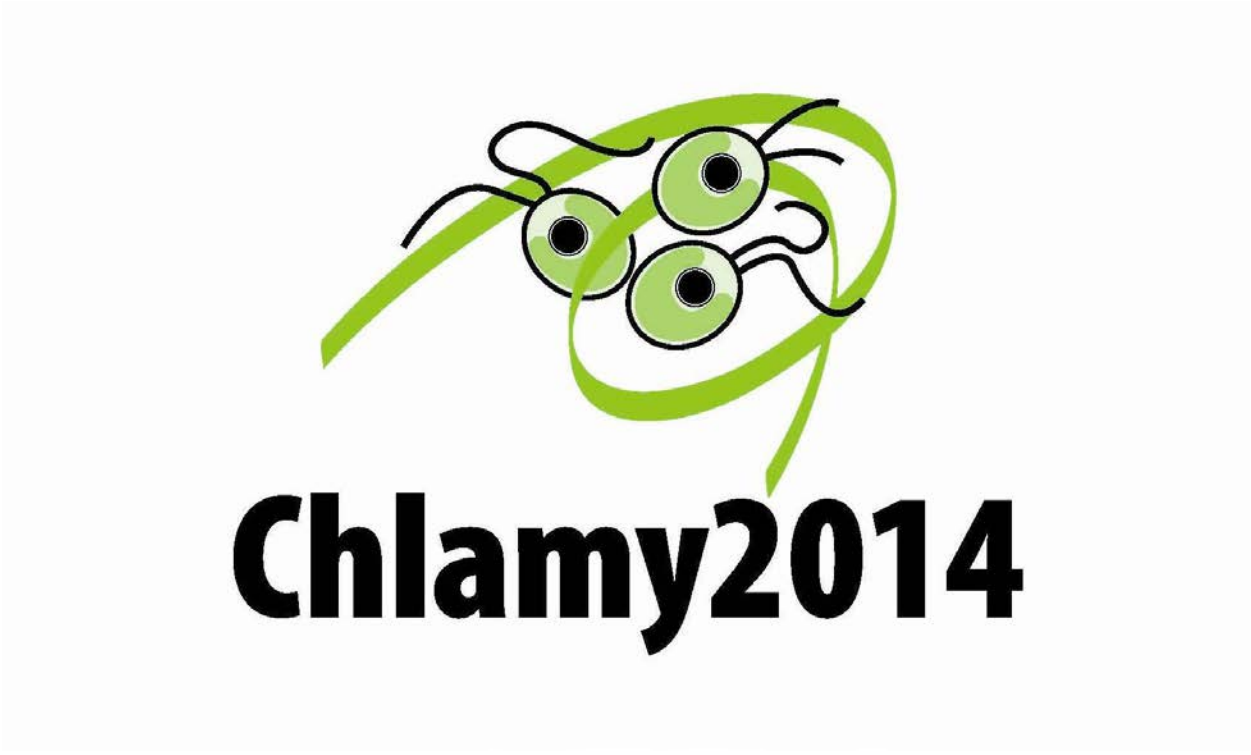
16th International Conference on the

Cell and Molecular Biology of *Chlamydomonas*

June 8-13, 2014
Asilomar Conference Center, Pacific Grove, CA, USA

Program and Abstracts



16th International Conference on the Cell and Molecular Biology of *Chlamydomonas*

June 8-13, 2014
Asilomar Conference Grounds
Pacific Grove, California

Program and Abstracts

Organizers:
Kris Niyogi, University of California, Berkeley
Winfield Sale, Emory University
Marilyn Kobayashi, University of California, Berkeley

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GENERAL INFORMATION AND EXHIBITS

Registration Desk

The Conference registration desk will be open according to the following schedule:

Date	Time	Location
Sunday, June 8	4:00 pm – 9:00 pm	Surf and Sand
Monday, June 9	8:00 am – 12:00 pm	Surf and Sand

Instructions for Speakers

All platform sessions will be held in Merrill Hall. Please arrive 30 minutes before the start of your session with your presentation on a flash drive to load on the MAC meeting computer.

Poster Sessions

All posters will be displayed in Merrill Hall. All authors should put up their display on Monday morning and take it down by by 12:00 midnight on Thursday night. Two authors will share a 4 x 8 ft (1.2 x 2.4 m) poster board. Each poster should be no wider than 4 feet (1.2 m). Authors will present according to the schedule listed below. Posters will also be on display during evening socials. The meeting does not take responsibility for posters that are not removed on time.

Poster Session A: Monday, June 9	4:00 pm – 6:00 pm	Even-numbered posters
Poster Session A: Tuesday, June 10	4:00 pm – 6:00 pm	Even-numbered posters
Poster Session B: Wednesday, June 11	4:00 pm – 6:00 pm	Odd-numbered posters
Poster Session B: Thursday, June 12	4:00 pm – 6:00 pm	Odd-numbered posters

Social Events

Welcome Reception: Sunday, June 8: Meet friends and colleagues at the Opening Mixer in Merrill Hall immediately following the Perspectives Lectures, from 9:00 pm – 12:00 midnight. There will be a cash bar.
Evening Socials: Monday, Tuesday and Thursday there will be socials in Merrill Hall from 9:00 pm – 12:00 midnight. There will be a cash bar.
Conference Banquet: Wednesday, June 11: A reception and banquet will be held at the Barbeque Area Bonfire Pit with the reception/cash bar starting at 5:30pm. The banquet will begin at 6:30pm.

Internet Access

WiFi is available in Phoebe Hearst Social Hall and the guest rooms. Internet access is not available in Merrill Hall.

Exhibitors

Bulldog Bio, Inc.
Email: info@bulldog-bio.com
URL: www.bulldog-bio.com
Bulldog Bio provides quality products for life science research. On display will be the NEPA21 Electro-Kinetic Transfection System that has been proven to be THE most efficient way to deliver nucleic acids into *Chlamydomonas.* Check out why the NEPA21 is Japan's most popular electroporator.

Life Technologies
Email: lora.daisley@thermofisher.com
URL: www.thermofisher.com
Life Technologies offers a wide range of tools and products for all your research needs. Our GeneArt® algae expression and engineering kits for *Chlamydomonas reinhardtii* and *Synechococcus elongatus* are the first commercially available genetic modification and expression systems for photosynthetic microalgae. Find more information at Lifetechnologies.com/algaeexpression.

Singer Instruments
Email: contact@singerinstruments.com
URL: www. Singerinstruments.com
Singer Instruments' **ROTOR HDA** is a bench top robot for the ultra-fast manipulation of microbial colonies, enabling systematic high-throughput genetic and genomic analyses in yeast, bacteria and algae. Our **dissection microscopes** are ideal for the quick and precise manipulation of microbial spores.

SCHEDULE OF EVENTS

Sunday, June 8	
4:00 pm - 9:00 pm	**Registration**
6:00 pm - 7:00 pm	**Dinner**
7:15 pm - 7:30 pm	**Opening Remarks**
7:30 pm - 9:00 pm	**Perspective Lectures** by Kent Hill (University of California, Los Angeles) and Christoph Benning (Michigan State University) *Chairs:* Kris Niyogi, University of California, Berkeley; and Winfield Sale, Emory University
9:00 pm - 12:00 midnight	**Welcome Reception**
Monday, June 9	
7:30 am - 8:30 am	**Breakfast**
8:00 am - 12:00 noon	**Registration**
8:30 am - 10:00 am	**Emerging Technologies** *Chair:* Martin Spalding, Iowa State University
10:00 am - 10:30 am	**Break**
10:30 am - 12:00 noon	**Assembly of the Flagellar Axoneme** *Chair:* Susan Dutcher, Washington University School of Medicine
12:00 noon - 1:00 pm	**Lunch**
1:15 pm - 4:00 pm	**Light, Carbon, and other Environmental Responses** *Chair:* Michael Schroda, University of Kaiserslautern
4:00 pm - 6:00 pm	**Poster Session A and Exhibitors**
6:00 pm - 7:00 pm	**Dinner**
7:15 pm - 9:00 pm	**Circadian Rhythm, Cell Cycle, and Development** *Chair:* James Umen, Donald Danforth Plant Science Center
9:00 pm - 12:00 midnight	**Social**
Tuesday, June 10	
7:30 am - 8:30 am	**Breakfast**
8:30 am - 9:45 am	**Biofuels** *Chair:* Chia-Lin Wei, DOE Joint Genome Institute
9:45 am - 10:15 am	**Break**
10:15 am - 12:00 noon	**Flagellar Length Regulation, Basal Bodies, and Transition Zone** *Chair:* Carolyn Silflow, University of Minnesota
12:00 noon - 1:00 pm	**Lunch**
1:00 pm - 4:00 pm	**Free Afternoon**
4:00 pm - 6:00 pm	**Poster Session A and Exhibitors**
6:00 pm - 7:00 pm	**Dinner**
7:15 pm - 9:00 pm	**Photosynthesis** *Chair:* Jun Minagawa, National Institute for Basic Biology
9:00 pm - 12:00 midnight	**Social**
Wednesday, June 11	
7:30 am - 8:30 am	**Breakfast**
8:30 am - 10:00 am	**Chloroplast Biogenesis and Function** *Chair:* William Zerges, Concordia University
10:00 am - 10:30 am	**Break**
10:30 am - 12:00 noon	**Metabolism (including Hydrogen)** *Chair:* Arthur Grossman, Carnegie Institution for Science
12:00 noon - 1:00 pm	**Lunch**
1:15 pm - 4:00 pm	**New directions in *Chlamydomonas* biology** *Chair:* José Luis Crespo, CSIC
4:00 pm - 6:00 pm	**Poster Session B and Exhibitors**
5:30 pm – 6:30 pm	**Reception**
6:30 pm - 9:00 pm	**Banquet**
9:00 pm - 12:00 midnight	**Social**

SCHEDULE OF EVENTS

Thursday, June 12	
7:30 am – 8:30 am	**Breakfast**
8:30 am - 9:45 am	**Beyond *C. reinhardtii* (Volvocales and other Algae)** *Chair:* Rachael Morgan-Kiss, Miami University
9:45 am - 10:15 am	**Break**
10:15 am - 12:00 noon	**Mechanism and Regulation of Flagellar Motility** *Chair:* David Mitchell, SUNY Upstate Medical University
12:00 noon - 1:00 pm	**Lunch**
1:00 pm - 4:00 pm	**Free Afternoon**
4:00 pm - 6:00 pm	**Poster Session B and Exhibitors**
6:00 pm - 7:00 pm	**Dinner**
7:15 pm - 7:30 pm 7:30 pm - 8:30 pm 8:30 pm - 9:00 pm	**Poster Awards and Announcements** **Keynote Lecture by Ursula Goodenough, Washington University** **Closing Remarks**
9:00 pm - 12:00 midnight	**Closing Social**
Friday, June 13	
7:30 am - 8:30 am	**Breakfast and Depart**

PLENARY SESSION LISTINGS

Sunday, June 8 7:15 pm–9:00 pm

Perspective Lectures

Co-Chairs: Kris Niyogi, University of California, Berkeley and Winfield Sale, Emory University

1 - 7:30

Motility and more: the flagellum of African trypanosomes. Kent L. Hill, University of Caliifornia, Los Angeles, CA.

2 - 8:15

Microalgal glycerolipid metabolism in the context of cell development and cell division. Christoph Benning, Michigan State University, East Lansing, MI.

Monday, June 9 8:30 am–10:00 am

Emerging Technologies

Chair: Martin Spalding, Iowa State University

181 - 8:30

TALE activation of endogenous genes in *Chlamydomonas reinhardtii*. Martin H. Spalding, Iowa State University, Ames, IA.

4 - 8:45

Genomic Resequencing of Chlamydomonas Laboratory Strains Reveals Corrected Phylogeny and Identifies Variant Genes. Sean D. Gallaher, University of California, Los Angeles, CA.

5 - 9:00

Using whole genome sequencing to identify mutations in *Chlamydomonas*. Susan Dutcher, Washington University School of Medicine, St Louis, MO.

6 - 9:15

A genome-wide mutant library to transform Chlamydomonas research. Xiaobo Li, Carnegie Institution for Science, Stanford, CA.

7 - 9:30

The Functional annotator tool. Michael Schroda, TU Kaiserslautern, Kaiserslautern, Rheinland Pfalz, Germany.

8 - 9:45

Analysis of the transgene expression potential of *Chlamydomonas* expression strains. Rouhollah Barahimipour, Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany.

Monday, June 9 10:30 am–12:00 noon

Assembly of the Flagellar Axoneme

Chair: Susan Dutcher, Washington University School of Medicine

9 - 10:30

A mutation in cullin, a scaffold for the ubiquitin E3 ligase, suppresses missense mutations in cytoplasmic dynein. Susan Dutcher, Washington University School of Medicine, St Louis, MO.

10 - 10:45

Functional analysis of the outer arm dynein assembly factors CCDC103 and FBB18/C21ORF59. Stephen King, University of Connecticut Health Center, Farmington, CT.

11 - 11:00

Novel properties of radial spoke assembly at the proximal axoneme. Lea M. Alford, Emory University, Atlanta, GA.

12 - 11:15

Direct Observation of IFT Turnover at the Flagellar Tip and Base using the Photobleaching Gate Assay. Ahmet Yildiz, University of California, Berkeley, CA.

13 - 11:30

Proteomic and single molecule studies on the role of D1bLIC in retrograde IFT and flagellar assembly. Mary Porter, University of Minnesota, Minneapolis, MN.

14 - 11:45

The ciliary inner dynein arm, I1 preassembles in the cytoplasm and requires IFT for transport in the cilium. Rasagnya Viswanadha, Emory University, Atlanta, GA.

Monday, June 9 1:15 pm–4:00 pm

Light, Carbon, and other Environmental Responses

Chair: Michael Schroda, University of Kaiserslautern

15 - 1:15

How Chlamydomonas responds to heat stress - a top-down Systems Biology approach. Michael Schroda[1,2], 1) TU Kaiserslautern, Kaiserslautern, Germany; 2) Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany.

16 - 1:30

Hypoxia signaling in *Chlamydomonas*. Anja Hemschemeier, Ruhr-University of Bochum, Bochum, Germany.

17 - 1:45

Phosphoprotein SAK1 is a regulator of acclimation to singlet oxygen in *Chlamydomonas reinhardtii*. Setsuko Wakao, University of California, Berkeley, CA.

PLENARY SESSION LISTINGS

18 - 2:00
Insights into ROS signal transduction pathways from algae to higher plants. Ning Shao[1,2], 1) Max-Planck-Institut für Molekulare Pflanzenphysiologie, Potsdam-Golm, Germany; 2) Chinese Academy of Sciences, Guang Zhou ,Guangzhou, China.

19 - 2:15
Safety in Numbers: The Molecular Basis of Predator Evasion Behavior in *Chlamydomonas reinhardtii*. Bradley Olson, Kansas State University, Manhattan, KS.

2:30 pm - **Break**

20 - 2:45
Nuclear proteome dynamics in acclimation to stress. Leonardo Magneschi[1,2], 1) University of Muenster, Muenster, Germany; 2) Alexander von Humboldt Stiftung/Foundation, Jean-Paul-Str., Bonn, Germany.

21 - 3:00
HLA3 and LCIA are associated with inorganic carbon transport in *Chlamydomonas reinhardtii*. Takashi Yamano, Kyoto University, Kyoto, Japan.

22 - 3:15
Two Differentiated Quiescent Cell Types of *C. reinhardtii*. Jannette Rusch, Washington University, St. Louis, MO.

23 - 3:30
Systems analysis of nitrogen sparing mechanisms in Chlamydomonas. Stefan Schmollinger, University of California, Los Angeles.

24 - 3:45
Acclimation responses of *Chlamydomonas reinhardtii* to sulfur deficiency. Munevver Aksoy, Carnegie Institution for Science, Stanford, CA.

Monday, June 9 7:15 pm–9:00 pm
Circadian Rhythm, Cell Cycle, and Development
Chair: James Umen, Donald Danforth Plant Science Center

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25 - 7:15
Identification of a Sizer Protein in *Chlamydomonas*. James Umen, Donald Danforth Plant Science Center, St Louis, MO.

26 - 7:30
Genetic characterization of *Chlamydomonas* cell cycle control. Frederick Cross, Rockefeller University, New York, NY.

27 - 7:45
Defects in a new class of sulfate/anion transporter links sulfur acclimation responses to intracellular glutathione levels and cell cycle control. Su-Chiung Fang, Academia Sinica, Tainan, Taiwan.

28 - 8:00
Transcriptional profiling of the *Chlamydomonas* cell cycle. James Matthew Zones[1,2], 1) The Donald Danforth Plant Science Center, St. Louis, MO; 2) University of California, San Diego, CA.

29 - 8:15
Coexpression analysis of a synchronized day/night cycling culture predicts isoform localization. Ian Blaby, University of California, Los Angeles, CA.

30 - 8:30
Dissection of the functional architecture of *Chlamydomonas* HAP2, the protein essential for gamete membrane fusion in plants and protists. Yanjie Liu, UT-Southwestern Medical Center, Dallas, TX.

31 - 8:45
Genome-wide analysis of gene regulatory networks in early zygote development of *Chlamydomonas reinhardtii*. Jae-Hyeok Lee, University of British Columbia, Vancouver, BC, Canada.

Tuesday, June 10 8:30 am–9:45 am
Biofuels
Chair: Chia-Lin Wei, DOE Joint Genome Institute

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32 - 8:30
Epigenetic interrogation of the algal lipid biosynthetic pathway. Chia-Lin Wei, DOE Joint Genome Institute, Walnut Creek, CA.

33 - 8:45
Dual specificity tyrosine-phosphorylation-regulated kinase DYRKP-1 negatively regulates starch and oil accumulation during nutrient deprivation. Miriam Schulz-Raffelt, CEA, CNRS, Saint-Paul-lez-Durance, France.

34 - 9:00
Substrate specificity and positional preference of *Chlamydomonas* diacylglycerol acyltransferases critical for stress-related biosynthesis of triacylglycerol. Jin Liu, University of Maryland Center for Environmental Science, Baltimore, MD.

35 - 9:15
The Path to Triacylglycerol (TAG) Obesity in the *sta6* Strain of *Chlamydomonas reinhardtii*. Ursula Goodenough, Washington University, St. Louis, MO.

36 - 9:30
Towards a new bioenergetic concept: Increasing the photorespiratory glycolate excretion in *C. reinhardtii* for bio-methane production. Anja Guenther, University of Leipzig, Leipzig, Germany.

PLENARY SESSION LISTINGS

Tuesday, June 10 10:15 am–12:00 noon
Flagellar Length Regulation, Basal Bodies, and Transition Zone
Chair: Carolyn Silflow, University of Minnesota

37 - 10:15
Characterization of the Uni1 protein and its localization on basal bodies. Carolyn Silflow, University of Minnesota, St. Paul, MN.

38 - 10:30
Using *Chlamydomonas* to elucidate the structure and function of the ciliary transition zone. Branch Craige, University of Massachusetts Medical School, Worcester, MA.

39 - 10:45
The role of *LF5* localization in the regulation of flagellar length. Paul A. Lefebvre, University of Minnesota, St Paul, MN.

40 - 11:00
A CDK-like protein kinase is a key regulator of flagellar disassembly. Junmin Pan, Tsinghua University, Beijing, China.

41 - 11:15
IFT74 is required for IFT-A / IFT-B interaction, but not for import of tubulin into flagella. Jason M. Brown, University of Massachusetts Medical School, Worcester, MA.

42 - 11:30
IFT-dependent and IFT-independent transport of tubulin in *Chlamydomonas* flagella. Julie Craft, University of Georgia, Athens, GA.

43 - 11:45
Comparative proteomic analysis of vesicles released from flagellar membrane with isolated flagellar membranes. Huan Long, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei, China.

Tuesday, June 10 7:15 pm–9:00 pm
Photosynthesis
Chair: Jun Minagawa, National Institute for Basic Biology

44 - 7:15
Chloroplast remodeling during state transitions in *Chlamydomonas reinhardtii* as revealed by non-invasive techniques in vivo. Jun Minagawa[1,2], 1) National Institute for Basic Biology, Okazaki, Japan; 2) CREST, Japan Science and Technology Agency, Chiyoda-ku, Tokyo, Japan.

45 - 7:30
Light harvesting in *Chlamydomonas reinhardtii* - between adaptation and acclimation. Roberta Croce, VU University Amsterdam, Amsterdam, The Netherlands.

46 - 7:45
Phosphorylation dependent reorganization of photosynthetic multiprotein complexes. Michael Hippler, University of Münster, Münster, Germany.

47 - 8:00
Photoprotection in Chlamydomonas revisited: role of light and metabolism. Dimitris Petroutsos, CEA, Grenoble, France.

48 - 8:15
Identifying a Novel Type of Violaxanthin De-epoxidase from *Chlamydomonas reinhardtii*. Zhirong Li, University of California, Berkeley, CA.

49 - 8:30
Functional analysis of PBC1, a protein conserved in the green lineage that is associated with the PSII core and involved in high light adaptation. Ligia S. Muranaka, TU Kaiserslautern, Germany.

50 - 8:45
Comprehensive Identification of Genes Responsible for Photosynthesis and the Carbon Concentrating Mechanism of *Chlamydomonas reinhardtii*. Leif Pallesen, Carnegie Institution for Science, Stanford, CA.

Wednesday, June 11 8:30 am–10:00 am
Chloroplast Biogenesis and Function
Chair: William Zerges, Concordia University

51 - 8:30
Past, present, and potential roles of Chlamydomonas research in chloroplast biology. William Zerges, Concordia University, Montreal, Quebec, Canada.

52 - 8:45
Interconnection between the chloroplast HSP70B chaperone system, VIPP1 and the Secretory Pathway in biogenesis of thylakoid membranes in *Chlamydomonas*. Mark Rütgers, TU Kaiserslautern, Germany.

53 - 9:00
Role of a novel SEC14 domain-containing protein in chloroplast lipid trafficking and photoautotrophic growth in *Chlamydomonas reinhardtii*. Jose Gines Garcia Cerdan, University of California, Berkeley, CA.

54 - 9:15
Intron *tscA* RNA is required for assembly of a chloroplast-splicing complex. Christina Marx, Ruhr-University Bochum, Bochum, Germany.

55 - 9:30
Nitric Oxide-triggered remodelling of chloroplast bioenergetics and thylakoid proteins upon nitrogen starvation in *Chlamydomonas reinhardtii*. Yves Choquet, Institut de Biologie Physico-Chimique, Paris, France.

56 - 9:45
Conditional depletion of the chloroplast ClpP1 protein activates nuclear genes involved in autophagy and chloroplast protein quality control. Silvia Ramundo[1,2], 1) University of California, San Francisco, CA; 2) University of Geneva, Geneva, Switzerland.

Wednesday, June 11 10:30 am–12:00 noon
Metabolism (including Hydrogen)
Chair: Arthur Grossman, Carnegie Institution for Science

57 - 10:30
The metabolic face of *Chlamydomonas* in the dark. Arthur Grossman, Carnegie Institution for Science, Stanford, CA.

58 - 10:45
Application of Phenotype Microarray to Improve Metabolic Network Modeling of *Chlamydomonas reinhardtii*. Hong Cai, A Center for Genomics and Systems Biolog, Abu Dhabi, UAE.

59 - 11:00
Identification of mutants with constitutive *HYDA1* expression using a motility screen. Carolyn Silflow, University of Minnesota, St. Paul, MN.

60 - 11:15
The role of pyruvate-ferredoxin-oxidoreductase in *Chlamydomonas reinhardtii* fermentative metabolism. Claudia Catalanotti, Carnegie Institution, Stanford, CA.

61 - 11:30
Discovery of a novel Complex I biogenesis factor regulating mitochondrial gene expression. Patrice Hamel, Ohio State University, Columbus, Ohio.

62 - 11:45
Exploiting thiamine regulation in *Chlamydomonas* for novel regulatory tools. Ginnie T. D. T. Nguyen, University of Cambridge, Cambridge, UK.

Wednesday, June 11 1:15 pm–4:00 pm
New directions in *Chlamydomonas* biology
Chair: José Luis Crespo, CSIC-Universidad de Sevilla

63 - 1:15
Control of ER stress-induced autophagy by ROS in Chlamydomonas. José L. Crespo, CSIC-Universidad de Sevilla, Sevilla, Spain.

64 - 1:30
***Chlamydomonas* Argonaute3 is the major catalytic engine of miRNA-mediated post-transcriptional gene silencing. Tomohito Yamasaki**, Kochi University of Technology, Kochi, Japan.

65 - 1:45
Computational prediction of AGO3 associated microRNAs and their targets in *Chlamydomonas reinhardtii*. Adam Voshall, University of Nebraska-Lincoln, Lincoln, NE.

66 - 2:00
Discovery of long non-coding RNAs via their distinct chromatin signature in Chlamydomonas. Daniela Strenkert, University of California, Los Angeles, CA.

67 - 2:15
A SUMO E2 conjugase mutant in *Chlamydomonas reinhardtii* reveals the role of SUMOylation during stress responses. Donald Weeks, University of Nebraska, Lincoln, NE.

2:30 pm - **Break**

68 - 2:45
Systems Biology in *Chlamydomonas reinhardtii*: A Case Study Using Contractile Vacuole Function and Osmoregulation as Example. Burkhard Becker, Universität zu Köln, Köln, Germany.

69 - 3:00
Evolution of sex in *Chlamydomonas* as a response to grazing. Hanna Koch, Max Planck Institute for Evolutionary Biology, Plön, Schleswig-Holstein, Germany.

70 - 3:15
Interkingdom Signaling Between *Chlamydomonas reinhardtii* and Bacteria. Sathish Rajamani, Bio-Labs, New Mexico Consortium, Los Alamos, NM.

71 - 3:30
RNA is a component of Chlamydomonas flagella and Tetrahymena cilia. Paul A. Lefebvre, University of Minnesota, St Paul, MN.

145 - 3:45
The *Chlamydomonas* S-nitrosylome. Samuel Morisse, Sorbonne Universités UPMC Univ Paris, France.

Thursday, June 12 8:30 am–9:45 am
Beyond *C. reinhardtii* (Volvocales and other Algae)
Chair: Rachael Morgan-Kiss, Miami University, Oxford, OH

72 - 8:30
The Antarctic *Chlamydomonas raudensis*: a case for advances in understanding photosynthetic stress adaptation in non-model organisms. Rachael Morgan-Kiss, Miami University, Oxford, OH.

73 - 8:45
An emerging unicellular green alga model system for studying regulation of photosynthesis. Melissa Roth, University of California, Berkeley, CA..

PLENARY SESSION LISTINGS

74 - 9:00
Body size, offspring number, and generation time in volvocine algae: regulation of interrelated traits during a major evolutionary transition. Deborah E. Shelton, University of Arizona, Tucson, AZ.

173 - 9:15
Discovery of *regA* Family Genes in non-*Volvox* Species. Zachariah Grochau-Wright, University of Arizona, Tucson, AZ.

76 - 9:30
The Genome of *Gonium pectorale*: Early evolutionary co-option of genes important for multicellularity occurred during the transition to colonial multicellularity. Bradley Olson, Kansas State University, Manhattan, KS.

Thursday, June 12 10:15 am–12:00 noon
Mechanism and Regulation of Flagellar Motility
Chair: David Mitchell, SUNY Upstate Medical University

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77 - 10:15
Axonemal dynein assembly mechanisms. David Mitchell, SUNY Upstate Medical University, Syracuse, NY.

78 - 10:30
Breaststroke flagellar photoresponse in *Chlamydomonas reinhardtii*. Kyriacos C. Leptos, University of Cambridge, Cambridge, United Kingdom.

79 - 10:45
Outer Dynein Arm activity is modulated by ethanol. Fan Yang, University of Mississippi Medical Center, Jackson, MS.

80 - 11:00
***IDA6* encodes a conserved subunit required for assembly of the N-DRC and several inner arm dyneins. Mary Porter**, University of Minnesota, Minneapolis, MN.

81 - 11:15
CCDC39 and CCDC40 are needed for assembly of the N-DRC for motility and length control. Susan Dutcher, Washington University School of Medicine, St Louis, MO.

82 - 11:30
Pf32p, a novel central-pair-projection protein required for flagellar beating with a regular periodicity. Masafumi Hirono. University of Tokyo, Tokyo, Japan.

83 - 11:45
Noisy rhythms of a eukaryotic flagellum. Kirsty Y. Wan, University of Cambridge, Cambridge UK.

Thursday, June 12 7:15 pm–9:00 pm
Poster Awards and Announcements
Keynote Lecture

7:15 Poster Awards and Announcements

7:30 Keynote Lecture

Ursula Goodenough, Washington University.

From Light to Sex to Motility to Obesity: Sharing Life with *Chlamydomonas* and the Chlamy Community.

Ursula Goodenough will lift up highlights of her 50 years of Chlamydomonas research, starting with photosynthesis and chloroplast biogenesis studies in Paul Levine's lab at Harvard, followed by studies of mating, flagellar architecture and signaling, cell-wall assembly, and lipid-body formation in her own lab, all with many terrific collaborators.

POSTER SESSION LISTINGS

Mechanism and regulation of flagellar motility

84
FAP174 is an MYCBP-1-like protein that interacts with a central pair A-Kinase Anchoring Protein 240. Jacinta D'Souza, UM-DAE Center for Excellence in Basic Sciences, Mumbai, Maharashtra, India.

Assembly of the flagellar axoneme

85
Life at the tip: IFT-independent transport and exchange of the ciliary tip protein EB1-GFP. Aaron J. Harris, University of Georgia, Athens, GA.

86
A *fap133* mutant gives new insights into dynein 1b. Yuqing Hou, University of Massachusetts Medical School, Worcester, MA.

87
Characterization of an *ift46* suppressor mutant shows that the N-terminus of intraflagellar transport protein IFT46 is critical for importing outer arm dynein into flagella. Yuqing Hou, University of Massachusetts Medical School, Worcester, MA.

88
The motility mutant *pf23* is defective in the DYX1C1 gene and fails to assemble the ciliary inner arm dyneins. Winfield S. Sale, Emory University, School of Medicine, Atlanta, GA.

Flagellar length regulation, basal bodies, and transition zone

89
The uncovering of several new deflagellation genes and the identification of ADF1. Paul Buckoll, Simon Fraser University, Burnaby, BC, Canada.

90
Quantitative proteomic analysis of differentially expressed proteins in a *LF* mutant and wild type cells of *Chlamydomonas reinhardtii*. Dilani Gunasena, Oklahoma State University Center for Health Sciences, Tulsa.

91
Transcriptome analysis of *shf* mutants. Aidan Huene[1,2], 1) Oral Roberts University, Tulsa, OK; 2) Oklahoma State University Center for Health Sciences, Tulsa, OK.

92
Axonemal tubulin polyglutamylation modulates flagellar length by enhancing tubulin turnover at the flagellar tip. Tomohiro Kubo[1,2], 1) University of Massachusetts, Worcester, MA; 2) Graduate School of Science, University of Tokyo, Tokyo, Japan.

93
Identification of the SHF1 gene using a "directed" whole genome sequencing approach. Dylan D. Smith, Oklahoma State University, Tulsa, OK.

94
Role of GSK3 and LiCl in flagellar regeneration and elongation. Limei Wang, Tsinghua University, Beijing, China.

Photosynthesis

95
Microalgal photosynthesis coupled to cell motion in a photobioreactor: kinetic model and dynamic simulation. Claude Aflalo, Ben Gurion University, Midreshet Ben Gurion, Negev, Israel.

96
Molecular characterization of lutein-deficient *Chlamydomonas* mutant with altered non-photochemical quenching. KwangRyul Baek, Hanyang University, Seoul, South Korea.

97
Isolation and Characterization of a High Pmax Mutant of *Chlamydomonas reinhardtii*. Yong Bai, University of California, Berkeley, CA.

98
Molecular Characterization of Chl*b*-lacking mutants in *Chlamydomonas reinhardtii*. Sandrine Bujaldon, CNRS/UPMC UMR7141, Paris, France.

99
A conserved rubredoxin in the thylakoid membrane is required for photosystem II accumulation. Robert Calderon, University of California, Berkeley, CA.

100
The fast and slow reversible fluorescence quenching (*in vivo*) of LHC complexes in *Chlamydomonas reinhardtii*. Emine Dinc, Vrije University Amsterdam, Amsterdam, The Netherlands.

101
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ABSTRACTS

1. Motility and more: the flagellum of African trypanosomes. **Kent L. Hill**. MIMG, UCLA, Los Angeles, CA.

Trypanosoma brucei is a lethal unicellular eukaryote that infects humans and other mammals in sub-Saharan Africa. A central feature of trypanosome biology is the parasite's single flagellum, which is an essential and multifunctional organelle that provides cell propulsion, controls cell morphogenesis and directs cytokinesis. Moreover, the flagellar membrane is a specialized subdomain of the cell surface that mediates attachment to host tissues and harbors multiple virulence factors. We will discuss the structure, assembly and function of the trypanosome flagellum, including canonical roles in cell motility as well as novel and emerging roles in cell morphogenesis and host-parasite interactions.

2. Microalgal glycerolipid metabolism in the context of cell development and cell division. **C. Benning**. Michigan State University, East Lansing, MI.

Glycerolipids are essential to all living cells as they provide the main building blocks for membranes enveloping the cell, but also organelles in eukaryotic cells ranging from chloroplast to cilia. In addition, triacylglycerols sequestered in lipid droplets provide safe storage for fatty acyl groups when cells experience adverse conditions, e.g. nutrient deprivation. The ability of microalgae to synthesize and store large quantities of triacylglycerols has become a prominent engineering target, because increasing the triacylglycerol content of microalgae will enhance the energy density and the quality of the biomass as feedstock for feed, food, and fuels. A key problem hampering maximization of triacylglycerol production in the algal biomass is the inverse relationship between cell growth and division on one hand, and triacylglycerol accumulation on the other. Nitrogen deprivation induces a quiescent state in Chlamydomonas, when cell divisions temporarily come to a halt and triacylglycerol biosynthesis is induced. Upon refeeding nitrogen, the cells exit quiescence, resume growth and remobilize triacylglycerol. Thus, lipid metabolism is intricately interwoven with other cellular processes making its engineering a daunting task. As a first step, we are striving to gain a thorough mechanistic understanding of triacylglycerol biosynthesis, i.e. the enzymes involved, its integration into overall cellular metabolism, and its regulation during the cell cycle. Towards this end we are applying genomic and genetic approaches in reference microalgae, primarily Chlamydomonas, but more recently also Nannochloropsis. Mutants of Chlamydomonas with deficiencies in enzymes and regulatory factors required for triacylglycerol biosynthesis have already provided important new insights into specific aspects of microalgal lipid metabolism including the role of enzymes involved in lipid turnover, a lipid transporter, and a transcription factor orchestrating the adjustment of transcriptional networks during entry and exit of quiescence.

3. Chlamy in the light: technologies for today's discoveries. **Sabeeha S. Merchant**. Institute for Genomics and Proteomics, UCLA, Los Angeles, CA.

Chlamydomonas has served for over half a century as a reference organism for fundamental discoveries in photosynthesis, ciliary biology and sexual reproduction. Biochemical and genetic approaches dominated the literature until a decade ago when genomic methodologies opened up new avenues of investigation, especially with respect to gene discovery and gene expression. Today's approaches include high throughput screening of indexed collections of mutants, quantitative proteomics and transcriptomics, phylogenomics and reverse genetics by both conditional knock-down and knock-out approaches. Sequencing of laboratory strains reveals considerable strain diversity, representing genotypes in the founder strains, which contributes to phenotypic variation in individual studies. Understanding chromatin modifications and the role of non-coding RNAs in the cell cycle, gametogenesis and cilium formation and function is a new area of interest.

4. Genomic Resequencing of Chlamydomonas Laboratory Strains Reveals Corrected Phylogeny and Identifies Variant Genes. **Sean D Gallaher**[1], Sorel T Fitz-Gibbon[2], Sabeeha S Merchant[1]. 1) Chem & Biochem, UCLA, Los Angeles, CA; 2) MCDB, UCLA, Los Angeles, CA.

The majority of laboratory strains of *Chlamydomonas reinhardtii* in use today can trace their lineage to soil samples collected by G.M. Smith in 1945. In 2007, a reference genome was published that was generated from the DNA of one such laboratory strain called "CC-503". Since then, this 120-Mb sequence has been used as the reference genome in countless studies; over 700 articles have cited that publication. The availability of a mature reference genome has been a boon to Chlamydomonas scientists. However, observable phenotypic differences between the laboratory strains suggest that there may be a great deal of unaccounted genetic diversity, and that this variation could be a confounding factor in Chlamydomonas research.

To that end, we embarked on a project to resequence over 40 commonly used laboratory strains, representing all branches of the Chlamydomonas family tree. We utilized the Illumina high-throughput sequencing platform to sequence 100+100 bp paired-end reads of approximately 300 bp genomic fragments, and aligned the resulting reads to the CC-503 reference genome with BWA. Variants, including single nucleotide variants (SNVs), insertions, deletions, and chromosomal rearrangements were identified using a combination of Pindel, BreakDancer, and SnpEff.

We observed that between any two strains, there were as few as 1000 and as many as 500,000 SNVs. Distinct patterns in the distribution of these SNVs were evident, with more closely related strains having similar SNV haplotypes. Using these patterns, we generated a phylogeny of the Chlamydomonas laboratory strains that corrected misconceptions about the relatedness of certain strains. Additionally, we were able to identify many thousands of genes that differ from the reference for strains belonging to certain clades in corrected phylogeny. The availability of this data should be a great benefit to the Chlamydomonas community, as it will allow researchers to positively identify their strains, to make informed choices about which strains to use in their experiments, and to more accurately interpret their results.

5. Using whole genome sequencing to identify mutations in *Chlamydomonas*. Huawen Lin, David Granas, Alison Albee, Paul Cliften, **Susan Dutcher**. Genetics, Washington Univ Sch Med, St Louis, MO.

Forward genetics allows the identification of mutants with phenotypes of interest and the mechanistic understanding of biological process. While gene lesions generated by insertional mutagenesis can be identified by Southern or PCR-based approaches, mutations induced by radiation and chemical mutagenesis relied on time-consuming meiotic mapping. Recently, single nucleotide polymorphism (SNP) discovery by whole genome sequencing (WGS) provides a fast and efficient method to pinpoint the mutations (Lin *et al*., 2013). Our WGS identified between 22,000 and 1,500,000 changes for a total of 2,500,000 noncausative changes in 17 strains. We have made a SNP database, and have used the database with WGS to identify the causative mutations in *FLA9, IMP4, AGG1, PF7*, and *PF8* as well as suppressors of the flagellar assembly mutant, *fla24*. For the *sup1* and *sup2* mutations, we sequenced four and three alleles, respectively, and screened for a gene that had alterations in all four strains. A pooled mapping strategy can be used to identify the map position of a mutant. The *pf27* mutant was crossed to CC-1952. WGS with DNA from a pool of fourteen *pf27* progeny from this cross was performed. Regions that are unlinked to the mutation will show SNPs from both the parent and the highly polymorphic strain. This heterozygosity in the SNPs will produce a ratio of 0.5 for unlinked regions relative to the reference. Regions that are tightly linked should produce a higher frequency of SNPs that are similar to the reference and should show an extended region with a ratio of near zero. Examination of the seventeen chromosomes shows that every chromosome has a region that has a low ratio of heterozygosity; these regions correspond to the centromeric regions. However, only chromosome 12 has an extended region with a low ratio that extends from 5.8 Mb to 7.9 Mb in Version 5.3 of Phytozome for *pf27*. This region was confirmed by dCAPs mapping (Alford et al., 2013). Finally, we have incorporated algorithms for detecting larger insertions and deletions in the genome and find that there are significant numbers in the collection of strains. To ask about the effects of DNA transformation on genome integrity, we sequenced one strain obtained after transformation using 200 ng of plasmid DNA. We find that it has three unlinked insertion sites that contain small pieces of the bacterial DNA used in the transformation. This suggests to us that insertional strains may require multiple rounds of outcrosses to remove these other mutations. Whole genome seqeuncing offers an easy alternative to insertional mutagenesis in *Chlamydomonas* that allows for the examination of conditional mutatoins as well as null mutations.

6. A genome-wide mutant library to transform Chlamydomonas research. **Xiaobo Li**, Weronika Patena, Ru Zhang, Spencer Gang, Sean Blum, Nina Ivanova, Rebecca Yue, Arthur Grossman, Martin Jonikas. Department of Plant Biology, Carnegie Institution for Science, Stanford, CA.

The green alga *Chlamydomonas reinhardtii* is the leading single-celled eukaryotic model for plant biology, however, few mutants are available for studies of genes of interest. We have developed a high-throughput approach that enables the creation of a genome-wide library of indexed mutants in Chlamydomonas. Individual strains are arrayed to 384-agar format and propagated using robotic transfer technology. The genomic location of the mutagenic insertion in each strain is determined using high-throughput methods based on next generation sequencing. To date, we have generated a proof-of-concept library, which contains high-confidence mutants for ~10% of all Chlamydomonas genes. Based on these results, we are moving towards generating the full-scale library, covering approximately 80% of Chlamydomonas genes with at least one allele.

This transformative resource will be made available to the Chlamydomonas research community as a tool to elucidate the functions of uncharacterized genes implicated in photosynthesis, biogenesis of flagella and basal bodies, lipid metabolism, carbon concentration and other important cellular processes. One question that we are addressing with this library is which genes are required for triacylglycerol biosynthesis under stress conditions.

Note: XL, WP, RZ, SG and SB contributed equally to this research.

7. The Functional annotator tool. T. Mühlhaus, A. Lüdemann, **M. Schroda**. Molecular Biotechnology & Systems Biology, TU Kaiserslautern, Kaiserslautern, Rheinland Pfalz, Germany.

Using controlled vocabularies for functional annotation or functional ontology simplifies the exploration and interpretation of high-throughput data by humans and machines. The MapMan ontology is represented as a hierarchically structured tree that comprises 35 main biological categories grouping 1854 functional categories (bins). It follows the paradigm of trying to assign a protein/gene to as few bins as possible. Functional analysis based on hierarchically structured annotations can directly and consistently be used for cross-species and cross-experiment comparisons. In addition to the MapMan ontology, the Functional annotation tool features a protein localization ontology. This ontology also is designed as a hierarchical tree structure and currently comprises 13 main localization terms and 49 sub-localization terms. The Functional annotation tool is a comprehensive data analyzing environment running as a web based cloud service tool within the IOMIQS framework that allows users to work with the ontologies in an interactive manner. The main functionalities of the application are: (1) Batch gene/protein identifier mapping and functional annotation. (2) Gene/protein database searching. (3) TreeMap based ontology visualization and interactive browsing. (4) Ontology term enrichment - testing and visualization. (5) Annotation of MapMan protein/gene functions and assignment of localization ontologies according to the user's expertise. (6) Addition of protein/gene synonyms. (7) Addition of protein/gene descriptions as free text. Entries made are tracked and immediately active. The tool is available at http://iomiqsweb1.bio.uni-kl.de/.

8. Analysis of the transgene expression potential of *Chlamydomonas* expression strains. **Rouhollah Barahimipour**, Juliane Neupert, Ralph Bock. Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany.

The green alga *Chlamydomonas* has become an important model organism for both basic research and biotechnology. However, harnessing the full potential of this microalga has been hampered by the presence of a strong silencing mechanism that affects the expression of nuclear transgenes. In a genetic screen for mutants with improved transgene expression properties, we have recently isolated two expression strains (UVM4 and UVM11) that show a dramatically increased expression level of various transgenes (*GFP*, *vYFP* and *LUC*). To further characterize these expression strains and their capacity to express different transgenes, we have begun to systematically investigate the effects of G/C content, codon adaptation and gene length on the efficiency of transgene expression in strain UVM11 and a wild-type strain. Testing a series of different *YFP* reporter genes, our results revealed that codon optimization is important, even for the expression strain. A/T-rich gene variants could not be expressed, neither in the wild type nor in the expression strain. Increasing the G/C content of the transgene to the level in the nuclear genome improves expression, but is not sufficient to obtain high expression levels. We also designed gene variants that allowed us to distinguish between the impact of G/C content and that of codon usage on expression efficiency. By fully optimizing codon usage, we were able to achieve protein accumulation levels of up to 1.2% of the total soluble protein in transformed UVM11 clones. Using the *Chlamydomonas* expression strains and engineering transgenic DNA sequences according to our observations will facilitate new applications in molecular biology and biotechnology.
Key words: *Chlamydomonas reinhardtii*, expression strains, transgene expression, codon usage, G/C content, gene length, *YFP* variant.

9. A mutation in cullin, a scaffold for the ubiquitin E3 ligase, suppresses missense mutations in cytoplasmic dynein. **Susan Dutcher**, Nicholas Nauman, Katherine Ferkol, Huawen Lin. Genetics, Washington Univ Sch Med, St Louis, MO.

Ubiquitination plays an important role in many cellular processes that include cell cycle regulation, apoptosis, and ER quality control. Polyubiquitination is generally a signal that targets proteins for degradation. Huang and Rosenbaum (2009) showed that many flagellar proteins become ubiqinated during flagellar resorption and early stages of mating, as well as in mutants in retrograde IFT genes and motors at the permissive temperature. The *fla24* mutant is caused by a leucine to proline change; this amino acid lies in an alpha helix at the base of the buttress in cytoplasmic dynein. It reduces the amount of DHC1b and D1bLIC at both the permissive and restrictive temperatures (Lin *et al.*, 2013). We screened for intragenic revertants and extragenic suppressors of the flagellar assembly defect at 32C. We have identified intragenic events at amino acid 3423 that change the proline back to leucine, serine, or arginine as well as intragenic revertants in the linker arm, which the mechanical amplifier of the dynein motor. These will allow us to probe the role of the linker in dynein activity. In addition, we found mutations in 5 other genes that suppress the mutant phenotype (*SUP1-SUP5*) based on pairwise recombination analysis. The *SUP1* locus has 37 alleles, *SUP2* has three alleles, and *SUP3-SUP5* each have a single allele. The *SUP3* locus carries a mutation in cullin, the scaffold needed to assemble the E3 ubiquitin ligase complex. Cre17.g734400 is one of three cullins in the Version 5.3 Phytozome genome and is likely to be a homolog of the cullin1 or cullin 2 family by phylogenetic analysis. The *sup3* missense mutation rescues the flagellar assembly defect of two different cytoplasmic dynein alleles (*fla24* and *dhc1b-3*), but does not suppress two null alleles in cytoplasmic dynein. The *sup3* mutation also does not suppress motility phenotypes of mutations in genes for axonemal proteins (DRC4, katanin p60, katanin p80, RSP3, DIC2) or the flagellar assembly defect of the anterograde motor, FLA10. Thus, it is not a general suppressor of axonemal defects. Using antibody to ubiquitin, immunoblots of isolated flagella show that the *fla24*; *sup3* double mutant flagella lacks the high level of ubiquinated proteins observed in the flagella of the *fla24* single mutant. We postulate that the *sup3* mutation acts to prevent the degradation of the IFT machinery by blocking ubiquitination. We are currently testing if this cullin is neccessary for ubiquitintion during in flagellar resorption and mating.

10. Functional analysis of the outer arm dynein assembly factors CCDC103 and FBB18/C21ORF59. **Stephen King**, Ramila Patel-King. Molecular Biology and Biophysics, Univ Connecticut Health Ctr, Farmington, CT.

Defects in CCDC103 and FBB18/C21ORF59 have been identified as causing primary ciliary dyskinesia in both zebrafish and humans due to the loss of outer dynein arms, and in the case of FBB18/C21ORF59 some inner arm structures as well (Panizzi *et al* 2012; Austin-Tse *et al* 2013). *Chlamydomonas* CCDC103 (Pr46b) is an ~29 kDa protein that is present in both cytoplasm and flagella. In cytoplasmic extracts, CCDC103 migrates in two large complexes of ~1 MDa and 300 kDa. In flagella, it is very tightly associated with the axoneme and can only be extracted with 0.5-1 M KI which essentially solubilizes the entire structure. Immunofluorescence revealed that CCDC103 is arrayed along the entire axonemal length but with an apparent concentration at the distal tip which may represent either an accumulation of this protein or enhanced antibody accessibility. CCDC103 is present in wildtype amounts in axonemes from mutants lacking the outer arm, docking complex or ODA5p indicating that it does not require any of these other structures for axonemal assembly. CCDC103 has rather extraordinary biophysical properties with a T_m of ~80^{o}C; it even refolded to its apparent native conformation following heating at 100^{o}C for 2 mins in 1% SDS. CCDC103 migrates as a mixture of monomers and dimers upon SDS-PAGE of freshly prepared gel samples of either axonemes, cytoplasmic extracts or recombinant protein; in stored samples, it forms very high-order oligomers that are SDS/DTT/heat resistant. Recombinant CCDC103 binds to taxol-stabilized microtubules and shows an apparent preference for actively assembling microtubules. In contrast, FBB18 is present in the flagellar matrix and migrates as a single small complex of ~60 kDa. In wildtype flagella, the amount of FBB18 present is low, but both flagellar and cytoplasmic levels are dramatically increased in mutants (*e.g*. *pf18*) exhibiting severe flagellar motility defects. These levels further increased in a time-dependent manner when a *fla10 pf18* double mutant (gift of David Mitchell) was placed at the restrictive temperature. Thus, FBB18 may be part of a feedback mechanism employed by the cell when motility is significantly impaired.

ABSTRACTS

11. Novel properties of radial spoke assembly at the proximal axoneme. **Lea M. Alford**[1], Alexa L. Matteyses[1], Emily L. Hunter[1], Huawen Lin[2], Susan K. Dutcher[2], Winfield S. Sale[1]. 1) Cell Biology, Emory University. 465 Whitehead Building, 615 Michael St., Atlanta, GA 30322; 2) Department of Genetics, Washington University School of Medicine. Box 8232, 660 S. Euclid Ave., St. Louis, MO 63110.

The overall goal is to determine mechanisms of assembly of the ciliary axoneme, focusing on assembly of the radial spoke. The 12S radial spoke precursor assembles in the cell body before being transported by intraflagellar transport (IFT) and docked as a fully assembled 20S radial spoke structure onto the axoneme (Qin et al., 2004; Diener et al., 2011). However, details of entry into and transport within the cilium are not well understood. We analyzed the paralyzed mutant *pf27* that is deficient in axonemal radial spokes. Using whole genome sequencing and molecular mapping strategies, we confirmed the location of *PF27* on chromosome 12, but we have not yet identified *PF27*. Notably, we determined that in *pf27* radial spokes are fully assembled 20S spoke complexes, but are localized only to the proximal quarter of the axoneme. Based on *in vitro* reconstitution studies, the *pf27* axoneme is not defective in radial spoke docking, but rather is likely defective in radial spoke transport. Consistent with a failure in radial spoke transport, radial spoke proteins are greatly reduced in the membrane-matrix of *pf27* cilia. Rescue of radial spoke assembly in temporary dikaryons with wild-type cells first occurs at the distal end of the pf27 cilium and then proceeds toward the proximal end of the axoneme. Importantly, this rescue is independent of established proximally assembled radial spokes. Furthermore, in regenerating *pf27* cilia radial spokes assemble asynchronously from other axonemal components, presumably at the proximal end. From these results we postulate that PF27p is required for IFT-mediated transport of radial spokes by IFT. Moreover, *pf27* reveals that diffusion of radial spoke precursors may be sufficient for entry to the cilium and assembly on the proximal axoneme, but that this process of assembly is quite inefficient.

12. Direct Observation of IFT Turnover at the Flagellar Tip and Base using the Photobleaching Gate Assay. **Ahmet Yildiz**. Molecular Cell Biology, University of California Berkeley, Berkeley, CA.

Tracking the dynamics of individual molecules and complexes in cilia is a challenging task due to their crowdedness and complexity. We developed a photobleaching gate assay that photobleaches a cilium and allows only a single fluorescent particle to enter at a time. We used this method to detect the arrival and departure of single IFT trains and motor complexes at the flagellar base and tip in *Chlamydomonas*. We observed that a single anterograde train remains at the tip for 3 s on average in two rate-limiting steps and splits into multiple retrograde trains. In the steady-state, multiple anterograde trains coexist at the tip and they mix with each other to form retrograde trains. Retrograde trains usually follow a separate track than the anterograde train, suggesting that IFT trains detach from microtubules during the remodeling at the tip. Kinesin-2 motors dissociate from IFT trains at the tip and are recycled back to the cell body by diffusion. Furthermore, we selectively bleached IFT trains in one flagellum and monitored the fluorescence recovery in both flagella. We observed that flagellar base is an open system, which allows exchange of particles between flagella. On the basis of these results, we propose new models for mixing and remodeling of IFT particles at the flagellar tip, coordination of IFT motors and flagellar length control.

13. Proteomic and single molecule studies on the role of D1bLIC in retrograde IFT and flagellar assembly. A. Schauer, J Reck, K VanderWaal, R Bower, D Tritschler, C Perrone, **M Porter**. GCD, University of Minnesota, Minneapolis, MN.

Retrograde IFT is driven by a cytoplasmic dynein known as DHC1b. Dynein motors are associated with IC and LC subunits that contribute to motor stability and cargo binding, but identity and function of such subunits are incompletely understood. We previously identified a LIC subunit as a component of the retrograde motor (Perrone et al., 2003). *d1blic* mutations are associated with flagellar assembly defects and accumulation of IFT particles (Hou et al., 2004), but the phenotype is not as severe as those observed with *dhc1b* or *fla14* mutations (Pazour et al., 1998, 1999; Porter et al., 1999). To better understand D1bLIC function, we analyzed IFT in a *d1blic* null mutant, a GFP-tagged rescued strain, and several strains in which *D1bLIC* expression was reduced. Knockdown to ~20% of wt levels reduced the frequency and velocity of retrograde IFT with minimal effects on anterograde IFT or flagellar length. Knockdown below 10% resulted in more severe defects in retrograde IFT and flagellar length and regeneration, similar to the *d1blic* null. iTRAQ analysis identified >700 proteins in wild-type and mutant flagella, but only a small subset were significantly altered in the mutant. Several membrane plus matrix polypeptides, including IFT and BBSome subunits, were elevated. However, a smaller subset of proteins were reduced in the mutant; these include novel polypeptides that may interact with the retrograde IFT motor and/or influence cell behavior. Transformation of *d1blic* with *D1bLIC-GFP* rescued the assembly defects and increased the velocity and frequency of retrograde IFT. D1bLIC-GFP copurifies with other subunits of the retrograde motor during dynein extraction, sucrose density gradient centrifugation, and immunoprecipitation. FRAP analysis revealed that the D1bLIC subunit undergoes a rapid transition from anterograde to retrograde transport at the flagellar tip similar to that described for IFT subunits. The D1bLIC-GFP construct will therefore be a useful reagent for the characterization of mutations in several genes that regulate retrograde IFT, flagellar assembly, and ciliary signaling.

14. The ciliary inner dynein arm, I1 preassembles in the cytoplasm and requires IFT for transport in the cilium. **Rasagnya Viswanadha**[1], Emily Hunter[1], Ryosuke Yamamoto[1], Lea Alford[1], Maureen Wirschell[2], Winfield Sale[1]. 1) Cell Biology, Emory University, Atlanta, GA; 2) Biochemistry, University of Mississippi, Jackson, MS.

To determine mechanisms of assembly of flagellar/ciliary dyneins, we focused on the inner dynein arm, I1. Fractionation of cytoplasmic extracts from wild-type (WT) cells revealed that I1 dynein assembles as a 20S complex prior to entry into the ciliary compartment. The WT cytoplasmic 20S complex appears to have the same composition as the isolated axonemal I1 complex. However, based on *in vitro* reconstitution assays, and unlike axonemal I1 dynein, the 20S cytoplasmic I1 dynein is not competent to bind axonemes. Analysis of cytoplasmic extracts from *ida7* (IC140-null) revealed that the intermediate chain subunit IC140 is required for assembly of the 20S cytoplasmic I1 complex. We used cytoplasmic complementation in dikaryons to test the hypothesis that I1 dynein is transported to the distal tip of the cilia for assembly in the axoneme. In dikaryons formed between WT and I1 dynein mutants, rescue of I1 assembly efficiently occurred and began at the distal tip of the mutant cilium. In contrast to other dikaryon combinations, I1 dynein assembly was

significantly delayed in dikaryons between *ida7* and *ida3*. Unique to the *ida7* x *ida3* dikaryon combination, cyclohexamide completely blocked rescue of I1 dynein assembly, indicating new synthesis of either or both IC140 and IDA3p is required for rescue. To test the hypothesis that I1 dynein is transported to the tip by IFT, we took advantage of the kinesin-II mutant, *fla10^{ts}* to conditionally disrupt IFT. In dikaryons formed between *fla10^{ts}* and *fla10ida3^{ts}* at permissive temperature (21°C), I1 dynein is transported to the tip. However at restrictive temperature (32°C), when kinesin-II is rendered inactive, I1 transport to the tip is completely inhibited. Thus, I1 dynein complexes require kinesin-II for transport to the distal tip prior to assembly in the axoneme. We postulate that Ida3p is required for I1 dynein transport.

15. How Chlamydomonas responds to heat stress - a top-down Systems Biology approach. D. Hemme[1,2], D. Veyel[2], T. Mühlhaus[1,2], F. Sommer[1,2], J. Jüppner[1,2], A. Unger[3], M. Sandmann[4], I. Fehrle[2], S. Schönfelder[2], M. Steup[4], S. Geimer[3], J. Kopka[2], P. Giavalisco[2], **M. Schroda[1,2]**. 1) Molecular Biotechnology & Systems Biology, TU Kaiserslautern, Kaiserslautern, Germany; 2) Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany; 3) Biology/Electron Microscopy, University of Bayreuth, Bayreuth, Germany; 4) Institute of Biochemistry und Biology, University of Potsdam, Potsdam-Golm, Germany.

We applied a top-down systems biology approach to obtain a better understanding of how *Chlamydomonas reinhardtii* acclimates to thermal stress. For this, we shifted cells from 25°C to 42°C for 24 hours and allowed them to recover at 25°C. We monitored protein dynamics via ^{15}N shotgun proteomics and quantified 1985 proteins. Furthermore, we analyzed levels of 63 polar and 187 lipophilic metabolites and monitored cytological and physiological parameters. Principal component analysis allowed distinguishing five heat stress response phases: (1) a very early response phase characterized by cell cycle arrest, depletion of central metabolites and the accumulation of potential compatible solutes. (2) An early response characterized by the accumulation of molecular chaperones, the decline of cytosolic ribosomes, the *de novo* synthesis of lipids with saturated fatty acids and the deposition of unsaturated fatty acids in lipid bodies. (3) A late response during which the capacities of photosynthetic oxygen evolution and respiration decrease, LHCs uncouple, aberrant structures at nodal points of thylakoid membranes appear, and ROS scavenging enzymes accumulate. (4) A recovery phase during which DNA synthesis resumes and most stress-induced changes relax. (5) A modified growth phase where cells resume growth and division although most stress-induced changes have not yet fully relaxed.

16. Hypoxia signaling in *Chlamydomonas*. Melis Düner, Dennis Huwald, Thomas Happe, **Anja Hemschemeier**. Dept Photobiotechnology, Ruhr-University of Bochum, Bochum, NRW, Germany.

In anaerobiosis, *Chlamydomonas* develops a complex fermentative metabolism that involves the generation of molecular hydrogen as well as products typical for bacterial mixed-acid fermentation such as formate. The microalga can grow under hypoxic conditions in the light and tolerates anaerobiosis in the dark for quite some time. In a recent study applying RNA-Seq we showed that the acclimation to dark-anoxia results in massive changes of the transcriptome (1). To date, only one transcription factor that activates a sub-set of genes in anoxia has been confirmed. This is the COPPER RESPONSE REGULATOR 1 (CRR1), the master regulator of the copper deficiency response of *Chlamydomonas* (2, 3). In dark-anoxia, however, only a fraction of the transcriptional changes depend on CRR1 as inferred from transcript profiles of *crr1* mutants (1). This indicates the presence of additional regulatory factors. We have recently shown that a so-called 2-on-2 helices- or truncated hemoglobin (THB) as well as nitric oxide (NO) are important signaling components during the hypoxic response of *Chlamydomonas* (4). The algal genome encodes for at least twelve THBs, which are quite unusual in length and domain arrangement. Furthermore, at least six genes coding for heme/NO-binding or soluble guanylate cyclases (sGCs), know from mammals to confer NO signals via cGMP generation, were identified (5). Here, new insights into *Chlamydomonas* THBs as well as the role of a sGC in the hypoxic response will be presented.

1. A. Hemschemeier *et al.*, *Plant Cell* **25**, 3186 (2013)
2. J. Kropat *et al.*, *PNAS* **102**, 18730 (2005)
3. M. Castruita *et al.*, *Plant Cell* **23**, 1273 (2011)
4. A. Hemschemeier *et al.*, *PNAS* **110**, 10854 (2013)
5. A. de Montaigu *et al.*, *Plant Cell* **22**, 1532 (2010)

17. Phosphoprotein SAK1 is a regulator of acclimation to singlet oxygen in *Chlamydomonas reinhardtii*. **Setsuko Wakao[1]**, Brian Chin[1], Heidi Ledford[1], Rachel Dent[1], David Casero[2], Matteo Pellegrini[2], Sabeeha Merchant[3], Krishna Niyogi[1,4]. 1) Plant and Microbial Biology, University of California, Berkeley, CA 94720; 2) Molecular, Cell, and Developmental Biology, University of California, Los Angeles, CA 90095; 3) Chemistry and Biochemistry, University of California, Los Angeles, CA 90095; 4) Howard Hughes Medical Institute.

Growth of photosynthetic organisms depends on light energy, which in excess causes oxidative damage to the cell if not managed properly. Singlet oxygen is a highly toxic form of reactive oxygen species that is predominantly generated at the reaction center of photosystem II. We have previously found that the unicellular green alga *Chlamydomonas reinhardtii* is capable of acclimating specifically to singlet oxygen and herein describe a mutant defective in acclimation, *singlet oxygen acclimation knocked-out 1* (*sak1*). Analysis of genome-wide changes in RNA abundance during acclimation to singlet oxygen by RNA-seq revealed that SAK1 is a key regulator of many of the gene expression response during acclimation. The *SAK1* gene encodes an uncharacterized protein with a domain conserved among chlorophytes and present in some bZIP transcription factors. The SAK1 protein level was induced by exposure to singlet oxygen, and this was accompanied by phosphorylation of SAK1. Subcellular fractionation indicates the protein to be predominantly in the cytosol where it possibly plays a role in a phospho-relay. Candidate regulatory genes that were up-regulated during acclimation were

identified from our RNA-seq data and their functional roles are being tested by knock-down using artificial microRNA. The interacting partners of SAK1 are being searched by coimmunoprecipitation followed by mass spectrometry.

18. Insights into ROS signal transduction pathways from algae to higher plants. **N. Shao[1,2]**, G. Duan[1], R. Bock[1]. 1) Max-Planck-Institut für Molekulare Pflanzenphysiologie, 14476 Potsdam-Golm, Germany; 2) Plant Gene Engineering Center , South China Botanical Garden, Chinese Academy of Sciences, Guang Zhou ,Guangzhou 510650, China.

Reactive oxygen species (ROS) have recently emerged as important cellular signalling molecules controlling development, initiating cellular stress responses and cell death in a wide range of organisms. Relatively little is known about the molecular mechanisms underlying the perception of ROS and initiation of cellular responses in eukaryotes. To explore this facet of ROS signaling transduction, we developed the combination of high-throughput forward genetic screens with a ROS-inducible reporter in the unicellular green alga *Chlamydomonas* and reverse genetic analyses in *Arabidopsis*. Searching for possible ROS sensor proteins, we identified a small zinc finger protein, METHYLENE BLUE SENSITIVITY (MBS), that is required for induction of singlet oxygen–dependent gene expression and, upon oxidative stress, accumulates in distinct granules in the cytosol. Loss-of-function *mbs* mutants produce singlet oxygen but are unable to fully respond to it at the level of gene expression. Knockout or knockdown of the homologous genes in the higher plant model *Arabidopsis thaliana* results in mutants that are hypersensitive to photooxidative stress, whereas overexpression produces plants with elevated stress tolerance. Together, our data indicate an important and evolutionarily conserved role of the MBS protein in ROS signaling and provide a strategy for engineering stress-tolerant plants. The combination of sensitive genetic screens in *Chlamydomonas* with subsequent functional analyses in both algae and higher plants provides a powerful strategy to dissect ROS responses and identify new players in the cellular networks controlling ROS homeostasis, sensing and signaling. The identification of MBS provides proof of principle for this approach and the many other mutants isolated in our genetic screen will provide a rich source for future investigations into the mechanisms of ROS signaling.

The author gratefully acknowledges the support of K.C. Wong Education Foundation, Hong Kong.

19. Safety in Numbers: The Molecular Basis of Predator Evasion Behavior in *Chlamydomonas reinhardtii*. **Bradley Olson**, Christopher Berger, Sarah Cossey, Nicole Richardson. Div Biology, Kansas State Univ, Manhattan, KS.

The current view of multicellular evolution is that ecological pressures such as predation and/or scarce resources drive genetic permanence of cellular cooperation. It has been presumed that the simplest mechanism to evolve multicellularity is clonal development, where incomplete mitosis and/or meiosis cause resultant cells to remain attached. Since clonal development is thought to be easy to evolve the presumption is that this mechanism underlies multicellular evolution in most taxa, including plants and animals. Aggregative cooperation is another mechanism of multicellular evolution that is observed in social amoeba, slime molds, and Rhizaria, where cells in the environment aggregate in response to stress, which provides them safety in number, and motility advantages. As this behavior is thought to be genetically complex, the assumption is that aggregative cooperation has a higher barrier for its evolution and thus is a special case. However, we found that predation of *Chlamydomonas* cells results in cellular aggregative cooperation within minutes of predator treatment. We have found that interaction of *Chlamydomonas* with a predator causes the algae to release a chemical signal into the media that when added to untreated *Chlamydomonas* cell causes them to aggregate. Moreover, *Chlamydomonas* will only aggregate with genetically similar strains, not other species with in the same genus, nor other closely related algae. This suggests that the aggregation signal elicits a self-self recognition mechanism for aggregation. We have characterized this chemical signal and it reveals a surprising conclusion, that laboratory strains of *Chlamydomonas* communicate important information about the environment and respond accordingly. Subsequently, we have performed RNA-seq on a time-course of predator treatment and identified genes important for the predator response. In summary, our data reveal an unrealized mechanism by which unicellular *Chlamydomonas* communicates the presence of predators and allows them to interpret this signal to determine if they should aggregate, resulting in groups that are more resistant to predation. Finally, these data question aggregative cooperation may be a more common mechanism of multicellular evolution than currently thought.

20. Nuclear proteome dynamics in acclimation to stress. **L. Magneschi[1,2]**, R. Bayersdorf[1], D. Nikolova[1], C. Fufezan[1], M. Hippler[1]. 1) Institute of Plant Biology and Biotechnology, University of Muenster, Muenster, Germany; 2) Alexander von Humboldt Stiftung/Foundation, Jean-Paul-Str. 12, 53173 Bonn, Germany.

The green alga *Chlamydomonas reinhardtii* is characterized by an extreme metabolic flexibility, as revealed by its ability to grow in presence of different carbon sources and to cope with a wide range of abiotic stresses. However, little is still known on the mechanisms and the molecular players allowing algae to acclimate to distinct environmental conditions. We undertook a sensitive proteomic approach to dissect the nuclear proteome of the green alga *Chlamydomonas reinhardtii* under different environmental and metabolic setups. First, we developed a high-purity nuclei isolation protocol for Chlamydomonas. Second, HPLC-ESI MS/MS was used to analyze both nuclei and whole cell samples from metabolically (^{14}N or ^{15}N) labeled cultures subjected to anoxia, iron starvation, or distinct trophic conditions. This array of samples permitted us to identify stress-specific or low abundant nuclear proteins, thereby enhancing the nuclear proteome dataset. Moreover we devised an innovative bioinformatic approach which, based on our quantitative proteomics data, allowed us to determine the nuclear or cellular localization of several thousand proteins, their stress-specific or general pattern of expression and their phosphorylation status. Candidate nuclear proteins significantly up-regulated under the investigated stress conditions were chosen for further physiological and molecular characterization. AmiRNA knock-down lines were produced and employed to assess the role of these proteins in the acclimation to the respective stresses.

21. HLA3 and LCIA are associated with inorganic carbon transport in *Chlamydomonas reinhardtii*. **Takashi Yamano**, Emi Sato, Hiro Iguchi, Hideya Fukuzawa. Graduate School of Biostudies, Kyoto Univ, Kyoto, Japan.

The supply of inorganic carbon (Ci; CO_2 and HCO_3^-) is one of the environmental limiting factors for photosynthesis. To overcome the difficulty in acquiring Ci at low-CO_2 concentrations, most microalgae induce an active transport system called the carbon-concentrating mechanism (CCM) to increase the Ci concentration around the pyrenoid, a specialized Rubisco-enriched structure in the chloroplast. *Chlamydomonas reinhardtii* has been used as a model organism for studying the CCM. Although several candidate genes responsible for Ci uptake have been identified by transcriptome analyses, their functional evaluation has been difficult due to their overlapping functions in Ci transport, and some of their subcellular localization has not been validated experimentally. Here, the subcellular localization of two candidate Ci transporters, LCIA and HLA3, were revealed to the chloroplast envelope and the plasma membrane, respectively, by immunofluorescence analysis and membrane fractionation. Furthermore, we used an inducible overexpression system with LCIA and HLA3 to characterize their functions in the Ci transport system. Although single overexpression of LCIA or HLA3 in high-CO_2 conditions did not affect the photosynthetic characteristics of the cells, simultaneous overexpression of these genes led to increases in photosynthetic Ci affinity, Ci accumulation, and CO_2 fixation. We also revealed that newly isolated LCIA insertion mutant grown in LC conditions showed decreased photosynthetic Ci affinity especially in high pH conditions, where HCO_3^- is the predominant form of Ci available. Our results demonstrate that LCIA and HLA3 are cooperatively associated with Ci transport thorough the chloroplast envelope and plasma membrane, respectively.

22. Two Differentiated Quiescent Cell Types of *C. reinhardtii*. **Jannette Rusch**, Robyn Roth, Tuya Wulan, Ursula Goodenough. Washington University, St. Louis, MO.

Cycling log-phase cells are likely to be in the minority in the native soil habitats of *C. reinhardtii,* with most cells instead in one of 3 dormant states: 1) quiescence due to an acute dilution/withdrawal of one or more nutrients -- our lab model being the sudden imposition of nitrogen starvation (-N) to log-phase cells; 2) quiescence due to a gradual depletion of nutrients -- our lab model being cultures entering stationary phase (SP); or 3) sexual zygotes. Both -N and SP cells rapidly differentiate into gametes, but those that fail to find mating partners enter holding patterns that allow long-term viability. Our studies document that -N and SP cells each adopt distinctive states of differentiation.

Chloroplasts SP cells remain fully green and maintain intact thylakoids, as assessed by quick-freeze deep-etch EM, whereas -N cells become chlorotic and their thylakoids are dismantled.

Rubisco levels By western blot, the small subunit of Rubisco is undetectable in -N cells after 12 h and the large subunit by 4 days, whereas both signals remain at log-phase levels in SP cells for 8 days.

APG3 and APG8 levels By western blot, both autophagy-related enzymes do not show strong increases in signal until 5 days -N, whereas strong increases accompany the transition into SP.

Lipid body (LB) formation -N cells initiate copious LB production, whereas very few LBs are produced by SP cells. Adding acetate to SP medium augments LB formation somewhat, but levels remain low compared with -N cells.

Vacuoles A particularly distinctive feature of SP cells is their rapid generation of numerous vacuoles with a fibrillar matrix and few membranous inclusions, which greatly enlarge with time. By contrast, after 3 days, -N cells produce vacuoles with a watery matrix and large membrane inclusions. The SP vacuolar membrane appears to derive largely from the outer chloroplast envelope, with contributions from golgi vesicles; the membrane encircles “islands” of cytoplasm that have denatured into the fibrillar configuration. The SP vacuoles are not acidic when assessed with pH-sensitive dyes, suggesting that they may store denatured, but not proteolyzed, protein. When SP cells are provisioned with acetate, the vacuoles disappear.

The following abstracts present additional studies of autophagy: Chang et al, Goodenough et al, Plancke *et al.*, and Ramundo *et al*.

23. Systems analysis of nitrogen sparing mechanisms in Chlamydomonas. **Stefan Schmollinger[1]**, Timo Mühlhaus[2,3], Nanette R. Boyle[1], Ian K. Blaby[1], David Casero[4,6], Tabea Mettler[3], Jeffrey L. Moseley[5], Janette Kropat[1], Frederik Sommer[2,3], Daniela Strenkert[1], Dorothea Hemme[2,3], Arthur R. Grossman[5], Mark Stitt[3], Matteo Pellegrini[4], Michael Schroda[2,3], Sabeeha S. Merchant[1,6]. 1) Department of Chemistry and Biochemistry, UCLA; 2) Molecular Biotechnology and Systems Biology, TU Kaiserslautern; 3) Max Planck Institute of Molecular Plant Physiology, Potsdam; 4) Department of Molecular, Cell, and Developmental Biology, UCLA; 5) Department of Plant Biology, Carnegie Institution for Science; 6) Institute of Genomics and Proteomics, UCLA.

In order to understand the acclimation of the metabolism of *Chlamydomonas reinhardtii* to nitrogen limitation we performed a multi-systems analysis within the first 48 hours of N deprivation. We used RNA-Seq to probe the transcriptome and untargeted quantitative LC-MS/MS to investigate the proteome. In addition the output is anchored to cellular physiology through measurements of cell growth, Chl content, C and N content, photosynthetic and respiratory capacity, and key metabolite pools.

In the presence of acetate, respiratory metabolism is prioritized over photosynthesis; consequently the N-sparing response targets proteins, pigments and mRNAs involved in photosynthesis and chloroplast function over those involved in respiration. Encoding RNAs and proteins of the Calvin-Benson cycle are reduced in N-deficient cells resulting in the accumulation of cycle intermediates. Additionally, tetrapyrrole biosynthesis decreased early, while chlorophyll degradation was induced, resulting in a total reduction of chlorophyll content per cell. Both cytosolic and chloroplast ribosomes were reduced, but via different mechanisms, reflected by rapid changes in abundance of RNAs encoding chloroplast ribosomal proteins but not cytosolic ones. RNAs encoding transporters and enzymes

for metabolizing alternative N sources increase in abundance, as is appropriate for the soil environmental niche of Chlamydomonas. A comparative inventory of the N-replete vs. N-deplete proteome indicated that abundant proteins with a high N content are reduced in N-starved cells while the proteins that are increased have a lower than average N content. This previously-unreported sparing mechanism contributes to a lower cellular N/C ratio, and suggests an approach for engineering increased N-use efficiency.

24. Acclimation responses of *Chlamydomonas reinhardtii* to sulfur deficiency. **Munevver Aksoy**, Arthur Grossman. Department of Plant Biology, Carnegie Institution for Science, Stanford, CA.

Sulfate (SO_4^{2-}) is the most abundant sulfur (S)-compound in the nature. While animals are incapable of reducing and assimilating SO_4^{2-}, it can be taken up by photosynthetic organisms, reduced and incorporated into organic compounds. However, even when abundant in the environment, SO_4^{2-} can be bound to soil particles where it is not readily accessible to plants and microbes. We have been studying the acclimation of *Chlamydomonas reinhardtii* (Chlamydomonas throughout) to S deficiency. S-deprived Chlamydomonas exhibits elevated scavenging of S from both external and internal sources as well as decreased growth and photosynthetic activity. We have identified several factors that are involved in the S acclimation process. There appears to be a two tiered transcriptional response to S deprivation; activation of first tier genes does not require *de novo* protein synthesis, whereas activation of second tier genes requires *de novo* protein synthesis. The gene set associated with first tier regulation includes three of the high affinity SO_4^{2-}transporters. Transcripts from these genes exhibit increased accumulation within 30 min of exposure of the cells to S-deficient conditions; they remain elevated for over 24 h. The second tier genes encode proteins that are involved in S-recycling and that may alter the architecture of the cell to limit utilization of S resources (e.g. synthesize proteins with low S amino acid content). An insertional mutant disrupted for a gene designated *ARS73a* fails to upregulate second tier genes. *ARS73a* itself is a first tier response gene. The *ARS73a* gene product exhibits no motifs that suggest its precise function, although its function is currently being examined using biochemical and molecular approaches. Recently, we discovered that genes encoding putative vacuolar transporter chaperons (Vtc's) appear to be essential for the acclimation of Chlamydomonas to S deprivation; cells mutated for these genes do not accumulate extracellular arylsulfatase and die more rapidly than wild-type cells during S-deficiency. These mutations also impact cell survival under conditions of phosphorous (P) deficiency, although the *vtc* mutants develop normal phosphatase activity when deprived of P. Yeast Vtc proteins have been shown to be involved in polyphosphate synthesis. Currently, we are trying to understand the potential link between polyphosphate metabolism and S stress responses of Chlamydomonas. We are also beginning to elucidate protein-protein interaction pathways necessary for acclimation to S deprivaton.

25. Identification of a Sizer Protein in *Chlamydomonas*. Y. Li[1,2], B.J.S.C. Olson[1,3], C. Lopez-Paz[4], D. Liu[4,5], G. Anderson[1], **J. Umen[4]**. 1) Plant Biology Laboratory, Salk Institute, La Jolla, CA; 2) Dept of Horticultural and Plant Sci, Univ of Fla, Gainesville, FL; 3) Division of Biology, Kansas State Univ, Manhattan, KS; 4) Donald Danforth Plant Sci Ctr, St Louis, MO; 5) Plant and Microbial Biosci Prog, Wash Univ, St Louis, MO.

Cell size is coupled to the cell division cycle in diverse eukaryotes, but size checkpoint control is relatively unexplored. *Chlamydomonas* divides by multiple fission and uses a sizer mechanism to couple mother cell size to division cycle number. We identified a size regulator, CDKG1, which is a D cyclin dependent kinase that phosphorylates the retinoblastoma-related (RBR) protein MAT3 as its key substrate. Loss of CDKG1 causes inadequate mitotic division and results in large daughters, while mis-expression of CDKG1 results in small daughters. CDKG1 is nuclear-localized and is produced in mother cells just prior to division. As cells divide CDKG1 is eliminated in a step-wise process until it finally disappears at the end of cell division. The concentration of CDKG1 in pre-mitotic cells is set by mother cell size, and provides a link between cell size and the extent of cell division. Cell-size-dependent accumulation and consumption of rate-limiting cell cycle regulators such as CDKG1 is a potentially general mechanism for cell size control.

26. Genetic characterization of *Chlamydomonas* cell cycle control. Frej Tulin, **Frederick Cross**. Rockefeller University, New York, NY.

We have initiated a screen for mutations specifically disrupting cell cycle control in *Chlamydomonas*. Robotically assisted microbiological methods allow efficient recovery of UV-induced temperature-sensitive lethals. From these, candidate cell-cycle-specific mutants were selected by time-lapse microscopy, based on the criterion of wild-type cell growth rate combined with first-cycle failure of cell division ('*div*' phenotype). *div* mutants were sorted into ~60 complementation groups, and causative mutations identified by next-generation sequencing of bulked segregant pools.

Almost *DIV* genes had clear *Arabidopsis* sequelogs, and included genes required for DNA replication and chromosome segregation. We also identified ts mutations in conserved cell cycle control machinery: the cyclin-dependent kinases *CDKA* and *CDKB*, two subunits of the anaphase-promoting complex, and the mitotic kinases Aurora B and Mps1.

Phenotypic analysis of the mutants provides an outline of global cell cycle control, in which CdkA is central for cell cycle initiation, and CdkB is critical later, for execution of mitosis. Our model suggests a possible negative feedback control architecture in which CdkA activates CdkB, and CdkB represses CdkA. Transcriptome analysis by RNAseq in wild type and mutants indicated a broad program of transcriptional regulation through the cell cycle, which both regulates and is regulated by core cell cycle machinery such as CdkA and CdkB.

Most *DIV* genes were homologous to genes implicated in cell cycle control in yeast and animals. However, some of the *DIV* genes have strong sequelogs in *Viridiplantae* but not in yeast or animals. For example the *BSL* protein phosphatase family likely has an uncharacterized essential role in *Arabidopsis*; our results in *Chlamydomonas* demonstrate an essential and specific role for BSL phosphatase in entry into mitosis.

27. Defects in a new class of sulfate/anion transporter links sulfur acclimation responses to intracellular glutathione levels and cell cycle control. **Su-Chiung Fang**[1], Chin-Lin Chung[1], Chun-Han Chen[1], James Umen[2]. 1) Biotechnology Center in Southern Taiwan, Academia Sinica, Tainan, Taiwan; 2) Donald Danforth Plant Science Center, St. Louis, MO, 63132, USA.

Glutathione redox cycling plays an important role in cell cycle control. However, the underlying mechanisms connecting glutathione to cell cycle progression remain elusive. *smt15-1* was previously isolated as a partial suppressor that alleviates the cell cycle defects caused by loss of the retinoblastoma tumor suppressor related protein (RBR) encoded by the *MAT3* gene in *Chlamydomonas reinhardtii*. *smt15-1* single mutant also had a cell cycle defect leading to a small-cell phenotype. SMT15 belongs to a previously uncharacterized sub-family of putative membrane localized sulfate/anion transporters that contain a SUL1 domain and are found in a widely distributed subset of eukaryotes and bacteria. Although we observed that *smt15-1* has a defect in adaptation to sulfur-limited growth conditions, *sac* mutants that are severely defective for acclimation to sulfur limitation do not have cell cycle defects and cannot suppress *mat3*. Moreover, *smt15-1,* but not *sac* mutants, over-accumulates glutathione. In wild-type cells glutathione levels were found to fluctuate during the cell cycle with highest levels in mid-G1 phase and lower levels during S and M phases, while in *smt15-1* glutathione levels remained elevated during S and M. Importantly, manipulating cellular glutathione by overexpressing the glutathione synthetase encoding gene, *GSH2,* recapitulated the small-size phenotype of *smt15-1* mutant, indicating a role of glutathione on size-mediated cell cycle control. Our data suggest a role for SMT15 in maintaining glutathione homeostasis that affects the cell cycle and sulfur acclimation responses.

28. Transcriptional profiling of the *Chlamydomonas* cell cycle. **James Matthew Zones**[1,2], Sabeeha Merchant[3], James G. Umen[1]. 1) The Donald Danforth Plant Science Center, St. Louis, MO, 63132; 2) Department of Biology, University of California San Diego, CA 92093; 3) Department of Chemistry and Biochemistry, University of California Los Angeles, CA 90095.

The multiple fission mitotic cycle of *Chlamydomonas* is characterized by a prolonged G1 (growth) phase that is followed by rapid alternating rounds of S phase and mitosis (S/M). A high degree of synchrony can be achieved in cultures grown phototrophically in a diurnal cycle allowing the investigation of periodic gene expression that is either influenced by light-dark conditions or under cell-cycle control. We used RNA-Seq to characterize genome-wide cell-cycle/periodic gene expression in synchronized *Chlamydomonas* growing under highly reproducible conditions in a photobioreactor. Replicate wild type cultures were densely-sampled at 1 hour or 30 minute intervals throughout an entire light-dark cycle. Global gene expression patterns of replicate samples were well-correlated and 90% of genes with detectable expression (corresponding to 78% of the predicted transcriptome) were significantly differentially expressed during the time course. 11,524 genes were grouped into 6 major clusters and 151 sub-clusters with distinct patters of expression. Clusters with similar expression patterns were then functionally characterized and also examined for the presence of sequence motifs that might be involved in cis-regulatory control of their expression. Genes involved in related biological processes showed coordinated diurnal regulation patterns including those for photosynthesis and lipid metabolism. Some especially interesting clusters include those containing flagellar protein encoding genes that separate into four groups with distinct expression patterns: mid G1 phase, early S/M, late S/M, and post-mitotic. Another interesting example is the set of genes that encode cytoplasmic ribosomal proteins with peak expression at the beginning of the dark phase during and after cell division. This pattern is dramatically different from the genes that encode chloroplast-localized and mitochondrial-localized ribosomal proteins which are expressed in early G1 phase and throughout the light phase respectively. Nearly all cell cycle related genes have high amplitude peak expression times during early S/M, but an interesting exception is the gene encoding the Cyclin D4 protein which is expressed during late G1 phase and not during mitosis. To date, this study is the most-highly resolved characterization of cell-cycle/periodic gene expression for a photosynthetic eukaryote and will provide a valuable resource for the study of many aspects of *Chlamydomonas* biology.

29. Coexpression analysis of a synchronized day/night cycling culture predicts isoform localization. **Ian Blaby**[1], James Zones[2], Sabeeha Merchant[1,3], James Umen[2]. 1) Department of Chemistry and Biochemistry, University of California Los Angeles, CA 90095; 2) Donald Danforth Plant Science Center, St. Louis, MO 63132; 3) Institute of Genomics and Proteomics, University of California Los Angeles, CA 90095.

Chlamydomonas reinhardtii is a premier organism for studying the molecular genetics of photosynthesis. Since it is able to utilize organic carbon in the form of acetate and remain viable in the dark (heterotrophic growth) photosynthetic genes that would be essential in other model organisms, such as Arabidopsis, can be disrupted in Chlamydomonas. This metabolic versatility has arisen as a consequence of the duplication of many genes, and pathways, involved in central carbon metabolism. For example, the latest genome assembly contains 5 predicted phosphoglucomutases, 4 predicted phosphoglycerate kinases and 4 predicted glycerol-3-phosphate dehydrogenases. However, it is unknown to which specific pathway, or compartment, the proteins encoded by each isoform are localized.

Here, we take a novel approach by analyzing gene co-expression in a synchronized cell culture, grown in a periodic day/night light regime. The high temporal resolution of the data has enabled us to make protein localization predictions on the basis of co-expression with genes specific to particular pathways. Comparison of our results with Predalgo, an *in silico* localization prediction tool that employs independent methods, are in high agreement.

30. Dissection of the functional architecture of *Chlamydomonas* HAP2, the protein essential for gamete membrane fusion in plants and protists. **Yanjie Liu**, William Snell. Department of Cell Biology, UT-Southwestern Medical Center, Dallas, TX.

Cell-cell fusion between gametes is a defining step during fertilization in eukaryotes, yet we know little about the cellular and molecular mechanisms of the membrane fusion reaction. HAP2, an integral membrane protein found in protists, plants, and several

multicellular animals, is the first broadly conserved gamete-specific protein shown by gene disruption to be essential for the membrane fusion reaction. HAP2 (also known as GCS1) acts at a step after species-specific membrane adhesion, presumably in merger of the lipid bilayers, but its molecular function is unknown. Although the HAP2 ectodomain is conserved, the cytoplasmic portion of the protein varies across evolutionary groups. In the murine malaria pathogen, *Plasmodium berghei*, HAP2 lacking the cytoplasmic domain is sufficient for fusion, indicating that the ectodomain can behave as an autonomous functional unit. Similarly truncated *Arabidopsis* HAP2, which lacks fusion-essential, positively charged motifs, however, fails to rescue *Arabidopsis* gamete fusion, but the role of its cytoplasmic domain is unknown. Here, we show that the cytoplasmic portion of HAP2 in *Chlamydomonas* regulates HAP2 location and function. Unlike in *Plasmodium* and *Arabidopsis*, HAP2 in *Chlamydomonas* is localized at a fusogenic apical membrane patch representing ~ 0.1% of the cell surface area. HAP2 lacking the cytoplasmic portion fails to localize to the fusogenic patch and fails to rescue fusion. Mutation of a cysteine dyad near the transmembrane domain preserves HAP2 cell surface expression and localization, but substantially impairs fusion activity. Thus, the autonomy of the HAP2 ectodomain in the membrane fusion reaction has been reduced during evolution as the cytoplasmic portion has evolved to regulate both function and location. To dissect the function of the ectodomain, we also modified a highly conserved region (Pfam 10699) in the ectodomain of HAP2. We found that some of the mutations on the conserved residue abrogated HAP2 surface expression and function. Experiments in progress will swap HAP2 portions of ectodomains between *Chlamydomonas* and *Plasmodium falciparum* to test for conservation of function with the goal of using *Chlamydomonas* as an expression system for generating a transmission-blocking malaria vaccine. This work is supported by NIH grant GM56778 and a UTSW High Impact/High Risk grant.

31. Genome-wide analysis of gene regulatory networks in early zygote development of *Chlamydomonas reinhardtii*. S. Joo[1], Y. Nishimura[2], M.-H. Wang[1], E. Cronmiller[1], **J.-H. Lee[1]**. 1) Dept of Botany, University of British Columbia, Vancouver, BC, Canada; 2) Dept of Botany, Kyoto University, Kita-Shirakawa, Kyoto, Japan.

Sexual reproduction, one of the inventions shared by all major eukaryotic lineages, entails the cycling of two key events: gametic cell fusion generating diploids, and meiosis producing haploid progeny. *C. reinhardtii* represents a unique model to study this process in a unicellular context. In *C. reinhardtii,* the fusion of *minus* and *plus* gametes initiates zygote development by allowing the heterodimerization of two sex-specific transcription factors, Gsm1 and Gsp1, which is both necessary and sufficient to produce viable meiotic progeny from a diploid cell (Lee et al. Cell 133:829). To investigate details of how the Gsm1/Gsp1 heterodimer coordinates diverse downstream events for the dramatic transformation of fused gametes into dormant spores, we analyzed global transcriptomes of early-stage zygotes in wild type and *GSP1*-null backgrounds. The results show the following. 1) The Gsm1/Gsp1 heterodimer controls the majority of early zygote-specific genes at the transcriptional level. 2) A subset of zygote-specific genes related to cell-wall assembly exhibits a complex expression pattern suggesting an additional level of regulation, possibly post-transcriptional, that is still dependent on the Gsm1/Gsp1 heterodimer. 3) Cellular differentiation events entail a surge of remodeling programs at multiple levels, including the proteasome complex, organelle-targeted DNA-modifiers, and epigenetic factors that function in both small RNA- and methylation-dependent regulation. 4) Assembly of new zygotic walls is accompanied by up-regulation of secretory machinery, plasma membrane-anchored hydroxyproline-rich glycoproteins, and callose synthases whose expression is limited to the zygote stage. Our transcriptome analysis has confirmed the dominance of the Gsm1/Gsp1 heterodimer in initiating zygote development, and revealed putative molecular components involved in unicellular differentiation process. Their study should enable understanding the evolutionary pathways leading to the multicellular complexity of land plants.

32. Epigenetic interrogation of the algal lipid biosynthetic pathway. **Chia-Lin Wei**. DOE Joint Genome Institute, Walnut Creek, CA.

The potential of microalgae as a major biofuel producer is largely compromised by the limited knowledge of the regulatory mechanisms controlling lipid accumulation. We adopted a time-resolved integrative epigenomic, transcriptomic and metabolomic analyses to decipher the regulatory mechanisms controlling growth and lipid accumulation in model algae *Chlamydomonas reinhardtii*. Candidate key transcription factors at the core of the transcription regulatory pathways that control lipid accumulation were identified and studied in detail by chromatin immuno-precipitation to dissect their target gene networks. Genes predicted to represent master regulators were perturbed followed by detailed metabolic profiling. Our results suggest the complex interplay between multiple cellular components in unicellular green algae and provide foundational underpinnings for their large-scale engineering, increasing their potential as future biofuel producing organisms.

33. Dual specificity tyrosine-phosphorylation-regulated kinase DYRKP-1 negatively regulates starch and oil accumulation during nutrient deprivation. **Miriam Schulz-Raffelt[1]**, Vincent Chochois[1], Pascaline Auroy[1], Emmanuelle Billon[1], David Dauvillée[2], Yonghua Li-Beisson[1], Gilles Peltier[1]. 1) CEA, CNRS, Aix-Marseille Université, Institut de Biologie Environnementale et Biotechnologie, CEA Cadarache, F-13108 Saint-Paul-lez-Durance, France; 2) Unité de Glycobiologie Structurale et Fonctionnelle CNRS UMR 8576, Université des Sciences et Technologies de Lille, Villeneuve d'Ascq, France.

Growth and biomass productivity of photosynthetic organisms are both strongly affected by nutrient limitation. A general response to nutrient deprivation is a stop in cell division and a massive accumulation of energy rich reserve compounds such as starch and triacylglycerols. Deciphering signaling networks linking nutrient and energy status to metabolic and cellular activities is a major challenge to understand adaptation of photosynthetic organisms to their environment and further improve productivity for the production of biofuel. From the screen of an insertional mutant library of the model alga *Chlamydomonas reinhardtii* aiming at identifying regulatory processes that control reserve accumulation, a mutant strain affected in starch degradation (*std1*) was isolated. The impaired gene was identified as encoding a dual-specificity tyrosine-phosphorylation-regulated kinase (DYRK), a protein family so far characterized

only in animals and yeasts. Phylogenetic analysis revealed that STD1 belongs to a new plant-like subgroup of the DYRK protein family (named DYRKP), distinct from other DYRK subgroups (DYRK1, DYRK2 and Yak1). Biochemical analyses showed that the *std1* mutant accumulates much more starch and oil than its wild-type progenitor in response to nutrient deprivation under photoautotrophic conditions. At the meantime, the *std1* mutant maintained higher photosynthetic activity and an increased biomass production. The *Chlamydomonas std1* mutant constitutes the first *dyrk* mutant of the green lineage reported so far. The STD1/DYRKP-1 is proposed to act as a negative regulator of cellular processes that link the nutrient and energy status of photosynthetic cells to cellular growth and accumulation of reserves. These data will be discussed in relation to the genetic improvement of microalgae for the production of biofuel.

34. Substrate specificity and positional preference of *Chlamydomonas* diacylglycerol acyltransferases critical for stress-related biosynthesis of triacylglycerol. **Jin Liu**[1,2], Danxiang Han[3], Kangsup Yoon[4], Qiang Hu[4], Yantao Li[1,2]. 1) Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Science, Baltimore, MD, 21202 USA; 2) Institute of Marine and Environmental Technology, University of Maryland Baltimore County, Baltimore, MD, 21202 USA; 3) Department of Applied Sciences and Mathematics, Arizona State University Polytechnic campus, Mesa, AZ, USA; 4) Center for Microalgal Biotechnology and Biofuels, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei 430072, China.

Microalgal triacylglycerol (TAG) has been recognized as a promising biodiesel feedstock. However, the pathways and molecular mechanisms underlying TAG biosynthesis remain poorly defined in microalgae. Diacylglycerol acyltransferase (DGAT), which catalyzes the last step of TAG assembly, is thought to be a rate-limiting step for TAG accumulation in plant and yeast. In this study, we present a detailed functional and biochemical analysis of DGATs in *Chlamydomonas reinhardtii*. Of the six putative *DGATs*, *CrDGAT2a*, *CrDGAT2b*, and *CrDGAT2c* demonstrated the ability to complement the TAG deficient phenotype of the *Saccharomyces cerevisiae* TAG-less mutant, with mutants expressing *CrDGAT2a* producing the highest level of TAG. All three DGATs showed transient up-regulation at transcriptional level under nitrogen deprivation conditions while CrDGAT2a was also up-regulated at translational level. In order to unravel the substrate specificity of the CrDGATs, we developed a novel radiolabeling-independent *in vitro* DGAT assay. Of the ten acyl-CoAs investigated, CrDGAT2a preferred unsaturated acyl-CoAs especially polyunsaturated ones. Intriguingly, DGAT2a also had a strong activity on C20:5-CoA, which does not exist in *C. reinhardtii*. Similar to CrDGAT2a, CrDGAT2b showed a strong activity on C20:5-CoA. In contrast, CrDGAT2c preferred C16-CoAs. As for the substrate DAG, all three CrDGATs preferred C18:1 over C16:0 in the *sn-2* position. But substitution of C18:1 with C16:0 in the *sn-1* position had little impact on the enzyme activity. Suppression of *CrDGAT*s resulted in the reduction of differential TAG species and an overall TAG less phenotype in *C. reinhardtii*. These results suggested that the three CrDGATs possess distinct acyl-CoA specificity and may cooperate together, possibly with acyl-CoA independent pathways, to contribute to the stress associated TAG accumulation in *C. reinhardtii*. *CrDGAT2a*, which plays a major role in TAG biosynthesis under N starvation conditions, is a promising target of genetic engineering for possible manipulation of TAG. The high activity of CrDGAT2a to incorporate C20:5 into TAG suggests the biotechnological applications in microalgae or higher plants for improving EPA-rich TAG productio.

35. The Path to Triacylglycerol (TAG) Obesity in the *sta6* Strain of *Chlamydomonas reinhardtii*. **Ursula Goodenough**[1], Jae-Hyeok Lee[2], Sabeeha Merchant[3], James Umen[4]. 1) Washington University, St. Louis, MO; 2) University of British Colombia, Vancouver, BC; 3) University of California, Los Angeles, CA; 4) Danforth Plant Science Center, St. Louis, MO.

When the sta6 (starch-null) strain of *Chlamydomonas reinhardtii* is nitrogen (N)-starved in acetate and then "boosted" after 2 days with additional acetate, the cells become "obese" after 8 days, with triacylglycerol (TAG) lipid bodies filling their cytoplasm and chloroplasts. To assess the transcriptional correlates of this response, *sta6* and the starch-forming *cw15* strain were subjected to RNA-Seq analysis during the 2 days prior and 2 days post boost, and transcriptomes were compared with those previously obtained in the Merchant/Pellegrini labs under a different set of culture conditions.

"Robust" biochemical pathways, like starch, fatty-acid, and TAG biosynthesis, were identified wherein patterns of expression of the relevant genes are largely concordant regardless of genetic background or culture conditions. Also identified were 21 "sensitive" genes, encoding products operating in several pathways, including the glyoxylate and Calvin-Benson cycles, gluconeogenesis, and the pentose phosphate pathway, that are apparently influenced by on-going carbon flux; their expression is coordinated but varies within strains and between conditions, suggesting that they play a role in monitoring and responding to N-depletion in particular biosynthetic/metabolic contexts. 13 of these "sensitive" genes are strongly responsive to the cell's acetate status, including 3 candidate acetate permease-encoding genes in the GPR1_FUN34_YaaH superfamily that are strongly boost-up-regulated.

A cohort of 64 autophagy-related genes is down-regulated by boost. We propose that this is linked to microscopic observations showing that non-boosted cells initiate an autophagocytic response at 48 h -N, accompanied by diminished TAG accumulation, that is not initiated in boosted cells.

4 genes—encoding a diacylglycerol acyltransferase (DGTT2), a glycerol-3-P dehydrogenase (GPD3), and two genes annotated as lipases (Cre03.g155250 and Cre17.g735600)—are selectively up-regulated in *sta6* and may feature in chloroplast lipid-body production. We further propose that the disruption of starch synthesis in *sta6* creates a glucose-6-P "backflow" that feeds into chloroplast lipid-body formation.

36. Towards a new bioenergetic concept: Increasing the photorespiratory glycolate excretion in C. reinhardtii for bio-methane production. **Anja Guenther**[1], Theresa Quaas[1], Susann Reinert[1], Susanne Heithoff[3], Mark Fresewinkel[2], Torsten Jakob[1], Christian Wilhelm[1]. 1) University of Leipzig, Institute of Biology, Johannisallee 23, 04103 Leipzig, Germany; 2) KIT, Institute of Life Science Engineering, Fritz-

Haber-Weg 2, 76131 Karlsruhe, Germany; 3) University of Bremen, Institute for Environmental Process Engineering, Leobener Str. 33, 28359 Bremen, Germany.

Most of the current microalgae based projects for biofuel production rely on the formation, harvest and processing of biomass. The harvest of the biomass and the conversion processes are extremely energy consuming so that current technologies show a barely positive energy balance. We present a new concept focusing on the active excretion of glycolate in microalgae as a substrate for bacterial biogas production without biomass harvest and minimal refinement as well as different strategies to maximize the glycolate yield. Glycolate is an early stable product of the photorespiratory pathway. In C. reinhardtii its excretion is restricted to a short time period (3-6 h) and to a moderate excretion rate. To identify the physiological limitations of this process we characterized the physiology of various available C. reinhardtii mutants under different photorespiratory conditions and the influence of different inhibitors on the glycolate excretion rate. As a result we show that a knock-out of the carbon concentration mechanism (CCM) enables the cells for long-term excretion of glycolate at a moderate rate. In contrast, the knock-out of the glycolate dehydrogenase (GYD) results in an increase of the glycolate rate in short time experiments, but becomes inhibited after the activation of CCMs. To merge the phenotype of those two mutations we designed an artificial microRNA targeting the GYD mRNA. Appling this amiRNA to wild type cells we could show that it causes a similar phenotype as presented from the GYD mutant. Adopting this construct to different CCM mutants emerged in double mutants that show both, an increased rate and prolonged stability of glycolate excretion. Combing these data with the results from the bacterial compartment, which metabolizes glycolate into CO2 and methane, we are able to estimate the area-based, annual biogas production which shows for the first time a high potential of a net energy gain from photon to tanks.

37. Characterization of the Uni1 protein and its localization on basal bodies. M. LaVoie[1], J. Salisbury[2], P. Ranum[1], P. Lefebvre[1], **C. Silflow[1]**. 1) Dept. Plant Biology, University of Minnesota, St. Paul, MN; 2) Dept. Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN.

Mutations in the *UNI1* gene result in significant numbers of flagellaless and uniflagellate cells. Because failure of flagellar assembly disproportionately affects the younger of the two basal bodies, located *cis* to the eyespot, the mutation appears to delay the process of basal body maturation (Huang et al., Cell 19:745, 1982; Holmes and Dutcher, J. Cell Sci. 94:273, 1989). Mutant cells have varied ultrastructural defects including altered amounts and placement of transition zone materials, failure in precise transition from triplet microtubules to doublet microtubules at the distal end of the basal bodies, and in transitional fibers that facilitate docking at the plasma membrane (Piasecki and Silflow, Mol. Biol. Cell 20:368, 2009; O'Toole and Dutcher, Cytoskeleton 71:108, 2014). Similar defects are associated with mutations in the *UNI2* gene that encodes a coiled-coil domain phosphoprotein with amino acid sequence similarity to mammalian Cep120. The structural defects are more pronounced in *uni1uni2* double mutants, which are mostly aflagellate. We cloned the *UNI1* gene using map-based cloning. It encodes a protein of 3017 amino acids; nearly all the protein is predicted to be of intrinsically disordered sequence, based on SPINE-D and other algorithms. Expression of an HA epitope-tagged wild-type Uni1 protein in mutant cells rescues the phenotype to wild-type flagellar number. We have used super-resolution microscopy to examine the localization of the Uni1 protein on basal bodies. Co-localization with antibodies against centrin showed discrete spots of Uni1 protein at both ends of the distal striated fiber linking the two basal bodies, with no reproducible difference between the ends. Cells with a *uni2* mutation have decreased levels of Uni1 protein at this location, suggesting that it may be involved in recruitment or stabilization of the Uni1 protein. In contrast to the Uni2 protein, localization of the Uni1 protein to probasal bodies was not detected. Positioning of Uni1 protein on basal bodies is consistent with its role in enabling basal bodies to gain competence for flagellar assembly.

38. Using *Chlamydomonas* to elucidate the structure and function of the ciliary transition zone. **B. Craige**, J. Awata, J. M. Brown, Y. Hou, G. B. Witman. UMass Medical School, Dept. of Cell and Developmental Biology, Worcester, MA.

The transition zone (TZ), the region at the base of cilia and flagella where the triplet microtubules of the basal body transition into doublets, contains a large number of highly conserved proteins whose cognate genes are often mutated in human ciliopathies. The specific molecular functions of these proteins, and how defects in them lead to ciliary dysfunction, are largely unknown. Using *Chlamydomonas* mutants null for genes encoding TZ proteins, we are elucidating the functional, structural and biochemical consequences of disruption of TZ integrity. Mutants that lack the TZ proteins CEP290, CC2D2A, or NPHP4 have altered flagellar protein content; *cep290* and *cc2d2a* mutants also have defects in flagellar assembly. CEP290 and NPHP4 define distinct structural and functional subdomains of the TZ; NPHP4 is localized to the distal part of the TZ in close association with the membrane, whereas CEP290 is concentrated in the proximal TZ between the doublets and the membrane. The *cep290* mutant has reduced numbers of the 'Y connectors' that link the TZ microtubules to the TZ membrane. In contrast, *cc2d2a* and *nphp4* mutants have normal Y connectors and TZ architecture, indicating that while these two proteins are required for normal flagellar content, they are dispensable for TZ structural integrity. A *cep290, nphp4* double mutant displays total loss of the TZ Y connectors and severe defects in flagellar assembly and morphology. The *cep290* mutant flagella have abnormal levels of intraflagellar transport (IFT) proteins as well as defects in the velocity and frequency of retrograde IFT. In contrast, flagella of the *nphp4* mutant have normal IFT particle movement and normal IFT protein levels. This indicates that abnormal IFT might contribute to the defects in flagellar assembly and content in some TZ mutants (*cep290*) whereas defects in the flagellar content of other TZ mutants (*nphp4*) occur despite apparently normal IFT. These studies are yielding insight into how defects in the TZ lead to ciliary dysfunction and disease in humans.

39. The role of *LF5* localization in the regulation of flagellar length. L-W. Tam, P. Ranum, **P. Lefebvre**. Dept Plant Biol, Univ Minnesota, St Paul, MN.

The length of Chlamydomonas flagella is controlled by a series of protein kinases identified by the phenotypes of long-flagella (*LF*) mutants *LF2*, *LF4* and *LF5*. *LF4*, a MAP kinase, and *LF5*, a kinase in the CDKL5 family, localize to the flagella, whereas the *LF2* gene

product localizes exclusively to the cytoplasm. *LF2* is always found in close association with the proteins encoded by *LF1* and *LF3*, in a complex known as the length regulatory complex (LRC). We have recently discovered that the LRC may control flagellar length, at least in part, by controlling the localization of *LF5*. In the flagella of wild-type cells, the *LF5* protein (LF5p) is found exclusively at the very proximal end of the flagella, but only in the flagella and not the transition zone. Mutations in *lf1*, *lf2* and *lf3* cause proximal localization of LF5p to be lost. In these mutants LF5p is either distributed along the length of the flagella, or even concentrated at the flagellar tip. The proximal localization of LF5p requires not only the action of the LRC, but also its own protein kinase activity. When kinase-dead, epitope-tagged versions of *LF5* were prepared by in vitro mutagenesis and introduced into *lf5* mutant cells by transformation, the modified protein is still localized to the flagella , but it neither rescues the mutant phenotype nor localizes to the proximal end of the flagella. Surprisingly the localization defect of the mutant protein could not be rescued by the wild-type *LF5* protein, because when the kinase-dead construct was transformed into wild-type cells, in the presence of the wild-type LF5p, the kinase-dead protein was still localized along the length of the flagella and not at the proximal end. The regulation of *LF5* by proteins of the LRC cannot be the sole control of length, however, as *lf5* null mutants have substantially shorter flagella than *lf4* null mutants and several mutant alleles of *lf1*, *lf2* and *lf3*.

40. A CDK-like protein kinase is a key regulator of flagellar disassembly. Z. Hu, Y. Liang, **J. Pan**. School of Life Sciences, #413 Renhuanlou, Tsinghua University, Beijing, Beijing, China.

Primary cilia disassemble prior to G1-S transition to control cell cycle progression. In contrast to cilia assembly, the mechanism underlying cilia disassembly remains poorly understood. Moreover little is known about how cilia shortening is coupled with cell cycle progression. Because of the genetic advantage of Chlamydomonas and conservation of the cilia shortening pathway, we initiated a large scale screen for mutants defective in cilia disassembly in Chlamydomonas. We have identified a flagellar shortening (fs) mutant fs1 with null mutation in a gene encoding a CDK-like kinase. We find that flagellar shortening is actually regulated by two-phasic regulation in flagellar length dependent manner. FS1 is only required for the disassembly of around the first half of flagellum. FS1 is activated by autophosphorylation in the cell body and its kinase activity is required for flagellar shortening. Loss of FS1 prevents rapid phosphorylation of CALK and also induces earlier appearance of phosphorylated CrKinesin13 in the flagella, a microtubule depolymerase previously shown to be required for flagellar disassembly. Thus, FS1 is a key regulator of flagellar shortening pathway and cells use two distinct modules to coordinate flagellar disassembly.

41. IFT74 is required for IFT-A / IFT-B interaction, but not for import of tubulin into flagella. **Jason M. Brown**, Deborah A. Cochran, Tomohiro Kubo, George B. Witman. Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, MA.

Intraflagellar transport (IFT) is the movement of “trains” of particles and their associated cargos anterogradely and retrogradely within the flagellum. IFT trains include the complexes IFT-A and IFT-B, and sub-stoichiometric cargo adapters such as the BBSome, all of which have been implicated in disease. The specific functions of some IFT proteins are becoming clear through *in vitro* binding assays and mutant analyses. For example, the positively-charged N-terminus of the IFT-B protein IFT74 has recently been shown *in vitro* to form part of a tubulin-binding domain with IFT81. However, the *in vivo* function of IFT74 is still unknown. By studying mutants either completely lacking IFT74 or expressing truncated versions of the protein, we have begun to determine specific IFT74 functions *in vivo*. Analysis of the null mutant, which lacks flagella, revealed that IFT74 is required to stabilize IFT-B, but not IFT-A. In the null background, expression of IFT74 lacking 130 amino acids of the charged N terminus stabilized IFT-B and promoted slow assembly of nearly full-length flagella. This suggests that there is more than one tubulin-binding site on IFT-B and that we have reduced the rate of flagella assembly by disrupting one of the sites. A further truncation lacking IFT74 amino acids 1-196 (including part of coiled-coil 1) also stabilizes IFT-B, but the cells form only short (~4 microns long) flagella. As in wild type, IFT-B still localizes to the transition fibers at the base of the flagellum, but IFT-A no longer co-localizes with IFT-B there. The short flagella have almost no IFT detectable by DIC microscopy, have greatly reduced IFT-A, and accumulate stalled but intact IFT-B. This suggests that some IFT-B but almost no IFT-A is being injected into the flagella, and that most of the IFT-B that does enter the flagella cannot be returned to the flagellar base in the near absence of IFT-A, which is thought to be necessary for retrograde IFT. Therefore, the N-terminus of IFT74 is required for association of IFT-A and IFT-B and import of IFT-A into flagella. The flagella also have decreased BBS4, suggesting that IFT-A is necessary for BBSome import into flagella.

42. IFT-dependent and IFT-independent transport of tubulin in *Chlamydomonas* flagella. **Julie Craft**, Karl Lechtreck. Cellular Biology, University of Georgia, Athens, GA.

The microtubular scaffold of the axoneme is the defining feature of cilia and flagella. *C. reinhardtii* possesses two motile flagella with 9+2 axonemes and ~350,000 tubulin dimers are required to assemble a flagellum of ~12 µm length. IFT81 and IFT72, two proteins involved in intraflagellar transport (IFT), bind tubulin dimers *in vitro* suggesting that tubulin may be a cargo of IFT (1). To analyze *in vivo* how tubulin is transported into and inside flagella, superfolder GFP-α-tubulin was expressed using a modified version of the recently introduced pBR25-tubulin vector (2). In whole cells, the expression levels of the GFP-tagged α-tubulin were ~20% or less of that of the endogenous α-tubulin. The epitope-tagged tubulin was imported into flagella, incorporated into axonemes, and post-translationally modified suggesting that GFP-a-tubulin is a useful marker to study tubulin transport. Total internal reflection fluorescence (TIRF) imaging revealed that GFP-α-tubulin entered flagella by IFT and by diffusion. To determine how tubulin interacts with IFT, modified α- and β- GFP-tubulins are expressed and analyzed *in vivo*; this ongoing project revealed that a C-terminally truncated GFP-α-tubulin is still transported by IFT and assembled into the axoneme. Diffusion propelled GFP-α-tubulin with velocities of ~10 µm/s along the flagella and continued in the absence of IFT revealing that tubulin can enter cilia independently of IFT. During flagellar growth, IFT-based transport of GFP-α-tubulin was increased ~15x over steady-state frequencies. Similarly, GFP-α-tubulin entry by diffusion was augmented in regenerating flagella. Fluorescence recovery after photobleaching (FRAP) analysis revealed a strong increase in the concentration of soluble GFP-α-

tubulin in regenerating over steady-state flagella. We propose that cells regulate the concentration of soluble tubulin in flagella by adjusting tubulin loading onto IFT particles and controlling IFT-independent entry of diffusing tubulin. A high intraciliary concentration of soluble tubulin is likely to boost axonemal assembly; IFT-based translocation of tubulin to the tip could further increase its local concentration at growing end of the axoneme.

(1) Bhogaraju et al. (2013) *Science* 3 41:1009-12; (2) Rasala et al. (2013) *Plant J*.74:545-54.

43. Comparative proteomic analysis of vesicles released from flagellar membrane with isolated flagellar membranes. **Huan Long**[1], Fan Zhang[1], Dennis Diener[2], Joel Rosenbaum[2], Kaiyao Huang[1]. 1) Key Laboratory of Algal Biology, Institute of Hydrobiology, C.A.S, Wuhan, Hubei, China; 2) 2.Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06520.

Cilia/flagella are microtubule-based organelles emanating from the surface of most eukaryotic cells. In addition to the well-established motile and sensory functions of these organelles, small ectosome–like vesicles release from the flagellum and carry a specific biological-active protease which can digest the mother cell wall during mitosis. In order to study the mechanism of the vesicle releasing, we purified the ectosome–like vesicles from the medium and isolated the flagellar membrane, and then used the Isobaric Tags for Relative and Absolute Quantitation method (iTRAQ) to compare the protein composition of these two membrane fractions. We found that the axonemal proteins and Intraflagellar Transport proteins were enriched the flagella membrane and the glyco-membrane proteins, proteases, GTPases, ubiquitinated proteins and proteins of Endosomal Sorting Complexes Required for Transport (ESCRT)were enriched in the ectosome–like vesicles. The unique protein composition of the ectosome-like vesicle suggested vesicle pinching off is a regulated process. In order to confirm these data we fused the GTPases, ESCRT, ubiquitin proteins with luciferase and expressed the fusion proteins in wild type cells of Chlamydomonas, Indeed, the luciferase activity of the fusion proteins were all detected in the medium. Interestingly, the activity of luciferase increased dramatically during the flagella shortening and mating process. Knockdown of ESCRT proteins result in decreasing the mating efficiency and the defect of flagella shortening. These data suggested the vesicles pinching off might also participate in the flagellar disassembly and mating. Supported by NSFC (#3 1171287).

44. Chloroplast remodeling during state transitions in *Chlamydomonas reinhardtii* as revealed by non-invasive techniques in vivo. Gergely Nagy[1,2,3], Renáta Ünnep[2], Ottó Zsiros[4], Ryutaro Tokutsu[5,6], Kenji Takizawa[5], Lionel Porcar[3], Lucas Moyet[7,8,9,10], Dimitris Petroutsos[7,8,9,10], Győző Garab[4], Giovanni Finazzi[7,8,9,10], **Jun Minagawa**[5,6]. 1) Paul Scherrer Institute, Laboratory for Neutron Scattering, 5232 Villigen PSI, Switzerland; 2) Wigner Research Centre for Physics, Institute for Solid State Physics and Optics, Hungarian Academy of Sciences; 3) Institut Laue-Langevin, BP 156, F-38042, Grenoble Cedex 9, France; 4) Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, POB 521, H-6701, Szeged, Hungary; 5) National Institute for Basic Biology (NIBB), Nishigonaka 38, Myodaiji, Okazaki 444-8585, Japan; 6) CREST, Japan Science and Technology Agency, Chiyoda-ku, Tokyo 102-0076, Japan; 7) Centre National de la Recherche Scientifique, UMR 5168 Laboratoire de Physiologie Cellulaire et Végétale; 8) Université Grenoble Alpes; 9) Institut National Recherche Agronomique (INRA); 10) Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA), Institut de Recherche en Sciences et Technologie du Vivant (IRTSV), CEA Grenoble, F-38054 Grenoble, France.

Algae respond to changes in light quality by regulating the absorption capacity of their photosystems (PSs). These short-term adaptations use redox-controlled, reversible phosphorylation of the LHCII to regulate the relative absorption cross-section of the two PSs, referred to as state transitions. It is acknowledged that state transitions induce substantial reorganizations of the PSs. However, their consequences on the chloroplast structure are more controversial. Here, we investigate how state transitions affect the chloroplast structure and function using complementary approaches for the living cells of *Chlamydomonas reinhardtii*. Using small-angle neutron scattering, we found a strong periodicity of the thylakoids in state 1, with characteristic repeat distances of ∼200 Å, which was almost completely lost in state 2. As revealed by circular dichroism, changes in the thylakoid periodicity were paralleled by modifications in the long-range order arrangement of the photosynthetic complexes, which was reduced by ∼20% in state 2 compared with state 1, but was not abolished. Furthermore, absorption spectroscopy reveals that the enhancement of PSI antenna size during state 1 to state 2 transition (∼20%) does not match with the decrease in PSII antenna size (∼70%), leading to the possibility that a large part of the phosphorylated LHCIIs do not bind to PSI, but instead form energetically quenched complexes. Altogether these noninvasive in vivo approaches allow us to present a more likely scenario for state transitions that explains their molecular mechanism and physiological consequences.

45. Light harvesting in *Chlamydomonas reinhardtii* - between adaptation and acclimation. Bartlomieij Drop[1], Sathish Yadav[2], Caner Unlu[3], Egbert Boekema[2], Herbert van Amerongen[3], **Roberta Croce**[1]. 1) VU University Amsterdam, Amsterdam, the Netherlands; 2) University of Groningen, Groningen, the Netherlands; 3) Wageningen University, Wageningen, the Netherlands.

The outer light-harvesting system of *Chlamydomonas reinhardtii* is composed of 20 Lhc gene products, nine belonging to the antenna of Photosystem I (PSI), and eleven primarily to that of Photosystem II (PSII). In addition, light harvesting in PSI and PSII is regulated by the process of state transitions that in response to changes in light quality/quantity balances the excitation between the two photosystems by shuttling light-harvesting complexes (Lhc) between them. This process, which in plants involves 15% of the LHCII, was proposed to involve 80% of the Lhcb complexes in this alga. Finally, it was shown that *C. reinhardtii* is also capable of non-photochemical quenching, but only upon acclimation to high light which triggers the expression of LhcSR. I will present our recent data on the characterization of the photosynthetic membranes C.r, obtained by integrating biochemical, structural and spectroscopic measurements. We show that both PSI and PSII supercomplexes are far larger than the complexes of higher plants [1,2], but despite this the overall trapping efficiency is very similar. State transitions seem to work differenty than in plants, with most of the LHCII antenna becoming

functionally disconnected from PSII but only part of it connects to PSI [3]. The largest PSI-LHCII complex, contains two LHCII trimers and one monomer in addition to the nine Lhcas [4].

References
1.Drop B., Webber-Birungi M., Fusetti F., Kouřil R., Redding KE, Boekema EJ and Croce R. (2011) "Photosystem I of *C.r.* is composed of nine Light-harvesting complexes (Lhca) located on one side of the core". *J. Biol. Chem.* 286: 44878-44887.
2.Drop B., Webber-Birungi M, Yadav S., Filipowicz-Szymanska A., Fusetti F., Boekema E.J. and Croce R. (2014) "Light-harvesting complex II (LHCII) and its supramolecular organization in *C.r.*" *BBA–bioenergetics*, 1837 (2014) 63–72
3.Ünlü C., Drop B., Croce R. and van Amerongen H. (2014) "State transitions in C.r. strongly modulate the size of Photosystem II but not of Photosystem I" *PNAS,* 111, 9, 3460 - 3465.
4.Drop B., Yadav S., Boekema E.J. and Croce R. (2014) "Consequences of state transitions on the structural and functional organization of Photosystem I in the green alga C.r.*i*" *Plant Journal*, in pres.

46. Phosphorylation dependent reorganization of photosynthetic multiprotein complexes. Sonja Verena Bergner, Martin Scholz, Kerstin Trompelt, Philipp Gäbelein, Johannes Barth, Christian Fufezan, **Michael Hippler**. Institute of Plant Biology and Biotechnology, University of Münster, Münster 48143, Germany.

Light is essential for photosynthesis but excess photons must be de-excited to avoid photodamage. In plants, qE, a component of non-photochemical quenching (NPQ), is required for thermal dissipation of excess absorbed light energy. In *C. reinhardtii,* LHCSR3 was shown to be crucial for functional qE[1]. Increased cyclic electron flow (CEF) around PS I is known to promote qE induction by formation of a trans-thylakoid proton gradient. Functional CEF depends on a reorganization of the major protein complexes in the thylakoid membrane, leading to the formation of a PS I-cytochrome b_6f supercomplex [2 3].
During state transitions, inducing the qT component of NPQ, light harvesting proteins (LHC) are redistributed between PS II and PS I. In *C. reinhardtii*, this phenomenon has been shown to be dependent on LHC II phosphorylation by the STT7 kinase [4]. Although occurring under similar conditions, CEF supercomplex formation is not depending on STT7 kinase activity [5]. In an effort to find a possible interconnection, we analyzed both phenomena in WT and stt7 deficient strains using quantitative mass spectrometry, phosphopeptide enrichment and mass spectrometric identification. These analyses were done on thylakoid membranes and isolated photosynthetic multiprotein complexes and led to the identification of 1,370 phosphoproteins and 2,498 distinct phosphopeptides. These results give new insights in STT7-dependent phosphorylation. They further indicate that non-phosphorylated LHCSR3 preferentially binds to the CEF supercomplex, while formation of the complex is promoted in the absence of STT7, pointing towards phosphorylation dependent regulation of CEF supercomplex organization. The presented data strengthens the understanding of the interplay between external stresses and the regulation of photosynthetic capacity via structural reorganization within the thylakoid membrane.

1 Peers, G. *et al. Nature* **462**, 518-521 (2009).
2 Iwai, M. *et al. Nature* **464**, 1210-1213 (2010).
3 Terashima, M. *et al. PNAS* **109**, 17717-17722, (2012).
4 Depege, N. *et al. Science* **299**, 1572-1575 (2003).
5 Takahashi, H. *et al. Nat Commun* **4**, 1954, (2013).

47. Photoprotection in Chlamydomonas revisited: role of light and metabolism. **D. Petroutsos[1]**, R. Tokutsu[2], S. Flori[1], D. Karageorgou[1], A. Greiner[3], M. Mittag[4], P. Hegemann[3], J. Minagawa[2], G. Finazzi[1]. 1) Cell and Plant Physiology Laboratory, CEA Grenoble, France; 2) Division of Environmental Photobiology, National Institute for Basic Biology, Okazaki, Japan; 3) Institute for Experimental Biophysics, Humboldt University, Berlin, Germany; 4) Institute of General Botany and Plant Physiology, Friedrich Schiller University, Jena, Germany.

Light is an essential factor in plants not only because it fuels carbon assimilation via the Calvin cycle, but also because it acts as a signal in several processes related to plant growth and environmental responses. In this work, we have addressed this double role of light in the case of photoprotective responses, using the model algae *Chlamydomonas reinhardtii*. We focused on NPQ (the major photoprotection mechanism in plants and algae) measuring fluorescence changes and the accumulation of LHCSR3, the photosystem 2 subunit triggering NPQ in this alga. We found that accumulation of LHCSR3 in high light is controlled not only by the intensity, but also by the quality of light. Thanks to a genetic analysis, we have identified the nature of the responsible actor for this regulation. Metabolic screening revealed that NPQ is also modulated by specific metabolites. Overall, these data allow revisiting the fundamental concepts of photoprotection, providing a link between light perception by specific photosensors, light utilization for metabolism and excess light dissipation via NPQ.

48. Identifying a Novel Type of Violaxanthin De-epoxidase from *Chlamydomonas reinhardtii* . **Zhirong Li[1]**, Rachel M. Dent[1], Graham Peers[2], Wiebke Apel[1], Scarlett Yang[1], Krishna K. Niyogi[1,3]. 1) Howard Hughes Medical Institute, Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA; 2) Department of Biology, Colorado State University, Fort Collins, Colorado 80523, USA; 3) Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720-3102, USA.

We have previously identified mutants defective in the xanthophyll cycle and qE in the unicellular green alga *Chlamydomonas reinhardtii* and the model plant *Arabidopsis thaliana* by using video imaging of Chl fluorescence (Niyogi et al., 1997; Niyogi et al., 1998). The *npq1* mutants are unable to convert violaxanthin to zeaxanthin in high light**.** Although the *Arabidopsis npq1* mutant was shown to affect the *violaxanthin de-epoxidase (VDE*) gene (Niyogi et al., 1998), the molecular basis of the *Chlamydomonas npq1* mutant has been

mysterious, because the *Chlamydomonas* genome lacks an obvious ortholog of the *VDE* gene found in plants and other algae. Therefore, we mapped the *npq1* mutation (Anwaruzzaman et al., 2004) and recently succeeded in identifying the *NPQ1* gene by map-based cloning and complementation. Surprisingly, the gene encodes a putative FAD-dependent oxidoreductase that is homologous to a different, previously characterized carotenoid biosynthetic enzyme, suggesting that NPQ1 represents a novel type of VDE. By complementing the *Arabidopsis npq1* mutation, we showed that the *Chlamydomonas* NPQ1 protein is indeed a functional VDE. NPQ1 protein is associated with the thylakoid membrane in both *Chlamydomonas* and *Arabidopsis*. Protease protection assay indicated that NPQ1 protein is located on the stromal side of the thylakoid membrane in *Arabidopsis*, which differs from the plant-type VDE that is located in the thylakoid lumen. (Supported by the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy, FWP number 449B.).

49. Functional analysis of PBC1, a protein conserved in the green lineage that is associated with the PSII core and involved in high light adaptation. **Ligia S Muranaka**[1], Mark Rütgers[1], Sandrine Bujaldon[2], Anja Heublein[3], Frederik Sommer[1], Torsten Möhlmann[4], Ekkehard Neuhaus[4], Stefan Geimer[3], Fabrice Rappaport[2], Francis-André Wollman[2], Michael Schroda[1]. 1) Molekulare Biotechnologie & Systembiologie, TU Kaiserslautern, Germany; 2) Institut de Biologie Physico-Chimique, Unité Mixte de Recherche 7141, Centre National de la Recherche Scientifique/ Université Pierre et Marie Curie, Paris, France; 3) Zellbiologie/Elektronenmikroskopie, Universität Bayreuth, Germany; 4) Pflanzenphysiologie, TU Kaiserslautern, Germany.

In normal day conditions photosynthetic organisms are exposed to different light intensities which can be higher than needed to saturate their photosynthetic capacity. In this case they need to be able to induce several regulation mechanisms to optimize photosynthesis and avoid photooxidative damage. Here we report on the characterization of PBC1, a protein of yet unknown function conserved in the green lineage that is induced by high light. Fractionation studies revealed that PBC1 is associated with the stromal side of thylakoid membranes. Based on blue native PAGE, sucrose density gradient centrifugation, and isolated photosystem II (PSII) particles from a D2-His-tagged strain, we could demonstrate a strong association of PBC1 with the PSII core complex. Electron micrographs of *PBC1*-amiRNA strains exposed to high light revealed severe thylakoid swelling, and immunoblot analysis, a delayed induction of LHCSR3. Biophysical data suggest an increased sensitivity of *PCB1*- amiRNA strains to high light, which was also observed in preliminary results on a *pbc1* knock-out mutant in *Arabidopsis thaliana*. In summary, our results indicate a possible role of PBC1 in cell adaptation to high light.

50. Comprehensive Identification of Genes Responsible for Photosynthesis and the Carbon Concentrating Mechanism of *Chlamydomonas reinhardtii*. **Leif Pallesen**, Greg Reeves, Weronika Patena, Saman Parsa, Rachel Purdon, Martin Jonikas. Department of Plant Biology, Carnegie Institution for Science, Stanford, CA.

Meeting the global food and energy demands in the coming decades will require innovative and sustainable solutions. Both food and biofuel require photosynthesis to harness abundant solar energy and store it in biomass via carbon fixation. The efficiency of photosynthesis is often limited by the availability of CO_2. Interestingly, the alga *Chlamydomonas reinhardtii* enhances photosynthetic efficiency with a genetically encoded Carbon Concentrating Mechanism (CCM) that actively concentrates inorganic carbon (CO_2 and HCO_3^-) inside the cell. The specific objective of this research is to comprehensively identify the genes required for photosynthesis and a subset of genes constituting the CCM. Toward this end, we have developed a quantitative high-throughput growth phenotyping pipeline. We have screened >120,000 mutant stains and isolated several thousand with deficient photosynthetic growth in air. A functional CCM is required for efficient phototrophic growth in air, but not at elevated CO_2 levels (*ie.* 3% CO_2). As such, several hundred candidate CCM mutants were identified that exhibit deficient phototrophic growth in air (0.04% CO_2) (CCM dependent) that is rescued by 3% CO_2 (CCM independent). A deep-sequencing based approach was used to identify the mutation sites of strains of interest. The comprehensive identification of photosynthesis and CCM genes expands our understanding of these processes and opens the door to engineering more efficient plant crops for increased food and biofuel production.

51. Past, present, and potential roles of Chlamydomonas research in chloroplast biology. **William Zerges**, Uniacke James, Marco Schottkowski, Matthew Peters, Yi Sun, James Dhaliwal, Yu Zhan. Biology Department, Concordia University, Montreal, Quebec, Canada.

This introduction to the session “Chloroplast Biogenesis and Functioning” will have three themes. The first will be the historic contributions of Chlamydomonas research to the genetics, molecular biology, and cell biology of chloroplasts. The second theme will be recent contributions of Chlamydomonas chloroplast research to a blossoming field of bacterial cell biology as well as discoveries that are specific to chloroplasts. This discussion will include a review of the cytological organization of the biogenesis of photosynthetic thylakoid membranes. The third theme will be open questions and problems of chloroplast biology.

52. Interconnection between the chloroplast HSP70B chaperone system, VIPP1 and the Secretory Pathway in biogenesis of thylakoid membranes in *Chlamydomonas*. **Mark Rütgers**[1], Lígia Muranaka[1], Mareike Possienke[2], Karolin Dorn[2], Anja Heublein[3], Stefan Geimer[3], Michael Schroda[1]. 1) Molekulare Biotechnologie & Systembiologie, TU Kaiserslautern, Germany; 2) Institute of Biology II / Plant Biochemistry, University of Freiburg, Germany; 3) Zellbiologie/Elektronenmikroskopie, Universität Bayreuth, Germany.

Recent results indicate a structural role of the vesicle-inducing proteins in plastids 1 (VIPP1) within thylakoid centers. These centers are located at the origin of multiple thylakoid membrane layers and are considered as sites at which the biogenesis at least of photosystem II occurs. Previous data also revealed a dynamic character of VIPP1 oligomeric states that is realized by the chloroplast HSP70B-CDJ2-CGE1 chaperone system. As reverse genetic approaches to knockdown *CDJ2* failed, we overexpressed non-functional CDJ2 to gain a dominant negative phenotype. Dominant negative strains accumulate VIPP1 higher oligomeric structures and display increased levels of HSP70B. Furthermore, we found that CDJ2 interacts with cpSECA, the motor protein of the thylakoidal secretory pathway.

Conditional knockdown of *SECA* leads to cell cycle arrest, lipid droplet accumulation and, as observed for the CDJ2 dominant negative phenotype, a strong induction of VIPP1, VIPP2 and HSP70B. These data support a role of the HSP70B-CDJ2-CGE1 chaperone system in coordinating Sec-mediated protein translocation at thylakoid centers.

53. Role of a novel SEC14 domain-containing protein in chloroplast lipid trafficking and photoautotrophic growth in *Chlamydomonas reinhardtii*. **Jose Gines Garcia Cerdan**[1], Alizée Malnoë[1,2], Alexander P. Hertle[1,2], Krishna K. Niyogi[1,2]. 1) UC Berkeley-HHMI, Berkeley, CA; 2) Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA.

From a large collection of non-photoautotrophic (acetate-requiring) insertional mutants of *Chlamydomonas*, we have isolated a mutant that exhibits severe light sensitivity. The mutant is unable to grow in low light (25 µmol photons m^{-2} s^{-1}) even on medium containing acetate, yet dark-grown cells exhibit a quantum efficiency of PSII (F_V/F_M) that is comparable to that of the wild type. Tetrad analysis indicated that there was only one DNA insertion, and the mutant phenotype co-segregated with the antibiotic selectable marker used for the DNA insertional mutagenesis. The insertional mutagenesis led to a deletion of several kb encompassing multiple genes, however, only complementation with one of these genes, encoding a SEC14 domain-containing protein, restored photoautotrophic growth. SEC14 domain-containing proteins participate in regulation of phospholipid metabolism in yeast and are found only in eukaryotes. We expressed the recombinant SEC14-like protein in *E.coli* and purified it by affinity chromatography. In lipid binding assays performed *in vitro*, SEC14-like binds to phosphatidic acid, a metabolic intermediate for synthesis and degradation of phosphatidylglycerol (PG), and to PG itself. The SEC14-like proteins bear a predicted amino-terminal chloroplast transit peptide (ChloroP). Following chloroplast sub-fractionation experiments, we localized SEC14-like proteins preferentially in the stromal fraction using a SEC14-like specific antibody. When mutant cultures were shifted from dark to light, enhanced photoinhibition of PSII was observed. Biochemical and biophysical characterization of the mutant and its light induced photoinhibition are in progress. Based on our results, we propose a role for the SEC14-like protein in chloroplast lipid metabolism that is essential for photoautotrophic growth.

54. Intron *tscA* RNA is required for assembly of a chloroplast-splicing complex. **Christina Marx**[1], Olga Reifschneider[1], Jessica Jacobs[1], Franziska Hundt[2], Dirk Wolters[2], Ulrich Kück[1]. 1) General and Molecular Botany, Ruhr-University Bochum, Bochum, Germany; 2) Analytical Chemistry, Ruhr-University Bochum, Bochum, Germany.

The maturation of the *psaA* transcript from the green alga *Chlamydomonas reinhardtii* is dependent on various *trans*-acting factors. The tripartite *psaA* gene, encoding one of two apoproteins of photosystem I, is flanked by group II intron sequences. Two *trans*-splicing reactions are necessary to generate the mature transcript. In addition to the *tscA* RNA which is involved in the splicing of the first intron, several nucleus-encoded factors are important for the *trans*-splicing reaction of *psaA*.

Previous studies indicated that proteins acting on the first *trans*-splicing reaction (Raa1, Raa3, Raa4 and Rat2) are associated in a ribonucleoprotein complex (RNP) together with unknown proteins. They were purified using a Raa4::TAP tag fusion protein (R4T). Here we show data with an alternative fusion protein (Rat2::TAP tag), that was applied for stringent purification conditions to identify protein subunits that belong to the core of the chloroplast splicing complex.

Furthermore we analyzed the photosynthesis deficient mutant L135F. Using Southern analysis and complementation studies, we identified *RAT2* as the gene responsible for the mutant phenotype. To analyze the effect of *tscA* on RNP assembly, we generated a *tscA* deletion in the R4T strain (R4TΔtscA). After performing TAP experiments with R4TΔtscA, we were able to show that deletion of *tscA* RNA prevents the assembly of the first *trans*-splicing complex. We further identified several splicing factors acting on the second splicing reaction. This data support our hypothesis that a supercomplex is the source for at least two subcomplexes, which promote *trans*-splicing of two group II introns.

55. Nitric Oxide-triggered remodelling of chloroplast bioenergetics and thylakoid proteins upon nitrogen starvation in *Chlamydomonas reinhardtii*. **Yves Choquet**[1], Lili Wei[1], Benoit Derrien[1], Arnaud Gautier[2], Laura Houilles-Vernes[1], Alix Boulouis[1], Denis Saint-Marcoux[1], Alizée Malnoë[1], Fabrice Rappaport[1], Catherine De Vitry[1], Olivier Vallon[1], Francis-André Wollman[1]. 1) UMR 7141, Inst Biologie Physico-Chimique, Paris, France; 2) UMR 8640, École Normale Supérieure, Département de Chimie, Paris, France.

Starving microalgae for nitrogen sources is commonly used as a biotechnological tool to boost storage of reduced carbon into starch granules or lipid droplets, but the accompanying changes in bioenergetics have been little studied so far. Here, we report that the selective depletion of Rubisco and cytochrome b_6f complex that occurs when *Chlamydomonas reinhardtii* is starved for nitrogen in the presence of acetate and under normoxic conditions is accompanied by a marked increase in chlororespiratory enzymes, which converts the photosynthetic thylakoid membrane into an intracellular matrix for oxidative catabolism of reductants. Cytochrome b_6f subunits and most proteins specifically involved in their biogenesis are selectively degraded, mainly by the FtsH and Clp chloroplast proteases. This regulated degradation pathway does not require light, active photosynthesis, or state transitions but is prevented when respiration is impaired or under phototrophic conditions. To better understand the mechanism leading to these losses, we further studied the metabolism of nitrogen-starved cells. We demonstrate that nitrogen-starved cells produce NO, as shown using NO-sensitive fluorescence probes visualized by confocal microscopy. We provide genetic and pharmacological evidence that NO production from intracellular nitrite governs the degradation of the cytochrome b_6f complex, as this degradation is respectively decreased or increased upon addition of an NO-scavenger or of two distinct NO producers. Furthermore, the cytochrome b_6f complex is preserved under phototrophic conditions or in *nit1*$^-$ mutants, two experimental situations preventing significant production of NO.

56. Conditional depletion of the chloroplast ClpP1 protein activates nuclear genes involved in autophagy and chloroplast protein quality control. **Silvia Ramundo**[1,2], David Casero[3], Timo Mühlhaus[4], Dorothea Hemme[4], Frederik Sommer[4], Michèle Crèvecoeur[2], Michèle Rahire[2],

Michael Schroda[4], Jannette Rusch[5], Ursula Goodenough[5], Matteo Pellegrini[6], Maria Esther Perez-Perez[7], José Luis Crespo[7], Olivier Schaad[8], Natacha Civic[8], Martin Jonikas[9], Peter Walter[1], Jean David Rochaix[2]. 1) Department of Biohemistry and Biophysics, University of California, San Francisco, CA; 2) Departments of Molecular Biology and Plant Biology, University of Geneva, Geneva, Switzerland; 3) Institute for Genomics and Proteomics, University of California, Los Angeles, CA; 4) Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany; 5) Department of Biology, Washington University, St Louis, MO; 6) Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA; 7) Instituto de Bioquimica Vegetal y Fotosintesis, CSIC-Universidad de Sevilla, Sevilla, Spain; 8) Genomics Platform, University of Geneva, Geneva, Switzerland; 9) Department of Plant Biology, Carnegie Institution for Science, Palo Alto, CA.

All the proteases and chaperones involved in plastid protein quality control are encoded by the nucleus with exception of the catalytic subunit of the evolutionarily conserved serine-protease ClpP. Unlike its E. coli ortholog, this chloroplast protease is essential for cell viability. In the green unicellular alga *Chlamydomonas reinhardtii*, a selective and gradual depletion of ClpP leads to a progressive alteration of chloroplast morphology, causes formation of vesicles and induces extensive cytoplasmic vacuolization, reminiscent of an autophagy phenotype. Moreover, it triggers a specific accumulation, both at the RNA and protein level, of nuclear-encoded chloroplast small heat shock proteins, chaperones, proteases and proteins involved in thylakoid maintenance. This result suggests the existence of a chloroplast-to-nucleus signaling pathway involved in organelle quality control, conceptually similar to that observed in the endoplasmic reticulum and in mitochondria. In order to elucidate this retrograde signaling pathway at the mechanistic level, we have now built reporter strains that will be soon used in a genetic screen.

57. The metabolic face of *Chlamydomonas* in the dark. **Arthur Grossman[1]**, Wenqiang Yang[1], Claudia Catalanotti[1], Tyler Wittkopp[1,2], Sarah Sarah D'Adamo[3], Matthew Posewitz[3]. 1) Department of Plant Biology, Carnegie Institution for Science, Stanford, CA; 2) Stanford University, Department of Biology, Stanford, CA; 3) Colorado School of Mines, Department of Chemistry and Geochemistry, Golden, CO.

In the natural environment photosynthetic organisms can spend half of their time in the dark. In both soil and lake environments, dark conditions may be linked to hypoxia or even anoxia. Elucidating metabolic processes that occur under both dark oxic and hypoxic/anoxic conditions is critical for understanding ecosystem energetics and for farming alga to produce food, fuel and nutraceuticals. We have been exploring the responses of Chlamydomonas to both dark oxic and dark anoxic/hypoxic conditions. Fermentation metabolism occurs when the cells are exposed to anoxic conditions, with the catabolism of pyruvate to products that include formate, acetyl-CoA, acetate, CO_2, H_2 and ethanol. Enzymes that play a dominant role in the anoxic catabolism of pyruvate include pyruvate formate lyase, pyruvate ferredoxin oxidoreductase, hydrogenase, phosphate acetyltransferase, acetate kinase and alcohol dehydrogenase. Elimination of each of these fermentative reactions in specific mutant strains leads to the use of alternate reactions that help eliminate the reductant generated during glycolytic energy production; this metabolic rerouting may result in the synthesis of lactate, glycerol and tricarboxylic acid (TCA) cycle products such as succinate (generated through entry of pyruvate into the reverse TCA cycle). Furthermore, even dark growth in the presence of acetate under oxic conditions requires specific redox components, including a 'dark critical' ferredoxin. Overall, our recent studies demonstrate the metabolic flexibility of Chlamydomonas and how little we know about regulation of physiological/metabolic processes in the algae under both dark oxic and oxygen-limiting conditions.

58. Application of Phenotype Microarray to Improve Metabolic Network Modeling of *Chlamydomonas reinhardtii*. **H. Cai**, A. Chaiboonchoe, B. Dohai, D. Nelson, A. Jaiswal, K. Salehi-Ashtiani. New York University Abu Dhabi, Division of Science and Math, and New York University Abu Dhabi Institute, Center for Genomics and Systems Biology; Abu Dhabi, UAE.

Genomic, transcriptomic, and proteomic studies have vastly improved our understanding of microorganisms at the genic level, addressing the question of "what might be there". However, the question of "what actually is there and what is it doing" remains unclear due to the lack of functional and phenotypic analysis of predicted genes. High-throughput Phenotype Microarrays (PMs) make it possible to test thousands of cellular phenotypes simultaneously and efficiently on 96-well microplates. In each single well, cell respiration and redox chemistry is used as a universal reporter for cell growth. The presence of computationally predicted gene functions can be tested and genome annotation can be improved by comparing closely related strains or cell lines; metabolic properties can be analyzed to optimize bioprocesses, as well as gaining a comprehensive overview of pathway functions in microorganisms. The PM technology has not been reported for metabolic phenotyping of microalgae previously.

In this study, we pioneered the application of Biolog Phenotype Microarray profiling in *Chlamydomonas reinhardtii* research to provide experimentally derived information for metabolic network validation and refinement. A protocol is developed and adapted to profile cellular phenotypes of *C. reinhardtii* CC-503. Nearly 1,000 assays were conducted for carbon, nitrogen, phosphorus and sulfur source utilization, peptide nutrient stimulation, osmotic stresses and pH tolerance. All plates were incubated at 30 °C, for up to 7 days and read with an Omnilog system every 15 min. "OPM" software package based on R was used and adapted to facilitate data interpretation. Kinetic curves and statistical analysis are being carried out to visualize the metabolic properties. Our results not only validated a good number of presenting metabolites but also identified a significant number of novel metabolites that is not accounted for in our current in silico model. The study provides a novel high throughput method to bridge the missing links between genomics and metabolomics.

59. Identification of mutants with constitutive *HYDA1* expression using a motility screen. X. Sun, P. Lefebvre, M. LaVoie, **C. Silflow**. Dept. Plant Biology, University of Minnesota, St. Paul, MN.

In nature, Chlamydomonas cells routinely experience anoxic conditions to which they respond with extensive changes in metabolic pathways. The expression of [FeFe]-hydrogenase enzymes encoded by the *HYDA1* and *HYDA2* genes and the production of hydrogen are components of the metabolic repertoire for response to hypoxia. Relatively little is known about the molecular mechanisms used by *Chlamydomonas* cells to detect low O_2 levels, to transduce the hypoxia signal, and to initiate large-scale changes in gene expression. Using a reporter gene system composed of a *HYDA1* gene promoter fused to the coding sequence of the HA-tagged Radial Spoke Protein 3 (*RSP3*) gene required for flagellar motility, we have developed a genetic screen to identify transacting factors in the pathway for regulating expression of the *HYDA1* gene. When transformed with the reporter gene, cells with a mutation in the *RSP3* gene (*pf14*) are immotile in aerobic conditions but become motile in anoxic conditions. We selected for chemically-induced mutations that allow cells to swim constitutively, even in aerobic conditions. The mutant motile strain B6-F shows upregulated expression of the HA-tagged Rsp3 protein. The strain also shows a ca.15-fold increase in transcript levels for *HYDA1*, but *HYDA2* transcript levels are not changed. When compared to the parent strain, the transcriptome of the B6-F mutant shows a greater than three-fold increase in transcripts for more than 50 genes, including those encoding proteins involved in anaerobic metabolism and the copper-deficiency response. We cloned the wild-type *CHC1* (Constitutive expression of Hydrogenase and Copper-responsive genes) gene and demonstrated that it can rescue the motility phenotype of B6-F cells, causing them to become immotile. The immotile strains also show rescue of the altered gene expression phenotype for *HYDA1*, *HYDG*, *FDX5*, *CYC6*, and *CPX1* genes, as shown by qPCR. The *CHC1* gene may function in the same pathway as the *CRR1* gene, as shown by analysis of progeny from *chc1* x *crr1* crosses. Expression of the reporter gene requires the *CRR1* wild-type allele together with the *chc1* allele. The data suggest that the *CHC1* gene encodes a negative regulator in the *CRR1* pathway. The *chc1* mutant cells respond to anoxia by increasing the transcript levels of the *HYDA1* and *HYDA2* genes further, in a manner similar to that of wild-type cells, indicating that the pathway in which *CHC1* functions is only partially responsible for the response to anoxia.

60. The role of pyruvate-ferredoxin-oxidoreductase in *Chlamydomonas reinhardtii* fermentative metabolism. **C. Catalanotti[1]**, W. Yang[1], S. D'Adamo[2], A. Atteia[3], R. van Lis[3], W. Inwood[4], M. Kobayashi[4], K. Niyogi4[4], M.C. Posewitz[2], A.R. Grossman[1]. 1) Carnegie Institution, Stanford, CA; 2) Colorado School of Mines, Golden, CO; 3) Centre National de la Recherche Scientifique-Aix-Marseille Université, France; 4) University of California, Berkeley.

Oxygen is used by aerobic microbes for the efficient production of ATP through oxidative phosphorylation. Nevertheless, it is common for microbes to experience hypoxic and even anoxic conditions in the natural environment. To maintain viability during hypoxia/anoxia, cells activate fermentation pathways that allow them to recycle/re-oxidize the NADH that is produced during the catabolism of glucose (mostly via glycolysis), which sustains the production of ATP. Re-oxidation of NADH involves the transfer of electrons to suitable acceptor molecules that can then be excreted from the cell. While aerobic respiration can generate over 30 ATP molecules per molecule of glucose oxidized, anaerobic or fermentative pathways only generate ~2 or 3 ATP molecule per molecule of glucose catabolized.

There is little known about the specific role(s) of the different branches of fermentation metabolism in algal physiology and how the algae sense and respond to a changing oxic environment. To elucidate fermentation pathways in the unicellular green alga *Chlamydomonas reinhardtii*, we generated mutants defective for specific fermentation reactions using both insertional mutagenesis and TILLING. Among the mutants characterized are strains disrupted for the genes encoding pyruvate formate lyase, pyruvate-ferredoxin-oxidoreductase (PFR1), alcohol dehydrogenase, hydrogenases A1 and A2, hydrogenase EF, phosphate acetyltransferase, acetate kinase and ferredoxin 5. The TILLING approach yielded five different mutant alleles of *PFR1*; a number of the alterations in the PFR1 amino acid sequence are non-synonymous substitutions in close proximity to the active site of the enzyme and are likely to impact the activity of the enzyme. Characterizations of the various mutants have revealed pathways for the fermentative elimination of reductant that had not be observed in WT *C. reinhardtii* cells under standard laboratory conditions, and demonstrate the enormous metabolic flexibility that this alga has evolved forfunctioning in hypoxic/anoxic conditions. (Research funded by Grant No. DE-FG02-12ER16338).

61. Discovery of a novel Complex I biogenesis factor regulating mitochondrial gene expression. Nitya Subrahmanian[1], Claire Remacle[2], **Patrice Hamel[1]**. 1) Department of Molecular Genetics, Ohio State University, 500 Aronoff Laboratory, 318 W. 12th avenue, 43210 Columbus, Ohio,; 2) Genetics of Microorganisms Laboratory, Department of Life Sciences, Université de Liège, B-4000, Liège, Belgium.

Mitochondrial Complex I (CI), a proton pumping NADH:ubiquinone oxidoreductase, is the most complicated enzyme of the respiratory chain. The assembly of this multimeric enzyme, is an intricate process that is poorly understood. The objective of this study was to identify novel factors involved in CI biogenesis using *Chlamydomonas reinhardtii* as an experimental model system. From a forward genetic screen conducted by insertional mutagenesis, the *amc11* mutant with a CI assembly defect was isolated. The mutation in *amc11* was mapped to a novel gene that encodes a unique protein with no conserved domains. The role *AMC11* in CI assembly was confirmed by complementation studies. In order to understand the basis for the CI deficiency due to loss of AMC11, the mitochondrial transcript levels encoding for the five CI subunits was determined. Interestingly, the accumulation of only the *nd4* transcript was reduced in the *amc11* mutant, the levels of which are restored upon complementation with the *AMC11* gene. Also,substitution of mitochondrial *nd4* coding sequence with a reporter in the *amc11* mutant results in loss of reporter protein levels with little impact on the accumulation of the reporter transcript. From previous studies on mitochondrial *nd4* mutants it is known that loss of ND4, a CI subunit of the membrane arm, is characterized by the accumulation of a labile sub-complex which is also the assembly defect observed in *amc11.* Thus,

the forward genetic screen has led to the discovery of AMC11, a novel nuclear-encoded protein required for the stability and translation of the mitochondrial *nd4* transcript.

62. Exploiting thiamine regulation in *Chlamydomonas* for novel regulatory tools. **Ginnie T. D.T. Nguyen**, Mark A. Scaife, Chloë E. Scott, Alison G. Smith. Department of Plant Sciences, University of Cambridge, Cambridge, UK, CB2 3EA.

Microalgae, such as the model green alga *Chlamydomonas reinhardtii,* are emerging as a metabolic engineering platform for the production of biofuel and high-value compounds. In comparison with other organisms like *Escherichia coli* and yeast, applications in algae are still limited due to lack of genetic elements to regulate gene expression, protein function, and metabolism (1). Our lab is studying thiamine metabolism in *C. reinhardtii*, and as a result has identified regulatory elements, which are thiamine pyrophosphate (TPP) dependent riboswitches (2). Riboswitches are regions in the mRNA to which a ligand - in this case TPP - binds, altering the secondary structure and thereby affecting expression of the mRNA. The *C. reinhardtii THI4* and *THIC* riboswitches mediate this effect by causing alternative splicing of the transcript. We have been investigating whether TPP riboswitches can serve as a new, robust and universal regulatory system for metabolic engineering in eukaryotes.

In detail, we have developed several constructs in which the regulatory mechanism of two TPP riboswitches are evaluated *in vivo* by their effect on the expression of a reporter gene. By this means, we have been able to dissect the important elements of TPP riboswitches and splicing mechanism. Through a rational approach, we have generated a collection of novel regulatory tools that respond to specific ligands, and propose a guideline of how to use thiamine as a repressible and reversible regulator element in controlling transgene expression in *C. reinhardtii*. This work has also revealed unexpected complexity in the endogenous thiamine biosynthetic pathway, in particular that two *C. reinhardtii* riboswitches showed differential sensitivity to thiamine biosynthesis intermediates (3).

1.Rosales-Mendoza, S., Paz-Maldonado, L. M. T., & Soria-Guerra, R. E. (2012). *Plant Cell Reports*, *31*(3), 479 – 94.
2.Croft, M., Moulin, M, Webb, M. E., & Smith, A. G. (2007). *PNAS*, *104*, 20770–5.
3.Moulin, M., *Nguyen, G. T. D. T., Scaife, M. A., Smith, A. G., & Fitzpatrick, T. B. (2013). *PNAS*, *110*(36), 14622–7.

63. Control of ER stress-induced autophagy by ROS in Chlamydomonas. Marta Pérez-Martín[1], María Esther Pérez-Pérez[2], Stéphane D. Lemaire[2], **José L. Crespo[1]**. 1) Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, 41092 Sevilla, Spain; 2) Centre National de la Recherche Scientifique, Sorbonne Universités UPMC Univ. Paris 06, UMR8226, Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, Institut de Biologie Physico-Chimique, 75005 Paris, France.

Autophagy is a complex catabolic process by which eukaryotic cells recycle or degrade internal constituents in a membrane-trafficking pathway. Our laboratory has previously shown that autophagy is conserved in Chlamydomonas and that this degradative process is activated in response to a range of stress conditions including nutrient limitation, oxidative stress, accumulation of unfolded proteins in the endoplasmic reticulum (ER), or photo-oxidative damage (1, 2, 3). In this study, by analyzing specific autophagy and ER stress markers, cell survival and ROS production, we provide experimental evidence connecting ROS generated during ER stress and autophagy activation. Treatment of Chlamydomonas cells with the ER stressor tunicamycin resulted in autophagy induction (1). Our results indicate that tunicamycin also led to the upregulation of ER resident ERO1 oxidoreductase and protein disulfide isomerases as well as to the production of ROS and higher levels of oxidized glutathione in the cell (4). Moreover, we found that the antioxidant glutathione partially suppressed autophagy in ER-stressed cells (4), further supporting a prominent role of ROS in the control of autophagy (5).

(1) Pérez-Pérez ME, Florencio FJ and Crespo JL (2010) *Plant Physiol* 152: 1874-1888
(2) Pérez-Pérez ME and Crespo JL (2010) *Autophagy* 6(4): 562-563
(3) Pérez-Pérez ME, Couso I and Crespo JL (2012) *Autophagy* 8: 376-388
(4) Pérez-Martín M, Pérez-Pérez ME, Lemaire SD and Crespo JL (submitted)
(5) Pérez-Pérez ME, Lemaire SD and Crespo JL (2012) *Plant Physiol* 160: 156-16.

64. *Chlamydomonas* Argonaute3 is the major catalytic engine of miRNA-mediated post-transcriptional gene silencing. **Tomohito Yamasaki**, Takeshi Ohama. Department of Environmental Systems Engineering, Kochi Univ of Tech, Kochi, Japan.

MicroRNAs (miRNAs) are 20-24 nucleotide (nt) noncoding RNAs that play important regulatory roles in a broad range of eukaryotes by pairing with mRNAs to direct post-transcriptional repression. The mechanistic details of miRNA biogenesis and miRNA-mediated post-transcriptional regulation have been well documented in multicellular model organisms. However, this process remains poorly studied in *Chlamydomonas reinhardtii*. Argonaute (AGO) proteins recruit miRNAs to form the core of silencing effector complexes and *Chlamydomonas* encodes three AGO proteins (AGO1-3). AGO1 is more divergent than the other paralogs and AGO1 appears to be involved in transposon silencing. On the other hand, given the striking amino acid sequence similarity between AGO2 and AGO3, they might be functionally redundant, and they had been suspected to be involved in the canonical miRNA-mediated gene silencing. In this study, we isolated *AGO2* mutant (*ago2-1*) and *AGO3* mutant (*ago3-1*). Interestingly, RNA blot and small RNA-seq analyses revealed that the most of miRNAs were significantly decreased in *ago3-1*, but not in *ago2-1*. In according with this finding, miRNA-mediated target mRNA cleavage was severely impaired only in *ago3-1*. Moreover, virtually no 12-nt long cleavage product of passenger strand miRNA was detected in *ago3-1*, suggesting that AGO3 mediates slicer-dependent unwinding of miRNA duplex. Our results suggest that AGO3 plays a principal role in miRNA-mediated gene silencing. In addition, we isolated two mutants defective in miRNA biogenesis. *DUS13* (*dull slicer 13*) mutant (*dus13-1*) accumulates no 12-nt long cleavage product of passenger strand miRNA, suggesting that *dus13-1* was impaired in

the ability to slice passenger strand miRNAs. *DUS16* mutant (*dus16-1*) exhibited a substantial increase in primary-miRNA accumulation and a depletion of mature miRNAs, suggesting that the processing of primary-miRNA was impaired in *dus16-1*. Analysis of these unique mutants will likely uncover the mechanistic detail of the miRNA biogenesis pathway in *Chlamydomonas*.

65. Computational prediction of AGO3 associated microRNAs and their targets in *Chlamydomonas reinhardtii*. **Adam Voshall**[1], Eun-Jeong Kim[1], Etsuko N. Moriyama[1,2], Heriberto Cerutti[1,2]. 1) School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE; 2) School of Biological Sciences and Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE.

While the production of microRNAs (miRNAs) by *Chlamydomonas reinhardtii* has been established for several years, little is known about how they target transcripts for regulation or what role they play in cellular processes. Additionally, a number of miRNA sequences have been deposited in miRBase but a recent re-evaluation of these *Chlamydomonas* miRNAs suggested that many might actually correspond to endogenous small interfering RNAs (e.g., multiple products from a long hairpin precursor without corresponding star sequences and/or lacking stable 5' processing). To characterize functional miRNAs in *Chlamydomonas*, we identified small RNAs associated with Flag-tagged-AGO3 by affinity purification and deep sequencing. Using a stringent set of criteria for canonical miRNA annotation, we found 38 suitable precursor miRNAs, which produce 44 unique miRNA sequences including 10 previously identified *C. reinhardtii* miRNAs and 34 novel ones. We also attempted to identify miRNA target transcripts, based on the complementarity of the miRNAs and the respective binding sites. Recent results with reporter constructs have indicated that *Chlamydomonas* miRNAs may regulate target genes through either transcript cleavage or translation repression. Thus, potential targets were divided into two categories depending on the extent of complementarity, those likely to be regulated through cleavage and those likely to be regulated through translational repression. The search for cleavage targets identified 102 transcripts, including 13 with perfect mRNA:miRNA matches. However, only 9 transcripts (5 with perfect matches) showed at least a 2-fold up-regulation of expression in a mutant strain deficient in miRNA biogenesis. The search for translational repression targets, which used complementarity criteria more stringent than those experimentally required for a reduction in target protein levels, identified 466 transcripts. Our efforts are now aimed at trying to validate some of the predicted miRNA targets.

66. Discovery of long non-coding RNAs via their distinct chromatin signature in Chlamydomonas. **D Strenkert**[1], S Cokus[1], SD Gallaher[1], JM Zones[2], JG Umen[2], M Pellegrini[1], SS Merchant[1]. 1) Institute of Genomics and Proteomics, University of California, Los Angeles; 2) Donald Danforth Plant Science Center St. Louis, Missouri.

Recently, it has become evident that RNAs, other than messenger RNAs (mRNAs) are capable of regulating biological processes just like proteins do. Besides the well-studied small non-coding RNAs like siRNAs and microRNAs, longer transcripts exist, that are called long non-coding RNAs (lncRNAs) that fulfill diverse biological functions. We analyzed the transcriptome of synchronized Chlamydomonas cells during a dark / light cycle. RNA sequencing showed that a large fraction of the genome is expressed periodically during the Chlamydomonas cell cycle (Zones et al. unpublished). We assume that if long non-coding RNAs exist in Chlamydomonas, we should be able to capture them during the diurnal life cycle. Bioinformatic tools enabled us to create a list of candidate lncRNAs, including one that we are calling "cRe-nc-v0.216A". In an experiment, we sampled cells during the transition from dark to light, and verified that "cRe-nc-v0.216A" is up-regulated during the end of the dark period of the Chlamydomonas life cycle. Chromatin- Immunoprecipitation (ChIP) is a powerful tool for investigating genomic regions in terms of either their association with transcription factors and/or histones. Coupled with deep sequencing it allows the untargeted investigation of binding sites of DNA binding proteins across the whole genome. Long non-coding RNAs harbor a very specific chromatin signature. This so-called K36-K4 chromatin domain was described previously to be associated with longer transcripts that are biologically meaningful but harbor no translational potential in mammalian cells. By correlating K36-K4 domains with the transcriptomic data sets, we are able to discriminate between transcriptional noise and biologically significant transcripts within our set of candidate lncRNAs. Therefore, we performed ChIP with an antibody specific for trimethylated H3K4 (H3K4me3) on samples taken during different time points of the Chlamydomonas cell cycle, followed by deep sequencing. When we mapped H3K4me3 binding sites to the Chlamydomonas genome, we observed enrichment of H3K4me3 at the promoter regions of coding genes, as has been observed for higher plants and mammals. Strikingly, we identified loci of H3K4me3 in the Chlamydomonas genome that are not annotated as promoter regions of coding genes. For example, the potential lncRNA candidate cRe-nc-v0.216A, shows enrichment of H3K4me3 and is therefore most likely biologically meaningful. In future work, we will employ reverse genetics of cRe-nc-v0.216A with inducible artificial microRNAs to enable a more detailed functional analysis of this lncRNA.

67. A SUMO E2 conjugase mutant in *Chlamydomonas reinhardtii* reveals the role of SUMOylation during stress responses. Amy Knobbe[1], Kempton Horken[1], Thomas Plucinak[1], Eniko Balassa[2], Heriberto Cerutti[2], **Donald Weeks**[1]. 1) Dept Biochemistry, Univ Nebraska, Lincoln, NE; 2) School of Biol. Sci., Univ Nebraska, Lincoln, NE.

Post-translational modification of proteins in eukaryotic cells by SUMO is involved in a diverse array of cellular processes, including various stress responses. In the unicellular green alga *Chlamydomonas reinhardtii* SUMOylation of multiple high molecular weight proteins is induced in response to abiotic stress. We have discovered and characterized a SUMO E2 conjugase deletion mutant in *C. reinhardtii*, *mut5* that fails to modify proteins with SUMO in response to multiple stress conditions. This failure to SUMOylate generally results in a reduced tolerance to a given stress. Complementation of CrUBC9 mutants with the deleted gene demonstrates that CrUBC9 is solely responsible for SUMOylation under stress conditions, and reveals that Mut5 predominantly localizes to the nucleus. Although *mut5* shows negative growth phenotypes under stress conditions, it has no observable phenotypes under non-stress conditions. We will present evidence that this is likely because the SUMOylation of proteins under non-stress conditions in *C. reinhardtii* is carried out by a second SUMO conjugase - a situation likely unique to *C. reinhardtii* and closely related algae.

68. Systems Biology in *Chlamydomonas reinhardtii*: A Case Study Using Contractile Vacuole Function and Osmoregulation as Example. **Burkhard Becker**, Karin Komsic-Buchmann. Biozentrum Köln, Universität zu Köln, Köln, Germany.

Systems Biology aims to model and discover emergent properties of biological systems. We applied a systems biology approach to osmoregulation and contractile vacuole function in *Chlamydomonas*. We will present a model of CV function/osmoregulation in *Chlamydomonas*. The model incorporates cellular growth as well as osmotic stress and allows prediction of the relative changes in metabolite/protein concentrations upon environmental challenges. The latter can be experimentally tested. Major characteristics of the osmoregulatory system and predictions of its behavior upon osmotic stress will be presented.

69. Evolution of sex in *Chlamydomonas* as a response to grazing. **Hanna Koch**, Martha Valiadi, Lutz Becks. Evolutionary Ecology & Community Dynamics, Max Planck Institute for Evolutionary Biology, Plön, Schleswig-Holstein, Germany.

Understanding the evolutionary advantage of sexual reproduction and its maintenance remain key areas of focus within evolutionary biology. A leading hypothesis is that sex may confer an advantage to populations exposed to novel or stressful environments, as recombination can increase genetic variation, thereby facilitating higher rates of adaptation. We used experimental evolution to compare differences in adaptation between sexual and asexual prey populations and link fitness changes to phenotype and genotype for ecologically-relevant traits. This was done using the predator-prey model system of rotifers, *Brachionus calyciflorus*, feeding on an algal prey, *Chlamydomonas reinhardtii*. *Chlamydomonas* is an ideal system to use in sex studies as it can reproduce both asexually and sexually, the induction of sex can be easily manipulated in the laboratory, and its sexual cycle has been studied in detail. We combined experimental evolution with functional genomics to a) compare adaptive differences in sexual and asexual prey populations exposed to predation, and b) determine the genetic basis of an adaptive defense trait in the prey of this predator-prey system. For the experimental evolution study, asexual and sexual prey treatments, with and without rotifer predation, were carried through alternating growth and sexual cycles for a total of 12 growth and 6 sexual cycles. Our results show an evolutionary advantage of sex in prey populations exposed to rotifer predation as they had a greater increase in mean population fitness compared to asexual treatments. In the sexual prey-rotifer treatments, mean population fitness levels were higher or close to that of the controls. This trend was not seen in the asexual treatments. We also tested for the short and long-term effects of sex by comparing mean and variance in fitness (growth rate and grazing resistance) for asexual and sexual offspring. Our results revealed a higher mean fitness (short-term effect) and higher variance in fitness (long-term effect) in sexual offspring. One evolutionary adaptation to rotifer predation is growth in colonies that are too large to be ingested. We compared the genome of an evolved Chlamydomonas colonial clone to that of an ancestral clone, and compared the transcriptome of each genotype under relevant conditions. The two genotypes showed significant differences in gene expression patterns with respect to genes related to the flagellum, cell wall proteins, and to sensing of extracellular stimuli. At the genome level, the colonial genotype had 199 unique mutations with 18 predicted to have 'high' functional effects. These data are consistent with the modification of cell structure unique to the evolved colonial genotype.

70. Interkingdom Signaling Between *Chlamydomonas reinhardtii* and Bacteria. **Sathish Rajamani**[1], Max Teplitski[2], Richard Sayre[1]. 1) Bio-Labs, New Mexico Consortium, Los Alamos, NM, USA; 2) Department of Soil and Water Sciences, University of Florida, Gainesville, FL, USA.

Bacterial cell-population sensing or quorum sensing (QS) through the production, secretion and detection of small molecular signals for their coordinated gene expression behavior is a widespread attribute in the bacterial community. Well-studied examples of QS molecules include Acyl Homoserine Lactones (AHLs) produced by Gram-negative bacteria. AHLs control the expression of unique genes through their interactions with transcription factors, including the LuxR family of protein receptors. Disruption of QS results in significant alteration in important bacterial physiological functions including virulence, biofilm formation, symbiosis, etc. Importantly, other organisms in a shared environment can sense and manipulate QS signaling behavior to gain advantage over the niche. Eukaryotes, including plants and algae have also been shown to utilize varied strategies to alter bacterial behavior. For instance, plants and algae can produce molecules that mimic QS signals and act as agonist or antagonists of QS signal. To understand the nature and significance of the QS mimic molecules in interkingdom signaling between eukaryotes and bacteria, we used *Chlamydomonas reinhardtii* to study bacterial QS signal interference and gene expression modulations by compounds produced by algae. Through our investigations, we have identified QS mimic activities produced by *Chlamydomonas* that impact bacterial QS. Interestingly, depending on the algae growth media, we also found that production of QS mimic activity can be substantially altered. *Chlamydomonas* cultured in minimal media accumulated a large diversity of separable mimic activity fractions. However, when cultured under carbon-rich media, they predominantly produced Lumichrome, a vitamin Riboflavin derivative as bacterial QS stimulatory mimic. Importantly, lumichrome is a structurally unrelated molecule to AHL and has no conserved homoserine lactone ring. When a *Chlamydomonas* AHL stimulatory QS mimic activity was applied to *Sinorhizobium melilotti,* it was found to differentially alter the proteome of the organism than its native AHL signal. Furthermore, AHL mimic activities from algae, with the exception of lumichrome, were inactivated by enzyme AHL lactonase that specifically hydrolyzes the homoserine lactone ring of AHLs. Expression of AHL lactonase in *Chlamydomonas* resulted in substantial reduction of algal AHL QS mimics and increased bacterial growth and biofilm formation in algal cultures. Characterizing these mimic structures and biochemical pathways will help explain the significance of these interkingdom signaling interactions.

71. RNA is a component of Chlamydomonas flagella and Tetrahymena cilia. **P. Lefebvre**[1], P. Ranum[1], L.-W. Tam[1], W. Dentler[2]. 1) Dept Plant Biol, Univ Minnesota, St Paul, MN; 2) Dept Molecular Bioscience, Univ Kansas, Lawrence, KS.

Purified Chlamydomonas flagella when analyzed on polyacrylamide gels contain several major bands in the range of 125 to 300 nucleotides when stained with ethidium bromide. The bands are present in flagella purified from gametes and vegetative cells of 5 different Chlamydomonas strains. The bands were released from axonemes by extracting flagella with NP-40, suggesting that they are membrane/matrix-associated RNA. The identity of these bands as RNA was established by digestion with RNase but resistance to digestion with DNase. RNA-sequence analysis of flagella or individual bands excised from gels revealed these bands to represent specific subfragments of 18s, 28s and 5s ribosomal RNAs. We are developing procedures to confirm the identity of these bands and to identify the proteins with which they are associated. Remarkably, nearly identical bands were found in detergent-extracted cilia purified from Tetrahymena, which suggests that these novel RNAs may be important and possibly universal flagellar and ciliary components.

72. The Antarctic *Chlamydomonas raudensis*: a case for advances in understanding photosynthetic stress adaptation in non-model organisms. **Rachael Morgan-Kiss**[1], Sarah Stahl[1], Wei Li[1], Jenna Dolhi[1], Andor Kiss[1,2]. 1) Microbiology, Miami University, Oxford, OH; 2) Center for Bioinformatics & Functional Genomics, Miami University, Oxford, OH.

Photosynthetic organisms occupy almost every ecological niche on our planet and must therefore survive environmental stresses that can accelerate the development of photooxidative processes. For example, photosynthetic microorganisms in polar habitats such as Antarctica have developed mechanisms to persist and thrive under permanent low temperatures. Cold-induced photooxidative processes have been thoroughly described for several model plants and algae; however, most studies have focused on chilling-sensitive or cold-tolerant species that are exposed to transient low temperatures in their natural habitats. Photosynthetic organisms adapted to permanent cold must balance temperature-independent reactions of light energy capture/transduction with downstream temperature-dependent metabolic processes such as carbon fixation; however, the underlying mechanisms are poorly understood. *Chlamydomonas raudensis* UWO241 (UWO241) is a psychrophilic green algal species and is a member of the photosynthetic community that provides the majority of fixed carbon for ice-covered Antarctic lakes. As a consequence of long term exposure to abiotic stress (low temperature, extreme shade/blue light, hypersalinity, nutrient deprivation, high oxygen tension), UWO241 exhibits a miriad of adaptive strategies, including unbalanced photosystem stoichiometry, high rates of cyclic electron transport, polyunsaturated membrane lipids, as well as significant oil body accumulation. In this presentation, these past studies on physiology and photobiology will be linked with new transcriptome-based data which indicate that this “non-model” organism possesses many gene products associated with survival under permanent stress, including antioxidant pathways and carbon concentrating mechanisms. Gains in photosynthetic metabolism that benefit mankind will include engineering solutions derived from organisms such as *Chlamydomonas raudensis* UWO241 that have solved extreme living conditions through unique adaptations.

73. An emerging unicellular green alga model system for studying regulation of photosynthesis. **Melissa Roth**[1], Sean Gallaher[2], Shawn Cokus[3], David Lopez[3], Matteo Pellegrini[3], Sabeeha Merchant[2], Krishna Niyogi[1,4]. 1) Plant and Microbial Biology, UC Berkeley; 2) Department of Chemistry and Biochemistry, UCLA; 3) Department of Molecular, Cell and Developmental Biology, UCLA; 4) Howard Hughes Medical Institute.

Studying fundamental processes in a simple organism may provide insights into the basic regulatory mechanisms in the green plant lineage. The unicellular green alga *Chromochloris zofingiensis* (formerly of the genus *Muriella* or *Chlorella*) has extraordinary photoprotection capacity, produces the commercially valuable ketokerotenoid astaxanthin, and is of interest as a biofuel feedstock. Because of its value in understanding basic regulation of photosynthesis, we are developing a molecular toolkit for *C. zofingiensis*. We have found that *C. zofingiensis* has a relatively small genome, about half the size of *Chlamydomonas reinhardtii,* and a reduced number of genes. Compared to *C. reinhardtii* and *Arabidopsis thaliana*, *C. zofingiensis* has respectively 4-fold and 2-fold higher photoprotective capacity, as assessed from measurements of nonphotochemical quenching of chlorophyll fluorescence (NPQ). The major NPQ pathway of quenching excess energy, qE, is turned on and off rapidly on a timescale of seconds to minutes, and is important for coping with rapid changes in incident sunlight. In *C. zofingiensis*, qE has two phases including a rapid initial increase and a slow subsequent rise. Experiments with nigericin, which disrupts the pH gradient, suggest that the rapid initial rise in NPQ immediately after illumination is most likely caused by a high transient pH. Experiments with inhibitors and varying the amount of dark-acclimation time prior to NPQ measurements (to allow for zeaxanthin re-epoxidation) suggest that the slow subsequent rise in NPQ is caused by the formation of zeaxanthin. The *C. zofingiensis* molecular toolkit will help elucidate how high photoprotection capacity in algae is achieved.

74. Body size, offspring number, and generation time in volvocine algae: regulation of interrelated traits during a major evolutionary transition. **Deborah E. Shelton**, Richard E. Michod. Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ.

Volvocine algae have an asexual cycle in which a number of cell divisions occur in rapid succession following a prolonged period of growth. In unicellular *Chlamydomonas reinhardtii*, the average number of rounds of cell division per cycle, *n*, increases approximately linearly with increasing light intensity for cells grown in constant light. This reaction norm reflects regulation of cell number, a trait that is thought to be among the first to have changed during the unicellular-to-multicellular transition in volvocine algae. We propose a model in which cells first grow for a constant time period during which divisions cannot occur. Based on the cell size after this time period, each cell then establishes a target volume and divisions are initiated when the target volume is reached. This model reconciles cell- and population-level observations of the behavior of *C. reinhardtii* cells in constant light. We also present an optimality analysis of the *n* reaction norm on light intensity. We focus on the impact of two largely endogenous factors: time needed to switch between growth and divisions and size-dependent cell growth. The combination of this cost and benefit can give a reaction norm in which optimal *n* increases as a function of increasing light intensity (as is seen in *C. reinhardtii*). Finally, we explore the way in which dependence of cell growth rate on colony size could favor lower *n* for a given light intensity in colonies compared to unicells. If this effect is indeed relevant to early-

branching colonial volvocines, then *n* regulation in these simple colonies would be adaptive specifically at the group level. This suggests the possibility that group-level individuality in the sense of specifically group-level adaptedness emerged very early in the volvocine transition to multicellularity in spite of the fact that group-level individuality in the sense of indivisibility of the higher-level unit emerged later.

75. Evolution of cellular mechanisms of embryo inversion that enabled volvocine algae to transition from flat to spherical colony. P. R. V. Elvira[1], **I. Nishii[1,2]**. 1) Temasek Life Sciences Laboratory, Singapore; 2) Department of Biological Sciences, NUS, Singapore.

Understanding the evolution from unicellularity to multicellularity entails an analysis of a set of organisms with varying levels of complexity. The volvocine algae are an ideal group as they offer an array of species with increasing number of cells per colony, such as: *Chlamydomonas* (1 cell), *Gonium* (8-16 cells in a flat colony), *Pandorina* (16 cells, spherical), *Eudorina* (32 cells, spherical), *Pleodorina* (64-256 cells, spherical) and *Volvox* (thousands of cells, spherical). Our aim is to use morphogenesis in explaining their varied colonial morphologies. During embryogenesis, spherical colonial species undergo inversion after a series of incomplete cytokinesis resulting in a curved cell sheet with flagella pointing inward. In *V. carteri*, inversion is achieved when the cells, linked to each other by cytoplasmic bridges (CBs), change cell shape while moving against CBs. The cell shape changes have been shown to be microtubule (MT)-dependent and the concurrent cell migration is powered by a kinesin, InvA. Before inversion corn-shaped cells are linked to each other above the equatorial region. When inversion starts they gradually become spindle-shaped. As they elongate to become flask-shaped, cells migrate against CBs until they are linked at the tips of the elongated stalks and the embryo inverts. This inverted embryo with flagella outside allows for the growth of a motile, spherical colony. To examine if the same mechanisms are true for smaller inverting species we analyzed cell shape changes using cell morphometry and MT-staining, as well as InvA localization in *P. morum*, *E. elegans* and *P. japonica*. We found that all three species also changed shape, but shorter than that of *V. carteri,* with cell lengths related to the embryonic cell number. For the three species, InvA was co-localized with the CBs, against which cells migrated like that of *V. carteri*, suggesting its key role for inversion. Further, the knockdown of *P. morum invA* by RNAi prevented the embryo from being a spherical colony but it developed as a flat colony resembling that of non-inverting *G. pectorale*. These results show the mechanisms of inversion in volvocine species with distinct levels of complexity, suggesting how flat colonies led to spherical ones.

76. The Genome of *Gonium pectorale*: Early evolutionary co-option of genes important for multicellularity occurred during the transition to colonial multicellularity. **Bradley Olson[1]**, Erik Hanschen[2], Tara Marriage[1], Patrick Ferris[2], Takashi Hamaji[3], Hisayoshi Nozaki[3], Atsushi Toyoda[4], Asao Fuiyama[4], Pierre Durand[5], David Smith[6], Richard Michod[2]. 1) Div Biology, Kansas State Univ, Manhattan, KS; 2) Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ; 3) University of Tokyo, Tokyo, Japan; 4) National Institute of Genetics, Japan; 5) University of Witswaterstrand, Johannasberg, South Africa; 6) University of Western Ontario, Canada.

The evolution of multicellular organisms is a major evolutionary state transition that has occurred at least twenty-five independent times in all domains of life, yet the molecular basis of the transition is unknown. The Volvocine algae, including the unicellular *Chlamydomonas reinhardtii* and its multicellular relatives such as colonial *Gonium* and *Volvox*, have been used as an important model system for multicellular evolution. The sequencing of the *Chlamydomonas* and *Volvox* genomes revealed that they were remarkably similar with a few notable differences that were attributed to *Volvox* specific functions. However, by sequencing the genome of the colonial multicellular alga *Gonium pectorale* we have found that nearly all the major genomic changes found *Volvox* were already present in the *Gonium* genome. For example these gene families have expanded in *Gonium* and *Volvox*: a sex limited retinoblastoma cell cycle regulator (*MAT3*), cyclin D1 family of cell cycle regulators, most of the extracellular matrix proteins, all families of transcription factors. Taken together these data suggest that genes important for multicellularity were co-opted early during the transition to colonial multicellularity. The only gene that did not evolve in *Gonium*, is the terminal cell differentiation factor *regA*. In a second approach, we synchronized the cell cycles of *Chlamydomonas* and *Gonium* and performed RNA-sequencing analysis to determine the genome-wide expression pattern of all genes. From this analysis we have found that many of the genes that are expanded in *Gonium* compared to *Chlamydomonas* have altered cell cycle regulation. Moreover, we have identified five candidate genes, two transcription factors and three cell-cell adhesion factors, whose sequences are similar between all Volvocales, but whose expression patterns have been altered in *Gonium* suggesting importance for multicellularity. In summary, using an evolutionary genomics approach we have several genes important for multicellularity in the Volvocine algae.

77. Axonemal dynein assembly mechanisms. Anudariya Dean, Paurav Desai, Judy Freshour, **David Mitchell**. Dept Cell & Dev Biol, SUNY Upstate Med Univ, Syracuse, NY.

Known loci that affect outer dynein arm (ODA) assembly in *Chlamydomonas* encode ODA subunits (*oda2, 4, 6, 9, 11, 12, 15*), axonemal docking site proteins (*oda1, 3, 14*), an accessory docking complex (*oda5*), IFT-associated transport factors (*oda16*) or cytoplasmic assembly factors (*oda7, pf13, pf22*). Here we report on positional cloning and characterization of two additional *oda* loci, *ODA8* and *ODA10*. We rescued both mutations using HA-tagged transgenes and show that both ODA8p (an LLR protein) and ODA10p (a coiled coil protein) fractionate nearly equally between cytoplasm and flagella. In dikaryon rescue experiments, pair-wise fusion of gametes carrying either of these mutations or *oda5* fails to restore wild type motility (Kamiya, JCB 107:2253-2258, 1988), suggesting that all three loci function in the same pathway. Consistent with this model, ODA8p is highly reduced in *oda5* and *oda10* flagella but not in cytoplasm indicating that ODA8p is dependent on ODA5p and ODA10p for its flagellar assembly, not its cytoplasmic stability. Conversely, neither ODA5p (Wirschell et al. MBC 15:2729-2741, 2004) nor ODA10p depend on ODA8p for their flagellar assembly, but they depend on each other for stability and co-IP from extracts, suggesting a direct interaction. Some (ODA8p) or all (ODA10p) of the flagellar protein was

axoneme-associated but only ODA10p was quantitatively extracted with 0.6M NaCl. When desalted extracts were mixed with *oda10* mutant axonemes, both dynein and ODA10p bound to the axonemes. However, ODA10p did not co-sedimented with the 23S ODA complex, and was not required for ODA binding to axonemes. Similar experiments show that ODA8p is not needed for in vitro binding of ODA to *oda8* axonemes. Unexpectedly, ODA complexes in *oda8* and *oda10* mutant cytoplasm display greatly reduced binding affinity to axonemes, indicating that ODA8p and ODA10p may be required in the cytoplasm to maintain assembly-competent outer dynein arms. To follow assembly in vivo, we have labeled an ODA subunit (IC2) with fluorescent protein tags. Confocal IF of assembly factors and TIRF microscopy of ODA assembly in wild type and mutant backgrounds is being used to reveal mechanisms of assembly.

78. Breaststroke flagellar photoresponse in *Chlamydomonas reinhardtii*. **Kyriacos C Leptos**, Kirsty Y Wan, Raymond E Goldstein. Applied Mathematics and Theoretical Physics, University of Cambridge, Cambridge, United Kingdom.

Phototaxis, the change of direction of swimming towards light, is of prime importance to photosynthetic microorganisms like *Chlamydomonas reinhardtii*. In this biflagellated alga, phototaxis is achieved by differential regulation of its two flagella, which are named *cis* and *trans* for their relative position to the cell's eyespot, so that the organism can steer towards light.
To further our understanding of this differential regulation, we conduct high-speed video microscopy on single *Chlamydomonas* cells in the presence of a blue-light stimulus and extract information about the two flagella using in-house image analysis tools. We describe how flagellar waveform, per-beat envelope area, and per-beat frequency vary as a function of light-stimulus intensity. We also perform experiments on a non-phototactic mutant that shows loss of differential regulation of the two flagella.

79. Outer Dynein Arm activity is modulated by ethanol. **Fan Yang**[1], C. Scarbrough[1], LA. Fox[2], J. Pavlik[3], WS. Sale[2], J. Sisson[3], M. Wirschell[1]. 1) Biochemistry, Univ Mississippi Medical Center, Jackson, MS; 2) Emory University, Dept. of Cell Biology, Atlanta, GA; 3) University of Nebraska Medical Center, Pulmonary, Critical Care, Sleep and Allergy Division, Omaha NE.

Diverse evidence indicates that alcohol abuse results in an increased incidence of pulmonary infection due to impaired mucociliary clearance. Analysis of motility in isolated mammalian airway ciliary axonemes has revealed that alcohol directly impacts normal motility driven by the ciliary dynein motors. Since the mechanism likely involves the disruption in axonemal protein phosphorylation, we hypothesized that alcohol directly alters conserved axonemal protein kinases or phospho-regulatory proteins that control the dynein motors. To test this hypothesis, we utilized the experimental advantages offered by the model genetic organism, *Chlamydomonas* to: [a] determine the precise effects of alcohol on ciliary dynein activity; and [b] identify conserved axonemal phosphoproteins that are altered or impaired by alcohol exposure. Based on analysis of swimming speed in live cells or in reactivated axonemes, we found that ethanol (0-100 mM) significantly inhibits ciliary motility in wild- type *Chlamydomonas* in a concentration-dependent manner. These results indicate that alcohol directly impacts a mechanism that is part of the axonemal structure. Taking advantage of informative mutant *Chlamydomonas* cells that lack specific ciliary dynein isoforms, we found that ethanol impacts the activity, or regulation, of the "outer dynein arm". Consistent with this finding, ethanol (10 mM) exposure results in a significant reduction in ciliary beat frequency, a parameter of ciliary movement that requires normal outer dynein arm function. Furthermore, using a phospho-threonine specific antibody, we determined that the phosphorylation state of DCC1 of the Outer Dynein Arm-Docking Complex (ODA-DC) is altered in the presence of ethanol. Moreover, using Oda-mutants that lack specific HC motor domains, we have determined that ethanol impacts the β-HC of the outer dynein arm. We are testing the hypothesis that the DCC1 phosphoprotein is part of an alcohol-sensitive mechanism that controls outer dynein arm activity.

80. *IDA6* encodes a conserved subunit required for assembly of the N-DRC and several inner arm dyneins. R Bower[1], D Tritschler[1], K VanderWaal[1], E O'Toole[2], T Heuser[3], D Nicastro[3], **M Porter**[1]. 1) University of Minnesota, Minneapolis, MN; 2) University of Colorado, Boulder, CO; 3) Brandeis University, Waltham, MA.

The nexin-dynein regulatory complex (N-DRC) is a group of polypeptides thought to coordinate dynein activity and interconnect doublet microtubules (Piperno et al., 1994; Gardner et al., 1994; Heuser et al., 2009; Lin et al., 2012), but its precise role in flagellar motility is poorly understood. We analyzed several *drc* mutants in *Chlamydomonas* to identify novel N-DRC subunits, to study N-DRC assembly, and to probe N-DRC function. To better understand the relationship between the N-DRC and the inner dynein arms, we characterized the *IDA6* gene. *ida6* is a motility mutant that fails to assemble dynein *e* and is associated with reduced levels of tektin, similar to the *drc* mutant, *pf3* (Kato et al., 1993; Gardner et al., 1994; Yanagisawa & Kamiya, 2004). Electron microscopy and 2D averaging revealed a major defect in assembly of the N-DRC, and so we tested candidates in the flagellar proteome (Li et al., 2004; Pazour et al., 2005) and identified the DRC2 subunit, FAP250, as the *IDA6* gene product (Austin-Tse et al. 2013). Mutations in the zebrafish and human orthologue CCDC65 are linked to defects in ciliary motility and primary ciliary dyskinesia. More recently we identified *sup-pf5* as another *drc2* mutation. DRC2 co-extracts, co-purifies, and co-IPs with other DRC subunits (Bower et al., 2013). iTRAQ labeling and MS/MS identified >10 axonemal proteins that are missing or reduced in *ida6* and *sup-pf-5,* and are restored in rescued strains. Spectral counting of dynein heavy chains (DHCs) revealed deficiencies in DHC8 and several other DHC isoforms. Cryo-electron tomography and 3D averaging further demonstrated that both *ida6* and *pf3* display similar defects in the assembly of the N-DRC and inner dynein arms, plus additional irregularities in the positioning of radial spokes. Thus *IDA6* encodes FAP250, which corresponds to DRC2, and DRC2 together with DRC1 plays a key role in assembly at the junction between the N-DRC, the radial spokes, and the dynein arms. Current studies focus on identifying the components that specify the binding sites for DRC1 and DRC2 on the outer doublet.

81. CCDC39 and CCDC40 are needed for assembly of the N-DRC for motility and length control. Huawen Lin, Zhengyan Zhang, **Susan Dutcher**. Genetics, Washington Univ Sch Med, St Louis, MO.

The two coiled-coil proteins, CCDC39 and CCDC40, were first identified as causative mutations in primary ciliary dyskinesia patients. Cilia from patients show disorganized microtubules and missing inner dynein arms (Merveille *et al*., 2011). In *Chlamydomonas*, the *PF7* and *PF8* loci encode CCDC40 and CCDC39, respectively, based on both reversion and rescue experiments. The *pf7* mutation results in a premature stop codon in exon 4 and the *pf8* mutation results in a premature stop codon in exon 6. In *Chlamydomonas*, these mutants have paralyzed flagella in which the outer doublets fall out of the ring of nine doublet microtubules into the center of the flagellar axoneme by electron microscopy in a fraction of the cross-sections. Both *pf7* and *pf8* assemble very short flagella in various media conditions. Based on the human patients, it was suggested that CCDC39 and CCDC40 are part of the nexin link, we used sliding assays and immunoblots to N-DRC proteins to test this hypothesis in *Chlamydomonas.* Wild-type axonemes incubated with 0.1mM ATP slide along their entire length after treatment with protease, but show no sliding with just 0.1mM ATP. Axonemes from *pf7* and *pf8* with 0.1mM ATP and no protease show splaying of the microtubules with attachment at one end of the flagella remaining as has been observed for other N-DRC mutants (Bower *et al*., 2013). The intact end after sliding is the proximal end based on staining with antibodies to a minor dynein heavy chain (DHC11), which is found only in the proximal region of the flagella. By immunoblots, CCDC39 is greatly reduced or absent in isolated *pf7* and *pf8* axonemes, respectively. Immunoblots with antibodies to DRC1, DRC2, DRC3, DRC4, DRC7, and DRC11 show a reduction or absence of all of these proteins in isolated pf7 and pf8 mutant axonemes. CCDC39 does not appear tightly associated with other N-DRC proteins and is not reduced or absent in these N-DRC mutants (Bower et al., 2013). CCDC39 and perhaps CCDC40 appear to be an additional subassembly of the N-DRC. Recently, we isolated a suppressor mutation of a *pf7*; *pf8* double mutant that restores flagella length but not motility to the double mutant strain. Its effects on the N-DRC are being characterized.

82. Pf32p, a novel central-pair-projection protein required for flagellar beating with a regular periodicity. Takumi Wada[1], Kota Abe[1], Haru-aki Yanagisawa[2], Toshiyuki Oda[2], Takuji Nakamura[1], Masahide Kikkawa[2], **Masafumi Hirono[1]**. 1) Dept Biological Sci, Univ Tokyo, Tokyo, Japan; 2) Dept Cell Biology and Anatomy, Univ Tokyo, Tokyo, Japan.

The central pair (CP) microtubules constitute a regulatory system that coordinates the dynein activities to produce regular flagellar beating. Previous studies using mutants or RNAi strains have shown that lack of certain projections from the CP causes a variety of motility defects. However, the function of each projection is not well understood. A novel *Chlamydomonas* mutant, *pf32*, that we recently isolated has defects in the CP structure and displays an interesting phenotype. Positional cloning using AFLP mapping revealed that this mutant has a small insertion that leads to a premature stop codon in a gene coding for a coiled-coil protein, Pf32p. It is widely conserved among organisms that have motile cilia/flagella, but not in *Drosophila* and *Trypanosoma*, whose flagella beat only slowly. Western blot analysis of several known CP mutants using a specific antibody revealed that Pf32p is a component of the C1 microtubule of the CP. In averaged cross section images of the *pf32* axoneme, the CP lacks two projections (C1c and C1e) located adjacent to the major projection C1a. The lack of these projections causes a displacement of the C1a tip. The mutant flagella beat with a low beat frequency but apparently with a normal waveform. However, high-speed video imaging showed that the two flagella in *pf32* cells frequently lose synchrony, leading to frequent changes in swimming direction. Observation with a uniflagellated double mutant, *pf32uni1*, suggested that the asynchronous beating is due to a failure to produce periodic beating in a single flagellum. These results suggest that the CP is important for generating regular periodicity in flagellar beating.

83. Noisy rhythms of a eukaryotic flagellum. **Kirsty Y Wan**, Raymond E Goldstein. Department of Applied Mathematics and Theoretical Physics, University of Cambridge, Wilberforce Road, Cambridge CB3 0WA, UK.

The eukaryotic flagellum is a remarkably sensitive cellular protrusion. Through precise, dynamic modulation of internal force-producing dyneins, flagellar beating can be sustained with unfailing rhythmicity but yet is capable of rapid responses to stimuli. Digitally resolving waveforms of the beating flagella of pipette-held *Chlamydomonas* cells, we assess *in vivo* flagellum sensitivity to noise and perturbations.

Flagellar waveform tracking has given us access to unprecedented spatiotemporal resolution, and long-time in-focus visualization of flagellar dynamics is made permissible by our experimental set-up. We find that low-dimensional limit cycles projections of flagellum oscillations are intrinsically stable, under static background conditions. Whilst stable, flagellar beating is inherently noisy! Highly quantitative measures of flagellum noise defined using mathematical tools suggest that waveform noise is maximal at transitions between the power and recovery stroke phases of a stereotypical beat cycle. Detailed analyses over thousands of continuous beat cycles reveal consistent negative correlation between envelope and frequency of flagellar beating. As signatures of dynamic complexity, time series of interbeat intervals exhibit fractal correlations spanning hundreds of beats, hinting at possible long-range modulation by calcium fluctuations. Further, by forcing individual beating flagella with externally-applied load, we establish flagellar stability to imposed perturbations.

These results evidence subtle as well as dynamic mechanisms of flagellar regulation that are related to the stochastic action of motor dyneins from which force and bend propagation arise. Periodic, and highly reproducible flagellar beating can thus be sustained in *Chlamydomonas*, against a cacophony of background thermal noise, intracellular biochemical processes, or even fluctuations in photon irradiance, and, without an obvious pacemaker.

84. FAP174 is an MYCBP-1-like protein that interacts with a central pair A-Kinase Anchoring Protein 240. **Jacinta D'Souza[1]**, Venkatramanan Rao[1], Ruhi Sarafdar[1], Twinkle Choudhary[1], Priyanka Sivadas[2], Pinfen Yang[2]. 1) UM-DAE Ctr for Excellence in Basic Sciences, Mumbai, Maharashtra, India; 2) Marquette University, Wehr Life Sciences, P.O. Box 1881, Milwaukee, WI 53201-1881, (414) 288-7355.

Known to be a part of the central pair, the current study embarks on identifying the A-kinase anchoring protein 240 (AKAP240). Since the sequence of AKAP240 remains unknown, an indirect approach was undertaken to identify it. Literature curation revealed MYCBP-1 as an AKAP interactor in other organisms; based on which an *in silico* search of the flagellar proteome identified an MYCBP-like protein (FAP174). The gene was cloned, the recombinant protein produced in *E. coli* was purified to homogeneity and antibodies were generated. An immunoblotting with flagellar mutants specifically localized FAP174 to the C2 microtubule. Protein-protein interaction assays showed that FAP174 interacted, albeit weakly, with a well established AKAP97 (RSP3). Whereas, using an overlay assay, FAP174 interacted with AKAP240 from the flagellar axonemes. *Chlamydomonas* cells expressing HA-tagged FAP174 were generated and immunoprecipitation with both anti-HA and anti-FAP174 antibodies showed that FAP174 interacts with AKAP240 and several other flagellar proteins. Our studies establish unequivocally that the C2 localized FAP174 interacts with AKAP240, now identified as FAP65.

85. Life at the tip: IFT-independent transport and exchange of the ciliary tip protein EB1-GFP. **Aaron J. Harris**[1], Yi Liu[2], Pinfen Yang[2], Karl Lechtreck[1]. 1) Cellular Biology, University of Georgia, Athens, GA; 2) Department of Biological Sciences, Marquette University, Milwaukee, WI.

Cilia and eukaryotic flagella are microtubule-based organelles that function in motility and signaling. Flagellar assembly and maintenance depend on intraflagellar transport (IFT), a bidirectional protein transportation system in flagella (Kozminski *et al*.1993). Recent data revealed that the axonemal protein DRC4-GFP is a cargo of IFT and largely depends on IFT for ciliary entry. However, it remains unclear which ciliary proteins utilize the IFT pathway for entry into cilia. Here, we analyzed the microtubule plus-end binding protein EB1, which was shown to track the ends of axonemal microtubules during flagellar maintenance, growth, and resorption (Pendersen *et al.* 2003). EB1-GFP accumulated at the ciliary tip and was also present along the ciliary shaft and along the cytoplasmic microtubules in the cell body. *In vivo* imaging of GFP-tagged EB1 revealed that the protein moved largely by diffusion inside cilia; transport by IFT was rarely observed (anterograde frequency <0.01 EB1-GFP/min). We used fluorescence recovery after photobleaching (FRAP) analysis to investigate the exchange rate of EB1-GFP at the ciliary tip. In steady-state cilia, the EB1-GFP signal at the tip fully recovered within 2-3 min post bleaching indicating a rapid exchange of the entire EB1-GFP pool at the ciliary tip with EB1-GFP freshly entering the cilia from the cell body. In regenerating cilia, recovery at the tip progressed faster than in steady-state cilia as expected for a protein that diffuses for a shorter distance along cilia to the tip. To determine if IFT was required for EB1-GFP dynamics, the protein was expressed in *fla10-1*, a temperature-sensitive mutant of kinesin-2, in which IFT can be switched-off by a temperature shift. In the absence of IFT, EB1-GFP was fully exchanged at the ciliary tip at a rate similar to that seen in wild-type cells under the same conditions. Our data suggest that EB1-GFP, which is expected to form a dimer of about 120 kDa, constitutively enters and exits cilia in an IFT-independent manner. Our data indicate that diffusion is sufficient to rapidly translocate cytoplasmic proteins of this size to the ciliary tip.

86. A *fap133* mutant gives new insights into dynein 1b. **Y. Hou**[1], S. M. King[2], K. F. Lechtreck[3], G. B. Witman[1]. 1) Dept. of Cell and Developmental Biology, Univ. of Massachusetts Medical School, Worcester, MA; 2) Dept. of Molecular, Microbial, and Structural Biology, Univ. of Connecticut Health Center, Farmington, CT; 3) Dept. of Cellular Biology, Univ. of Georgia, Athens, GA.

Flagella are assembled and maintained via intraflagellar transport (IFT), in which particles are carried from the cell body to the tip of the flagellum by kinesin-2 (anterograde IFT) and then back to the cell body by cytoplasmic dynein 1b (retrograde IFT). Dynein 1b contains at least a heavy chain (DHC1b), two intermediate chains (FAP133 and FAP163), a light-intermediate chain (D1bLIC), and a light chain (LC8). Mutations in the human homologues of DHC1b, FAP163 and FAP133 all cause Jeune asphyxiating thoracic dystrophy. Here we report the first *fap133* mutant in *Chlamydomonas*. The mutant was created by insertional mutagenesis with a hygromycin-resistance gene cassette. The cassette inserted into the first intron of the *FAP133* gene; FAP133 is still expressed but at such a greatly reduced level that western blotting detected none in whole-cell lysates and only trace amounts in isolated flagella. Flagella length in the mutant varies from short stumpy to near normal. DHC1b and D1bLIC levels are significantly reduced in the whole-cell lysate, indicating that FAP133 is important for dynein 1b stability; however, the reduction appears to be less than for FAP133. The residual dynein subunits still localize mainly in the peri-basal body region as in wild type, suggesting FAP133 is not required for dynein 1b to get to this region. DHC1b, D1bLIC and FAP163 levels are also reduced in the mutant flagella. Anterograde IFT is only mildly affected, but retrograde IFT velocity and frequency are respectively about one-half and one-fifth that of wild type. Consequently, the flagella accumulate IFT particles. We rescued the mutant with FAP133-HA and used an anti-HA antibody to immunoprecipitate FAP133-HA from the flagella membrane-plus-matrix fraction. All the FAP163 but only some of the DHC1b and D1bLIC were co-immunoprecipitated, indicating that FAP133 and FAP163 are tightly associated as a subcomplex within dynein 1b. In addition, two dynein light chains (Tctex1 and Tctex2b) were specifically co-immunoprecipitated with FAP133, thus identifying them as novel subunits of dynein 1b.

87. Characterization of an *ift46* suppressor mutant shows that the N-terminus of intraflagellar transport protein IFT46 is critical for importing outer arm dynein into flagella. **Y. Hou**, G. B. Witman. Dept. of Cell and Developmental Biology, Univ. of Massachusetts Medical School, Worcester, MA.

Cilia and flagella are assembled via intraflagellar transport (IFT). The IFT machinery is composed of motors and a set of 21 IFT proteins, which form two large complexes (complex A and complex B) that bind and transport IFT cargo, including axonemal precursors. We previously described an insertional mutation in the gene encoding IFT46, an IFT complex B protein; the mutant, *ift46-1*, is able to assemble only very short flagella due to instability of complex B. We also reported a spontaneous suppression that caused the phenotype to change conditionally (i.e., when stressed) from nonmotile short stumpy flagella to longer motile flagella lacking most outer dynein arms. Here, we show that the spontaneous suppression is due to an insertion of a transposon into the *ift46-1* allele. The insertion causes the expression of a hybrid protein whose first 10 AAs come from the transposon sequence and whose remaining 239 AAs are identical to

the C terminus of IFT46, which normally has 344 AAs. Rescue experiments confirm that the last 239 AAs of IFT46 stabilize IFT complex B and are sufficient for conditional rescue of *ift46-1*. Biochemical experiments show that the outer dynein arm defect in the rescued cells is due to a failure to import outer arm dynein into flagella. Transport of outer arm dynein into flagella is mediated by the IFT cargo adapter ODA16 (Ahmed et al., 2008); in the *ift46-1* cells rescued by IFT46 AA 239-344, the level of ODA16 in flagella also is drastically reduced. Therefore, IFT46 AA 1-104 are necessary for flagellar import of ODA16, which may form a link between outer arm dynein and IFT46. However, *in vitro* binding experiments detect interaction between ODA16 and IFT46 AA 105-344 but not IFT46 AA 1-104. We hypothesize that the N-terminus of IFT46 regulates binding of IFT46 to ODA16 and that when the IFT46 N-terminus is absent, IFT46 can no longer bind ODA16 and efficiently transport outer arm dynein into flagella.

88. The motility mutant *pf23* is defective in the DYX1C1 gene and fails to assemble the ciliary inner arm dyneins. Ryosuke Yamamoto[1], Stephen M. King[2], **Winfield S. Sale**[1], Susan K. Dutcher[3]. 1) Dept Cell Biol, Emory Univ Sch Medicine, Atlanta, GA; 2) Dept. of Molecular Biology and Biophysics, Univ. of CT Health Science Center, Farmington CT; 3) Dept. of Genetics, Wash. Univ. Sch of Medicine, St. Louis MO.

Studies have revealed that the ciliary axonemal dyneins are pre-assembled in the cytoplasm before being transported into cilia and docking on the axonemal microtubules. Pre-assembly of the axonemal dyneins requires a number of non-dynein-subunit proteins. The details for how these proteins contribute to dynein pre-assembly are lacking. Here, we show that the paralyzed ciliary mutant *pf23* is defective in the *Chlamydomonas* homologue of DYX1C1, a gene once thought to be linked to dyslexia, but now found to be linked to ciliary motility. *Chlamydomonas* has a single DYX1C1 homologue, of which expression and sequence were confirmed from the cDNA, revealing a predicted protein structure similar to the human DYX1C1. It is up-regulated 7-fold following deflagellation. The link between *pf23* and DYX1C1 includes several observations. First, *pf23* and the DYX1C1 gene map near each other on chromosome 11. Second, the DYX1C1 gene has a ~500bp deletion in *pf23* that removes the predicted 5th exon. Third, immunoblots of whole cell samples revealed an altered form of DYX1C1 in *pf23* (Mw of wild-type DYX1C1 = ~ 95 kDa, while Mw of *pf23*-type DYX1C1 = ~ 92 kDa). Immunoblots also revealed DYX1C1 is not found in the cilium. Fourth, BACs that bear the DYX1C1 gene rescued the *pf23* phenotype, and immunoblots of the rescued strains displayed both wild-type and mutant-type DYX1C1. Together, these data indicate that the DYX1C1 gene is defective in *pf23*. Genetic and phenotypic analysis has confirmed that the *pf23* cilia are paralyzed and the axonemes are defective in assembly of multiple inner arm dyneins, while the assembly of the outer arm dynein, N-DRC, radial spokes and central pair apparatus appear to be unaffected. We postulate that PF23/DYX1C1 operates, along with other previously identified dynein pre-assembly factors, for the folding and/or assembly of the inner arm dynein heavy chains.

89. The uncovering of several new deflagellation genes and the identification of ADF1. **Paul Buckoll**, Laura Hilton, Fabian Meili, Jaime Kirschner, Julie Rodriguez, Courtney Choutka, Lynne Quarmby. Simon Fraser University, Burnaby, BC, Canada.

'pH shock' causes *Chlamydomonas* cells to deflagellate. In response to intracellular acidification, a Ca^{2+} influx occurs that ultimately activates severing of the axonemal microtubules at a site distal to the flagellar transition zone. Our previous insertional mutagenesis screen for deflagellation-defective mutants produced multiple alleles of *fa1* and *fa2*, which are defective in axonemal severing, and multiple alleles of *adf1*, which are defective in acid-induced Ca^{2+}-influx. Both *FA1* and *FA2* were successfully cloned and encode a scaffolding protein and a NIMA-related kinase, respectively, but the identity of *ADF1 w*as elusive, until now.
The original screen did not uncover genes predicted based on the physiology. We expected, but did not find, *fa* mutants defective in a Ca^{2+}-sensor or in a microtubule severing protein. With the availability of whole genome sequencing (WGS) strategies that would allow us to rapidly identify point mutants, we undertook a new screen of UV-mutagenized cells, seeking temperature-sensitive deflagellation mutants using a phototaxis-based enrichment protocol. We recovered 29 new mutant strains, including two new alleles of *fa1*, four new alleles of *fa2*, fourteen new alleles of *adf1*, and temperature-sensitive alleles of two new deflagellation genes, *adf2*, and *fa3*.
We had previously mapped *ADF1* to a 450 kb region of chromosome 9, and WGS of three new alleles and one old allele of *adf1* revealed that all four alleles carry mutations in a TRP channel that lies at the heart of our mapped region, consistent with our prediction of a calcium-permeant channel in the acid deflagellation pathway. Mapping and WGS strategies are being employed to identify causative mutations in *adf2* and *fa3*.

90. Quantitative proteomic analysis of differentially expressed proteins in a *LF* mutant and wild type cells of *Chlamydomonas reinhardtii*. **Dilani Gunasena**, Nedra Wilson. Department of Anatomy & Cell Biology, Oklahoma State University Center for Health Sciences, Tulsa, OK 74107.

Chlamydomonas reinhardtii actively maintain flagella at a specific length. Long Flagella (*LF*) genes are essential for regulation of flagellar length and mutations in five genes are known to produce flagella that are longer than wild type. Label-free quantitative proteome analysis using LC-MS/MS was used to analyze the differentially expressed proteins in flagella and cell bodies of a *lf* mutant and wild type cells. To do this, cell bodies and flagella were obtained from wild-type and *lf* cells. Equal amounts of protein from each sample were then subjected to LC-MS/MS analysis. As expected for an analysis of equal amounts of cell body or flagellar protein, there was no significant difference in the protein level observed for alpha tubulin, beta tubulin, pyruvate kinase or actin. Other proteins, however, showed an interesting difference in levels between wild-type and *lf* cells. For example, the quantitative comparison of flagella indicates an 26-42 fold increase in the levels of some proteins in the *lf* mutant such as intraflagellar transport proteins 81, 74/72, 139, and 144 while others such as phosphoglycerate mutase and isocitrate lyase are decreased. Interestingly, ubiquitin-activating enzyme E1

demonstrated a 7 fold decrease in protein quantity in the *lf* mutants compared to wild type flagella. In addition, several flagella associated proteins exhibited up regulation in the *lf* mutant compared to wild type although some showed down regulation. In contrast to the differences in protein levels seen in flagella, the comparison of cell body proteome did not reveal a significant difference in most of the proteins. Currently, we are extending our proteomic analysis with additional mutants defective in the regulation of length as well. In addition, two-dimensional difference gel electrophoresis and phosphoproteome analysis will be performed to further characterize the differential proteome of wild type and *lf* mutants. These studies will provide further information in understanding the mechanisms involved in flagellar length control mechanism.

91. Transcriptome analysis of *shf* mutants. **Aidan Huene**[1,2], Nedra Wilson[2]. 1) Dept Biology and Chemistry, Oral Roberts University, Tulsa, OK; 2) Dept Anatomy and Cell Biology, Oklahoma State University Center for Health Sciences, Tulsa, OK.

Chlamydomonas has two equal length flagella that are actively maintained at an appropriate length. To date, three genes have been identified that when mutated result in cells that assemble half-length or short flagella (*shf*). In addition, when grown in the presence of acetate, *shf* mutants have an aflagellate phenotype. Preliminary ultrastructural analysis of *shf* cells grown in acetate reveals the presence of membrane blebs in the region of the flagella. Interestingly, in these cells, there are no detectable basal bodies or axonemal structures. To begin to better understand how the process of length regulation has been disrupted in *shf* mutants, we are utilizing transcriptome profiling of wild-type cells and *shf* mutants. Specifically, we are examining the differences between the transcriptomes of cells with steady-state flagella as well as cells that are undergoing flagellar assembly. In addition, we are currently examining the transcriptome profiles of cells grown in minimum and minimum + acetate media.

92. Axonemal tubulin polyglutamylation modulates flagellar length by enhancing tubulin turnover at the flagellar tip. **Tomohiro Kubo**[1,2], Masafumi Hirono[2], Takumi Aikawa[2], Ritsu Kamiya[2,3], George B. Witman[1]. 1) Dept. Cell and Developmental Biology, Univ. of Massachusetts, Worcester, MA; 2) Dept. Biological Science, Graduate School of Science, Univ. of Tokyo, Tokyo, Japan; 3) Dept. of Life Science, Faculty of Science, Gakushuin Univ., Tokyo, Japan.

The *C. reinhardtii* mutation *ssh1* (suppressor of short flagella 1) suppresses the short-flagella phenotype of mutants lacking multiple axonemal dyneins (LeDizet and Piperno, 1985). It has been used in several biochemical and physiological studies to produce longer flagella in mutants that would normally assemble only short flagella. Recently, we found that *ssh1* is an allele of *TPG2*, which encodes FAP234, a flagella-associated protein that forms a complex with the polyglutamylase TTLL9 encoded by *TPG1* (Kubo et al., 2013). Mutations in FAP234 (*tpg2* and *ssh1*) and TTLL9 (*tpg1*; Kubo et al., 2010) both reduce axonemal tubulin polyglutamylation and promote flagellar growth in *pf23*, a short-flagellated mutant lacking multiple inner arm dyneins. The polyglutamylation defects do not seem to affect intraflagellar transport (IFT), because the amount and motility of IFT particle proteins are normal in *tpg2* and *tpg1* flagella. Hence, we explored the possibility that tubulin polyglutamylation deficiency elongates flagella by affecting the dynamics of axonemal microtubule turnover. We found that double mutants of *tpg2* or *tpg1* and *fla10*, a temperature-sensitive mutant of the IFT anterograde motor kinesin-II, underwent slower flagellar shortening than the *fla10* mutant at restrictive temperature, suggesting that polyglutamylation deficiency decelerates tubulin disassembly at the flagellar tip. Moreover, tubulin incorporation into the flagellar tip was suppressed in the polyglutamylation-deficient flagella, as assessed by immunofluorescence microscopy of quadriflagellated zygotes produced between *tpg2* and the double mutant of *tpg2* and a strain expressing HA-tagged alpha-tubulin. Therefore, polyglutamylation deficiency reduces axonemal tubulin turnover. Taken together, these results indicate that polyglutamylation deficiency reduces tubulin disassembly at the flagellar tip, shifting the tubulin assembly/disassembly balance towards assembly. We propose that this causes the flagellar elongation in dynein-lacking mutants.

93. Identification of the SHF1 gene using a “directed” whole genome sequencing approach. **Dylan D Smith**, Nedra Wilson. College of Health Sciences, Oklahoma State University, 1111 W 17th St, Tulsa, OK.

Chlamydomonas maintains tight control over the length of flagella. Much progress has recently been made in the identification of genes that when mutated result in the assembly of abnormally long flagella. The genes responsible for the short flagella (*shf*) phenotype, however, have remained elusive. These *shf* mutants assemble flagella that are half the length of wild-type flagella. In addition, *shf* mutants have no obvious swimming defects unlike mutants with defective dynein arms. Jarvick et al, previously mapped *shf1* genetically to LG VI, 5 cM from the centromere and 32 cM from the mt locus. Comparing the genetic map and molecular maps for this area, we identified 3 molecular probes (S813, CNA46, and CNA80) that lie within this region of LG VI. Examination of the contigs for these probes identified 4 overlapping BACS that completely cover this region. Each BAC was transformed into *shf1-266* and transformants were screened for length of flagella. BAC 14j9 (from the CNA80 probe) was able to restore flagella back to wild-type length. Currently, we are using a targeted enrichment of the LG VI genome covered by BAC 14j9 from the various alleles of *shf1* coupled with next generation sequencing to identify the affected gene. In addition, transcriptome analysis will be performed to further examine the effect of the *shf1* mutation. Similarly we are analyzing the proteomes of *shf1* and wild-type cells to identify proteins that are differentially expressed in *shf1* cells.

94. Role of GSK3 and LiCl in flagellar regeneration and elongation. **L. Wang**, J. Pan. School of Life Sciences, Tsinghua University, Beijing, Beijing, China.

LiCl induces flagellar or ciliary elongation in Chlamydomonas as well as in mammalian cells. The molecular target of LiCl is widely regarded as glycogen synthesis kinase 3b (GSK3b). However, it remains controversial whether the effect of LiCl on cilia elongation was mediated by GSK3b. We reexamined possible role of GSK3b and LiCl during flagellar elongation as well as flagellar regeneration. A

miRNA construct against GSK3b driven by nitrate inducible promoter was transformed into Chlamydomonas wild type strain 21gr. Immunoblot analysis showed that more than 80% of GSK3b was reduced after one day under inducible condition and none GSK3b was detectable after two days. In the absence of GSK3b expression, cells grew apparently normally and cells possessed normal length of flagella. By adding LiCl to GSK3b depleted cells, however, flagella could elongate similar to GSK3b non-depleted cells. These data indicates that GSK3b may not be involved in LiCl induced flagellar elongation and LiCl may have other targets to control flagellar elongation. During flagellar regeneration, the relative level of phosphorylated GSK3b (active GSK3b) was reduced after deflagellation and gradually increased to normal level towards completion of flagellar assembly. GSK3b RNAi cells, which could regenerate full length flagella, showed slower initial flagellar regeneration rate. However, LiCl treatment after deflagellation only allowed assembly of flagella shorter than half-length. These data indicate that GSK3b functions in flagellar regeneration but may not be regulating flagellar elongation. The effect of LiCl on flagellar elongation and regeneration is likely not mediated by GSK3b.

95. Microalgal photosynthesis coupled to cell motion in a photobioreactor: kinetic model and dynamic simulation. **Claude Aflalo**. Microalgal Biotechnology Lab, Ben Gurion University, Midreshet Ben Gurion, Negev, Israel.

Modeling approaches for algal growth are needed to describe dense illuminated microalgal cultures, within which the light intensity varies in space through interaction with pigmented cells. In addition to the photosynthetic reactions, the motion of cells in the variable light field must be accounted for.

A cyclic three-state model serves as the standard formalism for photosynthesis, in which a (generic) **resting** reaction center (O) is first **activated**, and then irreversibly **inhibited** by photon absorption. The *activated* reaction center (C) may return to the *resting* state, after either unproductive energy dissipation, or productive energy transduction; the *inhibited* reaction center (I) may also recover to the *resting* state after a separate repair process.

This model was tested on two cases: (i) *static* illuminated suspension at steady state, (ii) *dynamic* exposure to variable light in a mixed suspension (flashing mode), simulated by random cell motion. While the former represents a well-defined reference state (approximated at low cell density), the latter relates better to real life in a photobioreactor (substantial density).

At moderate to high irradiance/low cell density, the static approximation holds and the model is able to fairly account for experimentally observed behavior. However as cell density is increased, it fails to reproduce the experimentally observed reduction in photosynthetic rate. This lack of fit cannot be amended by allowing for cell motion. Furthermore, critical dynamic aspects of microalgal growth - such as its dependence on mixing - are not supported by the model.

Only a more elaborate model involving the sequential absorption of four photons yielding a fully activated reaction center, coupled to simulated stochastic motion of particles can satisfactorily mimic the experimental results for high density cultures.
The results of simulations using both models will be discussed in terms of their implications on microalgal growth in commercially realistic photobioreactors.

96. Molecular characterization of lutein-deficient *Chlamydomonas* mutant with altered non-photochemical quenching. **KwangRyul Baek**, Minjung Kim, EonSeon Jin. Dept. of Life science, Hanyang University, Seoul, South Korea.

In microalgae, photoprotective thermal dissipation of the absorbed excess light energy is called non-photochemical quenching (NPQ). The conversion of the carotenoid violaxanthin to zeaxanthin is directly involved in xanthophyll cycle–dependent NPQ regulation; lutein is also known to play a direct role in qE generation. To better understand the relationship between xanthophyll pigments and NPQ, we constructed *Chlamydomonas reinhardtii* mutant libraries by random insertional mutagenesis and screened them for mutants with defects in xanthophyll metabolism and altered NPQ by imaging PAM and HPLC analysis. We isolated a lutein-deficient mutant that had reduced NPQ and increased photochemical quenching (qP). Despite the absence of lutein, this mutant had an increased xanthophyll pool size and significantly higher levels of violaxanthin than the wild type. It also had a truncated-antenna phenotype characterized 50% higher chlorophyll a/b ratio than that of the control. By using Southern analysis and TAIL PCR, we identified a putative target gene as an unknown gene. We are now performing a complementation test to rescue the phenotype.

97. Isolation and Characterization of a High Pmax Mutant of *Chlamydomonas reinhardtii* . **Yong Bai**[1], Graham Peers[2], Krishna K. Niyogi[1,3]. 1) Howard Hughes Medical Institute, Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA; 2) Department of Biology, Colorado State University, Fort Collins, Colorado 80523, USA; 3) Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA.

The actual efficiency of photosynthesis is significantly lower than the theoretical efficiency, mainly due to a lower maximal rate of photosynthesis (Pmax) and dissipation of excess absorbed light energy. Increasing the efficiency of converting absorbed light energy into biomass energy is critical for meeting future needs for food and fuel. The factors that control the maximum rate of photosynthesis (P_{max}) are not completely understood. Our lab is using the unicellular green alga, *Chlamydomonas reinhardtii*, as a model organism to isolate mutants that are either impaired or enhanced in photosynthesis. Recently, we have begun to characterize mutants with a higher light- and CO_2-saturated rate of photosynthesis (high P_{max} phenotype). One such mutant, called *hpm1,* has been selected for further study. Measurements of oxygen evolution showed that, under both low light and high light culture conditions, *hpm1* has at least a two-fold higher P_{max} value compared to the wild-type 4A+ strain. Genetic analysis has shown that the high P_{max} phenotype of *hpm1* is caused by a single, nuclear mutation. Next-generation sequencing technology is currently employed to map the mutation. (supported by the Howard Hughes Medical Institute and the Gordon and Betty Moore Foundation)

98. Molecular Characterization of Chl*b*-lacking mutants in *Chlamydomonas reinhardtii*. **Sandrine Bujaldon**[1], Natsum Kodama[2], Subramanyam Rajagopal[3], Yuichiro Takahashi[2], Francis-André Wollman[1]. 1) CNRS/UPMC UMR7141, Institut de Biologie Physico-Chimique, Paris, France; 2) Okayama University, Japan; 3) University of Hyderabad, India.

We characterized two nuclear mutants of Chlamydomonas lacking Chl*b*, BF3 and pg27 respectively isolated by Bennoun (1) and Picaud (2). These were compared with a chl*b*-less mutant, CBS3, isolated by Tanaka (3) that harbors a deletion that compromises expression of eight genes including the CAO gene responsible for the conversion of Chl*a* to Chl*b* by Chlorophyll Oxygenase. In genetic crosses, we show that the three mutants are allelic. Sequencing the CAO gene in BF3 and pg27 indeed showed the presence of a premature stop codon, respectively in the first and second exon of the CAO gene. Complementation of the CAO mutation in BF3 restored Chlb synthesis and a wild type phenotype. The three CAO mutants have a low fluorescence yield, reduced chlorophyll content per cell, and a smaller PSII antenna size. Their preliminary biochemical characterization points to a specific loss in three minor PSI subunits located near LHCa and a lower stability within the membranes of several LHCa and most LHCb subunits of the peripheral antenna complexes. During thylakoid preparation, most LHCb subunits were more easily cleaved by proteinases, suggesting that the structure of LHCb complexes might be modified by the absence of Chl*b*. Regarding the PSI peripheral antenna, we show that LHCa4 is drastically decreased, with most of the other LHCa proteins being reduced by 50% on a PSI basis. The phenotype of these mutants is light dependent with a more severe chlorophyll deficiency when grown in very low light (5µE) than in moderate light (20-50µE), a conditional phenotype that we attribute to the light-controlled accumulation of a specific subset of LHCa subunits.

(1): de Vitry C. and Wollman F-A. (1988) Changes in phosphorylation of thylakoid membrane proteins in light-harvesting complex mutants from *Chlamydomonas reinhardtii*. vol 933, p 444-449.
(2): Picaud A. and Dubertret G. (1986) Pigment protein complexes and functional properties of tetratype resulting from crosses between CP1 and CP2 less Chlamydomonas mutants. Photosynthesis Research. 7, 221-236.
(3): Tanaka A, Ito H, Tanaka R, Tanaka NK, Yoshida K and Okada K (1998) Chlorophyll *a* oxygenase (*CAO*) is involved in chlorophyll *b* formation from chlorophyll *a*. Proc Natl Acad Sci USA 95: 12719-1272.

99. A conserved rubredoxin in the thylakoid membrane is required for photosystem II accumulation. **Robert Calderon**[1], Catherine de Vitry[2], José García-Cerdán[1], Alizée Malnoë[1], Ron Cook[1], Rachel Dent[1], James Russell[1], Cynthia Gaw[1], Francis-André Wollman[2], Danja Schünemann[3], Krishna Niyogi[1]. 1) Howard Hughes Medical Institute, Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA; 2) Institut de Biologie Physico-Chimique, Unité Mixte de Recherche 7141, Centre National de la Recherche Scientifique - Université Paris 6, 75005 Paris, France; 3) AG Molekularbiologie Pflanzlicher Organellen, Ruhr-Universität-Bochum, 44801 Bochum, Germany.

We have isolated a Chlamydomonas mutant that has no detectable photosystem II (PSII) activity, whereas other components of the photosynthetic electron transport chain were still functional. This defect was shown to be due specifically to the absence of a gene, RBD1, encoding a thylakoid membrane-bound iron-sulfur protein known as a rubredoxin. Examination of Synechocystis and Arabidopsis mutants lacking the homolog of RBD1 revealed PSII-specific phenotypes, supporting the hypothesis that this rubredoxin has a conserved role in PSII-containing organisms, in contrast to previous reports indicating its role was in the assembly of photosystem I (PSI). Given that PSII subunits are rapidly regraded in the absence of this rubredoxin, we hoped to accumulate PSII assembly intermediates by creating a double mutant which lacks RBD1 and has an inactive form of the chloroplast protease FtsH. We have found that not only does the inactivation of FtsH permit accumulation of PSII subunits in the rbd1 mutant, but that these subunits assemble into a highly photosensitive PSII complex capable of low levels of oxygen evolution. Experiments are underway to determine the structural and functional characteristics of the PSII from the double mutant and to uncover the precise role of this rubredoxin in the assembly or stability of PSII. (Supported in part by the Philomathia Foundation and the Gordon and Betty Moore Foundation)

100. The fast and slow reversible fluorescence quenching (*in vivo*) of LHC complexes in *Chlamydomonas reinhardtii*. **Emine Dinc**, Lijin Tian, Roberta Croce. Department of Physics and Astronomy, Faculty of Sciences, Vrije University Amsterdam, 1081 HV Amsterdam, The Netherlands.

It is known that the qE process is triggered by low luminal pH and the Light harvesting complex stress-related protein (LhcSR) has recently been identified as the key component in the activation of an energy dissipation mechanism that leads to qE in the model organism *C. reinhartii*. Using RR5 transformant, reaction centers free cells were created that contain mainly LHCII or LHCII+LhcSR under different light conditions. These cells were used to study the fluorescence quenching properties of LHCs complexes *in vivo*.

101. The BSD2 homolog in *Chlamydomonas reinhardtii* is a polysome-associated chaperone of nascent polypeptides encoding the Rubisco large subunit. **L. Doron**, N. Segal, M. Shapira. life sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

Expression of the CO_2-fixation enzyme Ribulose-bisphosphate carboxylase/oxygenase (Rubisco) is subject to light-induced regulation. A cysteine-rich protein was previously identified in bundle-sheath cells of maize (BSD2) as being essential for Rubisco expression. The *Chlamydomonas reinhardtii* orthologue is a small protein (17 kDa) expressed in chloroplasts. It possesses four CXXCXGXG elements that are typical of DnaJ, yet it lacks any of the other conserved domains of this chaperone. BSD2 comigrates with the *rbcL* transcript on heavy polysomes, and both shift to the lighter fractions under oxidizing conditions that stop translation of Rubisco LSU. The association with *rbcL*-translating polysomes supports the possibility that BSD2 is a chaperone that promotes the *de novo* synthesis of Rubisco LSU. Furthermore, BSD2 migrates at the very top fractions in a *C. reinhardtii* premature-termination mutant of Rubisco LSU. The chaperone activity of BSD2 is exemplified by its ability to prevent aggregation of chemically denatured Citrate Synthase (CS); as well as

that of the Rubisco LSU chains *in vitro*. BSD2 could also prevent the precipitation of reduced b-chains *in vitro*, in an Insulin Turbidity test. However, unlike in the CS assay, a cys to ser replacement impaired this activity, indicating that BSD2 functioned as a chaperone of denatured CS, while in the Insulin Turbidity test its role was most probably to hold the reduced thiol groups together, preventing the precipitation of b-chains. Thus BSD2 combines both a chaperone "holdase" function, with an ability to interact with free thiols, combining both features to protect the newly synthesized Rubisco LSU chains.

102. The nucleus encoded TPR protein Mac1 is a novel regulator of PSI gene expression in the chloroplast. **Damien Douchi**[1], Linnka Lefebvre-Legendre[1], Xenie Johnson[2], Jean-David Rochaix[1], Michel Goldschmidt-Clermont[1]. 1) Department of Botany and Plant Biology, University of Geneva, Switzerland; 2) UMR 7141, CNRS/Université Pierre et Marie Curie, Institut de Biologie Physico-Chimique, Paris, France.

For 3.5 billion years, photosynthesis has been the primary mechanism for biomass production on earth through the conversion of light energy to chemical energy. In several organisms it is used for biotechnology to produce high value molecules. Harvesting light energy in the chloroplast photosynthetic machinery is a dangerous process that can produce reactive oxygen species (ROS) which are toxic for the cell. In order to optimize photosynthesis and minimize ROS production, the cell must adapt to fluctuating conditions such as light quantity and quality, nutrient availability or temperature. The assembly and activity of photosynthetic machinery is tightly regulated and involves coordinated crosstalk between the genomes of the chloroplast and the nucleus. In the green alga *Chlamydomonas reinhardtii*, RNA metabolism is an important component in the regulation of photosystem assembly in the chloroplast. Many of the chloroplast-localized mRNAs encoding subunits of the photosynthetic machinery are controlled by nucleus-encoded factors. This work describes a novel regulator of the photosystem I subunit PsaC, which contains the F_A and F_B iron-sulfur clusters responsible for the reduction of ferredoxin. We demonstrate that this novel regulator, Mac1, is a nucleus-encoded TPR-containing protein which stabilizes the 5'UTR of *psaC* mRNA. In conditions of iron limitation that lead to a decrease of PSI, we observe a reduction in the amount of the PsaA protein which correlates with a similar decrease of its messenger RNA. The strong diminution of PsaC protein is accompanied by a lesser decrease of its mRNA, suggesting a protease-driven process. The degradation of *psaC* mRNA is matched by a proportional degradation of the Mac1 protein which seems consistent with the implication of Mac1 in the stabilization of *psaC*.

103. Strategies of *Chlamydomonas reinhardtii* to cope with fluctuating light. **Martina Jokel**[1], Gilles Peltier[2,3], Yaghut Allahverdiyeva-Rinne[1], Eva Mari Aro[1]. 1) University of Turku, Department of Biochemistry, Molecular Plant Biology, Biocity 6th floor, Turku 20520, Finland; 2) CEA, Direction des Sciences du Vivant, Institut de Biologie Environnementale et de Biotechnologie, Laboratoire de Bioénergétique et Biotechnologie des Bactéries et Microalgues, CEA Cadarache, Saint-Paul-lez-Durance, F-13108 France; 3) Aix Marseille Université, UMR Biologie Végétale et Microbiologie Environnementale, Saint-Paul-lez-Durance, F-13108 France.

We have investigated the role of flavodiiron proteins (FDP), and the proton-gradient-regulator 5 (PGR5) as electron valves in photosynthetic light reactions under fluctuating light conditions, which is the natural environment for aquatic organisms.
FDPs are present in Archea and Bacteria, being involved in detoxification of NO and/or O_2. Phylogenetic analysis shows that genes encoding FDPs are present also in oxygenic photosynthetic organisms like cyanobacteria, green algae, mosses and ferns. The cyanobacterium *Synechocystis* PCC 6803 (hereafter, *Synechocystis*) possesses 4 genes encoding FDPs (Flv1 - Flv4 proteins). Recent studies demonstrated that Flv2/Flv4 heterodimer is involved in the photoprotection of PSII in cells acclimated to air level CO_2. Whereas, Flv1 and Flv3 proteins function in a Mehler-like reaction, donating electrons from downstream of PSI directly to molecular oxygen and thus play an important role as a strong sink of excess electrons. Besides, Flv1 and Flv3 enable the growth of cyanobacteria under fluctuating light conditions by protecting PSI against photodamage.

Phylogenetic analysis revealed two genes (*flvA* and *flvB*) in the genome of *C. reinhardtii* with high homology to *Synechocystis flv1* and *flv3* genes. RTq-PCR studies demonstrated an upregulation of both *flv* genes under high light, during the shift from high to air-level CO_2, during S-deprivation and under fluctuating light. However, we did not observe a strong phenotype from FLVA and FLVB knock-down mutants under fluctuating light. However, we found that the growth of the *Δpgr5* mutant of *C. reinhardtii* exhibits impaired growth phenotype under fluctuating light, suggesting an important role for this protein in regulation of electron flow during fast changes in light intensities. This is in line with higher plants like *Arabidopsis* that is missing the *flv* genes. Instead, it posesses PGR5 to safeguard PSI under fluctuating light. We discuss the coordinated function of both FLV and PGR5 proteins in *C. reinhardtii* in the regulation of the photosynthetic flow under fluctuating light.

104. Heme *c*i of the cytochrome *b6f* complex- an adaptation to oxygenic environment. **Alizée Malnoë**[1,2,3], Jacqueline Girard-Bascou[1], Frauke Baymann[4], Daniel Picot[5], Jean Alric[1,6], Daniel Olof Persson[7], Francis-André Wollman[1], Fabrice Rappaport[1], Catherine de Vitry[1]. 1) CNRS/UPMC UMR7141, IBPC, 13 rue Pierre et Marie Curie 75005 Paris, FRANCE; 2) Howard Hughes Medical Institute, Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA; 3) Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA; 4) CNRS/AMU UMR7281, BIP, 13402 Marseille, FRANCE; 5) CNRS/UP7 UMR 7099, IBPC, 13 rue Pierre et Marie Curie 75005 Paris, FRANCE; 6) CEA/AMU, IBEB, 13108 Saint-Paul-Lez-Durance, FRANCE; 7) Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Copenhagen, DENMARK.

Cytochrome bc_1 and b_6f complexes are key players in bioenergetic electron transfer chains. Their quinol oxidoreductase activity contributes to the formation of a proton motive force through the Q-cycle. Structural data has shown that b_6f contains three additional cofactors not found in bc_1: one *β*-carotene, one chlorophyll *a* and a singular heme, named c_i, located in the quinone reduction site (Q_i). Using genetic approaches in *Chlamydomonas*, we combined mutations that prevent covalent attachment of heme c_i with a mutation in the thylakoid protease FtsH1. This strategy enabled the accumulation of a b_6f complex lacking heme c_i in the Q_i site, thus resembling a bc_1

complex, and has shed light on the functional requirement of this heme in oxygenic photosynthesis. Preliminary data show that a b_6f complex strictly lacking heme c_i impairs phototrophic growth. This result points to a role for heme c_i in modifying the Q_i site so as to protect it from an oxygen-rich environment. Furthermore, we found that alteration of the heme c_i maturation pathway resulted in the presence of a Zn-porphyrin at the Q_i site in place of heme c_i. These findings raise the question of a possible role of the maturation pathway in selecting the right cofactor or, alternatively that the non-covalent attachment of heme triggers the loss of iron and subsequent replacement by zinc. (Supported in part by the Agence Nationale de la Recherche and the CNRS).

105. *In vitro* and *in vivo* analysis of LHCII proteins in *Chlamydomonas reinhardtii*. **Alberto Natali**. VU University, Amsterdam, Netherlands.

Chlamydomonas reinhardtii contains nine Major Light-Harvesting Complexes (LHCBM) and several minor LHC proteins. Although the main role of the LHCs is to harvest light and to transfer excitation energy to the reaction center, some of these complexes are suggested to have additional important roles in *C. r*. In particular LHCBM1 and LHCSR are involved in non-photochemical quenching (NPQ), the mechanism developed by plants and algae to protect themselves from photodamage, while LHCBM5 seems to be important for state transitions, the process that maintains the excitation balance between the Photosystem I and II. As a first step in determining the properties of the individual LHCBM proteins and relating them to their function, we study these complexes *in vitro*. However, all LHCs perform their function in the thylakoid membrane in combination with other complexes. Thus to fully understand the role played by the individual LHCs in NPQ and state transitions, to detect their interaction partners and to get insight in the molecular mechanisms of those processes it is necessary to look at the complexes “at work” and thus directly in the membrane. At present we are exploring different strategies, from mutation analysis *in vivo*, to overexpression of tagged proteins that will give us the possibility to still get molecular details while analysing the system at a high level of complexity. Preliminary results will be presented.

106. Self-adjusting light harvesting antenna: New ways to deal with the hazards of harnessing sunlight. **Sangeeta Negi**[1], Zoee Perrine[2], Anil Kumar[2], Amanda Barry[3], Richard Sayre[1,3]. 1) New Mexico Consortium,Los Alamos,NM; 2) Phycal,Inc St. Louis,MO; 3) Los Alamos National Lab, Los Alamos,NM.

At high-light intensities photosynthetic efficiency is compromised due to saturation of photosynthetic electron transfer processes resulting in possible photodamage. Plants and algae have evolved various strategies to protect themselves from photodamage including adjusting the size of light harvesting complexes and their distribution between photosystems as well as dissipating excess energy by non-photochemical quenching mechanisms. Recently, we have generated transgenic algae that are capable of self-modulating the size of the LHC complex as a function of light intensity. This was accomplished by using a redox sensitive, translational inhibitor, NAB1(Nucleic acid binding protein 1), to control the expression of the gene involved in Chl b synthesis (Chl a oxygenase,CAO). In high-light intensities NAB1 protein binds to its respective mRNA binding site of the CAO gene which catalyzes the synthesis of Chl b and inhibits translation of the CAO gene resulting in a reduced PSII peripheral antenna size. In contrast,under low-light intensities repression by NAB1 expression is reduced and Chlb synthesis increases resulting in an increase in PSII peripheral antenna size. Consistent with our hypothesis these lines show near wild-type antenna size when grown in low-light (Chl a/b ratio 3.1-3.4) and smaller antenna size (Chl a/b ratio 5.4-5.7) in high-light. These lines also show higher photosynthetic rates compared to wild type (2.7-3 fold). Interestingly, an increase in the levels of photoprotective pigments, zea and lutein, was also observed in high-light grown NAB CAO transgenics relative to wild type resulting in higher de-epoxidation. We also examined and compared growth rates of these lines with wild type and complemented wild type in identical bench-top photo bioreactors (Phenometrics) that simulate a pond environment.The NAB CAO lines had up to a 2-fold higher growth and biomass accumulation than wild type. In summary, results presented here demonstrate that algae with self-adjusting antennae have improved photosynthetic efficiency and growth in real-world growth conditions.

107. Deficiency in cyclic electron flow in a *Chlamydomonas pgrl1* knock-out mutant is compensated by combined increases in mitochondrial cooperation and oxygen photoreduction. Kieu-Van Dang[1], Julie Plet[1], Dimitri Tolleter[1], Martina Jokel[2], Pierre Richaud[1], Yagut Allahverdiyeva[2], Xenie Johnson[1], Jean Alric[1], **Gilles Peltier**[1]. 1) CEA, CNRS, Aix-Marseille Université, Institut de Biologie Environnementale et Biotechnologie, CEA Cadarache, Saint-Paul-lez-Durance, F-13108 France; 2) Laboratory of Molecular Plant Biology, Department of Biochemistry, University of Turku, FI-20014, Turku, Finland.

Biomass productivity of photosynthetic organisms is by driven metabolic reactions of CO_2 fixation fueled by NADPH and ATP, the soluble energy carriers produced by photosynthetic electron transport reactions. In addition to linear electron flow that operates from PSII to PSI, cyclic electron flow (CEF) around PSI has been proposed to participate in the supply of extra-ATP for optimal functioning of photosynthesis. In order to determine the contribution of CEF to microalgal biomass productivity, we study here photosynthesis and growth performances of a knock-out *Chlamydomonas reinhardtii* mutant (*pgrl1*) deficient in PGRL1-mediated CEF. We show that steady-state biomass productivity of the *pgrl1* mutant, measured in photobioreactors operated as turbidostats, is similar to its wild-type progenitor under a wide range of illumination and CO_2 concentrations. Based on the effect of mitochondrial respiratory inhibitors on chlorophyll fluorescence parameters and photosynthetic O_2 exchange measured using a Membrane Inlet Mass Spectrometer, we conclude that increased cooperation with mitochondrial respiration and increased oxygen photo-reduction downstream of PSI (Mehler reaction) allow the supply of extra-ATP for photosynthesis in the *pgrl1* mutant, resulting in a normal biomass productivity under steady state conditions. A marked accumulation of flavodiiron proteins FlvA and FlvB was observed in the mutant in comparison to the control strain, suggesting that Flv-mediated O_2 photoreduction is involved in the compensation mechanism. When high light pulses (1 min duration) were supplied to the algal culture, biomass productivity of the control strain increased, but the mutant was unable to take advantage of the additional light supply. We conclude that compensation mechanisms allow to maintaining a normal productivity at steady state, but are unable to respond to a fast increase in the ATP demand.

108. Alterations of photosynthetic apparatus during photoautotrophic growth of *Chlamydomonas*. **M Sandmann**[1], O Reich[1], M Steup[2]. 1) University of Potsdam, Institute of Chemistry, Potsdam, Germany; 2) University of Potsdam, Institute of Biochemistry and Biology, Potsdam, Germany.

The strain CC1690 of *Chlamydomonas reinhardtii* was synchronized under photoautotrophic conditions using continuous light-dark cycles. During fast light-driven growth, the photosynthetic capacity has to be retained to achieve an optimal reproduction of the cells. As a first approximation, the increasing cellular volume could cause a proportional change in the cellular content of all constituents of the photosynthetic apparatus. Results obtained in this study are basically consistent with this assumption. We observed, however, evidence for a more complex mode that ensures high photosynthetic capacity. Based on analyses of pigment composition and the photosynthesis capacities, an increase of the core components of the photosynthetic apparatus was found early in each light period. The chlorophyll a/b ratio increases reaching a maximum at the first quarter of the light phase. Detailed analyses of the light saturation curves of photosynthesis using the Talling model (Talling, 1957) reveal alterations in the light saturation parameter IK. Thus, the accumulation of the various constituents of Photosystem II and the light harvesting complexes differ. These results are consistent with data obtained by distributed light sensing. This technique permits the incident light to be monitored inside the cell suspension with a high temporal, spectral and spatial resolution. Time-dependent measurements revealed that the light intensity was reduced by approximately 35% during light phase. In addition, spectral properties of the light changed. The changes observed in the optical properties of the photosynthetic apparatus appear to directly match the altered spectral properties of the light that penetrates the cell suspension. During growth, the cells seem to optimize their photosynthetic apparatus to cope with changing light conditions.

Talling, J. F. 1957. Photosynthetic characteristics of some freshwater plankton diatoms in relation to underwater radiation. New Phytol. 56: 29-5.

109. Improved photoautotrophic growth of chloroplast transformants in which chloroplast *ycf4* gene was overexpressed. S. Nellaepalli[1,2], H. Kuroda[1,2], **Y. Takahashi**[1,2]. 1) The Graduate School of Natural Science and Technology, Okayama Univ, Okayama, Japan; 2) JST-CREST.

Two chloroplast-encoded factors, Ycf3 and Ycf4, are conserved from cyanobacteria to plants and are essential for PSI complex assembly in the green alga *Chlamydomonas reinhardtii* (1). In particular Ycf4 forms a large complex that interacts with some newly synthesized PSI proteins and thus is postulated to be required for initial assembly steps of PSI complex (2). In the present study, we have generated chloroplast transformants in which Ycf4 fused with HA tag at its N-terminus (N-HA-Ycf4) using chloroplast expression vector (3). Immunoblotting revealed that the N-HA-Ycf4 transformants accumulated N-H-Ycf4 protein 20 times more than the control strain. The N-HA-Ycf4 cells accumulated PSI at normal level and were able to grow photoautotrophically. Then we analyzed the cell growth of the N-HA-Ycf4 transformants under heterotrophic, mixotrophic, and photoautotrophic conditions to investigate the effects of the overexpression on cell growth under different conditions. Growth curve analyses exhibited that heterotrophic growth of the N-HA-Ycf4 cells grew slightly more slowly than that of control cells. In contrast, increase in photoautotrophic growth of N-HA-Ycf4 cells was observed under photoautotrophic conditions with light of 50, 200 and 700 µmole photons m^{-2} s^{-1}. We conclude that the overexpression of N-HA-Ycf4 improves photosynthetic performance of *Chlamydomonas* under photoautotrophic conditions.

1. E Boudreau, Y Takahashi, C Lemieux, M Turmel and J-D Rochaix, EMBO J. 16 (1997) 6065-6104.
2. S Ozawa, J Nield, A Terao J Stauber, M Hippler, H Koike, J-D Rochaix, and Y Takahashi, The Plant Cell 21 (2009) 2424-2442.
3. L Michelet, L Fefebvre-Legendre, S E. Burr, J-D Rochaix, and M Goldschmidt-Clermont, Plant Biotechnology J. (2010) 1-12.

110. Regulation of LHCSR3 expression in *Chlamydomonas reinhardtii*. **Ryutaro Tokutsu**[1], Dimitris Petroutsos[2], Shinichiro Maruyama[1], Andre Greiner[3], Tilman Kottke[4], Maria Mittag[5], Peter Hegemann[3], Giovanni Finazzi[2], Jun Minagawa[1]. 1) Division of Environmental Photobiology, National Institute for Basic Biology, Okazaki, Japan; 2) Cell and Plant Physiology Laboratory, iRTSV, CEA Grenoble, France; 3) Institute for Experimental Biophysics, Humboldt University, Berlin, Germany; 4) Department of Chemistry Physical and Biophysical Chemistry, Bielefeld University, Bielefeld, Germany; 5) Institute of General Botany and Plant Physiology, Friedrich Schiller University, Jena, Germany.

Photosynthesis converts light energy to electrochemical energy. To avoid photodamage under excessive light, photosynthetic organisms have developed mechanisms to dissipate excess light energy, via a mechanism called Non-Photochemical-Quenching (NPQ). In *Chlamydomonas reinhardtii*, such NPQ mechanism involves induction of LHCSR3 protein. In this study, we focused on the regulation of LHCSR3 gene and protein expression. When we measured the action spectrum of both NPQ and LHCSR3 expression by using Okazaki Large Spectrograph (Okazaki, Japan), both were not only depending on the photosynthetic activities but also on the wavelength of the incident light. Moreover, the capacity of light capturing and carbon fixation strongly affected the induction of both NPQ and LHCSR3 expression, implying that availability of carbon source around the cells can also be a trigger of light energy dissipation. From these results, we propose a hypothetical model of signal transduction of NPQ induction in *Chlamydomonas*.

111. Characterization of Rubisco assembly-dependent regulation in *Chlamydomonas reinhardtii*. **Wojciech Wietrzynski**[1], David B. Stern[2], Francis-André Wollman[1], Katia Wostrikoff[1,2]. 1) UMR7141, CNRS-UPMC, IBPC, Paris, France; 2) Boyce Thompson Institute for Plant Research, Ithaca, NY 14853, USA.

RuBisCO (Ribulose 1,5-biphosphate Carboxylase/Oxygenase) is essential for carbon fixation through the Calvin-Benson cycle, a process fueled by the NAPDH reducing power and ATP produced by the photosynthetic chain activity. The chloroplast localized holoenzyme consists of 8 large (LS) and 8 small (SS) subunits, which assemble in a given stoichiometric ratio despite their dual genetic origin: LS is

encoded by the chloroplast *rbcL* gene, while SS is encoded by the nuclear genome. Their assembly is concerted, as absence of either one of the subunits leads to a lack of RuBisCO accumulation. While small subunit undergoes proteolysis in absence of LS, previous studies (Khrebtukova and Spreitzer, PNAS, 1994) showed that LS synthesis is reduced in absence of SS in the green alga *Chlamydomonas reinhardtii*. Here we present evidence that in *C. reinhardtii* LS synthesis is autoregulated by its status of assembly, as it is observed in higher plants, thereby allowing further mechanistic investigations on this simpler model organism. To understand possible roles of RuBisCO associated proteins and/or describe novel components involved in *rbcL* gene expression, we will describe the generation of mutants affecting different RuBisCO related chaperones as well as the characterization of an epitope-tagged MRL1 protein, a PPR required to stabilize *rbcL* transcript.

112. Characterization of CPLD49, a GreenCut redox protein implicated in cytochrome *b6f* complex accumulation and the delay of 'senescence' in *Chlamydomonas reinhardtii*. **Tyler Wittkopp**[1,2], Xenie Johnson[1], Wenqiang Yang[1], Mark Heinnickel[1], Krishna Niyogi[3], Francis-André Wollman[4], Arthur Grossman[1,2]. 1) Dept of Plant Biology, Carnegie Institution for Science, Stanford, CA; 2) Dept of Biology, Stanford University, Stanford, CA; 3) Dept of Plant and Microbial Biology, University of California, Berkeley, CA; 4) Institut de Biologie Physico-Chimique, Paris, France.

The GreenCut is a group of proteins present in *Chlamydomonas reinhardtii* with orthologs in other green lineage, photosynthetic organisms, but that are not present in non-photosynthetic heterotrophs (Merchant et al, *Science*, 2007). Nearly half of the 597 GreenCut proteins have not been assigned a specific function, but many are likely to be involved in chloroplast processes including photosynthesis, chloroplast biogenesis, and plastid metabolism (Karpowicz et al, *J. Biol. Chem.*, 2011). CPLD49 belongs to the "redox" category of uncharacterized GreenCut proteins. It is a soluble, thylakoid-associated protein that contains a saccharopine dehydrogenase (SDH) domain. In plants and animals, SDH is involved in lysine degradation, a process that may generate a number of key metabolic intermediates and signaling molecules. A Chlamydomonas mutant with a disruption in the *CPLD49* gene model was previously identified (Dent et al, *Plant Phys*., 2005). The *cpld49* mutant cannot grow photoautotrophically and displays reduced photosynthetic performance relative to wild type (WT). This decline in photosynthesis is correlated with a significant reduction in the amount of the cytochrome b_6f complex. In WT, the CPLD49 polypeptide associates with thylakoid membranes, although it does not co-migrate with solubilized cyt. b_6f complex on a sucrose gradient. High light (HL) treatment of WT cells eliminates the association of CPLD49 with thylakoids. Interestingly, CPLD49 expression increases in HL and as cells enter stationary phase. These phenotypic observations in WT and the *cpld49* mutant suggest that the CPLD49 protein may be released into the stroma where it could have a role in delaying the 'senescence' response, a process that involves loss of cyt. b_6f complex. The exact function of CPLD49 has yet to be determined, but it is intriguing to speculate that this conserved protein may modulate a pool of metabolites that serve a regulatory function at the interface of photosynthesis, amino acid metabolism, stress responses, and senescence.

113. Quantitative growth measurement of individual mutants in pooled culture of *Chlamydomonas* to identify mutants deficient in photosynthesis and photoprotection. **Ru Zhang**, Nina Ivanova, Leif Pallesen, Luke Mackinder, John Nguyen, Weronika Patena, Xiaobo Li, Rebecca Yue, Spencer Gang, Sean Blum, Arthur Grossman, Martin Jonikas. Plant Biology, Carnegie Institution for Science, Stanford, CA. 94305, USA.

Photosynthesis is a critical process; however, many aspects of it remain poorly understood, especially its biogenesis and regulation. The unicellular green alga *Chlamydomonas reinhardtii* is a great model to study photosynthesis. Previously, *Chlamydomonas* mutants deficient in photosynthesis were usually identified by plate screens with an acetate-requiring phenotype (grow in dark with acetate but not in light by photosynthesis). The plate screen has led to many important discoveries of photosynthesis; however, it has two challenges: (1) the traditional methods to map the insertion sites are inefficient, limited to a dozen of mutants at once; (2) the mutant phenotypes are mostly identified by eye, in low throughput and not quantitatively. We have developed a powerful tool, ChlaMmeSeq (***Chla****mydomonas* **Mme**I-based insertion site **Seq**uencing), for high-throughput genotyping and phenotyping in a genome-wide collection of *Chlamydomonas* mutants. This strategy generates 20 bp genomic flanking sequences by the transforming cassette and allows us to map tens of thousands of mutants simultaneously in one pool. Validation of ChlaMmeSeq with individual mutants showed that 70% of the uniquely mapped flanking sequences correctly indicated the insertion sites. Importantly, ChlaMmeSeq is also reproducible and quantitative, allowing quantification of relative abundances (thus growth rate) of individual mutants in pooled culture. It can reliably detect a 10% reduction in growth rate in 14 generations and a 20% reduction in 7 generations. In addition, ChlaMmeSeq enables the generation of an indexed library of *Chlamydomonas* mutants. The library currently has 10,000 mutants, each with a unique 20 bp genomic flanking sequence, serving as a tag to track the growth rate of each mutant in pooled cultures. The combination of the indexed mutant library and ChlaMmeSeq enables pooled screens for high-throughput and quantitative identification of mutants deficient in photosynthesis and photoprotection, including mutants with strong phenotypes (such as those deficient in the biogenesis of photosynthesis), but also those with moderate phenotypes (probably deficient in the regulation of photosynthesis). Mutants with interesting phenotypes can be picked easily from the indexed mutant library based on the unique flanking sequences for further characterization. The pooled screen can also be used for other abiotic stresses, such as high temperature or nutrient deficiency.

114. Molecular insights into the functional role of the rubredoxin domain-containing protein RBD1 in the assembly of Photosystem II. **Jose Gines Garcia Cerdan**[1], Robert H. Calderon[1], Danja Schünemann[2], Krishna K. Niyogi[1,3]. 1) UC Berkeley-HHMI, Berkeley, CA; 2) AG Molekularbiologie Pflanzlicher Organellen, Ruhr-Universität-Bochum, 44801 Bochum, Germany; 3) Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA.

Rubredoxins are small iron-sulfur proteins [1Fe-0S] that participate in electron transfer reactions in many microbial organisms. Recently, we have found that mutations affecting a membrane-bound rubredoxin called RBD1 in several oxygenic photoautotrophs leads to a severe impairment of photoautotropic growth due to a defect in photosystem II (PSII) accumulation (Calderon et al. 2013, J. Biol. Chem. 288: 26688-26696). Further investigation has shown that the RBD1 protein is localized in the thylakoid membrane, anchored by a single trans-membrane helix, and that the rubredoxin domain is exposed on the stromal side of the membrane. RBD1 co-localizes with PSII subunits as assayed by blue native (BN)-PAGE, yeast split-ubiquitin and co-immunoprecipitation analyses in Arabidopsis. Furthermore, BN-PAGE analyses of thylakoid membranes isolated from D1-less and D2-less Chlamydomonas strains resolved co-migration of accumulated PSII pre-complexes with RBD1. We have also generated a yellow-in-the-dark (*y5*) and RBD1-less double mutant to study the *de novo* biogenesis of thylakoid membranes in the absence of RBD1. Interestingly, RBD1 protein accumulates in the dark-grown *y5* single mutant cells, implying an early role for RBD1 in the novo assembly of PSII. (Supported in part by the Gordon and Betty Moore Foundation)

115. A factor involved in chloroplast group II intron *trans*-splicing exhibits intrinsic RNase activity. **Jessica Jacobs**, Christina Marx, Olga Reifschneider, Stephanie Glanz, Ulrich Kück. General and Molecular Botany, Ruhr Univ Bochum, Bochum, Germany.

Chlamydomonas reinhardtii is widely used for analysis of nucleus-encoded factors that are thought to promote the maturation of chloroplast precursor RNAs. To elucidate the function and composition of ribonucleoprotein complexes that are presumably part of a transcript specific chloroplast spliceosome, we are studying the expression of the chloroplast encoded *psaA* gene [1]. The *psaA* gene is separated into three exons, which are widely distributed over the plastom and flanked by consensus sequences typical for group II introns. The exons are transcribed individually and the major transcript is then assembled in *trans* [2]. Here, we present a novel *trans*-splicing mutant, which is affected in splicing of the second *psaA* intron. LMS (ligation mediated suppression) PCR and genomic complementation led to the identification of *RAA6*. The gene encodes a protein of 114 kDa and shares no sequence identity with other known proteins. The chloroplast localization of Raa6 was confirmed by confocal fluorescence microscopy, using a GFP-tagged fusion protein and cell fractionation experiments. Unexpectedly, we found during its functional characterization as putative RNA-binding protein that Raa6 degrades diverse transcripts. These results indicate that Raa6 is a group II intron splicing factor with intrinsic RNase activity.
[1] Jacobs, J., Marx, C., Kock,V., Reifschneider, O., Fränzel, B., Krisp, C., Wolters, D. and Kück U. (2013) Mol Cell Proteomics 12:1912-1925
[2] Jacobs J, Glanz S, Bunse-Graßmann A, Kruse O, Kück U (2010) Europ J Cell Biol 89: 932-93.

116. The structure of chloroplast nucleoids in the green alga *Chlamydomonas reinhardtii*. **Y. Kobayashi**[1], M. Takusagawa[1], M. Odahara[2], Y. Fukao[3], T. Shikanai[1], Y. Nishimura[1]. 1) Kyoto Univ., Kyoto, Kyoto, Japan; 2) Dep. Life Sci., Coll. Sci., St. Paul's Univ., Tokyo, Japan; 3) Plant Global Edu. Project, NAIST, Nara, Japan.

Chloroplasts and mitochondria evolved from prokaryotic endosymbionts. Therefore these organelles have their own genome DNA which is packed into small size called as nucleoid. Multiple studies in plants, yeast, and other non-photosynthetic eukaryotes indicated that organelle nucleoids are the platform for DNA replication and transcription and are the segregation unit of the organelle genome. Intriguingly, the localization and morphology of plastid nucleoids of higher plants differ between species and also change in response to developmental stage. However, little is known about the nucleoid proteins that mediate the control of plastid DNA quality, copy number, morphology or localization. It is also unclear to what extent posttrascriptional steps of gene expression, such as RNA transcription, processing, mRNA stability control and translation are associated with the nucleoids.

In this analysis, we aimed to reveal molecular constitution, the mechanism to maintain nucleoid structure and evolution of nucleoid. For this purpose, we decided to analyze cp nucleoids of the unicellular algae *Chlamydonas reinhardtii*. We established the techniques for isolation of nucleoids preferentially and then the proteome were analyzed by using HTC-PAL/ Paradigm MS4 system coupled to LTQ Orbitrap XL mass spectrometer. Our analysis revealed that chloroplast nucleoids consisted of a variety of proteins related to DNA bending, recombination, transcription, RNA processing, protein quality control and so on. This result indicates that nucleoids are not only the architecture to pack DNA into small size, but also the platform to accomplish central dogma in chloroplasts. Furthermore, it has been suggested that the structure of nucleoids in *C. reinhardtii* is the hybrid of both prokaryotic nucleoid proteins and eukaryotic chromosomal proteins. Based on these findings, we would like to propose a model to explain the macroevolution of chloroplast nucleoids.

117. Identification and molecular characterisation of chloroplast ribonucleoprotein complexes involved in the second *psaA*-mRNA *trans*-splicing reaction. **Olga Reifschneider**[1], Linnka Legendre-Lefebvre[2], Christina Marx[1], Jessica Jacobs[1], Franziska Hundt[3], Dirk Wolters[3], Michel Goldschmidt-Clermont[2], Ulrich Kück[1]. 1) Department for General and Molecular Botany, Ruhr-University Bochum, Germany; 2) Department of Botany and Plant Biology and Department of Molecular Biology, University of Geneva, Switzerland; 3) Department of Analytical Chemistry, Ruhr-University Bochum, Germany.

The chloroplast *psaA* gene from green alga *Chlamydomonas reinhardtii* is split into three independently transcribed exons, which are flanked by consensus sequences of group II introns. The assembly of the mature mRNA via two *trans*-splicing reactions is highly dependent on nucleus-encoded factors. Recently we have shown that splicing factors involved in *trans*-splicing of the first *psaA* group II intron are part of a high molecular weight ribonucleoprotein complex. Here, data are presented to characterize a RNA-protein complex that is related to *trans*-splicing of the second group II intron. We have performed tandem affinity purification coupled to MudPIT to purify a protein complex containing previously characterized *trans*-splicing factors as well as not jet detected components. Direct protein interactions within the complex were analyzed by yeast two-hybrid analyses. Furthermore qRT-PCR analyses revealed that RNA corresponding to the second group II intron was copurified with the complex. Using gel filtration experiments it was shown that the

ribonucleoprotein particle has a size of about 500-600 kDa. These results confirm the presence of a 500-600 kDa protein complex that is involved in splicing of the second *psaA* group II intron.

118. Putative Cdc25 phosphatases in the cell cycle regulation of *Chlamydomonas reinhardtii*. Monika Hlavová, Mária Čížková, Hana Pitrunová, **Katerina Bisova**. Laboratory of Cell Cycles of Algae, Inst Microbiology ASCR, Trebon, Czech Republic.

CDC25 phosphatases are crucial positive regulators of cyclin-dependent kinases (CDKs) in fungi and the animal. However, their existence and function in the plant kingdom remains enigmatic even though the antagonistic Wee1 kinases are present in plants. We characterized three of four potential *CDC25* homologs present in the genome *Chlamydomonas reinhardtii*, *RDP1, 2, 3* (rhodanese-domain protein). Similarly to a putative *CDC25* homolog in *Arabidopsis thaliana*, the three proteins consist only of a catalytic domain and lack regulatory N-terminal extensions present in other systems. The three homologs showed distinct patterns of expression during progression of the cell cycle and had tyrosine-phosphatase activities as well as were able to activate *C. reinhardtii* CDK-cyclin complexes *in vitro*. Characterization of the inducible amiRNA and OX lines revealed a reproducible cell cycle related phenotype. In line with the results of phosphatase activity experiments the most pronounced phenotype was that of amiRDP2 and amiRDP3 lines while there were no significant differences in the case of amiRDP1. Both the *RDP2* and *RDP3* amiRNA lines showed a delay in the cell division. Surprisingly, the inducible OX lines also showed similar delay in the cell division. The results indicate *RDP2* and *3* are *bona fide* homologs of *CDC25* since they are involved in the timing of cell cycle progression. Furthermore, the cell cycle progression is dependent on tightly regulated expression level of Rdps since both downregulation and overexpression of the transcript affect the cell cycle duration.
This work was supported by AS CR (M200201205) and by projects Algatech and Algaman.

119. UV sensitivity variations in *C.reinhardtii* - a further investigation. **Vishalsingh Chaudhari**, Aniket Vyavahare, Swapan Bhattacharjee, Basuthkar Rao. Department of Biological Science, Tata Institute of Fundamental Research, Mumbai, Maharashtra, India.

Many photosynthetic organisms show variation in stress survival depending on light conditions. We observed a similar behavior in *C.reinhardtii*. A wild type Light grown culture (LC) is more sensitive to UV-C compared to dark incubated (DC, for 12hrs) culture. This behaviour is maintained in both photoautotrophic and mixotrophic growth conditions. We studied this light-dark switch of UV-C sensitivity in presence of Atrazine, a PSII inhibitor. Atrazine treatment made LC resistant like DC. 'Resistant' phenotype of LC+ Atrazine was in fact even stronger than DC. Similar observation was made with D1 (psbA) null mutant where LC was observed to be more resistant DC, a reversal of sensitivity. We monitored ROS levels in the paradigm to understand greater resistance conferred by blocking of PSII. Elevated ROS levels in Atrazine treated samples indicated that this higher resistance could be secondary effect of ROS signalling or a triggered SOS response. We provide an indirect support by showing better UV survival in samples given mild ROS treatment using H_2O_2 and Methyl Viologen. It has been previously reported that a circadian trained culture shows rhythmic variations in UV sensitivity. Our studies of UV sensitivity with circadian trained culture and free running rhythms indicated that this behaviour is non-circadian, contrary to a previous study. Atrazine treatment in circadian trained cultures strongly inhibited rhythmic behaviour and conferred a constitutive high resistance (slightly higher than dark phase). We show that atrazine treatment did not affect internal circadian rhythm for the period of observation. We suggest that circadian trained culture shows a rhythmic sensitivity as a response of alternating light-dark periods (or photosynthesis metabolism) and not as a function of circadian rhythm.

120. Cell-free extracts of *C.reinhardtii* exhibit excision repair activity against UVC damage in DNA. **Vishalsingh Chaudhari**, Vandana Raghavan, Basuthkar Rao. Department of Biological Science, Tata Institute of Fundamental Research, Mumbai, Maharashtra, India.

Chlamydomonas reinhardtii, as a prospective model organism for unravelling molecular mechanisms associated with DNA repair in plants and algae, remains largely under explored. An *in vitro* repair system from *C. reinhardtii* cell-free extracts can provide an excellent opportunity to realize this potential. We present here a methodology to prepare *C. reinhardtii* cell-free extracts that can efficiently repair UVC damage (Thymine-dimers) in DNA. We show using labeled nucleotides that *in vitro* repair activity is specific to Thymine-dimers. The repair activity driven nucleotide incorporation in UVC damaged supercoiled (SC) plasmid DNA marks that excision repair synthesis was followed by ligation of nicks in the cell-free extracts. The inhibitor profile of repair DNA polymerase involved in *C. reinhardtii in vitro* repair system is akin to animal rather than plant DNA polymerase. The repair reaction was found to be Aphidicolin sensitive but ddTTP insensitive. Extracts were also prepared from different time points of a rhythm traineComparison of relative efficiency revealed that excision repair is more efficient in dark phase than in light, which provides a rationale for observed rhythmic sensitivity against UVC in light-dark synchronous cultures. We believe this the first report of preparing the repair competent cell-free extract from *C.reinhardtti*. This methodology can aid further molecular characterization of excision repair pathway in *C. reinhardtii.*

121. Novel mutants affecting cell cycle regulation and DNA damage response in the green alga *Chlamydomonas reinhardtii*. **Monika Hlavova**, Maria Cizkova, Zoltan Turoczy, Katerina Bisova. Laboratory of Cell Cycles of Algae, Inst Microbiology AS CR, Třeboň, Czech Republic.

The eukaryotic cell cycle is controlled by complex of cyclin-dependent kinases and cyclins, their activity is controlled by de/phosphorylation mediated by regulatory proteins including inhibitory Wee1 kinase. A single homolog of *WEE1* kinase was identified in *Chlamydomonas reinhardtii* genome. Our preliminary results suggested it is involved both in the regulation of mitosis and in the response to DNA damage. The mechanism responsible for activation of *CrWEE1* expression either in normal cell cycle or in response to DNA damage is unknown. To unravel this mechanism we screened 20,000 insertional mutants to isolate those with altered expression of *CrWEE1* upon DNA damage. We recovered 14 *wer* (WEE1 regulation) strains with altered *WEE1* expression after DNA damage. The mutants fall into different groups based on changes in gene expression during the cell cycle and in response to DNA damage. We

characterized their cell cycle progression and sensitivity to different DNA damaging agents (zeocin, UV, cisplatin). Some of the mutants have altered cell cycle progression with either accelerated or prolonged cell division. Furthermore, we isolated mutant both sensitive and resistant to different DNA damage. The sensitivity of the mutants was connected to the absence of cell cycle progression delay in the presence of DNA damage suggesting the mutants have altered DNA damage checkpoint. The result further support Wee1 is involved both in the cell cycle regulation and in the response to DNA damage, particularly double strand breaks and UV irradiation.
This work was supported by Program Kontakt II (DNADAM, no. LH12145), AS CR (M200201205) and by projects Algatech and Algaman.

122. Promoters driving chloroplast gene expression in diurnal conditions. **Adam Idoine**, Alix Boulouis, Ralph Bock. Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany.

The plastome of *C. reinhardtii* is derived from a prokaryotic ancestor; however it has been domesticated by a eukaryotic cell. The regulation of its genes is hence thought to be a hybrid of prokaryotic and eukaryotic mechanisms. Bacterial gene expression is characterised by cotranscriptional translation, leading to great regulatory importance at the transcriptional level. This contrasts with the eukaryotic system, in which transcription and translation are uncoupled. Plastid transcripts have been shown to fluctuate in various conditions, however transcript levels have also been shown to be immaterial to translation rates. The relevance and mechanism for transcriptional control of chloroplast RNA species hence remains unclear.

Chloroplast transcript levels were measured under diurnal conditions (12 h light / 12 h dark). Strikingly, transcripts involved in the same process (transcription, translation or photosynthesis) behaved similarly over a 24 h cycle. All transcripts attained highest levels between midnight and midday; however, plastid encoded RNA polymerase transcripts peaked first within this window, followed by ribosome subunit transcripts, followed finally by photosynthesis-related transcripts. This matches perfectly to the theoretical demands of a cell establishing photosynthetic complexes after “waking up” from the night.

Analysis of the plastome sequence suggests that differences in promoters may be controlling the transcript levels. However, this would require more than the one currently annotated RNA polymerase sigma factor. Bioinformatics analysis has revealed additional unannotated candidate sigma factors. An analysis of potential destabilising elements in the 3' UTR is also presented.

123. Forward genetics study of the zygote differentiation mechanisms in *Chlamydomonas reinhardtii*. T. Kariwasam[1], S. Joo[1], D. Toor[1], E. Cronmiller[1], K. Noh[2], T. Wulan[2], U. Goodenough[2], **J.-H. Lee[1]**. 1) Dept of Botany, Univ. of British Columbia, Vancouver, BC, Canada; 2) Dept. of Biology, Washington Univ., St.Louis, MO, USA.

The sexual cycle of *Chlamydomonas reinhardtii* is under the control of two mating type loci, *plus* and *minus*, where the *minus* locus-specific transcription factor Mid dictates the sex determination program for *minus,* with the *plus* program operating by default. The *minus* dominance of *C. reinhardtii* has hampered a genetic approach to studying the zygote stage of the life cycle due to the impracticality of obtaining large numbers of homozygous mutants. To study molecular mechanisms of zygote development, we engineered a homothallic strain that becomes homozygous after self-mating, allowing mutant zygotic phenotypes to be directly monitored in cells starved for nitrogen for 24 hrs. Most zygote-defective mutants screened thus far fall into three phenotypic groups: retention of quadriflagellated cells (*Z*ygote *D*evelopment *D*efective); abnormal assembly of the zygotic wall (*Z*ygote *W*all *D*efective); and solitary zygotes (*Z*ygote *S*ocially *D*efective). In the first group, *zdd1* is found to have a large deletion that includes *GSP1*, encoding a homeoprotein necessary to initiate zygote development, indicating that the screen identifies relevant loci. The mutations identified in the other two groups are largely related to secretory pathways such as vesicular trafficking, *N*-glycosylation, and exocytosis. These novel mutants will provide invaluable tools to decipher the roles and regulation of individual pathways during the zygote differentiation. Interestingly, these zygote-defective mutants usually display no phenotype as vegetative cells, suggesting that a significant portion of eukaryote-specific gene families may have evolved in the context of sexual development.

124. Functional analysis of CRY-DASH1 in *Chlamydomonas reinhardtii*. **Wei Li[1]**, Lysett Wagner[1], Sabine Oldemeyer[2], Tilman Kottke[2], Maria Mittag[1]. 1) Institute of General Botany and Plant Physiology, Friedrich Schiller University Jena, 07743 Jena, Germany; 2) Physical and Biophysical Chemistry, Department of Chemistry, Bielefeld University, 33615 Bielefeld, Germany.

The *Chlamydomonas reinhardtii* genome encodes an animal-like cryptochrome (aCRY), a plant cryptochrome (pCRY) and two potential DASH-cyptochromes (named CRY-DASH 1 and 2, respectively). The focus of this work is on the functional analysis of CRY-DASH1. At first, the predicted cDNA models *CRY-DASH1-Chlre* and *CRY-DASH1-Augustus5* have been verified by cDNA cloning. A new model had to be constructed based on the sequencing results of two independent cDNA clones. A synthetic codon adapted gene of *CRY-DASH1* including a His-tag was expressed in *E. coli*. After purification of the His-tagged CRY-DASH1 protein by Ni-NTA and heparin columns, CRY-DASH1 was found as yellow flavoprotein. The purified protein was verified by LC-ESI-MS/MS analysis. In addition, spectroscopic analysis was done. 2 mg His-tagged CRY-DASH1 protein were purified and used for antibody production. The antibody recognizes the 62 kDa CRY-DASH1 protein in *C. reinhardtii*. CRY-DASH1 is expressed at different levels over the diurnal cycle. Currently, we investigate the localization of CRY-DASH1 and its potential role in light regulation and/or the circadian clock.

125. Involvement of a putative SUMO peptidase in size-mediated cell cycle control in *Chlamydomonas reinhardtii*. **Yang-Ling Lin**, Chun-Han Chen, Su-Chiung Fang. Academia Sinica, SinShih Dist, Tainan, Taiwan.

Small-ubiquitin-like modifier (SUMO) conjugation, or SUMOylation, plays an important role in cellular process of cell division. Defect in SUMOylation causes defects in cell cycle progression and cell proliferation. We previously identified a mutant, *smt7-1*, that partially suppresses the small-size defects caused by loss of the retinoblastoma tumor suppressor related protein (RBR) encoded by the *MAT3* gene in *Chlamydomonas reinhardtii*. Isolation of the full-length *SMT7* cDNA reveals that it encodes a novel SUMO peptidase (94 kDa) with extended N-terminal region followed by a conserved SUMO peptidase domain (pfam02902). Complementation of the *smt7-1mat3* mutant using a SMT7-3xHA construct confirmed that its expression was able to resume small-size of *mat3*. Based on *in vitro* assays, we showed the C-terminal portion of SMT7 functions as a SUMO peptidase. In addition, we observed that *smt7-1* mutant cells had slightly aberrant SUMOylation patterns comparing to wild type cells. Our preliminary data showed that SMT7 mRNA was regulated in a cell-cycle dependent manner. Immunoprecipitation experiments are under way to find *in vivo* targets of SMT7 that will shed light on how SMT7 regulates cell cycle.

126. Testing the constancy of the nuclear : cytoplasmic volume ratio in wild type and cell-size mutants of *Chlamydomonas*. **D. Liu**[1], J. Umen[2]. 1) Washington University, St. Louis MO; 2) Donald Danforth Plant Science Center, St Louis MO.

Chlamydomonas is an ideal organism for studying eukaryotic cell size control because its multiple fission cell cycle partially uncouples growth and cell division. Some models for cell size control involve changes in the nuclear:cytoplasmic volume ratio (N:C) [1]. In budding and fission yeast N:C is fixed meaning that nuclear and cytoplasmic volumes are tightly coordinated [2,3], but nuclear volume has never been systematically investigated outside of fungi. We took advantage of the large size range obtainable in synchronized *Chlamydomonas* cultures to measure N:C in live cells of different cell sizes using a ble-GFP fusion protein as a nuclear marker. Our data show that N:C stays fixed as cells grow by over ten-fold during G1 phase. Conversely, the nuclear volume halves with each round of cell division during multiple fission. These data mean that like the case in fungi, nuclear volume in *Chlamydomonas* is tightly coupled to cell size. An interesting corollary of these observations is that the concentration of DNA in the nucleus decreases dramatically in G1 phase as the nucleus grows but the amount of DNA and chromatin within it stays constant. *mat3-4* mutants are missing the retinoblastoma tumor suppressor homolog and are smaller than wild-type cells, but maintain a similar N:C as wild type over most of their size range. However, in *mat3-4* daughter cells the N:C ratio deviates from that in wild type and may be constrained by a lower limit to nuclear size. Ongoing work is aimed at measuring N:C in other cell size mutants to test whether there are other cases when the N:C ratio deviates from its wild type value.

1. Umen, J. G. The elusive sizer. *Curr Opin Cell Biol* **17,** 435–441 (2005).
2. Jorgensen, P. *et al.* The size of the nucleus increases as yeast cells grow. *Mol Biol Cell* **18,** 3523–3532 (2007).
3. Neumann, F. R. & Nurse, P. Nuclear size control in fission yeast. *J Cell Biol* **179,** 593–600 (2007).

127. Raptor and siRNA-mediated translation repression in *Chlamydomonas reinhardtii*. Tomohito Yamasaki[1,2], **Xinrong Ma**[1], Eun-Jeong Kim[1], Heriberto Cerutti[1]. 1) School of Biological Sciences and Center for Plant Science Innovation, University of Nebraska, Lincoln, Nebraska, USA; 2) Current address: School of Environmental Science and Engineering, Kochi University of Technology, Tosayamada, Kochi, Japan.

Small RNAs (~20-30 nucleotides in length) play important roles in regulating gene expression in eukaryotes. In *Chlamydomonas reinhardtii*, small interfering RNAs (siRNAs), derived from inverted repeat transgenes, can inhibit protein synthesis of targeted endogenous genes without or with only minimal mRNA destabilization. Moreover, siRNA-repressed transcripts remain associated with polyribosomes, indicating inhibition at a post-initiation step of translation. Interestingly, ribosomes associated with siRNA-repressed transcripts show reduced sensitivity to translation inhibition by certain antibiotics such as cycloheximide, both in ribosome run-off assays and in *in vivo* experiments. These results suggested that siRNA-mediated repression of protein synthesis might involve alterations to the function/structural conformation of translating ribosomes. However, the exact molecular mechanism remains unclear. By random insertional mutagenesis, we have isolated several mutants defective in siRNA-mediated translation repression in *Chlamydomonas*. Mut-19 displayed a 3' truncation of the raptor [regulatory associated protein of TOR (Target of Rapamycin)] gene. Transformation of this mutant with epitope-tagged raptor recovered siRNA-mediated translation repression and lowered the hypersensitivity of the mutant to rapamycin and torin1 inhibitors. RNA immunoprecipitation assays revealed that epitope-tagged raptor is associated with normally expressed transcripts whereas this association is reduced for mRNAs translationally repressed by siRNAs. The TOR kinase interacts with raptor to form a protein complex which plays a central role in the regulation of cell growth in response to environmental cues such as nutrient availability. Our findings suggest that siRNA-guided effector complexes may interfere with the TOR/Raptor function of stimulating mRNA translation.

128. Effect of resource limitation on asexual development of *Volvox carteri*. Alexandra Harryman, Jose Ortega Escalante, Jacob Kott, Michael Ishak, **Stephen Miller**. Deptartment of Biological Sciences, UMBC, Baltimore, MD.

While the effects of nutrient and light limitation on growth and development have been well studied in some green species, they are not well documented for the multicellular green alga *Volvox carteri*, despite the fact that it has long been hypothesized that the evolution of developmental complexity in this and related species was strongly influenced by ecological factors such as resource availability. Here we report findings from two studies that bear on this topic. First we documented the response of *V. carteri* to deprivation for light and two key macronutrients, phosphate and sulfate, to determine how and when growth and development are affected by these

environmental perturbations. The effects of light deprivation were obvious within 24 hours, while development of individuals deprived of phosphate and/or sulfate proceeded fairly normally for nearly two generations and did not terminate until the juvenile stage of the third generation. In no case was cell fate affected. Second, we analyzed a newly isolated, spontaneous Reg mutant derived from EVE that we named “partial Reg” (“pReg”). Under optimal growth conditions at low density, nearly all somatic cells of pReg develop normally, though in some spheroids one or a few somatic cells dedifferentiate then enlarge and develop as gonidia. However, the Reg phenotype of pReg is significantly enhanced in dense culture conditions and when pReg is grown with limiting amounts of macronutrients, including phosphate and sulfate. These observations suggest that while normally resource limitation has minimal effect on cell fate, there might be a cryptic pathway in *V. carteri* through which resource availability can alter somatic cell fate.

129. Actin-ring-independent cytokinesis in *Chlamydomonas*. **Masayuki Onishi**[1], Luke Mackinder[2], Martin Jonikas[2], John R. Pringle[1]. 1) Dept of Genetics, Stanford University, Stanford, CA; 2) Dept of Plant Biology, Carnegie Institution for Science, Stanford, CA.

Cytokinesis in animals and fungi involves a ring containing actin and myosin that is formed on the cytoplasmic face of the plasma membrane at the division site. This “contractile ring” has long been thought to provide the force for ingression of the cleavage furrow, through filament sliding between type-II myosin and F-actin. However, recent evidence suggests that many fungal and animal cell types do not actually require the motor activity of type-II myosin for successful furrow ingression. More importantly, the phylogenetic distribution of type-II myosin is confined to the narrow branch of unikonts (fungi, animals, and slime molds), yet most other eukaryotes also appear to divide by furrowing. Thus, we hypothesize that the common ancestor of all eukaryotes divided by furrowing independent of a contractile ring and that this ancestral mechanism of driving cleavage-furrow ingression is conserved among most modern eukaryotes.

Cell division in *Chlamydomonas* is carried out by furrowing, but it remains unclear whether this process involves a contractile ring. Although actin accumulates at the division site, it is not clear if this actin is filamentous, it has been reported that both cytochalasin-treated cells and a null mutant for the one conventional actin grow at nearly normal rates, and none of the three *Chlamydomonas* myosins is type II. We have further investigated actin behavior by fluorescently labeling F-actin in living cells expressing a Lifeact-Venus probe. In interphase, Lifeact-Venus localized to the mid-portion of the cell, forming a cage-like structure around the nucleus. This localization was sensitive to the actin-depolymerizing drug latrunculin A, confirming that it represents F-actin. In dividing cells, this localization disappeared, but no enrichment of Lifeact-Venus signal was observed in the cleavage furrow. One of the myosins have been successfully tagged with Venus and showed a similar localization pattern. Taken together, these results suggest that *Chlamydomonas* cytokinesis does not involve a contractile ring, so that genetic investigation of cytokinesis in this organism should shed critical light on the ancestral mechanisms of furrow formation.

130. Key Protein Domain Functions in Assembly and Placement of the Eyespot. **Mark Thompson**, Telsa Mittelmeier, Eric Figueroa, Carol Dieckmann. MCB, University of Arizona, Tucson, AZ.

The Chlamydomonas eyespot is a specialized sensory organelle that allows the cell to direct its movement towards an optimal level of light. The eyespot is assembled *de novo* after cell division in each daughter cell, at the equator of the cell near the tip of the daughter four membered rootlet (D4). We have identified proteins key to the assembly and placement of the eyespot including the channelrhodopsins (ChR1&2), EYE2, MIN1 and EYE3. Currently the focus is on understanding the role(s) of functional domains identified within these proteins. To investigate the role of the ChR photoreceptors in positioning and/or assembly of the eyespot, we expressed mCherry-tagged ChR1 in a strain lacking ChR1, and followed its localization in live cells via fluorescence microscopy. We found that the long C-terminal tail of ChR1 is not responsible for trafficking of the photoreceptor to its position at the eyespot; the carboxyl-truncated protein was able to localize to the wild-type position of eyespot formation. Currently we are generating a double photoreceptor mutant to parse out the role the highly redundant photoreceptor, ChR2, may play in aiding ChR1 localization. The EYE2 and MIN1 proteins both contain LysM (lysin motif) domains and are predicted to be localized to the region of the chloroplast envelope in the eyespot. We hypothesize that these domains play a role in protein-protein interactions that are necessary for proper assembly of the eyespot. We are testing this hypothesis by making mutations within these conserved domains and/or swapping them between the two proteins. EYE3 is a predicted ABC-1 family protein kinase that localizes to the pigment granule layers of the eyespot. To elucidate the role of the kinase activity in formation of pigment granule layers, we changed the active site Asp to Ala, creating a kinase dead mutant. This mutant does not have eyespots and is unable to phototax, suggesting that the kinase activity is essential for pigment granule and thus eyespot formation. Together these data will give us more insight into some of the molecular mechanisms underlying the localization and assembly of the eyespot. This work is supported by National Science Foundation grant MCB-1157795 to CD.

131. Functional analysis of an animal-like cryptochrome. **Sandra Wenzel**[1], Benedikt Beel[1], Tilman Kottke[2], Maria Mittag[1]. 1) Institute of General Botany and Plant Physiology, Friedrich Schiller University, Am Planetarium 1, 07743 Jena, Germany; 2) Physical and Biophysical Chemistry, Department of Chemistry, Bielefeld University, Universitätsstra□e 25, 33615 Bielefeld, Germany.

Cryptochromes were formerly known as UV-A and blue light photoreceptors with high sequence similarity to photolyases. Recently, an animal-like cryptochrome (aCRY) was identified in the green alga *Chlamydomonas reinhardtii* which gene expression is altered in response to not only blue light but also yellow and red light. This unique response of a flavoprotein in vivo has been attributed to the fact that the neutral radical of the flavin chromophore acts as dark form of the sensor, which absorbs in almost the entire visible spectral range (<680nm; Beel et al., The Plant Cell 2012, 24: 2992–3008). Since certain animal-like cryptochromes are involved in the entrainment of the circadian clock (e.g. in *Drosophila*), we started to investigate the role of aCRY in the input mechanism in *Chlamydomonas*. The clock-relevant subunit C3 of the RNA-binding protein CHLAMY1 displayed a phase-shifted transcript expression profile in the aCRY

mutant. First data of phase shift experiments of the phototaxis rhythm using red light show differences in the phase response curves between wild type and the aCRY mutant. Currently, we are also searching for possible interaction partners of aCRY by protein pulldown experiments.

132. Predator induced aggregation of *Chlamydomonas reinhardtii* by a diffusible signal. **Sarah Cossey**[1], Christopher Berger[1], Nicole Richardson[2], Bradley Olson[1]. 1) Kansas State University, Manhattan, KS; 2) University of Iowa, Iowa City, IA.

The evolution of multicellularity is a major evolutionary transition, occurring at least twenty five independent times across eukaryotic lineages. However, the ecological pressures that stimulate this transition are poorly understood. Chlamydomonas and its multicellular relatives show a linear progression of morphological complexity, from unicellular *Chlamydomonas reinhardtii* to colonial multicellular *Gonium pectorale* to *Volvox carterii* which is multicellular with differentiated tissues. It has been hypothesized that multicellularity evolved to increase organismal size thus, allowing it to evade predation. In the presence of predators such as *Daphnia,* we found that *C. reinhardtii* will rapidly form aggregates. However, in nutrient limited media we did not see aggregation. We hypothesize that the aggregation response seen in the predated *C. reinhardtii* has become genetically permanent in multicellular volvocine algae species. To test this hypothesis, we are characterizing the predator response in *C. reinhardtii* and exploring how it evolved in its multicellular relatives. First, bacteria can induce aggregation of *C. reinhardtii* cells, so we developed a method to prepare axenic *Daphnia* (removal of bacterial and fugal contaminants) such that their sole food source is *C. reinhardtii* cells. The axenic *Daphnia* then feed on the *C. reinhardtii* cells and a rapid aggregation response is observed. When media from the predator treated *C. reinhardtii* cells is added to untreated *C. reinhardtii* an aggregation response is also observed, suggesting that there is a mobile signal released into the media, causing this response. Interestingly, *G. pectorale*, a colonial multicellular relative of *C. reinhardtii* does not respond to predation by aggregation, suggesting that the aggregation response may be genetically permanent in this species. Here we will report the characterization of the signal that is responsible for this aggregation response. In summary, our data indicates that predation may have driven the evolution of multicellularity in the volvocine algae.

133. Chlamydomonas mutants that are hypersensitive to the TOR kinase inhibitor rapamycin show differential growth responses to carbon source. **Inmaculada Couso-Lianez**[1], Spencer Diamond[2], Garret Anderson[3], Jia Li[1], Jan Jaworski[1], James Umen[1]. 1) Umen Lab, Donald Danforth Plant Science Center, St Louis, MO; 2) UCSD, La Jolla, CA 92093; 3) Salk Institute, La Jolla, CA 92037.

Photosynthetic growth in plants and algae is driven by the fixation of inorganic carbon (CO_2/HCO_3) that can be a limiting resource. However, many green organisms like *Chlamydomonas* can also grow heterotrophically, or mixotrophically using a combination of photosynthesis and external reduced carbon. The relationship between these modes of growth is complex and involves partial suppression of photosynthetic metabolism and alterations in gene expression caused by the presence of acetate. Very little is known regarding possible signaling pathways that control the balance between organic and inorganic carbon metabolism in green algae. Because of its central role in governing eukaryotic cell growth, we focused on the potential role of TOR kinase in regulating carbon metabolism in *Chlamydomonas*. We conducted an insertional screen of ~60,000 *Chlamydomonas* lines to identify those that are hypersensitive to the TOR inhibitor rapamycin. Two recessive and non-allelic mutants, *rap1* and *rap2*, were isolated that have phenotypes related to carbon source metabolism. Both mutants exhibit much stronger growth inhibition than wild type in the presence of rapamycin, but their growth phenotypes on rapamycin are partially modulated by carbon source. The rapamycin sensitivity of *rap1* is partially rescued by acetate, while the presence of acetate exacerbates the rapamycin hypersensitivity of *rap2*. *rap2* has additional phenotypes that suggest an important role for the RAP2 protein in carbon partitioning. Ongoing work is aimed at dissecting the roles of RAP1 and RAP2 in regulating carbon partitioning and their relationship to TOR kinase signaling.

134. Characterization of NaCl stress inducible palmelloids in the green alga, *Chlamydomonas reinhardtii*. **Jacinta D'Souza**[1], Dolly Khona[1], Seema Shirolikar[2], Erik Hom[3], Manjushree Deodhar[4]. 1) UM-DAE Ctr for Excellence in Basic Sciences, Mumbai, Maharashtra; 2) 2Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai 400 005, India; 3) Department of Molecular and Cellular Biology, Harvard University, 520 Oxford Street, NW 469 Cambridge, Massachusetts 02138; 4) K. E. T's V. G. Vaze College of Arts, Science and Commerce, Mulund (E), Mumbai 400 081, India.

Chlamydomonas reinhardtii, a unicellular green chlorophyte is widely used to study abiotic stress responses. This organism is free living under favorable conditions but when exposed to organic acids, low pH medium or encounter with rotifers, it enters into a multicellular 'palmelloid' stage. The current study deals with characterization of palmelloids formed by this organism as a response to incorporation of NaCl in the growth medium. This phenomenon was found to be reversible as dissociation of these palmelloids was more rapid than its formation. Cells in palmelloids were non-motile though flagella were detected by immunofluorescence microscopy. Ultrastructural analysis by transmission electron microscopy revealed an increment in the number of autophagy vacuoles in the palmelloid cells. Remnants of excised flagella and transition zone were observed indicating mitotic division in these cells. There were also instances of vesicle like structures budding off from the flagellar membrane. Other physiological responses such as accumulation of starch and lipid granules and presence of extracellular polysaccharide mesh were analyzed qualitatively a quantitatively. Mass spectroscopy analysis of the cultured media supernatants after stress and de-stress events provided an insight in the probable role of these proteins in NaCl stress inducible responses.

135. Novel Retrograde Bilin Signaling Impacts Transition from Heterotrophic to Photoautotrophic Growth in *Chlamydomonas reinhardtii*. **Deqiang Duanmu**[1], Nathan Rockwell[1], David Casero[2,3], Tyler Wittkopp[4], Stefan Schmollinger[5], Rachel Dent[6], Matteo Pellegrini[2,3], Krishna Niyogi[6,7,8], Sabeeha Merchant[2,5], Arthur Grossman[4], Clark Lagarias[1]. 1) Molecular and Cellular Biology, University of California Davis; 2)

Institute for Genomics and Proteomics, University of California, Los Angeles; 3) Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles; 4) Department of Plant Biology, Carnegie Institution for Science, Stanford; 5) Department of Chemistry and Biochemistry, University of California, Los Angeles; 6) Department of Plant and Microbial Biology, University of California, Berkeley; 7) Howard Hughes Medical Institute; 8) Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley.

Changes in the developmental or metabolic state of chloroplasts can impact nuclear gene expression by retrograde signals, which are typically generated in the chloroplast, exported to the cytosol and act in the nucleus. Several of such signals have been characterized in plants. However, knowledge is limited about the mechanisms of retrograde signaling in *Chlamydomonas reinhardtii*. Here we show that bilin is a retrograde signal that targets a small network of gene expression to prepare cells for diurnal transition from heterotrophic to photoautotrophic growth. Biochemical evidences indicate bilin is synthetized in the chloroplast by the two enzymes: heme oxygenase (*HMOX1)* and bilin reductase (*PCYA)*. Bilin biosynthesis is blocked in the *hmox1* mutant and the photoautotrophic growth of the mutant was strongly impaired. Light-grown *hmox1* mutant has similar level of oxygen evolution rate as WT, consistent with the similar protein abundance of photosystem subunits between the mutant and the WT. Addition of exogenous biliverdin could be converted to bilin in *hmox1* mutant and thus rescue the light dependent chlorophyll-deficiency phenotype. RNAseq analysis reveals a small number of bilin-responsive genes in both dark-grown and transition cultures. Collectively, these data suggest *hmox1* mutation and the disrupted bilin biosynthesis impacts the transition from dark/heterotrophic to light/photoautotrophic growth in Chlamydomonas.
Bilins are cofactors of light harvesting phycobiliproteins or light sensing phytochromes.However, *Chlamydomonas reinhardtii* and other chlorophytes lack both phycobiliproteins and phytochromes. We therefore expect that such bilin retrograde signaling will be widely distributed amongst oxygenic photosynthetic organisms both with and without phytochromes, including land plants.

136. CO2-requiring mutants defective in CCM-induction generated by high frequency transformation with square electric pulses. Lyanyong Wang[1], Takashi Yamano[1], Masataka Kajikawa[1], Masafumi Hirono[2], Hiro Iguchi[1], **Hideya Fukuzawa[1]**. 1) Grad Sch Biostudies, Kyoto Univ, Kyoto, Japan; 2) Grad Sch Science, Univ of Tokyo, Japan.

Aquatic microalgae induce a CO_2-concentrating mechanism (CCM) to maintain photosynthetic activity under CO_2-limiting (low-CO_2, LC) conditions. Although the molecular mechanism of CCM has been studied, many aspects of CCM such as the CO_2-signal transduction pathways and carbon-transporters remain to be elucidated. In this study, we report the isolation of novel high-CO_2 (HC)-requiring mutants defective in the induction of CCM-components. Growth rates of 32,300 transformants were compared under HC and LC conditions using 96-well microtiter replica plates, and four mutants (H24, H82, P103, and P59) were isolated. The photosynthetic $K_{0.5}$(Ci) values, that is affinity against inorganic carbon, calculated by the photosynthetic O_2 evolution rates in three mutants (H82, P103, and P59) were increased to 525.6, 312.4, and 89.1 μM from 47.8 μM of wild-type, and accumulation of HLA3 and both LCIA and HLA3 were not detected in the H24 and H82, respectively. In the H82, a gene encoding a calcium-sensing receptor CAS was disrupted by insertion of the hygromycin cassette, and this insertion was tightly linked with the HC-requiring phenotype in the tetrad progenies. By transformation of a BAC clone containing the CAS, three candidate lines, which grow under air condition, accumulated significant level of LCIA, but CAS and HLA3 were not detected. Relationship between CCM activity and phenotype of those mutants will be discussed.

137. Impact of carbon nutrition on mechanisms of cellular iron homeostasis. **Anne Glaesener[1]**, Sabeeha Merchant[1,2]. 1) Chemistry and Biochemistry, University of California Los Angeles, Los Angeles, CA; 2) Institute of Genomics and Proteomics, University of California Los Angeles, Los Angeles, CA.

Iron is an essential micronutrient for all organisms because of its broad role as an essential cofactor for many cellular processes including redox reactions of photosynthesis, and respiration, among others. Because of its reactivity and low bioavailability, organisms need to maintain iron homeostasis by regulating iron uptake, distribution, and utilization. *Chlamydomonas reinhardtii* serves as an excellent experimental system, not only for studying basic biological and biochemical processes such as photosynthesis and metabolic pathways, but also for investigating aspects of iron metabolism and the interplay of metal nutrition with metabolic pathways. Several paradigms in iron homeostasis have been established in this alga, including remodeling of the photosystem, and preferential retention of some iron-dependent proteins in response to suboptimal iron nutrition. During the two major trophic states, photoautotrophic and photoheterotrophic, Chlamydomonas cells respond to iron-limitation differently. When grown under photoautotrophic conditions, the photosynthetic apparatus is more resistant to iron-deprivation induced loss, both on the level of photosynthetic activity and protein abundance. We investigate the impact of carbon source on mechanisms of iron uptake and distribution in Chlamydomonas, and how acetate supply affects iron uptake and storage, as well as the down-regulation of a subset of chloroplast iron-containing proteins and photosynthetic capacity in iron-limited cells. By monitoring changes in RNA and protein abundance and photosynthetic parameters during the transition from photoautotrophic to photoheterotrophic growth by adding acetate to iron-deficient cells, we can enhance the understanding of the dynamics of iron homeostasis in photosynthetic organisms.

138. Characterization of a Null Mutant of Isocitrate Lyase in *Chlamydomonas*. C. Plancke[1], H. Vigeolas[1], R. Höhner[2], R. Willamme[1], S. Roberty[1], P. Cardol[1], S. Hiligsmann[1], P. Thonart[1], G. Eppe[1], M. Hippler[1], **U. Goodenough[3]**, C. Remacle[1]. 1) University of Liege, Belgium; 2) University of Muenster, Germany; 3) Washington University, St. Louis, MO.

Isocitrate lyase (ICL) is one of the key enzymes of the glyoxylate cycle. A null *icl* mutant of *C. reinhardtii* has been isolated by insertional mutagenesis into the *ICL1* gene and characterized together with a complemented strain (*iclC*) obtained by integrating a wild-type copy of *ICL1* into the *icl* mutant.

We show that the *icl* mutant is unable to grow in the dark on acetate (heterotrophic conditions) and grows more slowly than wild type and *iclC* in the light + acetate (mixotrophic conditions). In the latter condition, reduced acetate assimilation and concomitant

reduced respiration are found. Using quick-freeze deep-etch EM, *icl* cells are indistinguishable from *iclC* cells under mixotrophic conditions with one striking exception: most *icl* mitochondria are devoid of cristae, with a few carrying 1-2 cristae, whereas all *iclC* mitochondria carry abundant, wild-type levels of cristae. Biomass composition analysis reveals an increase in total fatty acid content, including neutral lipids and free fatty acids, although no increase in *icl* lipid-body population is evident by EM. Quantitative proteomic analysis by $^{14}N/^{15}N$ labelling shows a strong decrease in the amounts of enzymes of the glyoxylate cycle and gluconeogenesis. In parallel, a shift of the TCA cycle towards amino acid synthesis is noticed, accompanied by an increase in free amino acids. The diminishment of glyoxylate cycle, gluconeogenesis, as well as the decrease in enzymes involved in β-oxidation of fatty acids in the *icl* mutant, are likely major factors that contribute to the remodeling of lipids in the *icl* mutant under mixotrophic conditions. In addition, elevation of the response to oxidative stress, with significantly augmented levels and activities of superoxide dismutase and ascorbate peroxidase and increased resistance to paraquat, are found. Despite increased levels of some autophagic enzymes such as APG8, there is no EM evidence of an autophagocytic response.

139. Biochemical characterization of "truncated" hemoglobins from *Chlamydomonas reinhardtii*. **Dennis Huwald**, Melis Düner, Thomas Happe, Anja Hemschemeier. Ruhr-University of Bochum, Bochum, NRW, Germany.

Recent genome-based primary structure analyses [1] indicate that *Chlamydomonas reinhardtii* possesses at least twelve hemoglobins with a 2-on-2 helix folding structure, also called truncated hemoglobins (THB). Truncated hemoglobins can be found in bacteria, plants and unicellular eukaryotes [2]. Although many of them have been studied biochemically and biophysically, the physiological function of only a few THBs is presently known [3]. Some of the *C. reinhardtii* THBs are unusually long and have uncommon domain structures. Except for THB8, which has been shown to interact with nitric oxide and which is proposed to be part of a signal transduction pathway under hypoxic conditions [4], most of the *Chlamydomonas* hemoglobins have not been studied at all and their functions remain unclear. We chose four of the *C. reinhardtii* THBs and overexpressed them heterologously in *Escherichia coli*. Studying these proteins and variants thereof via spectroscopic and biochemical methods revealed fundamental differences in the active sites of these hemoglobins, including the heme-iron coordination in the reduced state. Here, these findings will be presented and possible functions of the proteins will be discussed.

References

1. Hemschemeier, A. et al. Copper response regulator1-dependent and -independent responses of the *Chlamydomonas reinhardtii* transcriptome to dark anoxia. Plant Cell 25, 3186–3211 (2013).
2. Vuletich, D. A. & Lecomte, J. T. J. A phylogenetic and structural analysis of truncated hemoglobins. J Mol Evol 62, 196–210 (2006).
3. Vinogradov, S. N., Tinajero-Trejo, M., Poole, R. K. & Hoogewijs, D. Bacterial and archaeal globins - a revised perspective. Biochim Biophys Acta 1834, 1789–1800 (2013).
4. Hemschemeier, A. et al. Hypoxic survival requires a 2-on-2 hemoglobin in a process involving nitric oxide. Proc Natl Acad Sci U S A 110, 10854–10859 (2013).

140. Assessing the use of genetically engineered microalgae for the bioremediation of toxic metals. **Aniefon A. Ibuot**, Jon K. Pittman. Faculty of Life Sciences,University of Manchester, UK.

Toxic metals released into the environment as a result of industrial processes are of great concern. Algae have been considered a potential biological tool for the bioremediation of metals from metal-contaminated water due to its effective metal sequestering properties. However, these properties could be improved further by genetic engineering. Therefore in this work, different methods for manipulating metal uptake and tolerance in algae were examined. HMA4 is a metal transporter from the plant *Arabidopsis thaliana* which is a member of the HMA (Heavy Metal ATPase) family. AtHMA4 has been implicated in the transport of Zn^{2+} and Cd^{2+}, and was ectopically expressed in *Chlamydomonas reinhardtii*. Two types of AtHMA4-expressing strains were screened for improved metal accumulation and tolerance; FL lines expressing the full length AtHMA4 protein and CT lines expressing just the C-terminal metal binding tail of AtHMA4. AtHMA4 over-expression strains showed more metal tolerance and uptake abilities compared to the wild type. Another metal transport protein family studied are the Cation Diffusion Facilitator (CDF) family, known for providing significant tolerance in metals such as Mn^{2+} and Zn^{2+}. There are five CDF genes in *Chlamydomonas* (MTP1 – MTP5) which were cloned and functionally characterised by yeast heterologous expression. MTP4 was over-expressed in *Chlamydomonas*. These genetically engineered strains when screened for their specific metal tolerance and uptake revealed improved metal tolerance and uptake as compared with the wild type. Genetically engineered strains should therefore be adopted for bioremediation of toxic metals.

Keywords: Bioremediation, toxic metals, microalga.

141. Development of *Chlamydomonas reinhardtii* strains with improved photosynthetic activity and growth rate. **Jooyeon Jeong**, KwangRyul Beak, EonSeon Jin. Dept. of Life science, Hanyang University, Seoul, South Korea.

In photosynthesis, absorbed light energy is transformed by means of photochemical quenching (qP), non-photochemical quenching (NPQ) and chlorophyll fluorescence. We are interested in elucidating the mechanisms of regulation of the distribution of absorbed light energy between qP and NPQ. Understanding such mechanisms would provide clues to enhancing photosynthetic activity of microalgae. The objective of our study was to further improve strains which have high photosynthetic activity under any light conditions. We constructed *Chlamydomonas reinhardtii* mutant libraries by random insertional mutagenesis, and screened these libraries by measuring qP and NPQ. Out of 9,216 transformants generated, 62 mutants showed increased qP and decreased NPQ parameters. To analyze the

consequences of these changes in qP and NPQ, we monitored the growth rate of these mutants under various light conditions. Based on this analysis, we divided the mutants into three groups. The majority of mutants did not show any changes in their growth rate. However, the other two groups showed faster growth than the wild type. One group showed over 30% faster growth under low-light conditions. The other group exhibited more tolerance to high light and grew faster than the wild type under high light conditions. These mutants showed approximately 2 fold higher photosynthetic activity than the wild type. The mutants with the improved growth rate and photosynthetic activity are under investigation for molecular identification of genes linked to these phenotypes.

142. Towards Understanding Algal Truncated Hemoglobins – in vitro and in vivo Characterization of THB1. **Eric A. Johnson**, Selena L. Rice, Matthew R. Preimesberger, Juliette T. Lecomte. Biophysics, Johns Hopkins University, Baltimore, MD.

Truncated hemoglobins (TrHbs) belong to the hemoglobin superfamily of proteins and are found in a vast array of organisms – including bacteria, fungi and plants – yet the functional characterization of these proteins is still incomplete. Phylogenetic analysis of the *Chlamydomonas reinhardtii* genome has identified up to twelve genes containing truncated hemoglobin domains. The gene *THB1* encodes a single domain protein classified as a group I truncated hemoglobin (TrHb1). In prior *C. reinhardtii* studies, the gene product of *THB1* was detected within the flagella of the cell. In this work we have constructed a recombinant form of THB1 (rTHB1) and subjected it to structural analysis. Both optical and NMR spectroscopies demonstrate that rTHB1 binds a single ferric heme tightly, with coordination of the proximal histidine and a second protein ligand, likely tyrosine. The bound heme can be reduced to the ferrous state by spinach ferredoxin. Iron coordination in both ferric and ferrous state is pH-dependent. rTHB1 is also highly adept at exogenous ligand binding, especially diatomic molecules such as O_2 in the ferrous state and cyanide in the ferric state. The combined use of reduction and ligand binding was utilized to demonstrate that rTHB1 is capable of rapid, high-turnover Nitric Oxide Dioxygenase (NOD) activity, converting NO to NO_3^-. Companion work utilizing polyclonal antibodies raised against a portion of the THB1 peptide has confirmed the presence of THB1 within *C. reinhardtii*, but found that expression of the gene is linked to nitrogen content of the growth medium. When grown without nitrate, levels of THB1 drop significantly, and THB1 is not detected in strains of *C. reinhardtii* deficient in nitrate reduction. This link between nitrate metabolism and THB1 is significant given the NOD activity observed in vitro. Current investigations seek to link these results to cellular mechanisms and identify roles for TrHb proteins in unicellular algae.

143. Identification of proteins involved in contractile vacuole function in *Chlamydomonas reinhardtii* using a transcriptome approach. **Karin Komsic-Buchmann**, Burkhard Becker. Botanical Institute, University of Cologne, Koeln, Germany.

Under hypotonic conditions *Chlamydomonas reinhardtii* display two contractile vacuoles (CVs) working in osmoregulation. During diastole CVs increase continuously in size. In systole CVs expel the collected liquid and fragment into numerous vesicles. The small vesicles again fuse with each other and swell to a large CV.

Analysis of *Chlamydomonas* CC3395 exposed to different osmotic media revealed that cells alter their CV period rather than their CV size. However, CV size is related to cell size in *Chlamydomonas*. The regulation of the CV is unclear. Therefore, we tried to identify CV proteins using first qRT-PCR and second RNAseq. And indeed, qRT-PCR of cells of different osmotic media and a mutant strain lacking functional CVs (Osmo75, see Komsic-Buchmann et al. 2012) pointed to a regulation at the transcript level. Therefore, total RNA was isolated from *Chlamydomonas* CC3395 exposed to normal and strong hypotonic medium, Osmo75 and a rescued cell line of Osmo75 (Osmo75-SEC6GFP). RNA samples were sequenced (Ilumina HiSeq2000, 50 bp single reads, 18.9-23 mio reads per sample). The Ilumina data confimed the qRT-PCR results. First results on the identification of new CV proteins will be presented.

144. Nutrient availability and growth conditions influence the accumulation of neutral lipids in eukaryotic algae. **Michael McConnell**[1], Byeong-ryool Jeong[1], Amanda Kobayashi[1], Sean Awakuni[1], Heriberto Cerutti[2], Karin van Dijk[1]. 1) Department of Biology, Creighton University, Omaha, NE, USA; 2) School of Biological Sciences, University of Nebraska, Lincoln, NE USA.

Chlamydomonas reinhardtii accumulates high levels of neutral lipids under various abiotic stresses. Nitrogen-deprivation prompts the most notable and significant accumulation of lipids in the cell. As lipids accumulate while *C. reinhardtii* is starved for nitrogen the cells cease to divide. Our goal is to offer insight into how various growth conditions influence neutral lipid accumulation in *C. reinhardtii* and a number of high lipid-producing algal strains isolated from waters in the Midwest. We analyzed lipid accumulation under nitrogen deprivation conditions combined with other stressors including availability of reduced carbon sources, light quality and intensity, interruption of photosynthetic electron transfer, cell density and growth stage manipulation. We found that *Chlamydomonas* can accumulate lipid under nitrogen starvation in heterotrophic and mixotrophic modes; this has been confirmed by using chemicals and mutants to manipulate photosynthetic electron transfer efficiency in growing cells. Heterotrophic lipid accumulation is dependent on the initial density of the inoculum. We have isolated two algae, *Chlorella* sp. CU1-2a1 and *Micractinium* sp. CU 7-32 that grow better and accumulate more neutral lipids compared *Chlamydomonas*. We are currently further characterizing these strains.

145. The *Chlamydomonas* S-nitrosylome. **S. Morisse**[1], M. Zaffagnini[1,2], X.H. Gao[1], C.H. Marchand[1], S.D. Lemaire[1]. 1) Centre National de la Recherche Scientifique, Sorbonne Universités UPMC Univ Paris 06, UMR8226, Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, Institut de Biologie Physico-Chimique, Paris, France; 2) Laboratory of Plant Redox Biology, Department of Pharmacy and Biotechnology (FaBiT), University of Bologna, Bologna, Italy.

Protein S-nitrosylation, a post-translational modification consisting of the covalent binding of nitric oxide (NO) to a cysteine thiol moiety, plays a major role in cell signaling and is recognized to be involved in numerous physiological processes and diseases in mammals [1]. The importance of nitrosylation in plants and algae has emerged more recently [2-4]. The aim of this study was to expand our

knowledge on protein nitrosylation by performing a large scale proteomic analysis of proteins undergoing nitrosylation *in vivo* in *Chlamydomonas reinhardtii* cells under nitrosative stress. Using two complementary proteomic approaches, 492 nitrosylated proteins were identified. They participate in a wide range of biological processes and pathways including photosynthesis, carbohydrate metabolism, amino acid metabolism, translation, protein folding or degradation, cell motility and stress. Several proteins were confirmed *in vitro* by western blot, site-directed mutagenesis and activity measurements. Moreover, 392 sites of nitrosylation were also identified. These results strongly suggest that S-nitrosylation could constitute a major mechanism of regulation in Chlamydomonas under nitrosative stress conditions. This study constitutes the largest proteomic analysis of protein nitrosylation reported to date. The identification of 381 previously unrecognized targets of nitrosylation further extends our knowledge on the importance of this post-translational modification in photosynthetic organisms.

[1] Foster et al. (2009) Trend Mol Med 15 :391-404
[2] Astier et al. (2012) Free Radic Biol Med 53:1101-10
[3] Hemschemeier et al. (2013) Proc Natl Acad Sci USA 110:10854-59
[4] Wei et al. (2014) Plant Cell 26:353-72

146. Identification of potential components of the carbon concentrating mechanism in *Chlamydomonas reinhardtii* through insertional mutagenesis. **Bratati Mukherjee**, Marylou Machingura, Joanna Bajsa-Hirschel, Ananya Mukherjee, Susan Laborde, Nadine Jungnick, James Moroney, Dequantarius Speed, Julie Cronan. Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70802.

The availability of inorganic carbon is one environmental factor that affects photosynthetic output. The green alga *Chlamydomonas reinhardtii* is often exposed to fluctuating levels of carbon dioxide in its environment. The induction of a carbon concentrating mechanism (CCM) allows the alga to successfully acclimate to low CO_2 conditions without compromising photosynthetic output. The current model of the CCM includes several genes encoding proteins with potential roles in the regulation, uptake and inter-conversion of CO_2 and HCO_3^-. However, there are still several gaps in our understanding of the CCM. Hence, a better understanding of the roles of known CCM components and the discovery of new ones is required.

In order to achieve this, a large-scale insertional mutagenesis screen was undertaken. A mutant library (~30,000 colonies) was generated in the D66 background, through the random insertion of a DNA cassette with the antibiotic selective marker, *AphVIII*. This transformant library was then screened for altered growth under low CO_2. 200 colonies showing poor growth under low CO_2 were selected. Disrupted genes in these mutants were identified using an adaptor PCR technique. Insertions in over 30 genes that may have potential involvement in the *C. reinhardtii* CCM have been identified. Further characterization of these mutants will help in understanding the roles of these genes in the CCM *C. reinhardtii*.

Supported by a subcontract from the University of Illinois and a NSF award to JVM.

147. Comparative genomics in *Chlamydomonas* identifies an ancient nuclear envelope protein family essential for sexual reproduction in protists, fungi, plants, and vertebrates. **Jue Ning[1]**, Thomas Otto[2], Claudia Pfander[2], Frank Schwach[2], Mathieu Brochet[2], Ellen Bushell[2], David Goulding[2], Mandy Sanders[2], Paul Lefebvre[3], Jimin Pei[4,5], Nick Grishin[4,5], Gary Vanderlaan[1], Oliver Billker[2], William Snell[1]. 1) Department of Cell Biology, University of Texas Southwestern Medical School, Dallas; 2) Wellcome Trust Sanger Institute, Hinxton Cambridge CB10 1SA, United Kingdom; 3) Department of Plant Biology, University of Minnesota, St. Paul, Minnesota 55108, USA; 4) Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA; 5) Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA.

Our previous discovery that the broadly conserved protein HAP2 (GCS1) functioned in gamete membrane fusion in *Chlamydomonas* and the malaria pathogen *Plasmodium* led us to use a comparative transcriptomics strategy to search for additional conserved sexual reproduction genes. All previously identified *Chlamydomonas* fertilization-essential genes fell into related clusters based on their expression patterns. Out of several conserved genes in a minus gamete cluster, we focused on Cre06.g280600, an orthologue of the fertilization-related *Arabidopsis* GEX1. Gene disruption, cell biological, and immunolocalization studies show that CrGEX1 functions in nuclear fusion in *Chlamydomonas*. Moreover, CrGEX1 and its *Plasmodium* ortholog are essential for production of viable meiotic progeny in both organisms. Remarkably, we discovered that the genes are members of a large, previously unrecognized family whose first-characterized member, KAR5, is essential for nuclear fusion during yeast sexual reproduction. Our studies indicate that *Chlamydomonas* can be used to discover core components of sexual reproduction machinery that conserved across phyla.

Supported by NIH GM25661and GM56778, the Wellcome Trust 098051, the Medical Research Council G0501670.

148. A broader redox network in *Chlamydomonas reinhardtii* revealed by revisiting the proteome of thioredoxin targets. **M. E. Perez-Perez[1]**, A. Mauries[1], N. Tourasse[2], S. D. Lemaire[1], C. H. Marchand[1]. 1) Centre National de la Recherche Scientifique, Sorbonne Universités UPMC Univ Paris 06, UMR8226, Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, Institut de Biologie Physico-Chimique, 75005 Paris, France; 2) Centre National de la Recherche Scientifique, FRC550, Institut de Biologie Physico-Chimique, 75005 Paris, France.

Cysteine redox post-translational modifications play important roles in regulation and signaling pathways that allow living cells to sense environmental changes and trigger adaptative responses [1]. The global aim of our group is deciphering the redox network in

Chlamydomonas. During the last decade, proteins regulated by thioredoxins (Trx) [2], by glutathionylation [3, 4] or more recently by S-nitrosylation [5], have been identified using diverse proteomic strategies.

In the present study, we revisited our pioneering Trx targets identification work [2] by performing two complementary proteomic approaches [6]. On the one hand, Chlamydomonas Trx targets were purified by affinity chromatography using a column based on an active site mutant version of the cytosolic Trxh1 that is able to covalently retain Trx-interacting proteins. On the other hand, we analyzed the Chlamydomonas proteins enzymatically reduced by the Trx system composed of NADPH, Trx reductase and Trx. In the latter case, the newly exposed thiols were biotinylated and Trx targets were purified by avidin-based affinity chromatography.

Taking advantage of the recent improvements in mass spectrometry and also in Chlamydomonas genome annotation, we have identified more than one thousand putative targets. The identified proteins are involved in numerous cellular processes such as photosynthesis, translation, redox homeostasis or stress responses. Therefore, these results considerably broaden the importance of Trx-dependent redox regulation and signaling not only in Chlamydomonas but also in other photosynthetic eukaryotes.

[1] Go and Jones. (2013) J Biol Chem. 288:26512-20
[2] Lemaire et al. (2004) Proc Natl Acad Sci U S A. 101:7475-80
[3] Michelet et al. (2008) J Biol Chem. 283:21571-8.
[4] Zaffagnini et al. (2012) Mol Cell Proteomics. 11:M111.014142.
[5] Morisse et al. Antioxid Redox Signal. *In press*.
[6] Marchand et al. (2006) Proteomics. 6:6528-37.

149. The *Chlamydomonas UVS9* gene encodes an XPG homolog vital for DNA repair. Erin Deitsch[1], Erin Hibbard[2], **Jason Petersen[3,4,5]**. 1) The Center for Biological Control and Analysis by Applied Photonics (BCAAP), South Dakota State University, Brookings, SD; 2) Vassar College, Poughkeepsie, NY; 3) Avera Institute for Human Genetics, Avera Research Institute, Sioux Falls, SD; 4) Sioux Falls VA Health Care System, Sioux Falls, SD; 5) Sanford School of Medicine of the University of South Dakota, Sioux Falls, SD.

Ultraviolet (UV) light is potentially lethal or mutagenic to cells by inducing several forms of DNA damage including the cyclobutane pyrimidine dimer and 6-4 photoproduct. To cope with these, and other, forms of DNA damage, cells have developed mechanisms for repairing damaged DNA. A historic collection of UV-sensitive mutants of *Chlamydomonas* was generated as a means to better understand DNA repair. The *uvs9* (*uv*-sensitive 9) strain of *Chlamydomonas* was shown to be blocked in a form of DNA repair known as nucleotide excision repair (NER). It is estimated that over 40 proteins coordinate during NER to cleave the damaged DNA strand on each side of the damage, displace the damaged strand, and synthesize a replacement strand using the undamaged strand as template. To determine the molecular basis of the *uvs9* DNA repair defect, we used molecular mapping to localize the *uvs9* mutation to the left arm of chromosome 10. A candidate gene search in this region of chromosome 10 revealed a gene (Cre10.g429050) predicted to encode an XPG homolog. XPG is a structure-specific endonuclease that cleaves DNA strands 3' to sites of DNA damage during NER. Wild type DNA constructs containing Cre10.g429050 successfully complemented the *uvs9* UV-sensitivity and NER defect. The *UVS9* gene is composed of 17 exons and encodes a putative protein of 2,463 amino acids. Sequencing of the *uvs9* allele identified an amber mutation in the 9th exon, resulting in a truncated product of 1,802 amino acids. Amino acid sequence analysis of UVS9p identified a domain structure previously unrecognized in plant XPG homologs and provides a potential mechanism for protein/protein interaction for all XPG homologs.

150. Regulation of CCM genes in *Chlamydomonas reinhardtii* during conditions of light–dark cycles in synchronous cultures. Srikanth Tirumani[1,2], Mallikarjuna Kokkanti[2], Vishalsingh Chaudhari[1], Manish Shukla[1], **Basuthkar Rao[1]**. 1) Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, Maharashtra, India; 2) Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur 522510, Andhra Pradesh, India.

We have investigated transcript level changes of CO_2-concentrating mechanism (CCM) genes during light–dark (12 h:12 h) cycles in synchronized *Chlamydomonas reinhardtii* at air-level CO_2. CCM gene transcript levels vary at various times of light–dark cycles, even at same air-level CO_2. Transcripts of inorganic carbon transporter genes (HLA3, LCI1, CCP1, CCP2 and LCIA) and mitochondrial carbonic anhydrase genes (CAH4 and CAH5) are up regulated in light, following which their levels decline in dark. Contrastingly, transcripts of chloroplast carbonic anhydrases namely CAH6, CAH3 and LCIB are up regulated in dark. CAH3 and LCIB transcript levels reached maximum by the end of dark, followed by high expression into early light period. In contrast, CAH6 transcript level stayed high in dark, followed by high level even in light. Moreover, the up regulation of transcripts in dark was undone by high CO_2, suggesting that the dark induced CCM transcripts were regulated by CO_2 even in dark when CCM is absent. Thus while the CAH3 transcript level modulations appear not to positively correlate with that of CCM, the protein regulation matched with CCM status: in spite of high transcript levels in dark, CAH3 protein reached peak level only in light and localized entirely to pyrenoid, a site functionally relevant for CCM. Moreover, in dark, CAH3 protein level not only reduced but also the protein localized as a diffused pattern in chloroplast. We propose that transcription of most CCM genes, followed by protein level changes including their intracellular localization of a subset is subject to light–dark cycles.

151. Activation of the redox-regulated algal HSP33 that lost its Zn-binding activity during evolution. **Na'ama Segal**, Michal Shapira. Life Science, Ben-Gurion University of the Negev, Beer-Sheva , Israel.

Hsp33 is a bacterial redox-sensitive chaperone that protects unfolded proteins from aggregation, during oxidative stress combined with elevated temperatures. Since photosynthetic organisms are routinely exposed to light-induced oxidative stress in response to unbalanced illumination, we tested whether HSP33 from *Chlamydomonas reinhardtii* has a protective role under these conditions. The

algal HSP33 bears homology to its cyanobacterial ortholog, and is localized in the chloroplast. As expected, its expression increased in response to oxidative stress, even at ambient temperatures. Similar to the bacterial protein, the recombinant algal Hsp33 possesses independent chaperone activity, as indicated from its ability to prevent the aggregation of denatured citrate synthase *in vitro.* However, the mode of activation in the different organisms appears to vary. While the bacterial Hsp33 is activated following the release of a zinc ion from the zinc-binding center, the algal HSP33 does not bind zinc at all. The zinc binding site in the bacterial protein contains four conserved cysteine residues, C-X-C-X(27-32X)-C-X-X-C. However, the algal HSP33 has lost the first cysteine in the center, which is substituted by a Glycine residue **G**-X-C-X(33X)-C-X-X-C. Thus, although a resemblance is observed between the predicted structure of the algal protein as compared to the solved structure of the bacterial Hsp33, the two proteins vary in their modes of activation. While the bacterial protein is activated only upon its oxidation, the algal HSP33 is active both in its reduced and oxidized forms.
Hsp33 is widely conserved among prokaryotes and algae, but is not found in the genomes of lands plants. We discuss the potential evolutionary considerations that led to the disappearance of HSP33 during evolution, in the context of its function in lower photosynthetic organisms.

152. Abiotic Stress Induced PCD through Mitochondrian - Caspase Protease Pathway in *Chlamydomonas reinhardtii*. **Sirisha L Vavilala**, Mahuya Sinha, Jacinta D'Souza. Department of Biology, UM-DAE Centre for Excellence in Basic Sciences, Kalina Campus, Santacruz (E), Mumbai-400098, Maharashtra, India.

Environmental stress agents are known to induce production of reactive oxygen species (ROS) in cellular compartments thereby resulting in apoptosis in several metazoans. The obvious parallels in this machinery between higher plants and animals suggest an evolutionary origin. Various approaches have revealed candidate regulators in plants that show both similar and new properties compared with their animal counterparts. Of recent, a number of unicellular protists and fungi have been shown to die in a PCD-like manner. However, two related issues that remain elusive in these unicellular organisms are the key molecular players involved in the process and the precise physiological role of this cellular mechanism. A detailed study of PCD in unicellular photosynthetic organisms will provide important insight into intracellular and molecular cell death pathways which in multicellular organisms are difficult to investigate. In this present study, we investigate whether *C. reinhardtii* is capable of executing a PCD upon exposure to abiotic stress agents (50 µM menadione, 10 mM H_2O_2 and 200 mM KCl).

These stress agents caused a decreased cell survival (48-56 % for oxidative stress and 42% for osmotic stress) and biochemical analysis showed that oxidative stress caused a 2-fold increase in ROS production within 15 minutes; and, osmotic stress showed 1.5 fold increase within 30 minutes. Some hallmarks of apoptosis like DNA fragmentation detected by TUNEL and DNA laddering assay was observed within 6 h for oxidative stress while it was observed at 24 h for osmotic stress. However by addition of Zn^{2+} prevented DNA laddering. Both the stress conditions induced an alteration of the mitochondrial membrane potential. The Caspase-3-like activity however increased 6-fold under oxidative stress and 3-fold when exposed to osmotic stress. Similarly, the PARP-1 like protein cleavage also followed the same trend. These results clearly elucidate that these abiotic stress induce PCD through a mitochondrion-caspase protease pathway. When the kinetics of PCD was compared oxidative stress drives enhanced PCD over osmotic stress, which might have an evolutionary significance that is worth pursuing.

153. Trade-off between biomass production and *Non-Photochemical-Quenching* (NPQ) in *Chlamydomonas reinhardtii*. **Silvia Berteotti**, Matteo Ballottari, Roberto Bassi. Biotechnology, University of Verona, Verona, Verona, Italy.

Record biomass yield in photobioreactors (PBR) is about 20% with respect to optimal yield in lab scale in limiting light conditions and well below that measured in natural environments. Thus, domestication of algae is essential for obtaining an efficient conversion of solar light energy into biomass (Photon Conversion Efficiency, PCE) in PBRs. A major factor limiting the solar-to-biomass conversion efficiency is, likely, the thermal dissipation of excitation energy (called “Non-Photochemical Quenching” - NPQ): a photoprotective mechanism by which a significant fraction of photons is dissipated as heat. In green algae, the LHCSR protein has been reported to be responsible for the activation of the NPQ (Peers et al. 2009; Bonente et al. 2012). In this work we studied the correlation between the NPQ, light harvesting and biomass accumulation during autotrophic growth using the model organism, *Chlamydomonas reinhardtii* WT and the *npq4* mutant (Peers et al. 2009). *Npq4* has a reduced NPQ due to the absence of the most abundant protein, LHCSR3, isoform. The productivity of WT and *npq4* strains was estimated by growing them simultaneously in different conditions in a Multi-Cultivator system (Photon Systems Instruments) with batch airlift PBR, each of them representing an independent tubular PBR in small scale. Wide-range of different light and dark-light pulse conditions were tested, from 200 µmol photons m^{-2} s^{-1} to 800 µmol photons m^{-2} s^{-1}. Consistent with the direct correlation between LhcSR and NPQ induction (Bonente et al. 2011, Peers et al. 2009), we found that PCE decreased with increasing NPQ. The two conditions in which the highest PCE could be measured were: (A) continuous light of 200 µmol photons m^{-2} s^{-1}, and (B) light/dark cycles of 0.5 Hz with pulses of 400 µmol photons m^{-2} s^{-1}. In all tested light growth condition, *npq4* showed lower NPQ, higher biomass production and a better PCE than WT. The loss of all *LhcsR* gene (*npq4 1.1* mutant) due the same PCE of WT suggesting that a low level of NPQ activity is necessary for algal fitness in a dynamic light conditions. High light use efficiency in biomass production results from trade off between the ability for excess energy dissipation and the energy utilization for photosynthesis. On this basis we suggest that modulation of NPQ might be a promising strategy for increasing the algae productivity in PBRs.

154. Over-expression of the Calvin cycle enzyme FBPase in the *Chlamydomonas reinhardtii* chloroplast: effect on growth and biomass. **W. Dejtisakdi**, S. M. Miller. Department of Biological Sciences, UMBC, Baltimore, MD 21250 USA.

Chlamydomonas reinhardtii has great potential as a system for generating biofuels, pharmaceuticals, and other commercially valuable products. Our goal is to improve this potential by increasing photosynthetic output and biomass. Overexpression of the Calvin

cycle enzyme, fructose-1,6-bisphosphatase (FBPase), improves growth and biomass accumulation in higher plants, so we set out to determine if over-expression of FBPase has the same effect in *C. reinhardtii*. To this end, we generated a chloroplast expression vector for FBPase expression that includes the *C. reinhardtii FBP1* coding region synthesized with *C. reinhardtii* chloroplast codon-bias and flanked with *psbD* and *psbA* 5' and 3' regulatory sequences, respectively, and obtained transformants that integrated the vector properly into the chloroplast genome. These transformants accumulate increased levels of FBPase protein and 1.5-2 fold increased FBPase enzyme activity compared to the recipient strain. Analysis of growth and biomass accumulation of these transformants under several different culturing conditions indicates that over-expressing FBPase levels by up to two-fold in the *Chlamydomonas* chloroplast does not appreciably increase rate of growth or biomass.

155. Bio-hydrogen production in the green algae Chlamydomonas: effect of H2 partial pressure and acetate. **David Gonzalez-Ballester**, Jose Luis Jurado-Oller, Aurora Galvan, Emilio Fernandez. Bioquimica y Biologia Molecular, Universidad de Cordoba, cordoba, Cordoba, Spain.

We have investigated different approaches to optimize H_2 production in the unicellular green alga *Chlamydomonas reinhardtii*. The two most commons conditions used to achieve H_2 production in Chlamydomonas are sulfur deficiency and dark. However, we first studied different environmental conditions other that sulfur deficiency and dark (non-stress conditions) that could lead to H_2 production in this alga. One photosynthetic deficient strain (strain 704) showed an improved H_2 production under low light (LL). Light intensity was optimized and demonstrated that H_2 production under LL is linked to the PSII activity (H_2 production under LL in the presence of the PSII inhbitor DCMU is severely inhibited).

The effect of the H_2 accumulation in the headspace of the cultures was also investigated. We found that H_2 partial pressure is an important factor that can inhibit the H_2 bioproduction. By aerating the headspace of the cultures we have improved H_2 photo-production by 10 times.

Finally, we studied the effect of acetate in the culture media. We have demonstrated that cultures that were supplemented with acetate can improve H_2 production by 2 times relative to our reference cultures (LL, un-opened cultures). Moreover, continuous supplementation with acetate can result in a sustained H_2 photo-production.

Altogether, our results show an optimization of the bio-hydrogen production process in Chlamydomonas. By using the photosynthetic defective strain 704 under low light conditions, aeration of the headspaces of the cultures and supplementation with acetate we improved H_2photoproduction by more than 60 times. Moreover, opposite to most other described methods, our system for H_2-bioproduction can be sustained, and do not required pre-adaptation of the cultures or implementation of stress conditions that abolish biomass generation. In conclusion, our system for H_2 generation is more efficient, simpler and easier that previously known methodologies that can also lead to biomass production what can be used for other downstream biotechnological applications.

156. Comparison between nitrogen deprivation and high light as triggers for neutral lipid accumulation in *Chlamydomonas reinhardtii*. **Hugh D Goold[1,2]**, Stephane Cuine[2], Bertrand Legeret[2], Patrick Carrier[2], Pascaline Auroy[2], Brian Jones[1], Frederic Beisson[2], Gilles Peltier[2], Yonghua Li-Beisson[2]. 1) Faculty of Agriculture and the Environment, University of Sydney, Sydney, NSW, Australia; 2) CEA, CNRS, Aix-Marseille University, CEA Cadarache, Saint-Paul-lez-Durance, F-13108 France.

Neutral lipid accumulation by microalgae has recently regained considerable interest because these organisms are considered as a promising feedstock for the production of renewable biodiesel. Nitrogen deprivation is well described as a trigger for neutral lipid accumulation in various species of microalgae including Chlamydomonas. However, nitrogen deprivation provokes a stop in protein synthesis and cell division, therefore limiting microalgal biomass productivity. High light has also been reported as a trigger to induce oil accumulation. To gain insights into the differences in molecular mechanisms behind oil accumulation processes under nitrogen starvation to that of high light, in this communication, we report the lipidomic changes in cells subjected to the two stress conditions using the state-of-the-art UPLC-MS/MS-qTOF. Nitrogen deprivation was induced by replacing the dilution medium by a nitrogen free medium and high light by increasing the fluence rate from 150 to 800 mmol photons m^{-2} s^{-1}. *Chlamydomonas reinhardtii* were cultivated under phototrophic conditions in 1 L photobioreactors operated as turbidostats, thus allowing accurate control of experimental conditions and a determination of biomass productivity. Our results showed that despite intracellular TAGs were found to accumulate to lower levels in response to high light in comparison to nitrogen deprivation; the TAGs productivity was higher due to a persistent biomass production. Furthermore we will also report our characterization of the proteome and lipidome of the oil bodies isolated from both stress conditions.

157. Enhancement of starch accumulation in *Chlamydomonas reinhardtii* mutated in a maltose transporter. **S. Jang[1]**, Y. Yamaoka[1], H. Kim[1], D. Ko[2], J. Lee[2], T. Kurita[3], K. Kim[1], Y. Kim[1], W. Song[1], I. Nishida[3], Y. Lee[1,2]. 1) Department of Life Sciences, POSTECH, Pohang, South Korea; 2) Department of Integrative Bioscience & Biotechnology, POSTECH, Korea; 3) Division of Life Science, Graduate School of Science and Engineering, Saitama University, Japan.

Demands for alternative energy that can meet the need of growing world population have been increasing. Biofuel has been considered as a sustainable energy that can directly replace gasoline and diesel, since it is renewable and has chemical properties similar to the current fossil fuels. Microalgae are one of the promising biofuel producers.

We created mutant pools of CC-503 and CC-125 lines of the model green microalga *Chlamydomonas reinhardtii* by randomly inserting an antibiotic resistance cassette (*AphVII*) to their genomes. Approximately 20,000 mutants grown under normal and nitrogen-deprived (-N) conditions were individually screened for altered neutral lipid content using nile red fluorescence level as an indicator of intracellular lipid level.

A mutant showed low nile red fluorescence intensity per optical density at 750 nm (FI/OD_{750}). The mutant harbored the antibiotic resistance gene in *CrMEX1,* a maltose exporter-like protein 1 (Cre12.g486600.t1.2), and its *CrMEX1* expression level was decreased. The

mutant accumulated 3 to 5 fold higher level of starch than the wild type CC-503 under both nitrogen replete and deplete conditions. The starch accumulation in the mutant is similar to that in Arabidopsis *mex1* knockout mutant, which cannot export maltose from the chloroplast. Although FI/OD_{750} of the mutant was lower than that of the wild type, its lipid level was the same as that of the wild type when grown in TAP medium supplemented with 17.4 mM acetate. This might be due to higher OD_{750} value of the mutant, which is caused by the many shining starch granules in the mutant. These results suggest that *Chlamydomonas* use maltose transporter to export photoassimilates from chloroplasts, similarly as in terrestrial plants.

158. The fungicide fenpropimorph induces conversion of plastidial membrane lipids to triacylglycerols in *Chlamydomonas*. **H. Kim**[1], S. Jang[1], S. Kim[1], Y. Yamaoka[1], D. Hong[1], I. Nishida[3], Y. Li-Beisson[4], Y. Lee[2]. 1) Life science, POSTECH, Pohang, South Korea; 2) of Integrative Biology and Biotechnology, POSTECH, Pohang, South Korea; 3) Life Science, Graduate School of Science and Engineering, Saitama University, Saitama, Saitama, Japan; 4) Plant Biology and Environmental Microbiology, CEA-CNRS-Aix Marseille University, Saint-Paul-Lez-Durance, France.

Economic production of biofuel is a topic of intense interests in this era of global warming. Microalgae are promising platforms for biofuel production, because they accumulate high levels of lipids, and are not conventional food or feed for human and live stocks. However, the cost to produce algal oil is still very high, thus knowledge on how to cut down the cost is in great demand. Here, we report that a fungicide fenpropimorph rapidly induces high levels of neutral lipid accumulation in *Chlamydomonas reinhardtii*. When treated with fenpropimorph (10 μg mL^{-1}) for 1 h, *Chlamydomonas* cells accumulated triacylglycerols (TAGs) by 4 to 7 folds of the control untreated cells. The treatment with fenpropimorph did not induce changes in sterol content, which is known to be the mechanism of fungicidal effect of the drug in other organisms, suggesting a distinct mechanism of the drug-induced TAG accumulation. Microscopic observations revealed formation of lipid droplets in cells treated with the chemical, and collapse of the chloroplast. Analyses of the lipids using thin layer chromatography (TLC) and gas chromatography (GC) revealed that the accumulated TAGs were derived mainly from thylakoid membrane lipids. Thus this chemical induces conversion of plastidial membrane lipids to TAGs, a form of lipids more suitable for biodiesel than membrane lipids, in a time-saving, and thus less costly manner.

159. Induction of Triacylglycerol Turnover Through the *β*-Oxidation Pathway in *Chlamydomonas reinhardtii* Following Nitrogen Repletion. **joseph msanne**. University of Nebraska, Lincoln, Lincoln, NE.

The genetic and biochemical basis for triacylglycerol (TAG) turnover and the induction of the b-oxidation pathway has been explored in Chlamydomonas reinhardtii upon shift from nitrogen-deplete to nitrogen-replete growth conditions. b-oxidation pathway, taking place in peroxisomes and mitochondria, is the primary pathway implicated in fatty acid (FA) degradation. In Chlamydomonas, this pathway involves the enzymes acyl-CoA oxidase (AOX), dienoyl-CoA reductase/enoyl-CoA dehydrogenase, and ketoacyl-CoA thiolase (KAT). These remove two carbons from the acyl chain during each cycle and release an acetyl-CoA that is recycled in the chloroplast or mitochondria. Our assays show remarkable changes in transcript level following the switch to N-replete conditions, this is marked by an early induction in expression of the b-oxidation and starch degradation genes. We also observe a rapid decline in starch content, followed by reduction in TAG content upon N repletion. Understanding the changes in metabolic pathways involved in the biosynthesis and catabolism of FAs, TAG, and starch may provide a foundation for the molecular genetics required to direct metabolic engineering efforts to reduce TAG turnover and enhance the quantity and quality of microalgae-based oil.

160. Vectors for expression of alkane pathway enzymes in the *Chlamydomonas reinhardtii* chloroplast. **R. Park**, W. Dejtisakdi, S. Miller. University of MD, Baltimore County, Baltimore, MD 21250.

Alkanes are an important component of gasoline that are synthesized in some cyanobacteria and the chloroplast of some microalgae. In cyanobacteria, alkanes are synthesized from fatty acids in a two-step process catalyzed by the enzymes aldehyde decarbonylase (ADC) and acyl ACP reductase (AAR). We are attempting to express the *adc* and *aar* genes from the cyanobacterium *Nostoc punctiforme* in the chloroplast of *Chlamydomonas reinhardtii*. The codons for adc and aar were optimized for expression in the *C. reinhardtii* chloroplast, as were the 5' and 3' regulatory sequences. In order to integrate our gene construct into the *C. reinhardtii* chloroplast chromosome via homologous recombination, the insert DNA is flanked by sequences that target a gene free region in the chloroplast chromosome. We have transformants that have integrated plasmids with *adc* and with *adc*+*aar*. We are characterizing them for expression of epitope-tagged ADC and AAR. Ultimately we hope to apply these *Chlamydomonas* chloroplast transformation techniques to transform the chloroplast of more productive and resilient microalgae, such as *Chlorella*.

161. Fine-tuning transgene expression for ´designer´ biofuels production: a bottom-up approach. **Juan Rico**[1], Mark Scaife[1], Katherine Helliwell[1], Ginnie Nguyen[1], Saul Purton[2], Alison Smith[1]. 1) Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge, CB2 3EA, UK; 2) Institute of Structural and Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK.

There has been an enormous increase in research into microalgae for biofuel production over the past few years. However, many limitations exist before this can be a commercial reality. Biorefining approaches and production of high value products are likely to be key in generating income streams in the initial phase, but to realise the full potential of microalgae for biotechnology we need to increase the range of molecular tools available. In particular, establishing methods for regulated transgene expression is going to be essential to consolidate microalgae as a real alternative to conventional industrial biotechnology hosts. The advent of next generation sequencing combined with systems biology approaches, and the increasing number of transformable microalgal species, means that the field is poised for a period of rapid development.

Our research aims to improve methods for metabolic engineering of *Chlamydomonas reinhardtii* by taking two synergistic approaches. The first develops efficient and flexible workflows based on synthetic biology principles, establishing a library of standard parts that can be assembled rapidly in multiple permutations. The second takes advantage of our knowledge of algal metabolism to identify novel genetic elements that can be used to regulate transgene expression: promoters, 5' and 3'UTRs, riboswitches, 2A peptides, along with reporter genes and selectable markers. Using Gibson assembly, we can routinely assemble plasmids with multiple versions of 8 separate parts, to assess which provide optimal transgene expression. By this means, we screened 6 different short 3' UTRs, and found one in particular that gave more stable expression than many others, including some of the long 3´UTRs commonly used as for example *PSAD* gene terminator.

162. Determining *Chlamydomonas* TAG Yield Using a Commercial Serum Triglyceride Determination Kit. **Taylor Weiss**[1], Carrie Goodson[1], Jan Jaworski[2], Jia Li[2], Tuya Wulan[1], Ursula Goodenough[1]. 1) Washington University, St. Louis, MO; 2) Danforth Plant Science Center, St. Louis, MO.

Assays to assess microalgal triacylglyceride (TAG) content are time-consuming and require equipment and technical expertise that is often unavailable to groups interested in pursuing the development of biofuel technology. We have optimized a rapid and quantitative colorimetric TAG assay with *C. reinhardtii* using an inexpensive commercial kit developed to monitor serum TAG levels in human blood samples.

TAG quantitation commonly entails extracting cells with organic solvents, running the extract on thin-layer chromatography plates, scraping off the TAG band, converting to fatty-acid methyl esters (FAMEs), and quantitating FAMEs using gas chromatography mass-spec (GC-MS) or flame ionization detection (GC-FID), with peaks then integrated relative to an internal standard. An alternative quantitates the signal from BODIPY 493/503, which fluoresces in neutral-lipid environments, using an imaging flow cytometer. Research labs without access to such resources usually report their results using versions of Bligh and Dyer assays that measure both polar and neutral lipids and hence give no specific quantitative information about TAG content.

Using a TAG Determination Kit (Thermo Fisher Scientific) and a standard bench-top spectrophotometer, TAGs can be specifically quantitated colorimetrically through the detection of glycerol release by TAG lipase digestion, at a cost of ~$0.40 per sample (reagent and disposable cuvette).

Modifications to the kit protocol include: 1) optimizing reporter dye detection wavelength; 2) minimizing sample volume; 3) adding controls that take into account the absorbance of cellular pigments and endogenous glycerol; and 4) revising "average TAG" mass to reflect native organism profiles.

We have used this assay to compare TAG yields of starch-containing vs. starch-null strains and strains subjected to acetate boost. We document that the TAG yields of the *sta6* (starch-null) strain N-starved in high light and air + 2.5% CO_2 are only 25% those in acetate-boosted cells in low light and air. We also assess the merits/downsides of expressing TAG yields on the basis of dry weight vs. per-cell vs. per-volume-of-culture, and conclude that per-volume-of-culture is generally the most informative.

163. Suppression of the mutations in the gene *CHLH* encoding the large subunit of magnesium chelatase in *Chlamydomonas reinhardtii*. **Elena M Chekunova**[1], Elena B. Yaronskaya[2], Natalia V. Yartseva[1]. 1) St.-Petersburg State University, Saint-Petersburg, Russian Federation; 2) Institute of Biophysics and cell engineering, National Academy of Sciences of Belarus, Republic of Belarus.

Magnesium chelatase (MCh) is the first enzyme of chlorophyll branch in tetrapyrrole biosynthesis. It comprises three subunits: H, I and D, encoding the nuclear genes *CHLH*, *CHLI* and *CHLD*, respectively. Along with the fulfilling enzymatic functions, e.g., insertion of Mg2+ into the protoporphyrin IX (PP) molecule, the large subunit CHLH of MCh participates in the signal transduction from the chloroplast to the nucleus, and it is also involved in the pathways of hormonal and redox controls. The main goal of this study is to identify novel genes encoding unknown regulatory component of chlorophyll biosynthesis. The cells of the Chlamydomonas mutants chl1 and brs-1 defective in the gene *CHLH* are chlorophyll deficient, accumulate PP and form orange colonies growing in the dark. Several revertants exhibiting green phenotype in the dark and light conditions has been obtained in the result of the UV-irradiation of mutant cells. The genetic analysis of the revertants revealed that the mutations in two nuclear genes *SCH1* and *SCH2* lead to suppression of the mutant phenotype, and mutant alleles of the gene *SCH2* appeared to be closely linked to the gene *CHLH*. The activity of porphyrin biosynthesis system in the cells of the revertants has been analyzed by the determining of chlorophyll and heme contents as well as activities of MCh and 5-aminolevulinic acid (ALA)-synthesizing enzymes using HPLC techniques. Mutation in the suppressor genes *SCH1* and *SCH2* restored chlorophyll synthesis and increased activities of MCh and ALA-synthesis in the revertants. On the genetic background: chl1 and brs-1 the *Sch2*-mutations also lead to decreasing the growth rate of the revertants (cell doubling time – 43 h.), which formed small green colonies on the agar plates. These genetic and biochemical data suggest that *SCH1* and *SCH2* genes are involved in the regulation of MCh in C reinhardtii, and support the hypothesis about common regulation of MCh and ALA-synthesizing enzymes in photosynthesizing cells.

164. Functional differences between Chlamydomonas root-type FDX2 and leaf-type FDX1 may be attributed to a few amino acid residues based: link to H2 and NADPH photoproduction. **alexandra dubini**. national renewable energy laboratory, golden, CO.

Ferredoxins (FDX) are typically small, iron-sulfur cluster-containing proteins that are involved in a multitude of metabolic redox reactions. The green alga *Chlamydomonas reinhardtii* contains at least six chloroplastic [2Fe2S]-cluster FDXs, with FDX1 (or PetF) as the predominant isoform. FDX2 is highly similar to FDX1 and has recently been shown to interact with several known FDX1 interaction partners, such as the hydrogenases (HYDA1 and HYDA2), ferredoxin:NAD(P) reductase I (FNR1) and pyruvate:ferredoxin oxidoreductase (PFOR), albeit at lower catalytic rates. In order to determine and compare the characteristics of these two FDX isoforms and to identify

and study the nature of their interaction with other enzymes, we undertook biochemical and biophysical characterization of FDX1 and FDX2 proteins. These were characterized by UV/Vis, electron paramagnetic resonance (EPR), and CD spectroscopy. Both UV/Vis and EPR spectroscopy revealed typical paramagnetic signals for both FDXs and near identical spectra. We also report the first *Chlamydomonas reinhardtii* FDX structure. FDX2 crystal structure was solved at a resolution of 1.18 Å and was used to refine the current model for the FDX1 structure due to their high sequence similarity. The structural data were subsequently used to model the interactions of the FDXs with two well-known interaction partners, namely, HYDA1 and FNR1. Finally based on differences between the FDX1 and FDX2 structures, we also generated and purified point-mutated versions of the FDX2 protein (M62F, Δ95Y and the respective double mutant) and tested them in *in vitro* hydrogen and NADPH photo-production assays. The data show that probably only few amino acid residues are responsible for FDX1 and FDX2 functional differences, amongst them F62, Y95. The effect of these point mutations on H_2 and NADPH synthesis revealed possible roles for these residues in the reactions above.

165. Characterization of low-TAG accumulating mutant in *Chlamydomonas reinhardtii*. **M. Kajikawa**[1], Y. Sawaragi[1], H. Shinkawa[1], T. Yamano[1], M. Hirono[2], N. Sato[3], H. Fukuzawa[1]. 1) Grad. Sch. of Biostudies, Kyoto Univ., Japan; 2) Grad. Sch. of Sci, Univ. of Tokyo, Japan; 3) Grad. Sch. of Arts and Sci, Univ. of Tokyo, Japan.

Several microalgae accumulate large amount of triacylglycerol (TAG) under nitrogen (N) starvation condition. Therefore, microalgal oils have attracted interest as resources for renewable fuels. However, the regulatory mechanisms of the TAG production and accumulation are still largely unclear. Low-TAG accumulating mutant was screened from selection marker-tagged 20,000 mutant pools of the model green microalga *Chlamydomonas reinhardtii* by using fluorescence activated cell sorting (FACS) system. Here, we report to isolate a mutant with reduced TAG content and increased starch amount compared with the parental line following N deprivation. RNAseq analysis revealed that expression levels of 1,953 genes in the mutant under N deprivation for 8 h were decreased less than 50% of those in WT. In the group, genes involved in nitrogen assimilation (e.g. three ammonium-transporter genes, *AMT1A, AMT4* and *AMT6*), TAG biosynthesis (e.g. two acyltransferase genes, *DGTT1* and *PDAT1*) and starch metabolism (e.g. G6P isomerase and starch phosphorylase genes) were included. The large-scale expression change of genes involved in nitrogen assimilation to TAG and starch metabolisms suggests that sensing mechanism of the nitrogen is defective in the mutant. Causal gene of the mutant is a candidate of regulator in N sensing and TAG/starch accumulation mechanism.

166. Toward Mosquito Control with Green Algae: Expression of Cry Genes from *Bacillus thuringiensis israelensis* in the Chloroplast of *Chlamydomonas* . **Seongjoon Kang**, Obed W. Odom, David L. Herrin. Department of Molecular Biosciences, School of Biological Sciences, University of Texas at Austin, TX, 78712, USA.

Mosquitoes transmit a number of diseases, including West Nile, dengue and malaria. Chemical pesticides that target adult mosquitoes have undesirable effects on non-target organisms (including people), and are losing their effectiveness due to increasing resistance. *Bacillus thuringiensis* subsp. *israelensis* (Bti) produces a crystallous toxin during sporulation that is highly specific for Dipterans, especially mosquito larvae, which ingest it during feeding and wherein it damages their gut. Bti is sensitive to sunlight, however, and does not recycle, thus requiring frequent application. Green algae are an excellent - even preferred - food source for mosquito larvae, and could make an attractive alternative to Bti, if they could be engineered to kill feeding larvae. The Bti toxin is a synergistic combination of 3 large Cry proteins (4A, 4B and 11A) and a smallish cytolytic protein (Cyt1A), all of which have been expressed in certain prokaryotes, but not in a eukaryote. To evaluate the potential for chloroplast expression of the protoxins, we are using the copper-repressible system developed in *Chlamydomonas* by the Rochaix lab. Codon-optimized and C-terminal tagged versions of a truncated Cry4A gene and full-length Cry4B and Cry11A genes were synthesized commercially, and outfitted with a modified *psbD* 5' control region. The genes were inserted into the chloroplast genome of the photosynthetic *Cyc:Nac2*-containing strain, and the homoplasmicity of the transformants was confirmed by PCR. Using western blots of total cell protein with an epitope-tag antibody, accumulation of all 3 Cry proteins was observed when copper was removed from the medium. The highest protoxin levels were obtained with the truncated Cry4A strain, however,the Cry11A strain showed the strongest toxicity to *Aedes aegypti* larvae in a live-cell bioassay. These results suggest that it should be possible to obtain *Chlamydomonas* strains that express the Cry proteins in the chloroplast under normal (i.e., non-represssible) conditions, a necessary prerequisite for using them to control mosquitoes and black flies.

167. Carbonate and acetate metabolisms in *Chlamydomonas reinhardtii* by NMR. J. Chang[1], M. Singh[2], C. Goodson[3], N. van der Velde[1], A. Nguyen[1], U. Goodenough[3], **S.J. Kim**[1]. 1) Chemistry, Baylor Univeristy, Waco, TX; 2) Chemistry, Washington University, St. Louis, MO; 3) Biology, Washington University, St. Louis, MO.

The photosynthetic CO_2 contributions to carbon flux in *Chlamydomonas reinhardtii* strains *sta6* and *cw15* grown in nitrogen-replete and nitrogen-free media were investigated using solid-state NMR. Cells were grown in N-replete HSM media containing 40 mM sodium [^{13}C]bicarbonate and unlabelled 20 mM acetate to mid-log growth phase. Cells were then harvested and N-starved in N-free HSM containing unlabelled 40 mM sodium bicarbonate and 20 mM acetate. *C. reinhardtii* whole-cells containing ^{13}C (incorporated from ^{13}C bicarbonate) were harvested at 0 hr and at 48 hr of N-starvation. This generated two samples for each of the *C. reinhardtii* strains, which were then lyophilized and analysed using solid-state NMR. ^{13}C-Cross polarization Magic Angle Spinning (CPMAS), and solid-echo (SE) NMR experiments were used to analyse ^{13}C-carbon fluxes into the various carbons (such as starch, proteins and lipids) and to measure the total ^{13}C-labeled carbons present in the sample. The ^{13}C-CPMAS NMR of *sta6* shows a large accumulation of intracellular ^{13}C-bicarbonate during the log-growth phase, approximately double the amount found in *cw15*, which is utilized by the cells during N-starvation for the lipid biosynthesis. The ^{13}C-SE NMR of *sta6* confirmed the increased lipids originating from the intracellular ^{13}C-bicarbonate. A net loss of ^{13}C-intensity was observed by CPMAS and SE for both strains after 48-hr of N-starvation. This result was consistent with the loss of ^{13}C-

labeled spins originating from acetate observed in an earlier experiment, indicating an active autophagy during N-starvation to metabolize the cellular carbons.

168. Bimodal *C. reinhardtii* Network Co-evolution Revealed by Integrated Phylogenomics and Metabolic Network Analyses. **Kourosh Salehi-Ashtiani[1]**, Amphun Chaiboonchoe[1], Lila Ghamsari[2], Bushra Dohai[1], Patrick Ng[3], Ashish Jaiswal[1], Hong Cai[1], David Nelson[1], Xinping Yang[2], Jason Papin[4], Haiyuan Yu[3], Santhanam Balaji[5]. 1) New York University Abu Dhabi, Abu Dhabi, United Arab Emirates; 2) Center for Cancer Systems Biology (CCSB) and Department of Cancer Biology, Dana-Farber Cancer Institute, and Department of Genetics, Harvard Medical School, Boston, MA, USA; 3) 3Department of Biological Statistics and Computational Biology and Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY, USA; 4) Department of Biomedical, Engineering, University of Virginia, Charlottesville, VA, USA; 5) 5MRC Laboratory of Molecular Biology, Cambridge, UK.

Introduction: *Chlamydomonas reinhardtii* is a green unicellular alga that is studied as a model for optimization of algal-based commercial applications as well as systems level analyses of metabolic processes. We have recently reported the reconstruction of a genome-scale metabolic network model for *C. reinhardtii*, which herein is utilized to address the question of topological and functional interconnectivity with phylogenetic affinities of genes in the network.

Methods: The evolutionary affinities of genes in the *C. reinhardtii* network were defined for 13 eukaryotic lineages through sequence alignments in order to study topological and functional connectivity in relation to evolutionary affinity. Dynamic and static co-evolving gene pairs were identified by mutual information and phylogenetic profile distance measurements. Double gene deletion analysis was carried out *in silico* to predict synthetic metabolic interactions between genes in the network. Comparative analyses with metabolic networks of *Arabidopsis thaliana* and *Saccharomyces cerevisiae* were carried out to detect topological and functional conservation.

Results and discussions: We demonstrate that *C. reinhardtii* metabolic genes display a distinct bimodal dynamics with respect to their conservation and network topologies. Over half of the genes in the network were identified as being involved in either dynamic or static co-evolving relationships. Double gene deletion analysis indicates a non-random distribution of phylogenetic affinities between the interacting gene pairs with a marked tendency for the affinities to be distant. Furthermore, we demonstrate both pairwise conservation and rewiring of functions between Chlamydomonas, yeast, and Arabidopsis.

169. Isolation and characterization of lipid-accumulating mutants under photoautotrophic conditions using flow cytometer. **H. Shinkawa**, M. Kajikawa, H. Fukuzawa. Grad. Sch. of Biostudies, Kyoto Univ., Japan.

Chlamydomonas reinhardtii cells are often cultured mixotrophically in a TAP medium containing acetate that can be converted into triacylglycerol (TAG) under nitrogen-deficient (−N) conditions. It is known that higher levels of TAG are accumulated in *Chlamydomonas* cells under mixotrophic conditions than those under photoautotrophic conditions. Under −N conditions, the cells change metabolic pathways and cellular states (e.g. chlorophyll degradation, down regulation of protein synthesis, accumulation of starch and TAG and arrest of cell division). The molecular mechanism on partitioning of photosynthetically fixed carbon into TAG or starch under −N conditions has been poorly understood. To address these questions, it is important to identify factors regulating the carbon flow into TAG synthesis. In this study, 20 lipid-accumulating mutants of *C. reinhardtii* were isolated by using fluorescence-activated cell sorting under the photoautotrophic conditions with bubbling of air containing 5% CO_2 from 8,830 paromomycin-resistance tag lines. These mutants showed starch-less phenotype under the photoautotrophic and −N conditions. We quantified lipid amounts in these mutants under the photoautotrophic −N and the mixotrophic −N conditions, respectively. Among 20 mutants, two mutants accumulated approximately twice the amount of lipids under the photoautotrophic −N condition than that under the mixotrophic −N condition.

170. Identification of the chloroplast D-lactate dehydrogenase involved in pyruvate reduction in *Chlamydomonas reinhardtii*. **Hussein Taha[1,2]**, Steven J. Burgess[3], Justin A. Yeoman[1], Oksana Iamshanova[1], Peter J. Nixon[1]. 1) Department of Life Sciences, Imperial College London, London SW7 2AZ, UK; 2) Faculty of Sciences, Universiti Brunei Darussalam, Gadong BE1410, Brunei Darussalam; 3) Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, UK.

Under anoxic conditions the green alga *Chlamydomonas reinhardtii* excretes various metabolites including formate, acetate, ethanol, hydrogen and lactate. There is currently much interest in manipulating metabolism to improve the production of biohydrogen. How lactate is produced is unclear. Our bioinformatics analysis identified a candidate nucleus-encoded D-lactate dehydrogenase (Phytozome v9.1 ID: Cre07.g324550). Using an immunochemical approach we confirmed that the enzyme was oligomeric and targeted to the chloroplast. Analysis of the *Escherichia coli*-expressed enzyme confirmed that the enzyme was tetrameric and acted as an NAD^+-dependent D-lactate dehydrogenase (D-LDH) favouring the reduction of pyruvate. Interestingly the D-LDH was expressed in cells growing photoautotrophically under aerobic conditions. By using artificial microRNA technology, we successfully isolated 4 knockdown mutants with a protein reduction of greater than 80%. Effects on growth, sensitivity of photosynthesis to high light and production of biohydrogen will be presented.

171. Acetate Production Pathways in *Chlamydomonas reinhardtii* during Dark Anoxia and the Dominant Role of Chloroplasts in Fermentative Acetate Production. **Wenqiang Yang[1]**, Claudia Claudia Catalanotti[1], Sarah D'Adamo[2], Tyler Wittkopp[1,3], Cheryl Ingram-Smith[4], Luke Mackinder[1], Tarryn Miller[2], Kerry Smith[4], Martin Jonikas[1], Arthur Grossman[1], Matthew Posewitz[2]. 1) Plant Biology, Carnegie Institution for Science, Stanford, CA; 2) Colorado School of Mines, Golden, CO; 3) 3Stanford University, Stanford, CA; 4) Clemson University, Clemson, SC.

Chlamydomonas reinhardtii (Chlamydomonas throughout) displays broad flexibility to balance redox equivalents and generate energy when experiencing anoxic/hypoxic conditions. We isolated Chlamydomonas insertion mutants of acetate kinases (ACK1 and ACK2) and a phosphate acetyltransferase (PAT2, but not PAT1), which catalyze significant reactions when the alga reorients toward fermentation metabolism under anoxia/hypoxia. The generation of acetyl-CoA from pyruvate, followed by the catalysis of acetyl-CoA to acetate and ATP by PAT-ACK, is depicted below. The acetyl-CoA can also be reduced to ethanol, as shown.
The ACK1 and PAT2 proteins were localized to chloroplasts, while the ACK2 and PAT1 proteins are mitochondrial. The results showed that chloroplasts (PAT2 and ACK1) play a dominant role relative to mitochondria in producing acetate and sustaining fermentation metabolism during anoxia/hypoxia.

In vitro acetate kinase activity was not detected in the *ack1ack2* double mutant; however, surprisingly, this strain could still accumulate extracellular lower levels of acetate during anoxia, suggesting that Chlamydomonas uses other pathways for acetate synthesis in the absence of ACK activity. To identify enzymes responsible for acetate production in the *ack1ack2* double mutant, we searched the Chlamydomonas genome for gene models associated with acetate metabolism, including acetyl-CoA synthetase, aldehyde dehydrogenase, *acetate:succinate CoA-transferase, succinyl-CoA ligase* and acetyl-CoA hydrolase. Overall, our findings demonstrate that only modest alterations in accumulation of fermentative products are observed in the *ack1*, *ack2*, and *ack1ack2* mutants, and that acetate is not exclusively synthesized by the ACK pathway during anoxia. The results are discussed in the context of the control of fermentation metabolism and survival of Chlamydomonas under anoxia/hypoxia.

Research funded by DE-FG02-12ER16338 to A.R.G., DE-FG02-12ER16339 to M.C.P., and NSF Award #0920274, South Carolina Experiment Station project SC-1700340 and technical contribution #6207 of the Clemson University Experiment Station to K.S.S..

172. Evolution of a soma-determining gene. **P.J. Ferris**[1], E.R. Hanschen[1], Z.I. Grochau-Wright[1], B.J.S.C. Olson[2], R.E. Michod[1]. 1) Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ; 2) Division of Biology, Kansas State University, Manhattan, KS.

The *regA* mutation of *Volvox carteri* has a spectacular effect on soma determination: the ~2000 apparently somatic cells of the mutant spheroids soon regenerate into reproductive cells instead of undergoing cell death. *regA*, a member of the VARL (SAND) gene family, is thought to regulate chloroplast metabolism, thereby ensuring somatic differentiation. The phylogenetic tree of all predicted VARL domain genes in the *Chlamydomonas* and *Volvox* genomes shows that *regA* is one of four paralogs (the others are *rlsA*, *rlsB* and *rlsC*) whose only close *Chlamydomonas* relative is *RLS1*. The four paralogs are present in the genome as a tandem array, the *regA*-cluster. The genus *Volvox* is polyphyletic, spread among three clades in chlpDNA gene phylogenies. *V. carteri* is included in the largest *Volvox* clade; *Volvox gigas* and *Volvox powersii* are included within the *Eudorina* clade; and the EuVolvox form a sister clade with all the anisogamous volvocine species. Previous studies predicted that *Volvox* germ-soma evolved at least twice, so the evolutionary history of *regA* remains unknown.

Cosmid libraries were prepared from *V. gigas* and *Volvox ferrisii* (EuVolvox) genomic DNA, and probed with partial VARL gene sequences identified by gPCR using degenerate VARL domain primers. One cosmid from *V. gigas* contains *rlsA*, *regA* and *rlsB*; *rlsC* is on a separate cosmid. In *V. ferrisii*, an 80 kb contig generated from overlapping cosmids contains six VARL genes: *rlsD*, and five VARL genes that may correspond to the four paralogs in *V. carteri*, plus an additional paralog. Five non-VARL genes syntenic with *regA* or *rlsD* in *V. carteri* are also present in the contig. The presence of *regA*-clusters in all *Volvox* clades suggests that *regA*-clusters should exist in many volvocine species lacking soma. Attempts to demonstrate this are ongoing.

173. Discovery of *regA* Family Genes in non-*Volvox* Species. **Z. Grochau-Wright**[1], P. Ferris[1], E. Hanschen[1], B. Olson[2], R. Michod[1]. 1) Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ; 2) Division of Biology, Kansas State University, Manhattan, Kansas.

A major goal of evolutionary biology is understanding the genetic basis of evolutionary transitions in individuality (ETI), in which new higher level individuals arise from the integration of lower level units. The hallmark of the ETI from unicellularity to multicellularity is the evolution of germ-soma cellular differentiation. Somatic cells give up their reproductive ability and germ cells lose the ability to survive independently, therefore germ-soma differentiated colonies form a cohesive, irreducible evolutionary unit. *regA*, a transcription factor gene thought to regulate chloroplast biogenesis, is critical for normal somatic differentiation in the multicellular algae *Volvox carteri*. Previous work has shown that *regA* is part of a tandem duplication of four paralogs known as the *regA* gene cluster. Recent work has found that the *regA* gene cluster is present in two other *Volvox* species that do not share a recent common ancestor with *Volvox carteri*. This suggests that *regA* and the rest of the *regA* gene cluster should be present in multiple genera, including *Pleodorina* which has soma, and *Eudorina* which lacks somatic cells. To test this hypothesis we sequenced members of the *regA* gene cluster from two non-*Volvox* species: *Pleodorina californica* and *Eudorina elegans* var. *carteri*. Our results show that the genetic toolkit for somatic differentiation likely arose early in the evolution of multicellularity and is present in species that lack soma, suggesting that the evolutionary history of somatic differentiation is more complicated than previously thought.

174. New selectable markers for *Volvox carteri*. **Jose Ortega**,Owen Kwok, Stephen Miller. Biological Science, UMBC, Baltimore, MD.

Volvox carteri is an excellent model system for investigating developmental mechanisms and their evolution. Several selectable markers for nuclear transformation of *V. carteri* have been developed, including the *nitA* gene (encoding nitrate reductase) and genes for resistance to the antibiotics zeocin and paromomycin, but there are disadvantages to using each of these markers. For instance, *nitA* can only be used to transform Nit mutants, and transformants resistant to the antibiotic resistance markers can be difficult to select and/or are sometimes unstable. To improve nuclear transformation of *V. carteri*, we are developing vectors that provide stable, easily selectable resistance to the antibiotics hygromycin and blasticidin. Our lethal-dose experiments indicate that *V. carteri* is sensitive to both

antibiotics at a concentration of 20 mg/mL. We generated vectors with *Volvox*-specific regulatory sequences (*hsp70A*/*rbcS3* promoter and *rbcS2* 3' UTR) and codon-optimized hygromycin and blasticidin resistance genes from *Streptomyces hygroscopicus* that contain the first intron of the small subunit of the *rbcS2* gene included into the coding region of these markers. We are also testing additional 5' and 3' regulatory sequences, including cassettes from the b-tubulin, ferredoxin and ribosomal protein L14 genes.The hygromycin cassette with *hsp70A*/*rbcS3* and *rbcS2* regulatory sequences permitted simple selection for antibiotic resistance within ~10 days and when co-transformed with our standard *nitA* marker provided resistance to hygromycin and ability to grow with nitrate as sole nitrogen source. Thus we have developed at least one new, highly robust selectable marker gene for *V. carteri* transformation that should be useful for co-transformation experiments.

175. Microbial consortia: A way forward for the commercialisation of microalgae for industrial biotechnology? **C.J.A. Ridley**, E. Kazamia, A.G. Smith. Department of Plant Sciences, University of Cambridge, Cambridge, United Kingdom.

A major problem in algal biotechnology is productivity loss due to contamination by adventitious organisms, such as bacteria. But algal-bacterial interactions are prevalent in nature, and many algae require bacterial symbionts for the provision of key nutrients such as vitamins. We are investigating whether algal-bacterial symbioses can prevent algal culture "crash" via a model symbiosis between the green alga *Lobomonas rostrata* (*Chlamydomonadaceae*) and the rhizobial bacterium *Mesorhizobium loti*. This interaction is based upon the exchange of vitamin B12 from the bacterium in return for a fixed carbon source.

This interaction is stable, exhibits regulation of cell numbers, and is well described, making it an ideal model for study. We have identified a library of 25 bacteria isolated from non-axenic *L. rostrata* cultures via 16S rRNA sequencing, to which we have applied a fractional factorial experimental approach. Effects of bacteria on algal growth (axenic *L. rostrata* or co-culture with *M. loti*) are determined via ANOVA (Minitab, Minitab Inc.). Preliminary data suggest a deleterious effect of several *Pseudomonas* species, and a potentially beneficial interaction with the actinobacterium *Rhodococcus fascians*. By comparing data from axenic *L. rostrata* with data from *L. rostrata/M. loti* co-culture, we are able to explore the characteristics of the bacteria and to determine whether co-culture with *M. loti* offers any protective effects against contamination.

176. *Symbiodinium* Transcriptome and Global Responses to Changing Light Levels. **Tingting Xiang**[1], William Nelson[1,2], Jesse Rodriguez[3], Dimitri Tolleter[1,4], Arthur Grossman[1]. 1) Department for Plant Biology, Carnegie Institution for Science, Stanford, CA. 94305; 2) Maverix, 1670 South Amphlett Boulevard, Suite 214, San Mateo, CA 94402; 3) Stanford University, Biomedical Informatics Program, Stanford, CA 94305; 4) Division of Plant Science, Research School of Biology, The Australian National University, Canberra, ACT, Australia.

The symbiosis between the unicellular dinoflagellate (genus *Symbiodinium*) and their cnidarians host (e.g. corals, sea anemones) represents the foundation of coral reef ecosystems. The dysfunction of this symbioses imposed by environmental stress (e.g. high light and temperature) has led to global reef decline. Presently, there is still little known about *Symbiodinium* gene expression and mechanisms by which light impacts its association with its host. To address these issues, we generated a transcriptome from the axenic *Symbiodinium* strain SSB01. Here we report features of the transcriptome, including the occurrence and length distribution of the Spliced Leader (SL) sequences, the functional landscape of encoded proteins and the impact of light on gene expression. One of the largest, over-represented gene families in *Symbiodinium* SSB01 encodes the Regulator of Chromatin Condensation (RCC1), which may relate to the persistent condensed state of the dinoflagellate genome. The expression of many *Symbiodinium* genes appears to be significantly impacted by light. The most extensive changes in transcript abundances are observed when the algae are transferred from low light to darkness. Interestingly, transcripts encoding a number of cell adhesion proteins rapidly declined when the cells were placed in total darkness, which correlated with a dramatic change in the morphology of the cell surface that likely reflects the complexity of the extracellular matrix. Thus, light-sensitive cell adhesion proteins may play a role in establishing surface architecture, which may in turn alter interactions between the host and endosymbiont.

177. Developing the microdroplet platform for microalgal biotechnology. **Roshni J. Best**[1,2], Jie Pan[1], Chris Abell[1], Alison G. Smith[2]. 1) Department of Chemistry, University of Cambridge, Cambridge, United Kingdom; 2) Department of Plant Sciences, University of Cambridge, Cambridge, United Kingdom.

Microdroplets are typically 10-100 micron water droplets carried through a microfluidic device in an oil stream. Single *Chlamydomonas reinhardtii* cells can be encapsulated into microdroplets, and their growth and behaviour can be monitored within the droplet over several days[1]. Droplets can be manipulated and analysed in various ways using specialised microfluidic chips[2]. This provides a new platform for studying the stochastic behaviour and interactions of algae at an individual-cell level, rather than assuming that all cells behave identically, defined by the population average measured in bulk studies.

C. reinhardtii cells accumulate TAGs (triacylglycerides) as discreet lipid bodies when placed under nitrogen stress. This lipid accumulation process has been studied using different fluorescence-based single-cell screening techniques - microdroplet screening, flow cytometry and analysis of confocal microscopy images - to reveal the extent of stochasticity between cells of a supposedly homogeneous parent population. All three techniques reveal that there is considerable variation in the quantity of storage lipid per cell. The reliability and efficiency of these three single-cell screening methods has been compared.

1) Pan *et al.* - Quantitative tracking of the growth of individual algal cells in microdroplet compartments, Integrated Biology 2011
2) Microdroplet videos: http://youtu.be/ySubmd2FYOU, http://youtu.be/vwPO0coIOZU

178. Establishing CRISPR/Cas9 for Gene Targeting in *Chlamydomonas*. **Andre Greiner**, Lina Sciesielski, Peter Hegemann. Experimental Biophysics, Humboldt-University, Berlin, Berlin, Germany.

In the rapidly evolving field of gene targeting the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system, published in 2013 (Cong et.al., Science 2013), outperforms previous methods like ZFNs (Zincfinger-Nulceases) and Talens (Transcription Activator Like-Nucleases). CRISPR is a bacterial immune system preventing integration of foreign DNA, e.g. from bacteriophages, into the host genome. Small guide RNAs (gRNAs) including a 20bp target sequence, that serve as a template for target recognition, bind to an Endonuclease (Cas9) of the CRISPR system. Upon identification of foreign DNA sequences, Cas9 introduces a DNA double-strand break shearing the invading DNA.

The CRISPR immune system has now been adapted for gene targeting experiments. It has the outstanding advantage that, in contrast to ZFN and TALENs, for gene targeting experiments always the same enzyme (Cas9) can be utilized. Different genes are simply targeted by altering the gRNA target sequence. To facilitate the use of CRISPR in *Chlamydomonas*, the gRNA needs to be transcribed from RNA-Polymerase III since the transcript has to remain in the nucleus. In addition Cas9 expression and guidance to the nucleus are a prerequisite. Here we present our progress in adapting the system for *Chlamydomonas* and first results of gene targeting experiments. The poor rates of homologous recombination in *C. reinhardtii* are still one of the major drawbacks for its use as a model organism. In our last report (Sizova et al., Plant Journal 2013) we showed the successful deletion of the Channelrhodopsin-1 gene by application of Zincfinger-Nucleases in *Chlamydomonas*. Unfortunately, this method only worked in the non-motile strain CW15-302 (CW-4350). All our attempts to transfer the system to a motile strain failed. Strain 302 is known for its high transformation efficiency and high expression of heterologous genes. Therefore we were interested what makes a difference in this strain depicting it as the only alternative for directed gene targeting in *Chlamydomonas* at the moment. Genome sequencing of this strain revealed a mutation influencing nucleosome assembly.

179. Rapid constructing and screening artificial microRNA systems in Chlamydomonas reinhardtii. Jinlu Hu[1,2], Xuan Deng[1], Gaohong Wang[1], **Kaiyao Huang[1]**. 1) Key Laboratory of Algal Biology, Institute of Hydrobiology, C.A.S, Wuhan, Hubei, China; 2) University of the Chinese Academy of Sciences, Beijing 100039, China.

The unicellular green algae Chlamydomonas reinhardtii is a classic model to study flagella/cilia and photosynthesis. Recently it has been used for producing biopharmaceuticals and biofuel. Due to the low frequency of homologous recombination, the reverse genetic manipulation in Chlamydomonas is mainly dependent on the miRNA and siRNA-based knockdown methods. However, the difficulty in constructing the artificial miRNA vector, the laborious screening the knockdown transformants, and the undesired epigenetic silencing effect on exogenous miRNA constructs limit their application. In this communication, we established a one-step procedure to construct the artificial miRNA precursor by annealing eight ~40nt oligonucleotides. A luciferase gene was inserted between the promoter and the artificial miRNA precursor to monitor the expression of the miRNA and epigenetic effect on the miRNA construct. Our data demonstrated that the luciferase activity correlated well with the knockdown level of the target protein. The introns from Ribulose Bisphosphate Carboxylase/Oxygenase Small Subunit 2 can enhance both the luciferase activity and the knockdown effect by the miRNA. In addition, we built a fast screening and inducible artificial microRNA system with the promoter from the nitrate reductase. These results will facilitate the application of the artificial miRNA and provide new tools for studying the mechanism of epigenetics in Chlamydomonas. Supported by NSFC (#3 1171287).

180. High-throughput genotyping of *Chlamydomonas* mutants reveals random distribution of mutagenic insertion sites and endonucleolytic cleavage of transforming DNA. Weronika Patena*, Ru Zhang*, Ute Armbruster, Spencer Gang, Sean Blum, **Martin Jonikas**. Plant Biology, Carnegie Institution for Science, Stanford, CA.

We developed a novel tool, ChlaMmeSeq, which enables simultaneous mapping of large numbers of insertion sites in pools of *Chlamydomonas* mutants by Illumina sequencing. We applied ChlaMmeSeq to a mutant pool and mapped 11,478 insertions, covering 39% of annotated protein coding genes. We observe that insertions are distributed in a manner largely indistinguishable from random, indicating that mutants in nearly all genes can be obtained efficiently. The method is quantitatively reproducible, enabling its use for pooled enrichment screens. The data reveal that sequence-specific endonucleolytic activities cleave the transforming DNA, and allow us to propose a simple model to explain the origin of the poorly understood exogenous sequences that sometimes surround insertion sites. *These authors contributed equally.

181. TALE activation of endogenous genes in *Chlamydomonas reinhardtii*. Han Gao[1], David A. Wright[1], Ting Li[1], Yingjun Wang[1], Kempton Horken[2], Donald P. Weeks[2], Bing Yang[1], **Martin H. Spalding[1]**. 1) Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA; 2) Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE.

Transcription activator-like effectors (TALEs) are proteins secreted by certain plant pathogenic bacteria into host cells during infection. TALEs bind via a well-deciphered recognition code to specific sequences in the promoter region of targeted host genes, thereby activating the target gene's expression to the benefit of the pathogen. Gene activation induced by artificially designed TALEs (dTALEs) has been studied in multiple organisms, but has not previously been demonstrated in green algae, such as *Chlamydomonas reinhardtii*. In initial experiments, we generated and successfully expressed dTALEs targeting activation of two endogenous Chlamydomonas genes, *ARS1* and *ARS2*. Both target genes exhibited markedly increased expression induced by their respective dTALEs at both the transcript level and the protein level, which was confirmed by ARS colorimetric assays. This work demonstrates robust gene-specific activation induced by artificially designed dTALEs in Chlamydomonas, and also confirms the activity of the natural TALE activation domain in green algae. Furthermore, the frequency and efficiency of the induced expression demonstrate the potential of dTALEs as powerful tools for

targeted gene activation in Chlamydomonas. We are using dTALE-induced activation for fundamental biological research as well as for potential production of high value products in Chlamydomonas. Immediate target genes of interest include those encoding promising inorganic carbon transporter candidates involved in the Chlamydomonas CO_2-concentrating mechanism (CCM), and genes essential to the biosynthesis of potential high value products. Besides application of the dTALE technology in Chlamydomonas, our success with dTALE-induced activation may open new avenues to fast, high-throughput gene manipulation in other related organisms, such as other green algae and crop plants.

182. Flagellar adhesion triggers shedding of a unique, SAG1-C65-containing flagellar ectosome compartment. **Muqing Cao**, Jue Ning, Carmen Hernandez-Lara, Olivier Belzile, William Snell. Cell Biology Department, UTSouthwestern Medical Center, Dallas, TX.

The protein composition of the membrane of cilia and flagella is distinct from that of the plasma membrane and is proposed to be maintained by a diffusion barrier at the organelle base. Recently we showed that flagella adhesion-generated signaling in *Chlamydomonas* induced rapid redistribution of the flagellar signaling-related membrane polypeptide, SAG1-C65, from a plasma membrane-associated compartment to the peri-flagellar region and then into the flagellar membrane. Contrary to some models, flagellar entry of SAG1-C65-HA did not require the anterograde IFT motor. Here, we followed the fate of SAG1-C65 after its entry into flagella. When *SAG1-C65-HA plus* gametes were mixed with fusion-defective *hap2 minus* gametes, the amount of SAG1-C65-HA in the flagella membrane underwent a 10-20 fold-increase and remained high for many hours. On the other hand, when the gametes were mixed together in the presence of the protein synthesis inhibitor cycloheximide (CH), the amount of SAG1-C65-HA increased rapidly as in the control gametes, but after 2-3 hours nearly all of the SAG1-C65-HA was depleted from the flagella. Surprisingly, the SAG1-C65-HA in the cell bodies of the gametes in CH also was almost entirely depleted. The lost SAG1-C65-HA could be detected in the medium and was nearly quantitatively recovered as vesicles. TEM of non-adhering and adhering gametes showed that flagellar adhesion led to release of vesicles from the flagella. Resting gametes (i. e., gametes not undergoing flagellar adhesion) failed to release SAG1-C65-HA-containing vesicles; and, gametes activated by db-cAMP also did not release vesicles, even though large quantities of SAG1-C65-HA had moved to their flagellar membrane. Immunoblot analysis of the equal amounts of flagellar protein and vesicle protein showed that the vesicles were highly enriched in SAG1-C65-HA. Contrary to our expectations, the vesicles lacked all flagellar proteins we examined, including IFT proteins, tubulin, and the major flagellar membrane glycoprotein. We conclude that flagellar adhesion triggers formation of a unique flagellar membrane compartment that is shed into the medium in the form of flagellar exosomes. Thus, the high levels of SAG1-C65-HA in the flagellar membranes of adhering/signaling gametes represent a dynamic balance between membrane protein entry from the cell body and membrane protein release from the flagella as ectosomes. Supported by NIH GM25661 and UTSW.

183. High Throuput Phenotyping of New Chlamydomonas Species Isolated from Musaffah, UAE and New York, USA. **Amphun Chaiboonchoe**, Hong Cai, Kelly Dougherty, Ashish Jaiswal, David R Nelson, Marc Arnoux, Kourosh Salehi-Ashtiani. Division of Science and Math, New York University Abu Dhabi and Center for Genomics and Systems Biology (CGSB), New York University Abu Dhabi Institute, Abu Dhabi, UAE.

Biofuel production from microalgae has been receiving attention as an alternative energy resource due to its high biomass productivity and minimal land resource requirement. Soil algae face a wide spectrum of constraints in their environment, i.e, flooding, nitrogen and nutrient deprivations that may be specific to their local environment. Metabolic adaptation of algae to extreme environmental constraints are poorly understood. In this study, we report isolation of a number new Chlamydomonas species from New York, USA and Musaffah, UAE, and their high-throughput metabolic phenotype studies to explore the adaptive evolution of closely related green algae. Nearly 1,000 assays were conducted for carbon, nitrogen, phosphorus and sulfur source utilization, peptide nutrient stimulation, osmotic stresses and pH tolerance using the Omnolog Phenotypye Micorarray platform. Our results show that substrate utilizations vary widely among the algae isolates. Kinetic curves, heat maps and confidence interval analysis were generated to compare the metabolic properties. In general, the UAE isolates have a narrower range for nitrogen utilization, while as expected have a greater heat tolerance. Genome sequencing of these isolates indicated all to be members of Chlamydomonas genus and closely related to *C. reinhardtii*. These results offer insight on the relationship between environment and the evolvablility of algae and may help with the developments of region-specific algal optimization strategies.

184. Flagellar waveform affects bioconvection behavior of *Chlamydomonas reinhardtii*. **Azusa Kage**, Yoshihiro Mogami. Ochanomizu University, Tokyo, Japan.

Bioconvection is a collective motion of microorganisms arising from their swimming activity and the action of gravity. *Chlamydomonas* is extensively used for many experimental and theoretical studies on bioconvection, because of the high density yield of cell culture and a variety of its tactic response. Many studies have been done in relation to the photo-, the gravi-, and the gyrotactic properties of the cell, the last of which plays an inevitable role in bioconvection of *Chlamydomonas*.

Both theoretical and experimental studies so far have tended to focus on the onset process of bioconvection pattern formation, i.e., how the pattern is formed from the uniform initial state. From long-term experiments, Akiyama et al. (2005) found theoretically-unpredicted, spontaneous pattern transition in bioconvection of *Chlamydomonas reinhardtii*. Kage et al. (2013) further quantitatively described this pattern transition, and suggested that the transition might be caused by the changes in the motile activity, particularly flagellar beating patterns. This is based on the observation that the similar transition was induced by the changes in illumination intensity, although the *Chlamydomonas* cells are phototactically insensitive to the wavelength (>640 nm) of the illumination.

In this presentation, we show bioconvection behavior of the flagellar mutants of *Chlamydomonas reinhardtii*. *ida1* with impaired waveform but normal beat frequency showed rather different bioconvection behavior than that of the wild type (strain 137c), while *oda2*

with normal flagellar waveform but lower beat frequency did not. Individual swimming analysis showed that in both of the mutants the swimming speed was much lower than that of the wild type. These results suggest the important role of flagellar waveform in bioconvection behavior of *Chlamydomonas*, rather than swimming speed or beat frequency. It is therefore highly possible that the collective behavior of *Chlamydomonas* occurs in close relation to its motile properties, particularly flagellar waveform.

[References]
Akiyama, A., Ookida, A., Mogami, Y. and Baba, S. A. (2005). *J. Jpn. Soc. Microgravity Appl.*, 22, 210-215.
Kage, A., Hosoya, C., Baba, S. A. and Mogami, Y. (2013). *J. Exp. Biol.* 216, 4557-4566.

185. The *Chlamydomonas* genome encodes an active peptide processing enzyme. **D Kumar**[1], C Blaby-Haas[3], S Merchant[3], S King[1], R Mains[2], B Eipper[1]. 1) Molecular Biology & Biophysics, Univ of Connecticut Health Center, Farmington, CT; 2) Dept of Neuroscience, Univ of Connecticut Health Center, Farmington, CT; 3) Dept of Chemistry & Biochemistry, Univ of California, LA.

Peptides crucial for intercellular communication in multicellular organisms undergo extensive post-translational processing to become biologically active. C-terminal amidation, a modification often required for activity, is catalyzed by peptidylglycine α-amidating monooxygenase (PAM), a copper and ascorbate dependent enzyme that functions in the secretory pathway lumen. Glycine-extended peptides are converted to amidated products sequentially by peptidylglycine α-hydroxylating monooxygenase (PHM) and peptidyl-α-hydroxyglycine α-amidating lyase (PAL), the two enzymatic domains of PAM. PAM catalyzed peptide amidation occurs in organisms with a simple nerve net such as *Hydra* and in humans. A phylogenetic study revealed the presence of a PAM-like gene in *Chlamydomonas* (Attenborough et al, Mol Biol Evol. 2012 Oct;29(10):3095-109), suggesting that its evolution preceded multicellularity and nervous system development. We find that key residues required for catalytic activity are conserved in CrPHM and CrPAL and subcellular fractions prepared from *Chlamydomonas* display PHM and PAL activities. Specific activity in these fractions is comparable to that obtained in a neuroendocrine cell line. The properties of the *Chlamydomonas* enzymes resemble the mammalian enzymes; PHM activity is optimal in acidic conditions and is dependent on copper. Heterologous expression of CrPHM and CrPAL in a mammalian system yielded active PHM and PAL, demonstrating that the *Chlamydomonas* genome encodes active PAM. To elucidate the role of CrPAM, we generated an affinity purified polyclonal antibody to its C-terminal domain. Surprisingly, CrPAM shows a punctate distribution in cilia (flagella) and localizes to a structure in the cell body in *Chlamydomonas*. We are investigating the topology of CrPAM and the mechanism of its transport to *Chlamydomonas* cilia. Since peptide based signaling has not been reported in green algae and PAM is present in organisms with sensory cilia (such as *C. elegans*), we hypothesize that PAM plays a novel signaling role in cilia.

186. Chlamydomonas Resource Center. P. Lefebvre, C. Silflow, **M. Laudon**. Plant Biology, University of Minnesota, St Paul, MN.

The NSF supported Chlamydomonas Resource Center serves as a central repository to receive, catalog, preserve, and distribute high-quality and reliable wild-type and mutant cultures of the green alga Chlamydomonas as well as useful molecular genetic tools.
The poster will introduce the Chlamydomonas Resource Center with respect to history, past order and shipment statistics, cultures, plasmids, teaching kits, cDNA libraries, the molecular mapping kit, pricing and standard maintenance conditions.

187. Why and how to exchange the type I RubisCO in *Chlamydomonas reinhardtii* with a bacterial type II RubisCO. **Theresa Quaas**, Anja Günther, Torsten Jakob, Christian Wilhelm. Institute of Biology, University of Leipzig, Johannisallee 23, 04103 Leipzig, Germany.

The energetic capacity of the photosynthetic light reactions plants could utilize for carbon fixation is about 10 to 100 times higher as they effectively use due to the rate limiting metabolic reactions to produce cells. Up to now all attempts failed to improve the Calvin Cycle capacity by more or a faster RubisCO. *Chlamydomonas reinhardtii* is known to excrete glycolate under photorespiratory conditions. The excretion rate of glycolate seems to be not limited by metabolic reactions of producing new cells. If excreted glycolate is directly used in a biotechnological concept to produce methane in a second reactor compartment, RubisCO engineering might be a chance to overcome metabolic limitations in bioenergy production. The challenge is to stabilize the ratio between the oxygenation and the carboxylation reaction in *C. reinhardtii* to favor a constant and high glycolate excretion without completely depleting the cells in their carbon content and also to enhance the utilization of energy of the photosynthetic light reactions. Therefore, the presented approach is to express the bacterial type II RubisCO from *Rhodospirillum rubrum* in *C. reinhardtii* mutants that lack their native type I RubisCO. The aim is to investigate the differing catalytic properties of both enzymes for the O_2 and CO_2 fixation with measurements of photosynthetic rates and the impact on the glycolate excretion. We decided to transform the type II RubisCO into the nucleus of *C. reinhardtii*. The transformation success of the prospective RubisCO Type II expression vector was first tested with the fluorescence protein Kusabira-Orange (mKO1), with special focus on the chloroplast import of the target gene. First positive transformants for mKO1 were selected and analyzed by fluorescence spectroscopy as well as confocal laser scanning microscopy. Future work will be the physiological characterization of positive type II RubisCO mutants with respect to their carboxylation and oxygenation properties and the impact on the glycolate excretion.

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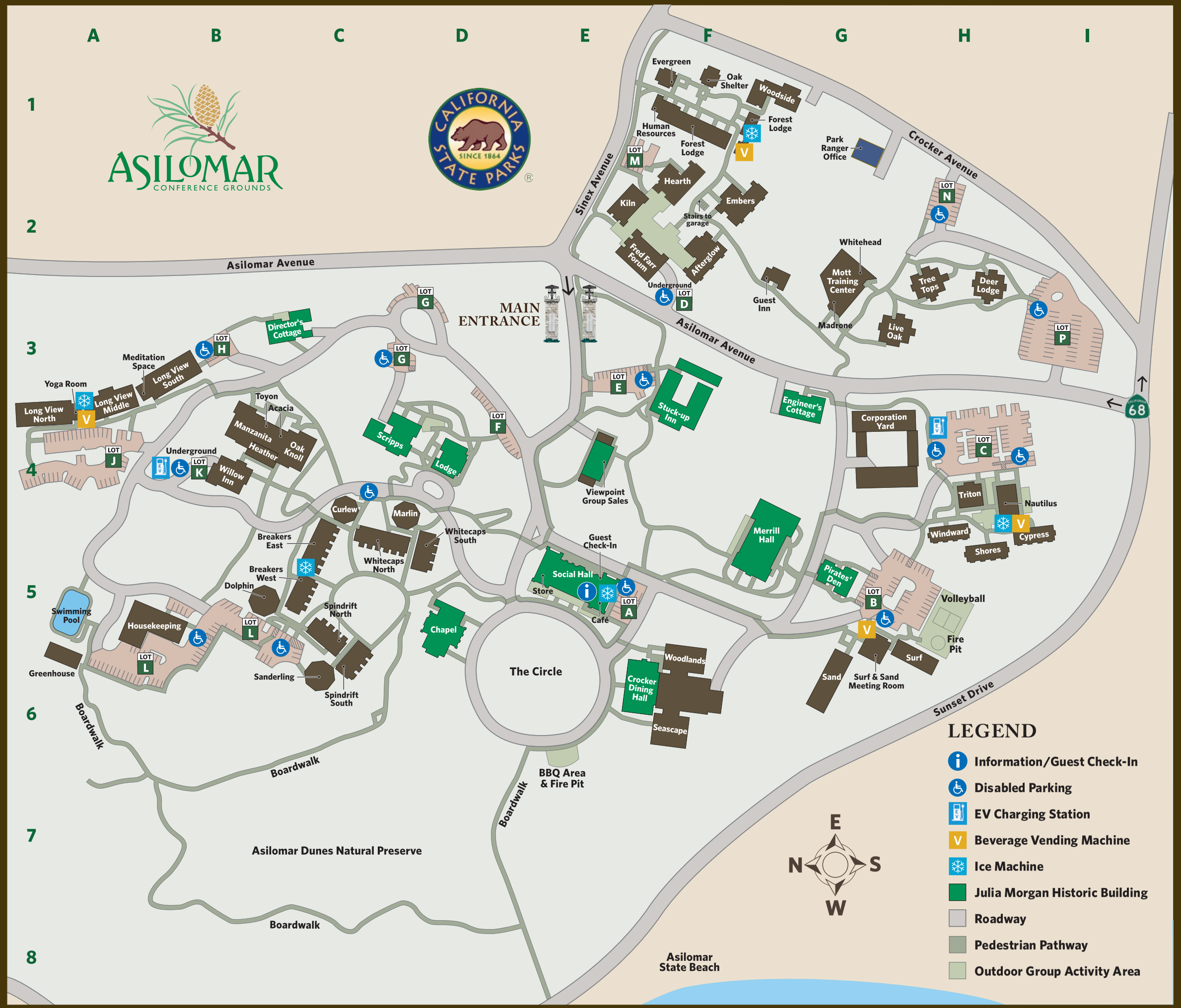

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