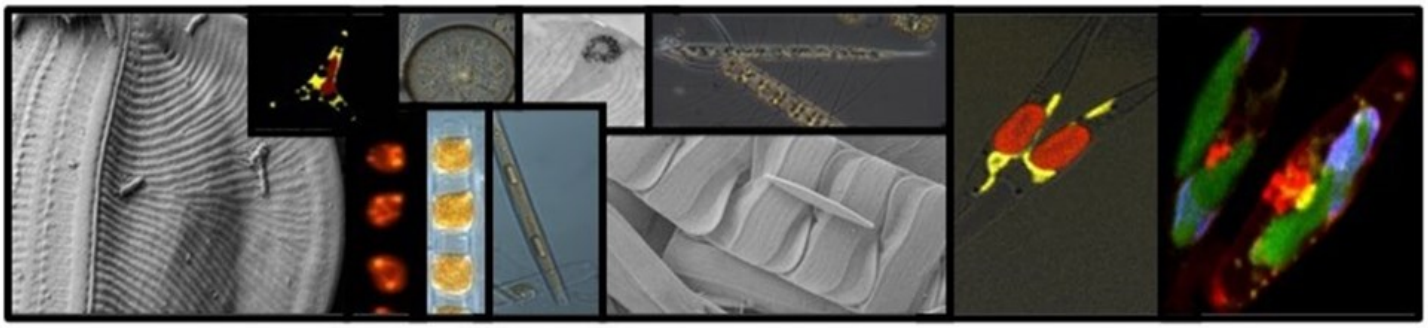
The Molecular Life of Diatoms 6

Virtually from San Diego, California

July 12 -14, 2021

Program and

Abstract Handbook



Organizers

Andrew Allen

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Scripps Institution of Oceanography

J. Craig Venter Institute, USA

Co-Organizers

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Moss Landing Marine Laboratories, USA

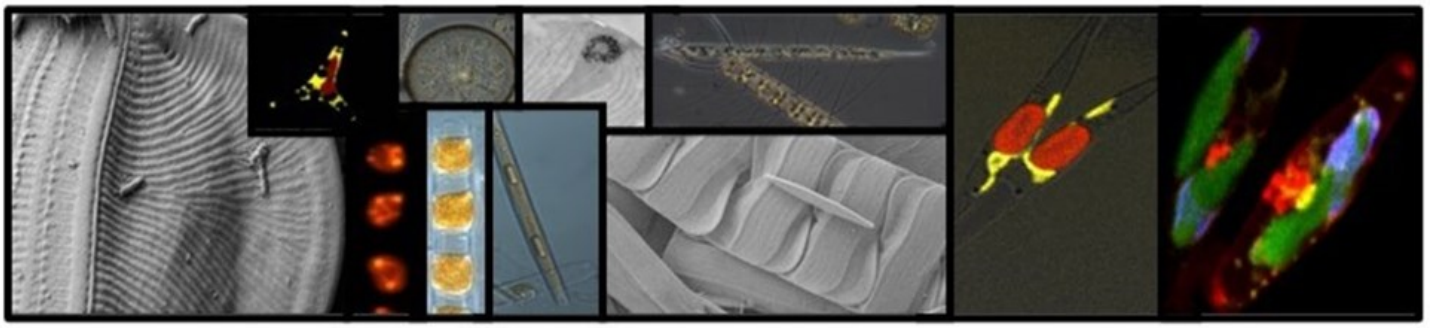
Harriet Alexander

Woods Hole Oceanographic Institution

Zoe Finkel

Mariella Ferrante

Stazione Zoologica Anton Dohrn of Naples



Co-Organizers

Chris Bowler

CNRS: French National Centre for Scientific Research

Thomas Mock

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Global Algae Innovations, Inc., USA

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Chinese Academy of Science, China

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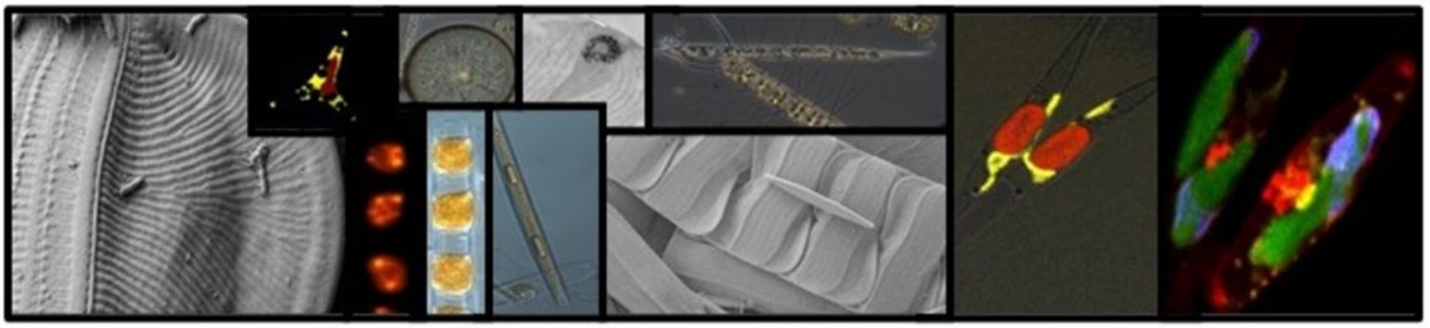
Tokyo University of Agriculture & Technology, Japan



Invited Speakers

Tom Delmont

Genoscope, France

Eveline Pinseel

University of Arkansas, USA

Elena Litchman

Michigan State University, USA

Erik Selander

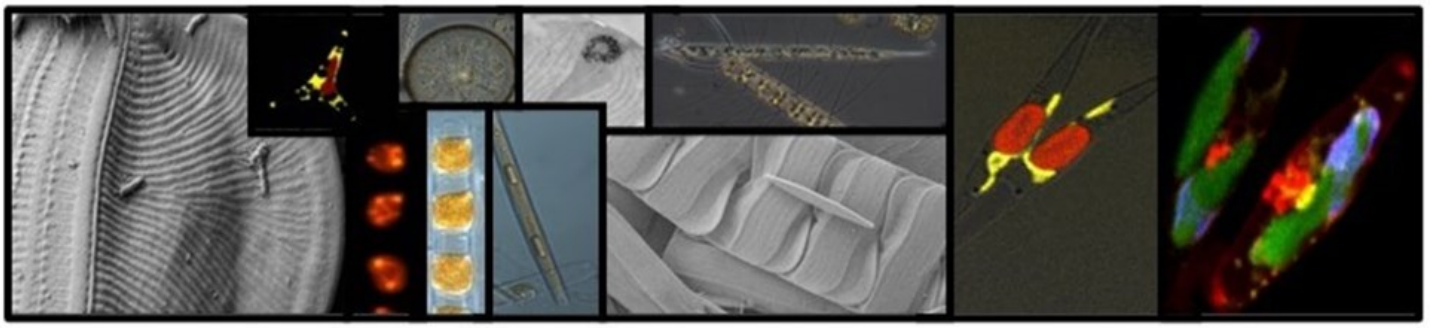
University of Gothenburg

Kim Thamatrakoln

Rutgers University, USA

Bryndan Durham

University of Florida, USA



Invited Speakers

Shady Amin

New York University Abu Dhabi

Uta Passow

Memorial University, Canada

Yuichiro Kashiyama

Fukui University of Technology

Katherine Helliwell

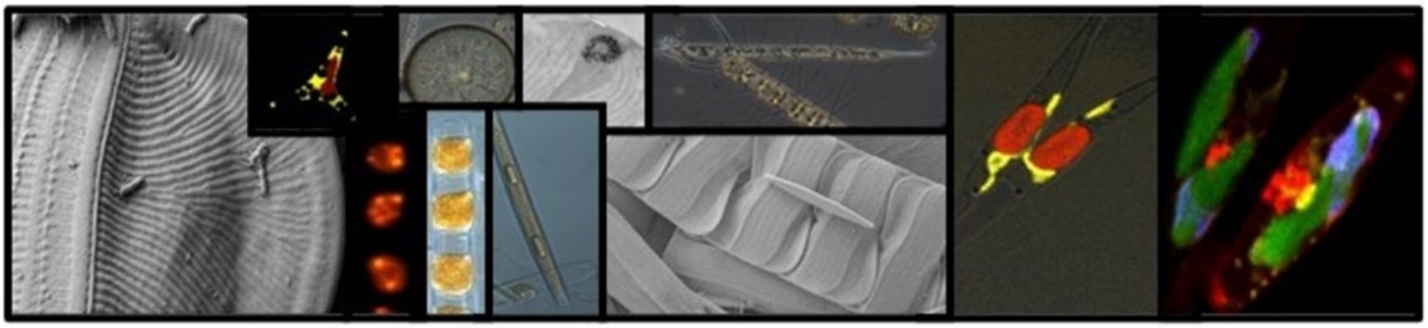
Marine Biological Association, Uni. of Exeter

David Hutchins

University of Southern California, USA

Sacha Coesel

University of Washington, USA



Invited Speakers

Atle Bones

NTNU: Norwegian University of Science and Technology

Gwenn Hennon

University of Alaska Fairbanks, USA

Stephanie Dutkiewicz

Massachusetts Institute of Technology, USA

Masao Adachi

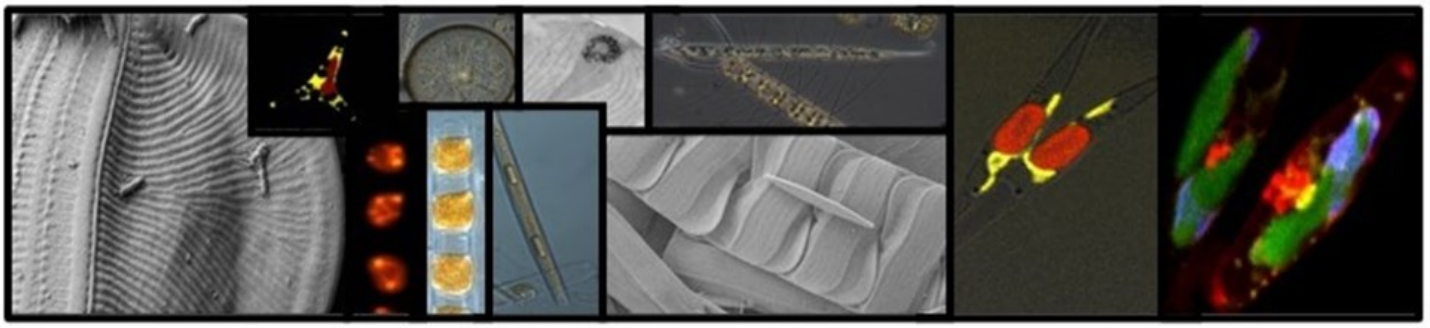
Kochi University, Japan

Bogumil Karas

The University of Western Ontario, Canada

Assaf Gal

Weizmann Institute of Science, Israel



Invited Speakers

Miroslav Oborník

Biology Centre, CAS, Institute of Parasitology

Graham Peers

Colorado State University, USA

Patrick Keeling

University of British Colombia, Canada

Dmitry Filatov

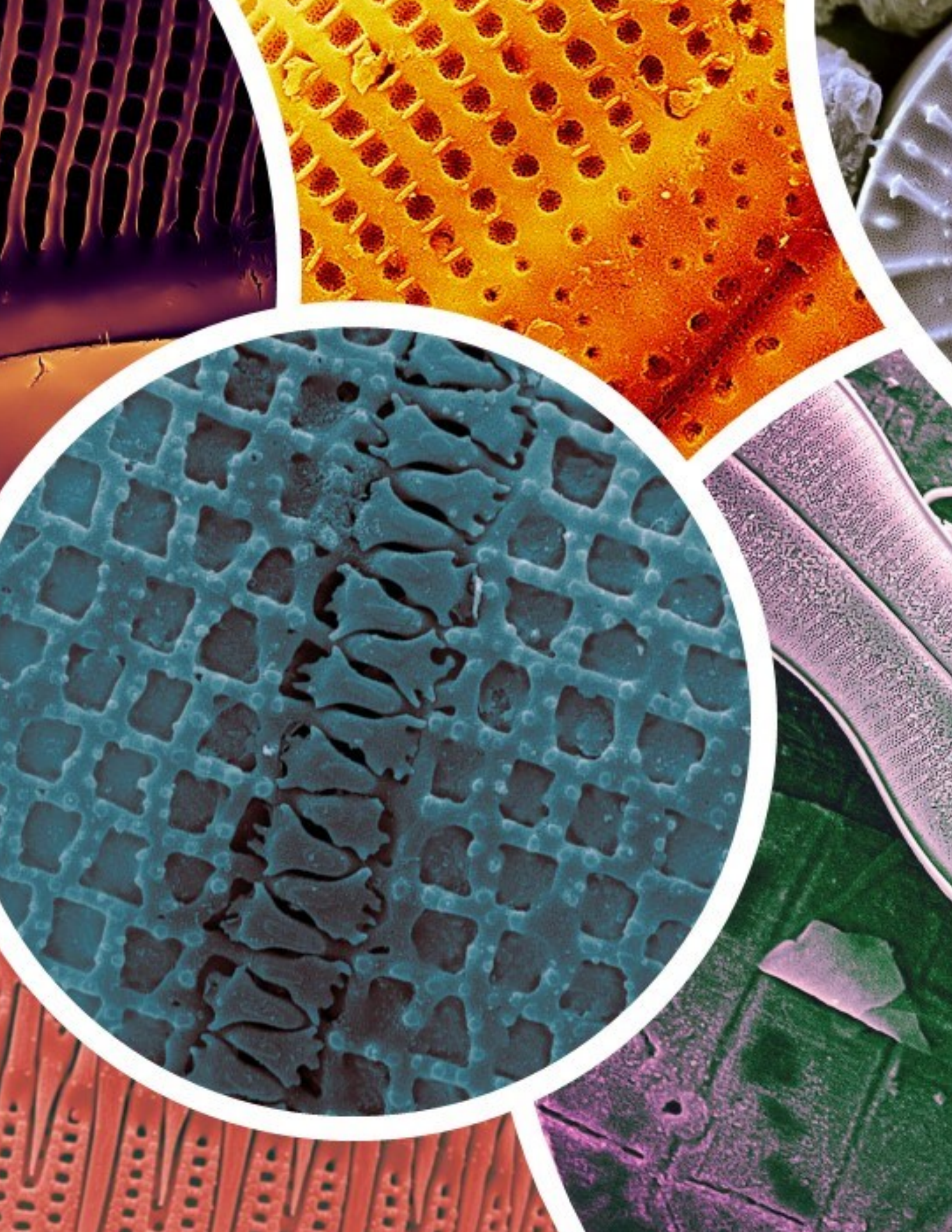
University of Oxford, UK

David Hutchins

University of Southern California, USA

Sacha Coesel

University of Washington,



Molecular Life of Diatoms 6
Conference Program

Monday July 12

2:00 – 2:40pm GMT Welcome and Opening Remarks
(Andy Allen)

Session 1: Ecology & Ecophysiology
(Moderator: Harriet Alexander)

2:40 – 3:00pm GMT Delmont
Environmental genomics of diatoms leads to new functional and evolutionary insights

3:00 – 3:20pm GMT Pinseel
Unravelling a natural evolutionary experiment: the colonization of low-salinity habitats in the Baltic Sea by the marine diatom Skeletonema marinoi

3:20 – 3:40pm GMT Litchman
Linking traits, evolution, and ecological interactions to understand diatom ecology

3:40 – 4:00pm GMT Selander
Grazer induced phenotypic plasticity provides insight into diatom evolution and molecular mechanisms

4:00 – 4:20pm GMT – Break

4:20 – 4:40pm GMT Thamatrakoln
Shunt or shuttle? Nutrient-driven biogeochemical consequences of diatom host-virus interactions

4:40 – 5:00pm GMT Durham
Exploring cooperative nutrient dynamics in diatom-bacterial interactions

5:00 – 5:20pm GMT Amin
How diatoms modulate bacteria in their microbiome

5:20 – 5:40pm GMT Passow
Diatoms and the biological carbon pump

5:40 – 6pm GMT Panel Discussion and Break
(Moderator: Zoe Finkel)

Poster Session 1
6 – 7:30pm GMT

Ecology

1. Upwelling-associated phytoplankton display resistance to ocean acidification
2. Diving into metapopulation genomics of the Chaetoceros genus
3. Growth-Stage Related Shifts in Diatom Endometabolome Composition Sets the Stage for Heterotrophic Bacterial Foraging
4. Exploring expression patterns of diatom silicon transporters under Si stress and competition
5. Survivors of the Sea: Using Transcriptomics to Elucidate the Survival Strategies of Century Old Diatoms in Sediment
6. Uncovering the diversity and seasonal patterns of algicidal bacteria in the Western English Channel
7. Growth dynamics of epizoic Achnanthes elongata and non-epizoic Psammodictyon panduriforme in co-cultures
8. The acquisition strategies of external bicarbonate by three plasma membrane SLC4 transporters under changing levels of CO2 limitation in the diatom, Phaeodactylum tricornutum.

Tuesday July 13

2:00 – 2:20pm GMT Opening Remarks
(Andy Allen)

Session 2: Ecophysiology & Cell Biology
(Moderator: Sarah Smith)

2:20 – 2:40pm GMT Kashiyama
How to digest delicious diatoms containing the phototoxic chlorophyll

2:40 – 3:00pm GMT Helliwell
A novel Ca2+ signalling pathway coordinates environmental phosphorus sensing and nitrogen metabolism in marine diatoms

3:00 – 3:20pm GMT Hutchins
Interactions between irradiance and temperature determine the niche of a novel low-light, cold-adapted nano-diatom from a wintertime temperate estuary

3:20 – 3:40pm GMT Coesel
Diel transcriptional oscillations of light-sensitive regulatory elements in open ocean eukaryotic plankton communities

3:40 – 4:00pm GMT Bones
Diatom Genomics and Gene Regulation

4:00 – 4:20pm GMT Hennon
Diatoms on thin ice: sensitivity and resiliency to anthropogenic change in the Alaskan Arctic

4:20 – 4:40pm GMT Dutkiewicz
Modeling marine diatom diversity and potential future changes at the global scale

4:40 – 5pm GMT Panel Discussion and Break
(Moderator: Mariella Ferrante)

5:00 – 6:00pm: Special Session on Community Tools
(Moderator: Chris Bowler)

Poster Session 2
6 – 7:30pm GMT

Cell Biology

1. Scarless GFP-tagging of native genes in Thalassiosira pseudonana
2. Prevalence of trypsin in marine phytoplankton and their functions in the integrative nitrogen and phosphorus signaling network
3. Valve morphogenesis in Amphitetras antediluviana Ehrenburg
4. Extracellular products from a flavobacterium induce transcriptional shifts of cell cycle and metabolism in a model diatom.
5. An integrative experimental and environmental atlas of diatom chloroplast transporters
6. Single-cell heterogeneity in response to environmental stress reveals pathways of cell fate regulation in marine diatoms
7. Investigating molecular mechanisms for phosphorus sensing in the marine diatom, Phaeodactylum tricornutum
8. Elucidating the microenvironment of mineral formation within silica deposition vesicles in diatoms

Wednesday July 14

2:00 – 2:20pm GMT Opening Remarks
(Andy Allen)

Session 3: Biotechnology & Evolution
(Moderator: Thomas Mock)

2:20 – 2:40pm GMT Adachi
Activities of promoters derived from four diatom-infecting DNA viruses in the marine diatom Phaeodactylum tricornutum

2:40 – 3:00pm Karas
Introduction to Synthetic Diatom Project

3:00 – 3:20pm GMT Gal
Using cryo electron microscopy to study diatom silicification

3:20 – 3:40pm GMT Obornik
Exploring the heme pathway in chromerid and diatom

3:40 – 4:00pm GMT Peers (Cell Bio)
Biosynthesis of fucoxanthin

4:00 – 4:20pm GMT Keeling (Evol)
How does endosymbiosis lead to new plastids?

4:20 – 4:40pm GMT Filatov (Evol)
Evolutionary genetics of marine phytoplankton: many questions, few answers

4:40 – 5pm Panel Discussion and Break
(Moderator: Jesse Traller)

Highlighted Talks from Poster Presentations
& Presentation of Poster Awards
(Moderator: Andy Allen)
5:00 – 5:15pm GMT TBD

5:15 – 5:30pm GMT TBD

5:30 – 5:45am GMT TBD

5:45 – 6pm GMT TBD

Poster Session 3
6 – 7:30pm GMT

Biotechnology

1. A functional PDAT encoded by unequally expressed allele enhances triacylglycerol accumulation under nitrogen starvation in Phaeodactylum tricornutum
2. High-efficiency transformation by electroporation and feasibility of CRISPR-Cas9-mediated gene editing in a centric diatom Chaetoceros muelleri
3. The heterologous expression of a green alga plastocyanin in Phaeodactylum tricornutum improves cell growth under iron-deficient conditions
4. The physiological role and structural arrangement of the mitochondria of Fistulifera solaris in the oil degradation process
5. An Analytical Approach for Understanding Light Modulation by Diatom Frustules
6. Digital holographic microscopy of morphological changes of diatom cellular contents induced by papain enzyme
7. Producing the SARS-CoV-2 Spike RBD antigen in the diatom Phaeodactylum tricornutum
8. A method for intact nuclei isolation from diatoms to facilitate the application of high-throughput technologies

Molecular Life of Diatoms 6
Conference Program

Monday July 12	Tuesday July 13	Wednesday July 14
Poster Session 1 (continued)	**Poster Session 2 (continued)**	**Poster Session 3 (continued)**
9. Cancelled	9. Exocytosis of diatom silica involves extensive membrane disintegration	9. Chloroplast genome engineering as a tool to improve the productivity of monoterpenes in the diatom Phaeodactylum tricornutum
10. Deciphering interactions between the marine benthic diatom Seminavis robusta and its microbiome using a multi-omics approach	10. Light sensing under the sea: diatom photoreceptors in Tara Oceans	10. Diatoms as promising hosts for monoterpene engineering
11. Pole to pole pattern of biogeography and acclimation in natural populations of marine diatoms	11. Comparative morphological and transcriptome analyses of valve plasticity induced under different salinity conditions in a centric diatom Pleurosira laevis	11. Proteomic analysis of the oil bodies with different sizes in the marine diatom Fistulifera solaris for oil body-engineering
12. Microdiversity and co-occurrence of diatoms and bacterial associates along a latitudinal gradient in the North Pacific	12. A FIB-SEM–based workflow to study diatoms light acclimation	12. Synthetic biology for the controlled production of high-value compounds in Phaeodactylum tricornutum.
Ecophysiology	13. Transporters dependent regulation of photosynthesis in Phaeodactylum tricornutum	13. Molecular drivers of sexual reproduction in Cylindrotheca Closterium
13. Essential amino acids in Lhcx proteins to confer the rapid photoprotection mechanism qE in the diatom Phaeodactylum tricornutum	14. The potential role of extracellular vesicles in stress response in marine diatoms	14. Metabolic flux analysis of the diatom Phaeodactylum tricornutum in response to nitrogen starvation
14. The Contribution of Proteorhodopsin to the Cellular Energy Budget of the Antarctic Diatom Pseudo-nitzschia subcurvata	15. Oxidative stress response during the resting cells formation in marine diatom Thalassiosira pseudonana"	15. Characterizing Ku70 knock outs and knock downs in Phaeodactylum tricornutum
15. Characterization and localization of a proteorhodopsin light-driven proton pump in Southern Ocean diatoms	16. Differential gene expression associated with sexual maturation of pennate diatoms	16. A regulatory cascade of phosphorus-nutrient strategies in the diatom Phaeodactylum tricornutum
16. Disentangling the roles of diatoxanthin and Lhcx1 in the nonphotochemical quenching of Phaeodactylum tricornutum	17. Low-Affinity Nitrate Transporters in diatoms, diNPFs: identification, evolution, structure and function	**Genomes and Genome**
17. Cancelled	18. Understanding the Cellular Role of Aureochromes, a New Type of Blue Light Photoreceptors, in Diatoms	17. Diurnal transcript profiling of the diatom Seminavis robusta reveals adaptations to a benthic lifestyle
18. Ecologically relevant metabolites produced by a genome-scale metabolic model of Thalassiosira pseudonana	19. Characterisation of membrane glycerolipid synthases in the model diatom Phaeodactylum tricornutum using CRISPR-Cas9 technology	18. DiatOmicBase, a portal to mine diatom omics
19. The evolution of morphology in diatoms: assessment of grazing pressure in long-term speciation.	20. Characterization of the Rubisco condensation protein PYCO1 from the pyrenoid of Phaeodactylum tricornutum	19. Polycomb Repressive Complex 1 in unicellular species: insights from diatoms
20. The roles of putative pyridoxal phosphate dependent transferase (PLP-DT) in nitrogen utilization and lipid synthesis in marine diatom Phaeodactylum tricornutum	21. A vitamin B12 physiological kaleidoscope in Phaeodactylum tricornutum	20. Eukrhythmic: leveraging the metatranscriptomic landscape to reproducibly detect and describe marine protistan communities
21. A tale of two Flavodoxins	22. Robust diatom growth data analysis	21. Evolution and adaptation of Fragilariopsis from the Arctic and Antarctic: Sequencing new strains and investigating adaptation to rising temperatures.
22. Regulation mechanisms of phosphate uptake in Phaeodactylum tricornutum and Thalassiosira pseudonana	23. Identifying Aureochrome-promoter interactions in Phaeodactylum tricornutum	22. Evolutionary analysis of DNA methyltransferases in unicellular eukaryotes: Insights from the model diatom Phaeodactylum tricornutum
23. Dynamic biofilm formation of diatoms by a transcriptomic study	24. Molecular mechanisms of survival to the polar night in the diatom Fragilariopsis cylindrus	23. Mitotic recombination between homologous chromosomes contributes to the genomic diversity in diatom clonal populations
24. Diatom Plasticity: Trends, Issues and Applications on Modern and Classical Taxonomy, Eco-Evolutionary Dynamics and Climate change	25. Circadian rhythms in the diatom Phaeodactylum tricornutum: regulatory function of bHLH/PAS proteins	24. The metabolic potential of the ocean
25. Differential expression analysis in the spore former Chaetoceros socialis during nitrogen starvation	26. Light intensity control on compound specific carbon isotope fractionation in cultures of Haslea ostrearia.	25. Genetic variation analysis provides insights into Pseudo-nitzschia multistriata population genomics
26. Species-specific bloom dynamics of Skeletonema in Ariake Sound, Japan: Development of Skeletonema species-specific qPCR	27. Molecular phylogeny of fucoxanthin-chlorophyll a/c proteins in the Chaetoceros gracilis genome suggests the diversification process of the light-harvesting complexes in the red algal lineage	26. Do core DNA repair enzymes regulate Diatom genetic diversity?
27. Investigating the Characteristics of Multipolar Diatoms in Response to Temperature and the Need for Fresh Environmental Isolates	28. Characterizing two nitrite reductase enzymes in P. tricornutum through CRISPR-Cas9 gene-knockout methods	27. The genome biology of Parmales (Bolidophyceae), a sister group of diatoms
28. Temperature sensing in diatoms: cold shock and Ca2+ signalling	**Evolution**	28. Multi-class predictions of intracellular locations of proteins in organisms with complex plastids
29. The Impact of Temperature on Vitamin B12 Use in the Polar Diatom Fragilariopsis cylindrus	29. Rhythm, topography and ecophysiology of horizontal gene transfer across the diatom pan-genome	**Engineering**
30. Molecular physiology of Antarctic diatom natural assemblages reveals multiple strategies contributing to their ecological success	30. Diatoms diversification in light of their ecological niche space	29. Synthetic promoters for control of gene expression in the model diatom Phaeodactylum tricornutum
	31. Using experimental evolution to reveal insights into how the (epi)genome and transcriptome changes due to a long-term temperature response of Thalassiosira pseudonana	30. The Cloning and Engineering of Diatom Mitochondrial Genomes in Yeast and Bacteria
	32. Investigating evolutionary adaptation of the polar diatom Fragilariopsis sp.	31. Modeling carbon metabolism of the diatom Phaeodactylum tricornutum due to genetic knockout of TAG degradation enzymes and to high light versus low light conditions
	33. Using a diatom model to study an unusual histone protein in Chromera velia	32. Towards a synthetic algal chloroplast: a streamlined platform for creating designer chloroplast genomes in Phaeodactylum tricornutum

Session to Abstract Cross Ref

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Poster Session	Abstract	Title	Authors
Poster Session 1 - 01	Abstract #03	Upwelling-associated phytoplankton display resistance to ocean acidification	Robert H. Lampe*, Tyler H. Coale, Kiefer O. Forsch, Samuel Kekuewa, Ariel J. Rabines, Hong Zheng, Erin M. Bertrand, Andreas J. Andersson, Katherine A. Barbeau, Andrew E. Allen
Poster Session 1 - 02	Abstract #07	Diving into metapopulation genomics of the Chaetoceros genus	Charlotte Nef*, Amin Madoui, Chris Bowler
Poster Session 1 - 03	Abstract #26	Growth-Stage Related Shifts in Diatom Endometabolome Composition Sets the Stage for Heterotrophic Bacterial Foraging	Malin Olofsson*, Frank X. Ferrer-González, Mario Uchimiya, Jeremy E. Schreier, Nicole Holderman, Christa B. Smith, Arthur Edison, Mary Ann Moran
Poster Session 1 - 04	Abstract #43	Exploring expression patterns of diatom silicon transporters under Si stress and competition	Evangelia Charalampous*, Nikolaj Gubonin, Camilla Karlsson, Anabella Aguilera, Hanna Farnelid, Daniel Lundin, Jarone Pinhassi
Poster Session 1 - 05	Abstract #53	Survivors of the Sea: Using Transcriptomics to Elucidate the Survival Strategies of Century Old Diatoms in Sediment	Matthew I. M. Pinder*, Olga Kourtchenko, Elizabeth K. Robertson, Volker Brüchert, Helle Ploug, Anna Godhe, Mats Töpel
Poster Session 1 - 06	Abstract #55	Uncovering the diversity and seasonal patterns of algicidal bacteria in the Western English Channel	Laura Branscombe*, Michael Cunliffe, Willie Wilson, Katherine Helliwell
Poster Session 1 - 07	Abstract #61	Growth dynamics of epizoic Achnanthes elongata and non-epizoic Psammodictyon panduriforme in co-cultures	Klara Filek* Lucija Kanjer, Peter Chaerle, Wim Vyverman, Sunčica Bosak
Poster Session 1 - 08	Abstract #73	The acquisition strategies of external bicarbonate by three plasma membrane SLC4 transporters under changing levels of CO2 limitation in the diatom, Phaeodactylum tricornutum.	Hermanus Nawaly*, Yoshinori Tsuji, Kazufumi Iwayama, Hiroki Ohashi, Hiroaki Matsui, Kensuke Nakajima, Yusuke Matsuda
Poster Session 1 - 09	Cancelled		
Poster Session 1 - 10	Abstract #82	Deciphering interactions between the marine benthic diatom Seminavis robusta and its microbiome using a multi-omics approach	Rita Bogorad*, Willem Stock, Cedric Hubas, Wim Vyverman, Graham J.C. Underwood, Koen Sabbe
Poster Session 1 - 11	Abstract #86	Pole to pole pattern of biogeography and acclimation in natural populations of marine diatoms	Juan Pierella Karlusich*, Karen Cosnier, Antoine Vallee, Fabio Rocha , Tara Oceans Coordinators, Chris Bowler
Poster Session 1 - 12	Abstract #90	Microdiversity and co-occurrence of diatoms and bacterial associates along a latitudinal gradient in the North Pacific	Rebecca S. Key*, Mary R. Gradoville, Bennett S. Lambert, Rhonda L. Morales, E. Virginia Armbrust, Bryndan P. Durham
Poster Session 1 - 13	Abstract #13	Essential amino acids in Lhcx proteins to confer the rapid photoprotection mechanism qE in the diatom Phaeodactylum tricornutum	Jochen M. Buck*, Peter G. Kroth, Bernard Lepetit
Poster Session 1 - 14	Abstract #24	The Contribution of Proteorhodopsin to the Cellular Energy Budget of the Antarctic Diatom Pseudo-nitzschia subcurvata	Kaylie Plumb*, Sarah Andrew, Alecia Septer, William Sunda, Brian M. Hopkinson, Adrian Marchetti
Poster Session 1 - 15	Abstract #29	Characterization and localization of a proteorhodopsin light-driven proton pump in Southern Ocean diatoms	Sarah Andrew*, William Sunda, Stephanie Smith, Brian Hopkinson, Alecia Septer, Adrian Marchetti
Poster Session 1 - 16	Abstract #32	Disentangling the roles of diatoxanthin and Lhcx1 in the nonphotochemical quenching of Phaeodactylum tricornutum	Dany Croteau*, Marianne Jaubert, Jean-Pierre Bouly, Angela Falciatore and Benjamin Bailleul
Poster Session 1 - 17	Cancelled		
Poster Session 1 - 18	Abstract #42	Ecologically relevant metabolites produced by a genome-scale metabolic model of Thalassiosira pseudonana	Helena van Tol*, Virginia Armbrust
Poster Session 1 - 19	Abstract #45	The evolution of morphology in diatoms: assessment of grazing pressure in long-term speciation.	Alessandra Petrucciani*, Alessandra Norici
Poster Session 1 - 20	Abstract #49	The roles of putative pyridoxal phosphate dependent transferase (PLP-DT) in nitrogen utilization and lipid synthesis in marine diatom Phaeodactylum tricornutum	Zichao Deng, Ruiping Huang, Xin Lin*
Poster Session 1 - 21	Abstract #56	A Tale of Two Flavodoxins	Shiri Graff van Creveld*, Sacha N. Coesel, Stephen Blaskowski, Ryan D. Groussman, Megan J. Schatz, Rhonda L. Morales, E. Virginia Armbrust
Poster Session 1 - 22	Abstract #57	Regulation mechanisms of phosphate uptake in Phaeodactylum tricornutum and Thalassiosira pseudonana	Hiroaki Matsui*, Toshiki Sugiyama, Yohei Fukuchi, Nanae Kimura, Kanako Maeda, Hermanus Nawaly, Yoshinori Tsuji, Yusuke Matsuda
Poster Session 1 - 23	Abstract #60	Dynamic biofilm formation of diatoms by a transcriptomic study	Ruqian Yang*, Bernard Lepetit, Peter G. Kroth
Poster Session 1 - 24	Abstract #69	Diatom Plasticity: Trends, Issues and Applications on Modern and Classical Taxonomy, Eco-Evolutionary Dynamics and Climate change	Lawrence Victor D. Vitug
Poster Session 1 - 25	Abstract #70	Differential expression analysis in the spore former Chaetoceros socialis during nitrogen starvation	Angela Pelusi*, Luca Ambrosino, Marco Miralto, Laura de Entrambasaguas Monsell, Maria Immacolata Ferrante, Marina Montresor

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Poster Session	Abstract	Title	Authors
Poster Session 1 - 26	Abstract #74	Species-specific bloom dynamics of Skeletonema in Ariake Sound, Japan: Development of Skeletonema species-specific qPCR	Kazuhiro Yoshida*, Hiroshi Oota, Takuya Iwanaga, Takayuki, Mine, Kei Kimura
Poster Session 1 - 27	Abstract #78	Investigating the Characteristics of Multipolar Diatoms in Response to Temperature and the Need for Fresh Environmental Isolates	Sam Coffin*, Matthew Davey, Alison Smith, Melody Clark
Poster Session 1 - 28	Abstract #79	Temperature sensing in diatoms: cold shock and Ca2+ signalling	Friedrich Kleiner*, Katherine Helliwell, Glen Wheeler, Colin Brownlee
Poster Session 1 - 29	Abstract #81	The Impact of Temperature on Vitamin B12 Use in the Polar Diatom Fragilariopsis cylindrus	Catalina Albury*, Elden Rowland, Erin Bertrand
Poster Session 1 - 30	Abstract #92	Molecular physiology of Antarctic diatom natural assemblages reveals multiple strategies contributing to their ecological success	Carly Moreno*, Natalie Cohen, Maggie Bernish, Yajuan Lin, Nicolas Cassar, Oscar Schofield, Adrian Marchetti
Poster Session 2 - 01	Abstract #04	Scarless GFP-tagging of native genes in Thalassiosira pseudonana	Irina Grouneva*, Luke Mackinder
Poster Session 2 - 02	Abstract #06	Prevalence of trypsin in marine phytoplankton and their functions in the integrative nitrogen and phosphorus signaling network	Yanchun You*, Senjie Lin
Poster Session 2 - 03	Abstract #08	Valve morphogenesis in Amphitetras antediluviana Ehrenberg	Mary Tiffany*, Bonnie Hurwitz
Poster Session 2 - 04	Abstract #09	Extracellular products from a flavobacterium induce transcriptional shifts of cell cycle and metabolism in a model diatom.	Zinka Bartolek*, Shiri Graff van Creveld, Sacha Coesel, E. Virginia Armbrust
Poster Session 2 - 05	Abstract #10	An Integrative Experimental And Environmental Atlas Of Diatom Chloroplast Transporters	Shun Liu*; Tomomi Nonoyama; Mattia Storti; Amandine Baylet; Giovanni Finazzi; Chris Bowler; and Richard G. Dorrell
Poster Session 2 - 06	Abstract #11	Single-cell heterogeneity in response to environmental stress reveals pathways of cell fate regulation in marine diatoms	Avia Mizrachi, Shiri Graff van Creveld, Rotem Haviv, Ester Feldmesser, Orr H. Shapiro, Chuan Ku, Robert E. Jinkerson, Shilo Rosenwasser, and Assaf Vardi
Poster Session 2 - 07	Abstract #12	Investigating molecular mechanisms for phosphorus sensing in the marine diatom, Phaeodactylum tricornutum	Yasmin Meeda*, Dr Adam Monier, Dr Glen Wheeler, Dr Katherine Helliwell
Poster Session 2 - 08	Abstract #14	Elucidating the microenvironment of mineral formation within silica deposition vesicles in diatoms	Lior Aram*, Diede de Haan, Assaf Gal
Poster Session 2 - 09	Abstract #15	Exocytosis of diatom silica involves extensive membrane disintegration	Diede de Haan*, Hadas Zehavi, Yoseph Addadi, Elena Kartvelishvily, Oz Ben Joseph, Lior Aram, Assaf Gal
Poster Session 2 - 10	Abstract #16	Light sensing under the sea: diatom photoreceptors in Tara Oceans	Carole Duchêne*, Juan Pierella Karlusich, Jean-Pierre Bouly, Chris Bowler, Angela Falciatore, Marianne Jaubert
Poster Session 2 - 11	Abstract #17	Comparative morphological and transcriptome analyses of valve plasticity induced under different salinity conditions in a centric diatom Pleurosira laevis	Shiho Kamakura*, Masahiko Idei, Matt Ashworth and Shinya Sato
Poster Session 2 - 12	Abstract #21	A FIB-SEM–based workflow to study diatoms light acclimation	Clarisse Uwizeye, Serena Flori*, Johan Decelle, Pierre-Henri Joneau, Benoit Gallet, Jean-Baptiste Keck, Davide Dal Bo, Christine Moriscot, Claire Seydoux, Fabien Chevalier, Nicole L. Schieber, Rachel Templin, Guillaume Allorent, Florence Courtois, Gilles Curein, Yannick Schwab, Guy Schoehn, Samuel Zeeman, Denis Falconet, Giovanni Finazzi
Poster Session 2 - 13	Abstract #22	Transporters dependent regulation of photosynthesis in Phaeodactylum tricornutum	Mattia Storti*, Claire Seydoux, Davide Dal Bo, Shun Liu; Richard Dorrell, Guillaume Allorent, Florence Courtois; Giovanni Finazzi
Poster Session 2 - 14	Abstract #27	The Potential Role Of Extracellular Vesicles In Stress Response In Marine Diatoms	Rotem Haviv*, Avia Mizrachi, Shifra Ben-Dor, Daniella Schatz, Assaf Vardi
Poster Session 2 - 15	Abstract #31	Oxidative stress response during the resting cells formation in marine diatom Thalassiosira pseudonana	Jun-Rong Liang*, Fan Hang, Qian-Qian Huang, Lu Huang, Gui-Fang Lin, Bin-Ying Shen, Chang-Ping Chen, Ya-Hui Gao
Poster Session 2 - 16	Abstract #33	Differential gene expression associated with sexual maturation of pennate diatoms	Darja Belišová*, Gust Bilcke, Lieven De Veylder ,Wim Vyverman
Poster Session 2 - 17	Abstract #39	Low-Affinity Nitrate Transporters in diatoms, diNPFs: identification, evolution, structure and function	Anna Santin*, Luigi Caputi, Antonella Longo, Maurizio Chiurazzi, Maurizio Ribera d'Alcalá, Monia Teresa Russo, Maria Immacolata Ferrante, Alessandra Rogato
Poster Session 2 - 18	Abstract #40	Understanding the Cellular Role of Aureochromes, a New Type of Blue Light Photoreceptors, in Diatoms	Robert Röllig*, Christian Wilhelm, Peter Kroth, Torsten Jakob

Session to Abstract Cross Ref

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Poster Session 2 - 20	Abstract #58	Characterization of the Rubisco condensation protein PYCO1 from the pyrenoid of Phaeodactylum tricornutum	Zhen Guo Oh, Warren Ang, Cheng Wei Poh, Soak Kuan Lai, Hoi Yeung Li, Shashi Bhushan, Tobias Wunder and Oliver Mueller-Cajar*
Poster Session 2 - 21	Abstract #64	A vitamin B12 physiological kaleidoscope in Phaeodactylum tricornutum	Shelby Newsad*, Gonzalo Mendoza, Marcel Llavero Pasquina, Katrin Geisler, Ellen Harrison, Alison Smith
Poster Session 2 - 22	Abstract #65	How to analyze diatom growth data in a robust way: an evaluation of mathematical methods for specific growth rate estimation using simulation trials	Olga Kourtchenko*, Kai T. Lohbeck, Björn Andersson, Tuomas Rajala
Poster Session 2 - 23	Abstract #66	Identifying Aureochrome-promoter interactions in Phaeodactylum tricornutum	Soo Hyun Im*, Sandeep Shrestha, Laura Weiss, Shvaita Madhuri, Bernard Lepetit, and Peter Kroth
Poster Session 2 - 24	Abstract #80	Molecular mechanisms of survival to the polar night in the diatom Fragilariopsis cylindrus	Juliette Laude*, Nathalie Joli, Lorenzo Concia, Flavienne Bruyant, Marine Beguin, Marie-Hélène Forget, Fredy Barneche, Marcel Babin, Chris Bowler
Poster Session 2 - 25	Abstract #87	Circadian rhythms in the diatom Phaeodactylum tricornutum: regulatory function of bHLH/PAS proteins	Alessandro Manzotti*, Raphael Monteil, Andrés Ritter, Rossella Annunziata, Antonio Emidio Fortunato, Soizic Cheminant-Navarro, Denis Jallet, Jean-Pierre Bouly, Angela Falciatore
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Poster Session 2 - 27	Abstract #93	Molecular phylogeny of fucoxanthin-chlorophyll a/c proteins in the Chaetoceros gracilis genome suggests the diversification process of the light-harvesting complexes in the red algal lineage	Minoru Kumazawa, Hiroyo Nishide, Ryo Nagao, Natsuko Inoue-Kashino, Ikuo Uchiyama, Yasuhiro Kashino, Jian-Ren Shen, Takeshi Nakano, Kentaro Ifuku
Poster Session 2 - 28	Abstract #77	Characterizing two nitrite reductase enzymes in P. tricornutum through CRISPR-Cas9 gene-knockout methods	Anne Schulberg*, James "Flip" McCarthy, Mark Mooseburner, Hong Zheng, Ariel Rabines, Rob Lampe
Poster Session 2 - 29	Abstract #02	Rhythm, topography and ecophysiology of horizontal gene transfer across the diatom pan-genome	Richard G Dorrell, Benoît Perez-Lamarque, Alan Kuo, Adrien Villain, Guillemette Audren de Kerdrel, Ouardia Ait-Mohamed, Kyle R Frischkorn, Zoltan Füssy, Fuhai Liu, Giselle McCallum, Andrew Watson, Nikola Zarevski, Igor R Grigoriev, Hélène Morlon, Guillaume Blanc, Connie Lovejoy, Chris Bowler
Poster Session 2 - 30	Abstract #28	Diatoms diversification in light of their ecological niche space	Sophia Lambert*, Richard Dorrell, Chris Bowler, Hélène Morlon.
Poster Session 2 - 31	Abstract #50	Using experimental evolution to reveal insights into how the (epi)genome and transcriptome changes due to a long-term temperature response of Thalassiosira pseudonana	Andrew Toseland*, Katrin Schmidt, Cock van Oosterhout, Thomas Mock
Poster Session 2 - 32	Abstract #72	Investigating evolutionary adaptation of the polar diatom Fragilariopsis sp.	Krisztina Sárközi*, Amanda Hopes, Reuben Gilbertson, Thomas Mock
Poster Session 2 - 33	Abstract #85	Using a diatom model to study an unusual histone protein in Chromera velia	Shun-Min Yang*, Ansgar Gruber, Kateřina Jiroutová, Miroslav Oborník
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Poster Session 3 - 02	Abstract #19	High-efficiency transformation by electroporation and feasibility of CRISPR-Cas9-mediated gene editing in a centric diatom Chaetoceros muelleri	Wenxiu Yin*, Hanhua Hu
Poster Session 3 - 03	Abstract #20	The heterologous expression of a green alga plastocyanin in Phaeodactylum tricornutum improves cell growth under iron-deficient conditions	Carmen Castell*, Pilar Bernal-Bayard, José M. Ortega, Mercedes Roncel, Manuel Hervás, José A. Navarro
Poster Session 3 - 04	Abstract #34	The physiological role and structural arrangement of the mitochondria of Fistulifera solaris in the oil degradation process	Yumika Kaneko*, Seiichiro Moriya, Yoshiaki Maeda, Tomoko Yoshino, Tsuyoshi Tanaka
Poster Session 3 - 05	Abstract #36	An Analytical Approach for Understanding Light Modulation by Diatom Frustules	Mohamed Ghobara*, Cathleen Oschatz, Peter Fratzl, Louisa Reissig
Poster Session 3 - 06	Abstract #37	Digital holographic microscopy of morphological changes of diatom cellular contents induced by papain enzyme	Kazuo Umemura*, Yuki Ide, Makoto Saito, Shigeki Mayama
Poster Session 3 - 07	Abstract #38	Producing the SARS-CoV-2 Spike RBD antigen in the diatom Phaeodactylum tricornutum	Sam Slattery*, Emily Stuckless, Dan Giguere, Arina Shrestha, Bogumil Karas, Greg Gloor, David Edgell

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Poster Session 3 - 09	Abstract #48	Chloroplast Genome Engineering As A Tool To Improve The Productivity Of Monoterpenes In The Diatom Phaeodactylum Tricornutum	Nicola Trevisan*, Sarah D'Adamo, Maria J. Barbosa, John van der Oost
Poster Session 3 - 10	Abstract #54	Diatoms as promising hosts for monoterpene engineering	Michele Fabris*, Jestin George, Alessandro Satta, Unnikrishnan Kuzhiumparambil, Lygie Esquirol, Rachelle Tran, Claudia E. Vickers
Poster Session 3 - 11	Abstract #59	Proteomic analysis of the oil bodies with different sizes in the marine diatom Fistulifera solaris for oil body-engineering	Marshila Kaha, Masayoshi Noda, Yoshiaki Maeda, Tomoko Yoshino, Mitsufumi Matsumoto, Tsuyoshi Tanaka
Poster Session 3 - 12	Abstract #63	Synthetic biology for the controlled production of high-value compounds in Phaeodactylum tricornutum.	Katrin Geisler, Caroline Faessler*, Patrick Hickland, Marcel Llavero-Pasquina, Alison G. Smith
Poster Session 3 - 13	Abstract #68	Molecular drivers of sexual reproduction in Cylindrotheca closterium	Sien Audoor*, Gust Bilcke, Katerina Pargana, Darja Belišová, Rossella Annunziata, Monia Russo, Maria Immacolata Ferrante, Klaas Vandepoele, Lieven De Veylder, Wim Vyverman
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Poster Session 3 - 15	Abstract #83	Characterizing Ku70 knock outs and knock downs in Phaeodactylum tricornutum	Emily Stuckless*, Julia Cholod, Samuel Slattery, Bogumil Karas, David Edgell
Poster Session 3 - 16	Abstract #94	A regulatory cascade of phosphorus-nutrient strategies in the diatom Phaeodactylum tricornutum	Senjie Lin*; Kaidian Zhang
Poster Session 3 - 17	Abstract #05	Diurnal transcript profiling of the diatom Seminavis robusta reveals adaptations to a benthic lifestyle	Gust Bilcke*, Cristina Maria Osuna-Cruz, Marta Santana Silva, Nicole Poulsen, Sofie D'hondt, Petra Bulankova, Wim Vyverman, Lieven De Veylder, Klaas Vandepoele
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Poster Session 3 - 21	Abstract #44	Evolution and adaptation of Fragilariopsis from the Arctic and Antarctic: Sequencing new strains and investigating adaptation to rising temperatures.	Amanda Hopes, Krisztina Sarkozi, Reuben Gilbertson, Kat Hodgkinson, Thomas Mock
Poster Session 3 - 22	Abstract #46	Evolutionary analysis of DNA methyltransferases in unicellular eukaryotes: Insights from the model diatom Phaeodactylum tricornutum	Antoine Hoguin, Ouardia Ait Mohamed, Catherine Cantrel, Chris Bowler, Auguste Genovesio, Fabio Rocha Jimenez Vieira, Leila Tirichine*
Poster Session 3 - 23	Abstract #52	Mitotic recombination between homologous chromosomes contributes to the genomic diversity in diatom clonal populations	Petra Bulánková*, Mirna Sekulić, Denis Jallet, Charlotte Nef, Cock van Oosterhout, Tom O. Delmont, Ilse Vercauteren, Cristina Maria Osuna-Cruz, Emmelien Vancaester, Thomas Mock, Koen Sabbe, Fayza Daboussi, Chris Bowler, Wim Vyverman, Klaas Vandepoele and Lieven De Veylder
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Poster Session 3 - 25	Abstract #67	Genetic variation analysis provides insights into Pseudo-nitzschia multistriata population genomics	Svenja Mager*, Francesco Manfellotto, Maria Valeria Ruggiero, Viviana Di Tuccio, Monia Russo, Marina Montresor, Remo Sanges, Mariella Ferrante
Poster Session 3 - 26	Abstract #71	Do core DNA repair enzymes regulate Diatom genetic diversity?	Reuben J Gilbertson*, Amanda Hopes, Cock van Oosterhout, and Thomas Mock.
Poster Session 3 - 27	Abstract #75	The genome biology of Parmales (Bolidophyceae), a sister group of diatoms	Hiroki Ban*, Shinya Sato, Shinya Yoshikawa, Kazumasa Yamada, Yoji Nakamura, Mutsuo Ichinomiya, Hisashi Endo, Romain Blanc-Mathieu, Akira Kuwata, Hiroyuki Ogata
Poster Session 3 - 28	Abstract #84	Multi-class predictions of intracellular locations of proteins in organisms with complex plastids	Ansgar Gruber
Poster Session 3 - 29	Abstract #01	Synthetic Promoters For Control Of Gene Expression In The Model Diatom Phaeodactylum Tricornutum	Tessama Kassaw

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Poster Session 3 - 32	Abstract #23	The Cloning and Engineering of Diatom Mitochondrial Genomes in Yeast and Bacteria	Ryan Cochrane*, Stephanie Brumwell, Maximillian Soltysiak, Arina Shrestha, Daniel Giguere, Samir Hamadache, Jennifer Davis, Jiayi Wang, Preetam Janakirama, Greg Gloor, David Edgell, Bogumil Karas
Poster Session 3 - 32	Abstract #91	Towards a synthetic algal chloroplast: a streamlined platform for creating designer chloroplast genomes in Phaeodactylum tricornutum	Emma Walker*, Mark Pampuch, Jennifer Davis, Ryan Cochrane, Gregory Gloor, Bogumil Karas
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Session 1 - 5	Kimberlee Thamatrakoln	Shunt or shuttle? Nutrient-driven biogeochemical consequences of diatom host-virus interactions	Kimberlee Thamatrakoln
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Session 1 - 7	Shady Amin	How diatoms modulate bacteria in their microbiome	Ahmed Shibl, Ashley Isaac, Michael Ochsenkühn, Anny Cárdenas, Cong Fei, Kristin Gunsalus, Christian Voolstra, Shady Amin*
Session 1 - 8	Uta Passow	Diatoms and the Biological Carbon Pump	Uta Passow
Session 2 - 2	Katherine Helliwell	A novel Ca2+ signalling pathway co-ordinates environmental phosphorus sensing and nitrogen metabolism in marine diatoms	Helliwell K. E.*, Harrison E., Christie-Oleza J., Rees A. P., Kleiner F. H., Gaikwad T., Downe J., Aguilo-Ferretjans M. M., Al-Moosawi L., Brownlee C., Wheeler G. L.
Session 2 - 3	David Hutchins	Interactions between irradiance and temperature determine the niche of a novel low-light, cold-adapted nano-diatom from a wintertime temperate estuary	David A Hutchins*, Kyla Kelly, Sophia Pei, Tatiana A. Rynearson, Joshua Kling
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Session 2 - 7	Stephanie Dutkiewicz	Modelling Marine Diatom Diversity and Potential Future Changes at the Global Scale	Stephanie Dutkiewicz
Session 3 - 1	Masao Adachi	Activities of promoters derived from four diatom-infecting DNA viruses in the marine diatom Phaeodactylum tricornutum	Takashi Kadono, Yuji Tomaru, Nao Sato, Kengo Suzuki, Koji Yamada, Masao Adachi*
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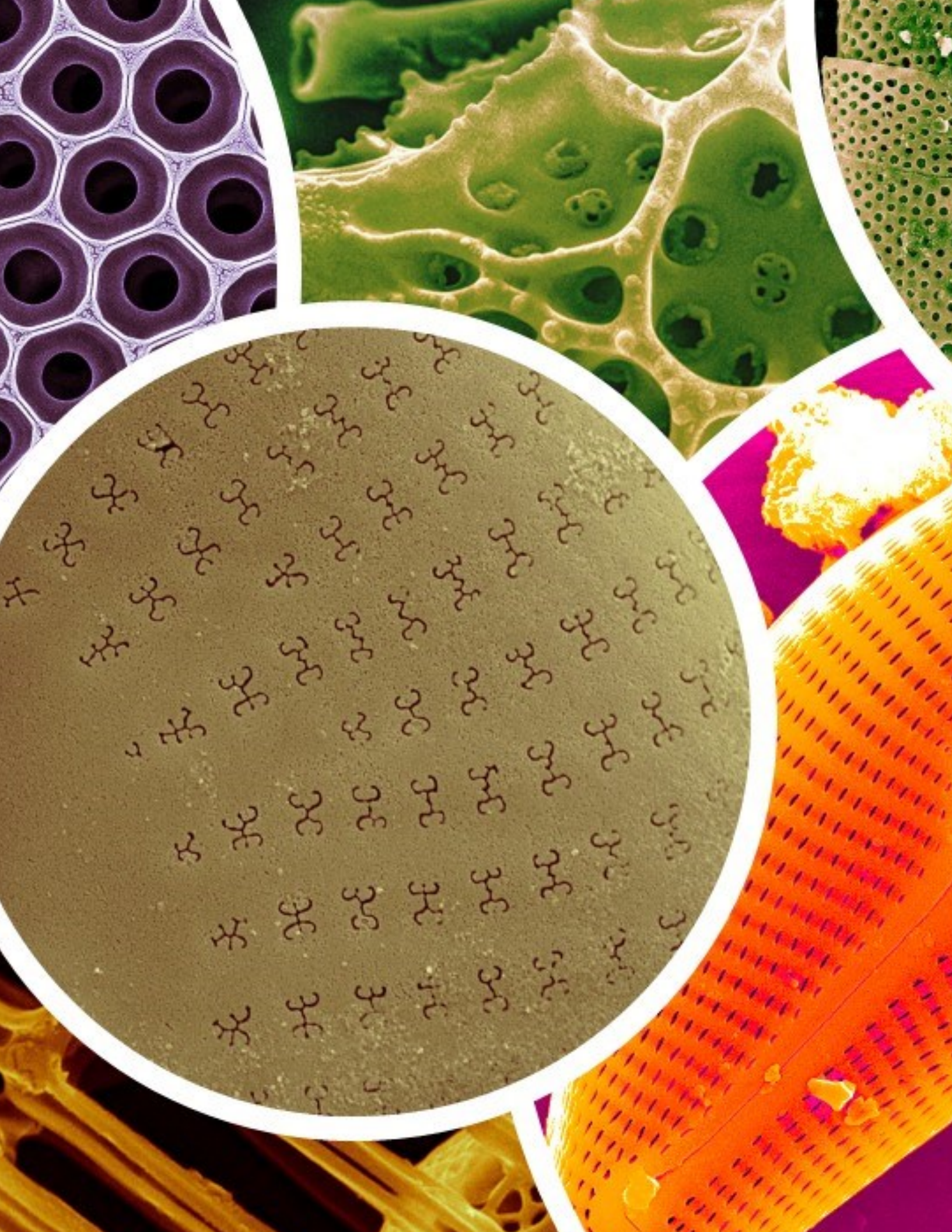
Session 2 - 1	Yuichiro Kashiyama	How to digest delicious diatoms containing the phototoxic chlorophyll	Yuichiro Kashiyama*
Session 3 - 4	Miroslav Oborník	Exploring the heme pathway in chromerid and diatom	Jitka Richtová, Lilach Sheiner, Shun-Min Yang, Ansgar Gruber, Luděk Kořený, Boris Striepen, Miroslav Oborník*
Session 3 - 6	Patrick Keeling	How does endosymbiosis lead to new plastids?	Patrick Keeling



1. Synthetic Promoters For Control Of Gene Expression In The Model Diatom Phaeodactylum Tricornutum

Tessema Kassaw

Tessama Kassaw

Colorado state university

Developing genetic approaches for efficient and scalable disruption or activation of gene expression has multifaceted advantages. It can aid in dissecting mechanisms governing cellular processes, studying gene function, enable high throughput genome-scale screens and assist in redirecting metabolic flux towards high-value metabolites. Chemical-inducible expression systems have been employed for precise dynamic control over genetically engineered traits. However, the current systems for controlled transgene expression in diatoms are limited to endogenous promoters that respond to different environmental factors. We developed a highly efficient, tunable, and reversible episome-based transcriptional control system in the model diatom Phaeodactylum tricornutum. We assessed the time- and dose-response dynamics of each expression system using a reporter protein (eYFP) as a readout. Using our circuit configuration, we found two inducible expression systems with high dynamic range and confirmed the suitability of episome expression platform for synthetic biology applications in diatoms. Addition of a chemical inducer (β-estradiol) to transgenic strains activates transcription with a dynamic range of up to ~180-fold. We demonstrated that our episome-based transcriptional control systems are tunable and reversible in a dose-and time-dependent manner. Using droplet digital PCR, we also confirmed inducer dependent transcriptional activation starts within minutes of inducer application without any detectible expression in the uninduced controls. The system described here expands the molecular and synthetic biology toolkits in algae and will facilitate future gene discovery and metabolic engineering efforts.

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2. Rhythm, topography and ecophysiology of horizontal gene transfer across the diatom pan-genome

Richard Dorrell

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Since the publication of the seminal Thalassiosira and Phaeodactylum genomes, it has been apparent that diatoms are evolutionary mosaics ; supported by networks of genes derived from endosymbiotic- and non-endosymbiotic horizontal gene transfers including red algal, green algal and bacterial partners. Owing to both the complexity of this signal, and the historical paucity of genomic and transcriptomic resources available for key eukaryotic and prokaryotic actors, many questions in the history of diatom gene transfers have remained until recently unresolved. These include the probable roles of other algal groups with secondary chloroplasts in gene transfers with diatoms ; the direction, cause and timepoints of probable mass transfer events into and out from diatom genomes; and the distinct functions performed by different classes of horizontally acquired genes in contemporary diatom biology.

We have used combined manual and automated resolution of thousands of gene trees across a densely-sampled reference dataset ; in silico and experimental localisation ; and transcriptional and environmental profiling incorporative of the Tara Oceans dataset to explore the dynamics of horizontal gene transfers across the diversity of diatom lineages. We can separate these transfers effectively into four categories : endosymbiotic gene transfers of genes encoding predominantly chloroplast-associated proteins from a chimeric eukaryotic alga into the ochrophyte common ancestor ; a mass transfer of genes encoding chloroplast-associated proteins from a pelagophyte or dictyochophyte relative of diatoms into haptophytes, and probably dinoflagellates ; a continuous wave of bacterial gene transfer across diatom evolutionary history, which may have occurred to a greater extent in diatoms than other algal groups, and serves principally to remodel the diatom secretome ; and recent and environmentally-adaptive horizontal gene transfers into individual diatom lineages involving both bacterial and eukaryotic partners, which may be biogeographically constrained, as in the case of ice-binding proteins independently acquired in Arctic-

and Antarctic-native algae. Our data serves to disentangle the convoluted evolutionary origins of some of the most successful algal groups in the modern ocean ; and identify new candidate genes for the experimental investigation of diatom fitness and adaptation to diverse marine environments.

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3. Upwelling-associated phytoplankton display resistance to ocean acidification

Robert Lampe

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Coastal upwelling regions are among the most biologically productive ecosystems in the ocean but may be threatened by amplified ocean acidification from rising atmospheric CO2. Increased acidification is hypothesized to reduce iron bioavailability for marine phytoplankton thereby expanding iron limitation and impacting primary production. Here we show from community to molecular levels that iron-stressed phytoplankton in an upwelling region exhibit resistance to acidification. Trace metal clean incubations were performed for up to four days and 1200 ppm pCO2; however, phytoplankton growth, nutrient uptake, and community compositions remained unchanged. Although variable, transcriptional responses suggest reduced cellular iron demand and alternative iron uptake pathways that may be less affected by acidification. By extrapolating these short-term experimental results and considering the success of many phytoplankton taxa within existing pH variability in coastal upwelling regions, we predict that acidification from average CO2 concentrations this century will not hinder continued high levels of productivity in these ecosystems.

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4. Scarless GFP-tagging of native genes in Thalassiosira pseudonana

Irina Grouneva

*Irina Grouneva, Luke Mackinder

University of York

Use of CRISPR/Cas9-based gene editing in diatoms has expanded their molecular toolbox considerably in recent years. Building on this progress we have developed a seamless knock-in method based on homology-directed recombination (HDR) that enables the introduction of a GFP tag to specific sites in the genome of the centric diatom Thalassiosira pseudonana.

Expression of all necessary components was achieved using bacterial delivered episomes containing Cas9, a sgRNA and 600bp long homology arms flanking GFP. We introduced targeted double-strand breaks (DSBs) in close proximity to the stop codon of each target gene. This triggered HDR repair, substituting the WT sequence for a GFP-containing template that has had the native stop codon removed and placed after the GFP. This results in GFP fusion proteins expressed from their endogenous genomic position under the control of all of their native cis (i.e. promoter, terminator) and trans regulatory elements. As proof of concept we targeted two CO2-concentrating mechanism-relevant genes: a bestrophin (95-99% editing efficiency) and a carbonic anhydrase (17% editing efficiency). Microscopy imaging localized the bestrophin to the pyrenoid, most likely in the pyrenoid traversing thylakoid membrane, implicating a role in HCO3- transport within the diatom CO2-concentrating mechanism. The carbonic anhydrase localized to the mitochondria confirming previous localization via random integration of an additional gene copy under the control of a constitutive promoter. The applications of this method in diatom research are far-reaching. It can be used not only for various localization studies but precise, tailored knock-ins, point and substitution mutations.

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5. Diurnal transcript profiling of the diatom Seminavis robusta reveals adaptations to a benthic lifestyle

Gust Bilcke

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Coastal regions contribute an estimated 20% of annual gross primary production in the oceans, despite occupying only 0.03% of their surface area. Diatoms frequently dominate coastal sediments, where they experience large variations in light regime resulting from the interplay of diurnal and tidal cycles. Here, we report on an extensive diurnal transcript profiling experiment of the motile benthic diatom Seminavis robusta. Nearly 90% (23,328) of expressed protein-coding genes and 66.9% (1124) of expressed long intergenic non-coding RNAs (lincRNAs) showed significant expression oscillations and are predominantly phasing at night with a periodicity of 24h. Phylostratigraphic analysis found that rhythmic genes are enriched in deeply conserved genes, while diatom-specific genes are predominantly associated with midnight expression. Integration of genetic and physiological cell cycle markers with silica depletion data revealed potential new silica cell wall associated gene families specific to diatoms. Additionally, we observed 1752 genes with a remarkable semidiurnal (12-h) periodicity, while the expansion of putative circadian transcription factors may reflect adaptations to cope with highly unpredictable external conditions. Taken together, our results provide new insights into the adaptations of diatoms to the benthic environment and serve as a valuable resource for diurnal regulation in photosynthetic eukaryotes.

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6. Prevalence of trypsin in marine phytoplankton and their functions in the integrative nitrogen and phosphorus signaling network

Yanchun You

Yanchun You*, Senjie Lin

Xiamen University

Prevalence of trypsin in marine phytoplankton and their functions in the integrative nitrogen and phosphorus signaling network

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Trypsin is an ancient protease best known as a digestive enzyme in vertebrate animals, but is less known in other organisms, and unexplored in phytoplankton. Here we report the wide distribution and active expression of trypsin genes in global ocean phytoplankton. Furthermore, using the marine diatom Phaeodactylum tricornutum as the model species we explore the function of trypsin in marine phytoplankton. Interestingly, our gene expression analyses showed that P. tricornutum trypsin 2 (PtTryp2) was down-regulated under N-depleted but up-regulated under P-depleted. Further, we created a PtTryp2-overexpressing (OE) and a PtTryp2-knockout (KO) strain, and conducted transcriptome profiling coupled with physiological analyses under different nutrient supply conditions. We observed opposite trends of responses to N-depleted and P-depleted conditions in PtTryp2-KO, and these trends were inversely switched or resecured by overexpression of PtTryp2. Additionally, PtTryp2 appeared to trigger down-stream N responses by repressing primary N starvation responses and to ameliorate P stress by activating phosphorus starvation induced (PSI) genes. Taken together, PtTryp2 may play a crucial role in shifting the set points at which N starvation- or phosphorus starvation- response is activated in face of environmental nutrient stress and, in feedback to the metabolic status, change the way cells commit to these different responses, all by tuning its expression level. The study reveals a novel function of trypsin and provides new insights into the upstream regulatory mechanism in phytoplankton to cope with environmental nutrient stress.

Key words: Phytoplankton, diatom, trypsin, nitrogen nutrient stress, phosphorus stress, nutrient homeostasis.

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7. Diving into metapopulation genomics of the Chaetoceros genus

Charlotte Nef

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Despite their abundance and diversity in marine ecosystems, comprising over 80,000 species and performing around 40% of total marine primary production, the microevolutionary structure of diatoms remains poorly understood. Exploring how closely related diatoms adapt to different oceanic ecoregions is particularly important given their short generation times, which may allow rapid adaptations to different oceanic environments through sexual recombination and drift; and their particular importance to marine regions such as the Arctic and Southern Oceans impacted dramatically by anthropogenic climate change. Here, we leverage 11 recently reconstructed metagenome-assembled genomes (hereafter referred as MAGs) from the Tara Oceans expedition to explore the evolutionary diversity of Chaetoceros metapopulations, the single most abundant diatom genus in the modern ocean and one of the most diverse.

We identified 15 Tara Oceans stations that presented sufficient coverage of the MAGs and investigated their distribution. The results confirm a prevalent distribution of Chaetoceros in the Arctic Ocean with a minor dispersal in the Pacific and Southern Oceans as well as in the Mediterranean. Consistent with previous studies, the genus was found to locally account to a maximum of 5% of metagenomic reads. Chaetoceros population connectivity was further investigated for 5 MAGs that were present in at least two sampling stations by identifying the single nucleotide variants (SNVs) associated to the different populations and computing the fixation index (hereafter referred as the Fst statistic), a proxy of the genetic distance based on their allelic frequencies. We observed moderate genetic structure in some Chaetoceros populations in the Arctic Ocean, suggesting genetic differentiation. Finally, the contribution of local environmental parameters in explaining the population structure was explored. Altogether, these analyses provide new insights and perspectives into diatom metapopulation genomics through the integration of metagenomic and environmental data.

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8. Valve morphogenesis in Amphitetras antediluviana Ehrenberg

Mary Ann Tiffany

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Amphitetras antediluviana is a marine epiphytic diatom that forms quadrangular chains. This species has not been cultured and, therefore, is understudied. Here, we provide the first study of valve development in A. antediluviana and compare valve development to other diatom species. Each valve has four ocelli at the corners with gently curving sides and a circular elevation in the center. In early valves, we observed a circular to oval annulus with radiating costae (virgae) that occasionally bifurcate before forming the first hints of the ocellus. The costae are interconnected by crossbars (vimines) to form areolae. Bars of silica form within the annulus, likely providing extra strength/support. Virgae at the periphery of the valve bend in the direction of the ocelli, eventually fusing to produce their rims. Once the rim is fully enclosed, small pores (porelli) start to form centripetally until they meet at the center, creating a nearly mature ocellus. Further development of the ocellus includes the addition of small and larger granules. Two types of cribra develop within the areolae. The most common form consists of small poroids supported by tiny struts on the edge of the cribrum. As in other species of centric diatoms, the struts repeatedly bifurcate to produce a mesh of poroids. In mature specimens, the poroids are most evident on the internal surface. The other type of cribrum begins with sturdier struts that broaden to become spathulate, adding flattened structures as they expand, and leaving slits instead of pores. The addition of a marginal flange occurs late in valve development. An image of a forming girdle band suggests that formation is unidirectional; Cribra later fill the rectangular pores. Future research is needed to determine how this species forms two very different types of cribra, possibly using transmission electron microscopy of dividing cells.

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9. Extracellular products from a flavobacterium induce transcriptional shifts of cell cycle and metabolism in a model diatom.

Zinka Bartolek

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Phytoplankton and bacteria form the base of marine ecosystems and their interactions drive global biogeochemical cycles. The effect of bacteria and bacteria-produced compounds on diatoms can range from synergistic to pathogenic and can affect the physiology and transcriptional patterns of the host diatom. Here, we investigate the physiological and transcriptional changes in the diatom Thalassiosira pseudonana induced by extracellular products of a known antagonistic bacteria Croceibacter atlanticus. Mono-cultures of C. atlanticus released compounds that inhibited diatom cell division and elicited a distinctive phenotype of enlarged cell sizes with multiple plastids and nuclei; similar to what was observed when the diatom was co-cultured with the live bacteria. The extracellular C. atlanticus metabolites induced transcriptional changes in diatom pathways that include recognition and signaling pathways, cell cycle regulation, amino acid production and chitin biosynthesis. These transcriptional changes explain the observed phenotype, and suggest that extracellular bacterial products can modulate the diatom's metabolism to better support bacterial growth.

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10. An Integrative Experimental And Environmental Atlas Of Diatom Chloroplast Transporters

Shun Liu

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Diatoms are an important group of marine algae, forming nearly one-half of contemporary eukaryotic phytoplankton, and responsible for one-fifth of total planetary photosynthetic activity. Diatoms dominate regions of the world ocean in which photosynthetic activity is compromised due to scarcity of the essential nutrient iron.

Diatom possess chloroplasts which were acquired by the secondary endosymbiosis of a red alga, are surrounded by four membranes, and are distantly related to those of plants. Chloroplasts, as the main organelles for photosynthesis, need transporters to mediate the exchange of ions and metabolites. Extensive chloroplast and mitochondria contact points have been observed in diatoms, suggesting the presence of intricate chloroplast-mitochondria cross-talk; however, the transporters related to this cross-talk remain undefined.

Using the model diatom Phaeodactylum tricornutum as a subject, we have identified a candidate gene, encoding a transporter that we have localized to chloroplast-mitochondria contact points. This gene is widespread across diatoms, but has no direct equivalent in plants. The expression pattern of this gene is strongly coregulated with the diatom mitochondrial genome. By generating heterozygous knockout mutant lines, we have found the knock out lines have phenotype related to iron.

To further understand the transporters activities of the diatom chloroplast, we have performed in silico analyses of 70 P. tricornutum chloroplast transporters found by genome-wide searches; considering similarity to Arabidopsis thaliana chloroplast transporters, transcriptional correlation to other metabolic pathways, and environmental expression trends using Tara Oceans data. We have identified one diatom chloroplast transporter whose expression is uniquely positively correlated with temperature, which may be explored as a future target for experimental investigation.

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11. Single-cell heterogeneity in response to environmental stress reveals pathways of cell fate regulation in marine diatoms

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Diatoms are photosynthetic microorganisms of great ecological and biogeochemical importance, forming vast blooms in diverse aquatic ecosystems. These blooms are characterized by rapid proliferation which is followed by a coordinated demise. The demise phase and the rapid turnover of phytoplankton were suggested to involve programmed cell death (PCD) that is induced by diverse environmental stressors. Nevertheless, little is known regarding the molecular mechanisms that mediate PCD in diatoms and phytoplankton in general. Current understanding of phytoplankton acclimation to stress is based primarily on population-level analysis, masking cell-to-cell variability. However, it is yet to be elucidated how a few cells survive the demise and serve as a seed for the next bloom. Here we investigated heterogeneity within isogenic diatom populations in response to oxidative stress, which mediates a wide range of environmental stress conditions. We combined flow cytometry and a microfluidics system for live-imaging microscopy to measure redox dynamics at the single-cell level. Using the redox-sensitive sensor roGFP, we measured in vivo oxidation patterns in the model diatom Phaeodactylum tricornutum. Chloroplast targeted roGFP exhibited a light-dependent, bi-stable oxidation pattern in response to H2O2 and high light, revealing two distinct subpopulations. The oxidized subpopulation was sensitive to the stress and subsequently died, while the reduced subpopulation survived. Oxidation of chloroplast targeted roGFP preceded commitment to cell death, and was used as a novel cell fate predictor. We used the variability in the response to H2O2 to identify genes involved in PCD or acclimation by performing transcriptome analysis of sorted subpopulations. These candidate genes are under an ongoing investigation using functional genomics approaches in P. tricornutum and mutants in an orthologous system in the green algae Chlamydomonas reinhardtii. In addition, based on these expression patterns, we are currently developing gene markers for sensitive detection of distinct physiological states in natural diatom blooms.

We propose that phenotypic variability within diatom populations can provide an ecological strategy to cope with rapid environmental fluctuations in the marine ecosystem.

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12. Investigating molecular mechanisms for phosphorus sensing in the marine diatom, Phaeodactylum tricornutum

Yasmin Meeda

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Marine diatoms are ubiquitous microalgae that contribute around one-fifth of global CO2 fixation. Phosphorus, an essential element needed for all living organisms, is often found in scarce supply in many marine ecosystems and can thus limit diatom growth and productivity. As diatoms compete with other phytoplankton, they have evolved metabolic mechanisms to cope with prolonged periods of phosphorus limitation. In addition, diatoms can rapidly sense when the phosphorus supply increases and regulate their metabolism accordingly. This suggests that diatoms have evolved sophisticated mechanisms for sensing environmental phosphorus availability. Recent evidence has identified that diatoms can sense phosphorus using a Ca2+-dependent signalling pathway, however, the molecular machinery underpinning this pathway remains unknown.

Here, we investigated the role of Ca2+-dependent kinases (CDPKS) in governing recovery responses of phosphorus-starved diatom cells to phosphorus resupply. Employing CRISPR-Cas9 gene-editing approaches, we generated gene knockout lines of PtCDPK1 in the marine diatom, Phaeodactylum tricornutum. We are now employing a range of physiological approaches to monitor the capacity of these mutants to grow in phosphorus limitation and resupply regimes. This work aims to provide new insight into the molecular mechanisms enabling this important group of marine algae to thrive in regions of pulsed nutrient supply.

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13. Essential amino acids in Lhcx proteins to confer the rapid photoprotection mechanism qE in the diatom Phaeodactylum tricornutum

Jochen Buck

Jochen M. Buck*, Peter G. Kroth, Bernard Lepetit

Light fluctuations are the common situation photosynthetic organisms have to cope with in nature. While low light may not supply sufficient energy for optimal fitness, supersaturating light goes along with formation of potentially toxic reactive oxygen species. To preempt high light induced photodamage, photosynthetic organisms evolved numerous photoprotective mechanisms. Amongst those, the energy-dependent fluorescence quenching (qE) provides a rapid mechanism to thermally dissipate excessively absorbed energy.

Diatoms thrive in all aquatic environments and thus belong to the most important primary producers on earth. qE in diatoms is provided by a concerted action of Lhcx proteins and the xanthophyll cycle pigment diatoxanthin. While the exact Lhcx activation mechanism of diatom qE is unknown, two lumen exposed acidic amino acids within Lhcx proteins were proposed to be regulatory switches upon light induced lumenal acidification. By introducing a modified Lhcx1 lacking these acidic amino acids into a previously established Phaeodactylum tricornutum Lhcx1-null qE knockout line, we demonstrate that qE is unaffected by those two acidic amino acids. Based on sequence comparisons with Lhcx4, shown previously to be incapable of providing qE, we perform domain swap experiments of Lhcx4 with Lhcx1 and identify two peptide motifs involved in conferring qE. Within the most essential motif, we identify one tryptophan with a major influence on qE establishment. This tryptophan is in close proximity to the diadinoxanthin/diatoxanthin binding site in the diatom Lhc crystal structure recently revealed. Our findings provide a structural explanation for the intimidate link of Lhcx and diatoxanthin in providing qE in diatoms.

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14. Elucidating the microenvironment of mineral formation within silica deposition vesicles in diatoms

Lior Aram

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A distinguishing feature of diatoms is their ability to form an intricate cell wall made of silica. Diatom silicification is a remarkable example of biological control over mineral formation and it strongly influences their physiology. The formation of the silica-based structures is linked to the cell cycle and usually occurs intracellularly in specialized compartments called silica deposition vesicles (SDVs). Despite many years of research, the structural and chemical properties of the microenvironment inside the SDV, which control the morphology and chemistry of biosilica, are unclear. Here we used electron tomography to directly visualize the intracellular process of silica formation within the SDV. Cultures of the diatom Thalassiosira pseudonana were synchronized to enrich the proportion of cells actively producing SDVs. Then the cells were cryo preserved at various stages during valve formation. 3D data sets of the native SDV architecture, with nanometer scale resolution, were collected. The tomograms show that the SDV membrane is tightly engulfing the forming mineral phase and expands simultaneously with silica deposition, until mature silica structure fills the SDV. By expanding and optimizing these in situ observations of the silicification process, we hope to establish a mechanistic understanding of cellular control over mineral formation in diatoms.

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15. Exocytosis of diatom silica involves extensive membrane disintegration

Diede de Haan

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Silicified cell walls of complex design are the most striking feature of diatoms. Since silicification in diatoms is generally an intracellular process, taking place inside a silica deposition vesicle (SDV), a membrane bound organelle, the cell walls have to be exocytosed after maturation. This exocytosis event is exceptional as the silicified cell wall elements are rigid and can be as large as half the cell surface. How diatoms maintain membrane homeostasis during cell wall exocytosis is a long-standing enigma. We investigated the membrane dynamics during cell wall formation and exocytosis in Stephanopyxis turris using both live-cell confocal microscopy and transmission electron microscopy (TEM). These two methods complement each other by offering both real-time cellular dynamics recorded in whole, living cells and high-resolution snapshots of regions of interest. To preserve cellular structures in their near-native state we prepared the cells for TEM by high-pressure freezing and freeze substitution. Our results provide detailed information on the ultrastructure and dynamics of the silicification process. During silica growth, the SDV membranes tightly enclose the mineral phase, creating a precise mold for the delicate geometrical silica patterns. Most interestingly, during exocytosis of the mature silica element, the proximal part of the organelle membrane becomes the new plasma membrane, and external membranes gradually disintegrate without signs for endocytic retrieval or extracellular repurposing. These observations suggest an extraordinary exocytosis mechanism used by diatoms to secrete their cell walls.

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16. Light sensing under the sea: diatom photoreceptors in Tara Oceans

Carole Duchêne

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Light is an essential source of energy and information for photosynthetic organisms. In the marine environment, the light field is structured by depth, as red and far-red light are quickly attenuated in the water column compared to blue and green. Accordingly, diatoms possess a wide array of blue and green photodetectors (cryptochromes, aureochromes, rhodopsins). More surprisingly, diatoms also possess red/far-red phytochrome photoreceptor (DPH), which regulate gene expression in response to far-red light (750nm) in the model diatom Phaeodactylum tricornutum. The physiological function of DPH for diatom life remains unknown. To address this question and the significance of such photoreceptor in the marine environment, we investigated the occurrence of DPH in the available genomes and transcriptomes of diverse marine diatoms and their global distribution in the meta-omics data generated from samples collected from a wide range of oceanic regions during the Tara Oceans expedition. Not all diatoms possess DPH, and in the open ocean, DPH was found only in diatoms belonging to the centric lineage. Interestingly, although centric diatoms are widely distributed in the oceans, including the tropical region, we could find diatoms possessing DPH only at higher latitude (from 30°), i.e. environments with low temperature and variable photoperiods. We synthesized and spectrally characterized 2 DPH genes found in the metagenomic data, as well as 5 DPH from phylogenetically- and geographically- diverse species. Similar to the DPH already characterized from model species, these “environmental” DPH absorb red/far-red light, supporting widely conserved sensing properties of this photoreceptor class in diatoms. Using the model diatom Phaeodactylum tricornutum, we have generated transgenic lines expressing YFP under the control of a DPH-regulated promoter. By measuring the YFP signal of cells exposed to different lights, we have been able to follow the DPH activity in vivo, and to start characterizing its in vivo photochemical properties, giving hints at the conditions in which it can be active in the marine environment.

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17. Comparative morphological and transcriptome analyses of valve plasticity induced under different salinity conditions in a centric diatom Pleurosira laevis

Shiho Kamakura

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Pleurosira laevis is an euryhaline diatom distributed around the world. Compère (1982) described several forms and varieties of P. laevis based on their morphology, including P. laevis f. laevis and P. laevis f. polymorpha, distinguished by their flat valve face or dome-shaped valve faces with protruding ocelli, respectively. In this study, we established 4 strains of P. laevis (3 of P. laevis f. laevis isolated from fresh or brackish waters, and 1 strain of P. laevis f. polymorpha isolated from a coastal area). Manipulating the salinity in culture showed that these P. laevis f. laevis and P. laevis f. polymorpha strains formed both flat (laevis type) and dome-shaped (polymorpha type) valves depending on the salinity. The morphological changes took place on the salinity boundary between 2‰ and 7‰ in all the strains. This valve shape plasticity required only 5 ‰ salinity difference to induce the dynamic morphological change. We then performed a comparative transcriptome analysis using the strain grown under salinity 2‰ and 7‰, expecting to identify genetic factors responsible for determination of each valve shape as well as the ones related to salinity responses. As a result, the expression of 7,899 genes was detected, including 1,913 differentially expressed genes between the two salinity conditions (FDR<0.01, Fold change>2). The functions of the differentially expressed genes included transposons, osmolyte synthesis, membrane transport and cytoskeleton elements.

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18. A functional PDAT encoded by unequally expressed allele enhances triacylglycerol accumulation under nitrogen starvation in Phaeodactylum tricornutum

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Phospholipid:diacylglycerol acyltransferase (PDAT) is the critical enzyme catalyzing the acyl-CoA-independent formation of triacylglycerols (TAGs), which serve as feedstock for biodiesel and can be accumulated at high levels in diatoms. The unique PDAT gene of the model diatom Phaeodactylum tricornutum strain CCMP2561 (Pt1) boasts 47 single nucleotide variants (SNVs) within protein coding regions of PDAT alleles, resulting in 17 amino acid differences between the encoded proteins (designated as PDAT1 and PDAT2). Similar SNVs in PDAT allelic sequences were also found in other accessions of P. tricornutum. Allele-specific expression (ASE) of PDAT gene was observed by the re-analysis of previously published RNA sequencing data and experimental validation. Overexpression of the allele PDAT2 in P. tricornutum could significantly enhanced the content of TAGs (by 44~74%) under nitrogen and/or phosphate limitation and only had minor impact on the growth. However, overexpression of the allele PDAT1 resulted in little increase in the TAG accumulation with marked inhibition of growth (to 77~79%). Heterologous expression in yeast also supported the significant differences between PDAT1 and PDAT2 in enzyme activity. Reconstructed PDATs were obtained by replacing the fragment in PDAT1 with the corresponding segment from PDAT2 and were also expressed in yeast. The results showed that the 7 amino acid variants at the N-terminal and the 3 amino acid variants at C-terminal between PDAT1 and PDAT2 had significant effects on PDAT activity. Knockout of PDAT significantly decreased TAGs content and promoted the growth slightly. PDAT knockout strains showed increases in the levels of phosphatidylcholine and monogalactosyldiacylglycerol, indicating these two lipids were the substrates of PDAT in P. tricornutum. The subcellular localization of PDAT in the innermost chloroplast membrane and high homology with green alga PDATs also support the substrate specificity of P. tricornutum PDAT. This study reveals a critical and unequal role of PDATs encoded by alleles in mediating TAG synthesis, and further suggests that ASE might be an important response to nutrient stress in diatoms.

Keywords: phospholipid:diacylglycerol acyltransferase, allele-specific expression, nitrogen, triacylglycerols, diatom

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19. High-efficiency transformation by electroporation and feasibility of CRISPR-Cas9-mediated gene editing in a centric diatom Chaetoceros muelleri

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Cosmopolitan Chaetoceros is one of the largest genera of marine diatoms, which serves as a major carbon contributor in waters and is also frequently used in aquaculture. Among the most used microalgae in this genus, Chaetoceros muelleri has the capacity to accumulate eicosapentaenoic acid and fucoxanthin and is considered as one of the most suitable microalgae for commercial exploitation. Presented here are methods for introducing of foreign genes into C. muelleri cells by electroporation and editing of the C. muelleri genome using CRISPR-Cas9 technology. We established a simple, rapid and effective genetic transformation system of C. muelleri by electroporation using nourseothricin, zeocin and blasticidin-S as screening markers, and transformants could be observed in 4-7 days. Three corresponding resistance genes, nourseothricin acetyltransferase (nat), bleomycin-resistance (ble) and blasticidin-S deaminase (bsr) driven respectively by C. muelleri promoters of fucoxanthin chlorophyll a/c binding protein (Lhcf4p) and acetyl-CoA acetyltransferase (ACATp), were successfully and stably expressed. The transformation was verified by southern blot and the expression of enhanced green fluorescent protein (eGFP) and β-glucuronidase (GUS), two exogenous proteins. For the construction of sequence specific CRISPR-Cas9 vectors applicable for C. muelleri, the expression of sgRNA, resistance gene (ble or bsr) and Cas9 gene were driven by the endogenous promoters of U6, ACATp and Lhcf4p respectively. Nitrate reductase gene (NR) and urease gene (URE) were used to verify the feasibility of CRISPR-Cas9 technology in C. muelleri. The resulted plasmid was transformed into the C. muelleri by electroporation, and mutants were confirmed by sequencing and functional analyses. NR knockout lines were initially generated, and then one NR knockout line was used as receptor for the URE knockout. The double-knockout lines were obtained, which could not be grown on media with nitrate/urea as the sole nitrogen source or with the two nitrogen sources. Our results showed that electroporation and CRISPR-Cas9 technology can be successfully applied to C. muelleri, and high transformation efficiency (up to 2900/108 cells) and gene editing efficiency (up to 87.5%) were achieved.

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20. The heterologous expression of a green alga plastocyanin in Phaeodactylum tricornutum improves cell growth under iron-deficient conditions

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We have investigated if the heterologous expression in the diatom Phaeodactylum tricornutum of a functional green alga plastocyanin can improve photosynthetic activity and cell growth. Transformed P. tricornutum strains were obtained expressing a single-mutant (E85K) of the plastocyanin from the green alga Chlamydomonas reinhardtii, that had previously shown to be the more effective in reducing P. tricornutum photosystem I in vitro. The C. reinhardtii E85K plastocyanin gene was placed under the control of the flavodoxin promoter, to ensure its expression in low iron conditions, in which the levels of native cytochrome c6 drastically decrease and become limiting for the photosynthetic activity. Monitoring of immunolabelled cells confirmed the detection of the heterologous plastocyanin in the chloroplast. Moreover, the presence of the holoprotein (i.e., with the copper active cofactor) was directly detected by its absorption spectrum in cell extracts. Our results indicate that even the relatively low intracellular concentrations of holo-plastocyanin detected (≈4 μM) are enough to promote an increased growth (up to 60%) under iron-deficient conditions as compared with the WT strain, measured as higher cell densities, content in pigments and active photosystem I, global photosynthetic rates per cell and even cell volume. In addition, the presence of plastocyanin as an additional photosynthetic electron carrier seems to decrease the over-reduction of the plastoquinone pool. Consequently, it promotes an improvement in the maximum quantum yield of both photosystem II and I, together with a decrease in the acceptor side photoinhibition of photosystem II –also associated to a reduced oxidative stress–, a decrease in the peroxidation of membrane lipids in the chloroplast, and a lower degree of limitation on the donor side of photosystem I.

In summary, the heterologous plastocyanin expressed in P. tricornutum appears to act as a functional electron carrier, alternative to the native cytochrome c6 under iron limiting conditions, and therefore promotes an improvement in cell growth compared to WT cells.

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21. A FIB-SEM–based workflow to study diatoms light acclimation

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Phytoplankton encompasses a huge diversity of eukaryotic unicellular organisms, issued by multiple endosymbiosis events, and belonging to distinct taxa. Their heterogeneous origin has led to a large biodiversity of phytoplankton, which comprises cells with different morphologies and sizes. Although they are exposed to extremely variable environments, phytoplankton cells are on average fast growing, suggesting that their metabolism should be highly flexible. Until recently, we didn't know if these original metabolic strategies were linked to singular cell topological arrangements, due to the lack of high-resolution imaging studies.

We developed a FIB-SEM–based workflow to generate 3D reconstructions of different eukaryotic microalgae representing major oceanic phytoplankton lineages (Uwizeye et al.,2021), suitable for quantitative morphometric analysis (surfaces and volumes) of organelles and subcellular structures. This workflow represents a valid procedure (open-source software, artefact-free, work-saving time), which can improve any environmental adaptation study.

Here, we present additional investigation of diatoms light-management using the pennate diatom Phaeodactylum tricornutum, to better understand the cellular and subcellular remodeling in response to external light changes.

Uwizeye, C., al. (2021) Morphological bases of phytoplankton energy management and physiological responses unveiled by 3D subcellular imaging. Nat Commun 12, 1049 (2021).

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22. Transporters dependent regulation of photosynthesis in Phaeodactylum tricornutum

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Photosynthesis is a key metabolism that emerged around 3.5 billion years ago shaping our planet environment and leading to development of new O2 dependent life forms. Photosynthetic includes a “light phase”, where photons drive an electron transport chain (ETC) than reduces NADP along with the formation of a proton motive force (pmf) for ATP synthesis. ATP and NADPH fuel the “dark phase” of photosynthesis, i.e. CO2 fixation. However, the achieved ATP/NADPH ratio is possibly insufficient to fuel the Calvin cycle and photosynthetic organisms adopt different strategies to cover the shortfall in ATP or to exploit the excess NADPH. While CEF (cyclic electron flow) and WWC (water-water cycle) likely adjust the ATP/NADPH ratio, plastid-mitochondria exchanges might readjust ATP and NADPH in diatoms, through a still elusive mechanism.

Our project aims to identify possible transporters involved in this coupling and elucidate the mechanism of photosynthetic regulation in diatoms. We combined a transcriptomic data survey and CRISPR-Cas9 technology to isolate knockout (KO) mutants for putative targets in Phaeodactylum tricornutum. A first one belongs to the MCF (mitochondrial carrier family), known to transport organic acids, sugars but also ATP and inorganic phosphate. This transporter seems to targeted to the plastid and the KO of the gene results in algae photosynthetically altered. Interestingly, the phenotype can be partially reproduced when respiration is blocked via chemicals. Another target, identified at IBENS, is a MFS (major facilitator superfamily) that localizes at the plastid-mitochondria interface. Its KO leads to a photosynthetic phenotype related to Iron-depletion, suggesting a role in maintaining cellular homeostasis. Finally, an evolutionary conserved cation antiporter seems to provide a fine-tuning of the plastidial pmf, to optimize light-use efficiency in response to different environmental stimuli.

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23. The Cloning and Engineering of Diatom Mitochondrial Genomes in Yeast and Bacteria

Ryan Cochrane

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Algae are attractive organisms for biotechnology applications, such as the production of biofuels and other high-value compounds due to their genetic diversity and metabolic processes. As new species are domesticated, rapid nuclear and organelle genome engineering methods need to be developed and optimized. To that end, the mitochondrial genomes of Thalassiosira pseudonana and Phaeodactylum tricornutum were cloned into Saccharomyces cerevisiae and transformed into Escherichia coli for analysis. The cloning experiment was repeated on a previously cloned and sequenced P. tricornutum mitochondrial genome and found a mutation rate of 1 mutation per 10 kbp associated with the cloning method. Next, the host burden of harbouring a mitochondrial genome from either T. pseudonana or P. tricornutum in S. cerevisiae and E. coli was assessed. While no substantial differences were observed in either microbe harboring a P. tricornutum mitochondrial genome compared to vector backbone only controls, both Escherichia coli growth rate and culture end-point density (OD600) were reduced when harboring a T. pseudonana mitochondrial genome induced to high plasmid copy number. Finally, the plasmid stability of both T. pseudonana and P. tricornutum's mitochondrial genomes were assessed in E. coli over approximately 60 generations. In total, 30 E. coli clones harbouring either a T. pseudonana and P. tricornutum were evaluated after 0 and 60 generations using a 6-amplicon diagnostic multiplex PCR. After, 0 generations all 30 E. coli clones harboring either species' mitochondrial genome showed successful amplification of all 6 amplicons. However, after 60 generations all 30 E. coli clones harboring a P. tricornutum mitochondrial genome had a complete genome compared to only 25 of 30 containing a T. pseudonana mitochondrial genome. In conclusion, the mitochondrial genomes of two diatom species were successfully cloned in yeast and bacteria and imposed no substantial growth burden. In E. coli, some plasmid instability was observed after 60 generations, suggesting additional analyses of mitochondrial genome integrity will be required following prolong propagation for downstream application. This study completes the first steps in developing a reproducible set of methods for cloning, manipulating, and installing synthetic organelle genomes.

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24. The Contribution of Proteorhodopsin to the Cellular Energy Budget of the Antarctic Diatom Pseudo-nitzschia subcurvata

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The proteorhodopsin (PR) protein is a membrane bound proton-pumping variant of rhodopsin that captures light via the chromophore retinal. The proton-pumping ability of PR is thought to power ATP synthase providing energy to the cell. PR is a widely distributed protein being found in many types of microbial organisms found in the surface ocean including phototrophs such as diatoms. A previous study examined the expression, distribution, and possible function of PR in the marine diatom Pseudo-nitzschia granii and found that it is more highly expressed during iron limitation and is more prevalent in cold, iron-limited waters. These findings, combined with newer evidence that PR's reaction rate is insensitive to temperature, suggests that PR could partially substitute for iron-rich conventional photosynthetic pathways under iron scarcity, especially in colder regions. We studied a PR-containing, Antarctic strain of the diatom Pseudo-nitzschia subcurvata with the aim of quantifying the portion of cellular energy generated through PR compared to conventional photosynthesis under varying iron concentrations. For comparison, the photosynthetic systems of non-PR containing polar diatoms, Synedra spp. and Chaetoceros socialis, were also examined under iron-replete and iron-limiting conditions. Photosynthetic productivity was measured via oxygen production, carbon uptake, and electron transport rates in all three diatoms. PR was quantified via mass spectrometry based quantitative proteomics and used to estimate energy generation rates from PR. The photosynthetic physiology of the PR containing diatom responded similarly to non-PR containing diatoms to iron limitation, with reductions in photosynthetic rates and efficiencies but maintenance of largely linear electron flow under all conditions. The estimated fraction of energy generated by PR in Pseudo-nitzschia subcurvata increased substantially (~10x) under low iron conditions, but always remained a small portion of total energy generation.

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25. DiatOmicBase, a portal to mine diatom omics

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DiatOmicBase, a portal to mine diatom omics

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Since the sequencing of the genomes of Thalassiosira pseudonana and Phaeodactylum tricornutum, a large number of genome-enabled datasets have emerged for these species, such as RNA-Seq and proteomics experiments, epigenomes, and ecotype variants. Unfortunately, because many of these resources were generated by different laboratories they are largely unconnected. Concerning P. tricornutum, gene annotations are deposited on JGI and ensembl websites, RNA-Seq experiments are available at the NCBI, pathway and metabolism databases are on DiatomCyc, and comparative genomics can be analyzed on Plaza.

To connect these disparate resources, we are developing DiatOmicBase. We have begun by gathering public omics resources for P. tricornutum, and will follow with other diatom species. We chose to build a gene-centered resource where users can find all the informations about one gene on one page: the gene annotations and domains, the gene transcriptomic profile and co-expression network, a genome browser with ecotype variants, histone and methylation marks, transposable elements and non coding RNAs, as well as RNA-Seq mapped read densities. We also developed a semi-automatically updated transcriptomic module to explore publicly available RNA-Seq experiments.

In this talk, we will show how the portal works and present the future directions that are being considered. We also hope to discuss with the diatom community about future needs. By developing a user-friendly but also the most comprehensive resource possible, we aim to value the work already done by the diatom community and thus facilitate future molecular investigations and enhance their reproducibility.

DiatOmicBase is available at: https://www.diatomicbase.bio.ens.psl.eu/

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26. Growth-Stage Related Shifts in Diatom Endometabolome Composition Sets the Stage for Heterotrophic Bacterial Foraging

Malin Olofsson

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Phytoplankton-derived metabolites fuel heterotrophic bacterial production in the global ocean, yet methodological challenges have limited knowledge of substrate transfer between these two microbial groups. In an experimental bloom study, the diatom Thalassiosira pseudonana was co-cultured with three heterotrophic bacteria representing taxa commonly associated with phytoplankton: Ruegeria pomeroyi, Stenotrophomonas sp., and Polaribacter dokdonensis. Diatom endometabolites were characterized by nuclear magnetic resonance (NMR) spectroscopy, while released exometabolites were predicted using bacterial transcriptome shifts as a proxy. Twenty-two diatom endometabolites were identified with high confidence during diatom growth, with nine increasing in concentration to the late stage of the bloom, eight decreasing, and five showing no variation through the bloom progression. These changes were supported by shifts in diatom gene expression and, in turn, by changes in bacterial gene expression for substrate uptake and catabolism. Taxon-specific bacterial substrate profiles identified distinct resource niches linked to different phases of the bloom for the three representative strains. Better understanding of temporal patterns of phytoplankton metabolite production and transfer to bacteria is key to untangling this almost invisible but pivotal step in ocean carbon cycling.

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27. The Potential Role Of Extracellular Vesicles In Stress Response In Marine Diatoms

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Extracellular vesicles (EVs) are produced and secreted by organisms from all kingdoms including bacteria, archaea, metazoans and protists, and are considered a new mode of intercellular communication, mediating cell response to biotic and abiotic stressors. In the aquatic system, microorganisms are challenged with stress conditions such as fluctuations in temperatures, light regime and nutrient deprivation. Hence, communication between these cells is particularly interesting due to its potential impact on acclimation to changing environmental conditions and synchronize population level behaviors. Recent studies suggest EVs important role in the marine environment, providing evidences for the release of EVs by marine cyanobacteria and their ability to support the growth of heterotrophic bacterial cultures, or demonstrating the signaling role of EVs produced during interactions between the microalga Emiliania huxleyi and its virus EhV. Yet, little is known about EVs biogenesis, function and mode of action in aquatic systems, and their role in communication requires further investigation.

To address EVs function in diatoms, we explored the evolutionary conservation of one of the mechanisms of vesicle formation and release, the Endosomal Sorting Complexes Required for Transport (ESCRT), responsible for diverse membrane deformation and budding events in the cell. Using a comparative genomic approach, we found that the model diatom Phaeodactylum tricornutum retained the core subunits of the ESCRT. By integrating eight publicly available transcriptomes of P. tricornutum, we examined the expression patterns of the different ESCRT components and detected significant alterations in response to oxidative stress, phosphate limitation and light/dark cycles, which might imply changes in vesicle formation and release. We further isolated and identified EVs form media culture and validated their presence using negative-stain transmission electron microscopy (TEM). In both P. tricornutum and T. pseudonana EVs accumulate over time, with a significant elevation in concentration in the transition between exponential and stationary growth and a relatively stable level with only mild increase throughout the stationary phase.

By combining laboratory-based research on EVs response and function in different stress conditions and exploring EVs in natural blooms population in the ocean, we aim to shed light on the role of EVs in cell-to-cell communication, ranging from the single cell level to population dynamics in an ecological context.

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28. Diatoms diversification in light of their ecological niche space

Sophia Lambert

Sophia Lambert*, Richard Dorrell, Chris Bowler, Hélène Morlon.

IBENS

Diatoms play an important role as “biological pump” and are responsible for the majority of carbon fixation in the ocean. They are globally distributed and specifically abundant and diverse in the polar region characterized by cold water, low salinity and high solar radiation. Despite the large number of studies focusing on understanding the current diversity pattern of diatoms few have considered historical component to explain the present-day diatoms diversity pattern. A recent study suggested that diatom clades exhibited heterogeneous diversification dynamic to distinct environmental drivers. Specific ecological characteristics of diatoms could explain this heterogeneity in diversification dynamic across diatoms clades. While characteristics of the ecological niche of diatoms also likely plays a role in their distinct diversification dynamics, this has rarely been explored. Here we propose to characterize the ecological niche breadth and position of diatoms and to evaluate if and how these characteristics have influenced their diversification rates.

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29. Characterization and localization of a proteorhodopsin light-driven proton pump in Southern Ocean diatoms

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Rhodopsins are ubiquitous retinal-based light harvesting proteins first identified in bacteria over two decades ago. Prior to their discovery, all light-driven metabolism was believed to occur through chlorophyll-based pigments such as those used in photosynthesis. Proton-pumping rhodopsins, called proteorhodopsin (PR), have been found to significantly contribute to light absorption in the upper oceans where photosynthetic rates are low due to light and/or nutrient limitation. Recently, PR was discovered in diatoms, however the biological function of this diatom-PR is still unclear. Its prevalence in polar diatoms from low-iron regions, such as the Southern Ocean, suggests a role in coping under iron limited growth conditions. Analysis of the transcriptomes from different temperate and polar diatom transcriptomes show that not all diatoms possess the PR gene, but high homology of the PR gene between species supports the claim that they are derived from a single horizontal transfer event, with subsequent gene losses. Amino-acid residues indicate that functional sites for proton pumping and binding of the retinal chromophore are conserved across diatom species, and that all possess a green spectrally tuned PR. To determine the subcellular localization of PR in the ecologically relevant Southern Ocean diatom Pseudo-nitzschia subcurvata, we developed an antibody against the C-terminal residues of the P. subcurvata PR gene. The antibody specificity was confirmed against P. subcurvata total protein extracts by western blotting, resulting in a single, abundant band of the predicted size (~ 27 kDa). We also confirmed specific activity of the antibody raised against the PR peptide from P. subcurvata against other diatom protein extracts by western blotting. Through immunofluorescent subcellular localization, we observed no evidence of co-localization of PR to the nucleus, mitochondria or chloroplasts, yet in some cells, multiple distinctive ring-like structures were observed, suggesting PR may be bound to the vacuolar membrane. Quantification of cellular PR levels in Fe-replete versus Fe-limited P. subcurvata cells using the PR antibody is currently underway.

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30. Polycomb Repressive Complex 1 in unicellular species: insights from diatoms

Xue Zhao

Xue Zhao*, Leila Tirichine

CEA-Grenoble

Polycomb group (PcG) proteins were initially identified as a set of regulators that involved with body segment development in Drosophila melanogaster. Studies on PcG proteins in multicellular organism including mammal and plant prove the vital role of PcG family in embryogenesis and cell development. Recent studies show that PcG proteins in unicellular species such as Paramecium tetraurelia and Phaeodactylum tricornutum have some novel features such as gene silencing and morphology regulation. However the knowledge of PRC1 complex in unicellular species remain ambiguous. Our preliminary result shows that RING1 homolog is the catalytic enzyme depositing H2AK119Ub in Phaeodactylum tricornutum even without the RAWUL domain which is conserved in multicellular RING1. Meanwhile, the crosstalk of H2AK119Ub with other active marks has been observed in Phaeodactylum tricornutum, for instance loss of PtRING1 leads to a decrease of a permissive histone mark H3K4me3 which has not been reported in other organisms. Evidence shows that another PcG protein PtLHP1 has demethylase activity towards H3K36me3. Our results indicate some unrevealed features of PRC1 complex in unicellular species. Further studies on composition and functions of PRC1 in Phaeodactylum tricornutum will provide new insights to understanding PcG complexes regulation in an evolutionary context.

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31. Oxidative stress response during the resting cells formation in marine diatom Thalassiosira pseudonana

Jun-Rong Liang

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The formation of resting cells is an effective strategy for some diatoms to cope with unfavorable growth environments and maintain long-term survival. However, little is known about the various regulatory processes during the formation of resting cells in diatoms. In this present study, the oxidative stress responses and related regulatory mechanism involved in the formation of resting cells in marine diatom Thalassiosira pseudonana were investigated using the physiological, biochemical, and proteomics methods. During the formation of resting cells under the conditions of dark and low temperature (4 °C) for 90 days, the levels of reactive oxygen species (ROS) and malondialdehyde (MDA) were significantly increased and reached a peak in the early stage (day 30). Correspondingly, the antioxidants (SOD, POD, GSH, CAT), and the de-epoxidation state (DES) in xanthophyll cycle reached the highest value in the early stage (all on day 30 except for CAT on day 15). The results show that ROS scavenging enzyme system and xanthophyll cycle involve in antioxidant stress responses in the early stage of resting cells formation. Proteomic analysis showed that most of antioxidant enzymes and proteins associated with xanthophyll cycle were down-regulated in mature resting cells, while proteins associated with phagocytosis were up-regulated. It suggests that phagocytosis may play an important role in antioxidant stress in the later stage of resting cell formation.

Proteomics data also showed that the expressions of the proteins involved in the chloroplast photosynthetic electron transport chain were restrained, which could lead to accumulate some ROS in mature resting cells. This result is consistent with biochemical observation that there were still some levels of ROS accumulation in mature resting cells compared with that of vegetative cells. Proteomics data also show that chrysolaminaran has not been accumulated in mature resting cells. However, the up-regulation of the proteins related to neutral lipid TAG biosynthesis suggests that neutral lipid could be used as primary energy for mature resting cells.

All findings indicate that the resting cells of T. pseudonana have an effective survival strategy and regulatory mechanism against stress, so as to maintain the long-term survival under an adverse growth environment.

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Key words: marine diatom, Thalassiosira pseudonana, resting cells, oxidative stress response, survival strategy

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32. Disentangling the roles of diatoxanthin and Lhcx1 in the nonphotochemical quenching of Phaeodactylum tricornutum

Dany Croteau

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Nonphotochemical quenching (NPQ) allows photosynthetic organisms to release excitonic pressure as harmless heat when photosystem II is exposed to potentially harmful supersaturating light. In the diatom Phaeodactylum tricornutum, NPQ deployment under excess light requires two molecular actors. First, NPQ is linearly correlated to the conversion of diadinoxanthin to diatoxanthin (DT) via the xanthophyll cycle in a seemingly Stern-Volmer-type quenching process. Second, NPQ maximal amplitude is also influenced by the amount of stress-related light harvesting proteins (Lhcx1). To deconvolute the respective roles of each molecular component, and to propose a quantitative and integrative model of NPQ, we produced a series of P. tricornutum mutants with ranging constitutive expression of the Lhcx1 isoform. We grew the wildtype and thirteen mutants under two light conditions with contrasting Lhcx1 and xanthophyll pools. We investigated NPQ induction and relaxation kinetics by measuring variable fluorescence over different light exposure protocols while sampling for pigments extraction to monitor xanthophyll cycle activity and for western blot to quantify the amount of Lhcx1. Our preliminary results demonstrate that, in all strains and conditions, NPQ remains proportional to DT, the slope being set by Lhcx1 accumulation. These results are compatible with 1) DT being involved in the formation of a Stern-Volmer homogeneous quencher and, 2) Lhcx1 binding DT and thereby determining the repartition between DT in photosystem II, participating to NPQ, and free DT in the thylakoid membrane.

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33. Differential gene expression associated with sexual maturation of pennate diatoms

Darja Belisova

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Cell size sensing is a fundamental but still incompletely understood mechanism in cell biology. In diatoms, a close monitoring of cell size is not only vital during the progression of the cell cycle, but it uniquely also plays a key role during life cycle regulation. It is well-established that only cells smaller than a species-specific Sexual Size Threshold (SST) can engage in sexual reproduction. How cells sense their SST, however, remains an enigma. Here, we present an RNA-seq experiment of two pennate diatoms, Cylindrotheca closterium and Seminavis robusta, to explore the nature of gene expression changes associated with the transgression of the SST. Both species have a well-documented life cycle and employ a different pheromone signaling system during mating 1–3. In our experiment, RNA of vegetative non-synchronized cells above and below the SST was sequenced. For both species, three genotypes per each mating type were used thus taking into consideration not only size, but also mating type (MT+/-) and possible size-MT interaction effects. Differential expression analysis showed that only 0.64 % of genes for C. closterium and 1.09 % of genes for S. robusta were differentially expressed for size and/or mating type. Among these, most genes were associated with small size of a specific mating type. Functional annotation illustrates enrichment in gene families containing DNA binding, receptor-like proteins and encoding extracellular molecules, as well as diatom-specific genes of unknown function. Based on our preliminary results, we conclude that both species show distinct changes in gene expression when crossing the sexual size threshold, even in the absence of mating partners, and that C. closterium and S. robusta activate different molecular pathways during sexual maturation.

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34. The physiological role and structural arrangement of the mitochondria of Fistulifera solaris in the oil degradation process

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Microalgal biofuel production has attracted much attention due to the increasing demands for alternative liquid fuel to reduce the carbon dioxide emission. We have investigated an oleaginous diatom, Fistulifera solaris, which shows high lipid accumulation (~65wt%), and high growth rate in large-scale outdoor cultivation systems. However, further improvement of lipid productivity is required for feasible applications. Consequently, in this study, we focused on the attenuation of oil degradation. Our understanding in the lipid degradation in diatoms is still in its infancy, although lipid synthesis metabolism has long been analyzed with multi-omics studies include genomics, transcriptomics, proteomics, and lipidomics. Transcriptomic analyses of F. solaris during the oil degradation process revealed that the β-oxidation pathway in the mitochondria played a major role in fatty acid catabolism and ATP production rather than that in peroxisomes. Next, we assessed whether this expression behavior is in line with the dynamics of organelles in the cells of F. solaris. The mitochondria and oil bodies were stained by TMRM and BODIPY 505/515, respectively, and the stained cells were observed with confocal laser scanning fluorescence microscopy. As a result, tubular mitochondria were observed in the cells. When the oil degradation was induced by nutrient depletion, the mitochondria tended to be localized around the two oil bodies at the polar regions of the cells. The image analysis was performed to quantitatively evaluate the contact between the mitochondria and oil bodies in the cells. The contact of both organelles increased. This result suggests that mitochondria likely assembled the contacting face with the oil bodies, and directly contributed to oil degradation. This observation is consistent with the aforementioned gene expression behavior, and unique to F. solaris. Although the generation of the contacting face between mitochondria and oil bodies were previously reported in animal cells, this is the first study to elucidate the direct connection between these organelles in diatoms during the lipid degradation. Our study provides new insight into lipid catabolism in diatoms, and also demonstrated the usefulness of F. solaris as a model for the analyses of organelle dynamics due to its simple structure composed of one chloroplast and two large oil bodies. As a future perspective, the molecular machineries involved in the assembly of the contacting face will be identified. This future study will identify the knockdown or knockout targets to decrease the lipid degradation in diatoms, leading to enhancement of biofuel production.

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35. Eukrhythmic: leveraging the metatranscriptomic landscape to reproducibly detect and describe marine protistan communities

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Metatranscriptome analysis, the processing and interpretation of transcribed sequences from a biological community, has emerged as a promising approach to answer questions about natural microbial communities without prior knowledge or bias. Metatranscriptomes are an accessible means to characterize a more complete suite of expressed genes. Despite the potential of this technology, processing methods are relatively new, and often focused on RNAseq data from single-organism transcriptomes or prokaryotic communities, not mixed eukaryotic systems. As more metatranscriptomes are collected in oceanographic surveys, it is crucial to generate metatranscriptome-specific tools to improve study reproducibility and comparability. Here, we introduce eukrhythmic, a Snakemake-based metatranscriptome processing pipeline designed to address common problems in analyzing marine or other environmental samples. Using multiple assembly methods and tandem sample processing, eukrhythmic minimizes assembly bias, and enables rapid collation of samples from multiple sites. The pipeline enables functional and taxonomic annotation with special consideration of microbial eukaryotes, and provides a curated assembly that can be integrated with metaproteomic data. We have applied this pipeline to multiple marine datasets, including a published dataset from Narragansett Bay, RI, and a diel metatranscriptomic time series from the Western Antarctic Peninsula. In the Narragansett Bay dataset, no less than 40% of mapped reads were from diatom species, and in the Western Antarctic Peninsula time series, the relatively diatom-poor diel samples were compared to nearby samples from the region with up to 60% diatom-derived reads. We discuss applications of our approach, and share insights gained from processing these rich marine metatranscriptomic datasets with eukrhythmic. In particular, we emphasize how metatranscriptomics can provide deep insight into the diversity and ecology of diatoms in two contrasting ecosystems.

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36. An Analytical Approach for Understanding Light Modulation by Diatom Frustules

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The diatom's siliceous frustules are well-known for their unique ultrastructure with ornamentation on the nanoscale, including their pores, which give these frustules exceptional properties, e.g., mechanical and hydrokinetic ones. During the last two decades, also some optical features have been observed, such as the light focusing ability of the valves of Coscinodiscus spp. or waveguiding associated with their girdle bands [1-2]. Several studies have suggested that such optical features could be relevant to the diatom's photobiology [2]. Moreover, their potential as photonic building blocks has been pointed out for several applications, e.g., optoelectronic devices [1-3]. Despite the sheer number of available diatom species, the frustule's optical properties of only a few species have been studied. We suggest, as an alternative to investigating the frustule's light interaction for each species separately - a time-consuming process - to focus on understanding the roles of the different optical components that comprise the frustule, and how they interfere. With this, the frustule's optical properties could be predicted, based on its structural components, to easier identify target species for specific photonic applications. As an example for this approach, we will present the analysis of how light of a range of wavelengths is modulated by the small pennate frustule of Gomphonema parvulum based on numerical analysis performed using COMSOL Multiphysics® 5.5. The structural properties and their statistical variations could be analyzed in 3D through a number of SEMs and FIB-SEMs. In conclusion, the observed light modulation induced by this small frustule could be explained when disassembling it into its different optical components, including; lens-, grid- and fiber-like structures, as well as thin slab elements, and combining the relevant interference patterns.

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37. Digital holographic microscopy of morphological changes of diatom cellular contents induced by papain enzyme

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In this work, we demonstrated holotomographic (HT) observation of morphology changes of living diatom cells induced by enzyme reactions. Living Cylindrotheca sp. cells in liquid culture medium were continuously observed a commercially available digital holographic microscopy in the presence and absence of papain enzyme. Papain is one of the major cysteine proteases which are widely used for various industrial applications such as food processing and pharmaceutical developments. For diatom studies, Horst et al. (2012) extracted triacylglycerols from diatom cells with crude papain and bromelain (J. Biotechnol., 162, 40-49).

A commercially available three-dimensional holographic microscopy (HT-2, Tomocube Inc.) was employed for the observation. Although the observation area was fixed in 80 x 80 □m, size of Cylindrotheca sp. cells was suitable for the observation. In the observation in the presence of papain, two □L of an activated papain solution (10 mg/mL, 20 mM phosphate buffer pH 7.0) was added to 18 □L of cell suspension in a specific dish for observation (tomodish, Tomocube Inc). For control experiments, two □L of the phosphate buffer solution was added to cell suspension. For 30 min observation at room temperature, there was no significant changes in diatom cell appearance. On the other hand, in the presence of papain enzyme, outflow of cellular contents from the frustule was clearly observed as a function of time. The three-dimensional cellular structure was reconstructed based of reflective index (RI) information of each pixel of the HT images. Thus, morphological change of diatom cells induced by enzyme treatment was well visualized as without any pretreatments. We hope our research provided helpful information for nanoscopic studies of living diatom cells.

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38. Producing the SARS-CoV-2 Spike RBD antigen in the diatom Phaeodactylum tricornutum

Sam Slattery

Samuel S. Slattery, Daniel J. Giguere, Emily Stuckless, Arina Shrestha, Lee-Ann Briere, Alexa Galbraith, Stephen Reaume, Mallory Frederick, Jeremy T. Lant, Ilka Heinemann, Patrick O'Donoghue, Liann Dsouza, Steve Martin, Peter Howard, Garth Styba, Martin Flatley, Bogumil J. Karas, Gregory B. Gloor, David R. Edgell

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The worldwide COVID-19 pandemic caused by the SARS-CoV-2 betacoronavirus has highlighted the need for reliable and scalable sources of viral antigen for uses in diagnostics, therapeutics, and basic biomedical research. Here, we adapt plasmid-based systems in the eukaryotic microalgae Phaeodactylum tricornutum to develop an orthogonal overexpression system for SARS-CoV-2 proteins. The receptor-binding domain (RBD) of the SARS-CoV-2 spike protein purified from whole cell extracts of P. tricornutum (algae-RBD) was found to be N-glycosylated by treatment with PNGase F and by mass spectrometry analyses. The algae-RBD was cross-reactive with anti-RBD polyclonal antibodies and inhibited binding of recombinant RBD purified from mammalian cell lines to the human ACE2 receptor. We also show that the algae-RBD can be conjugated to a lateral flow assay device to detect SARS-CoV-2 specific IgG antibodies from donor serum. P. tricornutum represents a cheap and scalable orthogonal system with minimal biocontainment requirements to produce SARS-CoV-2 or other coronavirus antigens for pandemic diagnostics.

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39. Low-Affinity Nitrate Transporters in diatoms, diNPFs: identification, evolution, structure and function

Anna Santin

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In the ocean, diatoms are continuously subjected to fluctuating nutrient concentrations, including nitrogen (N). Nitrate (NO_3^-) ion is among the most important N source for phytoplankton and its temporal and spatial variability affects their growth and distribution. To efficiently cope with NO_3^- availability, as other organisms, diatoms rely on a range of transmembrane transporters for NO_3^- uptake.

In this study, we provide the first characterization of the Nitrate/Peptide Transporter Family (NPFs) in diatoms. NPFs are well characterized in many organisms where they recognize a remarkably broad range of diverse substrates, ranging from di- and tripeptides in bacteria, fungi and mammals to a wide variety of molecules in higher plants, such as NO_3^- or phytohormones. To date, scarce information is available for diatom NPFs, diNPFs.

Using a multilevel approach which integrated -omics, phylogenetic, structural and expression analyses, we unveil an unexpected complexity of these transporters in diatoms, highlighting their relationship with the corresponding plant and bacterial transporters.

Sequence alignment and phylogenetic analysis revealed that diNPFs cluster in two clades, one of which lost in plants. With structural data available for both plant and bacterial NPFs, we obtained structural models for complete diNPF sequences, revealing that Clade I diNPFs have structural features that are found in bacterial NPF/POTs, while Clade II diNPFs are structurally closer to plant NPFs. This subdivision is supported by a different sub-cellular predicted localization which most likely reflects affinity to different substrates and different roles in the cell metabolism. Transcription analyses of diNPFs genes under different laboratory and environmental growth conditions suggest that diNPF diversification led to genetic adaptations that might contribute to diatoms plasticity, revealing once again their chimeric nature and their complex physiology.

In order to deepen if and how diNPFs divergence resulted in functional diversification, we generated loss of function Phaeodactylum tricornutum mutants using a CRISPR/Cas9 proteolistic approach. Preliminary characterization of the knock-out mutants is providing working hypotheses on their functional role. Biochemical characterization in a heterologous system, such as Xenopus laevis oocytes, is also planned with the aim to analyze the transport capability and substrate specificity. The obtained results will help understanding the genetic foundation regulating the extra- and intracellular transport system in diatoms and their adaptation strategies to environmental fluctuation.

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40. Understanding the Cellular Role of Aureochromes, a New Type of Blue Light Photoreceptors, in Diatoms

Robert Röllig

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Light is not only the driver of photosynthesis but has also influence on the acclimation to rapid changes in the natural environment caused by turbulences, depth and scattering material in the water column.

Especially in diatoms the acclimation capacity is highly relevant for their ecological success.

Photoreceptors required for sensing of different light quantities and qualities play a pivotal role in inducing regulatory mechanisms, including circadian clock and phototactic orientation.

One class of newly described blue light (BL)-absorbing photoreceptors are Aureochromes of which four were identified in the marine diatom Phaeodactylum tricornutum: PtAureo1a/b/c and PtAureo2.

Aureochromes are BL-induced transcription factors consisting of a LOV sensor domain binding Flavin and absorbing BL as well as a bZIP effector domain that binds DNA.

It has been shown previously that PtAureo1a and 1b are crucial for the light acclimation process under BL.

Comparative transcriptomic analysis of the P. tricornutum wild type (WT) and knock-out mutants deficient of PtAureo 1a (KO PtAureo1a) revealed up- or downregulation of 2/3 of all genes in the WT upon shift from red light (RL) to BL already after 10 minutes, indicating massive re-arrangement of cellular functions while most of these genes were not differently expressed in KO PtAureo1a showing that inactivation of PtAureo1a strongly reduces transcriptomic response and pointing out the importance of PtAureo1a for cellular light regulation in P. tricornutum.

This led to the question of how and why the inactivation of a single gene changed thousands of other genes: it is hypothesized that PtAureo 1a might be a “master switch” affecting (either alone or in cooperation with other Aureochromes) other transcription factors.

Additionally, it was demonstrated that during light quality shifts from RL to BL the metabolic profile of WT cells quickly reversed, with an inhibited carbohydrate biosynthesis and increased amino acid biosynthesis.

In this study we will show first data on comparative transcriptomic and metabolic analysis of KO PtAureo 1b, c and 2.

The correlation between transcriptome and metabolome profiling seeks to unravel the so far largely unknown functions of Aureochromes via precise physiological and molecular characterization and elucidate the genetic and metabolic network behind the BL-induced acclimation process in P. tricornutum.

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42. Ecologically relevant metabolites produced by a genome-scale metabolic model of Thalassiosira pseudonana

Helena Van tol

For over 60 years, Thalassiosira pseudonana has been a model organism for diatom molecular biology and physiology. Genome-scale metabolic models can provide mechanistic explanations for experimental observations by connecting genes to metabolic phenotypes. We used the genome annotation and published literature to re-construct a genome-scale metabolic model of T. pseudonana CCMP 1335. The model (iTps1432) represents 1,432 genes, 6,079 reactions, 2,792 metabolites, and six subcellular compartments. Flux Balance Analysis (FBA) was used to simulate steady-state growth under a range of light conditions (5, 60, 200 µmol photons m-2 s-1). Dynamic FBA was used to simulate growth in batch culture for the transition between nitrate-limitation to nitrogen starvation. iTps1432 was constrained with photosynthetic production measurements and nutrient uptake rates from a published chemostat and batch culture experiment. The objective function was adjusted with biomass composition measurements taken from the different conditions and over time. Our simulations indicate that cyclic electron flow could be more important for redox balance than energetic coupling between plastids and mitochondria under low light conditions. We also found that nitrate assimilation helps dissipate plastid reductants in nutrient-replete conditions, while sulfate assimilation helps balance redox when nitrate is limited, leading to the excretion of the osmolyte dimethylsulfoniopropionate (DMSP). We found that the character and quantity of metabolites excreted by iTps1432 depend on the conditions of redox balance, relationship between photosynthesis and respiration, and biomass re-modeling.

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43. Exploring expression patterns of diatom silicon transporters under Si stress and competition

Evangelia Charalampous

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Linnaeus University

Diatoms hold a special role in the ocean, linking the carbon to the silica cycle. Through the biosilicification process, they perform to build their most characteristic features, the silica cell walls (frustules). To form the frustules, diatoms take up silicon from the water, in the form of silicic acid using specialized proteins, the silicon transporters (SITs). The origin of the diatom SITs is linked to their evolution in which diversification was possibly driven by changes in dissolved Si concentrations during the history of the oceans resulting in SITs with different functional characteristics. On this basis, we used the two diatoms, Chaetoceros affinis (early branching, centric, multi-polar) and Cylindrotheca fussiformis (later branching, pennate), as model systems to test the performance of C. affinis SIT1 in different Si concentrations when alone (monoculture) and in competition with C. fussiformis. Species were selected based of their different SIT profiles and also different demands in Si concentrations for growth. The cells were grown in fed-batch cultures for four days. On the fifth day, a mixed culture was created, by mixing equal biovolume parts of the monocultures. The mixed culture was left for 24 hours so that the cells could adjust to the competitive for available nutrients environment. Cells from the cultures were harvested, re-suspended in artificial sea-water medium and used as inoculum to the four experimental treatments. RNA samples were taken 3 times with intervals of 1h after inoculation in order to identify the time-frame that C. affinis cells show the maximal SIT1 expression under Si stress and whether this response-time to Si starvation changed when in competition with other species. Our results show upregulation of the C. affinis SIT1 gene as a response to Si stress in monoculture (max after 1h) and when in a mixed community, although the upregulation was delayed in time (max after 2h). Competition with C. fussiformis resulted in downregulated SIT1 gene expression. We conclude that Si stress and competition with other species affect the regulation of the SIT1 in C. affinis, however the effect doesn't have the same direction resulting in a moderate combined effect. Identifying changes in the function of the diatoms SITs when exposed to different environmental factors will help us understand not only how each species utilises Si but also how the presence of other species affects these responses, providing insights to the complexity observed in the natural environment.

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44. Evolution and adaptation of Fragilariopsis from the Arctic and Antarctic: Sequencing new strains and investigating adaptation to rising temperatures.

Amanda Hopes

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University of East Anglia

Warming temperatures and melting of sea-ice is already a major issue in polar environments, especially in the Arctic. With diatoms acting as a key contributor to primary production in polar ecosystems, it is important to understand the effect of increasing pressures from climate change on diatoms and their ability to adapt.

We have carried out de-novo genome sequencing using a combined Illumina/Nanopore approach for 10 different strains of the key polar diatom Fragilariopsis, with strains collected from both Arctic and Antarctic sites. In addition, a long term evolution experiment has been conducted to see how each strain responds to a gradual increase in temperature from 0 – 12°C both physiologically and at a molecular level, including genomic, transcriptomic and epigenetic.

Here we present the first results from our comparative genome analysis of the 10 different strains and the long term evolution experiment. With the exception of 1102, which has a triploid profile, all sequenced strains appear to be diploid. Whilst 18s sequences suggest strains are Fragilariopsis cylindrus, there are pronounced genomic differences between strains, especially between those isolated from the Arctic and Antarctic, including differences in shared content and heterozygosity. Furthermore, strains responded differently to the increase in temperature, with some showing optimised growth at lower temperatures and others at mid-range temperatures. All strains struggled at higher temperatures, with only one strain maintaining growth past 10°C. Details of results from comparative genome analysis and the evolution experiment will be shown.

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45. The evolution of morphology in diatoms: assessment of grazing pressure in long-term speciation.

Alessandra Petrucciani

Alessandra Petrucciani,1*, Alessandra Norici,2

Università Politecnica delle Marche

The evolution of morphology in diatoms: assessment of grazing pressure in long-term speciation.

Alessandra Petrucciani, Alessandra Norici

Diatoms are important primary producers in oceans and affect global food webs. Species radiation under the synergic action of multiple selective pressures such as grazing, has deeply shaped their silica cell wall, which is less silicified in more recently evolved pennate diatoms.

Thus, the role of frustule in the prey-predator interaction was investigated in grazing experiments where diatoms differing in size and shape were exposed to copepods in monospecific and mixed cultures. Imaging Flow Cytometer analysis was newly applied to study phytoplankton functional and morphological features. This technique combines the power of a cytometer to discriminate cells basing on their size, complexity, and degree of fluorescence, to a microscope, collecting pictures of all the objects present in the sample and providing multiple high-quality single cell parameters. The four selected species (Thalassiosira pseudonana, Conticribra weissflogii, Cylindrotheca closterium, Phaeodactylum tricornutum) showed a species-specific response to grazing pressure in monospecific cultures. In particular, the smallest species T. pseudonana was the most affected by grazers in terms of growth, Si fluctuation and morphological modification. The biggest C. weissflogii elicited an acclimation response at day 1 but it did not last till the end of the stress, recovering the initial status quo and reaching the same cell density observed in the absence of copepods. A different response was observed in the pennate diatoms since growth parameters of copepod exposed cultures, although eaten by grazers, were unaltered as compared to the unexposed ones. Mixed cultures were established to mimic and investigate natural conditions where prey-predator interaction occurs together with competition for growth among algal species. For the centric diatoms, size played a key role in species selection by grazers: conversely from what observed in monospecific cultures, the smaller T. pseudonana was less eaten than the bigger C. weissflogii suggesting that competition for resources deeply affected prey-predator relation. When the pennate diatoms were added to the mixed cultures, centric diatoms were preferentially eaten. Possible explanations are here suggested confirming that grazing pressure had a role in diatom speciation leading to the development of new cell geometries and, consequently, of new defence strategies not based on strongly silicified frustules.

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46. Evolutionary analysis of DNA methyltransferases in unicellular eukaryotes: Insights from the model diatom Phaeodactylum tricornutum

Antoine Hoguin

Antoine Hoguin,1,2 Ouardia Ait Mohamed, 3, Chris Bowler, 4, Auguste Genovesio, 5, Fabio Rocha Jimenez Vieira,6, Leila Tirichine*

Cytosine DNA methylation is an important epigenetic mark in eukaryotes that is involved in the transcriptional control of mainly transposable elements in mammals, plants and fungi. Eukaryotes encode a diverse set of DNA methyltransferases that were iteratively acquired and lost during evolution. The Stramenopiles-Alveolate-Rhizaria (SAR) lineages are a major group of ecologically important marine microeukaryotes that include the main phytoplankton classes such as diatoms and dinoflagellates. However, little is known about the diversity of DNA methyltransferases and their role in the deposition and maintenance of DNA methylation in microalgae. We performed a phylogenetic analysis of DNA methyltransferase families found in marine microeukaryotes and show that they encode divergent DNMT3, DNMT4, DNMT5 and DNMT6 enzymes family revisiting previously established phylogenies. Furthermore, we reveal a novel group of DNMTs with three classes of enzymes within the DNMT5 family that diversified in diatoms and dinoflagelattes. Using a CRISPR/Cas9 strategy we demonstrate that the loss of the DNMT5 gene correlates with a global genomic depletion of DNA methylation in Phaeodactylum tricornutum. In addition, DNMT5:KOs also showed overexpression of transposable elements suggesting a direct repressive role of DNA methylation in diatoms. The study provides a pioneering view of the structure and function of a DNMT family in the SAR supergroup.

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47. A method for intact nuclei isolation from diatoms to facilitate the application of high-throughput technologies

Antonella Ruggiero

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In the last decade several centric and pennate diatom genomes have been released offering a fresh opportunity to discover diatom molecular secrets. This huge gain of knowledge has been complemented by the development of new molecular tools in various species. However, the peculiar composition of diatom frustule sets a limit for the direct application of a range of molecular techniques which are extensively applied to a number of model systems but innovative for phytoplankton.

For example, single cell RNA-seq, that in the last years has been broadly applied to uncover cell population heterogeneity, requires the separation and acquisition of individual cells, a challenging task in diatoms due to the presence of the rigid silica cell wall. A way to apply this kind of approach to diatoms can be the use of isolated nuclei as input.

We have recently developed a method to extract whole nuclei from different diatom species, two pennates, Phaeodactylum tricornutum and Pseudo-nitzschia multistriata,

and one centric, Chaetoceros diadema. This adaptable protocol combines treatment with acidified ammonium fluoride solution with low intensity sonication pulses. Moreover, by FAC-sorting or alternatively by sucrose/percoll gradient, it is possible to obtain an enriched nuclei sample.

Within this study we developed an easy-to-apply protocol potentially compatible with standard work flow for global and single cell profiling of genomes, transcriptomes and epigenomes. Epigenetic regulation is rising up as one of the key mechanisms

for diatoms plasticity in responding to environmental variability.

This protocol also aims to speed up applications in this new field for the diatom community. Currently we are using isolated nuclei, as starting material to optimize the ATAC-seq assay (Assay for Transposase Accesible Chromatin), which detects genome-wide chromatin occupancy, a desirable new method to be added to the repertoire of tools for the study of diatom gene regulation.

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48. Chloroplast Genome Engineering As A Tool To Improve The Productivity Of Monoterpenes In The Diatom Phaeodactylum Tricornutum

Nicola Trevisan

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Microalgae are emerging as promising candidates for the sustainable production of compounds that are difficult to source from nature and have complex chemical synthesis, such as monoterpenoids. Monoterpenoids are a class of natural molecules that contribute to the organoleptic properties of plants and have recently found industrial applications due to their broad range of biological activities, for example as flavourings, fragrances, pharmaceuticals, and high density jet fuels. Recent advances in synthetic biology have shown the potential to improve the yields of heterologously produced monoterpenoids in microalgae, in particular in the diatom Phaeodactylum tricornutum. The main characteristics that give P. tricornutum an edge compared to the currently used microbial platforms are its phototrophic growth capabilities and highly versatile metabolism, with high bioavailability of monoterpenoid precursors, such as isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). These molecules are in fact supplied through both the mevalonate (MVA) and the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathways, conversely to other microbial hosts which usually only have one. Ultimately, the possibility for compartmentalization of the synthesis of monoterpenoids in the chloroplast gives an ulterior advantage to P. tricornutum for containment of the compounds of interest without interfering with the rest of the cellular metabolism. The chloroplast represents an outstanding resource to be harnessed as a biofactory, thanks to the high gene-copy number, high pool of substrates, prokaryotic gene expression machinery, and efficient and predominant homologous recombination mechanisms. In this work, previously developed methods for the genetic tractability of the chloroplast of P. tricornutum will be optimized and used to express a variety of monoterpene synthases. Exploiting the chloroplast as a production factory is expected to greatly improve the monoterpenoid productivity in P. tricornutum.

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49. The roles of putative pyridoxal phosphate dependent transferase (PLP-DT) in nitrogen utilization and lipid synthesis in marine diatom Phaeodactylum tricornutum

Xin Lin

Zichao Deng, Ruiping Huang, Xin Lin

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Pyridoxal phosphate-dependent transferases (PLP-DTs) are involved in essential cellular processes such as amino acid and lipid metabolism by catalyzing transamination, decarboxylation and racemization. Previous studies implied that the putative PLP-DE (Phatr3_J55010) of Phaeodactylum tricornutum may play important roles in nitrate assimilation and in response to high CO2. However, the functions of PLP-DTs are poorly understood in phytoplankton. Here, we investigated the ecological and physiological roles of putative PLP-DT gene (Phatr3_J55010) in P. tricornutum by bioinformatic analysis and gene-editing technique. Putative PLP-DT homologs in phytoplankton were analyzed and Phatr3_J55010 knock-out mutant (Crispr-4-7) were obtained by CRISPR/Cas9 technique. The homologous genes of Phatr3_J55010 were found distributed in dinoflagellates, diatoms and haptophytes but not in prokaryotes. The growth rate and maximum biomass of Crispr-4-7 mutant were significantly decreased. Simultaneously, significant increase in soluble protein content and significant decrease in cellular neutral lipids was observed throughout the light period in Crispr-4-7 mutant. Our transcriptome data showed that genes correlated with nitrate reduction, nitrite reduction, carboxyl phosphate synthesis, urea degradation and malate dehydrogenation were significantly down-regulated in Crispr-4-7 mutant. In addition, genes involved in ribosome process, DNA replication, glutamate/glutamine synthesis, lipid synthesis and glycolysis, were also down-regulated in Crispr-4-7. Conversely, branched-chain amino acid degradation and arginine synthesis were promoted in Crispr-4-7. Furthermore, we co-cultivated wildtype and Crispr-4-7 mutant with heterotrophic bacteria Alteromonas sp. to investigate the potential role of Phatr3_J55010 in nitrogen exchange between diatoms and bacteria. Our results showed that the growth promotion effects of Alteromonas sp. on wildtype was significantly reduced on Crispr-4-7 mutant. In a word, PLP-DE has important implications in nitrogen utilization, cellular carbon/nitrogen balance and controlling carbon flow in lipid biosynthesis in P. tricornutum, which may contribute to the great success of diatoms in modern ocean.

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50. Using experimental evolution to reveal insights into how the (epi)genome and transcriptome changes due to a long-term temperature response of Thalassiosira pseudonana

Andrew Toseland

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The ocean environment is changing at an unprecedented rate with temperature having a demonstrable impact on diatom diversity and distribution. However, little is currently known about how diatoms will respond evolutionarily to ocean warming and how their responses might affect food webs and biogeochemical cycles.

In order to investigate the long-term response to temperature changes of the cosmopolitan diatom Thalassiosira pseudonana (Tp), we implemented an experimental evolution approach, selecting Tp, for approximately 300 generations at its upper (32°C), lower (9°C) and control (22°C) temperature limits under nutrient replete conditions.

In addition to measuring changes in cellular composition (carbon, nitrogen, phosphate, silicon and chlorophyll a) and physiological parameters, such as photosynthetic efficiency and size, we undertook genome resequencing, RNA-seq and bisulfite sequencing of Tp at multiple time points to measure genetic divergence, gene expression and methylation respectively. In comparison to our control, the greatest genetic divergence occurred in the 32°C samples. In addition to a higher spontaneous mutation rate, we observed large loss of heterozygosity (LOH) regions in several chromosomes. Differential methylation (both hyper and hypo) was also significantly higher under 32°C compared to our control and 9°C samples, roughly doubling at each sampled time point. We identified several clusters of coregulated, differentially expressed genes showing temperature-dependent expression.

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51. Characterisation of membrane glycerolipid synthases in the model diatom Phaeodactylum tricornutum using CRISPR-Cas9 technology

Nolwenn Guéguen

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INRAE

Glycerolipids are major components of membranes. In a eukaryotic cell, each subcellular compartment is characterized by its own glycerolipid composition. Membrane glycerolipids are also precursors for the production of triacylglycerol, a storage form for carbon in the cytosol. In photosynthetic organisms, some specific lipid classes are thus essential in the chloroplasts for the expansion of photosynthetic membranes (thylakoids) and the stability of the photosynthetic complexes. Whereas the membrane lipid metabolic pathways are well established in classical eukaryotic models, such as yeast, mammals or Arabidopsis, knowledge is scarce in unconventional models such as diatoms. This knowledge is missing for applied strategies, aiming at exploiting oleaginous diatoms for biotechnological purposes. Indeed the cell of diatoms has emerged from the combination of two eukaryotic cells, one non-photosynthetic, the other photosynthetic, via a process known as a 'secondary endosymbiosis'. Diatom cell thus contains a complex plastid, surrounded by four membranes, instead of two, which is physically connected with the nucleus. In the genome of the model diatom Phaeodactylum tricornutum, we identified three isoforms of enzymes predicted to synthesize classical chloroplast lipids. We addressed the role of each isoform on the biogenesis of subcellular membranes.

In order to characterise the exact function of each isoform in P.tricornutum, we used the genome editing CRISPR-Cas9 technology to knock-out gene expression. Phenotypic analysis was then conducted on the mutant strains generated, such as lipid content analysis by GC-FID (Gas Chromatography coupled with Flame Ionisation Detection) and LC-MS/MS (Liquid Chromatography coupled with Mass spectrometry), Transmission Electronic Microscopy (TEM) and photosynthetic efficiency measures. Preliminary results obtained on some isoforms regarding diatom cell development, architecture and physiology will be discussed.

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52. Mitotic recombination between homologous chromosomes contributes to the genomic diversity in diatom clonal populations

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Survival of species requires a delicate balance between maintaining the genome stability of its individuals and introducing genetic variability within the population. Typically, novel genetic combinations that contribute to the genetic variability within a population are created during meiosis, through the recombination of homologous chromosomes followed by their random assortment to the gametes. In contrast, in most eukaryotic mitotically dividing cells, recombination between homologous chromosomes (interhomolog recombination) is strictly suppressed, as it may result in potentially harmful loss of heterozygosity (LOH), copy number variation (CNV) and genomic rearrangements.

Here, following up on the observation that frequently more than two alleles of a gene can be isolated from vegetatively dividing diatom cultures started from a single cell, we quantified haplotype diversity of culture on basis of next-generation sequencing data and amplicon sequencing of selected loci. Our data demonstrated a rapid accumulation of multiple recombined haplotypes in Seminavis robusta and Phaeodactylum tricornutum cell cultures. Moreover, by comparing the genome of mother and daughter P. tricornutum cultures started from a single cell and separated by 30 days we detected four copy neutral LOH and three CNV events in four out of nine daughter cell cultures, further illustrating the rapid accumulation of genome diversity in clonal diatom lineages.

The generation of copy neutral LOH events requires an exchange of genetic information between homologous chromosomes. To estimate the rate of such recombination, we established a tractable endogenous readout system in P. tricornutum. We estimated that the rate of interhomolog recombination in this diatom model species is around ~4.2 events per 100 mitotic cell divisions per genome under standard growth conditions and further increases under stress.

The life cycle of many diatom species includes long periods of clonal reproduction, strongly limiting the possibility to fix polymorphisms in a homozygous state and to create recombinant genotypes through sexual reproduction. The observed high level of mitotic interhomolog recombination might represent a strategy to overcome these limitations, in such a manner increasing genetic diversity within clonal populations. This mechanism might provide a selective advantage that allows rapid adaptation to changing environmental conditions and could render mitotic interhomolog recombination beneficial for population dynamics, outweighing the risks for individual single cells.

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53. Survivors of the Sea: Using Transcriptomics to Elucidate the Survival Strategies of Century Old Diatoms in Sediment

Matt Pinder

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In response to adverse conditions, many species of diatoms are capable of forming resting stages, entering a dormant state and sinking down to the sediment, from which they can return to the water column and resume vegetative growth when more favourable conditions are restored. When resting stages become buried in the sediment, they can be isolated from sediment cores at depths amounting to decades or more of dormancy and, despite such long periods under dark, anoxic conditions, can be revived and cultured in the lab. While our current understanding can account for diatoms surviving for shorter time periods in this state, the mechanisms behind resting stage survival on decadal timescales, such as important metabolic pathways, is unclear.

To identify the processes behind this long-term survival, we are looking at differential expression in the marine diatom Skeletonema marinoi, which has been reported to survive for a century as resting cells. We sequenced RNA from two axenic strains of S. marinoi across seven timepoints - vegetative cells, and lab-induced resting stages which had been dormant for up to six months. Of the 17,383 gene models in the current version of the S. marinoi genome annotation, 816 were significantly upregulated in all vegetative-versus-resting comparisons for both strains, and 1,437 were significantly downregulated ($p < 0.05$, $|LFC| > 1$). GO enrichment analysis revealed upregulation in ribosome-related processes, and downregulation in cell division-related processes. In addition, 257 and 732 of the significantly up- and downregulated genes, respectively, have no functional annotation. Continued improvements to the S. marinoi genome annotation should help to highlight further relevant changes in gene expression which contribute to resting stage survival.

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54. Diatoms as promising hosts for monoterpene engineering

Michele Fabris

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Many bioactive commercial compounds - including high-value and in-demand pharmaceuticals - are derived from plant monoterpenes. Since these are naturally produced in extremely low amounts, sourcing them from plants is inefficient and associated with intense land and water use. To ensure low costs and sustainability to the process, their production in engineered microbes is being explored. However, despite examples of remarkable progress, this remains challenging because conventional production hosts do not naturally accumulate geranyl-diphosphate (GPP) - the isoprenoid precursor of monoterpenes.

Several peculiar features are emerging in the isoprenoid metabolism of diatoms and we have been exploring their suitability for biotechnological applications. We engineered Phaeodactylum tricornutum to produce plant monoterpenes and we determined that it accumulates pools of GPP. To better understand this unique trait and its potential, we investigated the metabolic hub that surrounds the biosynthesis of GPP and other prenylphosphates, which is central to cellular metabolism as it provides precursors to essential pathways, such as sterols and pigments biosynthesis. By identifying key enzymes, characterizing their subcellular localization and their catalytic specificity, we tracked the biosynthesis of GPP and other prenylphosphates in P. tricornutum.

Our findings provide new details on the metabolism of isoprenoids of diatoms, which is at the basis of formation and emission of volatile organic compounds and secondary metabolites, as well as novel elements to guide synthetic biology and metabolic engineering strategies, expanding the biotechnological potential of diatoms.

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55. Uncovering the diversity and seasonal patterns of algicidal bacteria in the Western English Channel

Laura Branscombe

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Diatoms are a major group of photosynthetic microalgae, which often dominate marine and freshwater phytoplankton communities. Due to their ubiquity and abundance in the oceans, and the ability of some species to form large, sometimes toxic blooms, diatoms often play a major role in shaping phytoplankton community composition.

As two of the major groups within the phytoplankton, the co-existence of diatoms with marine bacteria has resulted in a broad spectrum of complex interactions, ranging from synergistic to antagonistic. The importance of such diatom-bacteria interactions in shaping diatom growth, physiology and bloom regulation is becoming increasingly recognised, with a mounting number of studies indicating that diatoms may respond to the chemical signatures of their bacterial neighbours by altering their physiology or metabolism. In particular, antagonistic interactions between diatoms and algicidal bacteria can play a significant role in regulating diatom growth and bloom dynamics.

However, while there are numerous studies revealing the potential of algicidal bacteria as biocontrol agents for controlling harmful algal blooms, and as useful components in algal biotechnology systems, these studies reveal little about the nature of such interactions in natural environments. Thus, the diversity, abundance and ecological significance of such interactions in nature remains a significant knowledge gap.

In this study, we have established a robust plaque assay method to assess the diversity and abundance of interactions between a library of ecologically relevant diatoms and antagonistic bacteria in the Western English Channel. By employing this method over the course of an annual cycle, we have uncovered seasonal patterns of previously un-reported algicidal activity in a diverse range of bacterial lineages.

In addition, we have identified an algicidal bacterium with a broad host range, which persists in the environment throughout seasonal cycles. Moreover, we aim to characterise the physiological impacts of algicidal bacteria on a range of diatom host species, providing new insight into the potential ecological roles of algicidal bacteria in this highly productive coastal region.

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56. A Tale of Two Flavodoxins

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Flavodoxins and ferredoxins are small essential proteins that shuttle electrons in photosynthesis reactions. While ferredoxins are more efficient in electron transfer through the use of iron as a co-factor, flavodoxins are independent of iron by using flavin mononucleotide (FMN) as a co-factor. Diatoms often encode both flavodoxins and ferredoxins, and flavodoxin can functionally replace ferredoxin under iron limitation. The ratio of ferredoxin to flavodoxin has commonly been employed as a measure of iron limitation in natural phytoplankton communities. However, a recent global ocean survey found that unlike other major phytoplankton groups, diatoms tend to express flavodoxin in both high-iron and low-iron sites, a direct contradiction to current ferredoxin/flavodoxin ratio assumption that flavodoxin expression is specific to iron-limiting conditions. Phylogenetic analysis indicates that known diatom flavodoxins group as two clades: only clade II flavodoxins are induced by iron limitation, whereas clade I flavodoxins are induced as yet unknown triggers. Interestingly, ectopic expression of cyanobacterial flavodoxin in land plants, that encode only for the iron containing ferredoxin, led to general resistance to oxidative stress in addition to high tolerance to iron limitation. Therefore, we hypothesize that diatom-specific flavodoxins may have as yet undiscovered additional roles related to oxidative stress, a common downstream effect of multiple stresses. We analyzed metatranscriptome samples collected along an iron gradient in the North Pacific. Diatom clade II flavodoxins were detected at the low iron stations while clade I flavodoxins were detected at a high-iron location, specifically in morning samples, supporting the idea that the different flavodoxins clades may have different functions in the cell. To investigate the potential role of clade I flavodoxins in oxidative stress response, and the specificity of clade II to iron limitation, we analyzed the ferredoxins and flavodoxins transcript levels in 6 different diatom species, mostly open-ocean isolates, subjected to either iron limitation or hydrogen-peroxide-induced oxidative stress. CRISPR/Cas9 mediated knock-out of the single, clade I flavodoxin in Thalassiosira pseudonana led to hyper-sensitivity to oxidative stress, without affecting the response to iron limitation. Our results suggest that clade I flavodoxins have a unique role in tolerance to oxidative stress in diatoms.

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57. Regulation mechanisms of phosphate uptake in Phaeodactylum tricornutum and Thalassiosira pseudonana

Hiroaki Matsui

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Phosphorus is an essential element required as biological membrane, nucleic acids, apatite, and other phosphorylated compounds. Dissolved inorganic phosphate (Pi) is assimilated by photoautotrophs such as diatoms in the ocean, which is incorporated into marine food-chain and transported to the land by birds and anadromous fishes. Because Pi is non-volatility, photoautotrophs have an important role to transfer phosphate to generate counter gravity movement. However, the molecular mechanisms of phosphate assimilation in marine diatoms are still unclear.

Pi uptakes were characterized and compared between the pennate diatom, Phaeodactylum tricornutum and the centric diatom, Thalassiosira pseudonana. The uptake rates of Pi increased when cells were acclimated from Pi replete seawater (+P) to Pi deprived seawater (-P), and which activity reached at the maximum in –P after 6 days later. In the same Pi limited condition, external alkaline phosphatase (APase) activities increased after about 2 days lagged time and the activity increasing more after 7 days. These data suggest that Pi transport system constitutes an initial rapid phase and a later slow phase to gain organic phosphate with APase in general marine diatoms. Their Pi uptake rates were both saturated by 50 µM Pi in cells acclimated to -P, while +P grown cells required at least 200 µM for uptakes. The Pi uptake was independent of pH, but highly dependent on sodium ion, indicating that the major counter cation for Pi transportation is sodium ion in diatoms. Genome of P. tricornutum and T. pseudonana possess ten and five candidate genes encoding solute carrier (SLC) type Pi transporters, respectively. Transcriptional levels of PtSLC34-2, -5, and TpSLC34-2 were induced in -P than normal seawater. GFP tagging localization revealed that those orthologous SLCs were expressed at the plasma membrane. Knock-down or -out of TpSLC34-2 repressed the Pi uptake significantly despite culturing cells under –P, suggesting TpSLC34-2 mainly acquire phosphate in T. pseudonana under Pi deprivation.

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58. Characterization of the Rubisco condensation protein PYCO1 from the pyrenoid of Phaeodactylum tricornutum

Oliver Mueller-Cajar

Zhen Guo Oh, Warren Ang, Cheng Wei Poh, Soak Kuan Lai, Hoi Yeung Li, Shashi Bhushan, Tobias Wunder and Oliver Mueller-Cajar*

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The slow kinetics and poor substrate specificity of the key photosynthetic CO2-fixing enzyme Rubisco have prompted the repeated evolution of Rubisco containing compartments known as pyrenoids in diverse algal lineages and carboxysomes in prokaryotes. Inside these compartments actively transported bicarbonate is converted into CO2 gas, which saturates the carboxylase with its substrate. Using co-immunoprecipitation experiments in Phaeodactylum tricornutum we have identified the Rubisco linker protein PYCO1. Similar to the green algal Rubisco linker protein EPYC1, PYCO1 is intrinsically disordered, possesses repeats and is positively charged at physiological pH. However, it possesses no sequence similarity to EPYC1, as expected for convergent evolution of a red Rubisco containing pyrenoid. Fluorescent PYCO1 fusion proteins localize as a rod shaped structure in the diatom chloroplast, consistent with the shape of the pyrenoid defined by transmission electron microscopy. To test the hypothesis that PYCO1 is the diatom pyrenoid scaffold we produced pure protein in Escherichia coli. Recombinant PYCO1 protein undergoes homotypic liquid liquid phase separation in a salt dependent manner. Diatom Rubisco specifically partitions into PYCO1 condensates. Heterotypic PYCO1-Rubisco condensates can bind up to three Rubisco hexadecamers per PYCO1 protein. Rubisco carboxylase function is unaffected in the condensates. PYCO1 is highly mobile in homotypic condensates. In contrast PYCO1 condensates saturated with diatom Rubisco have greatly reduced dynamics, with both PYCO1 and Rubisco becoming immobile. Consistently, FRAP experiments indicate that PYCO1 is not mobile in vivo. A combination of Cryo-electron microscopy and site-directed mutagenesis data show that the KWSP motif found in PYCO1 repeats binds to small subunits at the entrance of the Rubisco hexadecamer's solvent channel. Analysis of mutant PYCO1 proteins show that both the "KWSP" tryptophan and another repeating tyrosine are essential for homotypic phase separation.

We speculate that the unusual material properties of the PYCO1-Rubisco condensate are necessary to support the unusual non-spherical shape of the Phaeodactylum pyrenoid. Careful characterization of multiple diverse Rubisco condensates will strengthen translational approaches aiming to introduce pyrenoids and other metabolic condensates into new host organisms.

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59. Proteomic analysis of the oil bodies with different sizes in the marine diatom Fistulifera solaris for oil body-engineering

Marshila Kaha

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The energy from fossil fuel is predicted to decrease in the future years, and in fact the combustion of fossil fuel has raised several environmental issues. Microalgal lipids have been considered as the next-generation feedstock for biofuel due to higher productivity as compared to plant oils, short growth cycle, and easy to scale up. The marine diatom Fistulifera solaris JPCC DA0580 has attracted immense attention as a promising producer of biofuel in particular bio-jet fuel. F. solaris, an oleaginous marine diatom has the capability to accumulate high content of lipids (~ 65wt%) However, the commercialization of microalgal oil remains challenging as the production cost is still high especially in harvesting and extraction processes that account for up to 70-80 % of the total production cost. To tackle this issue, a promising way is the secretion of fatty acids directly to the culture medium thereby avoiding the necessity for cell harvesting. In this study, we aim to associate triacylglycerol (TAG) lipase on the oil bodies in F. solaris to directly degrade TAG, resulting in a free fatty acid generation. Toward this goal, we performed proteomic analysis to identify the novel oil body-associated proteins, which can serve as signal sequences to transport TAG lipase on the oil bodies. The cell samples containing the oil bodies with different sizes were prepared by controlling the nutrition levels. Subsequently, the oil bodies were collected from the homogenized cells, followed by protein extraction and LC-MS/MS analysis. Based on the proteomic analysis, 32 proteins were predicted as novel oil body-associated protein candidates in F. solaris. Among these proteins, two candidate proteins were selected based on the functions involving in sterol metabolism and transcript expression, respectively. Next, as a model experiment, we engineered the oil bodies in F. solaris to associate a recombinant GFP fused with the identified oil body-associated proteins. As a result, the candidate proteins with GFP labeling showed localization on the oil bodies of F. solaris. Since GFP was stably anchored on the oil bodies in F. solaris, the identified proteins can be promising signal sequences to transport any functional proteins of interest on the oil bodies towards oil body engineering. In the presentation, the functions and impacts of the novel oil bodies-associated proteins on cell growth and oil body metabolism will also be discussed.

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60. Dynamic biofilm formation of diatoms by a transcriptomic study

Ruqian Yang

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Photoautotrophic biofilms, frequently found in intertidal zones, are aggregates of heterotrophic bacteria and phototrophic algae, in which diatoms are often a very abundant group. While biofilms can be advantageous for organisms living in them, they also may cause biofouling, damaging aquatic industry and transportation. In this study, we cultured the model diatom Phaeodactylum tricornutum together with a marine bacterium of Roseobacter. We observed bacteria-induced biofilm formation and morphological changes of P. tricornutum within 2 days after the start of the co-cultivation. To investigate mechanisms of the induction of biofilm formation on a molecular level, we studied P. tricornutum gene expression 30 min, 3 h, 24 h and 48 h after bacterial inoculation. Our analyses revealed that totally 1846 genes were found to be differentially expressed (DEGs: differentially expressed genes; absolute fold change >= 2, FDR p-value<0.05) during the entire co-cultivation. Between 30 min and 24 h after bacterial inoculation, we found 31 redox related DEGs as well as 60 transcription factors compared to axenic cultures, indicating that signal transduction is one of the first responses of the diatoms. After 48 h, we identified 312 DEGs in the presence of the bacterium. Contrarily to the first 24h, most of the DEGs at 48h were involved in metabolic pathways, such as photosynthesis and chlorophyll biogenesis and biosynthesis of unsaturated fatty acids. Our data indicate two stages of genetic responses during biofilm formation of P. tricornutum in the presence of the Roseobacter bacterium, including signal transduction and metabolic responses.

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61. Growth dynamics of epizoic Achnanthes elongata and non-epizoic Psammodictyon panduriforme in co-cultures

Klara Filek

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Biofilm-forming diatoms occur on external surfaces of marine vertebrates such as killer whales and loggerhead sea turtles. There are indications that certain diatom taxa are host-specific but to date, conclusive evidence is lacking. Loggerhead sea turtles (Caretta caretta) represent a peculiar habitat, as sea turtles exhibit complex behaviors such as ontogenetic shifts, courtship behavior, and long-distance migrations. Furthermore, the sea turtle carapace and skin have shown high potential for new diatom species discovery. The mechanisms underlying substrate specificity in putative turtle-specific (epizoic) versus non-specific (non-epizoic) diatom taxa remain unclear as some epizoic taxa have not yet been found on any other substrates in the turtles' environment, but seem to be easily cultured once isolated (e.g., Achnanthes, Poulinea, Proschkinia). In this study, we build on our previous work that focused on establishing monocultures and a polyphasic approach (morphology, molecular markers) to identifying diatoms from carapace and skin biofilms of incidentally caught loggerhead sea turtles in the Adriatic Sea. Morphological and DNA metabarcoding investigations of sea turtle-associated diatom assemblages regularly find putative epizoic Achnanthes elongata on different sea turtle species in high abundances regardless of A. elongata slower growth rates (in culture) and usually higher incidences of non-epizoic opportunists. We hypothesize that, in laboratory co-cultures, A. elongata and non-epizoic Psammodictyon panduriforme change their respective growth rates in favor of A. elongata. We suppose similar interactions could take place on the turtle carapace or skin, thus explaining an aspect of A. elongata prevalence on the sea turtle surfaces despite faster-growing opportunistic taxa. Although laboratory co-cultures cannot fully reflect the complex interactions between diatoms and other microorganisms on turtles' surfaces, they provide a basis for further exploration and interpretation of data obtained on epizoic diatoms. To test our hypothesis and investigate potential interactions among turtle-associated diatoms we assessed changes in growth and biomass of A. elongata and P. panduriforme by co-culturing. The co-cultivations were performed in cell culture plates and quantified by a multi-mode reader (bright field and CY5 fluorescence images). We present the initial results pointing to minor changes in growth rates of A. elongata when co-cultured with P. panduriforme, while P. panduriforme tended to grow slower in co-cultures than in monocultures.

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62. The metabolic potential of the ocean

Dominic Absolon

Dominic Absolon*, Katherine Helliwell, Alison Smith

University of Cambridge

The molecular life of diatoms, as with all life on earth is governed by metabolic processes. Recent global environmental surveys, such as the TARA oceans expeditions, have produced vast amounts of sequencing data. This explosion in available novel data now allows us to ask questions that have yet to be asked. For example, how have metabolic processes evolved in the marine environment? And where have diverging pathway branches split from a common ancestor or been introduced from horizontal gene transfer?

The diatoms belong to a particularly interesting group, the Stramenopiles. Interesting for many reasons, not least the diversity. With this diversity comes a range of cell type, lifestyles and metabolic capability. By analysing the metabolic potential of Stramenopiles, we will start to unravel the drivers of this diversity and identify key branch points between different groups. Here we offer some initial analysis of metabolic pathways in both MMETSP transcriptome data and TARA oceans metagenomic data.

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63. Synthetic biology for the controlled production of high-value compounds in Phaeodactylum tricornutum.

Katrin Geisler

Katrin Geisler, Caroline Faessler*, Patrick Hickland, Marcel Llavero-Pasquina, Alison G. Smith,

University of Cambridge

Microalgae like the marine diatom Phaeodactylum tricornutum are emerging biotechnological chassis for the sustainable production of high-value products such as terpenoids. Microalgae are naturally optimized to produce precursor molecules for terpenoid production, such as geranylgeranyl diphosphate (GGPP) and farnesyl pyrophosphate (FPP), because these compounds are already produced in high amounts to make photosynthetic pigments and membrane sterols, respectively. An additional benefit over fermentative hosts is that algae exhibit rapid growth rates in simple mineral salt solutions using light as energy input to fix CO2. Over the last years, we and others have domesticated a range of established and novel DNA parts (constitutive and inducible promoters, 5'UTRs, 3'UTRs, targeting peptides) based on the MoClo syntax that can be used for efficient and high throughput Golden Gate assembly for P. tricornutum.

We will present data on the influence of different DNA parts (e.g. introns, promoters) on transgene expression in P. tricornutum. We have tested and characterized standard parts to express a range of diterpenoid biosynthetic enzymes (diterpene synthases, cytochrome P450s) in this alga. Often it is desirable to regulate transgene expression using either tunable promoters or riboswitches. While a predicted thiamine riboswitch in P. tricornutum was found not to be functional, we have demonstrated tuneable regulation of transgene expression using a newly identified promoter. We have generated strains that show an increased protein abundance as well as diterpenoid titer with this tuneable promoter compared to strains in which the transgenes were expressed under the control of standard promoters. We have engineered the strains further by targeting rate-limiting enzymes involved in the biosynthesis of GGPP and could show an increase in diterpene titer.

In summary, our work shows that the adoption of synthetic biology approaches in P. tricornutum to metabolic engineering can help with strain selection and the production of terpenoids in this microalga.

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64. A vitamin B12 physiological kaleidoscope in Phaeodactylum tricornutum

Shelby Newsad

Shelby Newsad*,1, Gonzalo Mendoza,2, Marcel Llavero Pasquina,3, Katrin Geisler,4, Ellen Harrison,5, Alison Smith,6

University of Cambridge

Diatoms play an important role in the environment as they generate an estimated 20% of the world's oxygen and are lynchpins in oceanic microbiomes. B-vitamins influence microbiome community composition because many algae have B-vitamin requirements. Over 50% of surveyed diatoms require B12, a trait that is conferred by an absence of a B12-independent methionine synthase (METE), a core enzyme in C1-metabolism. To study this system, we are using model diatom Phaeodactylum tricornutum which has a well annotated genome sequence, can be easily transformed and is amenable to editing by CRISPR-Cas9. P. tricornutum is not B12 dependent because it has a METE gene, but this is repressed in the presence of a vitamin, as is the case in the green alga Chlamydomonas reinhardtii. We looked to compare responses of vitamin deprivation in P. tricornutum and C. reinhardtii to understand similarities in how relevant algae respond to nutrient stressors.

A B12-dependent strain of P. tricornutum was made through a partial METE sequence deletion with CRISPR-Cas9 and was physiologically characterised and compared to the wild-type strain. The effective dose required to support 50% growth (EC50) was found to be 4 ng/L B12 with excess vitamin amounts yielding similar growth rates to the wild-type strain. This is contrasted to the B12-dependent C. reinhardtii strains whose vitamin requirement varies depending on whether the media has a carbon source (58-88 ng/L B12 EC50 with carbon source; 9 ng/L B12 EC50 with no carbon source). Investigation of B12-relevant transcripts show more pronounced responses to vitamin deprivation in the B12-dependent strain, which has been similarly observed in the B12-dependent C. reinhardtii. Interestingly, the P. tricornutum wild-type increase in a B12-dependent methionine synthase isoform, METH, in the presence of B12, suggesting potential for a positive sensing mechanism. A B12-producing bacterial species (Halomonas sp.) was assayed for its ability to support growth of the strain and the two organisms appeared to form a mutualism via metabolic exchange. Lastly, a reporter strain was developed where the METE promoter sequence drove expression of fluorophore, mVenus. When this was introduced into the wild-type strain, it could be used to determine the specificity of different B12 variants.

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65. How to analyze diatom growth data in a robust way: an evaluation of mathematical methods for specific growth rate estimation using simulation trials

Olga Kourtchenko

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66. Identifying Aureochrome-promoter interactions in Phaeodactylum tricornutum

Soo Hyun Im

Soo Hyun Im*, Sandeep Shrestha, Laura Weiss, Shvaita Madhuri, Bernard Lepetit, and Peter Kroth

Diatoms can acclimate easily to variable light conditions. They possess a large repertoire of photoreceptors, converting light signals into cellular responses. In the diatom Phaeodactylum tricornutum, one of the blue light photoreceptors called aureochromes (AUREOs) has been shown to play an important role in light acclimation. Aureochromes possess a light-oxygen-voltage-sensing (LOV) domain and a basic region/leucine zipper (bZIP) domain, which makes them unique blue light receptors that also function as transcription factors. Four isoforms of aureochromes have been identified in P. tricornutum: PtAUREO1a, 1b, 1c, and 2. Transcription of the individual AUREOs is differentially regulated throughout the daily light/dark phases, suggesting that each PtAUREO might have a distinct function. However, also a cooperative functionality among AUREOs has been proposed, as the proteins are capable to form a homo-/hetero dimers. Since the light-induced gene regulation of PtAUREO1a knock-out mutant was almost completely inhibited under red light to blue light shift conditions, PtAUREO1a has been postulated to function as a master switch of gene regulation. However, it is still unclear how exactly PtAUREOs affect overall gene regulation in the cell. In this study, we used a yeast one-hybrid (Y1H) assay to identify interactions of PtAUREOs with their target promoters in order to determine which genes are directly regulated by PtAUREOs. As a result, we verified the self-regulation of PtAUREO1a and direct transcriptional activation of PtAUREO1a/1b for Ptaureo1c gene. Furthermore, we tested the interaction between PtAUREO1a and the promoter sites of other transcription factor genes. These findings may help us understanding how PtAUREOs affect the overall gene regulation.

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67. Genetic variation analysis provides insights into Pseudo-nitzschia multistriata population genomics

Svenja Mager

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Stazione Zoologica Anton Dohrn

The planktonic heterothallic diatom Pseudo-nitzschia multistriata alternates long phases of clonal replication with more episodic events of sexual reproduction, which occurs when strains of opposite mating type, MT+ and MT-, get in contact. P. multistriata demography and population genetics have been followed in the Gulf of Naples, Italy, in the past decade. In an atypical bloom in 2013, the appearance of a dominant MT+ genotype reduced genetic richness in the population, for reasons that could not be explained.

We resequenced 24 P. multistriata strains including two strains whose genotype dominated the bloom in 2013, and strains from different locations (Gulf of Naples, Adriatic Sea, Italy, and Gulf of Mexico, US), of different mating type, collected over different years. The variant information was used to produce a phylogenetic tree to investigate relationships between the strains. Scaffolds diverging in the level of heterozygosity between groups of strains were identified as well as fast evolving genes by nonsynonymous to synonymous nucleotide diversity estimations.

Sampling location showed stronger influence on strain clustering than isolation date, time in culture or mating type. The two strains from the Gulf of Mexico were genetically diverse from the rest and from each other, as indicated by high numbers of private SNPs.

While there were no scaffolds differing in heterozygosity level between mating types, such scaffolds were found for the dominant strains from the 2013 bloom as well as the strains from the Gulf of Mexico and the Adriatic Sea. By far most genes were located on the heterozygosity-specific scaffolds of the two dominant strains.

For diverse subgroups of strains (all strains, dominant strains, strains from different locations) between 69 and 191 genes were determined to be under positive selection, among them membrane transporters and genes involved in metabolism as well as gene expression and control.

Previous and current transcriptomics analyses of the early phases of sexual reproduction in P. multistriata provided indications on genes involved in signaling and recognition between opposite MTs, these will be analyzed to highlight possible sequence variation between groups of strains. Future explorations will also include the identification of large genomic rearrangements. Concluding, variant analyses in diverse strains can provide a promising source for advancing towards the understanding of evolution and functioning as well as adaptability in P. multistriata.

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68. Molecular drivers of sexual reproduction in Cylindrotheca closterium

Sien Audoor

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Ghent University

Sexual reproduction is pivotal for most diatom species as a strategy to circumvent death by extreme miniaturisation and to introduce genetic variation within a population. Sexual reproduction has been described for many species, revealing that the principal traits of the diatom life cycle are highly conserved (Chepurnov et al., 2002). However, the molecular basis underlying the process of sexual reproduction remains largely unknown. We aimed at identifying the main molecular drivers by comparing a transcriptomic data set generated during the sexual reproduction of Cylindrotheca closterium with available datasets and by applying gene editing for functional studies. C. closterium is a heterothallic pennate diatom species complex comprising of 5 clades and 2 strains belonging to the same clade were used for the generation of this dataset. Below the sexual size threshold (approximately 66 µm), cells become sexually mature and strains of opposite mating type pair after a pheromone-mediated search (Klapper et al., 2021). Once paired, parental cells form 2 haploid gametes each, resulting in 2 new initial cells after auxospore expansion. RNA-Seq transcriptomic data was obtained from dark-synchronised cultures at the peak moments of cell pairing, gamete/zygote formation and auxospore expansion, yielding gene expression information for 19858 genes. By comparing this data set with available data for Seminavis robusta and Pseudo-nitzschia multistriata, we identified 6 genes showing a conserved upregulated sex-related expression pattern, suggesting a key role during pennate diatom reproduction. Additionally, we observed 9 genes with a C. closterium-specific response during all stages of sex, over 100 genes with a strong response in 2 out of the 3 stages and around 50 genes with a strong response in only one stage of the sexual process. We are currently attempting CRISPR gene editing to elucidate the function of the conserved genes by the creation of knock-out mutants.

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69. Diatom Plasticity: Trends, Issues and Applications on Modern and Classical Taxonomy, Eco-Evolutionary Dynamics and Climate change

Lawrence Victor Vitug

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Diatoms exhibit phenotypic plasticity because of environmental changes. It is known that external and internal factors play an important role in changes in the morphology of diatoms. Several changes in morphology in diatoms may result in several morphotypes that have the same genotype. This paper aims to discuss the trends and issues covering diatom phenotypes that go beyond external and internal factors that affect morphological changes in varying/specific environmental conditions; it will also further explain the influence of biogeography, adaptive and evolutionary behavior, macromolecular composition, climate change, challenging the traditional species concept and molecular approaches that we might gain perspective and insights on diatom phenotypic plasticity. Using Phaeodactylum tricornutum and other diatoms as model species that exhibit phenotypic plasticity. Discussing the application of phenotypic plasticity in diatoms that undergo plasticity and an emerging field on eco-evolutionary dynamics that can be linked to phenotypic plasticity in diatoms.

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70. Differential expression analysis in the spore former Chaetoceros socialis during nitrogen starvation

Angela Pelusi

Chaetoceros socialis is a widespread marine planktonic diatom with a heteromorphic life cycle characterized by a vegetative phase in which cells form distinctive globular colonies and a resting one, in which cells are transformed into morphologically different, heavily silicified spores. Spores are produced within the parental cell where the two valves are synthetized after two subsequent mitotic divisions. Chaetoceros socialis can form spores in both nutrient deplete and nutrient replete conditions and it has been recently shown that spores can be a defense mechanism against viral attacks and that their formation is triggered by high cell density.

We report the preliminary results of a differential expression analysis obtained during the formation of spores in C. socialis. Cells were grown in nitrogen depleted medium (treatment) and collected at three time points: before spore appearance (day 2, T2), when spore formation started (day 3, T3) and when spores represented 76% of the total number of cells (day 4, T4). Cells at the beginning of the exponential growth phase were used as control. The de novo transcriptome contained 37,271 transcripts, while the differentially expressed genes (DEGs) dataset presented a total of 25,878 genes with Fold Changes (FC) >|1,5|, with the majority of them differentially expressed at T3 and T4, in which the number of spores increased. We will discuss the preliminary finding of key processes that seem strongly related to spore formation, such as silica transport and DNA replication. By using published data on the transcriptomic response of Thalassiosira pseudonana, a non spore-former centric diatom, to nitrogen starvation, we are also attempting to identify common and unique pathways to the two datasets, with the aim to distinguish the starvation response from the processes more exclusively related to the formation of spores.

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71. Do core DNA repair enzymes regulate Diatom genetic diversity?

Reuben Gilbertson

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University of East Anglia

Diatoms are the most diverse taxa group on the planet and are present in aquatic environments from urban rivers to Antarctic sea ice. The ability to adapt to this myriad of environments demands substantial genetic diversity within natural populations. How this diversity is produced is poorly understood. Using the model diatom Thalassiosira pseudonana I am researching how core genes in the DNA repair and recombination pathways facilitate genome evolution via mitotic recombination. Through CRISPR/Cas genome editing I have created cell lines lacking core genes in either homologous recombination (HR) or non-homologous end-joining (NHEJ). These cell lines displayed clear signs of disruption to the cell cycle, characteristic of DNA repair-deficient cultures missing cell cycle checkpoints due to an increased number of unrepaired DNA lesions. Despite the major disruption of DNA repair pathways, and subsequently recombination, all cell lines were able to recover full fitness while remaining deficient in the originally knocked-out gene(s). Using whole-genome and RNA sequencing, I aim to identify the mutational signatures in the genome that elucidate which DNA repair pathways were used to not only maintain genomic stability but also which resulted in a return to full fitness by making the disrupted pathway redundant. By using reverse genetics to knock out core genes in recombination pathways we will be able to further understand the impact mitotic recombination has on the evolution of diatom genomes.

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72. Investigating evolutionary adaptation of the polar diatom Fragilariopsis sp.

Krisztina Sárközi

Krisztina Sárközi*, Amanda Hopes, Reuben Gilbertson, Thomas Mock

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The Arctic Ocean is one of the most threatened ecosystems on the planet. Shifts in population size and diversity of key primary producers triggered by global warming may drive changes in biogeochemical cycles and affect higher trophic levels in ways unaccounted for by existing climate models. As single-celled microbes with large population sizes and high replication rates, phytoplankton species have considerable potential to adapt rapidly to changing environmental conditions. However, studies which link genomic and transcriptomic data to this adaptive potential are scarce. Here we present some preliminary results of our long-term experiment aimed at examining how adaptive evolution in different Fragilariopsis diatom species and strains, isolated in the Arctic and Southern Oceans, occurs in response to warming oceans.

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73. The acquisition strategies of external bicarbonate by three plasma membrane SLC4 transporters under changing levels of CO2 limitation in the diatom, Phaeodactylum tricornutum.

Hermanus Nawaly

Hermanus Nawaly,1*, Yoshinori Tsuji, 2, Kazufumi Iwayama,3, Hiroki Ohashi,4, Hiroaki Matsui,5, Kensuke Nakajima,6, Yusuke Matsuda,7

Kwansei Gakuin University

One of the key constraints on photosynthetic organisms in the aquatic environment is the availability of carbon dioxide (CO2). Diatoms, like other microalgae, use the CO2 concentrating mechanism (CCM) to meet the high [CO2] demand of CO2 fixing enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). It has been demonstrated that marine diatoms take up HCO3- either directly across the plasma membrane via a specific transporter or indirectly as CO2 via dehydration with external carbonic anhydrase. To date, the function of one plasma membrane HCO3- transporter, solute carrier (SLC) 4-2, has been identified in the marine diatom Phaeodactylum tricornutum, while there are few other functional studies so far carried out. This study looked into the functions of two putative plasma membrane SLC4s, PtSLC4-1 and PtSLC4-4, in the marine diatom P. tricornutum and discovered that these proteins are involved in HCO3- uptake across the plasma membrane. Overexpression mutants of each PtGSLC4-1 and PtSLC4-4G were found to stimulate HCO3- uptake and inhibited by 4,4'-diisothiocyanostilbene-2,2'-disulfonic (DIDS), a specific anion transport blocker. Furthermore, PtSLC4-1 required sodium ion concentrations of up to 100 mM, similarly to the PtSLC4-2. In contrast, PtSLC4-4 was much less specific to sodium than the other two PtSLCs. PtSLC4-4 demonstrated comparable HCO3- transport activity with potassium and lithium ions relative to that with the sodium ion. Transcript of PtSLC4-1 was the most abundant, thus was likely a major plasma membrane HCO3- transporter that works in a wide range of CO2 limitation, while PtSLC4-2 transcript was much less abundant. PtSLC4-4 did not show a significant increase in the transcript when cells were fully acclimated from 1% to atmospheric level CO2, but it was transiently increased to high levels during the initial acclimation stage from 1% to 0.002 % CO2, strongly suggesting that PtSLC4-4 is specifically used in cells at the severe CO2 limitation to take up HCO3- using cation less selectively.

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74. Species-specific bloom dynamics of Skeletonema in Ariake Sound, Japan: Development of Skeletonema species-specific qPCR

Kazuhiro Yoshida

Kazuhiro Yoshida*, Hiroshi Ota, Takuya Iwanaga, Takayuki, Mine, Kei Kimura

Saga University

Neritic diatoms not just contribute to primary production also fisheries/aquaculture in coastal waters. The cosmopolitan diatom Skeletonema are observed in any coastal waters except Antarctic waters and often form extensive blooms. Due to their significant availability in coastal waters, the ecology and physiology of Skeletonema, particularly S. costatum, have been actively investigated since the 19th century. However, recent rDNA and minute electron microscopic surveys reported that six cryptic species within S. costatum (sensu lato), which had been regarded as a single species. Even after these surprising taxonomical findings, the ecophysiology of these "new" Skeletonema has not been well-studied yet; it is thus needed to reevaluate the ecophysiological knowledge of Skeletonema. Using batch cultures of multiple Skeletonema species/stains, Kaeriyama et al. (2011) examined growth responses to temperature and irradiance. Although this work provides insights into the physiology of "new" Skeletonema species, their results also indicate that multiple species have quite similar physiological features. Another approach is to monitor each Skeletonema species in situ to track the species succession with environmental gradients. However, the manual enumeration of each Skeletonema species, by looking at faint morphological differences, is inapplicable for monitoring the dynamics of the coastal diatoms.

Here, we developed a novel molecular identification method, species-specific quantitative PCR (qPCR), for this genus, to quantify the abundance of each Skeletonema species. We applied the species-specific qPCR technique to natural coastal waters in Ariake Sound in western Japan, where multiple Skeletonema species form blooms throughout a year. To discuss the ecophysiology of Skeltonema species in-depth, we bi-weekly collected surface seawater from Ariake Sound. Using the highly-frequent monitoring samples, the annual dynamics of seven Skeletonema species were tracked. Three Skeletonema species (S. costatum, S. menzelli, and S. tropicum) formed blooms in summer, whereas the others increased their abundance in winter (S. japonicum, S. dohrnii, S. ardens, and S. grevillei). The summer species bloomed one after another, whereas the winter species showed multi-species blooms once sea surface temperature decreased. Moreover, their bloom dynamics differed among species; which showed (1) a single intense bloom event and (2) sustained moderate blooms. Our results demonstrated that Skeletonema species have different seasonality and bloom formation strategies. This study is the first to investigate the species-specific ecophysiology of Skeletonema in situ. We will also report the distribution of the "seed bank" of each Skeletonema species in the sediments in Ariake Sound.

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75. The genome biology of Parmales(Bolidophyceae), a sister group of diatoms

Hiroki Ban

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Parmales (Bolidophyceae) is a group of a unicellular eukaryotic phytoplankton that contains species with a cell wall made of silica as well as naked flagellated species. Recent studies revealed that parmales are a sister group of diatoms. Diatoms are one of the most diverse and successful groups of microalgae and sustaining the food web in the ocean. On the other hand, although parmales are widely distributed in the world's oceans from polar to subtropic, their diversity and abundance are extremely low compared to diatoms. A comparative study of parmales and diatoms has a potential to address several important issues, such as the physiological characteristics that made differences in ecological roles between them in the current ocean, the origin of cell walls and their early evolution.

In this study, we newly generated 8 parmales genome assemblies and performed a comparative genome analyses together with 5 diatoms genomes publicly available. A comparison of the gene composition showed that the parmales are phagocytotic mixotrophs and there was a large gene loss event related to phagocytosis at the root of the diatom lineage. Diatoms have more genes involved in nutrient uptake, such as nitrogen compound transporters and silicate transporters, compared to parmales, indicating that diatoms have efficient nutrient uptake ability. In addition, ISIP1 and FTR, which are involved in iron uptake, were not present in the parmales, but plastocyanin, which is present only in pelagic diatoms and works in poor iron conditions, was found in most of parmales. This result characterizes the iron metabolism of each group. Also, the expansion of cyclin and heat shock factor genes did not occur in the parmales. These results provide insight into the traits of the common ancestor and suggest that diatoms have evolved to adapt to a dynamic environment.

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76. Metabolic flux analysis of the diatom Phaeodactylum tricornutum in response to nitrogen starvation

Bo Wang

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As a metabolic response to nitrogen starvation, diatoms are able to accumulate a large amount of neutral lipids, triacylglycerols (TAG), which could serve as precursors to biofuels. In order to augment the TAG production in these photosynthetic microorganisms, we need to understand the global metabolic response of diatoms to nitrogen stress. Diatoms possess unique features in regulating nitrogen metabolism. It has been found that the expression of a large number of genes in the model diatom Phaeodactylum tricornutum (Pt) is sensitive to nitrate availability in the environment. In the current study, we investigate metabolic changes of wild-type Pt in response to removal of nitrate in the culture medium, as well as the metabolism of a nitrate reductase (NR) knock-out strain grown with nitrate. Leveraged by 13C metabolic flux analysis (MFA) techniques, we compare the central metabolism of three Pt cultures, i.e., WT with nitrate (WT/N+), WT without nitrate (WT/N−), and NR knockout with nitrate (NR/N+).

We found WT/N− accumulated much higher levels of TAG and total lipids than that of WT/N+, which is consistent with previous studies. Meanwhile, the chlorophyll, protein content and free amino acid levels inside WT/N− cells dropped substantially relative to that in WT/N+. In contrast, the carbohydrate content, urea and metabolites in the TCA cycle of WT/N− cells increased dramatically compared to that in WT/N+. Our results are consistent with previous findings that genes associated with urea cycle are upregulated while expression of urea-degrading urease is downregulated in WT Pt cells under nitrogen starvation conditions. Interestingly, although NR/N+ showed a biomass composition similar to that of WT/N−, its carbohydrate content was about 50% higher than that of WT/N−. 13C-labeling and targeted metabolomics revealed that NR/N+ cultures maintained smaller metabolite pool sizes in the TCA cycle and nitrogen assimilation pathways but exhibited higher labeling rates compared to WT/N−. Our findings based upon 13C-MFA will help us to understand how Pt adjusts its metabolism under nitrogen-stressed conditions, which will guide engineering efforts for enhancing lipid biosynthesis in Pt.

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77. Characterizing two nitrite reductase enzymes in P. tricornutum through CRISPR-Cas9 gene-knockout methods

Anne Schulberg

Anne Schulberg*, James "Flip" McCarthy, Mark Mooseburner, Hong Zheng, Ariel Rabines, Rob Lampe

Diatoms thrive throughout the world's oceans; they comprise 20-40% of all phytoplankton and are responsible for up to 40% of primary production. This ecological prominence is largely due to their superior competitive abilities in nitrate assimilation. Nitrite reductase (NiR) is the last enzyme in the nitrate assimilation pathway, producing bioavailable ammonium from which the cell constructs proteins and nucleotides among other critical components. Uniquely to diatoms, two distinct NiR enzymes within the chloroplast are functional; endosymbiont-derived NiR-A requires ferredoxin substrates, and exosymbiont-derived NiR-B requires NAD(P)H substrates. To describe the purpose and differentiation between the enzymes, knockout cell lines for both genes transcribing NiR have been produced in the marine model-diatom Phaeodactylum tricornutum by CRISPR-Cas9 knockout methods. The genes were identified by annotation and prior transcriptomic studies and disrupted via specific complementary gRNA design and attachment to the Cas9 endonuclease. The CRISPR knockout line for NiR-A (Phatr3_J12902), grows far more slowly on F/2 enriched with nitrate than the knockout line for NiR-B (Phatr_EG02296), whereas growth on F/2 enriched by ammonium for both lines remains unchanged. This disparity suggests environmental specialization between the two enzymes, perhaps induced by the varying light and oxygen availability within their marine environment. Dissimilatory nitrate reduction to ammonium (DNRA) has been investigated as a possible process employed by the uniquely non-algal derived NiR-B in anoxic conditions encountered either in oxygen minimum zones within the water column or in benthic sediment. Preliminary experiments comparing knockout cells' ammonium output in anoxic conditions and correlation studies of NiR-B expression with anoxic environmental conditions using global TARA data both suggest that DNRA in diatoms may be facilitated by this enzyme. This work enables future experimentation to elucidate the advantages conferred to the diatom through simultaneous expression of two seemingly redundant enzymes.

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78. Investigating the Characteristics of Multipolar Diatoms in Response to Temperature and the Need for Fresh Environmental Isolates

Sam Coffin

Sam Coffin*, Matthew Davey, Alison Smith, Melody Clark

British Antarctic Survey / University of Cambridge

Marine diatoms are prevalent in the polar regions, significantly contributing to primary productivity and ecosystem functioning. Polar diatom species are usually confined to the unique environments of the high latitudes. They are characterized by having optimal growth at low temperatures (<10°C) and have developed various genetic adaptations to cope with these extreme environments. However, the polar regions are some of the fastest changing regions on earth, experiencing elevated seawater temperatures, increased ice melt and decreases in salinity. As a result, it is important to understand how polar diatoms may be impacted by such changes, in particular, temperature, which drives metabolic rates and energy budgets. A common method for studying the effects of temperature is thermal performance curves (TPCs), which identifies temperatures that are optimal for growth and where growth declines. Here we present findings on the thermal tolerance of two diatom species Fragilariopsis cylindrus and Porosira glacialis from the Antarctic and the Arctic, taking advantage of the multipolar distribution of these species to investigate differences between the two regions. We found large differences in the thermal tolerances between the two regions in both species, demonstrating regional adaptation. Experimental work on polar specimens is vital to understanding how they will respond to environmental change, however, a barrier to this is the number of strains publicly available and the length of time they have been kept in culture collections, where they are often kept at temperatures above those they experience in their natural environment. Therefore, there is a need to provide fresh, more environmentally representative stocks. We also present recent work on the isolation and culturing of new environmental isolates of marine diatoms from the Western Antarctic Peninsula for better representation in experimental studies.

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79. Temperature sensing in diatoms: cold shock and Ca2+ signalling

Friedrich Kleiner

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Diatoms are a diverse collection of photosynthetically active microalgae with exceptional ecological importance. Temperature has already been identified as a key contributor to the wellbeing and distribution of diatoms, though nothing is known about the specific signal perception pathways helping diatoms to sense rapid changes in temperature. We examined the Ca2+ signalling activity in single P. tricornutum cells encoding the fluorescence-based Ca2+ biosensor RGECO in response to rapid temperature change and found highly reproducible cytosolic Ca2+ elevations in response to cold- but not heat shock. Ca2+ elevations were induced only by very rapid cooling, as we observed no signalling responses following a gradual decrease in temperature. Although the physiological role of these Ca2+ elevations is not yet understood, their highly graded nature and similarity to cold-induced Ca2+ elevations in plant models suggest a regulative role initialised by a yet unidentified Ca2+ channel. In inter-tidal and near-shore environments, rapid transitions in temperature are likely to coincide with large changes in other physical parameters, such as salinity. We find that the cold shock response interacts with the hypoosmotic shock signalling pathway to increase the survival of P. tricornutum to rapid changes in salinity. The findings expand our understanding of sensory perception in diatoms and indicate the potential for complex cross talk between diatom signalling pathways.

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80. Molecular mechanisms of survival to the polar night in the diatom Fragilariopsis cylindrus

Juliette Laude

Molecular mechanisms of survival to the polar night in the diatom Fragilariopsis cylindrus

Juliette Laude*, Nathalie Joli, Lorenzo Concia, Flavienne Bruyant, Marine Beguin, Marie-Hélène Forget, Fredy Barneche, Marcel Babin and Chris Bowler

High latitude regions are subjected to a unique light regime characterized by the annual instalment of a several months period of complete darkness, the polar night. After its onset, light reaching algae communities located under the sea ice drops below detectable limits. Despite such extreme conditions, marine polar ecosystems remain very productive and are generally dominated by one of the most widely spread lineages of photosynthetic organisms on Earth, diatoms. However physiological and molecular mechanisms allowing microalgae to survive several months of continuous darkness remain unclear. We seek to identify the key strategies that allow both survival through prolonged darkness and growth restoration when light becomes available in the pennate Fragilariopsis cylindrus, a prominent sea ice diatom. Transcriptome analysis of cultures grown under complete darkness for up to 3 months allowed us to identify key metabolic and genetic pathways involved in dark survival. A darkness specific transcriptional response is established with a delay of 3-7 days after switching off the light and shows a global reduction in transcriptional activity in the dark. Similarly, we witnessed about the same delay for recovery of a light acclimation state after switching the light back on. After transfer to darkness, cells stopped dividing and did not show any cell cycle progression apart from a slight enrichment in the G0/G1 phase over time. Moreover, cells cultivated for a prolonged period in darkness showed a drastic decrease in RNA polymerase II large subunit protein content, which is coherent with the reduced gene expression observed in the transcriptomic data. Taken together our results suggest that F. cylindrus enters a physiologically dormant state from 3-7 days of continuous darkness onward, characterized by cell cycle arrest, reduced transcriptional activity and progressive consumption of carbon reserves.

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81. The Impact of Temperature on Vitamin B12 Use in the Polar Diatom Fragilariopsis cylindrus

Catalina Albury

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The Southern Ocean shapes global climate and biogeochemistry, supporting large amounts of primary productivity via the upwelling of nutrient-rich deep water. The ecology of this dynamic polar environment is characterized by massive seasonal phytoplankton blooms which are often dominated by diatoms. However, phytoplankton in the Southern Ocean currently face stressors such as increasing sea surface temperatures and periodic nutrient limitation. There is evidence that vitamin B12, a cobalt-containing micronutrient, is limiting or colimiting to phytoplankton growth in the Southern Ocean. Vitamin B12 (also known as cobalamin) is only synthesized by a select group of bacteria and archaea, and over half of surveyed phytoplankton have an absolute requirement for vitamin B12. Our understanding of B12 quotas, how they vary with important environmental variables such as temperature, and the relevant molecular mechanisms that drive them, remains limited in diatoms. To address this gap in understanding, we designed an experiment to explore the effects of temperature on vitamin B12 use in an ecologically significant Southern Ocean diatom, Fragilariopsis cylindrus. F. cylindrus is a major component of sea ice and pelagic seasonal ice zone assemblages in the Southern Ocean. Cultures of F. cylindrus, with and without the addition of exogenous B12, were exposed to a variety of temperature treatments. Cellular carbon and nitrogen content remained relatively consistent with temperature, despite a mean 0.16 µm decrease in cell size per increased °C. Mass spectrometry-based assessments of cultures grown with the addition of exogenous B12 suggest that total per-cell B12 quotas range from 0.1-0.3 attomoles per cell, aligning with recent direct measurements of quotas in T. pseudonona. These quotas were significantly affected by temperature, and closely correlated with growth rate. Notably, the concentration of MetH protein, the primary known sink for cobalamin in diatoms, did not significantly change with temperature, indicating that other cellular mechanisms may underpin temperature-driven changes in cobalamin quotas. These findings also suggest that B12 use by F. cylindrus may increase with warming Southern Ocean temperatures, potentially leading to faster onsets of B12 limitation during bloom progression if production remains constant. These findings increase our understanding of vitamin B12 utilization and primary productivity in the face of a changing global climate.

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82. Deciphering interactions between the marine benthic diatom Seminavis robusta and its microbiome using a multi-omics approach

Rita Bogorad

Rita Bogorad*, Willem Stock, Cedric Hubas, Wim Vyverman, Graham J.C. Underwood, Koen Sabbe

UGent

Complex interactions between microalgae and bacteria, such as mutualism, parasitism and competition, shape the structure and functioning of photosynthetic microbial mats. Using experimental co-cultures, we aimed at obtaining a better understanding of the interactions and exchanges that underlie the association between the marine benthic diatom Seminavis robusta (SR) and bacteria that were recruited from two very different bacterial inocula, one from the brackish lagoon Veerse Meer (The Netherlands), the natural habitat of SR, and a second from a forest soil sample, an alien environment to SR. Metagenomics was used to reconstruct the genomes of the dominant members from the bacterial communities in both environments. Axenic SR was co-cultured with the bacterial inocula for eight growth cycles, after which the interactions between diatoms and bacteria were characterized through metatranscriptomes. The turnover of the bacterial communities was characterized by means of 16S rRNA and rDNA amplicon sequencing. At the end of the experiment, co-cultures were incubated in artificial seawater with an inorganic carbon-13 source to quantify the transfer rates of photosynthetically fixed SR carbon to the bacteria (fatty acids). SR grew equally well with both inocula. We hypothesized that by the end of the experiment the established bacterial communities would stay distinct taxonomically but would display convergence with respect to metabolic functions and carbon transfer rates. Preliminary results of all analyses will be presented.

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83. Characterizing Ku70 knock outs and knock downs in Phaeodactylum tricornutum

Emily Stuckless

Emily Stuckless*, Julia Cholod, Samuel Slattery, Bogumil Karas, David Edgell.

Western University

Phaeodactylum tricornutum is an emerging biotechnological platform, genetic manipulation of which would be accelerated by a more complete understanding of DNA repair pathways. Site-specific integration of genetic constructs by CRISPR into the chromosome requires homology directed repair (HDR) pathways, yet the predominant non-homologous end joining (NHEJ) pathways results in error-prone repair of breaks. To address this issue, it was hypothesized that downregulation of Ku70, a protein involved in the NHEJ repair pathway, would increase rates of homologous recombination occurring. This was first attempted by Cas9-mediated gene knock out, wherein several heterozygous deletions were identified as well as a homozygous in-frame 9 bp deletion (ΔΔ9), but no homozygous knock outs were found. One heterozygous knock out with a large 1002 bp deletion in Ku70 reverted to a wild type allele over time by gene conversion. To circumvent potential Ku70 lethality, a CRISPRi system was used to downregulate Ku70 expression. The ΔΔ9 mutant and CRISPRi knock downs had impaired growth rates, altered morphologies, and increased sensitivity to ionizing radiation as compared to the wild-type strain. Our data suggests that P. tricornutum Ku70 is a critical component of double-strand break repair. CRISPRi knockdown is an alternative to study the function of critical genes for which knockouts are difficult to obtain. In the future, we will use a traffic-light reporter system to monitor the ratio of HDR to NHEJ repair events using the Ku70 mutants as well as knockdowns of other DNA repair genes. Ultimately, by improving homologous recombination in P. tricornutum, whole gene pathways can be permanently integrated into the genome, allowing for large-scale production of engineered products.

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84. Multi-class predictions of intracellular locations of proteins in organisms with complex plastids

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Diatom plastids evolved by eukaryote-eukaryote endosymbiosis. This process led to a complex plastid ultrastructure, with a total of four membranes surrounding the stroma. The two innermost membranes correspond to the outer and inner envelope of primary plastids found in Archaeplastida. The second membrane from the outside (third from the inside) is considered to correspond to the former plasma membrane of the endosymbiont. Hence, the space between this second and third plastid membranes, the periplastidic compartment (ppc), is a remnant of the cytosol of the former endosymbiont.

Cell biological processes as well as metabolic reactions have been shown to take place in this compartment, however, genome wide predictions of the proteins targeted to this compartment were so far based on manual annotation work exclusively.

With the increase of published experimental data, this situation has changed. At least a subset of the ppc proteins can be predicted from genome data with high specificity. This allows for the estimation that at least 81 proteins are targeted to the ppc in Phaodactylum tricornutum (Pt), and 180 proteins in Thalassiosira pseudana (Tp). The discrepancy can only partially be explained by genome size (10.814 predicted proteins in P. tricornutum, vs 13.344 predicted proteins in T. pseudonana), and is supported by previous experimental studies on selected proteins. In contrast to the discrepancy in the number of predicted ppc proteins, the numbers of predicted proteins for plastids (1315 in Pt, 1338 in Tp) and mitochondria (545 in Pt, 475 in Tp) are more similar between the two species.

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85. Using a diatom model to study an unusual histone protein in Chromera velia

Shun-Min Yang

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Chromera velia is a photosynthetic alveolate, known as the most closely related phototroph to apicomplexan parasites. It has rhodophyte-derived complex plastids and mitochondria with tubular cristae. The mitochondrial and plastid genomes of C. velia are present in an unusual linear form, with so far unknown organization of the DNA. After the whole genome sequence of C. velia was published in 2015, the histone genes were annotated, and several histone variants were identified. One of the histone H2A variants in C. velia harbors an N-terminal extension resembling a mitochondrial transit peptide. Due to the lack of a genetic transformation system in C. velia, we used the diatom Phaeodactylum tricornutum, to localize the histone variants in the cell. We generated genetically transformed cell lines of P. tricornutum, for ectopic expression of the C. velia histone H2A variants fused to the green fluorescent protein (GFP). The resulting clones were selected and examined by confocal microscopy. Compared to the control, the histone H2A variant without pre-sequence, showed nuclear localization; while the variant with the pre-sequence was found in association with the plastids, and co-localized with DNA. This result suggests that the pre-sequence of the C. velia histone H2A variant likely leads the protein to the plastid where it might interact with the organellar DNA. Since histone proteins are rarely found in organelles and are absent from their bacterial ancestors, our finding underlines the unusual properties of organelle genomes in C. velia.

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86. Pole to pole pattern of biogeography and acclimation in natural populations of marine diatoms

Juan José Pierella Karlusich

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Diatoms constitute one of the most diverse and ecologically important groups of phytoplankton. Diatoms are found in most aquatic ecosystems and they are especially common in well-mixed coastal and upwelling regions and at high latitudes. Diatom species diversity correlates with a broad diversity of sizes, which ranges >9 orders of magnitude in cell volume, and it is expanded by the fact that many species form chains. The Tara Oceans circumnavigation collected plankton samples between 2009 and 2013 from the main oceanic regions using a standardized sampling procedure. Here, we report the combined analysis of two metabarcoding datasets (the V9 and V4 hypervariable regions of the 18S ribosomal DNA) from >850 seawater samples from 142 different geographical sites of the main ocean basins, covering surface and deep chlorophyll maximum across 5 size fractions (0.8-2000 µm). In addition, we report the diatom gene expression analysis for 476 of those samples (from 87 different geographical sites) in which metatranscriptomes were also generated. We use these approaches to characterise the diversity, abundance, and distribution (vertical and horizontal) of marine diatoms, as well as their transcriptional flexibility towards environmental factors. We show that V4 and V9 molecular data showed complementary patterns, related with differences in taxonomic resolution and reference databases. We describe the top abundant genera and show distinct communities of co-occurring diatom lineages. Finally, we show transcriptomic features associated with the displayed biogeography of the different diatom lineages. Overall, we provide a pole to pole update of biogeographical diversity and acclimation in natural populations of diatoms of the whole plankton size spectrum.

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87. Circadian rhythms in the diatom Phaeodactylum tricornutum: regulatory function of bHLH/PAS proteins

Alessandro Manzotti

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Like most organisms, diatoms experience daily light–dark changes and show rhythms of basic biological processes such that they occur at optimal times of the day. However, the mechanisms regulating these processes are still obscure, also because diatoms present no homologues of circadian clock components of other organisms. Thanks to high-throughput flow cytometry analysis we monitored robust oscillations in diatom cellular fluorescence, reflecting a synchrony in chloroplast ontogeny and cell cycle progression, which are closely coupled in diatoms. The persistence of these oscillations for at least five subjective days upon transfer from light:dark photo-regime (LD) to continuous light (LL) indicates the existence of a cell-autonomous circadian clock in this alga. Similar results were obtained by the follow up of dissolved oxygen levels in cultures, a marker of the metabolic balance between photosynthesis and respiration.Taken together, these results support a strong influence of the circadian clock on the regulation of P. tricornutum physiology. To identify the still unknown transcriptional network sustaining rhythmic gene expression, we completed a high-resolution profiling of the diurnal transcriptome in the diatom model system Phaeodactylum tricornutum. The analysis of mutant lines of the most oscillating P. tricornutum transcription factor uncovered the bHLH-PAS protein bHLH1a, renamed RITMO1, as a key regulator of cellular rhythmicity (Annunziata et al., PNAS 2019). By yeast two hybrid analysis, we recently identified another P. tricornutum bHLH/PAS protein, bHLH1b, as a physical interactor of bHLH1a, suggesting that these proteins may act together in regulatory network that generates biological rhythms in diatoms. RITMO1 and bHLH1b KOs show strongly altered rhythmicity of cellular fluorescence following LD to LL shift compared to the wild-type cells with a short-period phenotype and rapid dampening of oscillations, supporting the involvement of these proteins in circadian rhythm regulation. Phylogenetic analysis reveals a wide distribution of RITMO1-like proteins in the genomes of diatoms as well as in other marine algae, which may indicate a common function in these phototrophs. This study adds elements to our understanding of diatom biology and offers perspectives to elucidate timekeeping mechanisms in marine organisms belonging to a major, but under-investigated, branch of the tree of life.

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88. Light intensity control on compound specific carbon isotope fractionation in cultures of Haslea ostrearia.

Maria Luisa Sanchez Montes

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Highly branched isoprenoids (HBIs) are C25-long molecules synthesised by a reduced number of sea-ice diatoms [1]. HBIs have successfully been used in sea ice reconstructions in the Arctic (IP25) and the Antarctic (IPSO25) [1] and show to be robust qualitative sea ice proxies preserved in sedimentary records dating back as far as the Late Miocene [2]. To explore unique information about sea ice characteristics e.g. ice thickness/light penetration, novel tools such as compound-specific stable isotopes (δ13C and δ2H) may be useful. In the case of IP25 δ13C in the Arctic sea-ice, sediment traps and sediments, high values suggest that carbon fractionation is affected by sea-ice environments [3]. We present δ13C data of certain HBIs from Haslea ostrearia grown under different light settings, which aim to explore biochemical effects of Haslea ostrearia adaptation to different light settings [4]. Further efforts will focus on the investigation of both δ13C and δ2H of HBIs from sea-ice diatoms as part of the developmental work looking at new and complementary HBI derived tools for sea ice reconstructions.

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89. Modeling carbon metabolism of the diatom Phaeodactylum tricornutum due to genetic knockout of TAG degradation enzymes and to high light versus low light conditions

Amy Zheng

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The diatom Phaeodactylum tricornutum (Pt) has the ability to store up to 45% of dry cell weight as triacylglycerol (TAG), a neutral lipid and precursor to biodiesel1. To take advantage of this innate ability, we need to understand how metabolic pathways adjust to changing environmental conditions. The long-term goal of this project is to promote efficient production of high-value and fuel-related compounds through optimization of metabolic fluxes in Pt. Building upon our expertise in 13C metabolic flux analysis (MFA),2 our current goal is to develop novel experimental protocols and data analysis workflows to enable 13C MFA of Pt. We are currently investigating the metabolic adjustments of Pt to two variables, light and genetic knockout of TAG degradation enzymes, which strongly affect cell growth and lipid accumulation. In this presentation, we discuss the optimization of the isotope labeling experiments and metabolic models for these conditions. In our optimization, we observed phenotypes of the diatom during stationary phase and exponential phase at higher optical densities by looking at growth rate, chlorophyll content and metabolism. We discuss our optimization of the metabolic model and the biomass equation reflect the different experimental conditions. We also investigate the differences in phenotype and in metabolism when we knockout the Acyl-CoA dehydrogenase (ACAD) enzyme during nitrogen repletion after starvation. Our novel MFA analysis will include a new model that uses compartmentalization in conjunction with metabolic data generated from our tailored GC-MS and LC-MS/MS measurements to compare metabolism of different strains of diatoms and under different light conditions.

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90. Microdiversity and co-occurrence of diatoms and bacterial associates along a latitudinal gradient in the North Pacific

Rebecca Key

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University of Florida

Co-occurence of marine microbial eukaryotes and prokaryotes cultivates strong, taxon-specific interactions that influence carbon and nutrient dynamics on a global scale. Trophic interactions between phytoplankton and heterotrophic bacteria can influence rates of primary production as well as the cycling of other major elements like iron, nitrogen, and sulfur. The study of metabolic interactions between marine diatoms and bacteria has greatly enhanced our understanding of their importance in mediating biogeochemical cycling, but how these taxon-specific interactions modulate overall community structure and ecosystem function is still challenging to resolve. Advances in sequencing and bioinformatic approaches continue to aid in the identification of microbial interactions and host-specific partnerships. To uncover diatom-diatom and diatom-bacteria interactions, we characterized community composition patterns using 16S and 18S rRNA amplicon sequencing data collected along a latitudinal gradient in the North Pacific Transition Zone (NPTZ) in three different years. We show relative taxonomic abundances across the transect, as well as correlation networks between members of major diatom and bacterial clades. With these data, we plan to study correlations between microbial taxa against the nutrient landscape along the three NPTZ transects to further characterize environmental factors that drive tight associations between diatoms and associated bacteria.

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91. Towards a synthetic algal chloroplast: a streamlined platform for creating designer chloroplast genomes in Phaeodactylum tricornutum

Emma Walker

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Chloroplasts represent a unique opportunity for the metabolic engineering of microalgae. Characteristics of their prokaryotic-derived genomes and compartmentalized micro-environments render the organelle amenable to high levels of transgene expression and foreign protein accumulation. However, the development of the chloroplast as a synthetic biology tool has been limited by a lack of robust multigenic engineering techniques and methods for the targeted delivery of large DNA inserts. The objective of this project is to first explore two cloning approaches for creating synthetic chloroplast genomes: (i) in vivo homologous recombination in yeast and (ii) in vitro golden gate assembly. The marine diatom Phaeodactylum tricornutum will serve as the model organism for this study as it is an emerging chassis for the industrial synthesis of bio-products and it has an established set of molecular tools including DNA delivery via conjugation from E. coli. So far, we have PCR-amplified the 117 kb P. tricornutum chloroplast genome into 19 overlapping fragments ranging from 1.8 to 9.4 kb in length. We then cloned pairs or triads of overlapping fragments into plasmids containing bacterial/yeast artificial chromosome elements and IIS restriction enzyme recognition sites. This has yielded eight plasmids containing a chloroplast sequence of 11.8 to 17.6 kb in length that can be released upon digestion by the respective IIS restriction enzymes. Going forward, we will attempt to reconstitute the whole chloroplast genome from the captured fragments using the two cloning approaches listed previously. Once an efficient approach is established, we will explore novel methods for delivering whole synthetic genomes to the P. tricornutum chloroplast.

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92. Molecular physiology of Antarctic diatom natural assemblages reveals multiple strategies contributing to their ecological success

Carly Moreno

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NYU Abu Dhabi

The continental shelf of the Western Antarctic Peninsula (WAP) is a highly variable system characterized by strong cross-shelf gradients, rapid regional climate change and large blooms of phytoplankton, notably diatoms. These rapid environmental changes coincide with shifts in plankton community composition and productivity, food web dynamics and biogeochemistry. Despite progress identifying the important environmental factors influencing plankton community composition in the WAP, the molecular basis for variations in species abundance, metabolism and distribution remain largely unresolved. Here, we identify oceanographic variables along the WAP that influence eukaryotic phytoplankton composition and the metabolic profiles of phytoplankton with an emphasis on expression patterns of iron-related genes in diatoms as assessed through metatranscriptomic sequencing. Distinct phytoplankton communities and metabolisms closely mirrored the strong gradients in oceanographic parameters that existed from coastal to offshore regions. Diatoms were abundant in coastal, southern regions, where colder and fresher waters were conducive to a bloom of a large centric diatom, Actinocyclus. Members of this genus invested heavily in growth, energy production, carbohydrate and amino acid and nucleotide biosynthesis pathways, resulting in a uniquely expressed metabolic profile. We observed strong molecular evidence for iron limitation in shelf and slope regions of the WAP, where diatoms in these regions employed ISIP1, a geranylgeranyl reductase, aquaporins, and urease, among other strategies, while limiting the use of Fe containing proteins. The initial metatranscriptomic survey performed here revealed functional differences in diatom communities and provided further insight into the environmental factors influencing the growth of diatoms and their predicted response to future ocean conditions.

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93. Molecular phylogeny of fucoxanthin-chlorophyll a/c proteins in the Chaetoceros gracilis genome suggests the diversification process of the light-harvesting complexes in the red algal lineage

Minoru Kumazawa

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The genus Chaetoceros is among the largest genera of marine planktonic diatoms and comprises several hundred species. They can be found in saline environments such as seawater and saline lakes. Because of their excellent nutritional properties, Chaetoceros sp. have been used as feed for the aquaculture of shellfish and crustaceans during their larval stage. Additionally, Chaetoceros has been studied as a potential source of lipids for biofuels; Chaetoceros gracilis in particular contains high concentrations of lipids. Furthermore, C. gracilis is also useful for basic research on photosynthesis, because cell disruption to isolate protein complexes in thylakoid membranes is relatively easy in this strain. In fact, recent structural analyses using cryo-electron microscopy (cryo-EM) revealed the structures of photosynthetic supercomplexes in C. gracilis.

In this study, we report the genome database of C. gracilis: It is possible to perform similarity and motif search for ~15,000 predicted genes and their functional annotations and mRNA expression data are also available. The size and completeness of C. gracilis draft genome is comparable to those in model diatoms, Phaeodactylum tricornutum and Thalassiosira pseudonana; However, C. gracilis possesses ~1,000 unique orthologous gene groups. The genome data was also supplemented by the long-read transcriptomes with the PacBio Sequel system (IsoSeq).

Using our genome database, we obtained the entire set of fucoxanthin chlorophyll a/c-binding proteins (FCPs) in its light-harvesting complex (LHC) system. Molecular phylogeny suggests that FCPs in C. gracilis can be classified into 5 major subfamilies, including a novel subfamily. Each subfamily is characterized by its specific motifs that may be relevant to the unique pigment composition. Furthermore, our FCPs classification is well correlated with their localizations in the cryo-EM structures of photosystems, suggesting the conserved function specific to each subfamily. Further phylogenetic analyses using the genome data and transcriptome assemblies from other diatoms and microalgae on public databases indicate that the set of FCP subfamilies is mostly conserved in the red algal lineage including Stramenopiles and Haptista (Haptophyta). The above results are used to propose a hypothesis on the loss and gain of FCP/LHC subfamilies during evolutionary history of the red algal lineage.

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94. A regulatory cascade of phosphorus-nutrient strategies in the diatom Phaeodactylum tricornutum

Senjie Lin

Senjie Lin*; Kaidian Zhang

University of Connecticut

Phosphorus (P) is an essential nutrient for marine phytoplankton, the base of marine ecosystems. However, in various parts of the world's oceans, P-nutrient, primarily dissolved inorganic phosphorus (DIP), is limited. To maintain intracellular P homeostasis and population growth, phytoplankton have evolved a set of strategies to cope with P limitation, including increase of inorganic phosphate (Pi) transporters, induction of hydrolases for scavenging organophosphates, and reducing P demand by replacing phospholipids with sulfur- or nitrogen-lipids. Wherein, they mainly achieve this via alkaline phosphatases (APs) that hydrolyze phosphomonoesters, which account for ~75% of total DOP in the ocean, and other enzyme systems that hydrolyze phosphonates, which account for ~25% of total DOP. How phytoplankton such as diatoms regulate the expression and function of these enzymes for scavenging different sources of P, maintaining P homeostasis and responding to low-P stress is poorly understood.

Diatoms, which play a crucial role in the biogeochemical cycle, contribute ~40% of global primary production. Diatoms are relatively young in evolution and are widespread in various natural waters. Phaeodactylum tricornutum has been widely used as a model diatom species because its genome has been sequenced. Here, we use CRISPR/Cas9 to knock out two AP genes (PhoA and PhoD) and the gene of SPX protein, a P regulator previously known only in plants, and the perform transcriptome profiling to characterize their modes of functions. The main results are as follows:

1) Compensatory regulation between different AP genes. PhoA and PhoD gene expression compensates for each other after one is disrupted; the DOP-PhoA-P_uptake and the DOP_uptake-PhoD-P_pathways function interchangeably for different DOP substrates.

2) AP also functions, besides P-nutrient scavenging when DIP is limited, to constrain pigment biosynthesis, photosynthesis, fatty acid biosynthesis, and cell division, with implications in balancing metabolic processes and preventing premature cell division.

3) SPX is a negative regulator of P uptake and P-stress response. SPX regulation of P uptake and metabolism involves a phosphate starvation response gene (PHR) as an intermediate to combine a SPX-PHR-PSI central regulatory cascade.

This study is a primer of using the CRISPR/Cas9 genome editing technology to characterize how phytoplankton coping with phosphorus-nutrition. As the technique only recently began to be accessible for phytoplankton research, there is a wide-open field to apply the technique. These findings have important ecological implications regarding how varying P conditions can shape a phytoplankton assembly and how phytoplankton will respond or evolve to future ocean environments in the context of climate change. The mutants generated here will be a valuable resource for future studies to further dissecting the molecular machinery underlying phytoplankton acclimation and adaptation to P variability.

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95. Biosynthesis of Fucoxanthin

Graham Peers

Graham Peers

Colorado State University

Fucoxanthin is the main light harvesting carotenoid in diatoms and it also plays a role in photoprotection. While several pathways for fucoxanthin biosynthesis have been proposed, the actual process has remained elusive. We used a reverse genetics approach in Phaeodactylum tricornutum to create three mutants: vdl2, zep1 and crtiso5. These mutants exhibit a green color compared with the brown wildtype. High performance liquid chromatography analysis confirmed the change is due to the absence of fucoxanthin, along with the appearance of novel carotenoid intermediates. Reduced growth rates under low light conditions, photosynthesis-irradiance curves and functional absorption cross sections of PSII all suggested less effective light harvesting in these mutants compared to wildtype. The mutants also showed no capacity for non-photochemical quenching (NPQ). Overall, our results reveal the fucoxanthin biosynthesis pathway resulted from a series of gene duplications and neofunctionalizations.

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96. Exploring the heme pathway in chromerid and diatom

Miroslav Oborník

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Heme biosynthesis pathway is fundamental for almost all living organisms. Despite conserved function, the pathway's enzymes are located in different cellular compartments in various organisms. In general, their placements do not always reflect their evolutionary origins, as might be expected from the history of the endosymbiotic acquisition of the gene. Instead, an enzyme's location reflects multiple factors, including evolutionary origin, demand for the product, availability of the substrate, or pathway regulation. In eukaryotic phototrophs, the entire pathway, or most of it (in chromerids and rhodophytes), is plastid localized. However, most of the knowledge relies on in-silico predictions only. We investigated locations of heme pathway enzymes in Chromera velia and Phaeodactylum tricornutum using computational predictions and experiments. We localized selected heme pathway enzymes of C. velia via heterologous reporter gene expression in the apicomplexan parasite T. gondii and the diatom P. tricornutum and several enzymes of P. tricornutum by the homologous reporter gene expression in the diatom. The experimental localizations of the C. velia enzymes in heterologous models show substantial differences between the diatom and apicomplexan. In both C. velia and P. tricornutum, predictions are not always in agreement with experimental localizations. Our results show that targeting machinery of chromerids, diatoms, and apicomplexans are not fully compatible. Despite the supposed origin of the chromerid plastid in a stramenopile endosymbiont, diatoms very likely do not recognize cleavage sites in the bipartite targeting sequences in chromerid genes and deliver the enzymes to the periplastidial space or endoplasmic reticulum, except for the mitochondrial 5-aminolevulinate synthase. Even the apicomplexan parasite model, the believed descendant of phototrophic chromerids, does not interpret targeting pre-sequences properly, delivering the enzymes to the mitochondria, cytosol, and the apicoplast, again except for the mitochondrial 5-aminolevulinate synthase. Surprisingly, porphobilinogen deaminase, the only enzyme of the pathway displaying mitochondrial origin, is contrary to predictions and localization in other eukaryotic phototrophs, not plastid-localized in P. tricornutum.

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97. How to digest delicious diatoms containing the phototoxic chlorophyll

Yuichiro Kashiyama

Yuichiro Kashiyama,1*

Fukui University of Technology

The current biosphere on Earth is supported by oxygen-evolving photosynthesis, in which chlorophylls are essential factors. Diatoms operate this mechanism effectively and serve as the most important primary producers in the aquatic environment and thus are the most significant producers of chlorophylls. Most of the chlorophyll produced on land is degraded to colorless compounds by the PaO/phyllobilin pathway of the plant itself during senescence, whereas those produced in the ocean are converted to colored compounds called cyclopheophorbide enols (CPEs) and deposited into the sediments. Indeed, CPEs are ubiquitous and abundant in the aquatic environment. This metabolism, CPE-accumulating chlorophyll catabolism (CACC), is quite universal among protists that prey on microalgae by phagocytosis, and diatom predators are among the most typical. CPEs are non-phototoxic catabolites of chlorophylls, and CACC is an important biochemical strategy to prey on diatoms and other microalgae under ambient light, thereby providing the basis for promoting the marine food web. Interestingly, most of the “fossil chlorophylls” extracted from the Miocene diatomites are derivatives of CPEs, suggesting that the majority of marine planktonic diatoms are primarily in situ-predated by the CACC protists. In fact, in diatoms with overexpression of cytoplasmic chlorophyllase, CACC was inhibited by the rapid conversion of chlorophyll into chlorophyllide during the predatory process of amoeboflagellates, and the growth of predators was significantly suppressed. In addition to the identification of CACC-related enzymes that are expected to be universally shared by eukaryotes, it is necessary to clarify the degradative process of chlorophyll c (the only chlorophyll with a porphyrin skeleton rather than a chlorin skeleton), which is characteristically produced by diatoms and other marine algae.

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98. How does endosymbiosis lead to new plastids?

Patrick Keeling

Patrick Keeling

University of British Columbia

The origin of plastids and their spread to new eukaryotic lineages was a series of major evolutionary transitions that had a huge impact on global ecology and the tree of life. The recognition that these events were mediated by endosymbiosis was a major breakthrough in our understanding of plastid evolution and diversity, but what do we really mean when we say “by endosymbiosis”? There is no shortage of diagrams and explanations of this process in textbooks and papers in the field, but the term is often used more like “abracadabra” than it is like a mechanistic model of evolutionary change with specific functional details and testable implications. This is starting to change, and I want to review how a new way of looking at organelle origins by endosymbiosis has turned many of assumptions about the process, the order of events, and the expected outcomes upside-down. As an example, I will go through how this model was tested using data from tertiary plastids, which are relatively recent endosymbioses between dinoflagellate hosts and several different kinds of algae with secondary plastids (i.e., diatoms, cryptomonads, or haptophytes).

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99. Activities of promoters derived from four diatom-infecting DNA viruses in the marine diatom Phaeodactylum tricornutum

Masao Adachi

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Marine diatoms constitute a major group of unicellular photosynthetic eukaryotes. Diatoms are widely applicable for both basic studies and applied studies. Molecular tools and techniques have been developed for diatom research. Among these tools, gene promoters that drive transgene expression at a high level are very important for the metabolic engineering of diatoms. Among the available promoters, the fucoxanthin chlorophyll a/c binding protein (FCP) gene (fcp, referred to as light-harvesting complex containing fucoxanthin, Lhcf) promoter has been frequently used in biotechnological applications involving the transformation of diatoms. However, reports also suggest that fcp promoter activity may not be strong enough to overexpress introduced genes and promote significant increases in target products. Promoters from viruses that infect plants and mammals such as the cauliflower mosaic virus 35S promoter and cytomegalovirus immediate early gene promoter enable high constitutive expression of transgenes in plants and mammals, respectively.

Considering these issues, our group focused on promoters of marine diatom-infecting viruses (DIVs), including both DNA and RNA viruses, which have recently been isolated, and their genome sequences have been characterized. In silico analysis has revealed that the genomes of the DNA viruses among DIVs possess certain putative open reading frames (ORFs): a gene encoding the replication-associated protein (VP3), a gene encoding the structural protein (VP2), and genes of unknown function (VP1 and VP4). We recently investigated the activities of VP3 and VP2 genes' promoter regions (P1 and P2, respectively) in the marine diatom Phaeodactylum tricornutum with an objective of diatom metabolic engineering. However, to the best of our knowledge, the activities of VP1 and VP4 genes' promoter regions (P3 and P4, respectively) have not been characterized yet.

In this study, we analyzed the activities of P1–P4 promoters derived from four DNA viruses in P. tricornutum. P4 promoter activities showed a tendency to be higher than those of P1, P2, and P3 of all four DIVs. Consensus motif-finding algorithms revealed that each DIV promoter (P1, P2, P3, and P4) exhibits its own consensus sequence. Furthermore, we identified a consensus sequence in the promoters with high activity. P4 that showed the highest activity might be useful for future diatom metabolic engineering.

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100. Using cryo electron microscopy to study diatom silicification

Assaf Gal

Assaf Gal

Weizmann Institute of Science

The process of diatom silicification, which gives rise to their exquisite silicified cell walls, is under strict biological control. This is an inorganic polymerization process that occurs intracellularly within highly confined environments. Our understanding of the structural and chemical aspects of diatom silicification is still rudimentary, as conventional imaging and analytical tools are inadequate to resolve the native-state conditions related to mineral formation processes. In our work, we use a suite of cryo electron microscopy techniques in order to extract intracellular structural and chemical information with nanoscale resolution. 3D serial imaging and chemical mapping of whole Thalassiosira pseudonana cells, shows that the cells maintain high intracellular concentration of Si throughout the cell cycle. In addition, high-resolution tomography enables us to visualize in 3D the ultrastructure of silica deposition in various elements and species. In the species Chaetoceros tenuissimus we followed the formation of long silica needles and discovered a new mechanism for the deposition of silica outside the cell membrane. Overall, our direct approach to study the formation of diatom silica in situ by means of advanced microscopy tools is yielding detailed understanding of the cellular controls that shape the silicification process in diatoms.

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101. A novel Ca2+ signalling pathway co-ordinates environmental phosphorus sensing and nitrogen metabolism in marine diatoms

Katherine Helliwell

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Diatoms frequently form spatially extensive phytoplankton blooms, responding rapidly to increased availability of nutrients, including phosphorus (P) and nitrogen (N). Although it is well established that diatoms are common first responders to nutrient influxes in aquatic ecosystems, the mechanisms employed by diatoms for nutrient perception are poorly understood. Here, we show that P-limited diatoms use a Ca2+-dependent signalling pathway, not previously described in eukaryotes, to sense and rapidly respond to the critical macronutrient P. We demonstrate that P-Ca2+ signalling is conserved between a representative pennate (Phaeodactylum tricornutum) and centric (Thalassiosira pseudonana) diatom. Moreover, this pathway is ecologically relevant, being sensitive to sub-micromolar concentrations of inorganic phosphate and a range of environmentally abundant P forms. Notably, we show that diatom recovery from P limitation requires rapid and substantial increases in N assimilation and demonstrate that this process is dependent on P-Ca2+ signalling. P-Ca2+ signalling thus governs the capacity of diatoms to rapidly sense and respond to P resupply, mediating fundamental cross-talk between the vital nutrients P and N and maximizing diatom resource competition in regions of pulsed nutrient supply.

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102. How diatoms modulate bacteria in their microbiome

Shady Amin

Ahmed Shibl,1 Ashley Isaac,2 Michael Ochsenkühn,3 Anny Cárdenas,4 Cong Fei,5 Kristin Gunsalus,6 Christian Voolstra,7 Shady Amin,8*

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Diatoms rely on microbial communities for survival despite lacking specialized compartments to house microbiomes (e.g., animal gut). Microbial communities have been widely shown to benefit from diatom excretions that accumulate within the microenvironment surrounding phytoplankton cells, including beneficial and harmful bacteria. However, mechanisms that enable diatoms to nurture specific microbiomes by fostering beneficial bacteria and repelling harmful ones are mostly unknown. We hypothesized that diatom exudates may tune microbial communities and employed an integrated multi-omics approach using the ubiquitous diatom Asterionellopsis glacialis to reveal how it modulates its naturally associated bacteria. I will discuss how A. glacialis reprograms its transcriptional and metabolic profiles in response to bacteria to secrete a suite of central metabolites and two unusual secondary metabolites, rosmarinic acid and azelaic acid. While central metabolites are utilized by potential bacterial symbionts and opportunists alike, rosmarinic acid promotes attachment of beneficial bacteria to the diatom and simultaneously suppresses the attachment of opportunists. Similarly, azelaic acid enhances growth of beneficial bacteria, while simultaneously inhibiting growth of opportunistic ones. Initial transcriptomic studies on the beneficial bacterial response to azelaic acid indicates an uptake mechanism that involves a unique, putative C4-dicarboxylate transporter system that is widely distributed in the oceans. These results demonstrate the innate ability of diatoms to modulate select bacteria in their microbial consortia, similar to higher eukaryotes, using unique secondary metabolites that regulate bacterial growth and behavior inversely in different bacterial populations.

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103. Linking traits, evolution, and ecological interactions to understand diatom ecology

Elena Litchman

Elena Litchman

Michigan State University

Exploring how traits are shaped by evolution and ecological interactions can bring novel insights into diatom ecology. Here I present several examples of such studies. Incorporating silica into cell wall was a major evolutionary innovation in diatoms that enhanced their ecological success. We recently showed that an astounding diversity of cell shapes in diatoms is much greater than in other phytoplankton groups. Silica frustules may be key to this diversity by allowing cells to overcome mechanical constraints and achieve dimensions not possible in the absence of the rigid cell wall. These different shapes, including extremely elongated ones, can affect other traits, from nutrient uptake to grazer susceptibility, increasing the cell's surface area and helping reduce grazing pressure. Thus, evolutionary changes in some traits can also help diversification in other traits, modifying the interactions of diatoms with other species and the environment. In a series of other studies, we explored how evolution on shorter time scales may also alter traits, experimentally investigating diatom adaptation to high temperature. We found that major thermal traits evolved but nutrient limitation impeded adaptation to high temperature, possibly due to a trade-off between high temperature survival and nitrogen requirements. These studies demonstrate that the feedbacks between trait evolution, the environment, and ecological interactions shape diatom ecology on different time scales.

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104. Diatoms on thin ice: sensitivity and resiliency to anthropogenic change in the Alaskan Arctic

Gwenn Hennon

Kyle Dilliplaine, Gwenn Hennon*

University of Alaska Fairbanks

Sea-ice diatoms are a crucial part of the Arctic food web, especially in spring when phytoplankton are light-limited. Climate change is rapidly reducing the sea-ice extent, thickness and snow cover in the Arctic, allowing for more shipping and offshore oil exploration and increasing the chance of an accidental crude oil spill. Thinning and bare ice also increases irradiance to the base of the ice and the water column, which is predicted to increase primary productivity and shift species composition to favor phytoplankton in the Arctic. Here we report on differences in photophysiology and crude oil sensitivity of four sea-ice diatom isolates from land fast ice near Utqiaġvik, Alaska. We observed that biofilm forming sea-ice diatoms (Synedropsis sp.) were low-light specialists, while diatoms capable of living in both sea-ice and water column (Attheya sp. and Fragilariopsis sp.) grew faster overall and reached maximum growth at higher irradiances. The biofilm forming diatoms were resistant to even high levels of crude oil exposure, while Fragilariopsis sp. had significant decreases in growth rates with crude oil contaminated media particularly at higher irradiances. These results suggest that the sea-ice diatoms favored by thinning ice are also more susceptible to crude oil spill impacts.

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105. Diel transcriptional oscillations of light-sensitive regulatory elements in open ocean eukaryotic plankton communities

Sacha Coesel

Sacha Coesel, Shiri Graff van Creveld, Bryndan Durham, Ryan Groussman, Rhonda Morales, François Ribalet, and E. Virginia Armbrust.

University of Washington

The metabolisms of microbial communities in sun-lit oceans are tightly tuned to the day night cycle. Additionally, the photosynthetic organisms in these communities modulate non-rhythmic changes in light quality and quantity as they are mixed to different depths throughout the day. Here we follow a community of eukaryotic protists in the North Pacific Subtropical Gyre that are adapted to extreme high light intensities and oligotrophic growth conditions. The majority of these protists adopted either a heterotrophic or mixotrophic lifestyle, and diatoms are one of the few obligate photoautotrophic eukaryotic organisms found in this region, making up approximately 15% of the total protistan biomass. Environmental protists were found to transcribe genes encoding known and novel light-sensitive proteins that may serve as light-activated transcription factors, elicit light-driven electrical/chemical cascades, or initiate secondary messenger signaling cascades. Overall, blue-light sensitive photoreceptors of the cryptochrome/photolyase family, and Light-Oxygen-Voltage (LOV) domain proteins were most abundant at the transcriptional level. The greatest photoreceptor diversification occurred within Haptophyta and photosynthetic stramenopiles, including the diatoms, where the LOV domain was combined with different DNA-binding domains and secondary signal transduction motifs. Some small pennate diatoms additionally expressed high levels of ion pump-rhodopsins. Photoreceptors, such as phytochromes, appear to play minor roles in the North Pacific Subtropical Gyre. In this talk, I will discuss the diversity and regulation of light-sensitive proteins that may allow disparate groups of protists to respond to light and potentially synchronize patterns of growth, division, and mortality within the dynamic ocean environment. I will further highlight some significantly diel processes found at the transcriptional level for the diatoms adapted to these oligotrophic waters.

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106. Grazer induced phenotypic plasticity provides insights into diatom evolution and molecular mechanisms

Erik Selander

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Diatoms respond to grazer presence by expressing defensive traits. This grazer induced phenotypic plasticity can be used to gain insights into the selective pressures grazers exert on diatoms. Traits expressed in resistant phenotypes likely evolved in response to natural selection from grazers on evolutionary time scales. Here we explore phenotypic plasticity in diatom traits including colony size and production of harmful secondary metabolites. Colony size plasticity is accompanied by significant reductions in grazing mortality whereas the effect of phycotoxins such as domoic acid is less well understood. The cueing compounds from copepod grazers have been identified as a group of polar lipids called copepodamides. Copepodamides can be administered to induce defensive traits without the presence of grazers. Grazer induced traits can subsequently be evaluated in controlled grazing experiments and the molecular mechanism can be explored by comparing induced cultures with non-induced controls. We find that chain forming diatoms face opposing selective pressures from microzooplankton and mesozooplankton grazers, where microzooplankton favour larger, and mesozooplankton smaller colony size. We argue that grazing has likely been an important driver behind the evolution of some diatom traits such as colony size and colony size plasticity. Pharmacological blockers in combination copepodamide stimulation suggest that the signal transduction pathway in diatoms involves G protein coupled receptors and cAMP as a second messenger.

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107. Exploring cooperative nutrient dynamics in diatom-bacterial interactions

Bryndan Durham

Bryndan Durham, Katherine Heal, Angela Boysen, Sacha Coesel, Ryan Groussman, Rebecca Key, Anitra Ingalls, Virginia Armbrust

University of Florida

Microbial transformation of carbon in the surface ocean accounts for the largest flux of organic matter in the ocean. Often, molecules are exchanged through taxon-specific trophic networks wherein individual metabolites, or groups of metabolites, are synthesized and consumed by particular producers and consumers, respectively. Here, we used liquid chromatography-mass spectrometry (LC-MS)-based metabolomics approaches to taxonomically categorize metabolites across 42 microbial taxa that include dominant lineages of eukaryotic phytoplankton and heterotrophic bacteria. Eukaryotic phytoplankton, in particular diatom taxa, show distinct metabolite composition in osmolytes, sulfur- and nitrogen-containing metabolites, sugars, and amino acid derivatives. With this information in-hand, we explore environmental metatranscriptomic and metabolomic data in the North Pacific Transition Zone (NPTZ) to track taxon-specific metabolic interactions between diatoms and associated bacterial groups. The NPTZ provides a natural latitudinal gradient in nutrient availability, allowing assessment of how nutrient landscape alters cooperative nutrient exchange among diatoms and bacteria. Enhanced taxonomic resolution of diatom metabolites and corresponding gene expression patterns improve our ability to resolve microbial interactions in surface ocean communities.

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108. Environmental genomics of diatoms leads to new functional and evolutionary insights

Tom Delmont

Tom Delmont

Genoscope

Marine planktonic eukaryotes play a critical role in global biogeochemical cycles and climate. However, their limited representation in culture collections limits our understanding of the evolutionary history and genomic underpinnings of planktonic ecosystems. Recently, we used 280 billion metagenomic reads from Tara Oceans to reconstruct and manually curate more than 700 abundant and widespread eukaryotic metagenome-assembled genomes ranging from 10 Mbp to up to 1.3 Gbp. This work provided two interesting "Diatom Environmental Genomics" insights. At the scale of the eukaryotic tree of life, classification of unicellular eukaryotic plankton based on functions encoded in their genes revealed four major groups connecting distantly related lineages, with one group connecting diatoms and the green algae. At the scale of individual species, we uncovered a rare evolutionary event in a blooming diatomic population in the Southern Ocean that might considerably change our perspective of the size of diatom genomics.

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109. Shunt or shuttle? Nutrient-driven biogeochemical consequences of diatom host-virus interactions

Kimberlee Thamatrakoln

Kimberlee Thamatrakoln

Rutgers University

The role of virus as ‘shunts’ – diverting particulate organic matter (POM) away from higher trophic levels and redirecting it into the microbial loop – has been the ecological paradigm since the 1990’s. However, emerging data now suggests viruses can serve as ‘shuttles’ – stimulating carbon export through processes that facilitate sinking, such as particle aggregation, fecal pellet production, or ballast production. As the largest group of siliceous organisms in the ocean and major contributors to primary production, the fate of diatom organic material and associated elements has critical implications for both the marine silicon cycle and biological pump. In this talk, I will present our findings that silicon-limited diatoms experience accelerated viral infection and mortality supporting a role for viruses in upper ocean silicon recycling. In contrast, iron limited diatoms exhibit delayed infection dynamics and reduced mortality which, together with previous findings that iron limitation increases ballast silica production, suggests a role for viruses in stimulating sinking and export flux. We have also observed that viral infection can induce diatom spore formation as a potential defense against viral-induced mortality and serving as an additional mechanism for export. This work highlights that the underlying ecophysiological response of diatoms to environmental conditions and infection play a fundamental role in determining whether viruses recycle diatom material in surface waters or shuttle it to depth.

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110. Introduction to Synthetic Diatom Project

Bogumil Karas

Bogumil Karas

The University of Western Ontario

Algal species are some of the best biological platforms for the production of biofuels, proteins, and medicines; yet, few genetic tools exist for genome-scale engineering. New developments in genome editing technologies such as CRISPR/Cas9 improved the efficacy of genome engineering in many organisms. However, a significant limitation of CRISPR/Cas9 is the relatively low number of possible simultaneous genome editing changes without unintended modifications. The only technology that currently provides the flexibility to create designer genomes is de novo synthesis of genome fragments, followed by assembly of whole genomes in yeast, and finally transplantation into the destination cell. This breakthrough technology was realized with the creation of the first synthetic cell at the J. Craig Venter Institute and, more recently, a synthetic organism driven by a minimal set of essential genes. However, this technology is limited to bacteria. To fill this gap, the Synthetic Diatom Project (SDP) was launched in 2020 to create superior methods for engineering nuclear and organellar algal genomes. During my talk, I will provide an update about the genetic tools recently generated for the eukaryotic alga Phaeodactylum tricornutum, including the improved telomere to telomere genome map, creation of synthetic mitochondria, and chloroplast genomes. I will also discuss the future goals of SDP.

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111. Diatom Genomics and Gene Regulation

Atle Bones

TBD

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112. Modelling Marine Diatom Diversity and Potential Future Changes at the Global Scale

Stephanie Dutkiewicz

Given the importance of diatoms to marine productivity and the silica cycle, as well as their role at the base of key marine foodwebs, it is important to explicitly include diatom in global scale biogeochemical/ecosystem models. This is particularly important when projecting what might happen to the marine carbon cycle and foodwebs as a result of global change. Models are constructed using laboratory studies to guide the representation of physiology and growth, and to suggest parameter values controlling nutrient uptake and photosynthetic rates. This talk hopes to stimulate a dialogue on how laboratory studies, and in particular molecular data, can be more strongly linked to help guide these models. Most earth system models include a single diatom "group" and project a strong decrease in their biomass in the future. Here we present results from a model (Darwin) that includes additional aspects of diatoms diversity, in particular a range in size classes, thermal norms, as well as exploring symbiosis with a nitrogen fixing organism. We show how these inclusions of additional diversity alters the modelled biogeography of diatoms. We also show that such diversity provides more nuanced projected changes in the future warmer ocean than previous models: shifts in size classes of diatoms rather than wholescale decline in diatom biomass, and shifts in biography due to alterations in silicic acid supply. Though the Darwin model provide a more complex landscape of diatom diversity it is never-the-less still much simplified relative to the richness of the real world. As such, the talk will focus on needs for future modelling endeavours to include other traits of diatoms (e.g. shape, chain formation), and how molecular data might help in these (and other) advancements in models.

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113. Evolutionary genetics of marine phytoplankton: many questions, few answers

Dmitry Filatov

Dmitry A. Filatov

University of Oxford, UK

Despite enormous ecological importance of marine phytoplankton generally and diatoms in particular, surprisingly little is known about the evolutionary genetics of their astronomically large populations and their capacity to adapt to changing conditions. Adaptation and speciation processes may work in rather different ways in small populations of terrestrial organisms (which have mainly been the focus of evolutionary genetics so far) and astronomically large populations of marine phytoplankton. For example, it can be argued that in very large populations any possible mutation arises multiple times every generation, potentially allowing adaptation to proceed very quickly. Furthermore, the efficacy of natural selection to fix adaptive mutations and eliminate maladaptive mutations is expected to be very high in large populations. Here I use genome- and transcriptome-wide sequence analyses in diatom and coccolithophore species to test whether these theoretical expectations reflect the real evolutionary dynamics of marine phytoplankton. I report that genetic diversity and codon bias (which are the proxies of short- and long-term effective population size, respectively) are surprisingly small in marine plankton populations even for species that are ubiquitous and abundant in the world oceans. The results indicate that evolutionary meaningful 'effective' population size of marine phytoplankton species may be comparable to that in better studied terrestrial organisms with modest population sizes, which suggests that evolutionary genetic processes in large marine populations may not be so different from the 'conventional' well studied species such as Drosophila or humans. However, the factors limiting genetic diversity and codon bias in astronomically large marine phytoplankton populations remain unclear and require further research.

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114. Interactions between irradiance and temperature determine the niche of a novel low-light, cold-adapted nano-diatom from a wintertime temperate estuary

David Hutchins

David A Hutchins*, Kyla Kelly, Sophia Pei, Tatiana A. Rynearson, Joshua Kling

University of Southern California

Diatoms have well-recognized roles in fixing and exporting carbon and supplying energy to marine ecosystems, but only recently have we begun to explore the diversity and importance of nano- and pico-diatoms. Here we describe a small (~5 µm) diatom from the genus Chaetoceros with a unique obligate specialization for low-light environments (< 120 µmol photons / m2 * sec-1), isolated from a wintertime temperate estuary (2° C , Narragansett Bay, RI). This diatom exhibits a striking interaction between irradiance and thermal responses whereby as temperatures increase, so does its susceptibility to light stress. Historical 18S rRNA amplicon data from our study site show this isolate was abundant throughout a six year period, and its presence strongly correlates with winter and early spring months when both light and temperature are low. Two ASVs matching this isolate had a circumpolar distribution in Tara Polar Ocean Circle samples, indicating its unusual light and temperature requirements are adaptations to life in a cold, dark environment. We expect this isolate's low light, psychrophilic niche to shrink as future warming-induced stratification increases both light and temperature levels experienced by high latitude marine phytoplankton.

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115. Diatoms and the Biological Carbon Pump

Uta Passow

Diatoms, Carbon Sequestration and the Biological Carbon Pump

The term Biological Carbon Pump encompasses the different biological processes that lead to the transport of carbon against a concentration gradient to the deep ocean. Carbon transferred from the surface ocean, where pCO2 is in equilibrium with the atmosphere, to below 1000 m depth, is frequently considered sequestered, because on average it takes 100 years for such a carbon molecule to return to the surface ocean.

One of the main transport pathways of the Biological Carbon Pump is the fixation of inorganic carbon into diatom biomass and the subsequent sedimentation of diatom aggregates to depths. Aggregate formation is an important part of the lifecycle of most bloom-forming diatoms. Because sinking velocity is largely a function of size, aggregate formation of diatoms makes diatoms and other aggregating taxa important contributors to gravitationally settling carbon flux. Sinking velocity of settling aggregates is central, as the ratio of settling velocity to degradation rate appreciably determines the fraction of the carbon that arrives at depth, as does grazing pressure on aggregates. The silica frustules in diatom aggregates provide excess (compared to seawater) density that is higher than that of organic matter, also contributing to high sinking velocities of diatom aggregates. Grazing, degradation and fragmentation, all lead to a reduction of the carbon flux with depth, with sinking velocity determining the time spend “on route”.

Changes in environmental conditions, e.g. due to climate change will cause shifts in phytoplankton composition, succession, bloom peaks and timing and related food web dynamics, most certainly impacting diatom growth, aggregation, sedimentation – and ultimately vertical carbon flux due to diatoms. Predicting the direction of change involves predicting the growth response of diatoms to anticipated environmental change (nutrients, irradiance, ocean acidification), as well as anticipating impacts on food web dynamics such as competitive strength or grazing pressure, and the consequences for aggregation, including exudation and TEP production, and sedimentation including all processes impacting flux attenuation.

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116. Unravelling a natural evolutionary experiment: the colonization of low-salinity habitats in the Baltic Sea by the marine diatom Skeletonema marinoi

Eveline Pinseel

The salinity gradient separating marine and freshwater environments represents one of the major ecological divides for prokaryotic and eukaryotic microbes. Yet, the mechanisms by which marine diatoms adapt to, and ultimately diversify in, freshwater environments are poorly understood. Here, we take advantage of a natural evolutionary experiment that has played out over the past 9,000 years in one of the world's largest brackish water bodies: the colonization of the Baltic Sea by the marine diatom Skeletonema marinoi. We collected eight genotypes of S. marinoi from across the Baltic Sea salinity gradient, exposed these to different salinities that mimicked the Baltic Sea salinity cline, and used RNA-seq to compare patterns of gene expression between low and high salinity treatments. Inclusion of multiple genotypes allowed us to characterize a shared response to low salinity among all genotypes as well as highlight intraspecific variation in the response to low salinity. We found that S. marinoi cells in low salinities have altered general energy metabolism, experienced increased oxidative stress and higher nutrient demands, and produced more storage compounds and fewer osmolytes. Inclusion of strains from eight different localities revealed that different genotypes differ significantly in their response to low salinity, both in the direction and magnitude of gene expression. These differences include fundamental cellular processes such as cell division, regulation of transcription and translation, and aerobic respiration. Altogether, our results reveal substantial variation in the response of different genotypes, highlighting an important source of biological variation associated with how diatoms respond and adapt to environmental change.