatic acclimation' (CA4) is linked to the occurrence of a small genomic island occurring in two distinct configurations: CA4-A and CA4-B. These two *Synechococcus* pigment sub-types occupy complementary ecological niches in the field [1]. Recently, we showed that both mechanisms involve a lyase/lyase-isomerase couple that compete to bind a chromophore to cysteine-83 of α-phycoerythrin-II i.e., either phycoerythrobilin in green light or phycourobilin in blue light; yet CA4-A and CA4-B differ since the first is triggered by blue light and the second by green light [2]. The CA4-A regulatory machinery has been partially deciphered and involves two transcriptional regulators (FciA and FciB) with diametric effects on CA4, as well as a short putative phage-like regulator FciC of yet unknown function [3]. Here, we used CRISPR mutagenesis to explore the functions of *fciA* and *fciB* homologs in the CA4-B island, which is devoid of *fciC*. While in the CA4-A system, FciA activates the expression of the lyase-isomerase gene *mpeZ* in blue light and FciB represses it in green light [3], we propose that in the CA4-B system FciB activates the expression of the lyase gene *mpeW* in green light while FciA represses it in blue light.

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Genetic insight into the cyanobacterial transmembrane component EcfTC

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Abstract text

The *synpcc7942_2341* gene constitutes an intriguing node of the cyanobacterial PipX synteny network. Its gene product shows sequence similarity with bacterial EcfT (Energy Coupling Factor, T subunit) homologs involved in micronutrient (vitamin or metal) import. The most recognizable sequence signature in all proteins from the EcfT family is composed of two short motifs (ARG-ARG) with invariant arginines at the center. These cytoplasmic motifs have been shown to anchor into the deep groove of the energizing EcftA/A´ components (ATPase subunits) of the transporters and are important determinants of complex stability and/or intramolecular signalling in ECFs. However, the cyanobacterial orthologs are no t associated with recognizable *ecfA* (neither with substrate recognition *ecfS* genes) in the genomes and, importantly, they display distinctive features, present in homologs from all oxygenic photosynthetic organisms. To acknowledge these differences cyanobacterial homologs of *ecfT* have been referred to as cyano-T. For simplicity, we are naming the gene *ecfTC*. In *Synechococcus elongatus* PCC7942 *ecfTC* is essential under all conditions tested. Depletion as well as overexpression of EcfTC affected cell growth, produced dramatic morphological defects that include altered distribution of photosynthetic pigments and altered ratios of chlorophyll/phycobilisome signals. Overexpression phenotypes were supressed or attenuated by inactivation of *pipX*. The *in vivo* effects of Arg to Ala or Lys substitutions at the TRA and VRG motifs provided additional insights into EcftC functions. In the light of these and other results we will discuss current approaches to study the role of cyano-T proteins and of the inferred regulatory connections between EcfTC and PipX.

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Regulatory connections of PipY, a cyanobacterial paradigm for pyridoxal-phosphate binding proteins

Lorena Tremiño, Antonio Llop, Trinidad Mata, Asunción Contreras

Departamento Fisiología Genética y Microbiología. Facultad de Ciencias. Universidad de Alicante, Apartado 99, E-03080 Alicante., San Vicente Del Raspeig, Spain

Abstract text

Interest in the family of pyridoxal-phosphate binding proteins (PLPBP) is fuelled by the existence of familial mutations causing vitamin B 6 -dependent epilepsy in humans.

These proteins, involved in the homeostasis of vitamin B 6 vitamers and amino/keto acids, share a high degree of sequence conservation and are represented in all three domains of life. PipY from Synechococcus elongatus PCC7942 is one of the best characterized PLBPB members and can be regarded as a paradigm for the protein family. The operonic association pipXpipY in cyanobacteria as well as other observations indicate a functional connection between PipY and PipX, a small protein involved in signalling the intracellular energy status and carbon-to-nitrogen balance, thus providing a unique opportunity to investigate the roles of PLPBP proteins in the context of cyanobacterial signalling networks. PipX forms complexes with other small regulatory proteins, transcriptional regulators, and translation-related factors to influence metabolic homeostasis and stress responses in cyanobacteria. To better understand PipY roles and its connections with PipX signalling we are studying the effect of a variety of pipY and pipX alleles on growth, viability, accumulation of polyphosphate granules and protein interactions. The pipY alleles being studied include point mutation derivatives targeting key residues, including “clinical mutations”. We will discuss our recent advances in the contexts of PipY/PLPBP roles and of functional connections between PipX and PipY.

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Suppressors analysis of *glnB* null mutants to gain insights into PipX toxicity

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Abstract text

PipX is a small protein whose complexes with PII and NtcA, the key regulators of carbon/nitrogen homeostasis, are characterised to a great detail. In *Synechococcus elongatus* PCC7942 PipX is toxic when overproduced or whenever the intracellular PipX/PII ratio is increased by genetic manipulations, conditions that prevent cell growth. Suppressor mutations at *pipX* have been found in null *glnB* mutants obtained in different laboratories. The importance of a relatively low PipX/PII ratio for survival is further supported by the finding that cyanobacterial genomes contain at least as many copies of *glnB* as of *pipX*. PipX toxicity is proposed to be the result of the abnormally over-binding of PipX to other partner(s) in absence of PII.

To identify the “toxic partners” of PipX we selected for segregated *glnB* mutants in *S. elongatus*. Since so far the best candidates to mediate this response are the transcriptional regulator NtcA and the ribosome-assembly GTPase EngA, we looked for mutations at either *pipX, engA* or *ntcA* genes in the segregated *glnB* mutants. An important proportion of the selected clones contained mutations at *pipX*, confirming that this gene is the main target for suppression of *glnB* null mutations. The rest of the clones were submitted to NGS sequencing. The results obtained in this suppression analysis and their implication for PipX functions will be discussed.

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SbtB: A substrate of FtsH4

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Abstract text

Authors: Surbhi Sharma, Vendula Krynicka

Membrane-bound FtsH proteases are widely found in prokaryotes, mitochondria, and chloroplasts, playing crucial roles in cell viability, and maintaining cellular homeostasis. Cyanobacteria, unlike most bacteria encoding single *ftsH* gene, possess four FtsH proteases (FtsH1-4) that form heteromeric (FtsH1/3 and FtsH2/3) and homomeric (FtsH4) complexes. However, the function and substrate repertoire of each complex is poorly understood. Recent data showed that FtsH4 plays a role in stress acclimation by regulating high light inducible proteins (Hlips) [1]. To identify new substrates of FtsH4, we established a substrate trapping assay in *Synechocystis* PCC 6803 using a proteolytically inactivated FtsH4 (hereafter, ^{trap}FtsH4). It revealed around 40 proteins enriched in the ^{trap}FtsH4 pulldown compared to active FtsH4, including seven proteins involved in the carbon concentrating mechanism (CCM) such as SbtA, SbtB, and CupA. Notably, SbtB and FvI proteins were exclusively copurified with ^{trap}FtsH4, indicating them as potential substrates. SbtB is a P_{II}-like signal transduction protein, unlike P_{II} proteins, it senses inorganic carbon (Ci) limitation by binding to cAMP and c-di-AMP, along with other adenine nucleotides and is degraded by FtsH4 [2]. Overexpression of FtsH4 shows reduced SbtB levels, slowing the growth of mutants sensitive to carbon depletion, while FtsH4 deletion led to SbtB accumulation and increased Ci levels and supports the growth of mutants. *In vivo* proteolytic assay confirmed FtsH4’s role in SbtB degradation. Furthermore, co-purification of FtsH4-His with Slr0374, crucial for CO_2 uptake, suggests FtsH4’s involvement in Ci acquisition.

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Funding

1. ERC Project Photoredesign (no. 854126)

116 POSTER

Endogenous clock-mediated regulation of intracellular oxygen dynamics is essential for diazotrophic growth of unicellular cyanobacteria

Anindita Bandyopadhyay, Annesha Sengupta, Thanura Elvitigala, Himadri B. Pakrasi

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Abstract text

The ability of unicellular diazotrophic cyanobacteria to perform nitrogen fixation and photosynthesis in the same cellular platform is an enigma that continues to intrigue biologists. The observation made decades ago, that unicellular cyanobacteria can perform nitrogen fixation under continuous light, provided the first clues to the existence of a circadian clock in prokaryotes. However, owed to their recalcitrance to any genetic manipulation, the clock-mediated segregation of processes remained largely unexplored in this group of prokaryotes. To investigate its function in these diazotrophs, we disrupted the circadian clock defined by the *kaiABC* genes in the now well established model strain *Cyanothece* 51142. Unlike non-diazotrophic cyanobacteria, *Cyanothece* 51142 exhibits conspicuous self-sustained rhythms in various discernable phenotypes, offering a platform to directly study the effects of the clock on the physiology of an organism. Disrupting the clock by deleting *kaiA* led to impairment in nitrogen fixation and growth under continuous light or long day length conditions. Under these conditions, the conspicuous endogenous rhythms in oxygen cycling observed in the WT was disrupted in the mutant, suggesting that a loss in the regulation of oxygen cycling is detrimental to nitrogenase function and growth. This work provides the first molecular evidence of the involvement of the circadian clock in segregating essential yet incompatible processes in unicellular diazotrophic cyanobacteria. Our findings suggest that the addition of KaiA to the KaiBC clock was likely an adaptation that ensured optimal nitrogen fixation as microbes evolved from an anaerobic to an aerobic atmosphere under nitrogen constraints.

Funding

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Characterization of a class II lanthipeptide from the cyanobacterium *Nostoc punctiforme* and investigation of its role in symbiotic interaction

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Abstract text

The terrestrial cyanobacterium *Nostoc punctiforme* is a versatile microorganism that can live freely in the soil and in symbiosis with various partners, such as mosses, cycads, lichen or *Gunnera* sp. The *Nostoc* symbioses can be classified as nutritional symbioses, whereby its role is nitrogen fixation. *N. punctiforme* is a promising producer of bioactive compounds as it harbours a multitude of cryptic biosynthetic gene clusters (BGCs) for secondary metabolites. A ribosomally synthesized and post-translationally modified peptide (RiPP) of the model strain *N. punctiforme* PCC 73102 that has shown to play a role in symbiosis with mosses is the class II lanthipeptide RiPP4. Its biosynthetic gene cluster (BGC) showed an early upregulation in chemical and physical interaction studies with the moss *Blasia pusilla*.

Since the product of the RiPP4 BGC could not yet be analytically detected in *N. punctiforme* itself, we aim to express the peptide heterologously in *E. coli* and in the filamentous cyanobacterium *Anabaena* PCC 7120. This approach will be used to characterize the involved class II lanthipeptide synthetase and can provide an avenue to design bioassays to further understand the role of RiPP4 in symbiotic interactions of *N. punctiforme* and *B. pusilla*.

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Elucidation of a signalling cascade regulating the specialized metabolism in *Nostoc punctiforme*

Sofia Kerestetzopoulou[1], Keishi Ishida[2], Julia Krumbholz[1], Christian Hertweck[2], Elke Dittmann[1]

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Abstract text

Nostoc punctiforme PCC 73102 is a filamentous nitrogen-fixing cyanobacterium that is known to be a prolific producer of bioactive natural products. Most of the secondary metabolite biosynthetic gene clusters (BGCs) are silent under normal laboratory conditions and thus do not give rise to significant amounts of secondary metabolites. Cell density-dependent factors play a role in the control of BGC expression in *N. punctiforme*. Our recent work on the high-density dependent up-regulation of BGCs led to the discovery of nostovalerolactone and nostoclide as global chemical mediators regulating the specialized metabolism of *N. punctiforme* [1]. However, we have not yet identified signals leading to the upregulation of the corresponding *nvl* and *ncl* BGCs themselves. We have therefore designed a bioactivity-guided strategy to identify the upstream signal to gain a more comprehensive picture about the regulation of the secondary metabolism in *N. punctiforme*. We will present transcription-based assays using RT-PCR and reporter mutants to identify the chemical mediator acting upstream of nostovalerolactone and nostoclide as well as our attempts to identify further regulatory proteins involved in the cell-density-dependent signalling cascade. With this study, we aim not only to understand the regulation of secondary metabolism, but also to use it to develop an inducible promoter system for heterologous expression of BGCs in filamentous cyanobacteria.

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Integrative -omics analysis of *Nostoc punctiforme* to chase their cryptic natural product diversity

Maria Miguel-Gordo[1], Sofia Kerestetzopoulou[1], Julia Krumbholz[1], Keishi Ishida[2], Roderich D. Süssmuth[3], Elke Dittmann[1]

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Abstract text

Cyanobacteria are fascinating organisms with the biosynthetic capacity to produce highly bioactive and structurally diverse secondary metabolites, including harmful toxins and approved drugs. Genome mining of the terrestrial symbiotic cyanobacterium *Nostoc punctiforme* PCC 73102 predicts the presence of several orphan biosynthetic gene clusters (BGCs) with the potential production of new natural products. However, pairing genes and metabolites remains challenging and several strategies have been applied to stimulate the transcription of BGCs.

The amenability of this strain to genetic modification allowed the construction of the mutant AraC_PKS1, with the AraC transcription factor overexpressed. This strain was able to upregulate the expression of its associated BGC, pks1, and led to determining the related metabolites, nostovalerolactone and nostoclides, but also other BGCs were upregulated [1]. This was possible using the high light and CO_2 cultivation conditions (HD-cultivation), which improves the growth producing more biomass of the filamentous bacteria, but also reprograms the transcription of the BGCs enhancing the metabolic production. These differences in the regulation of the secondary metabolism enable the detection of metabolites whose genes are silent in conventional conditions [2].

In this work, we combine genome mining, transcriptomic data, and untargeted metabolomic analysis including MS/MS feature-based molecular networking. We use this approach to compare the differences in the regulation of the BGCs and the metabolic production of different strains of *N. punctiforme* (wild type vs AraC_PKS1) and cultivation methods. This study presents an efficient method for the metabolic characterization of candidate metabolites and opens possibilities to study their biosynthetic pathways and bioactivities.

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Funding

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120 POSTER

A quantitative description of cyanobacterial phototrophic growth using flux balance analysis

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Abstract text

The metabolism of phototrophic microorganisms, including cyanobacteria, is an integral part of global biogeochemical cycles, and the capability of cyanosbacteria to assimilate atmospheric CO2 into organic carbon has manifold potential applications for a sustainable biotechnology. To elucidate the properties of phototrophic metabolism and growth, computational reconstructions of the genome-scale metabolic networks play an increasingly important role.

In this contribution, we present an updated reconstruction of the metabolic network of the cyanobacterium *Synechocystis* sp. PCC 6803 and its analysis using flux balance analysis (FBA). To overcome limitations of conventional FBA, and to allow for the integration of quantitative experimental analyses, we present a novel approach to describe light absorption and light utilization. Our approach incorporates explicit absorption of photons and allows us to describe photoinhibition and a variable quantum yield of photosynthesis. We show that the resulting model is capable of predicting quantitative properties of cyanobacterial growth, including photosynthetic oxygen evolution and the ATP/NADPH ratio required for growth and cellular maintenance, with results that are in excellent agreement with quantitative experimental measurements. Our approach retains the computational and conceptual simplicity of FBA and is readily applicable to other phototrophic microorganisms. We will discuss possible extensions of our approach, such as the incorporation of arbitrary light spectra and the description of medium-scale photobioreactors. It is shown that our approach can guide optimization of phototrophic metabolism and has manifold applications in bioengineering.

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Exploring acclimatation processes to far red light of endolithic isolated strains from Sahara Desert

Sergio Vasco-Francisco, Elvira Perona, **M Ángeles Muñoz-Martín**

Universidad Autónoma de Masdrid, Madrid, Spain

Abstract text

Extreme environments, as Sahara Desert, that allow us to explore the life limits, have an increasing interest in astrobiology studies able to compare biology strategies. In some areas, the aridity index is so high that the microorganism seek refuge inside the rocks. At these ambient cyanobacteria represent the primary producers, but they find difficulties to obtain nutrients, such as nitrate sources and luminous energy to develop photosynthesis, as the PAR (Photosynthetically Active Radiation) cannot penetrate inside the rocks and the incident light is enriched in far red light. In this study, the ability of 9 cyanobacterial strains, isolated from Sahara rocks, to perform photosynthesis under far red light after and before acclimatation has been explored, as well as the possible mechanism involved in this acclimatation by analyzing their photosynthetic pigments. Seven selected strains are unicellular (4 *Pseudoacaryochloris sahariensis,* 2 *Chroococcidiopsis* sp., and 1 *Gloeocapsopsis* sp.), one nonheterocitous filamentous (*Nodosillinea* sp.) and one heterocitous filamentous (*Scytonema hyalinum*). *P. sahariensis*, a recently described genus and species, is the most abundant taxa found in a cryptoendolithc environment in Sahara Desert but has been also found in chasmoendolithic, hypolithic and biocrust environments [1, 2] where light is reduced, so one of these strains has been sequenced in order to detect possible genes involved in the acclimatation to make photosynthesis under far red light. Other physiological strategies such as nitrogen fixation are also explored to understand the abilities of these strain in this specific environment.

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122 POSTER

Effects of T4-like cyanophage genes on cyanobacterial carbon metabolism

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Abstract text

Marine picocyanobacteria of the genera *Prochlorococcus* and *Synechococcus* are the most abundant photosynthetic organisms on Earth. They are responsible for approximately 25% of ocean primary production. The viruses infecting cyanobacteria (cyanophages) carry host-like genes encoding proteins involved in the Calvin cycle and the pentose phosphate pathway. These genes are thought to redirect carbon flux in the host cell from the Calvin cycle for carbon fixation and toward the pentose phosphate pathway for NADPH and ribose-5-phosphate production for use by the cyanophage for DNA replication. Despite the importance of the carbon metabolism process, the function of cyanophage carbon metabolism genes during infection and their effect on the host are poorly understood. We aim to shed light on the role of such cyanophage genes using Syn9 as our model phage and the marine *Synechococcus* sp. strain WH8109 as the host. We are currently investigating the effect of these genes on the host by heterologously expressing them in the host and by generating cyanophage mutants lacking these genes. Initial data show that expression of one of the cyanophage-encoded pentose phosphate pathway genes reduced the growth rate of the host. This study will improve our understanding of how these genes alter host metabolism during infection at the molecular and physiological levels.

Funding

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Sll1071, a novel auxiliary protein factor involved in the repair cycle of photosystem II in *Synechocystis* sp. PCC6803

Guillem Pascual-Aznar, Josef Komenda

Institute of Microbiology, Academy of Sciences of the Czech Republic, Trebon, Czech Republic

Abstract text

The assembly of photosystem II (PSII) is a step-wise process in which the different assembly modules combine into the fully functional dimeric PSII (PSII(2)) with the assistance of auxiliary protein factors. Especially the PSII core complex lacking CP47 (RC47) is enriched in auxiliary factors and since RC47 is an assembly intermediate as well as the product of the disassembly during the PSII repair, some of these factors may facilitate the repair, not the assembly. In this study, we focused on the product of the *sll1071* gene, which has been co-isolated with Psb28-containg RC47. The protein most probably contains N-terminal lumenal domain and three transmembrane helixes.

Mutant of the cyanobacterium *Synechocystis* PCC 6803 lacking Sll1071 shows high-light sensitivity and its cellular content of chlorophyll (Chl) and Photosystem I (PSI) is reduced by almost 30% in comparison with WT. It accumulates an abnormal amount of RC47 as well as the high-light inducible proteins but does not exhibit defects in the fully assembled PSII(2). In the strain lacking CP43 the subsequent removal of Sll1071 leads to overaccumulation of RC47 and further Chl depletion resembling the phenotype of the CP43-less mutant lacking FtsH2, the protease essential for selective D1 degradation during the PSII repair.

Although the exact mechanism of the Sll1071 action remains elusive, the accumulation of RC47 together with the decreased level of Chl, the apparently normal functioning of the PSII(2) and the phenotype similarity with the FtsH2-less mutant point to the role of Sll1071 in PSII repair.

Funding

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124 POSTER

Moonlighting function of cyanophycinase in *Synechocystis* sp. PCC 6803

Éva Kiss[1], Martin Moos[2], Jan Mareš[1,3], Lenka Tomová[1,3], Roman Sobotka[1,3]

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[3] *Faculty of Science, University of South Bohemia, Ceské Budejovice, Czech Republic*

Abstract text

Cyanophycin is a nitrogen-rich biopolymer synthetized by many cyanobacteria. It is built from the amino acids arginine and aspartate, and its accumulation is determined by the amount of arginine synthetized in the cell [1]. Cyanophycin-, and particularly arginine metabolisms comprise complex and poorly understood metabolic pathways in living organisms. To contribute to this field, we studied the cyanophycinase enzyme of *Synechocystis* sp. PCC 6803 (CphB) that catalyzes the first step of cyanophycin degradation (mobilization of N stockpile). We found that CphB interacts with Gun4 and ArgD that are essential proteins involved in the biosynthesis of tetrapyrrolic pigments and the amino acid, arginine, respectively [2]. Although Gun4 strongly influences the branch point of chlorophyll and heme synthesis, the deletion of CphB (Δ*cphB*) had only marginal effects on the accumulation of the heme-derived bilin and chlorophyll pigments. However, when the need for bilins decreased by a mutation eliminating the rods of phycobilin-containing antennae, significantly more chlorophyll was produced in the absence of CphB. These results suggest that CphB interferes with the regulation of the recently discovered bilin-dependent activity of Gun4 [3]. Despite of its effect on the accumulation of photosynthetic pigments, CphB did not seem to be essential for photoautotrophic growth. However, Δ*cphB* failed to upregulate the biosynthesis of arginine that was detrimental specifically when the cells were shifted to dark, heterotrophic growth. Our data reveals that CphB is a stimulator of arginine biosynthesis, which has key importance under none-photosynthetic conditions.

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CHLOROPHYLL-driven dimerization of FERROCHELATASE protects the translation apparatus in cyanobacteria

Divya Aggarwal, Jan Janouškovec, Josef Komenda, Roman Sobotka

Institute of Microbiology, Centre Algatech, Trebon, Czech Republic

Abstract text

Oxygenic phototrophs produce tetrapyrroles via a long, branched biosynthetic pathway. At the branching point, either magnesium chelatase inserts magnesium for chlorophyll (Chl) biosynthesis or ferrochelatase (FeCh) adds iron for heme biosynthesis. An intriguing feature of FeCh in oxygenic phototrophs is the C'-extension (Hlip-domain), which originates from an ancient fusion between FeCh and the HliC protein[1]. HliC is a transmembrane single helix, high light inducible protein (Hlip), which must dimerize to bind Chl and carotenoids[2]. Yet, the FeCh Hlip-domain role remains enigmatic. As recently shown, the FeCh in the cyanobacterium *Synechocystis* PCC6803 can dimerize via its Hlip-domain and binds Chl, β-carotene and zeaxanthin, in excess of free Chl in membranes[3]. To study pigment binding to FeCh, we prepared *Synechocystis* strains that possess point mutations in FeCh Hlip-domain preventing the Chl binding/ dimerisation. Interestingly, these strains are cold sensitive and cannot survive the combination of cold (18°C) and higher light (300 µE of photons). By using $^{[35]}S$ radiolabelling, 2D electrophoresis and protein pulldowns we found that under stress conditions, the mutant strains rapidly lose the ability to synthesize membrane proteins. Mutant showed unbound Chls accumulation in the membranes, PSII photodestruction, and accelerated dissociation of PSI trimers. Strains with low FeCh content or after treatment with a FeCh inhibitor showed similar phenotype. Our data suggest that FeCh dimerisation, promoted by the binding of free Chl, enhances FeCh activity *in vivo*. We hypothesize that this regulation is essential for the prompt arresting of Chl biosynthesis to prevent release of ROS near the translation apparatus.

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CANCELLED

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Dynamic regulation of the proton motive force in cyanobacteria

Lauri Nikkanen

University of Turku, Turku, Finland

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Abstract text

Photosynthetic electron transport generates an electrochemical membrane potential over the thylakoid membrane, known as the proton motive force (*pmf*). The *pmf* energises ATP synthase to produce ATP, but also has a role in controlling photoprotective mechanisms. The *pmf* comprises the ΔpH and the electric field-dependent Δψ components. Both are thermodynamically equal in driving ATP synthesis, but ΔpH is specifically needed for the induction of photoprotection by inhibition of PQH_2 oxidation at the Cyt b_6f, a process known as photosynthetic control.

Adjusting the *pmf* and its composition between ΔpH and Δψ is achieved by regulating the proton conductivity of the ATP synthase and by ion channels, pumps, and exchangers on the thylakoid membrane. These processes have remained poorly understood in cyanobacteria. However, as cyanobacteria are increasingly used for sustainable production of various value chemicals and biofuels, understanding and being able to rationally modulate these key regulatory mechanisms of photosynthesis is crucial.

We dissected the *in vivo* regulation of the *pmf* in *Synechocystis* sp. PCC 6803 by time-resolved measurements of the electrochromic shift (ECS) signal. Our results reveal that conductivity of the thylakoid membrane undergoes dynamic adjustment during changes in light intensity via a mechanism that depends on the thiol redox state of the cell. Interestingly, down-regulation of thylakoid conductivity is impaired in a mutant strain lacking the protein Pgr5. Pgr5 has been suggested to play a role in cyclic electron transport around PSI, but we show that its function is more likely related to regulation of the ATP synthase.

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Characterising the role of cyanophage plastocyanin

Jack Dickenson[1], Rich Puxty[1], Andrew Millard[2], Dave Scanlan[1]

[1] *University of Warwick, Coventry, United Kingdom*

[2] *University of Leicester, Leicester, United Kingdom*

Abstract text

Cyanophage infecting Prochlorococcus and Synechococcus are ubiquitous in marine systems. Genome sequencing and infection studies have demonstrated great diversity in cyanophage genome size and infection strategies and within these genomes are a number of host-like metabolic genes, named Auxiliary Metabolic Genes (AMGs). Cyanophage AMGs are diverse, encoding genes related to photosynthesis, nutrient depletion and central carbon metabolism. Intriguingly, some cyanophages are capable of inhibiting CO2 fixation while maintaining photosynthetic electron transport, supposedly to ensure consistent ATP production for phage replication. However, there is relatively little work focused upon characterising the function of specific cyanophage AMGs during host infection. We set out to characterise the role of the cyanophage-encoded plastocyanin gene, petE. Plastocyanin is a key electron transport protein in cyanobacterial respiration and photosynthetic electron transport chains and the high abundance of the petE gene in cyanophage genomes suggests an important role during infection. Using the freshwater strain Synechococcus elongatus spp. PCC7942 as a heterologous host, we generated knock-in mutants where the PCC7942 gene was replaced with the petE gene from the marine Synechococcus sp. WH7803 or cyanophage S-RSM4. When comparing photophysiology between mutants, the phage petE mutant shows functional differences to the host variant with affinity to other photosynthetic ETC components. We are following this up with infection experiments tracking photosynthetic electron transport and proteomic analysis to further understand how the phage plastocyanin assists infection.

Funding

ERC Advance Grant

Session 4:
Ecology and interactions with the Enviroment

129 INVITED TALK

Metagenomics reveals the biosynthetic potential of cyanobacteria-dominated environmental biofilms

Pedro N. Leão

Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Porto, Portugal

Abstract text

Cyanobacteria have the particular ability to grow in mats or biofilms visible to the naked eye in a variety of ecological settings. Such environmental biofilms are typically an assemblage of multiple cyanobacteria, as has been previously documented. This characteristic of cyanobacteria to generate environmental outgrowths that can be easily detected and collected and brought to the laboratory, is not common for the majority of other bacteria, and can be leveraged to obtain abundant eDNA for metagenomics studies and biotechnological applications. We have been carrying out, for a few years, sampling campaigns for the collection of visible biofilms of cyanobacteria in different environments, namely freshwater, marine and subaerial biofilms. We have used shotgun metagenomics on eDNA extracted from these samples to obtain cyanobacterial MAGs of high quality, as well as those of heterotrophic bacteria associated with the biofilms. In addition, we have looked into natural product biosynthetic gene clusters present in these cyanobacterial MAGs. Here, we will present our main findings from our analysis of over 70 cyanobacteria-dominated biofilms and 300 cyanobacterial MAGs. We will provide an overview of general trends observed in terms of cyanobacteria and heterotrophic bacteria diversity, biosynthetic gene clusters distribution and diversity, as well as some more focused analyses of specific biofilms with high natural product biosynthetic potential and the results of our initial attempts to capture this biosynthetic diversity through heterologous expression.

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Comparative and environmental genomics of α-cyanobacteria

Emile Faure[1], Anastasia Paupe[1], Gregory Kevin Farrant[1], Hugo Doré[2], Ulysse Guyet[1], Louison Dufour[1], Frédéric Partensky[1], **Laurence Garczarek**[1]

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Abstract text

α-cyanobacteria harbor a wide genetic and functional diversity and constitute highly pertinent biological models to study genomic bases of niche partitioning in aquatic ecosystems [1]. To tackle this question, we recovered all α-cyanobacteria genomes available in public databases, annotated them based on the rich, manually curated annotation of the Cyanorak *v2* database [2], and selected the best quality genomes and/or those filling diversity gaps. Comparative genomics allowed us to i) determine *in silico* phenotypes based on the presence/absence of genes involved in accessory metabolic pathways (e.g. nutrient transport/assimilation, ii) to feed CyanoMarks, a multi-marker database, allowing us to reconcile the taxonomies inferred from these different markers, and iii) to enlighten the evolutionary history of this radiation.

To examine the distribution of specific functions and identify new genes/metabolic pathways potentially involved in the adaptation to various ecological niches, these genomes were then used as references to recruit meta-omic reads covering a large variety of oceanic biomes as well as time-series. Each assemblage of picocyanobacterial ecotypes were shown to possesses a distinct gene repertoire and correlation network analyses led us to identify individual niche-specific genes. Integration of these data with gene synteny in reference genomes using a network approach was used to unveil clusters of adjacent genes in reference genomes, displaying differential environmental distribution (eCAGs), and are thus potentially involved in the same metabolic pathway [3]. Altogether, our analyses provided important insights into the complex interactions between vertical phylogeny, functions and habitats that shaped the community structure and evolution of these organisms.

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131 ORAL

Rhythm on the Beach: Cyanobacterial circadian clock controlled rhythmicity in a complex microbial mat community.

Henk Bolhuis

Royal Netherlands Institute for Sea Research (NIOZ), Den Hoorn, Texel, The Netherlands

Abstract text

To understand the ecological role of circadian rhythms in prokaryotes, we often move from an initial observation in nature to an in-depth analysis of isolated species under laboratory conditions. The other way around is less common: newly found insights on circadian control of microbial physiology and metabolic interactions are rarely tested in the original ecosystem. This is partly due to the general inability to use laboratory type analytical tools and controlled conditions in the environment. Thanks to novel developments in high throughput DNA and RNA sequencing we now have tools to determine the extant expression of individual genes. I apply metatranscriptomics at 4-hour time intervals to coastal microbial mats, unique millimeter scale ecosystems that are dominated by circadian clock-controlled Cyanobacteria. I describe not only the natural rhythm of Cyanobacteria but also how these rhythms resonate throughout the microbial mat community.

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Mixotrophy in marine picocyanobacteria: glucose uptake in *Prochlorococcus* and *Synechococcus*

José Ángel Moreno Cabezuelo, María Del Carmen Muñoz-Marín, Antonio López-Lozano, Guadalupe Gómez-Baena, Jesús Díez, **José Manuel García-Fernández**

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Abstract text

Marine picocyanobacteria were traditionally considered autotrophic organisms. However, different teams have shown that they can also take up organic compounds. We discovered that *Prochlorococcus* can take up glucose; our team has extensively characterized this process, identifying the transporter (encoded by the *glcH*gene) and studying its glucose uptake kinetics with a series of physiological studies. Using transcriptomic, proteomic and metabolomic studies, we have shown that glucose is assimilated and can induce significant changes in the metabolism of *Prochlorococcus* and marine *Synechococcus* when available in high concentrations [1]. Laboratory experiments indicate this is active transport, while studies in the field have demonstrated that wild *Prochlorococcus* take up glucose at significant rates, following circadian rhythms, which are opposite to those of the rest of the marine microbial population [2]. Metagenomic studies show that the abundance of the *glcH* genes is similar to that of core genes in these microorganisms, highlighting the importance in their evolution. We are currently analyzing the structure of the transporter to understand the molecular underpinnings of its high affinity for glucose and multiphasic kinetics [3]. We are also carrying out studies to compare the glucose uptake kinetics of the glucose transporters GlcH vs GlcP (the standard glucose transporter in freshwater cyanobacteria, encoded by *glcP*). Our results support the idea that marine picocyanobacteria are actually mixotrophs, contrary to initial expectations.

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Funding

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EXCRETE enables deep proteomics of the cyanobacterial extracellular environment

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Abstract text

Extracellular proteins play a significant role in shaping microbial communities which, in turn, can impact ecosystem function, human health, and biotechnological processes. Yet, for many ubiquitous microbes, such as cyanobacteria, there is limited knowledge regarding the identity and function of secreted proteins [1]. Here, we introduce EXCRETE (enhanced exoproteome characterization by mass spectrometry), a workflow that enables a deep description of microbial exoproteomes from a few hundred microlitres [2]. This workflow can be done in less than 1 hour, with no special equipment, and with an overall cost of less than 0.50€/per sample. Using cyanobacteria as a case study, we benchmark EXCRETE and show that extracellular protein identifications almost doubled in comparison to a traditional ultrafiltration/in-solution digestion method. Subsequently, we show that EXCRETE can be miniaturized and adapted to a 96-well high-throughput format. Application of EXCRETE to different cyanobacteria species (*Synechocystis* sp. PCC 6803, *Synechococcus* sp. PCC 11901, and *Nostoc punctiforme* PCC 73102) and extracellular matrices (salt-rich and, viscous, polysaccharide-rich samples) identified up to 85% of all predicted secreted proteins. Finally, functional analysis reveals that cell envelope maintenance and nutrient acquisition are central functions of the cyanobacterial secretome. Collectively, these findings challenge the general belief that cyanobacteria lack secretory proteins and point to a functional conservation of the secretome across freshwater, marine, and terrestrial species.

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Modeling microbial communities using biochemical resource allocation analysis

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Abstract text

Oxygenic photosynthesis, as originally evolved in cyanobacteria, is a critical biological process that drives primary production in most ecosystems. While cyanobacterial growth dynamics in axenic laboratory cultures are reasonably well understood, cyanobacteria evolved as parts of interconnected ecosystems, and understanding their physiology requires taking their interactions with other microorganisms into account.

Our objective is, therefore, to understand the emergence of interactions between photo- and heterotrophic microorganisms using computational models based on biochemical resource allocation analysis. We aim to characterize the prerequisites and energetic trade-offs governing cooperation, division of labor, and nutrient cycles in microbial communities.

Building on established methods, we make use of quantitative computational models of microbial growth and resource allocation to simulate co-cultures of photo- and heterotrophic organisms, where each microbial partner maximizes its growth rate. By examining the costs and benefits of these interactions, we outline a plausible evolutionary pathway for the emergence of metabolic dependencies between these microorganisms in marine environments. We show that co-cultures can result in long-term stable cultures with increased productivity compared to axenic growth.

In summary, the perspective of cellular resource allocation offers a unique opportunity to understand the constraints and energetic trade-offs that govern the emergence of dependencies between photo- and heterotrophic microorganisms.

Funding

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135 POSTER

Resistance mechanisms to UVR stress of two *Nostoc commune* strains from warm and cold deserts unveiled by differential gene expression

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Abstract text

Cyanobacteria can survive in extreme environments, such as drylands or polar regions, and are found in aquatic or terrestrial habitats around the world. Their different resistance traits to cope with extreme and contrasted environments, such as the production of pigments to absorb UV radiation (UVR), have been well studied. However, a better understanding of their ecophysiological responses is necessary to clarify how these processes work. In the present study, we analyzed the ecophysiological performance of two *Nostoc commune* strains under UVR. Strain ULC002 was isolated from a microbial mat in an Antarctic lake (Larsemann Hills, East Antarctica) and CANT2 from a biological soil crust in a semiarid region (limestone quarry in Gádor, SE Spain). To analyze their resistance to UVR, both strains were incubated for 3 days under a UV-B and –A radiation of 2 W/m^2 and 10 W/m^2, respectively, followed by a recovery period without UVR. The chlorophyll *a*, scytonemin and carotenoid contents were extracted from the cultures and measured with a spectrophotometer at different time points, as well as the photosynthetic efficiency (F_v/F_m, measured by Pulse-Amplified Modulation spectrophotometry). Both strains showed a decrease in F_v/F_m values under UVR but the chlorophyll *a* contents showed a different pattern, being more stable in UL002 than in CANT2. In view of these results, the RNA of each strain was extracted at three different time points and sequenced by Illumina Novaseq. We analysed the differential gene expression of both strains to understand the genetic basis of their ecophysiological responses to UVR.

Funding

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How does temperature influence microcystin-LR content in acclimated *Microcystis aeruginosa* PCC7806?

Pierre-Louis Lalloué, Fanny Perrière, Célia Kerbrat, Anne-Hélène Le Jeune, Alexandre Bec, Apostolos-Manuel Koussoroplis, Clarisse Mallet, Delphine Latour

Laboratoire microorganismes : génome et environnement, CNRS, UCA, Clermont-Ferrand, France

Abstract text

In a warming environment, cyanobacteria are anticipated to exhibit accelerated growth rates, thereby amplifying the frequency and severity of blooms. These proliferations pose various threats to the ecosystem, with cyanotoxins standing out as a major concern as they impact the aquatic food web and may have consequences for human health. Among cyanotoxins, microcystins have been extensively investigated due to their worldwide distribution and notable toxicity. Recently, numerous studies focused on the influence of environmental parameters, like temperature, on microcystin production. However, it remains insufficiently studied with conflicting research findings indicating both augmentation and diminution of production at warmer temperatures. To improve our understanding of how predicted increase in mean temperature might impact the intracellular microcystins content, we worked on cultures of *Microcystis aeruginosa* PCC7806 that had undergone full acclimatization to temperatures ranging from 17°C to 35°C. We assessed cell density every two days to estimate exponential growth rates. Cell cultures were harvested during mid-exponential growth for intracellular microcystin-LR quantification using HP-LC, coupled with cell volume measurements conducted under an optical microscope. Our results contribute to understand the divergent findings reported in literature, which likely depend on the manner in which microcystins are expressed. When expressed as intracellular microcystins per cell, our results reveal an increasing with decreasing temperature, consistent with recent publications [1]. However, when expressed per biovolume unit, the same data unveil a non-linear relationship, reaching an optimum at 26°C. This observation raises questions about the potential role of microcystins in temperature resistance of acclimated cells.

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137 POSTER

A light-driven competition between the picocyanobacterium *Merismopedia* and a larger cyanobacterium, *Microcystis*

Noémie Cheuvart[1,2], Benjamin Legrand[2], Benjamin Marie[3], Fanny Perriere[1], Apostolos-Manuel Koussoroplis[1], Anne-Hélène Le Jeune[1], Delphine Latour[1], Laurianne Paris[1]

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Abstract text

Picocyanobacteria are defined as cyanobacteria that do not exceed 2 µm. For a considerable period, they received limited attention because of their size, as they were more difficult to study than larger cyanobacteria. Nevertheless, due to their potential toxin production, their proliferation can result in bathing closures, causing significant economic impact. Currently, the ecology of freshwater picocyanobacteria remains relatively unexplored. As they are supposed to be more abundant in early summer in association with larger cyanobacteria, we decided to study the effect of light on co-culture conditions involving the picocyanobacterium *Merismopedia punctata* PMC142.05, and the larger cyanobacterium *Microcystis aeruginosa* PCC7806. We specifically chose these organisms due to their frequent co-occurrence in French lakes. Over a period of 35 days, we closely monitored their development under two light intensities (low: 14 and high: 34 µmol.s^{-1}.cm^{-2}) in co-culture and monocultures. Results showed that after 17 days, *Merismopedia* began to predominate in the co-culture under low light conditions, while under high light conditions, a contrasting trend emerged. For the initial 24 days, both species exhibited concurrent development, but subsequently, *Microcystis* took an advantage over *Merismopedia*. This shift in kinetic was unexpected as *Merismopedia* declined promptly, which was not observed in other conditions. This leads us to question the impact of metabolites excreted by *Microcystis* on the growth of *Merismopedia*. Furthermore, it encourages us to investigate the broader role of light as a triggering factor in the succession from picocyanobacteria to cyanobacteria observed between spring and summer in freshwater lakes.

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Emerging Nitrogen-Fixing Cyanobacteria for Sustainable Cotton Cultivation

Lucía Jiménez-Ríos, Alejandro Torrado, José Luis Gonzalez-Pimentel, Macarena Iniesta-Pallarés, Fernando Publio Molina-Heredia, Vicente Mariscal, Consolación Álvarez

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Abstract text

Among growing environmental concerns and the crucial need for sustainable agricultural practices, this study explores the transformative potential of nitrogen-fixing cyanobacteria as biofertilizers, especially in cotton cultivation. The excessive use of synthetic nitrogen fertilizers in modern agriculture highlights significant environmental challenges, including greenhouse gas emissions and water system contamination. Our goal is to provide an eco-friendly alternative by investigating the natural capabilities of cyanobacteria.

Applying advanced analytical methods, including the relevant use of metabarcoding, we conducted a comprehensive assessment of soil bacterial communities within cotton fields. This study focused on evaluating the diversity, structure, taxonomic composition, and potential functional characteristics of these communities. Focus was placed on the isolation and subsequent application of native N_2-fixing cyanobacteria strains obtained from cotton soils. The results underscored these strains' efficacy in promoting cotton growth through significant plant growth-promoting (PGP) activities, such as N_2 fixation, phytohormone production, Fe solubilization, and biofertilization potential of five isolated cyanobacterial strains, underscoring their efficacy in cotton.

This research introduces the idea of employing these cyanobacteria as natural, eco-friendly alternatives to synthetic fertilizers. Besides, reintegrating these beneficial species into agricultural ecosystems can improve crop growth while maintaining a balanced microbial environment, as well as promoting global sustainable agriculture [1].

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139 POSTER

ZepA, a novel exoprotein mediating zinc acquisition in *Anabaena/Nostoc* sp. PCC 7120

Cristina Sarasa-Buisan[1], Jesús A. G. Ochoa De Alda[2], Cristina Velázquez-Suárez[3], Miguel Ángel Rubio[3], Guadalupe Gómez-Baena[4], María F. Fillat[1], **Ignacio Luque**[3]

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Abstract text

Zinc is an essential nutrient for all living beings and in many bacteria zinc handling is controlled by Zur, a regulator of the FUR family. Previous research has shown that in cyanobacteria, Zur represses the expression of many genes encoding cytoplasmic, plasma membrane, periplasmic and outer membrane proteins. We have investigated the role of Zur in the composition of the exoproteome of *Anabaena/Nostoc* sp. PCC 7120 and we have identified a protein of unknown function, named ZepA, that becomes particularly abundant in the in the extracellular space under conditions of zinc scarcity or in a Δ*zur* mutant. Experimental evidence has shown that ZepA is processed upon secretion, binds extracellular zinc in a conserved pocket composed of three histidines and mediates transfer of this metal to the cytoplasm, probably through interaction with a specific receptor on the cell surface. Based on mathematical models developed for siderophores, we propose that ZepA would be most efficient at zinc acquisition in structured habitats (i.e. microbial mats, biofilms or soil), where diffusion of extracellular molecules is limited. This appears in line with the strong competition for nutrients in these environments, where ZepA would allow *Anabaena* to lock zinc away from competitors that lack the specific receptor for this protein. *zepA* orthologs are only present in a handful of species of distant bacterial lineages, including some cyanobacteria. Phylogenetic analysis suggest that ZepA evolved early and was likely present in ancient bacteria, consistent with the scarcity of zinc in ecosystem of the archaean eon previous to the GOE.

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Water temperature effect on phycocyanin probe signal and correction factor applied to three morphotypes to improve cyanobacteria field monitoring

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Laurianne Paris[1], Noémie Cheuvart[1,2], Mathilde Dutaut[2], Sylvain Coly[2,3], David Foltier[2], Benoît Gire[2], Marianne Coulon[1,2], Antoine Thouvenot[2], Delphine Latour[1], Benjamin Legrand[2]

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Abstract text

In a context of global warming and heavy anthropization of freshwater systems, cyanobacteria monitoring is becoming an essential priority. In recent years, submersible fluorescence sensors capable of measuring pigments and estimating cyanobacterial biomass in near-real time have been deployed on a large-scale. However, these tools have several limitations, such as the detection limit of pigments like phycocyanin or the impact of temperature on the measurements. In an attempt to overcome these shortcomings, we compared the effectiveness of a phycocyanin probe at different temperatures, first on three cyanobacteria cultures (*Microcystis*, *Dolichospermum* and *Synechocystis*) and then on a small eutrophic lake in continuous recording for three years.

In vitro results showed a strong correlation between cyanobacterial biomasses and phycocyanin probe measurements, with R^2 higher than 0.96, even for biomasses lower than hundreds of $mm^3.L^{-1}$. A saturation effect was demonstrated between 350 and 550 $mm^3.L^{-1}$, depending on the strain. In addition, laboratory tests on temperature highlighted the sensor's limitations, with a strong over- and underestimation at low (< 15 °C) and high (> 25 °C) temperatures, respectively. Given this discrepancy, a water temperature correction factor was determined for each strain and applied according to the predominant cyanobacteria group identified in the field during the three years monitoring. Signal quality improved considerably, and the stronger relationship found between the corrected signal and the biovolume of cyanobacteria obtained by microscopic enumeration enabled us to validate this temperature correction factor, offering the possibility of using it to better monitor cyanobacterial dynamics.

Funding

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Expression of fatty acid desaturase gene variants from different marine cyanobacterial ecotypes in the model organism *Synechococcus elongatus* PCC7942

Isabel Escribano Gómez[1], Mercedes Nieves Morión[2], Ángel López Urrutia[3], Laura Alonso Sáez[1]

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Abstract text

The marine picocyanobacteria *Prochlorococcus* and *Synechococcus* are two of the most abundant phototrophs on Earth, with a wide distribution across the oceans . However,different ecotypes of these genera are adapted to distinct environmental conditions,including temperature and light. Maintaining membrane fluidity is crucial for the physiology of these phototrophs, as their photosynthetic machinery is located in cellular membranes. Therefore, the capability to control membrane fluidity may be key to understand ing their capability to withstand changes in environmental conditions. Fatty acid desaturases are enzymes that introduce double bonds into specific positions of fatty acyl chains and are,thus, essential for modulating membrane fluidity. Previous studies have shown that the composition of fatty acid desaturases and the degree of unsaturation of the acyl moiety of their membrane lipids vary among different marine cyanobacterial ecotypes. Furthermore, some factors such as a cold temperature influence the activity of these enzymes . Here, we performed a functional study of fatty acid desaturases from different marine cyanobacterial ecotypes by heterologous expression on the freshwater strain *Synechococcus elongatus* PCC 7942 , which contains a streamlined repertory of desaturases. Our aim is to understand the specific role of different fatty acid desaturase gene variants in cyanobacterial membrane lipid remodelling and ecophysiology.

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Toward understanding the regulatory strategy of *Mastigocladus laminosus* UU774: Morphological and transcriptional landscape under nutrient, nitrogen and heat stress

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Aribam Geeta[1,2], Mayuri Mukherjee[1,2], Sucheta Tripathy[1,2]

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Abstract text

The present study aims to evaluate the molecular and morphological processes of *Mastigocladus laminosus* UU774, a thermophilic cyanobacterium isolated from an Indian hot-spring, in response to three different abiotic stresses: nutrient, nitrogen and heat. During the early stages of nutrient treatment (exposure to media for 2h, 18h and 3days), UU774 concentrates its resources on specific functions, as evidenced by the 6.7% and 25.7% genes that are commonly downregulated and upregulated, respectively, in the three timepoints. Few genes that are overexpressed are related to housekeeping functions like carbon and energy metabolism. The transcription profile of the genes involved in heterocyst formation revealed that heterocyst development in UU774 occurs in 24h. Considered the heterocyst commitment timepoint, HetP, the principal heterocyst commitment factor, is upregulated at 18h. When nitrogen was added to the medium, UU774 yielded distinct transcriptomic pattern at 2h, 18h and day3. On day3, the nitrogen fixing, DNA repair and carbon metabolism genes displayed inverted expression, in contrast to their usual up- and down-regulation in 2h and 18h timepoints. The presence of nitrogen in the medium causes a delay in heterocyst formation switch and the heterocyst generation process took longer than 24h to complete. Heat shock study suggested that UU774 survives heat treatment for 6 and 24h by overexpressing peptidases [1]. Extended heat treatment produces dormant cells with a diameter of 3-10 µm that are resistant to lysozyme and bear a physical resemblance to monocytes. When restored to ideal conditions, dormant cells can produce germlines, indicating their reproductive potential.

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143 POSTER

The Organic Nitrogen Diet of Freshwater Picocyanobacteria

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Abstract text

Freshwater picocyanobacteria (*Syn/Pro* clade) are an essential component of our waterbodies, substantially contributing to primary production in waters which are often limited or co-limited by nitrogen. Nevertheless, they remain relatively poorly understood ecologically and genomically, especially in comparison to their marine relatives. Likewise, cyanobacterial nutrient research has typically focused on inorganic sources of nitrogen (ammonium and nitrate), neglecting organic nitrogen, especially amino acids, which dominate the oligotrophic environments in which picocyanobacteria thrive [1]. However, it is becoming increasingly evident that organic nitrogen is bioavailable and an integral component of the freshwater nitrogen pool.

Comparative genomic analyses on 295 strains of picocyanobacteria was carried out to identify encoded organic nitrogen assimilation capabilities within the *Syn/Pro*, comparing mechanisms present in freshwater strains to those found in marine environments. We reveal diversity in the amino acid transporters encoded, alternative mechanisms for chitin assimilation, and identify knowledge gaps in AA degradation capabilities within the *Syn/Pro*. Growth assays were conducted to determine the growth capabilities of freshwater picocyanobacteria utilising organic nitrogen sources, followed by proteomic analysis to investigate the intracellular response to growth on selected amino acids. Our results display widespread amino acid bioavailability, without constraint of encoded amino acid transporters suggesting extracellular catabolism or novel transporter function. Proteomic analysis putatively revealed a mild stress response under all AA conditions and potential mechanisms for basic AA assimilation. Our results contribute to the understanding of how picocyanobacteria have come to dominate DON-rich oligotrophic environments, and how their molecular machinery influences picocyanobacterial communities across habitats.

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Proteomic and transcriptomic analyses of nitrate utilization in co-cultures of *Prochlorococcus* and *Synechococcus*

Yesica Melero-Rubio, **Antonio López-Lozano**, Guadalupe Gómez-Baena, José Manuel García-Fernández

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Abstract text

The marine picocyanobacterial *Synechococcus* y *Prochlorococcus* dominate the world's picophytoplankton, occupying a key position at the base of marine food webs and contributing substantially to maintain the carbon, oxygen and nitrogen cycles in the biosphere [1]. The spatial distribution of these two groups depends on different factors such as nutrient availability, light and temperature [2].

These microorganisms require nitrogen (N) as an essential nutrient for growth, being ammonium the preferred source. Access to N is a limiting factor for phytoplankton in the oceans [3]. The assimilation of nitrate, the most oxidized form of N, is of particular interest since it is an abundant N species in oceanic environments, although it is also a costly source for the cell to metabolize. Almost all *Synechococcus* strains are able to utilize both oxidized and reduced forms of N unlike most *Prochlorococcus*, lacking the machinery to assimilate nitrate.

In this work, we present a detailed study, including proteomic and transcriptomic analyses, of the response of two marine cyanobacterial strains to a change in N source. *Synechococcus* sp. WH8102 and *Prochlorococcus* sp. MIT9313, strains thriving in the oligotrophic areas of the ocean, were grown both isolated and in a mixed culture under two experimental conditions, starting with standard ammonium concentration and then switching to micromolar nitrate concentration as sole N source. Our study evaluates the strain-specific metabolicresponse to the switch in N source and whether the presence of a competitive strain modulates the range of such responses.

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Environmental factors modulate the essentiality of the circadian rhythm

Alfonso Mendaña[1], María Santos-Merino[2], Raquel Gutiérrez-Lanza[1], Marina Domínguez-Quintero[1], Danny Ducat[2], Raúl Fernández-López[1]

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Abstract text

Cyanobacteria generate reactive oxygen species (ROS) as a by-product of photosynthetic activity. It is generally accepted that ROS produced during the day must be removed by night metabolism [1, 2]. In species with a strong circadian character, like *Synechococcus elongatus* PCC 7942, several mutants in circadian control exhibit an accumulation of ROS, which was linked to the inability of these mutants to mobilize glycogen reserves during nighttime [1]. This redox crisis leads to a phenotype of darkness-induced lethality, and the inability to grow in diel conditions. Here we explore how light intensity and CO_2 levels modulate these phenotypes in different circadian mutants, showing that circadian control is dispensable in particular environmental conditions. Our results point to a potential adaptive role of the circadian cycle to the changing irradiation and CO_2 levels during Earth´s history.

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The sigma factor *sigC* is a sensor of light intensity in *Synechoccocus elongatus* PCC 7942

Marina Domínguez-Quintero, Alfonso Mendaña, Raquel Gutiérrez-Lanza, Juan Manuel Medina, Víctor Campa, Raúl Fernández-López

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Abstract text

Light intensity is one of the major environmental factors constraining growth in cyanobacteria. To survive, these autotrophic microorganisms must integrate external information on irradiance with the internal circadian cycle that dominates gene expression during day and night times. In *Synechococcus elongatus* PCC 7942, the cycle controls the expression of nearly 70% of the genome, and many sigma factors are expressed in a circadian fashion. Here we show that the sigma factor *sigC*, which was previously involved in circadian control, specifically responds to light intensity, repressing its expression in conditions of high irradiance. Our results identify this sigma factor as a crossroads where light intensity and circadian regulators converge, suggesting that circadian control may be intimately linked to environmental sensing.

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147 POSTER

From biology to ecology: The role of N_2 fixation

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Abstract text

In recent years, new insights have been gained into the biological mechanisms of N_2 fixation. The impact of these mechanisms on the ecological role of this process has not yet been quantified at the ecosystem level. The natural assumption, that the ability to fix N_2 leads to an advantage under low DIN conditions, has not been observed in Lake Müggelsee. This lake experienced a decline in DIN concentration from 1980 to the present day but the share of *Nostocales* in total phytoplankton biomass has decreased [1]. We hypothesize that 1) the costs associated with N_2 fixation outweigh its benefits at moderate N stress, and 2) that the strategies differ among the N_2-fixing species regarding the level of N_2 at which cells differentiate, resulting in different responses to reduced or pulsed nitrogen supply. This poster outlines a project that is just starting to explore this question. The project will include laboratory experiments, field data from Lake Müggelsee as well as a dynamic, molecular-level agent-based model of N_2 fixation in filamentous cyanobacteria based on the existing model of *Anabaena* developed previously by Hellweger et al. (2016) [2]. A reproduction of the observed long-term trend from Lake Müggelsee by the updated model will support our hypothesis. The model will then be used to analyze the effects of increased temporal variability of N stress caused by climate changes as well as to gain new insights regarding N and P management in lakes.

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Nitrogen transfer from nitrogen-fixing cyanobacteria to diatoms

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Abstract text

N_2-fixing cyanobacteria are important contributors of combined nitrogen in nutrient-poor oceanic areas, in which they promote the growth of other organisms including algae thus increasing primary productivity [1]. Some N_2-fixing cyanobacteria live in symbiosis with algae, which likely involves specific, direct mechanisms of N transfer, whereas in other associations, interactions likely involve more general transfer mechanisms. Nonetheless, whereas the concept of N enrichment of the ecosystem by N_2-fixing cyanobacteria is widely considered, possible mechanisms of N transfer are largely ignored. To address possible N transfer mechanisms, we are studying the relations between the model marine diatom *Phaeodactylum tricornutum* and marine cyanobacteria including the unicellular N_2-fixing strain *Crocosphaera subtropica* ATCC 51142 and the filamentous, heterocyst-forming strain *Anabaena* sp. ATCC 33047. We first set up conditions permissive for the growth of both partners (testing different growth temperatures under light/dark cycles in marine artificial seawater medium) and found that, in co-cultures incubated in media lacking combined-nitrogen, the yield of both partners was frequently higher than their yield in individual cultures, indicating positive mutual influences of the partners for growth. In addition to considering the release of N-containing compounds such as ammonia or amino acids by the N_2-fixing cyanobacteria, we are exploring a possible role of membrane vesicles as N vehicles. Membrane vesicles are now known to be widely produced by bacteria, and in the case of outer-inner membrane vesicles (OIMV), they can carry cytosolic content [2]. We have observed abundant membrane vesicles 40-150 µm in diameter in *C. subtropica* supernatants.

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Funding

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149 POSTER

Effect of the presence of *Anabaena* in stress tolerance of plants: influence of FUR proteins expression

Giuliana Oriana Morabito*, Alodia Liesa*, Jorge Guío, Irene Oliván-Muro, María Francisca Fillat, **Ana Alonso-Simón**

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Abstract text

Cyanobacteria are ubiquitous microorganisms in soil, and may offer various benefits to plants. Nitrogen-fixing species act as biofertilizers, while others aid plants in overcoming abiotic stresses, through extracellular polymeric substances secretion (e.g. polysaccharides) or biofilm formation. Previous research in our group demonstrated *Anabaena* PCC7120's ability to degrade lindane, a pesticide harmful to plant growth [1].

FUR (Ferric Uptake Regulator) proteins are a family of transcriptional regulators essential to face several stresses in prokaryotes [2]. The genome of *Anabaena* sp PCC7120 codes for three FUR paralogs: FurA, FurB (Zur) and FurC (PerR), which are global regulators and control metal homeostasis, nitrogen metabolism and the response to oxidative stress, among other processes [3].

In this work we want to find out if the presence of *Anabaena* cells may help rice (*Oriza sativa*) seeds to germinate and develop when subjected to two different abiotic stresses, namely the presence of lindane and an excess of copper. Accordingly, we have conducted different experiments to test the effect of increasing concentrations of lindane and copper on rice seeds, evaluating their germination and growth. We have also studied the effect of both compounds on *Anabaena* growth, including wild type and mutants showing overexpression and repression of diverse FUR proteins. Finally, we have tried various ways to culture rice together with *Anabaena* cells, in order to evaluate the putative beneficial effect of cyanobacteria on overcoming abiotic stress. Further analysis on the effects of different *Anabaena* variants showing deregulation of FUR proteins in seeds germinations are on the way.

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Study of the effect of temperature on different strains of marine *Prochlorococcus* and *Synechococcus*

Claudia Moyano-Bellido, Antonio López-Lozano, María Agustina Domínguez-Martín, José Manuel García-Fernández

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Abstract text

Prochlorococcus and marine strains of the genera *Synechococcus* are the most abundant photosynthetic organisms on Earth [1]. These microorganisms contribute greatly to ocean primary production and play a key role in regulating global biogeochemical cycles. *Prochlorococcus* strains, classified in HL (high light) and LL (low light) ecotypes, are distributed between latitudes 45°N and 40°S, while *Synechococcus* show a wider surface distribution than that of the genera *Prochlorococcus*, not thriving at the depths this latter genera reaches [2].

Distribution patterns for *Prochlorococcus* and marine *Synechococcus* are defined by different parameters, such as light, nutrient concentration, and temperature. This work is focused on temperature, one of these key parameters. In our work, the effect of temperature has been studied in independent *Synechococcus* and *Prochlorococcus* cultures as well as in *Synechococcus* and *Prochlorococcus* co-cultures. In order to study the effect of temperature on these cultures through time, flow cytometry has been used to measure the number of cells, while different parameters such as the fluorescence of specific pigments and absorbance were also determined. Furthermore, the expression of key genes involved in nitrogen metabolism, and the morphology of cells from these cultures using transmission electron microscopy (TEM) were also studied.

A comprehensive study of the effect of temperature on individual cultures and on co-cultures would allow us to predict the future distribution of cyanobacteria in the ocean, from the evaluation of the individual response of the *Synechococcus* and *Prochlorococcus* strains, to the potential competition between different ecotypes from these two genera.

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Cyanobacterial-Plant Symbioses: Bridging Basic Research with Agronomic Applications

Vicente Mariscal[1], Lucía Jiménez-Ríos[2], Macarena Iniesta-Pallarés[1], Ana Jurado-Flores[2], Fernando P. Molina-Heredia[1], José Luis González-Pimentel[2], Consolación Álvarez[1]

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Abstract text

Nitrogen-fixing cyanobacteria within the *Nostocales* order can establish symbiotic relationships with a variety of plant species. These organisms exhibit a high level of symbiotic competence, as evidenced by a single cyanobacterial strain's ability to engage in biological nitrogen-fixing (BNF) associations with multiple plant species [1]. Our research centers on exploring the diversity of cyanobacterial-plant symbioses, with a particular focus on rice as a model organism. We focus into the structural dynamics of these associations and elucidate the mechanisms underlying symbiotic communication [2]. Moreover, we highlight the emerging role of cyanobacterial species as bio-inoculants in BNF, aimed at enhancing soil fertility and crop yield. Through the analysis of the soil microbiome in rice paddies, we have gained insights into the bacterial diversity within the rhizosphere [3]. Additionally, we have isolated and characterized specific cyanobacterial strains for their potential as rice biofertilizers, demonstrating significant improvements in plant growth and yield, thereby substantiating the efficacy of cyanobacterial inoculants as biofertilizers [3]. This approach offers a sustainable alternative to conventional synthetic fertilizers, promoting eco-friendly agricultural practices.

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Signalling Pathways Involved the Symbiotic Communication between *Nostoc punctiforme* and *Oryza sativa*

Consolación Álvarez, Manuel Brenes-Álvarez, Fernando Publio Molina-Heredia, Vicente Mariscal

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Abstract text

The symbiotic cyanobacterium *Nostoc punctiforme* has been extensively used as a model to study symbiosis between cyanobacteria and plants, including cereals [1]. We studied the signalling mechanisms involved in recognition between the cyanobacterium and *Oryza sativa*, in order to provide knowledge of the symbiotic interaction. To do that, we conducted a quantitative proteomic analysis (SAWATH MS) at early stages of the pre-symbiotic process [2]. We found 470 proteins significantly increased in abundance in *N. punctiforme* and 104 in *O. sativa*. Proteins were linked to several biological functions, including signal transduction, adhesion, defence-related proteins, and cell wall modification. While some of the proteins have previously been associated with plant–microbe interactions, other proteins are newly described in this context. Our research uniquely demonstrated the induction in *N. punctiforme* of several proteins analogous to Nod biosynthetic enzymes from *Rhizobium* sp., likely participating in the biosynthesis of lipo-chitooligosaccharides (LCOs). LCOs are perceived in the plant host through the 'common symbiosis signalling pathway' (CSSP). *Oryza* mutants in genes in the CSSP showed a reduced colonization efficiency providing first insights on the involvement of the CSSP for the accommodation of *N. punctiforme* inside the plant cells. We generated a *N. punctiforme* mutant in one of the Nod proteins induced in response to the plant, resulting in a strain with significantly impaired symbiotic capabilities. This outcome reinforces our initial hypothesis regarding the role of cyanobacterial LCOs in symbiosis, providing a greater understanding of the symbiotic interaction between *Nostoc* and land plants [3].

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Methane and CO_2 consumption from waste gases by microbial communities in enriched seawater

Niels-Ulrik Frigaard[1], **Ernst Stefan Seemann**[2]

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Abstract text

The greenhouse gases methane (CH4) and carbon dioxide (CO2) occur as often undesirable waste products in various industries. We have explored how these gases may be consumed by microbial communities for potential conversion into useful biomass products.

Bioreactors with natural seawater were enriched with inorganic nutrients and injected with a continuous gas stream containing CH4 and CO2, either in the dark or in the light. Microbial communities established that contained methanotrophic bacteria consuming CH4 and cyanobacteria and microalgae consuming CO2. The microbial diversity was characterized using chemotaxonomic markers (pigments and isoprenoid quinones), and 16S and 18S ribosomal RNA gene amplicons sequenced by Nanopore technology. Due to the rapid evolution of Nanopore chemistry, no single data analysis pipeline is currently recommended. We discuss different workflows and taxonomic reference databases used to identify enriched microbial communities. The microbial diversity was drastically lower in the incubated cultures as compared to the natural communities. The growth of phototrophs increased remarkably under light conditions with an early dominance by cyanobacteria later overtaken by microalgae. In the enriched cultures, the most abundant microalgae were green algae related to *Picochlorum*, and the most abundant cyanobacteria were related to *Geitlerinema* sp. PCC 7105, *Symphothece* sp. PCC 7002 and *Cyanobium* sp. PCC 6307 (based on SILVA reference database). The growth of methanotrophs increased remarkably only in the dark.

We conclude that it may be optimal to physically separate dark-dependent CH4 consumption and light-dependent CO2 consumption, until methanotrophic microbes have been characterized that grow well in co-cultures with phototrophs.

Funding

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SulP-family bicarbonate transporter from an N_2-fixing cyanobacterial endosymbiont of an open ocean diatom

Mercedes Nieves-Morión[1], Rubén Romero-García[1], Sepehr Bardi[2], Luis López-Maury[1], Martin Hagemann[3], Enrique Flores[1], Rachel A. Foster[2]

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Abstract text

Diatom-Diazotrophic Associations (DDAs) contribute significantly to new and primary production in the world's oceans. These symbioses involve diatoms and N_2-fixing, heterocyst-forming cyanobacteria of the genus *Richelia*, both partners being photosynthetic. *Richelia euintracellularis* resides in the cytoplasm of *Hemiaulus hauckii*, whereas *Richelia intracellularis* is periplasmic in *Rhizosolenia clevei*. In the ocean, bicarbonate is taken up by phytoplankton to provide CO_2 for photosynthesis. The genomes of both *Richelia* endobionts (ReuHH01 and RintRC01, respectively) contain genes encoding SulP-family proteins, which are oxyanion transporters [1]. To study the possible involvement of these transporters in bicarbonate uptake, we used complementation of a *Synechocystis* sp. PCC 6803 mutant that has its five CO_2 uptake systems inactivated [2]. This mutant strain is unable to grow in air levels of CO_2. Three genes from RintRC01 and one gene and a DNA fragment containing four partial gene sequences from ReuHH01 were chemically synthesized, cloned under the control of a strong gene promoter and incorporated in the chromosome of the *Synechocystis* mutant. One gene from RintRC01, RintRC_3892, complemented the *Synechocystis* mutant to grow with air levels of CO_2 or with low bicarbonate concentrations. The complemented strain showed strong sodium-dependent, low affinity bicarbonate uptake, which, together with phylogenetic analyses, identified RintRC_3892 as a BicA protein [3]. Additionally, RintRC_3892 transcripts were consistently detected in environmental samples from three ocean basins (North Atlantic, South China Sea, and North Pacific gyres). No evidence for a bicarbonate transporter was found for ReuHH01, suggesting different strategies for inorganic carbon uptake in the periplasmic and cytoplasmic endobionts.

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Sustaining rice production: rhizosphere and bulk soil bacterial community dynamics in Doñana wetlands and biofertilization with N_2-fixing cyanobacteria

Macarena Iniesta-Pallarés, Consolación Álvarez, Fernando Publio Molina-Heredia, Vicente Mariscal

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Abstract text

Some parts of the Doñana wetlands (UNESCO World Heritage Site) comprise intensive rice cultivation areas. These Mediterranean marshes are the most productive rice-growing areas in Europe. However, long-term nitrogen fertilization in these croplands results in degradation of soil, water, and air quality, producing eutrophication and contributing to global warming. We examined the bacterial communities in these domesticated soils as they are key for plant health and productivity and have a strong influence on biochemical cycles. We analysed the bulk and rhizosphere soils during different stages in the growing season through metabarcoding analysis coupled with metabolic predictions and co-occurrence networks [1]. The diversity and richness of the bacterial population inhabiting the rhizosphere was much lower than in the bulk soil. Rhizosphere networks, which mostly rely on the phyla Proteobacteria and Cyanobacteria, revealed a high number of negative connections, indicating unstable bacterial communities that may be highly influenced by biotic and abiotic factors. The bulk soil conserved high bacterial diversity and richness that was stable throughout the growth period of rice. Bacterial diversity in rice rhizosphere is critical for enhancing plant growth and productivity. In these agricultural systems, nitrogen-fixing cyanobacteria show a promising biotechnological potential as biofertilizers, improving soil fertility while reducing the environmental impact of the agricultural practice. Thus, we directly studied these Andalusian paddy fields, exploring them to isolate endogenous N_2-fixing cyanobacteria, which were subsequently re-introduced in a field trial to enhance rice production [2]. Our results provide valuable insights regarding an alternative natural source of nitrogen for rice production.

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157 POSTER

Chemical fingerprints of local adaptation within *Limnospira platensis*

Théotime Roussel[1], Cédric Hubas[1], Charlotte Duval[1], Tarik Meziane[1], Claude Yéprémian[1], Jean-Paul Cadoret[2], Cécile Bernard[1], **Benjamin Marie**[3]

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Abstract text

Limnospira can colonize a wide variety of environments (*e.g.* freshwater, brackish, alkaline or alkaline-saline water) and develop permanent blooms that limit overshadowed adjacent phototrophs diversity, especially in alkaline and saline environments (Cellamare et al., 2018). Phylogenomic analysis of *Limnospira* allowed us to distinguish two major phylogenetic clades (I and II), but failed to clearly segregate strains according to their respective habitats in terms of salinity or of biogeography (Roussel et al. 2023). In the present work, we attempt to determine whether *Limnospira* display metabolic signatures specific to its different habitats, particularly brackish or alkaline-saline ecosystems, and question the impact of accessory genes repertoire on respective chemical adaptation.

The study of the metabolomic diversity of 93 strains of *Limnospira* of the Paris Museum Collection, grown under standardised culture conditions, showed clearly distinct chemical fingerprints that were correlated with the respective biogeographic origin of the strains. The molecules that most discriminate the different *Limnospira* geographic groups are sugars, lipids, peptides, photosynthetic pigments, and antioxidant molecules. Interestingly, these molecule enrichments might represent adaptation traits to the local conditions encountered in their respective sampling environments concerning salinity, light and oxidative stress. We hypothesize that within extreme environments, such as those colonized by *Limnospira*, flexible genes that are necessary for the adaptation to specific local environmental conditions (e.g. salinity, light, oxidative pressure) are selected, leading to the specific production of certain metabolites involved in stress defence mechanisms.

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Direct interaction between marine cyanobacteria mediated by nanotubes

Elisa Angulo-Cánovas[1], Ana Bartual[2], Rocío López-Igual[3], Ignacio Luque[3], Nikolai P. Radzinski[4], Irina Shilova[5], Maya Anjur-Dietrich[4], Gema García-Jurado[6], Bárbara Úbeda[2], José Antonio González-Reyes[7], Jesús Díez[1], Sallie W. Chisholm[4], José Manuel García-Fernández[1], **María Del Carmen Muñoz-Marín**[1]

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Abstract text

Nanotubes are membrane-coated tubular structures that connect adjacent cells allowing the direct transfer of biomolecules [1]. In bacteria, they were first identified in *Bacillus subtilis* and *Escherichia coli* [1] and later on marine heterotrophic bacteria [2]. These structures can function as conduits for transporting different metabolites, proteins, non-conjugative plasmids or can also play a structural role in biofilm formation [1].

Our group discovered for the first time the nanotubes structures in xenic and axenic cultures of the most abundant photosynthetic organisms in the ocean, *Synechococcus* and *Prochlorococcus* [3]. We have also observed them in natural samples where *Synechococcus* is abundant. We characterized them with several microscopy techniques (SEM, TEM, Imaging Flow cytometry). Moreover, we demonstrated the exchange of cytoplasmic material between cells via nanotubes using fluorescent molecules (such as calcein, or plasmid- encoded fluorescent proteins).

These results show marine cyanobacterial cells can be connected and interchange molecules using nanotubes. This discovery has important implications for the evolution and ecology of microbial life in the open ocean.

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Cyanobacterial communities of the Sør Rondane Mountains (East Antarctica) differ by substrate types

Valentina Savaglia[1,2,3], Benoit Durieu[1], Bjorn Tytgat[2], Josef Elster[4,5], Wim Vyverman[2], Elie Verleyen[2], Annick Wilmotte[1]

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Abstract text

Cyanobacteria of the Antarctic ice-free areas are ecosystem engineers due to their role as primary producers (Chrismas et al. 2018). Yet, cyanobacterial communities and their drivers within edaphic ecosystems of inland Antarctica are often overlooked. A previous study (Savaglia et al. 2024) describing microbial communities from around 100 soil samples of the western Sør Rondane Mountains (East Antarctica) showed that substrate types were major structuring factors and several cyanobacterial phylotypes were recognized as putative keystone taxa. Here, cyanobacterial diversity was further investigated. Amplicon sequencing was applied using cyanobacterial specific primers targeting the V3-V4 region of the 16S rRNA gene via the Illumina MiSeq platform and taxonomy was assigned using the CyanoSeq database. Our results confirmed that substrate types shape the cyanobacterial communities of this region, providing a higher taxonomic resolution compared to our previous study. Granite harboured the greatest cyanobacterial diversity with a high abundance of filamentous cyanobacteria (i.e. Leptolyngbyaceae, Gomontiellaceae, Microcoleaceae, Oculatellaceae, Nostocaceae), and also unicellular taxa (Cyanothecaceae). In contrast, cyanobacterial diversity was lower in the other investigated substrate types, which were often dominated by only one taxon. Specifically, marble was dominated by *Alitarella*, gneiss by *Cyanothece*, and the dry and oligotrophic Austkampane and Widerøefjellet moraines by Microcoleaceae and Gomontiellaceae, respectively. Moreover, many taxa were uniquely encountered in the moraines of Yûboku-dani Valley, the only site characterized by the presence of several lakes, suggesting a high endemicity degree. Overall, our data highlight the importance of using specific primers and up-to-date taxonomy to accurately draw conclusions about microbial ecology.

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Funding

This work was funded by the Federal Belgian Science Policy Office (BELSPO) under the BRAIN-BE program MICROBIAN project (BR/165/A1/MICROBIAN). VS and BD were supported by a PhD FRIA fellowship from the FRS-FNRS. AW is Senior Research Associate of the FRS-FNRS.

160 POSTER

Marine cyanobacteria extracellular vesicles

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Abstract text

Synechococcus is one of the most important cyanobacteria in marine ecosystems alongside *Prochlorococcus*. They are responsible for a significant part of the primary production in the ocean [1].

It has been shown that these cyanobacteria are able to produce membrane vesicles (MVs) and release them into the ocean [2]. These MVs can be even more abundant than the organisms producing them, and they can contain proteins, different metabolites, carbohydrates, RNA and DNA [2].

We have studied how different strains of *Synechococcus* behave under different stress conditions: light shock, nitrogen starvation and phosphorous starvation. We quantified the abundance of the vesicles by nanoparticle tracking analysis (*NanoSight*) during these experiments, showing an increased vesicle production per cell in the studied strains, notably under light shock.

Phosphorous starvation did not affect culture growth but it did cause an increased vesicle release; while nitrogen starvation caused growth decrease in *Synechococcus* sp. WH8102, WH7803 and PCC 7002. We are currently studying how MVs could impact the growth of cyanobacterial strains when they are supplemented to the cultures in nutrient-limiting conditions; as well as how MVs are integrated in recipient cells using stable isotope labelling coupled with NanoSIMS. Preliminary results indicate that vesicles provide a beneficial effect for the cultures in nutrient-limiting conditions.

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Funding

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161 POSTER

Two Antarctic *Nostoc* sp. strains from freshwater and terrestrial habitats show contrasting resistance mechanisms to desiccation and re-wetting

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Abstract text

Filamentous heterocystous cyanobacteria belonging to *Nostoc* genus are worldwide ecosystem engineers in dry and oligotrophic environments. In Antarctica, members of this genus are often dominant in both edaphic and lacustrine habitats. Yet little is known about their adaptation mechanisms enabling them to thrive such contrasting environments conditions, and during their dispersal between these habitats. Here we studied the response to short-term desiccation exposure and rehydration of two Antarctic terrestrial (ULC180) and freshwater (ULC008) *Nostoc* strains sharing 97.7% of ANI similarity. We compared the concentration of different pigments and osmolytes (i.e. trehalose and sucrose), the photosynthetic efficiency as well as the differential gene expression after RNA-seq (Illumina NovaSeq 2x150 bp) between the controls (T0) and the different treatments (D = after 3h of desiccation; RW1 = after 10 min of rehydration; RW2 = 24h of rehydration; RW3 = 72h of rehydration). Both strains reacted to dehydration by accumulating sucrose, whereas trehalose was present in lower concentrations. Only the freshwater strain showed a recovery in chlorophyll *a* content after 72h of rehydration. Transcriptomic profiles showed that both strains protected their cells during dehydration by inducing stress-related genes, such as those for the production of carotenoids, trehalose and nitrogen fixation, but these were significantly up-regulated only in the terrestrial strain. The latter one also responded with a higher number of up-regulated genes compared to the freshwater strain, including those necessary to protect the photosystem II from degradation (e.g. *psbA2* gene), enabling a stronger resistance to dehydration of ULC180 compared to ULC008.

Funding

VS was supported bya PhD FRIA fellowship from the FRS-FNRS. AW is Senior Research Associate of the FRS-FNRS. BRR was supported by the Special Funds for Research (University of Liège), the IPD-STEMA Program, and the Junta de Andalucía (PAIDI-DOCTOR 21_00571).

162 POSTER

Temperature and drought stress response association with genome diversity of cyanobacterium *Microcoleus*

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Abstract text

Our research investigates the link between drought stress response and genome diversity in the cyanobacterium *Microcoleus*. *Microcoleus vaginatus*, one of the most abundant soil cyanobacterium inhabiting semiarid and arid regions around the globe, exhibits remarkable resilience to desiccation. We investigated if the variations in drought stress tolerance exist among species and are linked to specific regions of the genome under selective pressure, potentially contributing to speciation events. To investigate this, we conducted laboratory experiments using *Microcoleus* strains and exposed them to drought stress in different temperatures, followed by rewetting. We selected 50 *Microcoleus* strains from Stanojković et al. 2024. Throughout the experiment, we monitored their metabolic activity and photosynthetic response. Additionally, we employed transcriptome sequencing to explore gene expression levels and their functional roles under drought stress, providing insights into the factors driving speciation and adaptation.

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163 POSTER

Using novel tools to determine carboxysome accessory components contributions to carbon fixation and oxygen sensitivity.

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Abstract text

Carboxysomes are unique among bacteria microcompartments in that they house the critical enzyme of the center metabolic pathway in carbon-fixing autotrophs. This demand creates selective pressure on the carboxysome and associated proteins to evolve rapid responses to environmental stressors. Despite our knowledge of the role of central carboxysome components in assembly, the function of accessory shell components widely found throughout carboxysome-containing bacteria phyla remains unknown. Furthermore, our current understanding of carboxysome shell as a selectively impermeable barrier lacks direct evidence. Here we utilize fluorescent microscopy and novel gas exchange techniques to elucidate accessory shell components' role in carbon fixation, oxygen sensitivity, and carboxysome function within the cyanobacteria Synechoccocus elongatus PCC7942.

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164 POSTER

Exploration of cyanobacterial–plant symbioses by confocal laser scanning microscopy

Ana Jurado-Flores, Consolación Álvarez, Lucía Jiménez-Ríos, Macarena Iniesta-Pallarés, Fernando P. Molina-Heredia, Vicente Mariscal

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Abstract text

Symbiotic cyanobacteria play a pivotal role in terrestrial ecosystems forming associations with plants across the major phylogenetic divisions. Notably, *Nostoc punctiforme* demonstrates exceptional host versatility, capable of establishing nitrogen-fixing relationships with a variety of plant species. This not only enhances plant growth but also contributes to agricultural productivity. Our research delves into the complex nature of cyanobacterial-plant interactions, examining both endophytic and epiphytic associations. Through the application of confocal laser scanning microscopy, we have detailed the colonization patterns of *N. punctiforme* in its associations with *Sphagnum palustre*, *Anthoceros agrestis*, and *Oryza sativa*. Our findings illuminate the diverse types of associations formed by *N. punctiforme*, which extend beyond a mere surface colonization to include penetration of root surfaces and epidermal cells of host plants. This investigation enhances our understanding of the underlying mechanisms of cyanobacterial symbiosis with terrestrial plants, emphasizing the significance of these interactions in promoting plant growth and development.

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165 POSTER

The hepatotoxin Microcystin shapes the microbiome of the cyanobacterium *Microcystis*

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Abstract text

Harmful cyanobacterial blooms formed *Microcystis* pose an increasing threat to freshwater bodies worldwide. Additionally, some *Microcystis spp*. produce and release a variety of secondary metabolites – many of them being highly toxic to humans and animals alike. Especially, the compound class of Microcystins (MCs) has shown to be hazardous to mammalian life due to their hepatotoxic characteristics. However, the biosynthetic genes encoding for the biosynthesis of MCs have evolved much earlier than eukaryotic life suggesting that MCs may serve purposes going beyond a simple defense mechanism. Still, the biological function to the producing organism *Microcystis* is not fully understood.

In their natural habitat, *Microcystis spp.* usually appear in communities with heterotrophic bacteria, suggesting the existence of specialized phototroph-heterotroph interactions. Our research is aimed to elucidate of the involvement of MCs in the mediation of directed phototroph-heterotroph interactions.

Using 16S-rDNA analysis and PCR-based discrimination of field-sampled single *Microcystis* colonies, we demonstrate that the presence of *mcyA* gene is indicative for lower alpha-diversity. Furthermore, we identified taxa on the species-level, that were significantly differentially abundant based on the presence/absence of the *mcyA* gene.

In longitudinal experiments with synthetic communities consisting of a MC-producing *Microcystis* WT and a non-producing mutant together with 21 heterotrophic isolates, we identified two bacterial isolates that showed reciprocal relative abundance with respect to toxin production. Using these two candidates in tri-partite experiments, we aim to further understand the role of MC for the specialized recruitment of heterotrophic partners as well as the nature of the specialized interaction.

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166 POSTER

Phage resistance in marine picocyanobacteria - a link to carbon export?

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Abstract text

Synechococcus and *Prochlorococcus* are the most abundant phototrophs in the oceans responsible for ca. 25% of global marine primary production. However, current estimates show that up to 40% of these organisms are killed by bacteriophages daily. Despite this, total cell numbers remain relatively stable which hints at potential counter mechanisms against phage infection.

To elucidate these phage resistance mechanisms, we carried out a co-evolution experiment between *Synechococcus* sp. WH7803 and S-CAM7 phages We discovered mutations in the host *galE* genes conferred resistance to the phage. To validate this, we generated *ΔgalE1*, *ΔgalE2*, and *ΔgalE1-ΔgalE2* double mutants in the *Synechococcus* sp. WH7803 host which also showed resistance to the S-CAM7 phages.

Bioinformatics analyses showed copies of the *Synechococcus* sp. WH7803 *galE* genes share similarities with the *galE* gene of *E. coli* that encodes for UDP-galactose 4-epimerase protein. We then verified this function through enzymatic assay in which purified GalE1 and GalE2 proteins convert UDP-Galactose to UDP-Glucose.

This protein is known to be involved in the biosynthesis pathway of cell surface structures. However, the *ΔgalE1*, *ΔgalE2*, and *ΔgalE1-ΔgalE2* double mutants showed similar lipopolysaccharide profiles and growth with the wild-type strain. Interestingly, the mutants display a sinking phenotype which has environmental relevance given such cells would contribute to carbon export to the deep ocean.

Session 5:
Biotechnology and Synthetic Biology

167 INVITED TALK

Biocatalytic Chemical Production Using Photosynthetic Cyanobacteria

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Abstract text

Photosynthetic cyanobacteria, with their low nutrient requirements and versatile metabolism, present a sustainable platform for producing targeted chemicals from sunlight and CO_2 (or N_2). Advances in synthetic biology tools continuously diversify this production platform, enabling the synthesis of a wide array of chemicals. Whole-cell biotransformation emerges as a promising technology, where cyanobacteria efficiently convert fed compounds into targeted chemicals through heterologously expressed enzymes, utilizing photosynthetically produced reducing equivalents and O_2. This utilization of cyanobacteria ensures the sustainable regeneration of cofactors through photosynthesis, addressing a major bottleneck in the biocatalyst field.

By applying our knowledge in photosynthetic electron transfer, we engineer electron flux to heterologous enzymes and study the effects of these artificial sinks on photosynthetic electron transport under various environmental conditions. Our data demonstrate that strong artificial sinks can outcompete alternative electron transport routes.

To further extend biocatalytic production, we entrapped engineered cells in biodegradable polymer scaffolds, transitioning photosynthetic production from suspension to solid-state (hydrogel) production, resembling an artificial leaf. This method addresses challenges associated with suspension cultures and enables long-term continuous production (from days to several months). Integration with 3D-printing and photocurable polymers offers scalability and the potential to enhance sustainability in the chemical industry. These advancements in utilizing photosynthetic cyanobacteria hold promise for a more environmentally friendly and economically viable approach to chemical production.

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Engineering *Syn*6803-based chassis for the expression of heterologous enzymes

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Paula Tamagnini[1,2,3], Catarina C. Pacheco[1,2,3], Filipe Pinto[1,2]

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Abstract text

Pharmaceutical and chemical industries provide most of society's daily used materials, however they are major polluters contributing significantly to carbon emissions and generating 5-100x more waste than product. In this context, biocatalysis became a promising approach to develop greener, more sustainable and cheaper chemical manufacturing with cyanobacteria emerging as alternative chassis to the heterotrophic workhorses currently used. Aiming at expressing industrially relevant heterologous enzymes, such as hydrogenases and monooxygenases [1], several Syn6803 mutants with streamlined photosynthetic electron flow were generated. Our targets included genes encoding putative competing electron sinks such as: flavodiiron proteins Flv1/3, NdhD2 subunit of NDH-1 complex, COX terminal oxidase and a native CYP120A1. Currently, the effectiveness of these chassis, in terms of electron flow redirection towards redox enzymes, is being evaluated.

In parallel, envisaging large-scale outdoors cultivation, Syn6803-chassis harboring a synthetic device for the production of the compatible solute glycine betaine were generated and tested. The presence of this device in a mutant deficient in the production of the native compatible solute glucosylglycerol (Δ*ggpS*) enhanced Syn6803 growth in 3% NaCl compared with the wild-type [2]. The effects of the impairment of putative carbon competing pathways, namely extracellular polymeric substances and glycogen, on glycine betaine production is being assessed. Moreover, we are also investigating the impact of increasing the availability of the precursor glycine.

References

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Funding

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169 ORAL

Nanofilaments for *de novo* organisation of heterologous enzymatic pathways in cyanobacteria

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Abstract text

Cyanobacteria are an attractive group of organisms for the development of sustainable industrial processes owed to their genetic tractability and fast phototrophic growth utilising sunlight as a “free” source of energy. Over the years, engineering cyanobacteria has advanced substantially and their metabolic capacity as cell factories is demonstrated by a large array of different products reported in the literature. Producing a certain molecule, often requires engineering of multi-enzyme, heterologous metabolic pathways. However, the efficient spatial organisation of heterologous enzymes within the complex cellular architecture of cyanobacteria remains challenging.

To address this challenge, we assembled *de novo* cytoplasmic protein-based nanofilaments in cyanobacteria of rod-shaped (*Synechococcus elongatus* UTEX 2973) and coccoid (*Synechocystis* sp. PCC 6803) cellular morphology. As a first step towards functionalisation, we co-localised a fluorescent reporter (mCitrine) with the nanofilaments through the fusion with an encapsulation peptide [1]. Next, we applied this nanofilament-based co-localisation approach to a two-enzyme pathway for the biosynthesis of ethanol in *Synechococcus elongatus*. Applying these strategies led to an increase in ethanol production of more than 25 times. Ongoing work is now focused on engineering nanofilament-cargo interactions to enhance specificity and improve control of stoichiometry. Our work shows that applying synthetic biology is a promising strategy to generate intracellular *de novo* metabolic hotspots and can contribute to advancing product biosynthesis in cyanobacterial chassis.

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Efficient multiplex genome editing of the cyanobacterium *Synechocystis* sp. PCC6803 via CRISPR-Cas12a

Wei Du

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Abstract text

Cyanobacteria have been extensively genetically modified to convert CO_2 to biochemical products. Despite some reports leveraging the CRISPR-Cas system to advance genetic engineering in cyanobacteria, we have carried out below improvements:

► Mob elements of RSF1010-based replicative plasmid were re-located to another suicide plasmid - “Mob plasmid”. Both replicative plasmid and Mob plasmid were transformed in the same *E. coli* for conjugation.

► transcription of both Cas12a and gRNA were enhanced by adjusting the direction of each promoter. Direct Repeats of the gRNA sequences were optimised to avoid the occurrence of simple short repeats. This is to enhance the reliability and robustness of such genetic sequences.

► template DNA fragment was provided as pure plasmids that can be introduced to *Synechocystis* via natural transformation. The *sacB* gene was inserted into the replicative plasmid to facilitate plasmid curing after genome editing.

With this optimised system, the replicative plasmid containing both Cas12a and gRNA is introduced to *Synechocystis* cells via conjugation to cut the circular chromosome. Template DNA plasmid that has meanwhile been assimilated will repair it and genetic modifications can then be achieved. We validated this system in *Synechocystis* by deleting the chromosomal neutral sites N5, N10 and N15, both respectively and collectively. With the *sacB*-sucrose approach for plasmid-curing, all deletions were simultaneously made markerless in just 4 weeks (instead of three months!). Moreover, we have applied this CRISPR-Cas12a system to remove seven targeted genes for an on-going project. We foresee this system will greatly facilitate future genome engineering in cyanobacteria.

171 ORAL

More blue than green: engineering *Synechocystis* sp. PCC6803 to produce bio-indigo

Giovanni Loprete[1], Caterina Martin[2], Marco Wilhelmus Fraaije[3], **Elisabetta Bergantino**[1]

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Abstract text

Indigo is the dye that gives to textiles a characteristic blue color. Its modern industrial production relies on chemical processes using aniline, sodium hydroxide, and chloroacetic acid as starting materials, generating significant amounts of waste and consuming large quantities of energy [1].

Efforts are underway to develop biological processes employing biocatalysis to reduce the environmental footprint associated with indigo production. Flavin-dependent monooxygenases (FMOs) are explored as biocatalysts in different configurations, i.e. as isolated recombinant enzymes or in whole-cell biotransformations using *E. coli* [2]. These oxidative enzymes catalyse the oxidation of indole to indoxyl, resulting in indigo formation. The reliance on costly redox cofactors like NADPH significantly hinders the industrial application of these systems for driving the reaction.

The utilization of the photosynthetic apparatus of cyanobacteria for NADPH regeneration is an efficient mean for overcoming this bottleneck and offers a sustainable method of generating commodity chemicals, by harnessing light energy, CO_2 and water [3]. By constitutively expressing an FMO in *Synechocystis* we enabled the conversion of indole into indigo by whole-cell biotransformation. Grown in batch and fed with indole as substrate, our transgenic cyanobacterial strain produced 0.12 g/L of bio-indigo, conveniently secreted in the growth medium.

Providing insights into the cultivation conditions that support optimal growth and productivity, and proposing a method for recovering the dye directly from the growth medium, we add valuable knowledge for scaling up the process.

The effectiveness and sustainability demonstrated in the laboratory-scale biotransformation using *Synechocystis* provide strong justification for further developments of the process towards industrial application.

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Continuous production of bioplastics from cyanobacteria microbiomes

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Beatriz Altamira-Algarra, Artai Lage, Eva González-Flo, **Joan García**

Universitat Politècnica de Catalunya-BarcelonaTech, Barcelona, Spain

Abstract text

The market for bioplastics is experiencing a steady growth. Polyhydroxyalkanoates (PHA) are the only bioplastics directly synthetized from microorganisms, but the high cost and the negative impacts of current production limit their industrial use. In this work we propose a novel production approach using cyanobacteria in the form of microbiomes obtained from environmental samples. Specifically, we will introduce a methodology consisting on a dual-phase photobioreactor operation which involves cycles of alternating 7-days cell growth and subsequent 7-days biopolymer accumulation. At the beginning of the growth phase, nutrients are added to the culture to allow biomass growth. In the accumulation phase, the PBRs are enclosed to prevent light penetration, and a supplement of acetate is added to enhance PHB production. At the end of this phase, a portion of the reactor content is purged and replaced with fresh medium with nutrients to begin a new repetition (growth/accumulation). We will show results from 4 different microbiomes cultured over 11 photobioreactor cycles, lasting over 160 days of operation. Initially, PHA yield was relatively low for all cultures (< 10 $\%_{dcw}$ PHA). However, as the experiment progressed, PHA production increased. Our strategic production methodology resulted in notable 25-28 $\%_{dcw}$ PHA over much of the 160 days, ranking among the highest values recorded by the cyanobacteria strains present in the studied microbiomes (*Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 6312). Notably a microbiome reached levels up to 40$\%_{dcw}$ PHA. This research signifies a novel approach towards maximizing the potential of cyanobacteria microbiomes.

Funding

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173 ORAL

Light regulation strategy on a phototrophic microbial community towards enhanced hydrogen and lipid production: meta-proteomics reveals the microbial interactions

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Helmholtz Centre for Environmental Research, Leipzig, Germany

Abstract text

The construction of synthetic phototrophic microbial consortia holds promise for sustainable bioenergy production. Nevertheless, it remains elusive whether strategies for efficient regulation of the synthetic communities towards products could be achieved. Applying tools of genetic engineering and light regulation, this study successfully constructed a synthetic community of phototrophs consisting of cyanobacteria *Synechocystis_acs* (acetate overproducing strain) and *Rhodopseudomonas palustris*, enabling a sustainable production of biohydrogen and fatty acids from carbon dioxide and nitrogen fixation. The regulation strategy of circular illumination effectually limited the level of oxygen in systems, ensuring the activity of nitrogenase. Further using a circular illumination of white light and infrared light promoted a significantly enhanced production of H_2 (1.29 μM/mL working volume) and fatty acids (especially C16 and C18). Analysis of elemental balance and proteomics suggests a potential acetate supply from *Synechocystis_acs* to *R. palustris*, and a probable ammonium provided in reverse. Infrared light significantly stimulated the proteins coding nitrogen fixation, carbohydrate metabolic process, and transporter in *R. palustris* in cocultures. Meanwhile, the photosynthesis-related proteins of *Synechocystis_acs* were upregulated the most with constant illumination of white light. This study demonstrated the successful construction of a phototrophic community towards the production of H_2 and fatty acids using genetic engineering and light regulation. Additionally, a deep insight into the interactions within the phototrophic community was revealed.

Funding

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Revealing the hidden life (and death) of cyanobacteria using time-lapse microscopy

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Abstract text

Metabolism is highly organized in space and time. In bacteria, this spatial and temporal organization of metabolism enables multiple, often competing, reactions to occur simultaneously. However, the architectural principles that give rise to bacterial cells, communities, and structures including colonies, mats, and artificial microbial assemblies are only beginning to be appreciated. Our interdisciplinary research lab harnesses cutting-edge synthetic biology tools, advanced live-cell imaging modalities, quantitative image analysis, and an integrated theoretical framework to investigate the regulatory and physical design principles underlying the spatiotemporal modulation metabolism in microbial populations at single and sub-cellular resolution.

We are particularly interested in the study of cyanobacteria because they are major primary producers and are unique in their ability to perform oxygenic photosynthesis, nitrogen fixation, CO_2 fixation, and biomineralization using light as the only energy source; these reactions are naturally optimized through spatial and temporal separation. These attributes make cyanobacteria ideal platforms to investigate the molecular architecture of microbial metabolism. In this talk, I will provide videographic evidence to reveal aspects of cyanobacterial life (and death) that have remained hidden from view-until now.

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Long-term evolution of a fast-growing cyanobacterium

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Abstract text

Cyanobacteria thrive in a diversity of environments, with a wide range of growth rates. Even strains from the same species often exhibit broad differences in environmental preferences and proliferation rates. The reasons for this phenotypic plasticity are poorly known. Here, we performed a long-term evolution experiment where we kept the model cyanobacterium *Synechococcus elongatus* under continuous high light illumination for 1200 generations. The evolved strain exhibited a 600% increase in fitness, growing at rates six time faster than its ancestral counterpart. Genome sequencing revealed three mutations fixed in the population, two of which replicated the fast-growing phenotype in the wild-type. A deletion in SasA, a key circadian regulator, was essential for fast growth. Transcriptomic and metabolomic analyses revealed that this mutation perturbed the circadian rhythm, while simultaneously locking the cell in a transcriptomic response to high-intensity illumination. A comparison with another fast-growing isolate, UTEX 2973, showed convergent transcriptomic states despite different driving mutations. Our results indicate that the circadian clock is key in the adaptation of *Synechococcus* to different environmental conditions, and that mutations in circadian regulation are essential in the generation of fast-growing strains.

Funding

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Cyanobacteria on the edge: How do they respond to very high CO_2?

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Abstract text

Cyanobacteria have adapted to sequester CO_2 from the atmosphere at very low concentrations and are often inhibited at concentrations exceeding 10% CO_2 in the gas phase. Our goals are to understand why cyanobacteria are inhibited under high CO_2 and to bioengineer the organisms such that they are tolerant to higher CO_2 concentrations. CO_2-tolerant strains have several biotechnological applications, such as cultivation in high-CO_2-containing flue gases.

A CRISPR interference knockdown mutant library of *Synechocystis* sp. PCC 6803 was screened for mutations that conferred either a growth-promoting or growth-impeding phenotype under extremely high CO_2 conditions (30% CO_2). The results showed that the knockdown of phycocyanin synthesis promoted growth in 30% CO_2 suggesting that these cells experience high light stress. In contrast, growth inhibition was observed in 30% CO_2 when knocking down a regulator of carbon utilization (*pmgA*), which points to the importance of carbon utilization and its regulation in the adaptation to extremely high CO_2 conditions.

In parallel, we used Illumina sequencing of RNA samples from *Synechococcus* sp. PCC 7002 under conditions of various CO_2 concentrations (up to 30% CO_2) to investigate which genes and pathways are affected by high CO_2 conditions in this strain. The results show that many cytoplasmic membrane proteins and some functions in photosynthesis (including photosystem I and phycobiliproteins) are regulated at 30% CO_2. Single and double CRISPRi knockdown mutants of several inorganic carbon pumps were constructed to compare growth in very high CO_2 (30% CO_2) and optimal conditions (4% CO_2).

Funding

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Thylakoid targeting improves expression and stability of a plant cytochrome P450 in the cyanobacterium *Synechocystis* sp. PCC 6803

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Abstract text

Plants produce a large array of natural products of interest for pharmaceuticals, nutraceuticals, food colourants and flavouring agents. Usually, these compounds are natively produced at low titres and involve complex biosynthetic pathways which often include cytochrome P450s (P450s). P450s are known to be difficult to express in traditional heterotrophic chassis. However, cyanobacteria have shown promise as a sustainable alternative for heterologous expression of plant P450s and light-driven product biosynthesis[1]. In this study, we explore strategies for improving plant P450 stability and membrane insertion in cyanobacteria. We have chosen as our model system the cyanobacterial model organism *Synechocystis* sp. PCC 6803 and the well-studied P450 CYP79A1 from the dhurrin pathway of *Sorghum bicolor*. Combinations of the P450 fused with different elements of PetC1 were tested. PetC1 is the major Rieske protein in the cytochrome b_6f complex and localises to the thylakoid membrane. All tested CYP79A1 variants led to oxime production. Interestingly, the relative oxime levels of variants using elements of PetC1 resulted in up to 20 to 25 times higher oxime levels compared to the unmodified CYP79A1.These findings are promising to improve heterologous P450 expression in cyanobacteria and can ultimately contribute to advancing recombinant plant natural product biosynthesis in cyanobacterial chassis.

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Activity of an O_2 tolerant hydrogenase, expressed in *Synechocystis*, depends on protein level, maturation and physiological conditions

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Abstract text

Coupling of hydrogenases with the cellular metabolisms bears the potential to fuel biotechnological processes or to produce H_2 with cyanobacteria via light driven oxygenic photosynthesis. One of the major challenges in using native cyanobacterial hydrogenases is their high oxygen sensitivity. We addressed this challenge, by the introduction of an O_2-tolerant hydrogenase from *Cupriviadus necator* into a phototrophic bacterium, namely the cyanobacterial model strain *Synechocystis* sp. PCC 6803 (1). We characterized the strain *Syn_Cn*SH$^+$ in detail and found that activity is limited by a low enzyme production and maturation. Therefore, we designed an advanced *Cn*SH expression system in *Synechocystis*, adapting the CyanoGate cloning system, whereby we achieved a higher level of synthesized protein and elevated activity *in vivo*. Interestingly, we found that maturation of the multi component protein complex limits activity and we implemented additionally the maturation apparatus of *C. necator* in *Synechocystis*. Furthermore, we determined limitations and improved enzyme activity by optimizing physiological conditions, in particular the light availability, substrate (H_2) supply and a sufficient electron sink (CO_2). Finally, we could show an efficient H_2 utilization capacity of the designed stains and we improved H_2 production in *Synechocystis.* We will present challenges that need to be addressed in future to develop a sustained H_2 biotechnology with phototrophic microorganisms.

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179 POSTER

Temporal transcriptomic profiling of the fast-growing and highly productive cyanobacterium *Synechococcus* sp. PCC 11901

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Abstract text

Synechococcus sp. PCC 11901 (PCC 11901) is a fast-growing marine strain of cyanobacteria that has a capacity for sustained biomass accumulation to high cell densities. Although several synthetic biology tools have now been developed to facilitate the engineering of this strain, the genetic determinants associated with high density growth in PCC 11901 are still unknown. Here, we will describe the outcomes of a recent RNA sequencing experiment to examine and compare the transcriptomes of PCC 11901 and the closely related model marine strain *Synechococcus* sp. PCC 7002 (PCC 7002) across eight different time points during a high-density growth regime, where the peak biomass yields for PCC 11901 exceeded that of PCC 7002 by 2.5-fold. Comparison of these datasets sheds new light on the genetic components and pathways associated with high density growth in PCC 11901 and those that comparatively restrict biomass yields in PCC 7002. Furthermore, we explored the transcriptomic landscape of PCC 11901 to identify novel putative neutral integration sites and native genetic elements (e.g. promoters) that could be used to significantly expand the available engineering toolbox for PCC 11901.

Funding

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The absence of RfbC and FucS modulates the amount and composition of the extracellular polysaccharides of *Synechocystis* sp. PCC 6803

João Pissarra[1,2], Marina Santos[1], Sara Bernardes Pereira[1], Sónia Santos Ferreira[3], Cláudia Nunes[4], Manuel A Coimbra[3], Ricardo Monteiro[1], Didier Cabanes[1], Rita Mota[5], Paula Tamagnini[1,6]

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Abstract text

Many cyanobacteria can produce extracellular polymeric substances (EPS). These substances can be attached to the cell (CPS) or be released into the extracellular environment (RPS). The EPS produced by cyanobacteria are mainly heteropolysaccharides, which exhibit an unusually high diversity of monosaccharides, including acetyl, methyl, and amino sugars, as well as peptide moieties and sulfate groups, which makes them attractive for several biotechnological/biomedical applications [1]. However, the limited understanding of the intricate mechanisms involved in cyanobacterial EPS production hinders their tailoring to better fit the industrial needs [2]. To better understand how to modulate and control the production of these polymers, the model cyanobacterium *Synechocystis* sp. PCC 6803 was used to engineer knockout mutants on genes encoding proteins putatively involved in the biosynthesis of the deoxyhexoses dTDP-L-rhamnose and GDP-L-fucose: *rfbC* (*slr0985*) and *fucS* (*sll1213*) [3]. A clumping phenotype can be observed for both mutants at low cell densities and the *fucS* mutant exhibit a growth impairment compared to the wild type. The *rfbC* mutant produces significantly less RPS than the wild type, but its monosaccharide composition showed no major differences. In contrast, the RPS monosaccharidic composition from the *fucS* mutant showed no detectable levels of fucose and rhamnose, while the CPS exhibit diminished levels of rhamnose. Currently, other mutants, namely a single deletion of another putative RfbC encoding gene (*slr1933*) and a double *rfbC* (*slr0985* and *slr1933*), are being generated and characterized.

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Assessing the stability of using growth-coupled fumarate as a substrate for malate production in *Synechocystis*

Loles Hoogerland, Wei Du, Filipe Branco Dos Santos

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Abstract text

Cyanobacterial CO_2 conversion holds great promise towards sustainability while challenges remain. One of them is the instability of production. When production imposes a fitness burden on the cell, revertants that eliminate this burden via spontaneous mutations tend to take over the population by impairing the overall productivity. A strategy to overcome this instability is via growth-coupled production. In the cyanobacterium *Synechocystis* sp. PCC6803, this has been explored for acetate [1] and fumarate [2]. It has been hypothesized that growth-coupled products could be used as substrates to synthesize downstream compounds. This was first tested with photoautotrophic malate production by *Synechocystis* mutants. [3] Here, the enzymes responsible for recycling malate (ME and Mdh) are knocked out. Preliminary experiments suggested that such constructs would be stable even when higher production levels of malate would be achieved via the overexpression of fumarase (FumC). Here, we are testing malate production stability in much more stringent conditions. We use tightly controlled turbidostats where we cultivate during many generations with a small propagation bottleneck (5%). There, growth rate is applied as the selection pressure. Results show stable production in both the *ΔmeΔmdh* and *ΔmeΔmdhΔNSI::fumC* strains for > 55 generations. This suggests that drawing from a growth-coupled pool does not impose a hampering burden on the cells. We plan on extending the product range with additional thermodynamically favourable reactions and investigate the influence of dark metabolism. Our results show that this is a promising strategy to extend the list of alternative compounds to be stably produced.

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A CRISPRi-dCas12a-mediated CRISPR interference for multiplex gene repression in cyanobacteria for isobutanol and 3-methyl-1-butanol production

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Abstract text

Cyanobacterium *Synechocystis* PCC 6803 exhibits promise for biochemical conversion, albeit gene deletion within *Synechocystis* presents challenges due to its time-consuming nature and potential lethality to cells. CRISPR interference (CRISPRi) represents an emerging technology leveraging the catalytically inactive Cas protein and CRISPR RNA (crRNA) to selectively repress sequence-specific genes without necessitating gene knockout, thus enabling the rewiring of metabolic networks in various prokaryotic cells. In this study, our objective was to enhance isobutanol (IB) and 3-methyl-1-butanol (3M1B) production by systematically assessing potential competing pathways in a *Synechocystis* strain engineered for IB/3M1B production using the newly developed CRISPRi-dCas12a system [1]. We targeted genes involved in photosynthesis efficiency, carbon storage compound synthesis, and those relevant to the consumption of precursors for IB and 3M1B biosynthesis. Prior to systematically characterizing the effects of repression on the proposed sixteen gene targets, we optimized cultivation conditions to ensure suitability for testing the CRISPRi-dCas12a system in the IB/3M1B-producing *Synechocystis* strain. Repression of ten target genes—including *ccmA*, *ppsA*, *ppc*, *gltA*, *acnB*, *accC*, *pdh*, *cpcB*, *ilvE*, and *sps*—led to significantly improved IB and/or 3M1B production per cell. Subsequently, combined inhibition of these effective targets was tested, revealing a synergistic effect. Our findings introduce a novel application of the CRISPRi-dCas12a system in *Synechocystis* for systematically and rapidly mapping potential competing pathways for IB/3M1B biosynthesis. The identified targets provide valuable insights for enhancing pyruvate-derived chemical bioproduction across various cyanobacterial species.

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Towards Cyanobacterial Photobiological Hydrogen Production. Engineering, expression and in vivo maturation of heterologous [FeFe] hydrogenases.

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Abstract text

Hydrogen is considered one of the key enablers of the transition towards a sustainable and net-zero carbon economy. Photobiological hydrogen production is considered one of the most promising technologies, enabling direct solar-to-H_2 energy conversion while avoiding the need for renewable electricity and rare earth metal elements, whose demands are greatly increasing due to the current simultaneous electrification and decarbonization goals. Photobiological hydrogen production employs photosynthetic microorganisms to harvest solar energy and split water into molecular oxygen and hydrogen gas, unlocking solar energy storage possibilities and a wider range of sunlight energy utilization. However, photobiological hydrogen production has to-date been constrained by several limitations. The presented research work focuses on one of the key innovations required to unravel photobiological hydrogen production: The expression and activation of heterologous [FeFe] hydrogenases in cyanobacteria, as well as [FeFe] hydrogenase engineering strategies to improve photobiological H_2 production in whole-cell sytems. Results not only shown improvements in H_2 production from engineered cyanobacterial strains but also provide key insight on the interaction of heterologous [FeFe] hydrogenases with the native cyanobacterial electron transport chain.

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Funding

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Redirecting photosynthetic electron flow in *Synechocystis* towards heterologously expressed enzymes

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Abstract text

In a society with growing food and energy demands, sustainability concerns and environmental consciousness are increasing. Pharmaceutical and chemical industries provide most of society's daily used materials, but are highly polluting, having a large carbon footprint and generating 5-100x more waste than product [1]. Cyanobacteria's ability to grow under minimal nutritional requirements and amenability to genetic manipulation make them promising candidates for performing chemical reactions in a more sustainable way, mitigating the current environmental impact of highly pollutant chemical and pharmaceutical industries. Recently, our group showed that *Synechocystis* sp. PCC 6803 (Syn6803) whole cells expressing a heterologous cytochrome P450 monooxygenase (CYP110D1) could selectively convert testosterone into 15β-hydroxytestosterone in a light-driven without the need for external sacrificial electron donors [2]. Building up on this work, we aim at streamlining the photosynthetic electron flow in Syn6803 towards heterologously expressed redox enzymes such as monooxygenases and hydrogenases. For this purpose, several single and double Syn6803 mutants were generated via double homologous recombination, using Syn6803 wild type and Δ*hoxYH* strains as genetic background. Our targets were genes encoding for several putative competing electron sinks such as: flavodiiron proteins Flv1/3, NdhD2 subunit of NDH-1 complex, COX terminal oxidase and the native CYP120A1. The electron flow is now being analyzed in the 5 single and the 5 double mutants: Δ*ndhD2*, Δ*hoxYH*Δ*ndhD2*, Δ*flv1,* Δ*hoxYH*Δ*flv1,* Δ*flv3,* Δ*hoxYH*Δ*flv3,* Δ*coxCDE,* Δ*hoxYH*Δ*coxCDE,* Δ*CYP120A1 and* Δ*hoxYH*Δ*CYP120A1*, by expressing the sensor protein CYP1A1, which activity will be monitored in a high-throughput manner measuring the conversion of 7-ethoxyresorufin into resorufin (fluorescence) [3].

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Developing new tools for efficient RNA-guided transposition in cyanobacteria from complex ecological communities

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Cristina Sarasa-Buisan[1], Erbil Güngör[2], Sergio Arévalo[1,2,3], Peter Lindblad[3], Enrique Flores[1], Sandra Nierzwicki-Bauer[4], Henriette Schlupmann[2]

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Abstract text

Numerous intriguing aspects of cyanobacterial biology from complex ecological communities are emerging, emphasizing the need for efficient genetic manipulation tools for cyanobacteria isolated from natural settings to explore genes influencing their physiological traits. Some of the challenges for their genetic manipulation include slow growth under specific culture conditions or even the inability to grow separated from a host such as the obligate symbiont from *Azolla* fern, *Nostoc azollae*. Moreover, certain species of cyanobacteria possess complex mucilaginous envelopes or protective sheaths that can potentially hinder effective genetic transformation or conjugation methods. To address these challenges, an effective gene transfer and editing system is required. Our approach, a CRISPR-associated transposition system (CAST), previously designed for *Anabaena* sp. PCC 7120 [1], involves its integration into a replicative plasmid under a strong inducible promoter, thereby reducing reliance on recipient machinery. In this study we describe efforts for the adaptation of CAST to some ecologically relevant cyanobacteria including the unicellular marine *Crocosphaera subtropica* and heterocyst-forming filamentous cyanobacteria such as the obligate symbiont *Nostoc azollae*, the facultative symbionts *Nostoc punctiforme* and *Richelia rhizosoleniae* SC01, and the fast-growing *Anabaena* sp. ATCC 33047. To monitor early conjugation efficiency, several CAST elements were optimized initially in *Anabaena* sp. PCC 7120, e.g., replacing the EYFP promoter with the strong *E. coli* P_{trc10}. Metabolically relevant genes including *nifK* and *amt* were used as sgRNA targets. This research could be foundational for precise genetic manipulation of cyanobacteria in symbiotic associations or complex ecological communities.

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Unlocking the biological CO_2 capture potential of fast-growing cyanobacterium *Synechococcus* sp. PCC 11901 via targeted bioengineering

José Ángel Moreno-Cabezuelo[1], Pierce Bozeat[1], Adrienne Kerley[1], Uma Shankar Sagaram[1], Alistair J. Mccormick[2], David J. Lea-Smith[3], David Kim[1]

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Abstract text

In the urgent quest to combat global warming, our groundbreaking initiative has redefined the potential of cyanobacteria to act as an efficient carbon sink. Through genetic engineering of a fast-growing cyanobacterium *Synechococcus* sp. PCC 11901 we focused on increasing a “carbon sink” within the cells by enhancing the demand for fixed carbon, thus effectively alleviating metabolic bottlenecks and supercharging the cells’ photosynthetic efficiency. These modifications included the targeted overexpression of AroF D174N, which is key for aromatic amino acid production, and shikimate kinase, a crucial enzyme for channeling carbon flux through the shikimate pathway. To further enhance carbon capture, we addressed fatty acid metabolism by engineering the overexpression of *tesA*, a key enzyme that increases the availability of free fatty acids, thus optimizing the carbon fixation process. Additionally, the knockout of CP12, which inhibits key enzymes in the Calvin Benson cycle under certain conditions, was employed to further boost CO_2 fixation and photosynthesis. Our experiments have shown enhanced CO_2 fixation rates and robust growth patterns of these engineered strains in comparison to the wild type, validating their potential for widespread deployment. This research paves a promising path for climate change mitigation, leveraging the optimized innate capabilities of fast-growing PCC 11901 strain to effectively reduce atmospheric CO_2 levels and move us closer to achieving global sustainability goals.

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Engineering RNA polymerase to construct biotechnological host strains of cyanobacteria

Otso Turunen, Tayyab Saleem, Juha Kurkela, Pauli Kallio, Taina Tyystjärvi

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Abstract text

Using cyanobacteria as biotechnological cell factories is increasingly explored. Economically feasible and carbon neutral large scale bioproduction, bioremediation and biotransformation require more robust and efficient host strains. We have engineered sigma factors of the model cyanobacterium *Synechocystis* sp. PCC 6803 to improve stress resistance and productivity. As main regulators of gene expression, modifying the of abundance of a single sigma factor can have both wide and specific effects on transcription patterns. Overexpression of the stress response sigma factor *sigB* gene under the strong *psbA2* promoter improved multiple stress responses [1]. The growth of SigB overexpression (SigB-oe) strain was accelerated under heat, solvent and oxidative stress, and the amount of protective carotenoids, HspA protein and non-photochemical quenching was increased. The yellow fluorescent protein production of SigB-oe was similar to the control strain in standard conditions, but surpassed it under oxidative stress. Currently, we are exploring the use of *Synechocystis* and the SigB-oe strain in nutrient collection from greenhouse wastewater. Initial experiments show increased nitrate and phosphate uptake and accelerated growth in wastewater for SigB-oe compared to the control strain.

We also aimed at controlling growth rate by overexpressing an extra copy of the primary sigma factor SigA or the stationary phase specific SigC factor under the *psbA2* promoter. Despite transcriptional up-regulation, post-transcriptional regulation allowed only moderate increase in SigA protein, leading to phenotype of SigA-oe resembling that of the control strain. SigC overexpression proved lethal to *Synechocystis*.

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Engineering cyanobacteria as the basis for a synthetic microbial community

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Abstract text

In this collaborative project, our goal is the *de novo* design of a synthetic microbial cross-kingdom community based on the well characterized and genetically amenable model organisms representing cyanobacteria (*Synechocystis* sp. PCC 6803, or *Synechococcus elongatus* PCC 7942), ascomycete (*Saccharomyces cerevisiae*) and basidiomycete fungi (*Ustilago maydis*).

Co-cultivation is based on the carbon source sucrose, which is produced by the phototrophic cyanobacterium using light and carbon dioxide. Sucrose secretion into the culture medium is achieved by inducible, heterologous expression of a sucrose permease gene in the cyanobacteria.

An important aspect for creating a synthetic community is to design and establish tools for the formation of stable co-cultures and the analysis/quantification of the microbial partners of the community. Thus, in this part of the project, we test different cultivation devices and photobioreactor setups for online monitoring of co-cultures as well as single-cell flow cytometry analysis for quantification of individual populations within the co-culture. Another important aspect for the formation of a synthetic microbial community is the characterization of the optimal cultivation conditions for cyanobacterial sucrose production and simultaneous growth of all co-culture partners. In order to track the carbon source sucrose and other important metabolites within the co-culture, we also intend to establish biosensors in cyanobacteria.

Exploiting these established tools, we will create a stable synthetic microbial community which will then be further used to characterize the nutrient exchange in microbial consortia with a special focus on carbon economics and logistics.

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Characterization of the *petRP system in cyanobacteria*

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Abstract text

After the Great Oxidation Event (GOE), iron availability decreased considerably, and photosynthetic organisms developed new mechanisms and proteins to cope with this new situation. One of these proteins was plastocyanin (PC), a type I blue-copper protein that can replace cytochrome Cc6 as a soluble electron carrier between cytochrome b6f and photosystem I (PSI). In most cyanobacteria the expression of these two proteins is regulated by the availability of copper in the medium [1]. This regulation is mediated by a transcription factor belonging to the BlaI/CopY family (PetR) and a membrane protease of the BlaR family (PetP) in cyanobacteria. The PetR transcription factor represses the expression of the *petE* (PC) gene and induces the expression of the *petJ* (cytochrome Cc6) [2]. Meanwhile, the membrane protease PetP controls PetR levels in presence of copper. New data related to how copper is detected and how the signal is transduced by the PetRP system will be presented by analysing site directed mutants of both proteins. We have also introduced the corresponding mutations in *Synechocystis* sp. PCC 6803 and analysed the levels of the PC, Cc6 and PetR proteins and transcriptional reporters fused to mVenus. Finally, we will present evidence conservation of the function of this system in *Anabaena* sp. PCC 7120.

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190 POSTER

Engineering and studying a synthetic cyanobacterial endosymbiont

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Abstract text

Primary endosymbiosis describes the internalization of a proto-cyanobacterium by a eukaryotic cell to become the ancestor of the plastids in eukaryotic photosynthetic organisms. We will recapitulate the events proposed by the “ménage à trois” (MAT) hypothesis that enabled the proto-cyanobacterium to become an endosymbiont[1]. The MAT-suggested main event was the linkage of carbon and energy metabolism. Due to the loss of the endosymbiont’s ability to efficiently store carbon as substrate for respiration and energy production, the endosymbiont accumulates carbon in excess during the day that the host consumes, while being dependent on the host’s energy supply during the night. Thus, a mutually beneficial relationship was achieved. To test this hypothesis, we will utilize markerless knockout mutants in the model cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* UTEX 2973. We will stepwise engineer them to synthetic organelles and cultivate them on microfluidic chips functioning as the host’s cytoplasm. Starting with ADP-glucose pyrophosphorylase (*glgC*) to impair the cyanobacterium´s ability to synthesize the carbon storage molecule glycogen, genes will be sequentially knocked out. According to our hypothesis, implementation of a hexose-phosphate transporter and an ATP/ADP antiporter will allow the compensation of carbon excess and energy deficiencies of the Δ*glgC* mutant strains. Changes in the levels of metabolites will be monitored by MS-based analytics and *in vivo* by the introduction of metabolite sensors. The effects of the changes to the transcriptome will be analyzed with RNA-seq, revealing possible targets for further knockouts.

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Engineering cyanobacteria as chassis for sustainable terpenoid production

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Abstract text

The looming climate and energy crisis has caused an ever growing interest in cyanobacteria as platforms for sustainable biotechnology, capable of carbon-neutral production of biofuels as well as high-value and commodity chemicals. Terpenoids are a large class of organic compounds with wide-ranging applications that can be produced photosynthetically by cyanobacteria.

In cyanobacteria, the precursors for terpene biosynthesis are generated via the methylerythritol-4-phosphate (MEP) pathway which is fueled by pyruvate and glyceraldehyde-3-phosphate. Given the suite of important roles of terpenoids for cyanobacterial viability, the metabolic flux through the MEP pathway is heavily regulated on various levels which can significantly counteract productivity.

The highly volatile 5-carbon terpene isoprene is the simplest representative of the terpenoids family and an ideal model compound to study terpenoid anabolism in cyanobacteria. While cyanobacteria do not produce isoprene naturally, expression of heterologous isoprene synthase genes conveys the ability to emit photosynthetically produced isoprene [1]. Here, we employ various strategies to enhance the isoprene productivity of *Synechocystis* sp. PCC 6803. First, conventional metabolic engineering, i.e. overexpression of endogenous, as well as heterologous MEP pathway genes aids at identifying bottlenecks that limit productivity. This approach is complemented with protein engineering to circumvent undesired metabolic regulation. Furthermore, by engineering transcriptional regulators and regulatory proteins we aim to establish a streamlined cellular framework that favours heterologous production over maintenance of cellular homeostasis, thus maximizing the carbon flux towards the compound of interest.

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Transcriptomic profiling of *Synechococcus* adaptation to 30% CO_2 exposure

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Abstract text

Green biotechnology applications of cyanobacteria—that use industrial flue gas as carbon source—are limited by a reduced growth phenotype due to the extremely high CO_2 exposure levels. In this study, we investigate the transcriptome of the model organism *Synechococcus* sp. PCC 7002 as it adapts to 30% CO_2 v/v aeration in comparison to its transcriptomic profile under 0.04% CO_2 aeration and nearly optimal carbon exposure (4 & 8% CO_2). We generated short-read RNA-seq data (n=4 replicates) from bacterial cultures at the same cell concentration. The majority of genes (2, 866) were identified as differentially expressed (DEGs) in at least one pairwise comparison between the 4 CO_2 concentration conditions (overall FDR < 5%). Up-regulation of photosynthetic activity and growth-beneficial metabolic pathways during optimal growth were enriched by DEGs (pathway enrichment analysis per pairwise comparison; FDR < 1%). A complementary protein-protein association network analysis indicates over-representation (FDR < 5%) of DEG clusters in the secretion system, carotenoid metabolism, and overall translation regulation. In support, we see evidence for over-representation (FDR < 5%) of DEGs with SignalP predicted signal peptides in the photosynthesis and carotenoid biosynthesis pathway. Our transcriptomic profiles provide a first characterization of the gene expression regulation to high CO_2 concentration adaptation of *Synechococcus*. We anticipate that future work will take outset in our list of DEGs to design gene mutational experiments to improve the growth phenotype of *Synechococcus* in biotechnological applications.

Funding

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Self-sustaining biofilms: Diazotrophic *Tolypothrix* sp. PCC 7712 as a potential cyanobacterial candidate for technical applications

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Abstract text

Cyanobacteria obtain energy from oxygenic photosynthesis. Their use of light as an energy and water as an electron source makes them particularly interesting as whole-cell biocatalysts. Biofilms enable cyanobacteria to reach higher cell densities than planktonic growth [1, 2]. A biofilm is formed by microbes that produce an extracellular matrix to attach themselves to phase boundaries. Only a few studies have been carried out on cyanobacterial biofilms in technical systems, and their potential value for industrial applications has not yet been fully explored. In this study, 6 cyanobacterial strains including a benchmarking strain *Synechocystis* sp. PCC 6803 have been evaluated and it was shown that diazotrophic *Tolypothrix* sp. PCC 7712, is capable of forming 'superior' biofilms, when coupled with *Pseudomonas taiwanensis* VLB120. The dual-species biofilms containing this strain exhibit high biofilm surface coverage, low detachment, and high biomass production, especially under N_2-fixing conditions. Co-localization of two species in biofilms may increase system stability through the exchange of nutrients within biofilms and the expression of proteins involved in surface and cell attachments and outer membranes, according to metabolome, proteome, and microscopy analyses. Co-localized cells were also more resistant to additional forces. We propose the diazotrophic cyanobacterium *Tolypothrix* sp. PCC 7712 is an ideal candidate for photo-biotechnology because it requires no nitrogen-containing compounds like ammonium or nitrate, adjusts its pigmentation according to the light available (chromatic complementary acclimation), and can partner with a chemoheterotrophic organism to increase its biofilm stability in the technical system.

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194 POSTER

Biostimulant properties of *Limnospira indica*'s extracts on plants for future Life Support Systems

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Abstract text

This research project, embedded within the European Space Agency's Micro-Ecological Life Support System Alternative (MELiSSA) initiative, seeks to enhance our understanding and capabilities in the domain of bioregenerative life support systems. The project hereafter, part of the ArtEMISS project, is dedicated to exploring the growth and metabolic activities of the cyanobacterium *Limnospira indica* under space conditions and its biostimulant properties.

Whitin the MELiSSA loop, *L. indica* serves as a primary producer for oxygen and food producers, as well as biostimulant source for vascular plants in space [1]. Through this study, the potential of *L. indica* as a biostimulant for germination and plant growth is being explored extending its utility beyond oxygen and food production to enhancing the growth and health of plants. This study presents the impact of *L. indica* culture growth under nitrate availability condition (N+) and nitrate deficiency condition (N-) on *Solanum lycopersicum*. These culture conditions are tested as they influence the polysaccharides production by *L. indica*, where polysaccharides are known to present interesting biostimulant properties. *L. indica* polysaccharides are therefore carefully characterised. Overall, the use of culture media of *L. indica* grown in absence of nitrate (N-) presented a significant positive impact on *Solanum lycopersicum* germination, growth and flowers production compared to water and other biostimulant extract used in this analysis. These results suggest that the metabolites produced by *L. indica* grown in absence of nitrate, such as polysaccharides [2] presented a positive impact on plants productivity in term of flowers [3].

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A closer look at the acetate pathways in *Synechocystis* PCC 6803; Effects of insertion of a heterologous phosphoketolase

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Abstract text

The exploitation of fossil resources leads to increased amount of CO_2 in the atmosphere and severe environmental issues. Therefore, the need for sustainable sources of not only fuels but also chemicals, such as acetate, is high . Acetate has several applications including in the food industry and as an intermediate for the production of other products. Several microorganisms, such as yeast, naturally produce acetate, especially under dark and anaerobic conditions. Cyanobacteria have the ability to capture and utilize CO_2 from the atmosphere leading to an efficient recycle of carbon, which makes them excellent candidates for acetate production. However, very few studies have been done addressing acetate production under photosynthetic conditions [1]. It is known, though, that acetate production is mainly based on the precursors acetyl-P and acetyl-CoA through the enzymes acetate kinase (acka) and acetyl-CoA hydrolase (ach) respectively. One way to increase the titer of these substrates is the insertion and expression of a phosphoketolase (PK). An increased production of 1-butanol through the precursor Acetyl-CoA, due to an enhanced carbon flux from the CBB cycle to acetyl-P and G3P has been observed [2]. We have both knocked out and overexpressed the enzymes in the acetate pathway, including phosphotransacetylase (pta), an enzyme that converts irreversibly acetyl-P to acetyl-CoA, in combination with the expression of a PK. The insertion of the PK increased the production of acetate 40 times compared to in WT cells, and the increase was further doubled when combined with overexpressing the pta.

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196 POSTER

Design and implementation of a NADP⁺/Ferredoxin-reducing O_2-tolerant hydrogenase in *Synechocystis*

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Abstract text

Currently, the search for clean energy sources to mitigate climate change while preventing the shortage of available fuel is pushing towards H_2 as an energy carrier. In this context, biotechnological hydrogenase-catalyzed H_2 synthesis driven by oxygenic photosynthesis is a desirable approach, as such H_2 production mainly relies on light, water, and CO_2 fixation. Yet, this biological process is hindered by the simultaneous release of O_2 being a strong inhibitor of most native NAD(P)H and ferredoxin (Fd) accepting hydrogenases. Previous studies showed the possibility to implement the O_2-tolerant NADH-dependent hydrogenase from *Cupriavidus necator* into *Synechocystis* sp. PCC 6803 [1]. The obtained results showed that electron transfer from the photosynthetic light reaction to the hydrogenase suffered from cofactor incompatibility, with a dependence of the hydrogenase on the photosynthetic electron currencies NADPH or Fd as preferred option.

To this end, a fusion of the hydrogenase module from C. *necator* with a NADPH/Fd binding module (diaphorase module) from *Synechocystis* was targeted. As an alternative strategy, we aim at changing the cofactor dependency of the NADH-accepting HoxF subunit from C. *necator* via targeted enzyme engineering.

After limited success of first efforts, we aim at a machine learning approach for *in silico* screening of suitable amino acid substitutions in order to improve NADPH and Fd binding as well as the binding between subunits of different origin.

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Engineering the cyanobacterium *Synechocystis* sp. PCC 6803 for the production of hydrocarbons

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Abstract text

Increasing energy demands is one of the major concerns in today's scenario. With the depletion of fossil fuels and their repercussions leading to global warming, finding an alternative to meet energy needs is of utmost importance. The limitations of the current production strategies of biofuels make them inadequate to cope with large-scale needs, thus effective practical solutions which meet the economical and production values are to be put forward. Alkanes of defined lengths act as a promising alternative for fuel needs. Cyanobacteria are preferred photosynthetic microorganisms for the production of various metabolites and biomolecules due to their genetic amenability. *Synechocystis* sp. PCC 6803, a model cyanobacterium has several layers of thylakoid membranes consisting of glyco and phospho-lipids with C16 and C18 fatty acids esterified to the sn-2 and sn-1 positions respectively. Recently, a photoenzyme FAP that converts saturated fatty acids into hydrocarbons has been discovered. This enzyme can be engineered to produce hydrocarbons. The objective is to mutate the desaturases *desA* and *desD* to increase the saturated fatty acids. Knockout deletional mutation of *desA* and *desD* was performed. It was observed that the *ΔΔdesAD* strain, had an increase in the ratio of saturated to unsaturated fatty acids. LipA which hydrolyses the phospholipids to release free fatty acids was overexpressed. The photoenzyme has been mobilized for overexpression which would act on the free saturated fatty acids to release hydrocarbons. This generated strain would produce hydrocarbons. Further, the yield of the hydrocarbon will be optimized for its extensive use in daily life.

Funding

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Reduction of genetic instability in cyanobacteria by the study of toxin-antitoxin systems as addiction genetic modules

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Abstract text

Cyanobacteria are organisms with biotechnological applications, although their genetic instability limits their potential. Genetic instability is often associated with the presence of mobile genetic elements (MGEs). One of the mechanisms that bacteria have developed to stabilize such MGEs are toxin-antitoxin systems (TAs) [1]. Among TA-systems, Type II comprises of two genes encoding proteins: a toxin that induces cell death, and an antitoxin that neutralizes the toxic effect.

We are focused on the cyanobacterium *Anabaena* sp. PCC 7120 which complex genome includes 6 plasmids, and a plethora of predicted MGEs and TA modules. First, we analyzed *Anabaena* genome *in silico*, identifying 56 predicted TAs. Then, we developed a method to analyze them in *Escherichia coli* identifying 7 different TAs: three encoded in plasmids, and four in the chromosome. Of the latter, two are encoded into DNA elements interrupting genes and that are excised during heterocysts differentiation [2]. Hence, we sought to determine whether TA modules play a role in stabilizing these MGEs, acting as addiction modules. For that purpose, we are developing a sophisticated genetic system in cyanobacteria because previous test in *E. coli* revealed that these systems may have host-restricted functionality. Our final goal is to reduce the genome of *Anabaena* to construct a cyanobacterial chassis. However, we would like to point out that during the chassis development process we are creating new genetic systems that may have interest and potential for research and biotechnological uses.

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- VII Plan Propio de Investigación y Transferencia de la Universidad de Sevilla, 2023. Ref: VIIPPIT-2023-II.2. y Atracción Investigadores Alto Potencial. Ref: VIIPPIT-2022-II.5.

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Using CRISPRi-seq screening to investigate the high biomass yielding phenotype of the fast-growing cyanobacterium *Synechococcus* sp. PCC 11901

Michael Astbury[1], Angelo Joshua A. Victoria[1], Christina Bugert[1], Pierce Bozeat[2], José Moreno-Cabezuelo[2], Adrienne Kerley[2], Uma Sagaram[2], David Kim[2], Alistair Mccormick[1]

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Abstract text

CRISPRi-seq is a high throughput technique for identifying genetic features that contribute to cell fitness in specific growth conditions. By constructing a pooled library of mutants carrying sgRNAs targeting most annotated genome features via an inducible dCas9, competitive growth analyses can identify mutants that are enriched or depleted [1]. Here, we have leveraged a range of genetic tools recently established in the fast-growing marine cyanobacterium *Synechococcus* sp. PCC 11901 [2] to develop a CRISPRi-seq screen for investigating the molecular basis of its high biomass yielding growth phenotype [3]. A library of approximately 20, 000 sgRNAs was constructed to target over 95% of the annotated genome features of PCC 11901, with most features targeted by 5 individual sgRNAs. Additionally, we have extended the CRISPRi-seq approach by designing sgRNAs to tile the intergenic space to 'fish' for unannotated features, such as non-coding RNAs that may contribute to the growth phenotype. Moving forward, we aim to exploit the results of the screen to identify genetic interventions that could lead a greater understanding of growth regulation in cyanobacteria and improved phenotypes for biotechnological applications, such as improved biomass yields for carbon capture utilization and storage applications.

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Engineering *Synechocystis* sp. PCC 6803 as an efficient host for whole-cell biotransformation

Michal Hubacek, Lauri Nikkanen, Yagut Allahverdiyeva

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Abstract text

Cyanobacteria show promise as hosts for whole-cell biotransformation platforms in which a heterologous enzyme utilises native reductants to fuel its activity. Various enzymes have been deployed, such as representatives of the Cytochrome P450 and the Monooxygenase families, which typically require NAD(P)H or reduced ferredoxin (Fd) as the electron donor. While these reductants are produced by photosynthetic light reactions, a complex regulatory network around the photosynthetic apparatus pits the heterologous enzymes in competition with native pathways. Flavodiiron proteins (FDPs), cyclic electron transport (CET) mediated by the NAD(P)H dehydrogenase-like complex, terminal oxidases and other metabolic pathways all rely on electrons supplied by photosynthetic light reactions. These alternative electron transport pathways have been frequently targeted in studies focusing on enhancing biotransformation efficiency with varying outcomes.

In our study, we use NAD(P)H-dependent ene-reductase and Baeyer-Villiger monooxygenases (BVMO) expressed in *Synechocystis* sp. PCC6803. We characterised the effects of the biotransformation reaction on the photosynthetic apparatus using state-of-the-art biophysical methods. We then used various strains with altered FDPs expression levels to enhance the biotransformation activity and decipher the effects of these modifications.

Our results demonstrate the potential of engineering cyanobacteria for application in whole-cell biotransformation and shine a light on the mechanisms and bottlenecks of the selected systems as well as their interactions with native photosynthetic reactions. We have significantly improved the efficiency of the reaction performed by BVMOs. Additionally, the results stress the importance of carefully characterising each enzymatic reaction and its effects on the cell's physiology before attempting targeted engineering of the photosynthetic apparatus.

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Cyanobacterial Biofilms for Photoelectrochemistry

Nathan Victor Wright, Conrad Mullineaux, Ana Jorge Sobrido

Queen Mary University of London, London, United Kingdom

Abstract text

Cyanobacteria can export high energy electrons to conductive substrates when exposed to light via external electron transfer (EET) resulting in photocurrentcurrent generation that can be utilised directly as electricity to power electronic devices, photoelectrochemistry or scalable micro-grid applications known as biophotovoltaics (BPVs) with a theoretical maximum efficiency up to 7.7 W m^{-2}.

Electrospun carbon nanofibre (CNF) working electrodes offer a cheap, Earth abundant platform as a conductive substrate for biofilm formation in BPVs. The resultant carbon-based semiconductor offers a high active surface area per unit mass and the carbon feedstock can be derived from waste biomass including lignin or cellulose.

EET mechanisms remain elusive. High energy electrons may transfer directly from the thylakoid membrane to the electrode, or via an endogenous mediator such as NADPH. Type 4 Pili (T4P), cell surface appendages composed of major and minor pilin subunits, crucial for motility and conjugation, have been shown to contribute indirectly to EET efficiency, most likely due to their role in organising the biofilm architecture or substrate adhesion. External polymeric substances (EPS) are another integral structural feature of biofilms, and we plan to investigate the various contributions of T4P and EPS to EET efficiency and adhesion as interfacial electron transfer remains a key limiting factor in BPV performance.

During this stage of the project I have been investigating the light stress tolerance of *Synechocystis* inside a BPV system. My recent research activity suggests a photoprotective role of the circuit, potentially as a terminal electron donor.

Funding

UKRI - EPSRC

202 POSTER

Out of the box: engineering *Synechocystis* sp. PCC6803 for the secretion of heterologous proteins via extracellular vesicles

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Abstract text

With the development of Biotechnology, cyanobacteria are being engineered as sustainable microbial cell factories of heterologous proteins, which upon expression usually accumulate in the cell. However, the extracellular space also matters. A good example is extracellular vesicles (EVs). These are non-replicative biogenic nanoparticles released to the extracellular medium from the parent's cell envelope. EVs carry and protect biomolecules to otherwise inaccessible targets [1]. Using different sets of promoters and secretion signals, we engineered *Synechocystis*, and obtained an optimized strain capable of expressing the reporter green fluorescent protein (GFP) and package it in EVs [2]. Cyanobacterial EVs revealed to be a promising antigen-carrier platform for fish, given that injection in seabass (a fish species of high economic value in the Mediterranean) of engineered GFP-loaded S6803 EVs can trigger immunoglobulin production against the reporter protein, to an extent higher than injection of purified GFP. Following the same methodology, S6803 was then engineered for the expression of antigens from a relevant pathogen in aquaculture facilities (*Mycobacterium marinum*), and their loading into EVs. However, Western blot analysis of the engineered strains revealed poor expression of the selected antigens, and absence in EVs. This could be related to low stability of recombinant proteins in S6803 upon translation. Currently, we are addressing this with alternative constructions and protein fusions, as well as attempting to express protein-antigens from other fish pathogens (e.g. *Vibrio* sp.). This work is an effort to stimulate more sustainable measures in aquaculture, by introducing cyanobacterial EVs as green nanocarriers for fish vaccination.

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Synthetic photorespiratory bypasses in *Synechocystis* sp. PCC 6803

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Abstract text

Due to the low catalytic rate and affinity towards its substrate CO_2 RuBisCO limits photosynthetic carbon fixation. Furthermore, the oxygenase side reaction leads to the production of toxic 2-phosphoglycolate (2PG). To ensure cell survival 2PG needs to be detoxified via photorespiration in which afore fixed CO_2 and NH_4^+ is released. Thus, photorespiration is supposed to be a wasteful pathway and limits cell growth. To overcome this, we aim to turn photorespiration into a carbon neutral or even carbon positive process by synthetic carbon fixation pathways including designed new-to-nature enzymes. In our project we are currently following two different strategies.

The first pathway is based on a β- keto-acid-cleavage enzyme (Kce). Together with designed and adopted enzymes a cycle was created to produce formate from bicarbonate and acetyl-CoA. Formate is than subsequently incorporated into photorespiration via the glycine/serine interconversion and the C1-metabolism, thereby compensating the loss of CO_2 during the detoxification of 2PG.

In a second approach we will create a photorespiratory bypass by implementing the so-called tartronyl-CoA Pathway ([1], TaCo). This bypass is based on a novel tartronyl-CoA carboxylase, which facilitate carbon fixation and 2PG detoxification within only 5 enzymatic steps instead of 11. This makes the TaCo pathway more energy-efficient than natural photorespiration and even leads to net carbon fixation. Both pathways are established in Synechocystis sp. PCC 6803 to check their compatibility with oxygenic photosynthesis.

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204 POSTER

Characterization of photoprotective mechanisms involving the Orange Carotenoid Protein in marine cyanobacteria

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Abstract text

Marine cyanobacteria are the main photosynthetic organisms inhabiting Earth, as they are responsible for a significant part of world's photosynthetically produced O_2 and play major roles in the global carbon cycle. Indeed, *Prochlorococcus* and *Synechococcus*, the most abundant marine strains, contribute with about 25 % of the primary production in the oceans [1].

As photosynthetic organisms, marine cyanobacteria have developed photoprotection mechanisms which enable them to survive in high-light conditions. The Orange Carotenoid Protein (OCP), highly conserved among the cyanobacteria phyla, is involved in these photoprotective systems [2]. Although this protein has been widely studied among freshwater cyanobacteria, not much has been elucidated about the mechanism occurring in marine strains.

Focused on the ecologically relevant marine cyanobacteria, *Synechoccoccus* sp., this work aims to shed light on the molecular machinery that harvests light energy and protects these primary producers from the toxic consequences of absorbing light when it cannot be converted to chemical energy. With this purpose, the *ocp* genes from different *Synechococcus* sp. strains were cloned and overexpressed in a heterologous system, phycobilisomes were isolated and analyzed alongside the OCP and the co-transcription of genes contained in the putative *ocp* operon was studied.

This work is particularly relevant when considering the consequences of climate change, as it impacts the photosynthetic machinery of marine cyanobacteria with implications on how this environmental crisis will evolve in the future.

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Funding

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Synthetic devices for the modulation of intracellular O_2 levels in *Synechocystis* sp. PCC 6803

Bárbara Pires[1,2,3], Catarina Coutinho Pacheco[2,3,4], Peter Lindblad[5], Paula Tamagnini[2,3,4]

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Abstract text

Oxygen (O_2) has been part of Earth's atmosphere for millions of years. Consequently, organisms have had to thrive in an O_2 rich environment. For aerobic organisms, it meant using O_2 for their advantage whereas anaerobic ones had to develop strategies to create an anoxic environment essential for survival. The presence of oxygen can impair the activity of several enzymes, such as hydrogenases [1], having a detrimental impact on production processes, namely hydrogen production. Therefore, and for biotechnological applications, mechanisms to improve the efficiency of O_2-sensitive processes are required. Thus, this work focuses on the design and construction of synthetic devices for the modulation of intracellular levels of O_2 in *Synechocystis* sp. PCC 6803 (*Synechocystis*) aiming at developing chassis for optimized H_2 production. In a previous work from our group, a laccase-based Oxygen Consuming Device (OCD) was successfully introduced into *Escherichia coli*, and it was possible to demonstrate that the device could consume O_2 *in vivo*, representing a proof of concept that these type of OCDs can generate an intracellular oxygen sink [2]. Following a similar approach, we will develop OCDs customized for *Synechocystis* based on enzymes involved in O_2 detoxification and scavenging processes, as well as enzymes that use O_2 for substrate oxidation. In addition, the modulation of the activity of *Synechocystis* native enzymes, like the respiratory chain terminal oxidases and the flavodiiron proteins, will be explored. In the design of the OCDs the use of inducible promoters, such as O_2-sensitive promotors, will be considered.

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Funding

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206 POSTER

Optimizing the production of the heterologous compatible solute glycine betaine in *Synechocystis*-based chassis

Manuel Correia[1,2,3,4], David Rodrigues[1,2], Daniel Santos[1,2], Catarina Pacheco[1,2,4], Paula Tamagnini[1,2,4]

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Abstract text

Compatible solutes are low-weight organic molecules that stabilize cell components under stress conditions. Among these, glycine betaine (GB) has high commercial value with applications in feed and pharmaceutical/cosmetic formulations. Currently, GB is obtained from sugar beets or by chemical synthesis, with low-yields or high-waste generation. Therefore, establishing an efficient and sustainable bioproduction of this compatible solute is highly desirable. Our research group successfully implemented the heterologous production of GB in *Synechocystis*-based chassis using a synthetic device (Ahbet) [1]. The presence of this device in a *Synechocystis* Δ*ggpS* strain (deficient in the production of the native compatible solute glucosylglycerol) enhanced its growth in 3% NaCl compared with the wild-type, and a production of 1.9 µmol GB/mg protein was achieved. Furthermore, extracellular polymeric substances (EPS) and glycogen were identified as carbon competing pathways [1]. The implementation of the Ahbet device in a strain impaired in EPS export (Δ*kpsM*), previously generated by our research group [2], led to a production of 3.8 µmol GB/mg protein in 3% NaCl. Supplementing the culture media with 6 mM glycine (GB precursor) further increased the production to 13 µmol GB/mg protein. Based on these results, new mutants are being generated, namely *Synechocystis* Δ*glgC* (deficient in the production of glycogen) and *Synechocystis* Δ*gcvT* (lacking the T protein of the glycine decarboxylase system, leading to intracellular accumulation of glycine). In parallel, and to further understand the different phenotypes, transcriptomic studies are being performed.

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Novel genome engineering tools in cyanobacteria for their growth using flue gases

Encarnación Díaz Santos, Pablo Sousa, Juan E. Pérez-Fernández, Carmen Pérez-Nieto, Ana I. López-Pérez, Alba M. Gutiérrez-Diánez, Luis G. Heredia-Martínez, Manuel Hervás, José M. Ortega, Mercedes Roncel, José A. Navarro, Luis López-Maury

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Abstract text

The strategy of coupling CO_2 fixation with the production of compounds of interest using cyanobacteria is a very promising approach to mitigate the effects of climate change. This will allow the development of sustainable and environmentally friendly green technologies, as well as the implementation of a circular economy to reuse waste from the different industrial processes. In this sense, the search for new alternative processes, in which biotechnology and synthetic biology are involved, must play a central role. Although cyanobacteria are easily genetically manipulated in comparison to other heterotrophic microorganisms, their genetic tools are still scarce and underdeveloped. We are currently implementing new genetic tools in the model cyanobacterium *Synechocystis* sp. PCC 6803: I) We have generated a barcoded transposon library to identify essential genes and that will allow us to sample the genetic landscape in this organism. II) We are implementing and optimizing the use of a compact CRISPR system based on Cas12f. III) We are investigating the use of alternative replicative plasmids to those based in RSF1010, that are so far from the most successful plasmids used in cyanobacteria, using the SEVA plasmid collection. We aim the implement these tools on order to select enhanced strains of the model cyanobacterium *Synechocystis* sp. PCC 6803 capable of growing using flue gases as a carbon source.

Funding

CYANOGAS: Genome engineering in cyanobacteria for their adaptation to the use of flue gases. Convocatoria 2021 - «Proyectos Transición Ecológica y Transición Digital» Agencia Estatal de Investigación, Ministerio de Ciencia e Innovación. Reference: TED2021-129165B-I00.

CYANOMET: Mechanisms of Metals Homeostasis in Cyanobacteria. Convocatoria 2020 - «Proyectos de I+D+i» Agencia Estatal de Investigación, Ministerio de Ciencia e Innovación. Reference: PID2020-112645GB-I00.

208 POSTER

Evaluation of noise in the distribution of synthetic-replicative plasmids in cyanobacterial populations

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Abstract text

Cyanobacteria have been long regarded as interesting organisms for biotechnological purposes. However, the repertoire of genetic tools for genome engineering in cyanobacteria is underdeveloped in comparison with heterotrophic bacteria. More precisely, the scarcity of genetic tools such as Synthetic-Replicative plasmids (SR-plasmids) in cyanobacteria, is curbing the development of this field.

We have developed a modular system to construct plasmids inspired by a previous approach (1). We work in two cyanobacteria strains: the filamentous cyanobacterium *Anabaena* sp. PCC 7120 and the unicellular *Synechocystis* sp. PCC 6803. The combinations of parts yielded 30 plasmids that contained a replication module, antibiotic resistance cassettes, and a *gfp* reporter placed under a constitutive promoter that was used to monitor their fate by fluorescence. *Anabaena* containing plasmids integrated in the chromosome showed a homogeneous GFP signal along the filaments. However, strains containing SR-plasmids showed an uneven distribution of GFP in cells along the filament, hinting at a variable copy number. The SR-plasmids were based on two different replicons: pDU1 and RSF1010, with variants of both. The RSF1010 plasmids have also showed heterogeneity in the unicellular strain. Our results indicate that the stability of SR-plasmids depends on both the origin of replication and the resistance cassette. Furthermore, a single plasmid bearing multiple resistance cassettes showed different stability depending on the antibiotic used for selection. Although further analyses are currently ongoing to elucidate the causes of SR-plasmid instability, our study constitutes an unprecedented approach to these mobile genetic elements with potential applications in genome engineering.

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Development of engineered devices based on cyanobacteria for energy harvesting

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Abstract text

Solar energy is an abundant resource distributed on the Earth’s surface and its use to generate electricity through photovoltaic devices is widespread. In this regard, nature provides valuable knowledge of materials, their functionality, and their assembly mechanisms to produce sustainable light harvesting (LH) structures [1]. One such example is bacterial photosynthesis, whose efficiency in capturing and transforming solar energy has inspired scientists to develop engineered LH (ELH) devices based on pigments from photosynthetic bacteria [2, 3]. As an alternative, we propose the use of cyanobacteria as a group of phototrophic bacteria. We seek conditions for embedding cyanobacteria in polymer nanofibers as the core architecture in the fabrication of an ELH device. A key step in devising this system is processing a viscous fluid, a polymer solution, by electrohydrodynamic methods to produce such nanofibers. Therefore, it is essential to investigate the interaction of cyanobacteria with the polymeric solution.

In this context, we chose *Anabaena* sp. PCC 7120 a strain that is capable of growing in the absence of combined nitrogen by fixing atmospheric N_2 in heterocysts. Preliminary results have shown that *Anabaena* embedded in aqueous polymer solutions of varying composition can survive and grow for several days under laboratory conditions (continuous light at 30 ºC). We are currently developing the best polymeric composition to control and maintain this interaction with cyanobacteria. This preliminary study lays the groundwork for the development of a protocol to manipulate a cyanobacteria-polymer system. To the best of our knowledge, our work is pioneering in the field.

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210 POSTER

Machine Learning-Aided Accelerated Evolution and High-Throughput Screening of RubisCO mutants for Carbon Fixation in Cyanobacteria

Axel Fredrik Knave, Paul Hudson

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Abstract text

Cyanobacteria, photosynthetic organisms, utilize sunlight energy to fix carbon dioxide via the Calvin-Benson-Bassham (CBB) cycle, converting it into essential sugars and molecules. One of the most important enzymes is the Ribulose-1, 5-bisphosphate carboxylase/oxygenase (RubisCO), responsible for the fixation of CO_2. RubisCO variants have displayed a trade-off between catalytic turnover (kcat) and selectivity of CO_2 over O_2 ($S_{C/O}$), i.e. enzymes with high CO_2 selectivity typically demonstrate low kcat.

In order to optimize $kcat_{,C}$ for carboxylation and $S_{C/O}$, we started to engineer a RubisCO variant with a relatively high starting $kcat_{,C}$ of 22 ± 1 s-1 derived from the soil bacterium Gallionella sp. We have designed thousands of RubisCO mutants containing both single and combinations of mutations.

Utilizing the cyanobacterium Synechocystis sp. PCC 6803 as a screening platform, we assessed the impact of amino acid substitutions on enzyme functionality. For screening, we developed a strain reliant on mutant enzyme activity via inducible CRISPRi repression targeting native RubisCO. Each mutant was labeled with a unique 20-nucleotide barcode downstream of the gene. Illumina sequencing can then be used to track the growth of each mutant enabling the library to be pooled and cultuvated in a single flask. The mutant library underwent cultivation under various conditions to evaluate enzyme properties comprehensively. Through this strategy, we aim to gather data that can be feed to machine learning systems to optimize and generate new RubisCO variants with enhanced catalytic efficiency and CO_2 selectivity, potentially improving carbon fixation efficiency in cyanobacteria.

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Exploring growth arrest strategies in cyanobacteria for long-term enhanced lactate production.

Emil Sporre, Axel Knave

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Abstract text

The production of value chemicals from photoautotrophic bacteria gives lower volumetric yields than heterotrophic bacteria due to light limitation already at cell densities of OD600 > 1. A promising strategy for circumventing this problem is to induce growth arrest, keeping the culture at optimal cell density while shifting carbon partitioning from growth to product. Here we investigate two strategies for growth arrest in lactate producing strains; CRISPRi repression of citrate synthase and overexpression of the signalling metabolite ppGpp. We explore the impact of these strategies on the cell by applying proteomics and metabolomics and compare these conditions to more natural growth arrest conditions such as nitrogen-/light limitation. Using this data, we aspire to create an overproducing growth arrest strain with high, consistent productivity.

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In vitro circadian gene expression

Mingxu Fang[1], Miron Leanca[2], Andy Liwang[3], Yulia Yuzenkova[2], Susan Golden[1]

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Abstract text

Organisms from bacteria to mammals have evolved biological clocks that coordinate physiology. Temporal information from a core oscillator that establishes a period near 24 h is translated into physiological changes via output pathways that control expression of genes with different peak times. The simplest organism whose circadian clock has been characterized, the cyanobacterium *Synechococcus elongatus* PCC 7942, has been used as a model to reveal the adaptive advantage that biological clocks confer to life in a rhythmic daily environment. Circadian gene expression in *S. elongatus* relies on rhythmic phosphorylation of a transcription factor, RpaA. To better understand how RpaA controls different promoters with distinct timing, we established a high-throughput *in vitro* transcription assay that leverages the aptamer Broccoli and *S. elongatus* RNA polymerase (SeRNAP). Using this assay, we were able to directly measure activation of transcription from some promoters by phosphorylated RpaA *in vitro*. Conversely, the binding of RpaA to other promoters blocks transcription by SeRNAP. These two mechanisms generate opposite phases of rhythmic gene expression. The repressor mechanism is amenable to coupling with heterologous transcription machinery. When an RpaA binding site was introduced downstream of a T7 promoter, we were able to couple the reconstituted *S. elongatus* clock to the T7 transcription system to achieve clock-regulated gene expression. The successful *in vitro* coupling of the clock with T7 transcription system provides a platform to understand how temporal information from the clock is transduced to gene expression. Additionally, it shows the potential to transplant the *S. elongatus* clock to other organisms.

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From sunlight to synthesis: Kinetic modelling of photosynthetic terpene metabolism reveals the theoretical limits of squalene production in *Synechocystis*

Andreas Nakielski[1,2], Yasemine Beyza Baran[1], Ilka Maria Axmann[1], Anna Matuszynska[2]

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Abstract text

Cyanobacteria show great potential as sustainable production hosts for the biosynthesis of terpenoid compounds from CO_2. They utilize the 2-C-methyl-D-erythritol 4-phosphate (MEP) for the production of their native photosynthetic pigments as well as commercially interesting secondary metabolites, such as the triterpene squalene. However, the production of squalene compete with photosynthetic pigments for intermediates, so increasing its synthesis rate may have a negative impact on cell growth. Thus, the objective of metabolic engineering is to both maximize the production of squalene and ensure a minimum of pigmentation for functional photosynthesis.

To explore the theoretical boundaries of terpenoid production in *Synechocystis*, we constructed a kinetic model of the MEP pathway and terpenoid synthesis based on experimental data. This model describes feedback regulation by intermediates and cellular growth depending on pigmentation. All carbon and energy for production originates from photosynthesis and the Calvin-Benson-Bassham cycle, described by a previously published model of photosynthesis.

We performed Metabolic Control Analysis (MCA) to identify key reaction steps that hold the control over the production pathway in different scenarios. Results of MCA were then used to optimize the model to yield the theoretical maximum in the light-driven production of terpenoids by *Synechocystis* while still accounting for cellular growth and constraints imposed by enzyme overexpression.

This theoretical study may be used to guide strain engineering for the enhanced production of any terpenoid compound in *Synechocystis* and can be used as a modular platform to simulate additional metabolic modifications and combine engineering and illumination strategies.

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Designing evolutionarily stable biotechnological strategies - coupling the synthesis of (heterologous) products to the fitness of the microbial host

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Abstract text

Human societies have evolved towards being mostly fueled by the oxidation of fossilized deposits leading to a net production of CO_2. We are faced with a global challenge of inverting this disturbing tendency. Processes based on photosynthetic microorganisms (*e.g.* cyanobacteria) that can convert CO_2 into target compounds, are very relevant and extensively investigated. Still, their implementation has remained limited by two main technical hurdles: *(i)* conventional metabolic engineering strategies tend to lead to unstable cell factories that lose their production ability when scaled to industrial levels[1]; and *(ii)* all of the alternative tactics proposed so far are generally limited to conditions that may not be applicable in industrial environments where different stresses and non-growth periods tend to occur. We have been successful tackling these challenges, by generalizing the concept of growth-coupling that we developed previously and extending it to “fitness coupling”. These new tactics are based on the combination of mathematical modeling with the development of genetic engineering tools and experimental cultivation techniques. They can be applied to produce a multitude of (heterologous) compounds. We have validated them in several instances, namely for *(i)* the synthesis of growth-coupled acetate; *(ii)* the production of fumarate around the clock in a dual-strategy exploiting the metabolism of the native circadian cycle; and *(iii)* the accumulation of mannitol using its properties as a compatible solute to counteract osmotic stress. The results and methodologies showcased here can be critical during scale-up towards the industrial application[2] of these much thought after, fully sustainable, production methods.

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Cyanobacterial production of the key environmental sulfur compound dimethylsulfoniopropionate

Andrew Curson[1], Keanu Walsham[1], Xiaoyu Zhu[1], David Pearce[1], Jinyan Wang[1], Beth Williams[1], Xiao-Hua Zhang[2], Jonathan Todd[1], David Lea-Smith[1]

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Abstract text

Dimethylsulfoniopropionate (DMSP) is a hugely influential organosulfur molecule that is produced in very large amounts by marine microbes. Through its conversion to the gas dimethyl sulfide (DMS), it has a critical role in the global sulfur cycle, returning large amounts of sulfur from the oceans to the land. DMS is also a signalling molecule for a range of organisms from bacteria to seabirds, and may affect climate through its role in cloud formation.

Although previous work had shown that cyanobacteria can produce or import DMSP (1, 2, 3), little was known about the range of organisms that can produce it and its contribution to global production, and nothing at all was known about the genes or enzymes involved in DMSP production in cyanobacteria. Here, we have grown different species of cyanobacteria and tested them for DMSP production under different environmental conditions, showing that some of these strains produce DMSP and that this production may be upregulated under different environmental stresses. We have identified a cyanobacterial MTHB methyltransferase, termed DsyC, that catalyses a key step in DMSP biosynthesis and have looked for the presence or expression of this gene in different marine environments as a marker for organisms that may be producing DMSP.

By determining the amount of DMSP that key marine cyanobacterial genera like *Trichodesmium*, *Synechococcus* and *Prochlorococcus* produce, and based on the distribution of the DMSP synthesis gene *dsyC*, we hope to estimate the contribution of cyanobacteria to the production of this hugely important and environmentally influential sulfur compound.

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Insights of Biophotovoltaics in a 3 Liter Microbial Fuel Cell

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Abstract text

Biophotovoltaic concept proposes harvesting electrical energy primarily from prokaryotic photosynthetic organisms through a microbial fuel cell[1]. The underlying phenomenon behind this technology is extracellular photocurrent from cyanobacteria when illuminated with optimum light intensity. Much of the research on this field has been concentrated on enhancing the rate of charge extraction across cyanobacterial cell walls through various fabrication techniques[2]. In line with our previous research of demonstrating MFC scale up projects, this demonstrates a pilot scale biophotovoltaic operation with a triple MFC used as a singular unit[3]. A *Synechocystis* PCC6803 loaded MFC and a BG11 electrolyte-based control MFC were operated. The biotic MFC was loaded with cyanobacteria in fed-batch mode for 11 months in pseudoanaerobic conditions with 60 W/m^2 irradiance of white light. The cathode was operated with compressed air in phosphate buffer (Ph7). Polarization data was collected at regular intervals. Various factors like cyanobacterium loading, organic loading, buffer concentration, flow rates in anode and cathodes were found to be influencing the electrode kinetics. A trend of gradual reduction in maximum power density from 23 µW/m^2 (light); 10.42 µW/m^2 (dark) on day 20 to 3.99 µW/m^2 (light); 3.37 µW/m^2 (dark) on day 210 was seen over time due to gradual electrode clogging. However, a consistent difference in magnitude of internal resistance between biotic (0.0046±0.000861 Ω in light and 244±30 Ω in dark) and abiotic MFC (31014±26900 Ω in light and 38227±47714 Ω in dark) reactors with lower standard deviation indicates presence of a unique biofilm configuration.

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Establishing an *in vivo* cascade in cyanobacteria for light-driven redox biocatalysis on gram scale

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Abstract text

Cyanobacteria are ideal host organisms for truly sustainable whole-cell biocatalysis, as both reduction equivalents and O_2 are provided *in vivo* via water oxidation with light as sole energy source.[1] This attracted the coupling of redox reactions, especially oxygenases, to the photosynthetic light reaction via NADPH and O_2 in recombinant cyanobacteria. Introducing heterologous reactions into microbial hosts often suffers from reactant toxicity. Based on a recombinant *Synechocystis* sp. PCC 6803 strain harboring a Baeyer-Villiger monooxygenase (BVMO),[2] we implemented the first artificial light-driven redox cascade for the conversion of cyclohexanone to the polymer building block 6-hydroxyhexanoic acid. BVMO and lactonase co-expression, both from *Acidovorax* sp. CHX100, enabled this two-step conversion with an activity of up to 63.1 ±1.0 U g_{CDW}^{-1} without accumulating inhibitory ε-caprolactone.[3] Thereby, one of the key limitation, i.e., reactant inhibition or toxicity, was overcome. Besides design of the two-step cascade in *Synechocystis*, scale-up to lab scale photobioreactors and process optimization will be presented. With this strategy, we achieved titers up to 3.11 ± 0.12 g L^{-1} 6-HA with product yields ($Y_{P/S}$) of 0.96 ± 0.01 mol mol^{-1}, illustrating the potential of producing this non-toxic product in a synthetic cascade. Fine-tuning the energy burden on the catalyst was found to be crucial, indicating a limitation by the metabolic capacity possibly being compromised by biocatalysis-related reductant withdrawal. Product and energy balancing revealed that the cells increased light conversion efficiency under biotransformation conditions. This study shows the feasibility of light-driven cascade operation in cyanobacteria and highlights respective metabolic limitations and engineering targets.

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