Tetrapyrrole Photoreceptors of Photosynthetic Organisms ICTPPO 2009

Asilomar Conference Center
July 26-31, 2009

International Conference on Tetrapyrrole Photoreceptors of Photosynthetic Organisms (ICTPPO 2009)

July 26-31, 2009
Asilomar Conference Center
Pacific Grove, CA

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AND WE THANK

Alison Byrum
Conference Coordinator
CEVS, UC Davis

Welcome to ICTPPO 2009

Dear colleagues,

We welcome you to the 2009 ICTPPO Conference and wish you a productive and enjoyable meeting during the next six days. This is the 9th ICTPPO Conference that has evolved considerably since the first conference held in 1991 at UC Davis in partial celebration of the retirement of Dr. Paul Castelfranco. A notable figure in the field of chlorophyll biosynthesis, and co-discoverer of the C5 pathway of tetrapyrrole biosynthesis, Paul also is an ordained minister and poet. Please take the time to talk with Paul during the first two days of the conference. Well into his eighties, Paul has retained a keen awareness of the heady days of tetrapyrrole biosynthesis before any of the genes were cloned.

We thank all who insisted that this meeting be sustained, particularly to Mamoru Mimuro who transferred the mantle of responsibility to us over a keg of sake in 2007, and to generous support from the US Department of Energy (BES) and the US Department of Agriculture (CSREES). Now that all of the hard work of organization is nearly complete, we look forward to its present incarnation and to your active and enthusiastic participation. With 10 notable plenary speakers, a selected number of short talks and poster sessions interspersed with free afternoons, this year's ICTPPO seeks to highlight the diversity of photosynthetic organisms and research on the structure, biosynthesis, turnover and functional importance of tetrapyrrole photoreceptors for light to chemical energy conversion in both non-oxygenic and oxygenic photosynthetic organisms. More than 40% students and postdoctoral fellows are in attendance. It is therefore clear that this field of study continues to attract young and dynamic researchers who will play a fundamental role in solving the twin issues of renewable energy production and reduction of global greenhouse gas emissions – both of which we expect will involve the exploitation of tetrapyrrole-based photosynthetic organisms.

The Monterey Bay and Peninsula possess among the richest and most diverse environments for photosynthetic and nonphotosynthetic organisms (including *Homo sapiens*) on the planet. We hope you can take advantage of this diversity by visiting the Aquarium or a few wineries during the Wednesday afternoon excursion. Please check with the Asilomar main desk for other activities and venues in the local area, or just hang out on the wind-swept Pacific coast alone or with a colleague to contemplate the challenge for oxygenic photosynthetic organisms to cope with high light, oxygen and photodynamic pigmentation all within the same cellular compartment.

Yours sincerely,

J. Clark Lagarias (Professor of Biochemistry and ICTPPO 2009 Chair)

R. David Britt (Professor of Chemistry and ICTPPO 2009 Co-Chair)

Special Event Times

Sunday	**3:00-6:00 PM**	**Registration & Poster Setup**
	10 PM-12:00 AM	**Bonfire Reception**
Tuesday	**1:30 pm**	**Group Photograph**
Wednesday	**1:00-5:30 PM**	**Excursions**
Thursday	**5:00-6:00 PM**	**Wine and Hors d'oeuvres Reception**
	6:00-7:30 PM	**Conference Banquet**
Friday	**9:00 AM**	**Departure after breakfast**
		Checkout by 12 noon

Typical Daily Program

7:30-8:30 AM	**Breakfast**
9:00-12:00 PM	**Morning Scientific Session**
12:00-1:00 PM	**Lunch**
1:00-5:00 PM	**MW: Recreation Time/Excursion**
2:00-5:00 PM	**TuTh: Afternoon Scientific Session**
5:00-6:00 PM	**M&T: Poster Sessions with Authors**
6:00-7:00 PM	**Dinner**
7:30-10 PM	**Evening Scientific Session**
10 PM	**Posters and Recreation Time**

SUNDAY, JULY 26TH

ALPHA SESSION, MERRILL HALL

7:30-7:45 PM Welcoming Remarks

7:45-8:30 PM Alpha Lecturer **Bob Blankenship**
Washington University in St. Louis
"Evolutionary Diversity of Photosynthetic Organisms"

8:30-8:45 PM Discussion

EVENING SESSION 1: NEW DEVELOPMENTS FROM YOUNG INVESTIGATORS

Discussion Leader: Antony F. McDonagh, UC San Francisco

8:45-9:00 PM **Markus Bröcker**
Technische Universität Braunschweig, Germany
"ATP-driven chlorophyllide formation by nitrogenase-like dark-operative protochlorophyllide oxidoreductase"

9:00-9:10 PM Discussion

9:10-9:25 PM **Paul Jaschke**
University of British Columbia, Vancouver Canada
"Zinc-bacteriochlorophyll in Rhodobacter sphaeroides: consequences for photosystem function and a proposed biosynthetic pathway"

9:25-9:35 PM Discussion

9:35-9:50 PM **Cheng Yao**
Arizona State University, Tempe USA
"Lifetimes of chlorophyll and chlorophyll-binding proteins in the cyanobacterium Synechocystis sp. PCC 6803"

9:50-10:00 PM Discussion

SUNDAY EVENING BONFIRE (10 PM - MIDNIGHT)

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MONDAY, JULY 27TH

MORNING SESSION 2: TETRAPYRROLE BIOSYNTHESIS & ITS REGULATION

9:00-9:45 AM Plenary Lecturer **Alison Smith**
University of Cambridge, Cambridge UK
"Regulation of tetrapyrrole biosynthesis in higher plants"

9:45-10:00 AM Discussion

10:00-10:30 AM Coffee Break

MONDAY, JULY 27TH (CONT)

10:30-10:50 AM **Robert Willows**
Macquarie University, Sydney Australia
"Subunit interactions and mechanism of the Rhodobacter capsulatus magnesium chelatase complex: Insights from enzyme kinetic studies"

10:50-11:00 AM Discussion

11:00-11:20 AM **Roman Sobotka**
Institute of Microbiology, South Bohemia Czech Republic
"Cyanobacterial ferrochelatases: structural and functional roles of the C-terminal domain containing a putative chlorophyll-binding motif"

11:20-11:30 AM Discussion

11:30-11:50 AM **Krishna Niyogi**
University of California, Berkeley USA
"Yellow-in-the-dark mutants identify novel nuclear genes required for light-independent chlorophyll biosynthesis in Chlamydomonas"

11:50-12:00 noon Discussion

MONDAY AFTERNOON POSTER SESSION: ODD NUMBERED POSTERS (5-6 PM)

EVENING SESSION 3: TETRAPYRROLE CATABOLISM & ITS REGULATION

7:30-8:15 PM Plenary Lecturer **Ayumi Tanaka**
Hokkaido University, Sapporo Japan
"Tetrapyrrole metabolism of photosynthetic organisms: degradative pathway & their regulation"

8:15-8:30 PM Discussion

8:30-8:50 PM **Thomas Mueller**
University of Innsbruck, Innsbruck Austria
"Chlorophyll breakdown in higher plants: unexpected diversity of catabolites"

8:50-9:00 PM Discussion

9:00-9:20 PM **Gopal K. Pattanayak**
University of Chicago, Chicago USA
"The role of Arabidopsis ACD2 in programmed cell death"

9:20-9:30 PM Discussion

9:30-9:50 PM **Shih-Long Tu**
Academia Sinica Institute of Plant & Microbial Biology, Taipei Taiwan
"Biosynthesis of phytobilins in green photosynthetic eukaryotes"

9:50-10:00 PM Discussion

TUESDAY, JULY 28TH

MORNING SESSION 4: THE PHOTOSYNTHETIC LIGHT HARVESTING APPARATUS

9:00-9:45 AM Plenary Lecturer **C. Neil Hunter**
University of Sheffield, Sheffield UK
"Architecture of the photosynthetic light harvesting apparatus"

9:45-10:00 AM Discussion

10:00-10:30 AM Coffee Break

10:30-10:50 AM **Alfred Holzwarth**
Max-Planck-Institut Bioanorganische Chemie, Muelheim a.d. Ruhr Germany
"Molecular structure of a chlorosome solved: alternating syn-anti bacterio-chlorophylls form concentric helical nanotubes in chlorosomes"

10:50-11:00 AM Discussion

11:00-11:20 AM **Antoine Royant**
Institut de Biologie Structurale, Grenoble France
"Crystal structure of plant light-harvesting complex shows the active, energy-transmitting state "

11:20-11:30 AM Discussion

11:30-11:50 AM **Wendy Schluchter**
University of New Orleans, New Orleans USA
"Phycobiliprotein biosynthesis in cyanobacteria: Structure and function of enzymes involved in bilin addition"

11:50-12:00 noon Discussion

ICTPPO 2009 GROUP PHOTOGRAPH (1:30 PM)

AFTERNOON SESSION 5: ELECTRON TRANSFER REACTIONS

2:00-2:45 PM Plenary Lecturer **Gary Brudvig**
Yale University, New Haven USA
"The light side: Photosynthetic energy/electron transfer"

2:45-3:00 PM Discussion

3:00-3:20 AM **Andrew Fisher**
University of California, Davis USA
"Structural insight into the radical intermediate of phytobilin biosynthesis by the ferredoxin dependent bilin reductase enzyme PcyA"

3:20-3:30 PM Discussion

TUESDAY, JULY 28TH (CONT)

3:30-3:50 PM **Stefan Stoll**
University of California, Davis USA
"Substrate radical intermediates in cyanobacterial bilin reductases"

3:50-4:00 PM Discussion

4:00-4:20 PM **Jürgen Moser**
Institute for Microbiology, Braunschweig Germany
"Chimeric nitrogenase-like enzymes of (bacterio)chlorophyll biosynthesis"

4:20-4:30 PM Discussion

TUESDAY AFTERNOON POSTER SESSION: EVEN NUMBERED POSTERS (5-6 PM)

EVENING SESSION 6: GENOMICS & SYNTHETIC BIOLOGY

7:30-8:15 PM Plenary Lecturer **Donald A. Bryant**
Pennsylvania State University, University Park USA
"Synechococcus sp. PCC 7002: a robust and versatile cyanobacterial platform for biofuels development"

8:15-8:30 PM Discussion

8:30-8:50 PM **Beverley Green**
University of British Columbia, Vancouver Canada
"Genomes of diatoms and other marine algae reveal some tetrapyrrole surprises"

8:50-9:00 PM Discussion

9:00-9:20 PM **Tohru Tsuchiya**
Kyoto University, Kyoto Japan
"Production of a novel chlorophyll in the chlorophyll d-dominated cyanobacterium Acaryochloris marina MBIC 11017"

9:20-9:30 PM Discussion

9:30-9:50 PM **Dror Noy**
Weizmann Institute of Science, Rehovot Israel
"Coupling a water soluble de-novo designed chlorophyll-binding protein to a natural allophycocyanin antenna"

9:50-10:00 PM Discussion

WEDNESDAY, JULY 29TH

MORNING SESSION 7: TETRAPYRROLE SIGNALING IN PLANTS

9:00-9:45 AM Plenary Lecturer **Joanne Chory**
The Salk Institute and HHMI, La Jolla USA
"Do tetrapyrroles play a role in retrograde signaling from the chloroplast?"

9:45-10:00 AM Discussion

10:00-10:30 AM Coffee Break

10:30-10:50 AM **Bernhard Grimm**
Humboldt University, Berlin Germany
"Do regulatory factors control distribution of 5-aminolevulinic acid for chlorophyll and heme synthesis?"

10:50-11:00 AM Discussion

11:00-11:20 AM **Tatsuru Masuda**
The University of Tokyo, Tokyo Japan
"Coordinated regulation of chlorophyll biosynthesis by light and phytohormones in Arabidopsis"

11:20-11:30 AM Discussion

11:30-11:50 AM **Jesse Woodson**
The Salk Institute, La Jolla USA
"A new genetic screen using Arabidopsis thaliana to identify proteins involved in chloroplast to nucleus organelle signaling"

11:50-12:00 noon Discussion

WEDNESDAY BOX LUNCH & EXCURSIONS

EVENING SESSION 8: LIGHT SIGNALING IN PLANTS & ITS REGULATION

7:30-8:15 PM Plenary Lecturer **J. Clark Lagarias**
University of California, Davis USA
"Exploiting a constitutively activated phytochrome allele to regulate plant development"

8:15-8:30 PM Discussion

8:30-8:50 PM **Noritoshi Inagaki**
National Institute of Agrobiological Sciences, Tsukuba Japan
"Characterization of a pale green phenotype observed in rice phytochrome B mutants grown under red light irradiation"

8:50-9:00 PM Discussion

WEDNESDAY, JULY 29TH (CONT)

9:00-9:20 PM **Baisnab Tripathy**
Jawaharlal Nehru University, New Dehli India
"Involvement of phytochrome A in near-etiolation photomorphogenesis in rice"

9:20-9:30 PM Discussion

9:30-9:50 PM **Pill-Soon Song**
Jeju National University, Jeju Korea
"Phytochrome A transgenic turf grasses"

9:50-10:00 PM Discussion

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THURSDAY, JULY 30TH

MORNING SESSION 9: BILIPROTEIN STRUCTURE, ASSEMBLY AND ENGINEERING

9:00-9:45 AM Plenary Lecturer **Roger Y. Tsien**
University of California and HHMI, San Diego USA
"Mammalian Expression of Infrared Fluorescent Proteins Engineered from a Bacteriophytochrome"

9:45-10:00 AM Discussion

10:00-10:30 AM Coffee Break

10:30-10:50 AM **Lars-Oliver Essen**
Philipps University, Marburg Germany
"Structural and functional analysis of two types of cyanobacterial phytochromes"

10:50-11:00 AM Discussion

11:00-11:20 AM **Wolfgang Gärtner**
Max-Planck-Institut Bioanorganische Chemie, Muelheim a.d. Ruhr Germany
"Conformational changes of phytochrome chromophore combining time- resolved absorption spectroscopy and solid state NMR techniques"

11:20-11:30 AM Discussion

11:30-11:50 AM **Jyotishman Dasgupta**
University of California, Berkeley USA
"Tracking structural changes within the bilin chromophore during light sensing in phytochrome using femtosecond stimulated Raman spectroscopy"

11:50-12:00 noon Discussion

THURSDAY, JULY 30TH (CONT)

AFTERNOON SESSION 10: BILIPROTEIN STRUCTURE, ASSEMBLY & ENGINEERING

Discussion Leader: Nicole Frankenberg-Dinkel, Ruhr-University Bochum Germany

2:00-2:20 PM **Rei Narikawa**
University of Tokyo, Tokyo Japan USA
"Spectral and structural characterization of a novel cyanobacteriochrome-type photoreceptor AnPixJ"

2:20-2:30 PM Discussion

2:30-2:50 PM **Beronda L. Montgomery**
Michigan State University, East Lansing USA
"Biliprotein-regulated photoperception and photomorphogenesis in cyanobacteria"

2:50-3:00 PM Discussion

3:00-3:20 PM **Jessica Wiethaus**
Ruhr-University, Bochum Germany
"Chromophorylation of phycoerythrin by the lyase CpeS in Prochlorococcus marinus"

3:20-3:30 PM Discussion

3:30-3:50 PM **Richard M. Alvey**
Pennsylvania State University, University Park USA
"Biosynthesis of unnatural biliproteins"

3:50-4:00 PM Discussion

RECEPTION & BANQUET (5:00-7:30 PM)

OMEGA SESSION, MERRILL HALL

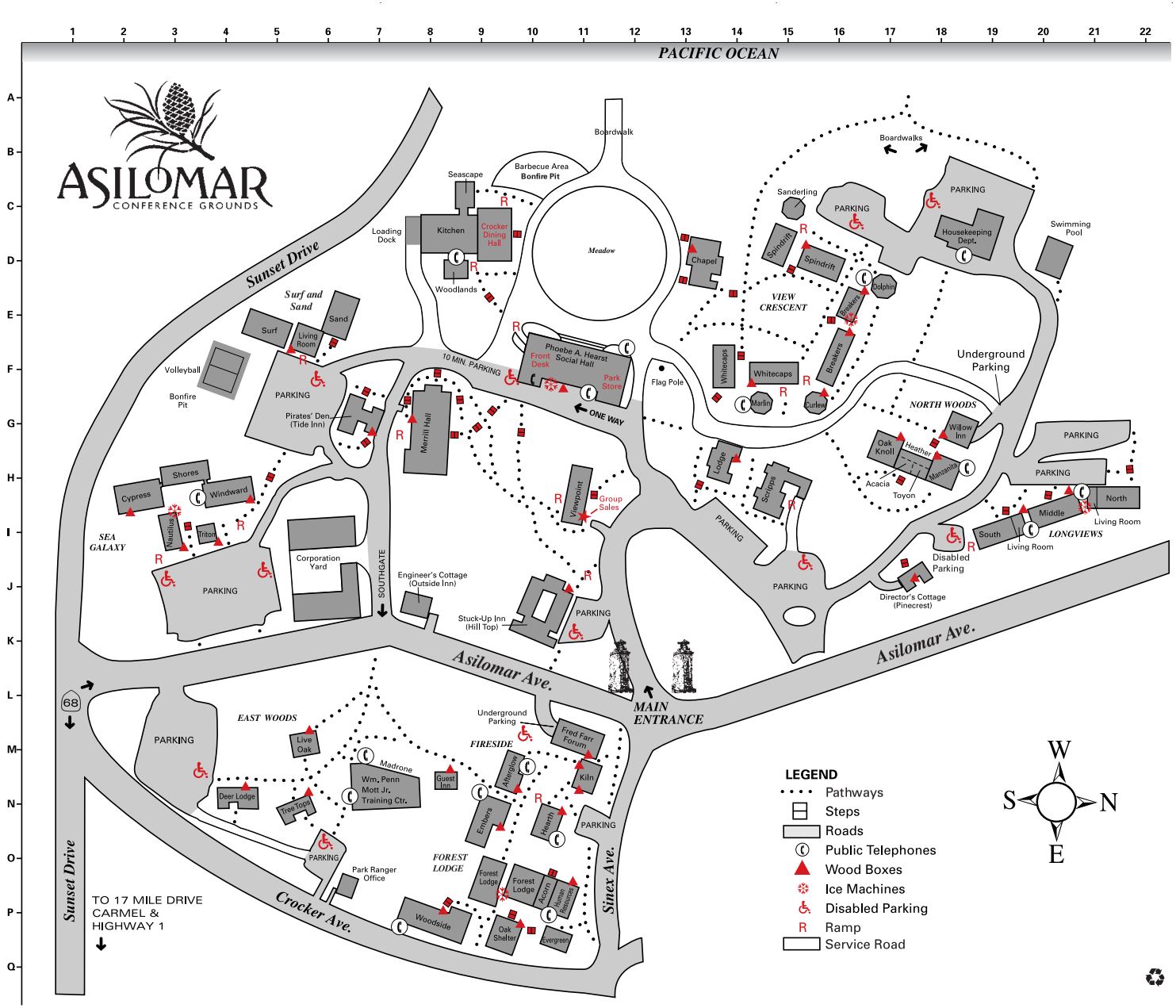
8:00-8:30 PM Business Meeting and General Discussion

8:30-9:15 PM Omega Lecturer **Thomas A. Moore**
Arizona State University, Tempe USA
"The evolution of engineered and artificial photosynthesis"

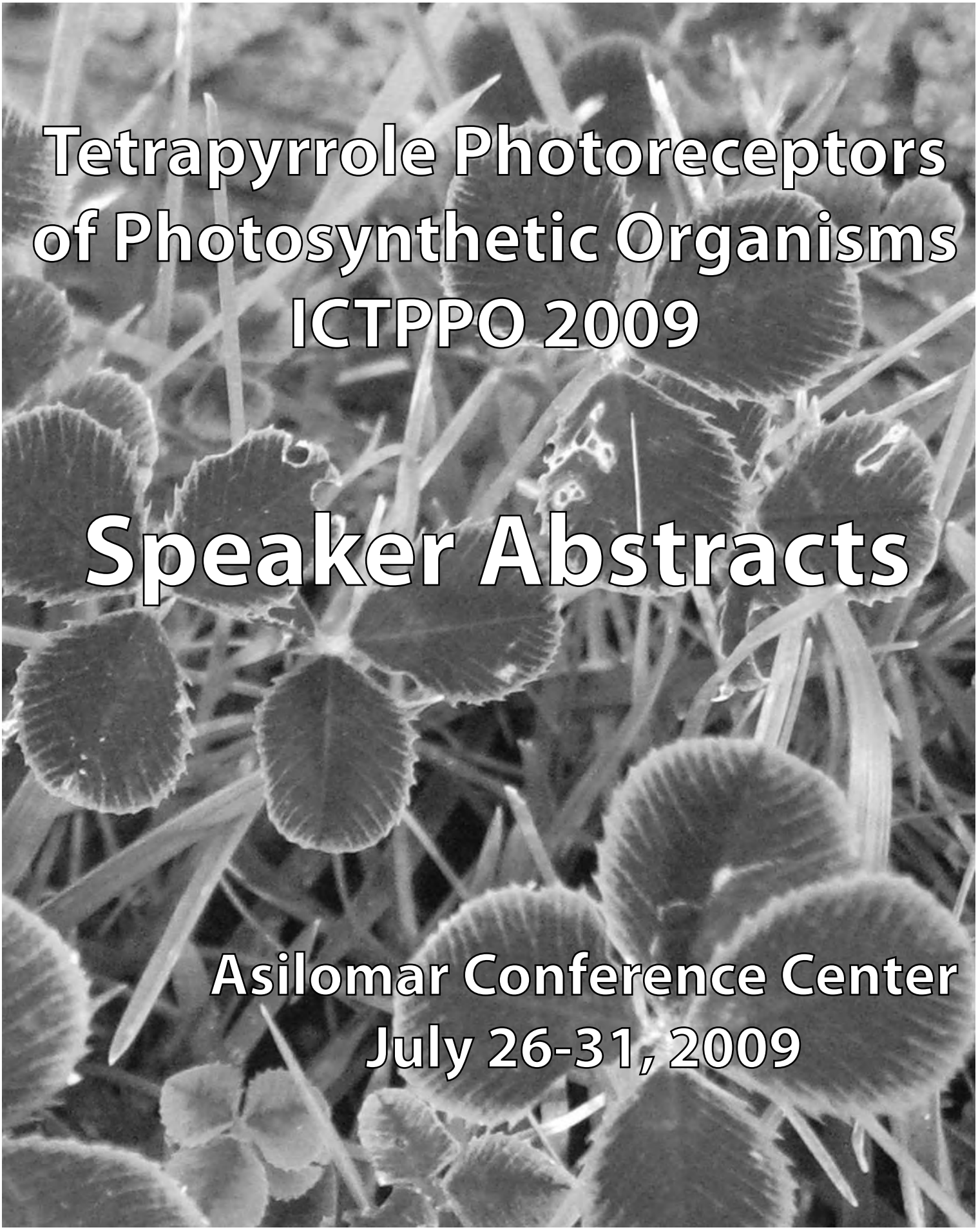
9:15-8:30 PM Discussion

FRIDAY, JULY 31ST

9:00 AM Departure after breakfast (***checkout by 12 noon***)



FACILITY	ROOM #	MEETING ROOMS	LOC.
Longviews (H-21)			
Longview North	101-110	North Living Room	H-22
Longview Middle	111-120		H-21
Longview South	121-130	South Living Room	I-19
North Woods (G-18)			
Willow Inn	1025-1036	Heather	G-19
Manzanita	1001-1012	Toyon	H-18
Oak Knoll	1013-1024	Acacia	H-17
View Crescent (E-15)			
Whitecaps South	801-808	Marlin	F-14
Whitecaps North	809-820		F-15
Breakers East	821-832	Curlew	F-16
Breakers West	833-840	Dolpin	E-16
Spindrift South	841-848	Sanderling	D-15
Spindrift North	849-856		D-16
Fireside (O-10)			
Afterglow	1301-1312	Fred Farr Forum	M-9
Hearth	1325-1336	Kiln	N-10
Embers	1313-1324		N-9
Guest Inn	901-903		M-8
Forest Lodge (M-10)			
Forest Lodge	1201-1211	Acorn	O-10
		Evergreen	P-11
Woodside	1212-1223	Oak Shelter	P-8
Historic Core			
Lodge	201-218		H-14
Scripps	301-323	Scripps Meeting Rm	H-15
Stuck-Up Inn (Hilltop)	401-414		K-10
Pirates' Den (Tide Inn)	501-510		G-6
Director's Cottage (Pinecrest)	1401-1403		J-18
Viewpoint		Viewpoint East	I-11
Group Sales Office			I-11
Merrill Hall			G-8
Chapel			D-14
Front Desk & Park Store / Phoebe A. Hearst Social Hall			F-10
Crocker Dining Hall		Crocker, Woodlands & Seascape	D-9
Engineer's Cottage (Outside Inn)			J-8
Fire Academy Offices			J-8
Sea Galaxy (G-4)			
Sand	605-610	Surf & Sand Room	E-6
Surf	601-604		E-4
Windward	701-708	Triton	H-4
Shores	709-716		H-3
Cypress	717-724	Nautilus	H-2
Eastwoods (M-4)			
Live Oak	1101-1110		M-5
Tree Tops	1111-1120		N-5
Deer Lodge	1121-1130		N-3
William Penn Mott Jr. Training Center			N-7
Madrone			M-7
Activity Locations			
Barbecue Area			C-10
Swimming Pool			D-21
Volleyball Court			F-4
Corporation Yard		Facilities	J-6
Housekeeping			D-19
Woodhaven – Park Ranger Office			O-7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Speaker Abstracts
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Robert E. Blankenship, Ph.D. Robert Blankenship grew up in southeast Nebraska. He graduated from Nebraska Wesleyan University in 1970 with a degree in Chemistry and then did graduate studies at the University of California at Berkeley, earning a PhD in Chemistry in 1975. He was a postdoctoral fellow from 1976 to 1979 at the University of Washington in Seattle. He was a faculty member in Chemistry at Amherst College in Massachusetts from 1979-85. In 1985 he moved to Arizona State University in Tempe, AZ where he stayed for 21 years. He served as Chair of the Department of Chemistry and Biochemistry at ASU from 2002-2006. In 2006 he moved to Washington University in St. Louis, where he is a joint Professor in the Departments of Biology and Chemistry. In 2007 he was named Lucille P. Markey Distinguished Professor in Arts and Sciences.

Dr. Blankenship has spent his entire career of more than 30 years researching the highly interdisciplinary subject of photosynthesis. This research has used a wide range of techniques ranging from magnetic resonance and ultrafast optical spectroscopy to genomics and molecular evolutionary analysis. He has worked on a large number of problems involving energy transfer and electron transfer processes in photosynthetic antenna and reaction center complexes. One of the hallmarks of his research program is that it emphasizes studying the mechanism of energy storage in the complete range of known organisms that do photosynthesis, with the goal of discovering the essential or irreducible aspects of how light energy is stored.

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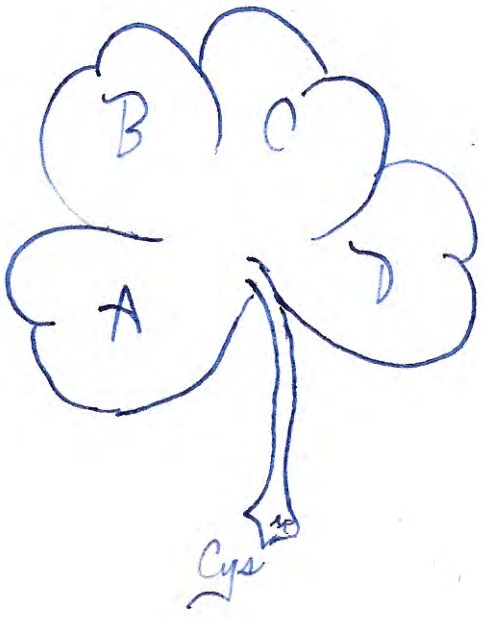
ALPHA PLENARY LECTURE. EVOLUTIONARY DIVERSITY OF PHOTOSYNTHETIC ORGANISMS

Biology and Chemistry, Washington University in St. Louis, St. Louis, Missouri, 63130, USA

Photosynthesis is a central biological process that has a long and complex evolutionary history. The photosynthetic machineries found in the existing groups of phototrophs have both common and divergent characters, suggesting an evolutionary process that combines de novo gene appearance, gene duplication, gene and pathway recruitment and loss, with both vertical and horizontal genetic transfer. The earliest phototrophs were almost certainly anoxygenic and were in existence on Earth by at least 3.4 billion years ago and possibly somewhat earlier. Oxygenic phototrophs were undoubtedly in existence by 2.4 billion years ago when free molecular oxygen, the waste product of oxygenic photosynthesis, began to accumulate in the atmosphere. These organisms may have been present up to several hundred million years before that time. Several lines of evidence, including molecular evolution analysis, structural comparisons, and biochemical and biophysical data, suggest that all modern photosynthetic reaction centers are derived from a single ancient common ancestor and that the anoxygenic phototrophs preceded oxygenic

ones. The transition from anoxygenic to oxygenic photosynthesis was accompanied by a number of evolutionary innovations, including multiple gene duplication and divergence events, modification of the pigment biosynthesis pathways from anaerobic to oxygen-requiring, invention of the oxygen evolution center and a dramatic increase in the reaction center protein subunit complexity. The evolutionary pathway that led to the current diversity of different types of phototrophs was not linear and involved significant amounts of horizontal gene transfer.



TP-DRIVEN CHLOROPHYLLIDE FORMATION BY NITROGENASE-LIKE DARK-OPERATIVE PROTOCHLOROPHYLLIDE OXIDOREDUCTASE

Markus Johannes Bröcker, Denise Wätzlich, Simone Virus, Hugo Scheer, Wolfhart Rüdiger, Miguel Saggu, Friedhelm Lendzian, Dieter Jahn, Jürgen Moser

Institut für Mikrobiologie, Technische Universität Braunschweig, Braunschweig, Niedersachsen, 38106, Germany

Chlorophyll and bacteriochlorophyll biosynthesis requires the two electron reduction of protochlorophyllide ring D by a protochlorophyllide oxidoreductase to form chlorophyllide. The light independent variant of this enzyme is the dark-operative protochlorophyllide oxidoreductase (DPOR) which plays an important role in the greening process of gymnosperms, algae and photosynthetic bacteria in the absence of light. We describe the characterization of the DPOR enzyme from *Prochlorococcus marinus* consisting of the subunits ChlN, ChlB and ChlL sharing significant sequence homology to the individual nitrogenase subunits NifD, NifK and NifH. DPOR catalysis is initiated by electron transfer from a "plant type" [2Fe-2S] ferredoxin onto the dimeric DPOR subunit $ChlL_2$ carrying an intersubunit [4Fe-4S] redox center coordinated by residues Cys124 and Cys158. Residue Lys37 in the phosphate binding loop (P-loop) and residue Leu153 in the switch II region of ChlL were found crucial for ATP-driven electron transfer from $ChlL_2$ onto the heterotetrameric subcomplex $(ChlNB)_2$. The transient interaction of $ChlL_2$ and $(ChlNB)_2$ was stabilized by using ADP-AlF_4- which is a transition state analog of ATP-hydrolysis. The subsequent [Fe-S] cluster dependent reduction of the protochlorophyllide substrate is unrelated to nitrogenase catalysis, since no molybdenum cotaining cofactor or P-cluster equivalent is employed. The $(ChlNB)_2$ complex was shown to carry two [4Fe-4S] clusters which were proposed to be ligated by residues Cys17, Cys42, Cys103 of ChlN and residue Cys95 of ChlB, respectively. From these results it was concluded that electrons from the [4Fe-4S] cluster of $(ChlNB)_2$ are directly transferred onto the substrate Pchlide at the active site of DPOR. The specific substrate recognition at the active site of $(ChlNB)_2$ was characterized by using 16 synthetic protochlorophyllide derivatives carrying specifically altered substituents on rings A-E.

ZINC-BACTERIOCHLOROPHYLL IN *RHODOBACTER SPHAEROIDES*: CONSEQUENCES FOR PHOTOSYSTEM FUNCTION AND A PROPOSED BIOSYNTHETIC PATHWAY

Paul R. Jaschke, Su Lin, Haiyu Wang, Mark L. Paddock, Aaron Tufts, James P. Allen, Federico I. Roselle, A. Grant Mauk, Neal W. Woodbury, J. Thomas Beatty

Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, V6T1Z3 , Canada

At the heart of photosynthesis lie the chlorophyll pigments responsible for absorption and transmission of light energy. The purple non-sulfur bacterium *Rhodobacter sphaeroides* is a model system for studying the biosynthesis of chlorophylls and the associated protein photosystem by virtue of its diverse metabolism, easily manipulated genetic material, and wealth of structural data. Recently, we studied a *R. sphaeroides* bchD (magnesium-chelatase) mutant to determine the properties of its photosystem in the absence of bacteriochlorophyll (BChl). Unexpectedly, the bchD mutant was found to synthesize an alternative form of BChl in which the central magnesium atom is replaced by zinc. This unusual zinc-bacteriochlorophyll (Zn-BChl) was found to assemble with the photosystem similarly to the normal magnesium-containing BChl (1). The reaction center (RC) isolated from the bchD mutant was found to contain Zn-BChl in the special pair and accessory BChl sites, but with blue-shifted absorption peaks relative to the wild type. Interestingly, spectroscopic measurements show that Zn-BChl binds in place of both bacteriopheophytins in the RC, resulting in a bacterial RC that contains 6 identical chlorophyll cofactors, analogous to the arrangement of chlorophylls in photosystem 1 of cyanobacteria and plants. Functional characterization of the isolated Zn-RC showed that electron transfer reactions proceed at >95% the rate and efficiency of the wild type (2). We hypothesize that electron flow occurs efficiently because the Zn-BChl bound in the bacteriopheophytin site has a more positive midpoint potential than the accessory and special pair Zn-BChls, due to a difference in the metal coordination state. Despite having a functional Zn-RC, this bchD mutant is incapable of photosynthetic growth. We attribute this absence of growth to the low amount of Zn-BChl produced in the cell, limiting photosystem development to only 4-7% of the wild type. The work described above will be summarized as an introduction, followed by presentation of data supporting a proposed Zn-BChl biosynthetic pathway beginning with the enzyme ferrochelatase, which shares the common precursor protoporphyrin IX with magnesium chelatase.

(1) Jaschke PR and Beatty JT. (2007) The photosystem of *Rhodobacter sphaeroides* assembles with zinc bacteriochlorophyll in a bchD (magnesium chelatase) mutant. *Biochemistry* 46: 12491-12500.
(2) Lin S, Jaschke PR, Wang H, Paddock M, Tufts A, Allen JP, Roselle FI, Mauk GA, Woodbury NW, and Beatty JT. (2009) Electron transfer in the *Rhodobacter sphaeroides* reaction center assembled with zinc bacteriochlorophyll. *Proc. Natl. Acad. Sci.* U S A. 106: 8537-8542.

Research supported by NSERC PGS-M and Discovery grants, a UBC Graduate Fellowship, NIH GM 41637, and NSF MCB0642260.

LIFETIMES OF CHLOROPHYLL AND CHLOROPHYLL-BINDING PROTEINS IN THE CYANOBACTERIUM *SYNECHOCYSTIS* sp. PCC 6803

Cheng Daniel Yao, Daniel C. Brune, Wim Vermaas

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There is a vast disparity between the lifetime of chlorophyll (about a week in *Synechocystis*) [1] and chlorophyll-binding proteins such as the PsbA (D1) protein (on the order of an hour). This disparity raises the question how assembly of photosynthetic complexes is orchestrated as accumulation of free chlorophyll in the cell would be harmful in the light and in the presence of oxygen and free polypeptides may not be stable in the membrane. In order to gain insight in the lifetimes of photosystem II (PSII) chlorophyll and proteins, we developed a combined stable-isotope labeling (15N) and mass spectrometry method to be able to follow both old and new pigments and proteins. Photosystem I-less *Synechocystis* cells were provided with 15N-ammonium nitrate at a specific time, and cells were harvested at specific intervals. PSII complexes, carrying a His tag on the CP47 subunit, were isolated from a cell extract, and the ratio of labeled and unlabeled protein and chlorophyll was determined. The PsbA (D1), PsbB (CP47), PsbC (CP43), PsbD (D2), PsbE and PsbF (cytochrome b-559), PsbH, PsbO, and Psb27 proteins were identified in the complex by mass spectrometry. The half-lives of PSII proteins ranged from 3 hours (D1) to 30 hours (PsbE and PsbO), but interestingly during the first nine hours of labeling the amount of unlabeled PsbE and PsbO protein present in PSII complexes increased by ~25% suggestive of a considerable pool of unincorporated polypeptide that was present at the time of labeling. A similar phenomenon was observed with chlorophyll (~35% increase). To determine the role that SCPs (small Cab-like proteins, which are associated with damaged PSII [2]) may play in this process, similar labeling was carried out with the PSI-less/SCP-less strain. In this strain the half-life of PSII chlorophyll was about 4-fold shorter than in the PSI-less mutant (as expected [1,2]) but interestingly in the PSI-less/SCP-less mutant no increase in unlabeled protein or chlorophyll was observed after labeled ammonium acetate was added. These results suggest that: (1) *Synechocystis* cells have reservoirs of a significant amount of chlorophyll and/or its precursors. (2) There is a pool of selected PSII polypeptides in the thylakoid membrane that are not incorporated into a PSII complex. And (3) SCPs may function both as reservoirs of chlorophyll precursors and chlorophyll in *Synechocystis* cells and in stabilizing non-incorporated PSII proteins.

1. Vavilin, D., Yao, D., and Vermaas, W. 2007. Small Cab-like proteins retard degradation of photosystem II-associated chlorophyll in *Synechocystis* sp. PCC 6803. *J. Biol. Chem.* 282, 37660-37668.
2. Yao, D., Kieselbach, T., Komenda, J., Promnares, K., Prieto, M.A., Tichy, M., Vermaas, W., Funk, C. 2007. Localization of the small Cab-like proteins in photosystem II. *J. Biol. Chem.* 282, 267-276.

ALISON G. SMITH, Ph.D. Alison Smith is Professor of Plant Biochemistry in the Department of Plant Sciences, University of Cambridge. She obtained a BSc in Biochemistry at the University of Bristol, and a PhD in Plant Biochemistry at Cambridge. Her research is focused on metabolism in plants, microbes and algae, in particular that for tetrapyrrole biosynthesis and vitamin metabolism. She has been instrumental in the identification and characterisation of many of the enzymes involved, and in determining the subcellular location and regulation of these pathways. More recently, she was responsible, together with Martin Warren (University of Kent), for the discovery of the widespread symbiotic interaction in which algae obtain vitamin B12 from bacteria, in exchange for fixed carbon. At the same time, her group has identified and characterised thiamine pyrophosphate-responsive riboswitches that regulate thiamine biosynthesis in algae.

Alison Smith is a founding member of the Algal Bioenergy Consortium, a group of several biologists and engineers in Cambridge and London, whose interests are the exploitation of microalgae for biofuel production. She is using synthetic biology approaches to manipulate biosynthetic pathways for fuel molecules in algae, and the photosynthetic apparatus in order to maximise lipid productivity. A parallel project is investigating the potential of algal cells in biophotovoltaic devices.

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PLENARY LECTURE 2. REGULATION OF TETRAPYRROLE BIOSYNTHESIS IN HIGHER PLANTS

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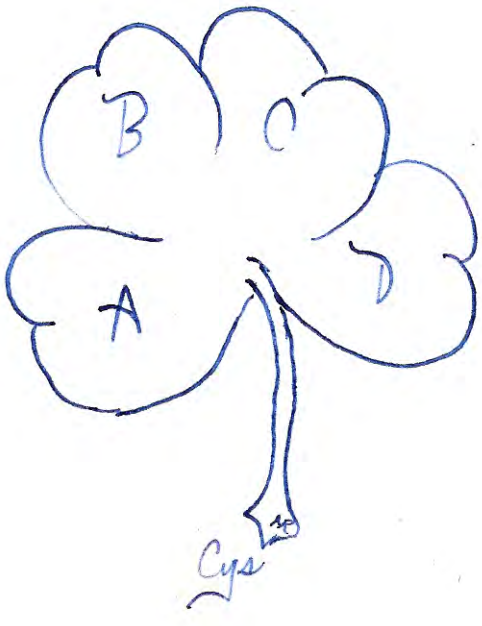
Higher plants synthesise four major tetrapyrroles, chlorophyll, haem, sirohaem and phytochromobilin, which contribute to a broad range of metabolic processes within the plant. These compounds are synthesised in the plastid via a single branched pathway, but at the same time their synthesis requires interaction with other metabolic pathways, and coordination with the production of the cognate apoproteins, the majority of which are encoded by the nucleus. Regulation of the tetrapyrrole pathway is therefore complex, not

least to ensure that there is no accumulation of intermediates, many of which are phototoxic. It is likely that reactive oxygen species generated through the photo-oxidation of various tetrapyrroles can activate a range of stress signalling pathways, suggesting that misregulation of the tetrapyrrole pathway may have much wider repercussions for cellular function. This is confirmed by phenotypes of mutants defective in one or more of the enzymes, and in transgenic plants with altered levels of the enzymes due to overexpression or antisense/RNAi constructs. Genes for the majority of the thirty or so enzymes of the pathway have been identified in *Arabidopsis thaliana*, and we have been studying their expression with microarray analysis in wild-type and transgenic/mutant plants, in combination with metabolite profiling of biosynthetic intermediates by LC/MS. This has enabled us to start to build predictive models of the pathway, which enables identification of key regulatory points. At the same time the tools have demonstrated that regulation of expression of LHC genes is not directly correlated to levels of the intermediate Mg-protoporphyrin IX, as had previously been suggested.



SUBUNIT INTERACTIONS AND MECHANISM OF THE *RHODOBACTER CAPSULATUS* MAGNESIUM CHELATASE COMPLEX: INSIGHTS FROM ENZYME KINETIC STUDIES

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The biosynthetic pathway for (bacterio)chlorophyll is initiated by magnesium chelatase (BchI, BchD, BchH). This first step involves insertion of magnesium into protoporphyrin IX, a process that requires ATP hydrolysis. The next step in (bacterio)chlorophyll synthesis is the SAM dependent methylation of magnesium protoporphyrin catalysed by BchM. Structural information shows the BchI and BchD subunits form a double-hexameric enzyme complex while BchH binds protoporphyrin IX and can be purified as BchH-protoporphyrin. Utilizing the *Rhodobacter capsulatus* magnesium chelatase subunits, data from continuous magnesium chelatase assays can be analyzed such that the BchD subunit is treated as the enzyme with both BchI and BchH-protoporphyrin IX as substrates. Michaelis-Menten type kinetics was observed with the BchI subunit whereas the BchH subunit exhibited sigmoidal kinetics with positive cooperativity (Hill coefficient of 1.85). The BchI:BchD complex had intrinsic ATPase activity and addition of BchH caused a large increase in ATPase activity. The stimulation of ATPase activity by BchH was concentration dependent and yielded sigmoidal kinetics which indicates that there is more than one binding site for the BchH subunit on the BchI:BchD complex. The increased ATPase activity initiated by the BchH subunit continued despite cessation of magnesium chelation activity and this indicates one or more secondary roles for ATP hydrolysis, and possibly an as-yet unknown switch required to terminate ATPase activity. One of the secondary roles for BchH stimulated ATP hydrolysis by a BchI:BchD complex is in the correct binding of protoporphyrin to BchH to form BchH-protoporphyrin which is subsequently capable of participating in magnesium chelation activity. These data suggest that ATP hydrolysis by the BchI:BchD complex causes a series of conformational changes in BchH to effect substrate binding, magnesium chelation and product release. Testing BchM as a potential moderator of ATP hydrolysis revealed that this subunit interacts with BchH in a 1:1 ratio and kinetic data suggest channeling between the magnesium chelatase and BchM.

CYANOBACTERIAL FERROCHELATASES: STRUCTURAL AND FUNCTIONAL ROLES OF THE C-TERMINAL DOMAIN CONTAINING A PUTATIVE CHLOROPHYLL-BINDING MOTIF

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Chlorophyll and heme share a common biosynthetic pathway branched at the point, where magnesium chelatase and ferrochelatase (FeCH) insert either magnesium for chlorophyll biosynthesis or ferrous iron for heme biosynthesis. An intriguing feature of cyanobacterial and chloroplast FeCH is the C-terminal extension, containing a putative chlorophyll-binding motif (CAB domain). This conserved CAB domain is connected to the FeCH catalytic core by a more variable spacer, however the functions of the spacer and the CAB domain are not known. In order to elucidate the role of both C-terminal domains we have constructed *Synechocystis* mutants expressing truncated versions of this enzyme. The Δh347 mutant lacking only the CAB domain exhibited increased accumulation of chlorophyll and decreased accumulation of FeCH substrate protoporphyrin IX, suggesting a regulatory function of the CAB domain in tetrapyrrole metabolism. Furthermore, by using 2D electrophoresis, protein purification and immunoprecipitation, the FeCH CAB domain was found to be responsible for the interaction of FeCH with a Photosystem II-related protein complex(es) containing chlorophyll binding antenna CP47, which has been shown previously to form a complex with the cyanobacterial small CAB-like proteins. Conversely, the Δh324 mutant lacking both the CAB domain and the spacer accumulated large quantities of protoporphyrin IX and grew much slower than the Δh347 strain. Indeed, measurements of FeCH in-vitro activities demonstrated a dramatic decrease in FeCH activity in the Δh324 mutant in comparison to control and to the Δ347 strain. Native purification of the 3xFLAG-tagged full-length and truncated FeCHs in *Synechocystis* and analysis of purified enzymes using gel filtration and native electrophoresis showed that whereas the full-length and the ΔH347 proteins form an active dimer, the ΔH324 FeCH is strictly monomeric. These results revealed an essential role of the ‘spacer' sequence for the dimerization of *Synechocystis* FeCH and suggested importance of dimerization for proper functionality of the enzyme. Together, these findings imply rather intrinsic structural role of the spacer region for the FeCH activity; in contrast to the CAB domain that appears to be a regulatory segment connecting the FeCH to the intracellular signaling network.

YELLOW-IN-THE-DARK MUTANTS IDENTIFY NOVEL NUCLEAR GENES REQUIRED FOR LIGHT-INDEPENDENT CHLOROPHYLL BIOSYNTHESIS IN *CHLAMYDOMONAS REINHARDTII*

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The enzymatic conversion of protochlorophyllide to chlorophyllide is light dependent in angiosperms, which explains why they are unable to green in the dark. In contrast, gymnosperms, mosses, many algae and photosynthetic bacteria have a light-independent protochlorophyllide reductase (LIPOR) that allows them to synthesize chlorophyll in the dark. Although all other chlorophyll biosynthetic enzymes are encoded in the nucleus in algae and plants, LIPOR is composed of three chloroplast-encoded subunits (ChlL, ChlN, and ChlB), which are homologous to the NifH, NifD, and NifK subunits of nitrogenase, respectively. Mutants of *Chlamydomonas reinhardtii* that are defective in LIPOR exhibit a yellow-in-the-dark phenotype (due to accumulation of carotenoids and lack of chlorophyll), and these classical y mutants were among the first mutants isolated in C. reinhardtii more than a half-century ago (1). In addition to chloroplast mutations affecting the chlL, chlN, and chlB genes, at least seven non-allelic nuclear y mutants have been characterized, all of which appear to affect the translation, assembly, and/or stability of the ChlL subunit of LIPOR (2). Using insertional mutagenesis, we have isolated a collection of new y mutants of *C. reinhardtii*. Genetic analysis and sequencing of insertion sites have enabled us to identify the genes that are affected in a classical mutant (y5) and a new mutant (y12). Y5 and Y12 are novel nuclear genes that encode chloroplast-targeted proteins necessary for LIPOR function. Analysis of these proteins will provide new insights into the regulation of expression, assembly, and/or stability of LIPOR.

1. Sager R (1955). Inheritance in the green alga *Chlamydomonas reinhardi*. Genetics 40: 476-489.

2. Cahoon AB, Timko MP (2000) Yellow-in-the-dark mutants of *Chlamydomonas* lack the CHLL subunit of light-independent protochlorophyllide reductase. *Plant Cell* 12: 559-568.

AYUMI TANAKA, Ph. D. Professor Ayumi Tanaka of the Institute of Low Temperature Science at Hokkaido University of Japan earned a bachelor's degree in Biology in 1977 and his doctorate in Botany in 1982, all from Kyoto University.

He has been a member of Hokkaido University since 1998. The major challenge of Tanaka's lab is to understand the basic processes of chlorophyll metabolism: They identified genes and enzymes involved in the metabolism in higher plants and cyanobacteria, re-evaluated the biosynthetic route and investigated its regulatory mechanisms. In addition, his lab has been elucidating the evolution of photosynthesis as well as various physiological processes of the plants such as germination, acclimation, senescence and cell death through the studies on chlorophyll metabolism. Agricultural application is also an important target for his lab. They attempts to modify chlorophyll metabolism to produce plants with some agricultural advantages, and to identify useful chemicals for controlling plant growth by monitoring their effects on chlorophyll metabolism. In conclusion, Tanaka sees everything through the small molecule of chlorophyll.

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PLENARY LECTURE 3. TETRAPYRROLE METABOLISM OF PHOTOSYNTHETIC ORGANISMS: DEGRADATIVE PATHWAYS & THEIR REGULATION

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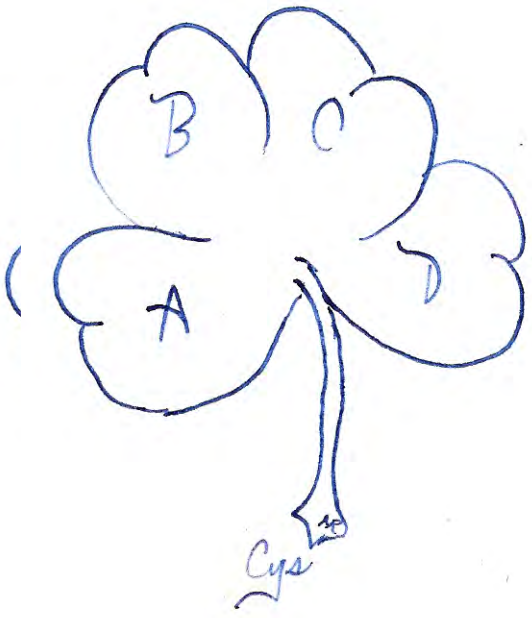
Chlorophyll molecules participate in the initial step of photosynthesis such as harvesting light energy and driving electron transport. During greening of etiolated tissues, chlorophylls are actively synthesized and finally incorporated into proteins. These chlorophyll-protein complexes are stoichiometorically assembled to form photosystems. During senescence, chlorophyll enters into degradation pathway after disassembly of the complexes into pigments and proteins. It had been considered that dephytylation of chlorophyll to chlorophyllide by chlorophyllase is the first step of chlorophyll degradation, however, recent study by Hörtensteiner's group showed that chlorophyllase is not involved in chlorophyll degradation pathway and proposed the following pathway; chlorophyll → pheophytin → pheophorbide. Although degradation pathway has now been determined and most of the enzymes identified, important questions remain to be answered.In this conference, I will discuss following topics based on mutant analysis and biochemical approach.

1. Disassembly of chlorophyll-protein complexes. Concerning the initial step of chlorophyll degradation, it is still unknown whether the disassembly of the complex is realized by proteases, chlorophyll degradation enzymes or other components such as chlorophyll chelating proteins. I will discuss the role of chlorophyll b reductase, which catalyzes the initial step of chlorophyll b degradation, in the LHCII disassembly process.

2. Regulation of chlorophyll degradation. Chlorophyll degradation requires reducing power, for example, pheophorbide a oxygenase and chlorophyll b reductase use reduced ferredoxin and NADPH, respectively. However, how reducing power is supplied for chlorophyll degradation is completely unknown. I will discuss the function of a novel flavoprotein which might be involved in the regulation of chlorophyll degradation.

3. Physiological function of chlorophyll degradation. We construct the CAO-overexpressing plants which accumulate a large amount of chlorophyll b. These mutants exhibited stay green phenotype. Interestingly, these mutants retain high protein level of RuBPCase and photosystems as well as mRNAs for these proteins during senescence. I will discuss whether retention of chlorophyll affects on the senescence process.



CHLOROPHYLL BREAKDOWN IN HIGHER PLANTS: UNEXPECTED DIVERSITY OF CATABOLITES

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In autumn, the de-greening of leaves (as a consequence of chlorophyll breakdown) and the emergence of the fall colors are highly visible signs of leaf senescence, a form of programmed cell death. The degradation of the green pigments in higher plants remained a remarkable mystery until about twenty years ago, but is believed now to follow a largely common and well-controlled catabolic path providing colorless products. When these chlorophyll catabolites were first identified in senescent leaves, they turned out to be colorless tetrapyrroles (typified as "nonfluorescent" chlorophyll catabolites - NCCs) which appeared to be the "final" degradation products. However, recent discoveries studying chlorophyll breakdown in leaves as well as fruit of higher plants revealed an unexpected divergence in the later stages of this chlorophyll degradation pathway. Our recent findings suggest chlorophyll breakdown to be more than a mere detoxification process. Two topics will be discussed to outline both, nature's biological diversity during leaf senescence and fruit ripening as well as the improved chemical methodology (NMR spectroscopy, mass spectrometry) to study such phenomena:

(i) Leaves and fruit of pear and apple trees accumulate identical nonfluorescent chlorophyll catabolites during senescence and ripening. They were found to be overlooked antioxidants.

(ii) Surprisingly ripening banana fruit [1] and senescing banana leaves accumulate different chlorophyll catabolites.

[1] Moser, S., Mueller, T., Ebert, M.O., Jockusch, S., Turro, N.J., Kraeutler, B. Blue Luminescence of Ripening Bananas. *Angew. Chem. Int. Ed.* 2008, 47, 8954-8957.

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THE ROLE OF ARABIDOPSIS ACD2 IN PROGRAMMED CELL DEATH

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Arabidopsis ACCELERATED CELL DEATH 2 (ACD2, also called Red Chlorophyll Catabolite Reductase) is a chloroplast protein with multiple functions. One of its proposed functions is to protect cells from porphyryn-related molecules like protoporphyrin IX (PPIX) that have photodynamic properties and cause cell death. Modulation of the levels of ACD2 strongly affects PPIX and pathogen-induced cell death: loss of ACD2 results in excessive cell death whereas it's over production is cytoprotective. Cell death is highly correlated with the production of hydrogen peroxide (H_2O_2) in mitochondria. The binding of ACD2 to PPIX-like molecules might prevent their photo activation or convert them to other less reactive molecules. Using a tryptophan fluorescence-quenching assay, we find that purified rACD2 binds to PPIX. In vitro ACD2 appears to reduce the generation of singlet oxygen ($^1O_2$), assayed by using the fluorescent $^1O_2$-reactive dye called Singlet Oxygen Sensor Green (SOSG), generated from photo activated PPIX. Using confocal microscopy and SOSG, we also visualized the generation of $^1O_2$ in mitochondria of PPIX-treated wild-type and light-treated acd2 protoplasts respectively. The $^1O_2$ quencher vitamin B6 partially rescued cell death in acd2. This suggests a role for mitochondrial $^1O_2$ in cell death activation that undergo cell death. To date, both $^1O_2$ and H_2O_2 in the mitochondria of cells are implicated to cell death activation. To test this further, we overexpressed and targeted the antioxidant enzymes, thylakoid ascorbate peroxidase and catalase exclusively to the mitochondria. This resulted in enhanced cell viability of the acd2 mutant, suggesting that in addition to $^1O_2$, mitochondrial H_2O_2 also contributes to cell death activation. The involvement of mitochondria in cell death is further implicated since exclusive targeting of ACD2 to mitochondria prevents cell death in acd2 mutant. From all the above observations we propose that the previously observed dynamic localization of ACD2 to mitochondria is important for protecting plants from cell death mediated by porphyrin-related molecules during stress and pathogen attack.

BIOSYNTHESIS OF PHYTOBILINS IN GREEN PHOTOSYNTHETIC EUKARYOTES

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Phytobilins are open-chain tetrapyrrole molecules that mainly function as chromophores of light-harvesting phycobiliproteins and light-sensing phytochromes in photosynthetic organisms. In the phytobilin biosynthesis pathway, biliverdin IXα is converted to different phytobilins by members of ferredoxin-dependent bilin reductase (FDBR) family with distinct double bond reduction activities. It has been shown for a long time that HY2 (LONG HYPOCOTYL 2) is the only FDBR in land plants which encodes a phytochromobilin synthase. However, we identified another homolog of FDBRs from moss *Physcomitrella patens*. The novel plant-type FDBR has the same enzymatic activity as PebA, the FDBR member commonly found in cyanobacteria and red algae that converts biliverdin IXα to 15,16-dihydrobiliverdin. Cyanobacterial homologs of PebA have been known to function in the biosynthesis of light-harvesting chromophore for photosynthesis, but the similar function seems to be lost in higher plants during evolution. We also found homologs of PebA in *Selaginella mollendorffii* (fern), *Ostreococcus* and *Micromonas* (green algae), suggesting that PebA is important in the green lineage from cyanobacteria to lower plants. PpPebA is localized in chloroplasts similar to PpHY2. PpPEBA is highly expressed at gametophore and regulated by light. As far as we know, this is the first evidence to show the phytobilin biosynthesis gene in eukaryotic photosynthetic organisms is light-modulated. Preliminary data showed gametophores of PpPEBA-knockout mutant developed pale-green phenotype under nutrient-limited condition compared to wild-type, revealing the importance of PpPebA in *Physcomitrella*. More functional analysis on PpPebA is in progress and will be presented in the meeting. We believe this discovery will provide the insight into the mechanism and evolutionary trend of light-harvesting or light-sensing system in photosynthetic organisms.

Christopher Neil Hunter, Ph. D. Professor C. Neil Hunter of the Department of Molecular Biology and Biotechnology, University of Sheffield, earned bachelor's degrees in biological sciences in 1975 from Leicester University, a doctorate in biochemistry in 1978 from Bristol University and a D. Sc., from Bristol University in 1996. He currently is the Krebs Professor of Biochemistry at Sheffield University, and has been a past recipient of prestigious SERC and EMBO Fellowships. Well known for his work on the structure and function of the photosynthetic apparatus of purple bacteria, he was one of the first to leverage the power of molecular genetics to these questions, while also making key contributions to the enzymology of tetrapyrrole and carotene metabolism in plants and photosynthetic bacteria. In recognition of his scientific contributions, he was elected Fellow of the Royal Society in May 2009.

C. Neil Hunter developed molecular genetic tools for *Rhodobacter sphaeroides* and used them to map and clone all of the photosynthesis-related genes in this model organism. He has pioneered the use of protein engineering for biophysical studies of light harvesting in bacteria identifying components corresponding to the red shifts in chlorophyll proteins, and providing the first spectroscopic data on isolated, membrane-bound reaction centers. The latter have facilitated crystallization and projection-structure-determination of the LH2, LH1 and LH1-RC complexes of *R. sphaeroides*. His laboratory has defined the order of assembly of the photosynthetic unit, and established the importance of carotenoids in LH assembly. C. Neil Hunter has been a major contributor to the enzymology of chlorophyll biosynthesis, notably magnesium chelatase (the first committed step), recently applying ultrafast techniques to study catalysis by NADPH:protochlorophyllide oxidoreductase with key collaborators. His recent work on nanofabrication and structural analysis of higher ordered order structures of photosynthetic light harvesting assemblies offers great potential for design of bio-inspired artificial photosynthetic systems.

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PLENARY LECTURE 4. ARCHITECTURE OF THE PHOTOSYNTHETIC LIGHT HARVESTING APPARATUS

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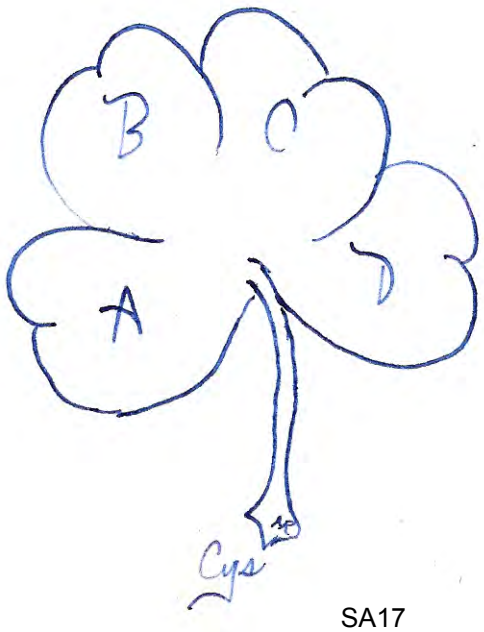
Light harvesting and energy trapping in photosynthesis is achieved by macromolecular membrane assemblies which bind thousands of chlorophylls, held in specific orientations and in close proximity to one another in order to ensure efficient energy transfer. The structures of light harvesting and reaction center complexes, determined by

crystallographic methods over the last 25 years, have been profoundly important in furthering our understanding of these early stages in photosynthesis. Thus, we know a great deal about the internal arrangements of chlorophyll-protein complexes that foster efficient harvesting of solar energy, its transmission to reaction centers, and trapping of this excitation energy as a stable charge separation. Given that many light harvesting and reaction center complexes are found grouped together in photosynthetic membranes, we need to understand the next level of structural information, namely the supramolecular organisation of individual complexes to form a 'photosynthetic unit'. The size and irregular structures of such assemblies preclude analysis by crystallographic and other averaging methods. However, in the simplest case of bacterial photosynthesis, atomic force microscopy has made inroads into this difficult structural problem, revealing the architecture of photosynthetic membranes in sufficient detail to allow docking of atomic structures into membrane maps. Combinations of atomic force microscopy, linear dichroism, cryo-electron microscopy and computational methods have allowed computation of models of whole membrane assemblies which can take into account spectroscopic data on energy transfer and trapping. Such models are starting to address the collective behaviour of whole membrane assemblies, to make predictions of the energy transfer and trapping behaviour of large-scale arrays, and to identify desirable design motifs for artificial photosynthetic systems. New surface chemistries and patterning methods are being developed to facilitate the creation of innovative architectures for coupled energy transfer and trapping. Nanometer and micron-scale patterns of photosynthetic complexes have been fabricated on self-assembled monolayers deposited on either gold or glass using near-field photolithographic methods. Such artificial light-harvesting arrays will advance our understanding of natural energy-converting systems, and could guide the design and production of proof-of-principle devices for biomimetic systems to capture, convert and store solar energy.



MOLECULAR STRUCTURE OF A CHLOROSOME SOLVED: ALTERNATING SYN-ANTI BACTERIOCHLOROPHYLLS FORM CONCENTRIC HELICAL NANOTUBES IN CHLOROSOMES

Alfred R. Holzwarth, Swapna Ganapathy, Gert T. Oostergetel, Piotr T. Wawrzyniak, Michael Reus, Aline Gomez Maqueo Chew, Francesco Buda, Egbert J. Boekema, Donald A. Bryant, Huub J. M. de Groot

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Chlorosomes are the largest and most efficient light-harvesting antennae found in nature, and they are constructed from hundreds of thousands of self-assembled bacteriochlorophyll (BChl) c, d or e pigments. Since they form very large and compositionally heterogeneous organelles, they had been the only photosynthetic antenna system for which no detailed structural information was available. In a novel approach, the structure of a member of the chlorosome class was determined and compared with the wild type to resolve how the biological light harvesting function of the chlorosome is established. By constructing a triple mutant, the heterogeneous BChl c pigment composition of chlorosomes of the green sulfur bacteria Chlorobaculum tepidum was simplified to nearly homogeneous BChl d. Computational integration of two different bio-imaging techniques, solid-state NMR and cryoEM, revealed a previously undescribed syn-anti stacking mode and showed how ligated BChl c and d self-assemble into coaxial cylinders to form tubular-shaped elements. A close packing of BChls via π-π-stacking and helical H-bonding networks present in both the mutant and in the wild type forms the basis for ultrafast, long-distance transmission of excitation energy. The structural framework is robust and can accommodate extensive chemical heterogeneity in the BChl side chains for adaptive optimization of the light-harvesting functionality in low-light environments. In addition, syn-anti BChl stacks form sheets that allow for strong exciton overlap in two dimensions enabling triplet exciton formation for efficient photoprotection (1).

(1) Ganapathy, S., Oostergetel, G.T., Wawrzyniak, P.K., Reus, M., Gomez Maqueo Chew, A., Buda, F., Boekema, E.J., Bryant, D.A., Holzwarth, A.R., and de Groot, H.J. (2009). Alternating syn-anti bacteriochlorophylls form concentric helical nanotubes in chlorosomes. *Proc. Natl. Acad. Sci.* USA 106, 8525-8530.

CRYSTAL STRUCTURE OF PLANT LIGHT-HARVESTING COMPLEX SHOWS THE ACTIVE, ENERGY-TRANSMITTING STATE

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The plant light-harvesting complex of photosystem II (LHC-II) collects most of the solar energy in the biosphere. LHC-II is the prototype of a highly abundant and highly conserved family of membrane proteins that fuels plant photosynthesis in the conversion of excitation energy into biologically useful chemical energy. In the past three decades, evidence has accumulated that LHC-II also plays an important role in the controlled dissipation of excess excitation energy under light stress conditions, in a process known as non-photochemical quenching (NPQ). In the centre of recent attention has been the energy-dependent component of NPQ (qE), by which plants adapt to rapidly fluctuating light conditions. The exact details of the qE mechanism are largely unknown, but it is widely believed to involve a conformational change of LHC-II that creates a quenching centre of two neighboring pigments within the complex. We performed a thorough investigation of the fluorescence properties of LHC-II crystals using a dedicated laboratory at the European Synchrotron Radiation Facility (1), so as to assess whether the structure reflects the energy-transmitting, or energy-dissipative, state. The strong, orientation-dependent fluorescence emission and comparatively long fluorescence lifetimes of single LHC-II crystals indicate that the complex is unquenched, and that therefore the crystal structure shows the active, energy-transmitting state of LHC-II (2). We propose that quenching of excitation energy in the light-harvesting antenna is due to the molecular interaction with external pigments in vitro or other pigment-protein complexes such as PsbS in vivo, and does not require a conformational change within the complex.

(1) Royant *et al* (2007) Advances in spectroscopic methods for biological crystals. Part 1. Fluorescence lifetime measurements. *Journal of Applied Crystallography* **40**:1105-1112 (2) Barros *et al* (2009) Crystal structure of plant light-harvesting complex shows the active, energy-transmitting state. *The EMBO Journal* **28**:298-306

PHYCOBILIPROTEIN BIOSYNTHESIS IN CYANOBACTERIA: STRUCTURE AND FUNCTION OF ENZYMES INVOLVED IN BILIN ADDITION

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Phycobiliproteins are soluble light-harvesting proteins and are highly fluorescent due to the addition of one or more linear tetrapyrrole chromophores (bilins) attached via a thioether linkage to cysteine residues within the α and β subunits, usually by enzymes called bilin lyases. Genes encoding putative bilin lyases in the cyanobacterium *Synechococcus* sp. PCC 7002 were identified and characterized using comparative genomics, reverse genetics, in vitro enzyme assays of the recombinant proteins, and in vivo heterologous expression in *E. coli*. Two new families of bilin lyases that are involved in phycobiliprotein biosynthesis in *Synechococcus* sp. PCC 7002, first identified in Fremyella diplosiphon as cpeS and cpeT, were characterized. There are three cpeS-like genes (denoted cpcS, cpcU, cpcV) and one cpeT-like gene (denoted cpcT) within the genome of *Synechococcus* sp. PCC 7002. The CpcS and CpcU proteins form a heterodimer (1:1) and catalyze the addition of phycocyanobilin (PCB) to Cys-82 on CpcB (β-phycocyanin) and most allophycocyanin subunits (ApcA, ApcB, ApcD, and ApcF). CpcT attaches PCB to Cys-153 on CpcB. The ApcE protein is a large linker protein (LCM99) with an allophycocyanin-like (AP-like) domain in the amino-terminal portion of the protein and several repeated linker domains which bind together the core of the light-harvesting complex. The AP-like domain of ApcE was shown to have intrinsic bilin lyase activity and to contain a region with similarity to CpcS/CpcU. Many cyanobacteria have one CpcS/CpeS protein that is capable of bilin attachment. We hypothesized that these proteins may form homodimers. Consistent with this, a 3D structure of CpcS-III from *Thermosynechococcus elongatus* BP1 (tll1699) was recently solved at 2.8 Å, and it crystallized as a homodimer. This is the first structure solved for any bilin lyase, and we have verified it is the Cys-82 bilin lyase for attaching PCB to CpcB, ApcA and ApcB by in vitro enzyme assays. Now, we hope to gain more mechanistic insight into how these enzymes catalyze the specific addition of bilins to their phycobiliprotein substrates.

Gary W. Brudvig, Ph. D. Professor Gary Brudvig of the Department of Chemistry at Yale University received a B.S. in Chemistry from the University of Minnesota in 1976 and a Ph.D. in Chemistry from the California Institute of Technology in 1981 where he worked with Sunney Chan on the metal centers in cytochrome c oxidase. Dr. Brudvig conducted his postdoctoral studies as a Miller Fellow at the University of California, Berkeley where he learned about photosynthesis while studying with Kenneth Sauer. Dr. Brudvig has been a member of the Yale faculty since 1982 and is currently the Eugene Higgins Professor of Chemistry as well as a Professor of Molecular Biophysics and Biochemistry. Professor Brudvig has published over 220 papers. His research examines the molecular basis of water oxidation in plant photosynthesis in order to understand how nature converts light energy into chemical energy. Using insights gained from the natural system, he is working to develop a system that will efficiently produce renewable fuel by using water-oxidation catalysts attached to nanostructured TiO_2.

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PLENARY LECTURE 5. THE LIGHT SIDE: PHOTOSYNTHETIC ENERGY/ELECTRON TRANSFER

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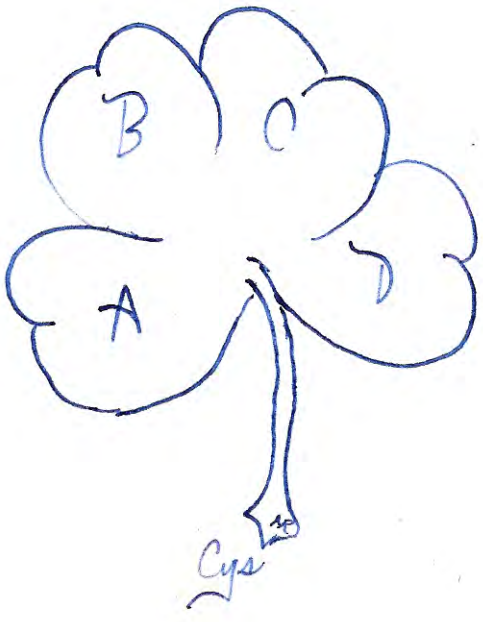
An overview of the light energy conversion processes in photosynthesis will be presented. Two specific research programs will be discussed: one on the water oxidation chemistry of photosystem II and another on the development of artificial systems for solar fuel production. Photosystem II, in all green plants, algae and cyanobacteria, uses light energy to split water into protons, electrons and O_2. In this reaction, Nature has solved the difficult chemical problem of efficient four electron oxidation of water to yield O_2 without significant amounts of reactive intermediate species such as superoxide, hydrogen peroxide and hydroxyl radicals. In order to use Nature's solution for the design of artificial catalysts that split water, insights from research on photosynthetic systems are being applied to develop biomimetic photochemical processes for water oxidation and fuel production.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STRUCTURAL INSIGHT INTO THE RADICAL INTERMEDIATE OF PHYTOBILIN BIOSYNTHESIS BY THE FERREDOXIN DEPENDENT BILIN REDUCTASE ENZYME PCYA

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Phytobilins are heme-derived linear tetrapyrroles that perform light sensing roles in oxygenic photosynthetic organisms including green plants, cyanobacteria, and red algae. When attached to proteins (biliproteins), phytobilins function as both light detectors and light-harvesting antennae. Since biliproteins are necessary for adaptation to a changing light environment, phytobilin synthesis is critical for survival of these organisms. The phycocyanobilin:ferredoxin oxidoreductase (PcyA) is a member of the ferredoxin-dependent bilin reductase (FDBR) family of catalysts. PcyA is responsible for the formation of phycocyanobilin - the direct precursor of the chromophores of the photoreceptor phytochrome and the phycobiliprotein photosynthetic antennae. PcyA catalyzes the four-electron reduction coupled with a four-proton transfer to the substrate biliverdin IXα to generate phycocyanobilin. Since the single-electron carrier ferredoxin furnishes the electrons and PcyA is void of any metals or cofactors, the reaction mechanism proceeds through one-, two-, and three- electron reduced intermediates. Electron paramagnetic resonance spectroscopy (EPR) was used to confirm the presence of the one- and three-electron reduced radical intermediates. We previously determined the crystal structure of wildtype PcyA in the absence of biliverdin substrate (Tu *et al,* 2007). Recent work reveals that altering critical residues near the active site (H88Q or D105N) can stabilize the radical intermediates for many hours, allowing the structural investigation of these radical intermediates. Crystals of both mutants have been grown that diffract x-rays to 1.3Å resolution. A surrogate reducing agent of sodium dithionite was used to soak crystals of a PcyA-biliverdin complex to generate the one-electron reduced radical intermediate. Single-crystal EPR experiments with soaked crystals confirm the existence of the dithionite-generated reduced radical (Stoll *et al,* 2009). The radical structure revealed the removal of an ordered water molecule in the active site. Additionally, small changes in the planarity of D-ring and A-ring pyrrole groups of the substrate were observed in the radical structure, which could explain the preference of D-ring reduction preceding A-ring.

Tu, S.-L, Rockwell, N. C., Lagarias, J. C., and Fisher, A. J. (2007). Insight into the radical mechanism of phycocyanobilin:ferredoxin oxidoreductase (PcyA) revealed by X-ray crystallography and biochemical measurements. *Biochemistry* **46**:1484-1494.

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SUBSTRATE RADICAL INTERMEDIATES IN CYANOBACTERIAL BILIN REDUCTASES

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Phycocyanobilin:ferredoxin oxidoreductase (PcyA) is a member of a group of cyanobacterial bilin reductases that regioselectively reduce one or two double bonds of biliverdin IXα to produce linear tetrapyrrole chromophores that are used to capture light for photosynthesis and to detect light in phytochrome photoreceptors. In these bilin reductases, the reductions proceed via substate-centered radical intermediates and involve successive steps of electron and proton transfers. The enzymes are highly unusual as they directly deliver the electrons from an extrinsic reductant to the substrate without the help of any cofactors. We present recent results from high-field EPR, ENDOR and ESEEM of the trapped radical intermediate in wild-type and mutants of PcyA from *Synechocystis* sp. PCC6803 together with density functional theory calculations that help to identify the exact nature of the radical intermediates and thus enable us to clarify the reaction mechanism.

CHIMERIC NITROGENASE-LIKE ENZYMES OF (BACTERIO)CHLOROPHYLL BIOSYNTHESIS

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The nitrogenase-like dark-operative protochlorophyllide oxidoreductase (DPOR) is involved in chlorophyll biosynthesis. Bacteriochlorophyll formation additionally requires the structurally related chlorophyllide oxidoreductase (COR). During catalysis homodimeric subunits L_2 of DPOR and X_2 of COR transfer electrons to their corresponding heterotetrameric catalytic subunits $(NB)_2$ and $(YZ)_2$, respectively. Various chimeric DPOR enzymes formed between recombinant subunits $(NB)_2$ and L_2 from *Chlorobaculum tepidum*, *Prochlorococcus marinus* and *Thermosynechococcus elongatus* were found enzymatically active indicating a conserved docking surface. Biotin label transfer experiments revealed the interaction of *P. marinus* L_2 with both subunits, N and B of the $(NB)_2$ tetramer. Based on these findings and on structural information from the homologous nitrogenase system a site-directed mutagenesis approach yielded ten DPOR mutants for the characterization of amino acid residues involved in protein-protein-interaction. Surface exposed residues Tyr127 of subunit L, Leu70 and Val107 of subunit N and Gly66 of subunit B were found essential for *P. marinus* DPOR activity. Next, the L_2 part of DPOR was exchanged with electron-transferring X_2 subunits of COR and $NifH_2$ of nitrogenase. Active chimeric DPOR was generated via combination of X_2 from *C. tepidum* or *Roseobacter denitrificans* with $(NB)_2$ from *C. tepidum*. No DPOR activity was observed for the chimeric enzyme consisting of $NifH_2$ from *Azotobacter vinelandii* and $(NB)_2$ from *C. tepidum*, *P. marinus* and *T. elongatus*.

Donald A. Bryant, Ph. D. Professor Don Bryant of the Dept. of Biochemistry and Molecular Biology at The Pennsylvania State University grew up on a dairy farm in Kentucky and earned his bachelor's degree in chemistry in 1972 from the Massachusetts Institute of Technology and his doctorate in molecular biology in 1977 from the University of California, Los Angeles. After postdoctoral training in microbiology at the Institut Pasteur in Paris (1977-1979) and in biophysics at Cornell University (1979-81), he joined the Penn State faculty in 1981 as an assistant professor. Rising through the ranks to associate professor (1986) and professor (1991), Dr. Bryant has held the Ernest C. Pollard Chair in Biotechnology since 1992. He also currently holds a partial appointment as Research Professor in the Dept. of Chemistry and Biochemistry at Montana State University (Bozeman, MT).

An author or co-author of 250 publications, since 1972 Bryant's research has focused on the biochemistry, physiology, molecular genetics, and genomics of chlorophototrophic bacteria, especially cyanobacteria and green sulfur bacteria. He is best known for his structural and functional studies on light-harvesting antenna systems, including phycobilisomes and chlorosomes, and on Type-I reaction centers. He recently described the first chlorophototroph from the phylum *Acidobacteria*. His laboratory has sequenced more than 30 genomes from chlorophototrophs belonging to five different bacterial phyla. The data have allowed the elucidation of pathways for the synthesis of bacteriochlorophyll *c* and various carotenoids, including the novel aromatic carotenoid, synechoxanthin. Recent studies in the Bryant laboratory have focused on using Photosystem I for biohydrogen production and on the development of the cyanobacterium *Synechococcus* sp. PCC 7002 as a platform for biofuels production.

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PLENARY LECTURE 6. *SYNECHOCOCCUS* SP. PCC 7002: A ROBUST AND VERSATILE CYANOBACTERIAL PLATFORM FOR BIOFUELS DEVELOPMENT

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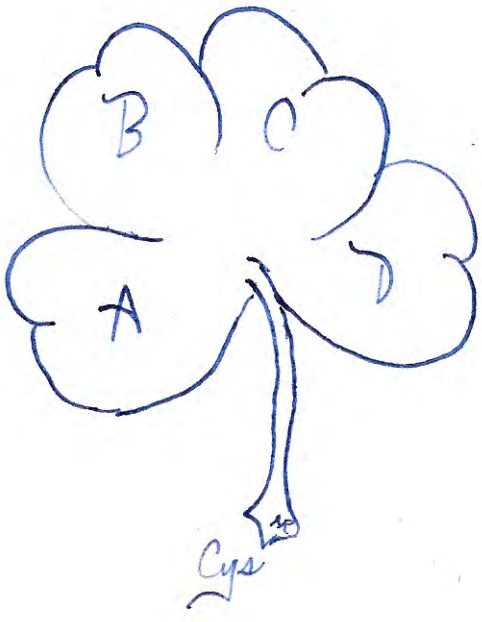
Synechococcus sp. PCC 7002 is a euryhaline, unicellular cyanobacterium that presents many opportunities as a platform for metabolic engineering for biofuels production. This cyanobacterium has the fastest known doubling time (~3 h under optimal conditions) and

is extremely tolerant of very high light intensities (up 3 suns). Importantly, this cyanobacterium is efficiently and naturally transformable with linear and circular DNA molecules, and homologous recombination is extremely efficient. *Synechococcus* sp. PCC 7002 can be grown heterotrophically in the absence of Photosystem I and Photosystem II with glycerol as the carbon and energy source. The *Synechococcus* sp. PCC 7002 genome is small and encodes about 3450 genes is small (~3.4 Mb). It has been completely sequenced and annotated and is available in public databases. The genome consists of 7 circular DNA molecules. The chromosome is 3.008 Mb, and the organism naturally harbors six plasmids that range in size from 4.8 kb to 186 kb. Although some plasmids (e. g., pAQ6 and pAQ7) have copy numbers per cell that are equal to the chromosome copy number, the smaller plasmids (pAQ1, pAQ3, pAQ4, and pAQ5 are present in present at copy numbers ranging from 3 to 10 times the copy number of the chromosome. Using these plasmids, systems for genetic complementation and protein overproduction have successfully been developed for this organism. For example, thirteen genes encoding a relatively oxygen-tolerant bidirectional hydrogenase and its assembly factors from *Ralsonia eutropha* have been introduced and successfully expressed. Transcriptomic, proteomic, and metabolomic analyses of *Synechococcus* sp. PCC 7002 are in progress. Examples of attempts to modify the metabolism of this organism will be discussed.



GENOMES OF DIATOMS AND OTHER MARINE ALGAE REVEAL SOME TETRAPYRROLE SURPRISES

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Thanks to the recognition of the ocean as an important controller of global climate, and interest in harmful algal blooms as they impact both humans and fish, genomic sequencing of marine algae is proceeding at an accelerating pace. We now have finished genomes of a centric and a penate diatom (*Thalassiosira pseudonana* and *Phaeodactylum tricornutum*). A third diatom (*Fragilariopsis cylindrus*) that lives in Antarctic ice is almost complete. There are also genomes for an amazing miniature heterokont (*Aureococcus anophagefferens*) and the haptophyte *Emiliania huxleyii*. Assembly of the cryptophyte *Guillardia theta* is in progress. Comparison of nuclear and plastid genomes of three diatoms showed several genes that were in different stages of transfer from plastid to nucleus (1). However, some genes appear to have gone missing altogether, e.g. the acsF gene that encodes the oxidative cyclase. It is not found in either nuclear or plastid genomes of the diatoms, and there is no sign of a bchE homolog either. At 2 um, *Aureococcus* is about the size of a well-fed *E. coli*. What is a nice little cell doing with 64 genes for Chl a/c proteins? They are not simple duplicates but appear to represent lineage-specific amplification of certain clades, possibly related to the presence of 19-hexanoyloxy fucoxanthin. Although all the algae with Chl c are the result of secondary endosymbiosis involving a red alga and an non-photosynthetic eukaryote, the red algal ferrochelatase is unrelated to those of diatoms and plants (2). It is becoming clear that the genomes of single-cell photosynthetic eukaryotes are dynamic entities. Endosymbiotic gene transfer from plastid to nucleus is still ongoing, and at least in the diatoms there has been extensive acquisition of bacterial genes (3). Recent insights from our work on new genomes will be discussed.

(1) M.-P Oudot-Le Secq, J. Grimwood, H. Shapiro, E. V. Armbrust, C. Bowler & B. R. Green. (2007) Chloroplast genomes of the diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*: Comparison with other plastid genomes of the red lineage. *Mol. Gen. Gen.* 277: 427-439

(2) M. Oborník and B. R. Green. (2005) Mosaic Origin of the Heme Biosynthesis Pathway in Photosynthetic Eukaryotes. *Mol. Biol. Evol.* 22: 2343-2353. (3) Bowler *et al* (2008). "The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes." Nature 456: 239-244

PRODUCTION OF A NOVEL CHLOROPHYLL IN THE CHLOROPHYLL d-DOMINATED CYANOBACTERIUM *ACARYOCHLORIS MARINA* MBIC 11017

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Acaryochloris spp. has chlorophyll (Chl) d as a major Chl and also a small amount of Chl a. In addition, minor pigments, i.e. Chl d' for photosystem (PS) I, pheophytin a for PS II, were detected in respective complexes isolated from the type strain, *Acaryochloris marina* MBIC 11017 (1, 2). This feature is quite unique and differentiates A. marina from other oxygenic phototrophs. Although widespread distribution of Chl d in the world was revealed, *Acaryochloris* spp. is the only known species that produces Chl d at this time, and the importance of this organism in aquatic primary production has been recognized. Recently, the complete genome of *A. marina* was sequenced (3), however, lack of a genetic technique prevented us from studying *A. marina* intensively by a molecular genetic approach. Thus, we tried to develop the technique for gene transfer into *A. marina*, and we succeeded in producing a few transformants by introduction of foreign genes. Among them, we focused on a transformant that expresses a gene involved in Chl biosynthesis. The pigment composition of the transformant was analyzed by HPLC, and the accumulation of a novel pigment was found. Based on the absorption spectrum, we estimated it to be a Chl derivative. The properties of the novel Chl in vivo will also be discussed.

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2. T Tomo, T Okubo, S Akimoto, M Yokono, H Miyashita, T Tsuchiya, T Noguchi and M Mimuro (2007) Identification of the special pair of photosystem II in a chlorophyll d-dominated cyanobacterium. *Proc Natl Acad Sci* USA, 104: 7283.
3. WD Swingley, M Chen, PC Cheung, AL Conrad, LC Dejesa, J Hao, BM Honchak, LE Karbach, A Kurdoglu, S Lahiri, SD Mastrian, H Miyashita, L Page, P Ramakrishna, S Satoh, WM Sattley, Y Shimada, HL Taylor, T Tomo, T Tsuchiya, ZT Wang, J Raymond, M Mimuro, RE Blankenship and JW Touchman (2008) Niche adaptation and genome expansion in the chlorophyll d-producing cyanobacterium *Acaryochloris marina. Proc Natl Acad Sci* USA, 105: 2005.

COUPLING A WATER SOLUBLE DE-NOVO DESIGNED CHLOROPHYLL-BINDING PROTEIN TO A NATURAL ALLOPHYCOCYANIN ANTENNA

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The phycobilisomes (PBSs) of cyanobacteria and red algae are sophisticated and dynamic systems for effectively capturing incoming solar energy. These, and other natural light-harvesting (LH) complexes inspire the development of effective LH units and their coupling to the photochemical catalytic centers in man-made solar energy conversion devices. Additionally, the PBSs provide fascinating and unique examples of supra-molecular assembly of multiple pigment and protein components into a single, stable structure. Our experience, and recent success in designing de novo, and constructing novel proteins that can self-assemble and incorporate various chlorophyll derivatives, have led us to investigate a new strategy for exploiting the exceptional LH properties and unique modular architecture of natural PBSs. It is based on designing and constructing hybrid modular photosystems that may be utilized for solar energy conversion either in vitro as stand-alone molecular devices, or in vivo by heterologous expression in bacteria. More specifically, we plan to fuse natural PBS linker proteins to de novo designed protein-pigment complexes. The linker serves as an anchor for recruiting PBS components that self-assemble and form a light-harvesting unit attached to the de novo designed protein. The latter may be designed either as functional reaction center analogs, or as a relay of excitation energy transfer. Our first prototype is a fusion between HP49, a de novo designed chlorophyll-binding four-helix bundle protein, and Lc, the low molecular weight protein linker of allophycoyanine (APC). Fusing the water-soluble HP49 and the hydrophobic Lc resulted in a water-soluble protein. We show that the fusion protein is bifunctional, and capable of binding chlorophylls at the HP49 domain, and self-assemble with APC at the Lc domain. Moreover, the increased solubility of the fusion protein circumvents the difficulties of APC-Lc in vitro reconstitution, which previously required partly denaturing conditions due to the hydrophobic nature of Lc. Importantly, the new design makes it possible to explore energy transfer between the APC-Lc domain and chlorophylls bound to the HP49 domain. It proves the feasibility of our hybrid modular design approach and opens many new possibilities for coupling artificial, customized protein constructs to PBSs.

Joanne Chory, Ph.D. Joanne Chory is an Investigator with the Howard Hughes Medical Institute and is Professor at The Salk Institute for Biological Studies, where she directs the Plant Biology Laboratory. She is also Adjunct Professor of Biology at the University of California, San Diego.

Dr. Chory is interested in identifying the mechanisms by which plants respond to changes in their light environment. She and her lab members use the reference plant, *Arabidopsis*, to identify components of the phototransduction pathways that link changes in the light environment with differential growth and global alteration of the transcriptome. Their genetic analyses indicate that light responses are not simply endpoints of linear signal transduction pathways, but are the result of the integration of information from a variety of photoreceptors acting through a complex network of interacting signaling components. Studies from the Chory lab have also revealed that steroid hormones and auxin are involved in light-regulated development of plants. They have identified the plant steroid receptor, a plasma membrane localized receptor protein kinase, as well as characterized several components in the signaling pathway that link recognition of the steroid at the cell surface to changes in gene expression in the nucleus. In studies of relevance to this meeting, the Chory lab has done a large forward genetic screen for components of retrograde signaling from damaged plastids, and identified 5 loci that implicate the tetrapyrrole pathway in this response.

A native of Massachusetts, Joanne received an A.B. degree in biology with honors from Oberlin College, OH, a Ph.D. in microbiology from the University of Illinois at Urbana-Champaign, and conducted postdoctoral research at Harvard Medical School. In 1988, she joined the faculty of the Salk Institute, where she has remained. Joanne has served on numerous advisory committees and editorial boards and has been the recipient of multiple awards. She is a member of the U.S. and German National Academy of Sciences, the American Academy of Arts and Sciences, an associate member of EMBO, and is a fellow of the American Association for the Advancement of Sciences.

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PLENARY LECTURE 7. DO TETRAPYRROLES PLAY A ROLE IN RETROGRADE SIGNALING FROM THE CHLOROPLAST?

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Chloroplasts of higher plants contain about 3000 proteins of which more than 95% are encoded by nuclear genes. This necessitates a tight coordination of gene expression that involves two-way signaling between these spatially separated genomes. Nuclear genes that

regulate chloroplast development and chloroplast gene expression provide part of this coordinate control. There is also compelling evidence that information flows in the opposite direction, from chloroplasts to the nucleus. Multiple chloroplast-derived signals have been proposed to regulate nuclear gene expression, yet almost nothing is known of their signaling pathways. We designed a mutant screen in Arabidopsis to analyze these retrograde signaling pathways, and identified mutations in 5 genes that express nuclear-encoded photosynthetic genes in the absence of proper chloroplast development. Four of these loci (gun2, 3, 4, and 5) encode enzymes in the tetrapyrrole pathway, mutations in which would be expected to reduce the levels of Mg-protoporphyrin IX, the first intermediate specific to the chlorophyll branch. Relying on old papers from the *Chlamydomonas* literature, as well as judicious use of inhibitors, feeding experiments, quantification of tetrapyrrole intermediates and the characterization of other tetrapyrrole pathway mutants, we proposed that accumulation of Mg-protoporphyrin IX in the plastid generates a signal that causes transcriptional repression of nuclear genes encoding plastid-localized proteins (1). Accumulation of Mg-proto IX appeared to be both necessary and sufficient to repress the expression of a large number of nuclear genes encoding plastid-localized proteins. Recently, several laboratories have questioned the validity of this model (2, 3). More precise tetrapyrrole measurements revealed that Mg-proto IX levels were not correlated with the expression of nuclear genes. In my talk, I will review the published data surrounding a tetrapyrrole-based signal. I will present our latest data in which new mutant screens again point to perturbations in the tetrapyrrole pathway as a primary signal for initiating retrograde signaling in plants.

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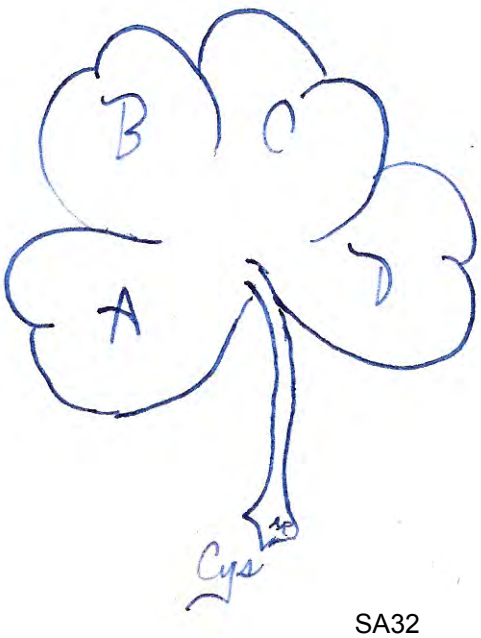
(2) Moulin, M., McCormac, A.C., Terry, M.J., and Smith, A.G. 2008 Tetrapyrrole profiling in Arabidopsis seedlings reveals that retrograde plastid nuclear signaling is not due to Mg-protoporphyrin IX accumulation, *PNAS* 105:15178-15183.
(3) Mochizuki, N., Tanaka, R., Tanaka, A., Masuda, T., and Nagatani, A., 2008, The steady-state level of Mg-protoporphyrin IX is not a determinant of plastid-to-nucleus signaling in Arabidopsis, *PNAS* 105:15184-15189.



DO REGULATORY FACTORS CONTROL DISTRIBUTION OF 5-AMINOLEVULINIC ACID FOR CHLOROPHYLL AND HEME SYNTHESIS?

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Control of tetrapyrrole biosynthesis plays an important role for the biogenesis of functioning chloroplasts. This essential metabolic pathway is regulated at different levels of expression and enzyme activation. Moreover, different amounts of the tetrapyrrole endproducts chlorophyll and heme are required under light/dark condition and during plant development. It is proposed that control mechanisms at the level of the rate limiting step of 5-aminolevulinic acid (ALA) synthesis ensure the channelling of metabolites towards the Mg or Fe branch of tetrapyrrole biosynthesis. GUN4 binds the chlorophyll intermediates Mg- and protoporphyrin, stimulates Mg chelatase activity and is implicated in plastidic retrograde signalling. We propose for GUN4 a role as a main key regulator for chlorophyll biosynthesis to integrate acclimation to environmental changes and endogenous stimuli, mainly at the post-translational level. During photoperiodic growth GUN4 deficiency prevents ALA synthesis for chlorophyll accumulation. All metabolic changes triggered by in the *gun4* knock-out mutant do not correlate with altered gene expression or hardly any change of protein abundance in tetrapyrrole biosynthesis. In a yeast two-hybrid approach a novel protein was identified showing protein interaction with *A. thaliana* GluTR. The new 30 kDa GluTR binding protein (GluTRBP) is localised in the plastidic stroma and attached to the thylakoid membranes. GluTRBP forms homodimers in plants and is present in almost all stages of plant development. Overproduction of GluTR and GluTRBP alters enormously tetrapyrrole biosynthesis leading to leaf necrosis. It is proposed that GluTRBP functions as an anchor protein for ALA synthesis in subcompartments of plastids and contributes to the redirection of tetrapyrrole metabolites. The deregulated control mechanism of ALA synthesis can be explained by means of analysis of gene expression and enzyme activities in the tetrapyrrole biosynthetic pathway under different growth conditions. A model is proposed to describe the involvement of GUN4 and GluTRBP in posttranslational regulation of ALA biosynthesis to sustain chlorophyll and heme synthesis under varying environmental conditions.

COORDINATED REGULATION OF CHLOROPHYLL BIOSYNTHESIS BY LIGHT AND PHYTOHORMONES IN ARABIDOPSIS

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For functional chloroplast biogenesis, light is prerequisite stimuli for coordinated transcriptional activation of key genes involved in photosynthesis and tetrapyrrole biosynthesis. Previously, we showed that in Arabidopsis roots, a light signaling transcription factor HY5 is essential for the chlorophyll biosynthesis as well as the chloroplast differentiation. Furthermore, cytokinin and auxin, respectively, positively and negatively affected the chlorophyll biosynthesis via transcriptional regulation. To obtain further insight, we generated promoter:GUS construct of CHLH, encoding the H subunit of Mg-chelatase, with (pCHLH-G:GUS) or without (pCHLHw:GUS) deletion of G-box (CTCGAG) which is presumed as HY5 binding site. Although pCHLHw:GUS showed normal GUS staining in roots, no GUS activity was detectable in pCHLH-G:GUS, suggesting the binding of HY5 to G-box is essential for the transcriptional activation in roots. We then observed histological localization of chlorophyll and HY5:YFP in Arabidopsis roots. Although the chlorophyll accumulation was limited in cortex cells, fluorescence from HY5:YFP was observed in almost all cells even in lateral roots. Cytokinin and auxin did not affect the HY5 protein stability. Thus, it is likely that HY5 is essential but not sufficient factor for chloroplast differentiation in Arabidopsis roots, and phytohormones may affect distinct pathway for transcriptional regulation. Recently, it is reported that GARP transcription factor GLK (golden2-like) controls the coordinate expression of the photosynthetic apparatus in Arabidopsis. Our transcriptome and qT-PCR analyses confirmed the phytohormones-dependent expression of GLK2 in Arabidopsis roots. Thus, it is probable that the coordinated expression of photosynthetic apparatus is regulated by two transcription factors, HY5 and GLK2, in Arabidopsis roots. On the contrary, in Arabidopsis cotyledons during photomorphogenesis, HY5 had only a limited function on chlorophyll biosynthesis. Mutant analyses, however, revealed that chlorophyll accumulation increased in auxin-signaling mutants *axr2*, *shy2* and *slr*, while decreased in a cytokinin receptor mutant *ahk2ahk3*. In auxin mutants, the enhanced chlorophyll accumulation was independent on the absence of HY5 or transcriptional activation of GLK. Consequently, the system of coordinated regulation of chlorophyll biosynthesis in roots is largely different from that of aerial organs, the complex regulation of which involves plural positive and negative regulatory factors originated from light and phytohormones signaling pathways.

A NEW GENETIC SCREEN USING *ARABIDOPSIS THALIANA* TO IDENTIFY PROTEINS INVOLVED IN CHLOROPLAST TO NUCLEUS ORGANELLE SIGNALING

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Although chloroplasts contain their own genomes, most of their genes were lost or transferred to the nucleus during evolution. Today, more than 95% of the approximately 3000 proteins present in chloroplasts are encoded in the nucleus, translated in the cytosol, and imported into the organelle post-translationally. This separation of genetic information necessitates the coordination of nuclear and organellar genome expression. Such regulation is accomplished, in part, by retrograde signals from the chloroplast to the nucleus to modulate gene expression in response to the status of the chloroplast. For example, when chloroplast biogenesis is blocked, a signal from the plastid is communicated to the nucleus to repress transcription of nuclear-encoded chloroplast proteins. Although the nature of this signal is not well understood, loss-of-function genetic screens have identified the tetrapyrrole biosynthetic pathway and several additional proteins as playing a role in chloroplast-to-nucleus organelle communication. To identify additional proteins involved and to further understand the role of tetrapyrroles in this signaling pathway, we devised a screen for gain-of-function mutants using activation tagging. *Arabidopsis thaliana* plants were mutagenized by the random insertion of T-DNAs that contain multimerized transcriptional enhancers from a viral promoter. Here we describe two recessive and one new dominant mutant allele that cause an impairment of organelle signaling when chloroplast biogenesis is blocked and discuss how they contribute to our understanding of organelle communication and its relationship to tetrapyrrole biosynthesis.

J. Clark Lagarias, Ph.D. Professor of Biochemistry, J. Clark Lagarias of the Department of Molecular and Cellular Biology at UC Davis earned bachelor's degrees in botany and chemistry in 1975 and his doctorate in chemistry in 1979, all from UC Berkeley. The recipient of the Paul K. and Ruth R. Stumpf Professorship in Plant Biochemistry in 1999, Dr. Lagarias was subsequently elected to the National Academy of Academy of Sciences in 2001. Known for his work in investigating the molecular and chemical bases of plant light perception, Lagarias' research interests hold great promise for improving crop yield through modification of their light responsiveness.

A member of the UC Davis faculty since 1980 and a co-investigator of the NSF Center for Biophotonics Science and Technology, Lagarias' research has focused on a group of light-receptor proteins called phytochromes. His lab has developed tools for producing phytochromes and phycobiliproteins in living cells, and has engineered fluorescent and constitutively active alleles of phytochrome for biotechnological and agricultural applications. The University of California has patented key technologies for producing phytochromes and phycobiliproteins in living cells derived from this work. A co-author of over 90 publications in peer-reviewed journals, Lagarias continues to make important basic discoveries in the development of light-modulated proteins that hold the potential for numerous biomedical, diagnostic and therapeutic applications.

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PLENARY LECTURE 8. EXPLOITING CONSTITUTIVELY ACTIVATED PHYTOCHOME ALLELES TO REGULATE PLANT DEVELOPMENT

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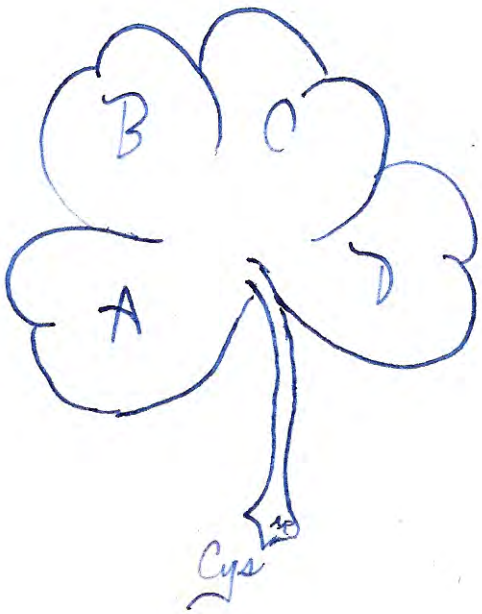
As a consequence of their sessile nature, plants must maximize the energy and nutritional resources available at the site where they are rooted. The most important of these resources is light, which sustains the process of photosynthesis. Through the diurnal cycle, the fluence rate can vary from zero at night to over 2000 μmol m^{-2} s^{-1} in full sunlight at midday. At high latitudes, day length also can widely throughout the year. Consequently, it is critical for plants to perceive the ambient light conditions to continuously optimize photosynthesis and to adjust the timing of flowering to anticipate the changes in season. Plants thus possess multiple families of photoreceptors that interpret the fluence rate, spectral quality duration and direction of light to facilitate adaptation at the cellular and organismal level. Among these, members of the red/far-red sensing phytochrome family arguably perform the most critical role as chlorophyll-containing antennae of plants are particularly optimized for gathering red

light. Phytochromes are biliproteins that can adopt photointerconvertible red and far-red light absorbing forms with distinct regulatory activities. Phytochrome photoconversion initiates changes in protein-protein interactions, intracellular protein translocation and protein degradation cascades that effect changes in gene expression, photosynthetic efficiency and changes in plant architecture and development. Many laboratories are engaged in research to elucidate these networks at the molecular level with the long-term goal being the ability to mitigate phytochrome-mediated responses deleterious to plant yield in high density agricultural settings. The recent discovery of a new class of dominant gain-of-function tyrosine-to-histidine (YH) phytochrome mutants (Su and Lagarias 2007 *Plant Cell* **19**:2124-2139) provides a powerful new tool to understand phytochrome signaling at the molecular level, but also the means to genetically engineer altered light sensitivity in crop plant species. Can we exploit these alleles to generate robust, environmentally sustainable crops of value to biofuels and plant-made products applications? Approaches to answer this question will be outlined in this talk.



CHARACTERIZATION OF A PALE GREEN PHENOTYPE OBSERVED IN RICE PHYTOCHROME B MUTANTS GROWN UNDER RED LIGHT IRRADIATION

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Rice phytochrome B (*phyB*) mutants grown under continuous red light irradiation (Rc) display a pale green phenotype whose seedlings contain only 20% of chlorophylls as compared with wild type (Nipponbare) seedlings and thylakoid membranes in their chloroplasts are poorly developed. It suggests that phyB-dependent signaling pathways are essential for complete development of chloroplasts. To approach the signaling pathways, we tried to isolate factors correlating with the phenotype through microarray analysis in which gene expression profiles in phyB mutants were compared with those in WT ones. We found that the gene for H subunit of Mg chelatase (OsChlH) was especially repressed in the phyB mutants, which seems to be the most probable cause for appearance of the phenotype. We carried out greening experiments in which seedlings grown in the complete darkness for 8 days were exposed to Rc and we examined transitions of transcript levels for genes involved in chlorophyll synthesis, chlorophyll precursor contents and chlorophyll accumulations during red-light induced greening. These results revealed that not only OsChlH but also OsGun4, both are activated by Rc irradiation, are significantly repressed in the mutants. In addition, contents of Mg protoporphyrin IX, a product of Mg chelatase, were severely diminished, too. These data suggest that the Mg chelatase activity fails in the phyB mutants under Rc irradiation, which might trigger to appear the pale green phenotype. I will also discuss physiological meaning of the pale green phenotype in terms of the typical shade avoidance responses.

INVOLVEMENT OF PHYTOCHROME A IN NEAR-ETIOLATION PHOTOMORPHOGENESIS IN RICE

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The synthesis of chlorophyll and the greening process in plants is mediated by cross talk between photoreceptors and phytohormones. Rice seedlings germinated and grown on germination paper in red light (400 μmoles photons m^{-2} s^{-1}) having their shoot-bottom exposed did not accumulate Chl. Rice seedlings grown under vermiculite in identical light regime having their shoot bottom covered in vermiculite were green and accumulated Chl. The perception of red light by the shoot bottom turned off the greening process and manifested near-etiolation morphogenesis. Nearly 50% suppression of the greening process was manifested by critical threshold of red light (150 μmoles photons m^{-2} s^{-1}). The red-light-induced near-etiolation response is substantially reversed by blue light. The suppression of photomorphogenesis was not a red-light-induced photo-bleaching effect or porphyrin induced photodynamic damage. The down-regulation of chlorophyll synthesis in red-light-grown non-green seedlings is accomplished by the down regulation of gene/protein expression of several enzymes involved in chlorophyll biosynthesis. Four-d-old homozygous *phyA* etiolated seedlings when transferred to red light accumulated substantially higher amounts of Chl than their WT background. This suggests that the near-etiolation photomorphogenesis in rice seedlings grown in red light having their shoot bottom exposed is mediated by high-irradiance response of PhyA. PHYB and PHYC could also be partially responsible for the suppression of photomorphogenesis. In the upstream signal transduction event the gene expression of heterotrimeric Gα and Gβ were down-regulated in seedlings grown without vermiculite having their shoot bottom exposed. The light signal perceived by PHYA present in shoot bottom activates downstream signaling cascade of CAM kinases resulting in phosphorylation of several soluble and thylakoid proteins leading to the suppression of greening process. Application of calmodulin antagonist W7 or TFP suppresses CaM kinases activity leading to down regulation of phosphorylation and restoration of greening process and chloroplast development.

PHYTOCHROME A TRANSGENIC TURF GRASSES

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Phytochrome A (phyA) is a far-red/shade light-sensing photoreceptor to suppress the shade-avoidance response (SAR) of plants to a shade stress. Previously, the PHYA gene has been introduced into crop plants such as tobacco, tomato, potato, rice, and wheat. In this study, we introduced the oat PHYA gene along with the bar gene into creeping bentgrass (*Agrostis stolonifera* L. cv. "Crenshaw") and Japanese lawngrass (*Zoysia japonica* Steud.). In addition, a hyperactive mutant of the PHYA gene, PHYA-Ser599Ala, was introduced into both types of turf grasses. Apparent phenotypes were compared to assess the suppression of SAR in different light environments. We also field-tested the phenotypes of transgenic Zoysia grass under natural conditions. In response to neighbor-cast shade stresses, the transgenic grasses exhibited phenotypes similar to those observed under lab conditions. The hyperactive phy A mutant transgenic grasses showed the highest chlorophyll content among various cultivars tested under greenhouse and outdoor conditions. Our genetically engineered turf grasses also contain the bar gene to confer herbicide-resistance on the plants. So, shade- and herbicide-tolerant turf grasses reported here offer economic advantages over wild-type grasses in reducing the frequency of mowing, irrigation, and herbicide application.

Roger Y. Tsien, **Ph.D.** was born in 1952, received his A.B. in Chemistry and Physics from Harvard College in 1972. He received his Ph.D. in Physiology in 1977 from the University of Cambridge and remained as a Research Fellow until 1981. He then became an Assistant, Associate, then full Professor at the University of California, Berkeley. In 1989 he moved to the University of California, San Diego, where he is an Investigator of the Howard Hughes Medical Institute and Professor in the Depts. of Pharmacology and of Chemistry & Biochemistry. His honors include First Prize in the Westinghouse Science Talent Search (1968), Searle Scholar Award (1983), Artois-Baillet-Latour Health Prize (1995), Gairdner Foundation International Award (1995), Award for Creative Invention from the American Chemical Society (2002), Heineken Prize in Biochemistry and Biophysics (2002), and shares of the Wolf Prize in Medicine (2004), Rosenstiel Award (2006), E.B. Wilson Medal of the American Society for Cell Biology (2008), and Nobel Prize in Chemistry (2008). He is a member of the National Academy of Sciences and the Royal Society. Dr. Tsien is best known for building molecules that report or perturb signal transduction inside living cells. These molecules, created by organic synthesis or by engineering naturally fluorescent proteins, have enabled many new insights into signaling via calcium, sodium, pH, cyclic nucleotides, nitric oxide, inositol polyphosphates, membrane and redox potential changes, protein phosphorylation, active export of proteins from the nucleus, and gene transcription. He is now developing new ways to target contrast agents and therapeutic agents to tumors and other sites of extracellular protease activity.

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PLENARY LECTURE 9. MAMMALIAN EXPRESSION OF INFRARED FLUORESCENT PROTEINS ENGINEERED FROM A BACTERIOPHYTOCHROME

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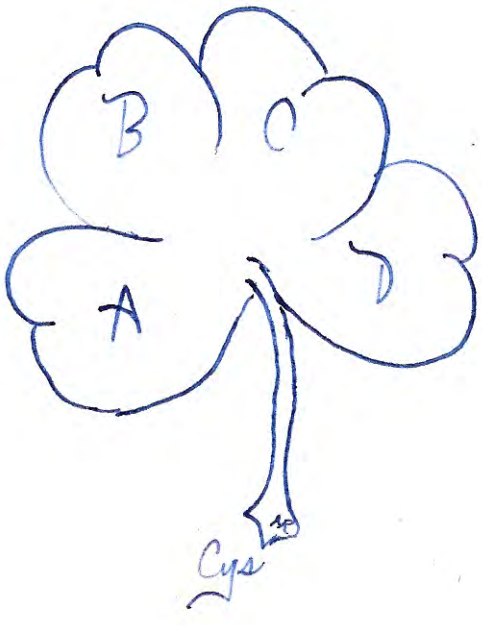
Green fluorescent protein (GFP) from jellyfish and its red homologs from corals have revolutionized many areas of molecular and cell biology, but the use of these visible FPs in intact animals, such as mice, has been handicapped by poor penetration of excitation light. Here we report that a bacteriophytochrome from *Deinococcus radiodurans*, incorporating biliverdin as the chromophore, can be engineered into monomeric, infrared-fluorescent proteins (IFPs), with excitation and emission maxima of 684 and 708 nm, respectively; extinction coefficient >90,000 M−1cm−1; and quantum yield up to 0.07 so far. IFPs express well in mammalian cells and mice and spontaneously incorporate biliverdin, which is ubiquitous as the initial intermediate in heme catabolism but has negligible fluorescence by

itself. Because their wavelengths penetrate tissue well, IFPs are suitable for whole-body imaging. Furthermore, IFPs can be used for in vivo quantitative fluorescence imaging and 3D reconstruction by fluorescence molecular tomography. The IFPs developed here provide a scaffold for further engineering.



STRUCTURAL AND FUNCTIONAL ANALYSIS OF TWO TYPES OF CYANOBACTERIAL PHYTOCHROMES

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The cyanobacterial phytochrome Cph1 from *Synechocystis* sp. PCC 6803 is highly homologous to plant phytochromes and can be overproduced as phycocyanobilin- (PCB) complexed holoprotein in suitably engineered *E. coli* strains. We crystallized and solved the structure of a variant that only misses the C-terminal effector domain, but comprises the complete sensory module at an effective resolution of 2.45 Å. Its overall structure is divided into a major lobe, consisting of the PAS / GAF bidomain and a minor lobe constituting of the PHY-domain. Both lobes are interconnected by a long α-helix and a tongue-like protrusion from the PHY-domain. The architecture of the PAS / GAF bidomain resembles the known phytochrome structures. The structure of Cph1Δ2 provides an unusual tripartite chromophore binding site, formed by the N-terminal α-helix, the GAF domain and a tongue-like feature protruding from the PHY-domain. Various mutants for analysing signal transfer from the PCB-binding site have been generated and spectroscopically analysed. The crystal structure of the Y263F mutant at 2.5 Å resolution shows like the wild type antiparallel pairing of the Cph1 monomers, but with altered arrangements of the PHY domains. Cph2 is the second family of phytochromes discovered in cyanobacteria. Unlike plant/Cph1-like phytochromes it lacks the N-terminal PAS as well as the PHY domains but has multiple GAF domains which can act as cofactor binding domains. We analysed the first two GAF domains of Cph2 from *Synechocystis* sp. 6803 which has three GAF domains with two cofactor binding sites. This phytochrome is unusual in the Cph2 family because it lacks the typical histidine kinase domain. Instead the output region consists of two GGDEF and an EAL domain which are supposed to regulate the c-di-GMP concentration. In contrast to the unclear function of Cph1 in *Synechocystis* sp. Cph2 deletion mutants show reduced growth under red light conditions. Furthermore Cph2 is supposed to inhibit phototaxis towards blue light. We produced PCB assembled Cph2 in vivo and showed that the first two GAF domains undergo photoconversion. The Pfr conformation is thermally instable and converts to Pr in the dark as a first order reaction. CD-spectroscopy as well as elution profiles of SEC revealed a great conformational difference between Pr and Pfr. Fluorescence measurements showed a typical fluorescence quantum yield for phytochromes, but an unusual low quantum yield of phototransformation. Lumi-R could not be detected by fluorescence measurements. Dark reversion and the huge Pfr to Pr ratio at the photoequilibrium suggest that the Pfr state is the physiologically active conformation.

CONFORMATIONAL CHANGES OF PHYTOCHROME CHROMOPHORE: COMBINING TIME-RESOLVED ABSORPTION SPECTROSCOPY AND SOLID STATE NMR TECHNIQUE

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The light induced reactions of the phytochrome chromophore have been followed by ultra-short (ps) absorption spectroscopy, employing structurally modified phycocyanobilin (PCB) derivatives (1). The formation of the initial photoproduct after light excitation (I700) follows multi-exponential kinetics, indicating heterogeneity already of the parent state Pr. Chromophores with a changed substitution pattern at ring D induce changes in the chromophore-protein interaction, evident from altered absorption properties of the (first) I700 photoproduct. Employment of uniformly or selectively labeled bilins allowed detecting selectively the chromophore conformation against the background of non-labeled protein by ^{13}C- and ^{15}N-solid state NMR. ^{15}N-labelling identified all four pyrrole nitrogen atoms as protonated in Pr and in Pfr (2). A comparison of ^{13}C-labelled PCB in the Pr and the Pfr state yielded greatest changes around ring D of the chromophore. The light-induced rearrangement of ring D causes steric constraints in the chromophore, which are compensated mostly by conformational rearrangements of ring C (3). Solid state NMR investigation of a pure Pfr state of the cyanobacterial phytochrome Cph1 (PAS-GAF-PHY construct), including back irradiation, yielded also the NMR spectra of two intermediates and thus gave insight into the Pfr-to-Pr conversion process. The data indicate that following the light-induced double bond isomerization the hydrogen-bonding network persists and a single bond rotation takes place. Only after these changes, also the hydrogen- bonding network rearranges and allows formation of the Pr state.

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TRACKING STRUCTURAL CHANGES WITHIN THE BILIN CHROMOPHORE DURING LIGHT SENSING IN PHYTOCHROME USING FEMTOSECOND STIMULATED RAMAN SPECTROSCOPY

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Photochemical interconversion between the red (Pr) and far-red (Pfr) absorbing form of the photosensory protein phytochrome initiates signal transduction transduction pathways in bacteria and higher plants that affect growth and development. The Pr-to-Pfr photoreaction is driven by an ultrafast bond isomerization at the C15=C16 methine-bridge within the bilin chromophore. Using femtosecond stimulated Raman spectroscopy (FSRS), we track the ultrafast nuclear dynamics during the isomerization by recording transient vibrational structural changes of the chromophore after excitation of Pr. The enhanced intensity of the C15-H hydrogen out-of-plane (HOOP) mode, and the appearance of red-shifted C=C stretch and N-H in-plane rock by 500 fs reveal the initial distortion of the C15=C16 bond to occur during the excited-state. In cyanobacterial phytochrome Cph1, the HOOP and skeletal modes evolve within 3 ps to a Lumi-R-like pattern in the excited-state, during which time 85% of the excited population relaxes back to Pr. The remaining 15% relaxes to the ground state Lumi-R photoproduct in 30 ps, revealing that the 15% photochemical quantum yield is determined within 3 ps after excitation [Dasgupta et al 2009 *Proc. Natl. Acad. Sci.* 106, 1784-1789]. Detailed vibronic analysis of the Pr absorption spectrum using resonance Raman intensities support 1) a homogenous photoexcitable Pr ground state population and 2) a moderate, but similar excited state displacement along the C=C stretch and the HOOP mode, emphasizing evolution on a multimodal nuclear coordinate during the ~150 fs Franck-Condon dynamics. To test these conclusions, more recent FSRS analysis of plant and cyanobacterial phytochromes substituted with isotope-labeled phycocyanobilin will be presented.

SPECTRAL AND STRUCTURAL CHARACTERIZATION OF A NOVEL CYANOBACTERIOCHROME-TYPE PHOTORECEPTOR ANPIXJ

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Phytochromes of plants, cyanobacteria and some bacteria are the photoreceptor superfamily that binds a linear tetrapyrrole and exhibits reversible photoconversion between the red-absorbing (Pr) and the far-red-absorbing forms. They exhibit photochemical isomerization between the C15-Z and C15-E isomers of the linear tetrapyrroles. GAF domain plays a crucial role in the function of the chromophore. Cyanobacteriochromes are the recently emerging photoreceptors in cyanobacteria whose GAF domains are related to but distinct from those of the phytochromes. Mutational studies in cyanobacteria have revealed that they are involved in various photoacclimation responses such as chromatic acclimation and phototaxis. GAF domains in the phototaxis regulator SyPixJ1 (from *Synechocystis* sp. PCC 6803) and TePixJ (from *Thermosynechococcus elongatus* BP-1) bind a phycoviolobilin and undergo the reversible photoconversion between blue-absorbing and green-absorbing (Pg) forms. Similar GAF domains identified in cyanobacterial genomes may function in novel photoreceptor proteins. Putative phototaxis regulator AnPixJ (from *Anabaena* sp. PCC 7120) binds PCB and shows reversible photoconversion between Pr and Pg forms (1). Time-resolved spectral analysis suggest that the Pr form of AnPixJ is almost equivalent to that of the phytochromes and starts a primary photoreaction with Z-to-E isomerization in a mechanism similar to that in the phytochromes, but is finally photoconverted to the unique Pg form. On the other hand, chromatic acclimation regulator SyCcaS (from *Synechocystis*) covalently binds PCB and shows similar reversible photoconversion between Pr and Pg forms (2). However, relationship of chromophore configuration and spectral properties is opposite between AnPixJ and SyCcaS. To understand the structural basis, we are now trying the X-ray crystallographic analyses (3). We determined the crystal structure of the GAF domain of AnPixJ in the Pr form (Narikawa *et al,* submitted). The backbone structure of the protein and the chromophore configuration are independently similar to those of the Pr form of bacterial phytochromes, although relative position of the chromophore bound to the protein is significantly deviated. Correspondingly, critical amino acid residues interacting with the chromophore are also diverged in position or orientation, and resultantly in the function. Together with the role of water molecules, a novel protonation mechanism of the chromophore is proposed. Unique features of a hydrophobic pocket to accommodate photoisomerization of the chromophore are discussed with regard to its unusual formation of the Pg form. We will discuss about structure-function relationship of the tetrapyrrole-based photoreceptors.

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2. Hirose Y, Shimada T, Narikawa R, Katayama M, & Ikeuchi M (2008) *Proc. Natl. Acad. Sci.* U. S. A. 105: 9528-9533.
3. Narikawa R, Muraki N, Shiba T, Ikeuchi M, & Kurisu G (2009) *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun*. 65: 159-162.

BILIPROTEIN-REGULATED PHOTOPERCEPTION AND PHOTOMORPHOGENESIS IN CYANOBACTERIA

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Photosynthetic organisms exhibit finely tuned abilities to sense and respond to changes in their ambient environment. The perception of light, i.e. photoperception, and the resulting metabolic and developmental changes that occur as a response to light signals are among the most important adaptations of any organism that uses light for carbon fixation and generation of reductant. The long-term research interest of my lab is to understand the dynamic molecular processes that photosynthetic organisms employ to adapt to changes in their photoenvironment. Our current research targets biliproteins, light-absorbing pigments centrally involved in both photosynthesis and the regulation of photomorphogenesis in cyanobacteria, algae, and plants. Photomorphogenesis is defined as the control of growth and development by light intensity and color. Related sensory biliproteins are involved in photomorphogenic responses of plants and cyanobacteria, including the optimization of photosynthetic efficiency and maximization of development and reproduction. We are investigating the regulation of these responses in the model plant Arabidopsis and the filamentous cyanobacterium *Fremyella diplosiphon*. Our studies in *F. diplosiphon* center on the role of the phytochrome-related biliprotein RcaE in regulating light-dependent changes in the protein composition of the photosynthetic light-harvesting complexes, i.e. phycobilisomes (PBS). Our recent results demonstrated that RcaE also regulates light-dependent changes in cell shape and filament morphology in *F. diplosiphon*. The light-dependent changes in PBS protein composition and cellular morphology occur maximally in response to green and red light and are core features of the light-dependent acclimation process known as complementary chromatic adaptation. Our ongoing studies employ molecular genetic, biochemical, and cell biological approaches to elucidate the roles of conserved motifs and domains of RcaE in its regulation of photomorphogenesis, as well as to identify and characterize downstream effectors involved in the RcaE signaling cascade and RcaE-dependent regulation of gene expression resulting in developmental changes. Our studies are leading to the identification of novel components centrally involved in the photoregulation of pigmentation and morphogenesis in *F. diplosiphon*.

CHROMOPHORYLATION OF PHYCOERYTHRIN BY THE LYASE CPES IN *PROCHLOROCOCCUS MARINUS*

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Phycobilisomes are the major light-harvesting complexes of cyanobacteria, rhodophytes and cryptophytes. These photosynthetic antenna proteins efficiently harvest light in the 'green gap' where chlorophylls absorb only poorly. This is achieved via linear tetrapyrrolic chromophores, known as bilins that are covalently bound via thioether bonds to specific cysteine residues of the apoproteins. Autocatalytic chromophore addition is rare whereas in most cases regio- and stereoselective chromophore attachment is supported by phycobiliprotein lyases. An increasing number of lyases has been identified in cyanobacteria but only few examples of the three known lyase types have been characterized in detail so far. Heterodimeric E/F-type lyases catalyze chromophore addition to the cysteine α-84 of phycocyanins and phycoerythrocyanins and can have an additional isomerase activity. T-type lyases serve the cysteine ß-155 binding site in phycocyanins and phycoerythrocyanins. The S/U-type in contrast acts as a more universal lyase and attaches bilins to cysteine α-84 of allophycocyanins and phycoerythrins and cysteine β-84 of all phycobiliproteins. In contrast to the majority of cyanobacteria, the unicellular marine cyanobacterium *Prochlorococcus marinus* MED4 uses an intrinsic divinyl-chlorophyll-dependent light-harvesting system for photosynthesis. Despite the absence of phycobilisomes this high light adapted strain possesses β-phycoerythrin (CpeB), a putative S-type lyase (CpeS) and enzymes for the biosynthesis of phycoerythrobilin (PEB) and phycocyanobilin (PCB). Here we spectroscopically characterize the chromophore binding properties of CpeB and CpeS. Both PEB and PCB assemble spontaneously to CpeB forming fluorescent complexes. The putative lyase CpeS covalently binds PEB as shown by affinity chromatography whereas PCB is attached non-covalently. Furthermore we present strong evidence for the chromophore transfer from the putative lyase CpeS to the phycobiliprotein CpeB. Spontaneous and lyase catalyzed chromophore attachment is compared via the spectroscopic features of the reaction products.

BIOSYNTHESIS OF UNNATURAL BILIPROTEINS

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Cyanobacteria employ a number of different linear tetrapyrrole/biliprotein combinations both as accessory pigments to augment their ability to utilize wavelengths of light that are not efficiently captured by chlorophyll and as sensors to perceive and respond to light conditions in their environment. Up to four different linear tetrapyrroles may be incorporated into various phycobiliproteins: phycocyanobilin, phycoerythrobilin, phycoviolobilin and phycourobilin. Phycobiliproteins without autocatalytic binding capabilities have been found to use specific lyases to ensure proper attachment of a given chromophore to its cognate apoprotein. When bound, each of these bilins confers unique spectral properties, which collectively enhance the ability of the cyanobacteria to exploit the light available in its environment. On the other hand, plants are thought to utilize biliproteins known as the phytochromes only as sensors. Plant phytochromes uniquely incorporate the bilin, phytochromobilin. In the last several years the components involved in the assembly of biliproteins have been characterized. This study was conducted to determine the factors involved in maintaining the specificity between phycobiliproteins and their cognate chromophores and to determine the effects on a cyanobacterium when chromophores are misincorporated into a non-cognate apoprotein. Using a dual-plasmid expression system in *E. coli*, we studied the effects of various combinations of bilin lyases and bilin biosynthetic enzymes, on the final holo-phycobiliprotein. We found that CpcA from *Synechocystis* sp. PCC 6803 can bind up to six different linear tetrapyrrole chromophores, including the plant-specific chromophore phytochromobilin and an A-ring isomerized version of phytochromobilin not known to occur naturally. This suggests that the specificity of attachment of a linear tetrapyrrole to its cognate apoprotein may be more general than once believed. The effects of potential mis-pairings of chromophores and apoproteins were then examined in *Synechococcus* sp. PCC 7002, a cyanobacterium that utilizes only phycocyanobilin. Site-specific integrations into the multi-copy plasmid pAQ1 were used to introduce the phycoerythrobilin or phytochromobilin biosynthetic genes into its genome. The strong cpcB promoter from *Synechocystis* sp. PCC 6803 was used for overexpression of these genes. This resulted in strains with readily apparent altered absorbance profiles and possibly altered light sensing as well, if these chromophores are also mis-incorporated into the prokaryotic type phytochromes of the cyanobacterium. These studies shed new light on the assembly and evolution of biliproteins, and the results have implications for the production of recombinant proteins with properties not found in natural proteins.

Thomas Moore, Ph.D. is a Professor of Chemistry and Biochemistry at Arizona State University and Director of the Center for Bioenergy and Photosynthesis at ASU. Professor Moore worked under the direction of Professor Pill-Soon Song for the Ph.D. degree from Texas Tech University. He served as President of the American Society for Photobiology in 2004 and received the Senior Research Award from the Society in 2001. Over the period 2005-2007, Professor Moore was awarded a Chaire Internationale de Recherche Blaise Pascal, Région d'Ile de France. He teaches undergraduate and graduate level biochemistry at ASU and lectures on bioenergetics, energy and sustainability at the CEA Saclay and Universitè de Paris Sud, Orsay. He has served on several Department of Energy Basic Research Needs Workshops including the DOE Basic Energy Sciences Grand Challenges Committee which produced "*Directing Matter and Energy: Five Challenges for Science and the Imagination*," outlining research priorities for the foreseeable future. Professor Moore and his long-time colleagues, Professors Ana Moore and Devens Gust, collaborate on research in artificial photosynthesis which is aimed at providing a deeper understanding of natural photosynthesis and the design, synthesis and assembly of bio-inspired constructs capable of sustainable energy production and conversion for human use.

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OMEGA PLENARY LECTURE. THE EVOLUTION OF ENGINEERED AND ARTIFICIAL PHOTOSYNTHESIS

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Because energy transduction is fundamental to biology, organisms have evolved elegant and diverse machinery for energy capture and utilization. The evolution of this machinery is driven by the selection of traits that most effectively marshal energy resources to achieve reproductive success of the organism. In humans, the demand on energy resources includes expanding our gross domestic product. This expansion, fueled by cheap energy, often trades high growth rates for efficiency – trades favored by cheap energy and a lack of understanding of environmental impacts of energy use. Now, it is clear that the profligate use of energy to grow the GDP is incongruous with the finite nature of the resource. The need to address this mismatch is crucial and will require high levels of conservation and efficiency. Nature's energy transduction processes offer both optimized systems and examples where intervention can make much-needed improvements. Parallels between

technological and biological energy transduction will be used to identify both circumstances, i.e., where nature can teach us and where we can teach nature (1). These parallels also set the stage for describing a major challenge which must be met in order to take advantage of the best features of biology and technology: coupling electromotive force (emf) provided by human technology to the phenomenal catalysts for the synthesis of energy-rich fuels found in nature (2). Addressing this challenge, several bio-inspired constructs for solar energy conversion have been assembled. In one, a photobiofuel cell uses artificial reaction centers, visible light and two enzymes to reform biological substrates such as ethanol into hydrogen. This system interfaces redox chemistry with emf at two junctions, and demonstrates two methods for coupling biological redox catalysts with emf (3). In a second system, solar-driven water oxidation using an IrO2 catalyst replaces anodic organic substrates; an external boost of about 0.3 V produces hydrogen at the cathode. Another construct uses a bio-inspired molecular heterojunction-based polymer to assemble a photovoltaic device (4). The control of energy flow in biological systems is crucial to their stability. We have developed energy and electron transfer-based models for incorporation of these features into artificial systems. Conducting the high oxidation potential necessary for water oxidation from sensitizer to catalyst presents challenges in electronic coupling and thermodynamics to control the flow of electrons. We have reported a bio-inspired system which accomplishes this by mimicking the elegant redox relay found in water-oxidizing photosynthesis (5).

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2. Biology and Technology for Photochemical Fuel Production, M. Hambourger, et al., *Chem. Soc. Rev.* (2009) 38: 25-35
3. [FeFe]-hydrogenase catalyzed H2 production in a photoelectrochemical biofuel cell, M. Hambourger, et al., *J. Am. Chem. Soc.* (2008) 130: 2015-2022
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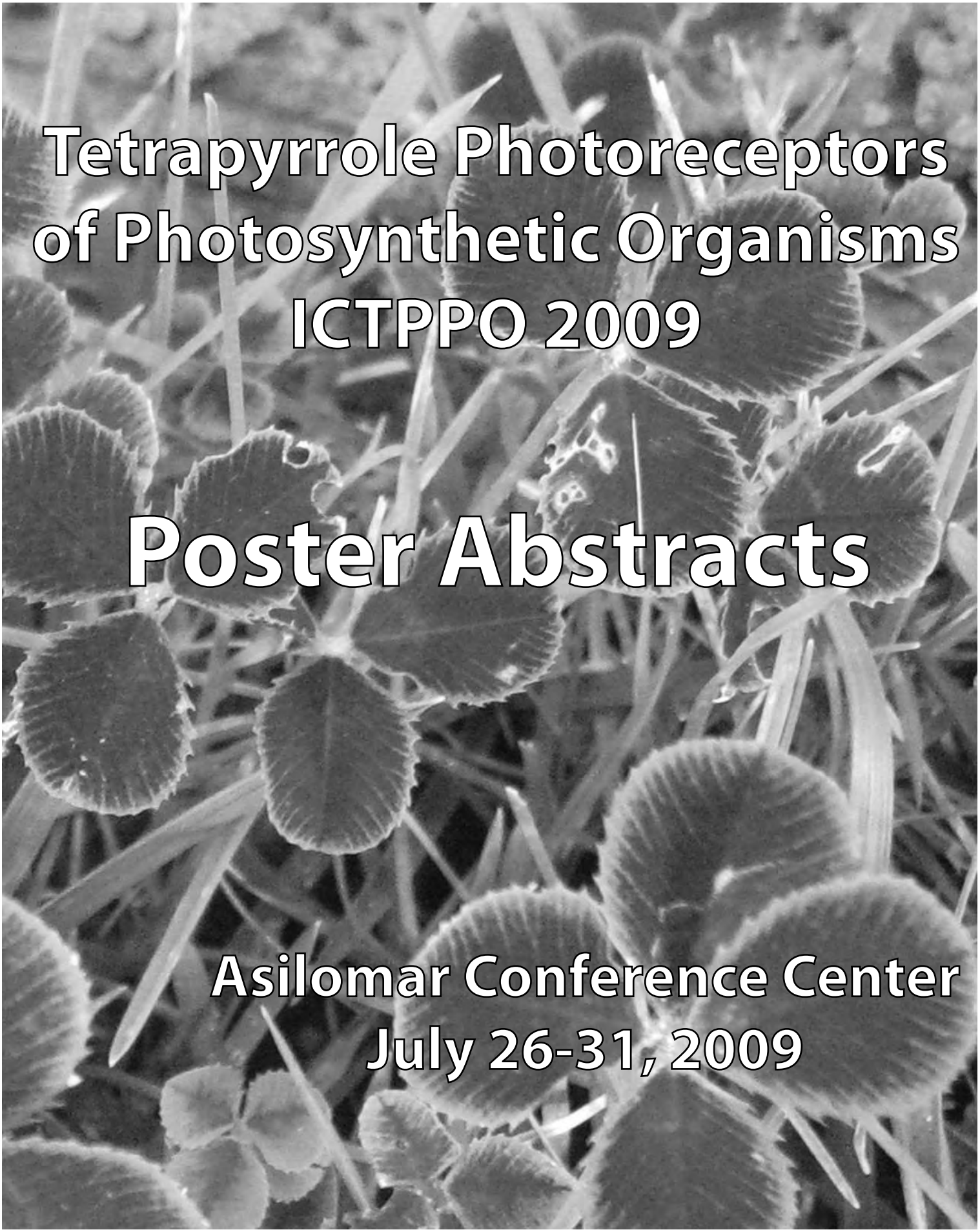

Tetrapyrrole Photoreceptors of Photosynthetic Organisms ICTPPO 2009

Poster Abstracts

Asilomar Conference Center
July 26-31, 2009

P1. EVOLUTION OF THE TETRAPYRROLE BIOSYNTHESIS IN EUKARYOTES THAT POSSESS PLASTIDS

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In order to reveal origins of the tetrapyrrole biosynthetic enzymes in eukaryotes, we performed detailed phylogenetic analyses of all genes of the pathway. Considering the results together with the current knowledge about the intercellular localization of this pathway in various eukaryotes, we estimate the effect of endosymbioses on the tetrapyrrole synthesis. Our results show that the origin of the pathway is mosaic in eukaryotes as a consequence of endosymbioses with either prokaryotic or eukaryotic cells that evolved into organelles. Photosynthetic plastids originated from a cyanobacterium that was engulfed by a heterotrophic eukaryote (so called primary endosymbiosis). However, many algae possess complex secondary plastids that arose from engulfed eukaryotes with primary chloroplast. In all photoautotrophic lineages, most genes of the tetrapyrrole pathway were transferred from the plastid into the nucleus and the original genes heve been replaced. The pathway is localized entirely in the chloroplast, where the bulk of its endproducts is needed. Exceptionally, the mixotrophic flagellate *Euglena gracilis* retained both pathways; one comes from the engulfed alga and serves for the production of chlorophyll and plastidial heme; the second pathway is from the ancestral heterotrophic cell and produces the heme for mitochondrion. Regarding the evolution of tetrapyrrole metabolism, another interesting group of organisms are the apicomplexan parasites that arose from a photosynthetic ancestor. These parasitic protists still harbor a remnant of a secondary plastid termed apicoplast and, similarly to photosynthetic eukaryotes, many genes involved in the heme synthesis show plastid origin and their products are targeted to the apicoplast. However, the localization of heme biosynthesis resembles more that of the primary heterotrophs; they synthesize 5-aminolevulinic acid by Shemin pathway and the final steps seem to be localized in the mitochondrion. Recently described photosynthetic alga *Chromera velia* is closely related to these parasites (1). Based on the sequence data from 454 genome sequencing and full-length cDNA sequencing, we identified 5-aminolevulinic acid synthase, but none of the enzymes of the alternative C5 pathway. It thus appears that *Chromera velia* uses the Shemin pathway for the tetrapyrrole biosynthesis, similarly to *Plasmodium*, the causative agent of malaria. Production of chlorophyll from glycine and succinyl-CoA instead of glutamate is much unexpected in a eukaryotic alga. Tetrapyrrole biosynthesis is intensively studied especially in apicomplexan parasites where it is believed to be a potencial drug target. A better understanding of the tetrapyrrole metabolism in Chromera could thus help in pursuit of an effective treatment for serious diseases such as malaria.

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P2. THE IN VIVO EFFECT OF GUN4 ON A HAEM/CHLOROPHYLL BRANCHPOINT ENGINEERED IN ESCHERICHIA COLI

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The first committed step of chlorophyll biosynthesis, the formation of Mg protoporphyrin IX (Mg proto) from protoporphyrin, is catalysed by the magnesium chelatase enzyme. The active enzyme contains three subunits, ChlH, ChlI and ChlD. We show that the *Synechocystis* sp. PCC 6803 genes encoding these proteins can be expressed in E. coli and a small accumulation of Mg proto can be observed in vivo. Therefore, a haem/chlorophyll branchpoint, much like the one present in photosynthetic organisms, has been engineered into *E. coli*. The in vivo accumulation of Mg proto can be improved by the addition of Mg2+ and δ-aminolaevulinic acid to the growth medium. In addition, the chelation of Mg assayed in vitro is enhanced in the presence of Gun4, a protein able to bind porphyrins. The *Synechocystis* ChlH protein has been shown to co-purify with Gun4, therefore an association in cyanobacteria has been confirmed. It is possible that Gun4 plays a part in controlling flux down the haem and chlorophyll pathways, as shown by in vitro enzyme kinetics. In order to test the regulatory effects of Gun4 in vivo, an *E. coli* strain expressing Gun4 along with the Mg chelatase proteins was created, and we show that this strain accumulates greatly increased amounts of Mg proto. The next reaction in the chlorophyll biosynthetic pathway, the conversion of Mg proto to Mg protoporphyrin monomethyl ester (MgPME), is catalysed by the methyltransferase enzyme, encoded by chlM. We show that the coupled expression of the Mg chelatase and methyltransferase enzymes in *E. coli* results in the in vivo accumulation of MgPME, confirmed by HPLC. This work shows that an in vivo *E. coli* system can be assembled which can be used to examine the haem/chlorophyll branchpoint and its regulation.

P3. IN PLANTA MAGNESIUM CHELATASE STUDIES: FOCUS ON THE CHLI AND CHLD SUBUNITS

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Mg Chelatase is a multi-subunit enzyme situated at a crucial point in tetrapyrrole biosynthesis. Positioned at the pathway branch where heme and chlorophyll biosynthesis diverge, it functions to insert Mg^{2+} into substrate Protoporphyrin IX (Proto IX), committing the intermediate into the chlorophyll biosynthetic branch. Though this enzyme has been under intense scrutiny for decades, its mechanism of assembly and its stoichiometry have yet to be demonstrated. Previous work focused on mutant studies and in-vitro analysis of subunits from *Rhodobacter* and *Synechocystis*, resulting in the current theory wherein subunit I functions as the ATP motor for the enzyme, D serves as a platform for enzyme assembly, and H binds the substrate Proto IX. Biochemical analyses of recombinant proteins revealed that subunit H binds Proto IX non-covalently, suggesting that H may be the catalytic subunit. However, recent studies have shown that BchH behaves as a substrate for the I:D complex, and suggest that enzyme control occurs via the D-subunit. Our studies aim to investigate assembly and action of Mg-Chelatase *in planta*. In plants, chelation may involve elements such as Gun4, a cofactor necessary for chlorophyll accumulation. We endeavored to circumvent the issue of a heretofore undiscovered constituent, using *Agrobacterium* to transiently express wild type and mutant Mg Chelatase subunits in *N. benthamiana* leaves. In a multidirectional approach, the tissue was then assayed for changes in chelatase activity, product accumulaton and gene expression. We are currently employing iTRAQ to investigate changes in the protein profile of infiltrated tissue.This work explores the action of Mg chelatase in an environment that we hope will expand our understanding of the higher plant enzyme.

P4. THE BARLEY MAGNESIUM CHELATASE 150-kDa SUBUNIT IS NOT AN ABSCISIC ACID RECEPTOR

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Magnesium chelatase catalyzes the first unique step in the biosynthesis of the chlorophylls, the energy-dependent insertion of Mg2+ into protoporphyrin-IX. It is a heteromultimeric protein composed of three subunits (in *Hordeum vulgare*: XanF, 150 kDa, XanG, 70 kDa, and XanH, 40 kDa). Abscisic acid is a hormone influencing various processes in plants, among them germination, post germination development, and stomatal aperture. The large magnesium chelatase subunit was recently identified as an abscisic acid receptor in *Arabidopsis thaliana* (1). We have evaluated whether the large subunit of barley (*Hordeum vulgare* L.) magnesium chelatase, XanF, could be a receptor for abscisic acid. The study involved several induced chlorophyll deficient mutants with defects identified at the gene and protein level. Magnesium chelatase mutants showed a wild-type response in respect to post-germination growth and stomatal aperture. To confirm the in vivo studies, the influence of abscisic acid on the activity of Mg-chelatase and the binding of the phytohormone to the subunit were analyzed in vitro. Abscisic acid had no effect on magnesium chelatase activity and binding to the barley 150-kDa protein could not be shown. These results question the role of the large magnesium chelatase subunit as an abscisic acid receptor.

Reference: (1) Shen *et al* 2006 The Mg-chelatase H subunit is an abscisic acid receptor. Nature 443:823-826.

P5. THE REGULATABLE EXPRESSION OF THE LIGHT-DEPENDENT PROTOCHLOROPHYLLIDE OXIDOREDUCTASE IN *SYNECHOCYSTIS* PCC 6803

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Stringent control of the chlorophyll (Chl) biosynthesis pathway is essential particularly for oxygenic organisms like plants and cyanobacteria that cope with the problem of photo-oxidation. To prevent accumulation of harmful 'free' tetrapyrroles, it is expected that Chl formation is tightly synchronized with synthesis of cognate apoproteins. In order to understand coordination between Chl and protein biosynthesis we prepared the *Synechocystis* pMON:por/Δpor/ΔchlL strain enabling to control Chl availability via regulatable expression of the light-dependent protochlorophyllide oxidoreductase (LPOR), a penultimate enzyme of this pathway. In the first step, we deleted the por gene coding for LPOR. Resulting Δpor mutant accumulated significantly less Chl per cell in contrast to dramatically increased carotenoid level and displayed strongly retarded growth rate even at moderate light (35 μE). As the second step, the por gene was introduced back into the Δpor strain using the pMON:por vector possessing the regulatable nirA promoter. This promoter is tightly repressed by ammonium and activated by nitrate providing nice repression/induction system. The phenotype of the pMON:por/Δpor strain was similar to wild type suggesting that the expression of the por gene from nirA promoter fully complemented the Δpor mutation. To eliminate contribution of the dark-operative POR (DPOR) to the Chl production we finally eliminated the chlL gene obtaining the pMON:por/Δpor/ΔchlL mutant producing Chl only by the regulatable LPOR enzyme. Although the Chl content decreased after DPOR deletion, growth rate of the final mutant remained similar to wild-type. In contrast, after 10 days of the LPOR repression by ammonia the strain retained only ~17% of Chl level in comparison to the wild type and growth rate was strongly affected. Surprisingly, although the Chl level was lower than in the Δpor mutant, we did not observe accumulation of carotenoids indicating an essential role of the LPOR enzyme even at very low concentration per cell. Biogenesis of membrane proteins under limited Chl availability was analyzed using S35 radiolabeling and 2D electrophoresis. It revealed overall very low membrane protein synthesis, however several proteins were found to be specifically over-expressed as an effect of Chl deficiency. In addition, total protein accumulation in membranes was also dramatically changed under analyzed conditions. Detailed analysis of the protein biosynthesis and tetrapyrrole metabolism in the pMON:por/Δpor/ΔchlL mutant will be presented.

P6. PIGMENT SHUFFLING IN PHOTOSYSTEMS BY OVEREXPRESSING CHLOROPHYLLIDE A OXYGENASE (CAO) INDUCES THE DELAY OF LEAF SENESCENCE IN *ARABIDOPSIS THALIANA*

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Chlorophyllide a oxygenase (CAO) is a Rieske-type monooxygenase that catalyzes conversion of chlorophyll a to chlorophyll b. CAO protein consists of three domains, namely, A, B, and C domains (Nagata et al., 2004). Previously, we demonstrated that the C domain alone has a catalytic function, the B domain is linker, and the A domain alone is involved in the regulatory mechanism of CAO protein level, in which mechanism the CAO protein level is maintained below a certain level (Yamasato et al., 2005). We made transgenic plants that overexpressing B, and C domains of CAO in *Arabidopsis thaliana* (we named this mutant as BC). Chlorophyll a/b ratio drastically decreased in BC plants compared with wild-type plants. Interestingly, BC plants showed strong stay green phenotypes during senescence stage. Furthermore, most photosynthetic proteins and CO_2 fixation capacity were retained during senescence in BC plants, suggesting that BC is a stay green mutant in which photosynthetic capacity was retained. The mechanism of delay of leaf senescence in BC plants will be presented.

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P7. HEME OXYGENASES OF *ARABIDOPSIS THALIANA* - TWO FAMILIES, TWO FUNCTIONS?

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Heme oxygenases (HOs) catalyze the oxidative cleavage of heme to biliverdin, iron and carbon monoxide. In plants this reaction yields biliverdin IXα (BV IXα) a precursor of the phytochrome chromophore and is thus essential for proper photomorphogenesis. *Arabidopsis thaliana* contains four putative HOs (HY1, HO2, HO3, HO4), of which only HY1 has been biochemically characterized so far. Higher plant heme oxygenases are encoded in the nucleus, but contain chloroplast translocation sequences at their N-termini. The transit peptides of the four putative Arabidopsis HOs are sufficient for chloroplast translocation as shown by GFP reporter gene fusions. HY1, HO3, and HO4 are monomeric enzymes and able to use spinach Fd as an electron donor. Addition of a 2nd electron donor led to ten times faster heme conversion. Furthermore, heme turnover is also promoted by light when spinach thylakoids are present. Obtained kinetic parameters (dissociation- (K_D) and Michaelis-Menten (K_M)- constants, also activation energy (EA)) for HY1, HO3, and HO4 do not differ significantly suggesting that all three heme oxygenases are able to contribute to phytochrome chromophore biosynthesis. To yield sufficient amounts of soluble HO2 a synthetic gene, adapted to the codon usage of *E. coli* was constructed. HO2 is also a monomeric enzyme and is unable to bind or degrade heme indicating that it is not a heme oxygenase. However, HO2 shows strong binding of protoporphyrin IX, a precursor of both, heme and chlorophyll biosynthesis. A possible function of HO2 might lay in the regulation of tetrapyrrole metabolism.

P8. COMPLEX FORMATION BETWEEN HEME OXYGENASE AND BACTERIOPHYTOCHROME IN *PSEUDOMONAS SYRINGAE*

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The plant pathogen *Pseudomonas syringae* pv tomato carries two bacteriophytochrome- (Bph, PsBph1 and PsBph2) and one blue light photoreceptor- (PsLOV) coding genes in its genome. Both bph gene products show sequence motifs indicative for the employment of biliverdin (BV) as chromophore and exhibit a histidine kinase motif as signalling domain. PsBph1 is found in an operon arrangement with a heme oxygenase-coding gene (PsHO1), whereas PsBph2 is followed in the genomic arrangement by a response regulator-coding gene. Apo-PsBph2 could not be loaded with chromophore, neither when expressed as apoprotein and incubated later with BV, nor via co-expression together with PsHO1. PsBph1, in contrast, showed typical features of a bacterial phytochrome, when incubated with BV (absorption maxima of Pr and Pfr states, 700 and 750 nm, respectively). However, yield of assembly and yield of light-induced conversion between Pr and Pfr state were moderate, even when a coexpression of PsHO1 and PsBph1 from two separate plasmids was performed. Excellent quality of recombinant protein, of chromophore loading and of photoreversibilty was only achieved, when the operon of PsHO1 and PsBph1 was expressed from the same plasmid. Under these conditions, the yield of recombinant bacteriophytochrome and of photoreversibility was optimal. Furnishing both genes with tag-encoding sequences (5'-tag of PsHO1 and 3'-tag of PsBph1) revealed, employing either of both tags for purification, the presence of both proteins. The represented data clearly demonstrate a complex formation of heme oxygenase (PsHO1) and bacterial phytochrome (PsBph1) during biosynthesis.

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P9. EXTRACTION AND QUANTIFICATION OF PROTOHEME FROM PLANT TISSUES

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In higher plants, predominant heme is protoheme (here referred as heme), which is required as prosthetic group of various hemoproteins. Furthermore, heme is known to function, as a negative feedback inhibitor of 5-aminolevulinate biosynthesis, as well as organelle-derived signal that regulates cellular processes, such as transcription and post-translational protein transport. Thus, to analyze physiological function, quantitative extraction and precise determination of heme are prerequisite. Classically, heme is extracted from plant tissues by successive extraction with neutral and/or ammonia-containing basic acetone, followed by HCl-containing acidic acetone (1). It is considered that non-covalently bound heme is extracted in acidic acetone, while heme extractable in neutral or basic acetone may represent putative free heme pool that is not bound to hemoproteins (1, 2). Recently, based on the ability of horseradish peroxidase (HRP) apo-enzyme to reconstitute with heme to form an active enzyme, we developed novel heme assay that is extremely sensitive and specific to heme (3, 4). By using this method, we re-evaluated the extraction method of heme from plant tissues. When plant homogenates were extracted with three distinct acetones, the level of heme extracted in basic acetone was intermediate to that in acidic acetone. No heme was extracted in neutral acetone. When authentic heme solution, which represents free heme, was added to the homogenates, total amount of heme was quantitatively recovered in acidic acetone, while marginal increase in heme was observed in basic acetone extract. When extracted from authentic hemoglobin and catalase, heme was quantitatively extracted in acidic acetone, while intermediate and negligible levels of heme were extracted in basic and neutral acetones, respectively. The levels of heme in basic acetone were consistent with dissolved protein contents, while heme was quantitatively extracted in acidic acetone irrespective to protein concentration. These results clearly showed that heme extractable in basic acetone originates from dissolved hemoproteins but not represents free heme. It is likely that initial basic acetone extraction may deduct total heme concentration that is quantitatively extractable by following acidic acetone. We also confirmed that, as well as authentic heme solution, the acid acetone extracted heme can completely reconstitute HRP activity after dilution. The endogenous levels of heme in Arabidopsis that deficient in tetrapyrrole or iron metabolism will also be presented.

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P10. MOLECULAR MECHANISM OF THE TWO-STEP REDUCTION CATALYZED BY PCYA

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Phycocyanobilin:ferredoxin oxidoreductase (PcyA) is a member of the ferredoxin-dependent bilin reductase family. PcyA catalyzes the reduction of biliverdin IXα (BV) in two sequential steps to produce 3Z/3E-phycocyanobilin, one of the major pigments in the phycobilisome, the light-harvesting antennae complex. Catalysis occurs via initial reduction of the D-ring vinyl group of BV to generate 181,182-dihydrobiliverdin IXα (18EtBV) followed by reduction of the A-ring of 18EtBV to generate 3Z/3E-phycocyanobilin. In each reduction step, two electrons are supplied by ferredoxin. This sequential reaction reflects stringent reduction regiospecificity controlled by PcyA. Here we have determined the crystal structures of PcyA from the cyanobacterium *Synechocystis* sp. PCC 6803 complexed with 18EtBV and the BV13 isomer of 18EtBV, at 1.48Å and 1.04Å resolution, respectively. Structural comparisons of PcyA-18EtBV and PcyA-BV adducts demonstrate that interaction between the carboxyl group of Glu76 and the vinyl group of BV D-ring is abolished following reduction of the D-ring vinyl group. This change, together with the protein environment of the vinyl group of the BV13 D-ring, indicates that Glu76 plays a catalytic role in proton transfer to the BV D-ring vinyl group. This hypothesis is further supported by the biochemical data of E76Q mutant protein of PcyA, which selectively impairs only first step of reduction, and by the structure of its complex with BV. All substrate-bound complexes have identical structure around the A-ring within experimental error and retain the activity of the second step of reduction. The mechanism by which the sequential reduction of PcyA is controlled will be discussed.

P11. RADICAL MECHANISM OF PHAGE-DERIVED PHYCOERYTHROBILIN SYNTHASE

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Phycoerythrobilin (PEB) is an open chain tetrapyrrole (phycobilin) which functions as a chromphore in the light harvesting structures of cyanobacteria, the phycobilisomes (PBS). There, the pink-colored pigment is attached to phycobiliproteins and helps transferring light energy to photosystem II. The biosynthesis of phycobilins starts with the oxidative cleavage of heme yielding biliverdin IXα (BV). This reaction is catalyzed by heme oxygenases. BV is then the substrate of a novel class of radical enzymes, the ferredoxin-dependent bilin reductases (FDBRs). The FDBRs do not possess any metal or organic cofactors and utilize ferredoxin as the electron donor. In cyanobacteria, the two major pigments phycocyanobilin (PCB) and PEB are synthesized via two independent reactions involving members of the FDBR family. PCB:ferredoxin oxidoreducatse (PcyA) catalyzes the formal four-electron reduction of BV to PCB and 15, 16-dihydrobiliverdin:ferredoxin oxidoreductase (PebA) and PEB:ferredoxin oxidoreductase (PebB) the sequential two-electron reduction of BV to PEB. Just recently another FDBR involved in PEB biosynthesis was discovered. Interestingly, PEB synthase (PebS) identified in the cyanophage P-SSM2 directly catalyzes the four-electron reduction of BV to PEB. The PebS mechanism seems to be exclusive to phage since all cyanobacteria known so far utilize two enzymes yielding PEB in two sequential reductions. Surprisingly, P-SSM2 has been found to solely infect strains of the genus *Prochlorococcus*, which are one of the major primary producers in the oceans and do not possess PBS. Here we show that PebS acts via a radical mechanism as shown for other bilin reductases suggesting a general mechanism for FDBRs. Mutant analyses support the mechanism that was proposed based on the crystal structure of substrate bound PebS.

P12. CRYSTAL STRUCTURE OF RED CHLOROPHYLL CATABOLITE REDUCTASE (RCCR) REVEALS THAT RCCR AND FERREDOXIN DEPENDENT BILIN REDUCTASE WERE EVOLVED FROM THE COMMON ANCESTOR

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The key steps in the degradation pathway of chlorophylls are the ring-opening reaction catalyzed by pheophorbide a oxygenase and the sequential reduction by red chlorophyll catabolite reductase (RCCR). During these steps the chlorophyll catabolites lose their color and phototoxicity. RCCR catalyzes the ferredoxin-dependent reduction of the C20/C1 double bond of red chlorophyll catabolite (RCC). RCCR appears evolutionarily related to the ferredoxin-dependent bilin reductase (FDBR) family, which synthesizes a variety of phytobilin pigments, on the basis of sequence similarity, ferredoxin dependency, and the common tetrapyrrole skeleton of their substrates. The evidence, however, is not robust; the identity between RCCR and FDBR HY2 from *Arabidopsis thaliana* is only 15% and the oligomeric states of these enzymes are different. Here, we report the crystal structure of *Arabidopsis thaliana* RCCR (AtRCCR) at 2.4 Å resolution. AtRCCR forms a homodimer, in which each subunit folds in α/β/α sandwich. The tertiary structure of AtRCCR is similar to those of the FDBRs, strongly supporting that these enzymes evolved from common ancestor. The two subunits are related by noncrystallographic two-fold symmetry, in which the α-helices near the edge of the β-sheet unique in AtRCCR participate in the intersubunit interaction. Amino acid residues involved in this intersubunit interaction are conserved in RCCRs from several plants but not conserved in FDBRs, reflecting the oligomeric states of RCCRs and FDBRs. The putative RCC-binding site, which is estimated by the superimpositions of AtRCCR onto the biliverdin-bound forms of FDBRs, forms an open pocket surrounded by the conserved residues among RCCRs. Two acidic residues, which stand opposite each other in the pocket, are likely to be involved in substrate binding and/or catalysis.

P13. SYNTHESIS OF OPEN TETRAPYRROLES BY PHOTO-OXIDATION OF CHLOROPHYLL DERIVATIVES AND THEIR OPTICAL PROPERTIES

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We have focused on chemistry of chlorophylls and bacteriochlorophylls, a family of light-harvesting pigments found in plants and photosynthetic bacteria, and prepared a variety of their derivatives by modifying peripheral substituents on chlorin and bacteriochlorin π-ring systems. The synthetic compounds are useful for elucidation of optical properties of natural pigments as well as of their biosynthetic routes. In enzymatic degradation of chlorophylls in senescent higher plants, chlorophyll-a molecule is converted to open tetrapyrroles. Similar oxygenolytic opening of the chlorin π-ring was reported in the photo-oxidation of chlorophyll derivatives [1,2]. More than one billion tons of chlorophyll are supposed to be biosynthesized and degraded every year on the earth [3], but the chlorophyll degradation is not fully revealed. Especially, such chlorophyll-derived open tetrapyrroles are little available and their absorption and fluorescence spectra were less measured. Here we report on synthesis of linear tetrapyrroles by photo-oxidation of chlorophyll derivatives possessing various substituents at the peripheral positions of the π-skeleton and their optical properties in a solution.

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P14. ZINC-BACTERIOCHLOROPHYLL IN *RHODOBACTER SPHAEROIDES*: CONSEQUENCES FOR PHOTOSYSTEM FUNCTION AND A PROPOSED BIOSYNTHETIC PATHWAY

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At the heart of photosynthesis lie the chlorophyll pigments responsible for absorption and transmission of light energy. The purple non-sulfur bacterium *Rhodobacter sphaeroides* is a model system for studying the biosynthesis of chlorophylls and the associated protein photosystem by virtue of its diverse metabolism, easily manipulated genetic material, and wealth of structural data. Recently, we studied a *R. sphaeroides* bchD (magnesium-chelatase) mutant to determine the properties of its photosystem in the absence of bacteriochlorophyll (BChl). Unexpectedly, the bchD mutant was found to synthesize an alternative form of BChl in which the central magnesium atom is replaced by zinc. This unusual zinc-bacteriochlorophyll (Zn-BChl) was found to assemble with the photosystem similarly to the normal magnesium-containing BChl (1). The reaction center (RC) isolated from the bchD mutant was found to contain Zn-BChl in the special pair and accessory BChl sites, but with blue-shifted absorption peaks relative to the wild type. Interestingly, spectroscopic measurements show that Zn-BChl binds in place of both bacteriopheophytins in the RC, resulting in a bacterial RC that contains 6 identical chlorophyll cofactors, analogous to the arrangement of chlorophylls in photosystem 1 of cyanobacteria and plants. Functional characterization of the isolated Zn-RC showed that electron transfer reactions proceed at >95% the rate and efficiency of the wild type (2). We hypothesize that electron flow occurs efficiently because the Zn-BChl bound in the bacteriopheophytin site has a more positive midpoint potential than the accessory and special pair Zn-BChls, due to a difference in the metal coordination state. Despite having a functional Zn-RC, this bchD mutant is incapable of photosynthetic growth. We attribute this absence of growth to the low amount of Zn-BChl produced in the cell, limiting photosystem development to only 4-7% of the wild type. The work described above will be summarized as an introduction, followed by presentation of data supporting a proposed Zn-BChl biosynthetic pathway beginning with the enzyme ferrochelatase, which shares the common precursor protoporphyrin IX with magnesium chelatase.

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P15. OBSERVATION, CHARACTERIZATION, AND POTENTIAL SIGNIFICANCE OF THE MN-OXO OLIGOMER MATERIAL IN SOLAR ENERGY CONVERSION

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The energy consumption in the world is expected to double in the next 50 years, and the energy costs have increased drastically in the last decade. In addition, fossil fuels produce carbon dioxide, which have significant negative impact on the environment and global warming. To address these issues, novel renewable carbon-free or carbon-neutral energy sources must be identified and replace the fossil fuels. Comparing all the energy options, solar energy is the most promising source. The key for transforming solar energy into an affordable energy source is the invention of water splitting catalysts from earth-abundant elements. Recently we observed the formation of a novel Mn-containing water splitting compound with high catalytic activity in water splitting reaction. The precursor of the catalyst is a dinuclear Mn(III/IV)-oxo compound with bounded terminal water molecules, "Brudvig catalyst," which is able to continuously catalyze the conversion of water to dioxygen in the presence of oxidant serving as a PSII functional model [Limburg, J., Vrettos, J. S., Liable-Sands, L. M., Rheingold, A. L., Crabtree, R. H., and Brudvig, G. W. (1999) *Science* 283(5407): 1524-7]. Our experiments showed that the Brudvig catalyst is unstable under UV radiation and thermal conditions. Varied the temperature from 20-85°C we found that as the temperature increases, the absorption at 400 nm of Mn-oxo dimer aqueous solution increases first and then decreases, indicating the structure of compound may altered in two different phases. The transition temperature is 60°C. The reaction was accompanied with formation of new products as observation of brown precipitates and disappearance of the colored Mn(III/IV)-oxo dimer from brown to colorless. Unexpectedly, the oxygen evolution measurements showed an activity increase by 80% after the decomposition reaction was completed. The results were reproducible. Further experimental results on the precipitate portion and supernatant portion showed that both samples are able to splitting water in the presence of oxidant. Thus, it is likely the two water-splitting catalysts were generated: one in solid form and one aqueous. The ratio of Mn content in the precipitate to in the supernatant is about 3 to 1 determined by atomic absorption spectrometry. Elemental analysis, FTIR, and X-ray absorption spectroscopic data indicated that the Mn-containing solid material is not manganese dioxide. We speculate that the solid material with high catalytic activity is a Mn-oxo oligomer compound. The catalytic activity of the Mn-oxo oligomer material in the presence of ozone was optimized under experimental conditions. The Mn-oxo oligomer material is thermal stable and may provide an unique opportunity for preparing catalytic nanomaterials and making Mn-based electrodes in solar cells. This may open a new route for using solar energy to split water in neutral pH and room temperature.

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P16. ASSEMBLY OF A BACTERIAL MEMBRANE ORGANELLE

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Cells require a source of energy, usually provided by membrane-protein complexes borne on infoldings of membranes elaborated within organelles such as mitochondria and chloroplasts, and within some bacteria. In order to study the biogenesis of these essential energy-yielding membranes we used the well characterised prokaryote *Rhodobacter sphaeroides* to establish the relationship between 'nascent' (UPB) and 'mature' (ICM) intracytoplasmic photosynthetic membranes. Both types of membrane were purified and their morphology, protein organisation, and function compared at the single membrane level using electron, atomic force and fluorescence microscopy. The proteomes of the early and mature, budded membranes reveal fundamental differences, with the more complex UPB proteome possessing many biosynthetic functions and the mature ICM devoted to its core photosynthetic function. Gentle lysis of cells containing histidine-tagged ICM, in the presence of a surface containing Ni-NTA and subsequent AFM of this surface, demonstrates the existence of at least some 'free-living' ICM vesicles within the bacterial cytoplasm. Thus, these vesicles, which possess the machinery for converting light energy into ATP, can be regarded as bacterial membrane organelles.

P17. LAF1 IS INVOLVED IN LEAF DEVELOPMENT OF ARABIDOPSIS

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Auxin affects a large number of important growth and developmental processes including of shoot and root branching in higher plants. Recent results have shown that the polar auxin transport inhibitors such as N-1-naphthylphthalamic acid, 9-hydroxyfluorene-9-carboxylic acid, and 2,3,5-triiodobenzoic acid affect leaf growth and leaf vein morphology. To investigate possible role of LAF1 (Long After Far-red light 1), a R2R3 Myb transcription factor and a downstream signal transducer in phyA signaling, in auxin transport, we examined leaf growth and vein patterning of *laf1* mutant under short day and far-red light conditions. Length of rosette leaf is shorter in *laf1* mutant and midvein is much broader in *laf1* mutant. Cell numbers are decreased in *laf1* mutant and leaf width is much narrow in *laf1* mutant, and petiole length is also shorter in *laf1* mutant. GUS assay revealed that LAF1 is much strongly expressed in root, in particular in main root tip and lateral root, and its expression is induced by auxin IAA in roots. Transcript levels of PIN (PIN-FORMED) and IAA genes encoding auxin efflux-related and response proteins are low in *laf1* mutant, which suggest that LAF1 may positively regulate expression of genes relating to auxin signaling through its transcriptional activity and auxin transport might be significantly blocked in *laf1* mutant. In addition, phytochrome A (phyA) degradation is delayed in *laf1* mutant under red light condition, suggesting that LAF1 regulates phyA stability and activity. Taken together, our results indicate that LAF1 involves in leaf and root developments through modulation of phyA signaling as a positive regulator of auxin signaling.

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P18. INDUCING PHYTOCHROME B SIGNALING WITHOUT ACTIVATION OF OTHER PHYTOCHROMES

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Phytochrome B (phyB) plays a dominant role in red light sensing in plants, with its regulatory role redundantly shared and modulated by other members of the phytochrome protein family. This redundancy has made it difficult to identify genes and gene products specific to the phyB signaling pathway. Recently, our laboratory identified a class of dominant constitutively active mutant alleles of phyB that faithfully recapitulate phyB-regulated gene expression networks in a light-independent manner (1, 2). By exploiting the chromophore-dependent activity of the Y276H allele (YHB) of Arabidopsis phyB, we are developing a bilin-inducible system to manipulate phyB signaling pathways in light-grown plants under conditions where other phytochromes are inactive. Through expression of the cyanophge bilin biosynthetic enzyme PebS to produce the unnatural bilin precursor phycoerythrobilin (3) in transgenic plants, YHB alleles are activated while wild-type alleles are photoinactive. In this way, signaling by phyB can be selectively activated in light-grown plants without activation of other phytochromes.

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P19. INORGANIC CARBON TRANSPORT AND ACCUMULATION IN THE CO2-CONCENTRATING MECHANISM OF *CHLAMYDOMONAS REINHARDTII*

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Aquatic photosynthetic microorganisms, including microalgae, have adapted to the variable and often-limiting availability of CO2, and inorganic carbon (Ci) in general, by development of inducible CO2 concentrating mechanisms (CCMs) that allow them to optimize carbon acquisition. Increased interest in microalgae as potential platforms for the production of biofuels and other bioproducts has fueled interest in understanding microalgal photosynthesis and the microalgal CCM as a potential source of ways to increase microalgal productivity. Significant advances in understanding the *Chlamydomonas reinhardtii* CCM have occurred with the aid of mutational approaches, recognition of the existence of multiple acclimation states and the availability of the *Chlamydomonas* genome sequence. As a result of these advances, an integrated picture of the functional components of the microalgal CCMs is beginning to emerge, including the identification of Ci transport candidates that function at either the chloroplast envelope or the plasma membrane, as well as other components, such as LCIB, that are critical to Ci accumulation but whose molecular functions are not clear.

P20. MAMMALIAN EXPRESSION OF INFRARED FLUORESCENT PROTEINS ENGINEERED FROM A BACTERIOPHYTOCHROME

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Green fluorescent protein (GFP) from jellyfish and its red homologs from corals have revolutionized many areas of molecular and cell biology, but the use of these visible FPs in intact animals, such as mice, has been handicapped by poor penetration of excitation light. Here we report that a bacteriophytochrome from *Deinococcus radiodurans*, incorporating biliverdin as the chromophore, can be engineered into monomeric, infrared-fluorescent proteins (IFPs), with excitation and emission maxima of 684 and 708 nm, respectively; extinction coefficient >90,000 M−1cm−1; and quantum yield up to 0.07 so far. IFPs express well in mammalian cells and mice and spontaneously incorporate biliverdin, which is ubiquitous as the initial intermediate in heme catabolism but has negligible fluorescence by itself. Because their wavelengths penetrate tissue well, IFPs are suitable for whole-body imaging. Furthermore, IFPs can be used for in vivo quantitative fluorescence imaging and 3D reconstruction by fluorescence molecular tomography. The IFPs developed here provide a scaffold for further engineering.

P21. ENGINEERING THE GAF DOMAIN OF CYANOBACTERIOCHROMES FOR MAMMALIAN EXPRESSION

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We are studying cyanobacteriochromes, a cyanobacterial family of biliprotein photoreceptors containing GAF domains related to those of phytochromes. We are currently working on the characterization of isolated GAF domains from the five class II (blue/green) cyanobacteriochromes encoded in the genome of the model thermophilic cyanobacterium *Thermosynechococcus elongatus*: Tlr0924, Tll0569, Tll0899, Tlr0911, and Tlr1999. We have successfully expressed several of these truncations and have also purified a C499D mutant truncation of the Tlr0924 GAF domain. As in full-length protein, this mutation ablates normal blue/green photochemistry, resulting in a red fluorescent protein. We are currently introducing the equivalent mutation into the other GAF domains to choose the shortest, brightest clone for mammalianization of such a red fluorescent reporter. Compared to earlier reports of phytochrome-based fluorescent reporters, cyanobacteriochromes offer the promise of a much smaller reporter (about 160 amino acids) and do not contain the knotted PAS/GAF bidomain that limits phytochromes to N-terminal fusion constructs. Compared to other red-fluorescent markers such as monomeric DsRed and mCherry, we believe biliprotein reporters will exhibit substantially improved resistance to photobleaching. We plan to optimize the DNA sequence of the chosen mutant cyanobacteriochrome for mammalian expression and fuse it to actin to monitor the actin cytoskeleton during infection of lymphocyte cells with GFP-labeled HIV.

P22. STRUCTURAL BIOLOGY OF A RED FLUORESCENT PHYTOCHROME

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The three dimensional structure of phytochrome from *Deinococcus radiodurans* provided an opportunity to design structure-based variants of this red light photoreceptor with altered photocycle properties (1). The most dramatic of these are a family of fluorophores with substitutions in the Asp207 position. Given that these novel proteins use a tetrapyrrole chromophore available within cells (biliverdin and/or protoporphyrin IX) and have emission maxima above 700 nm, they are extremely promising biotechnology tools. We have solved the crystal structure of the Asp207His variant of the chromophore-binding domain of *D. radiodurans* phytochrome in order to understand the molecular nature of its fluorescence. An altered hydrogen bond network within the biliverdin-binding pocket is observed. His207 forms a direct hydrogen bond to the chromophore A ring oxygen, and an interaction between Tyr263 and the now-missing Asp207 is altered. Additionally, with the goal of decreasing the size of this fluorophore and facilitating its use as a fluorescent tag in vivo, we changed several residues in the native coiled-coil dimer interface. Most significantly, Leu311 and Leu314 were replaced with glutamate to introduce charge repulsion. Structural characterization of the resulting dimer-interface variants is underway. (1) Wagner J.R., Zhang J., von Stetten D., Gunther M., Murgida D.H., Morginski M.A., Walker J.M., Forest K.T., Hildebrandt P., and Vierstra, R.D., Mutational analysis of *Deinococcus radiodurans* bacteriophytochrome reveals key amino acids necessary for the photochromicity and proton exchange cycle of phytochromes. *J. Biol. Chem.* 283:12212-12226, 2008.

P23. CYANOBACTERIOCHROME CCAS REGULATES PHYCOERYTHRIN ACCUMULATION IN *NOSTOC PUNCTIFORME* THAT PERFORMS GROUP II CHROMATIC ADAPTATION

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Cyanobacteria utilize phycobilisome (PBS) as a light harvesting antenna. Typically, PBS contains two major tetrapyrrole-bound proteins, green-absorbing phycoerythrin (PE) and red-absorbing phycocyanin (PC). Certain species are able to raise PE/PC ratio under green light and to reduce under red light, which has been called "complementary chromatic adaptation". It is known that a group regulates only PE synthesis (group II) while another group regulates both PE and PC synthesis (group III) (1). Recent studies have revealed that cyanobacteria possess unique phytochrome-related photoreceptors called "cyanobacteriochrome" (2). Cyanobacteriochrome CcaS perceives green and red light with covalently-bound phycocyanobilin and is suggested to regulate the expression of PBS linker gene, cpcG2, in *Synechocystis* sp. PCC 6803 that possesses PC but not PE (3). Interestingly, a ccaS ortholog gene is clustered with linker and regulatory genes of PE in the genome of *Nostoc punctiforme* ATCC 29133, that performs group II chromatic adaptation.

In this study, we disrupted the ccaS gene and cognate response regulator gene, ccaR, in N. punctiforme. Relative absorption spectra and transcriptional profile of ccaS and ccaR mutants suggested that CcaS induces the expression of phycobilisome linker genes, cpeC,cpcG2 and cpeR, under green light, possibly by phosphorylation of CcaR. Additionally, CcaS is suggested to repress the expression of these genes under red light, possibly by dephosphorylation of CcaR. Relative absorption spectra of triple mutant of cpeC,cpcG2 and cpeR suggests that green light-induced expression of these genes is essential for PE accumulation. On the other hand, the expression of PC genes is not affected in these three mutants. From these results, we conclude that CcaS and CcaR govern PE accumulation by the transcriptional regulation of cpeC,cpcG2 and cpeR in N. punctiforme. This results provide new insights into molecular mechanism and evolution of two types of the chromatic adaptation in cyanobacteria.

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P24. CHARACTERIZATION OF PHOTOCHEMICAL PROPERTIES OF CYANOBACTERIOCHROME TEPIXJ

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Cyanobacteria harbor many putative GAF-containing photoreceptors that may bind a linear tetrapyrrole as a chromophore (cyanobacteriochromes) in addition to typical phytochromes. One of them, TePixJ of *Thermosynechococcus elongatus* BP-1 is essential for phototaxis. Previously, we reported spectral properties of the GAF domain of TePixJ (denoted TePixJ-GAF) that was expressed in *Synechocystis* PCC 6803. Purified TePixJ-GAF showed reversible photoconversion between the 433nm (Pb form) and 531nm-absorbing forms (Pg form) (1). In contrast with Pr and Pfr forms of *Synechocystis* phytochrome Cph1 carrying phycocyanobilin (PCB), Pb and Pg of TePixJ-GAF are extremely blue-shifted (approximately 150~200nm). We also reported that molecular mass of the chromophore of TePixJ was identical to PCB but was different in spectral properties (1). Moreover, we carefully compared TePixJ with Cph1 after denaturation with acidic urea. The spectral properties of TePixJ chromophore were clearly different from those of Cph1 PCB, but were very similar to those of phycoviolobilin (PVB), an isomer of PCB (2). Here, we performed in vitro and in vivo reconstitution experiments to study assembly of TePixJ-GAF holoprotein. In the early phase of in vitro reconstitution, free synthetic PCB was covalently incorporated into the apoprotein with concomitant accumulation of a photoactive intermediate holoprotein, which shows nearly reversible photoconversion between Pb-like blue-absorbing form (Pb* form) and Pg-like green-absorbing form (Pg* form). Further incubation at 50°C resulted in slow conversion to the final form carrying spectral properties similar to the native TePixJ. Denaturation analysis revealed that the chromophore of the initial intermediate form was PCB, whereas that of the final form was PVB. In vivo reconstitution was performed in E. coli, which co-expresses TePixJ-GAF apoprotein and two enzymes (Ho1, PcyA), which synthesize PCB from heme. The holocomplex thus prepared possessed a mixture of PCB and PVB as a chromophore. Further incubation at 50ºC allowed additional isomerization from PCB to PVB was detected. There is a second conserved cysteine residue (Cys494: TePixJ numbering) among blue-green photoreversible cyanobacteriochromes (i.e. Tlr0924 (3)). Mutation of Cys494 resulted in mis-assembly of a red-absorbing form, which did not exhibit photoconversion. Then, we studied functioning of a cysteine residue by FTIR measurement of the native TePixJ-GAF. Light-induced difference spectra of FTIR revealed a green light-induced crosslinking of a free SH group of possibly Cys494. These results suggest that the light-induced crosslinking of a cycteine residue (probably Cys494) to the PVB (probably at C10) takes place in the assembly of Pb that is extremely blue-shifted compared with the typical phytochrome. In conclusion, the in vitro reconstitution proceeds as two steps: the fast incorporation of PCB to form Pb*, which ligates PCB at the canonical cysteine residue and the second cysteine residue, and the slow isomerization of PCB to PVB, leading to assembly of the final photoreversible holoprotein. The extreme blue shift of TePixJ can be explained by reversible adduct formation between the chromophore and Cys494.

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P25. PHOTOREACTION OF A BLUE/GREEN REVERSIBLE PHYTOCHROME-LIKE PHOTORECEPTOR STUDIED BY LOW-TEMPERATURE SPECTROSCOPY

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Phytochromes are well-known photoreceptors mediating photomorphogenetic responses in plants. Plant phytochromes and some of bacteriophytochromes covalently bind a linear tetrapyrrole as a chromophore, and show red/far-red reversible photoconversions. PixJ1 is a phytochrome-like photoreceptors necessary for positive phototaxis in a unicellular cyanobacterium *Synechocystis* sp. PCC6803. We have already reported that PixJ1 protein covalently binds a linear tetrapyrrole and show a novel reversible photoconversion between a blue (Pb, absorption maximum at 435 nm) and a green (Pg, absorption maximum at 435 nm) absorbing forms [1]. In order to determine the photoconversion pathways, Histidine-tagged chromophore-binding (GAF) region of PixJ1 was coexpressed with enzymes involved in the phycocyanobilin (PCB) synthesis in *E. coli* as described previously [1], and photoreactions from Pb to Pg and also from Pg to Pb were investigated by measuring low-temperature light minus dark UV-Visible absorption difference spectra. During the photoreaction from Pb to Pg, primary photoreaction takes place below 77 K, and two intermediates were detected upon temperature rise above 200 K. The final product, Pg, was observed at above 240 K. During the backward photoreaction from Pg to Pb, on the other hand, three intermediates were detected above 100 K and the final product, Pb, appeared at 230 K. All the three intermediates of PixJ1 show bath-chromic shifts, in contrast to those of plant-type phytochromes showing blue-shifts during the photoconversion from Pfr to Pr. It is assumed that photoconversion of PixJ1 involves Z-Eisomerization around methine bridge between rings C and D of the chromophore like plant-type phytochromes. The present results of the low-temperature spectroscpy show obvious differences in the spectral features between PixJ1 and plant-type phytochromes suggesting diverse photoreaction mechanisms between them. Molecular basis for this diversity will be discussed.

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P26. TIME-DEPENDENT DFT ON PHYTOCHROME CHROMOPHORES: A WAY TO FIND THE RIGHT CONFORMER

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A theoretical approach based on the time dependent density functional theory (TDDFT) is implemented together with a polarizable continuum model for incorporating the bulk effect of the surrounding environment to estimate the excitation energies for different chromophores found in phytochrome. We realized that a crystallographic structure without geometry optimization as template for the calculations is critical to account for the steric effect of the apoprotein over the chromophore. Through an analysis of the theoretical spectra we predicted that phycocyanobilin inside the cyanobacterial phytochrome Cph1 likely adopts a semicyclic conformation ZZZssa (C5-Z,syn;C10-Z,syn;C15-Z,anti) (1), which was then confirmed experimentally from a crystallographic structure by x-ray diffraction (2). Analogously, we implemented the method to phytochromobilin, i.e. the chromophore of plant phytochrome, with results that suggest a ZZZssa structure too. Therefore, we assume that theoretical spectra based on TDDFT seem to give us a good way to predict the right conformer. Furthermore, we assess the ratio of the spectral bands on the theoretical spectra (Q-band over the Soret band or Q/S ratio) finding that the spectroscopic Q/S index indeed is directly related to the conformational isomerism of the chromophore.

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P27. ULTRAFAST DYNAMICS IN THE BLUE/GREEN LIGHT ABSORBING CYANOBACTERIOCHROME TLR0924

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The goal of this research is to identify and characterize, on a molecular level, the ultrafast (fs-ns) dynamic events leading to the activation of biliprotein photosensors. The recently static photochemical characterization of the cyanobacteriochromes superfamily of bilin-proteins that absorb blue and green light, as opposed to the red and far red light absorbing cyanobacteriochromes more commonly seen, provides a powerful opportunity for generating valuable knowledge about the nature and diversity the initial photochemical mechanisms in signal transduction in cyanobacteriochromes. One such example is Tlr0924 from the thermophilic cyanobacterium *Thermosynechoccocus elongatus* that photoswitches from a green state to a blue state and vice versa. Presented here is a fs-ps understanding of the mechanism of photoconversion. Rockwell *et al* modeled the photochemical mechanism for Tlr0924 to include four states and thermal equilibria in addition to the photoinduced reactions (Rockwell *et al* 2008. A second conserved GAF domain cysteine is required for the blue/green photoreversibility of cyanobacteriochrome Tlr0924 from *Thermosynechococcus elongatus*. *Biochemistry* 47, 7304-7316). The protein acts as a photoswitch wherein the blue absorbing species will convert to the green absorbing species upon absorbing blue light, and vice versa. Using ultrafast dispersed transient absorption measurement, we resolved the rapid kinetics of both forward and reverse relaxation processes. Our results show an unusual process for the conversion of the blue-absorbing PbS state into the green-absorbing Pg state via heterogeneous ground-state population that appear to evolve into a common excited-state intermediate. To our knowledge, this is the first ultrafast characterization of any class cyanobacteriochrome GAF domains to date.

P28. ROLES FOR THE BILIN PROPIONATES IN THE ASSEMBLY AND PHOTOCONVERSION OF HOLOPHYTOCHROMES

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In plants and cyanobacteria, biliverdin IXα (BV) can be reduced by ferredoxin-dependent bilin reductases (FDBRs) to yield phytobilin pigments including phycocyanobilin (PCB), which are then incorporated into photosensory proteins such as phytochromes and into cyanobacterial phycobiliprotein light-harvesting complexes. To explore the roles of the propionate moieties in bilin metabolism, biliverdin and PCB monoamides were incorporated into the model phytochromes DrBphP and Cph1, which form spectroscopically distinct Pfr states. Both phytochromes exhibit unusual Pr adducts with the appropriate 8-monoamide, demonstrating a role for the 8-propionate in assembly of bilin with apophytochrome. By comparison, the 12-propionate is dispensable for photoconversion in DrBphP, but may play a role in tuning the pK¬a of the bilin ring system and hence its spectral sensitivity. The 12-propionate is also dispensable for primary photochemistry in Cph1, but it is required for Pfr formation. These findings further underscore the structural and mechanistic differences between the two classes of phytochrome photochemistry.

P29. PRODUCTION OF A NOVEL CHLOROPHYLL IN THE CHLOROPHYLL d-DOMINATED CYANOBACTERIUM *ACARYOCHLORIS MARINA* MBIC 11017

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Acaryochloris spp. has chlorophyll (Chl) d as a major Chl and also a small amount of Chl a. In addition, minor pigments, i.e. Chl d' for photosystem (PS) I, pheophytin a for PS II, were detected in respective complexes isolated from the type strain, *Acaryochloris marina* MBIC 11017 (1, 2). This feature is quite unique and differentiates A. marina from other oxygenic phototrophs. Although widespread distribution of Chl d in the world was revealed, *Acaryochloris* spp. is the only known species that produces Chl d at this time, and the importance of this organism in aquatic primary production has been recognized. Recently, the complete genome of *A. marina* was sequenced (3), however, lack of a genetic technique prevented us from studying *A. marina* intensively by a molecular genetic approach. Thus, we tried to develop the technique for gene transfer into *A. marina*, and we succeeded in producing a few transformants by introduction of foreign genes. Among them, we focused on a transformant that expresses a gene involved in Chl biosynthesis. The pigment composition of the transformant was analyzed by HPLC, and the accumulation of a novel pigment was found. Based on the absorption spectrum, we estimated it to be a Chl derivative. The properties of the novel Chl in vivo will also be discussed.

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P30. CHROMOPHORYLATION OF PHYCOERYTHRIN BY THE LYASE CPES IN *PROCHLOROCOCCUS MARINUS*

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Phycobilisomes are the major light-harvesting complexes of cyanobacteria, rhodophytes and cryptophytes. These photosynthetic antenna proteins efficiently harvest light in the 'green gap' where chlorophylls absorb only poorly. This is achieved via linear tetrapyrrolic chromophores, known as bilins that are covalently bound via thioether bonds to specific cysteine residues of the apoproteins. Autocatalytic chromophore addition is rare whereas in most cases regio- and stereoselective chromophore attachment is supported by phycobiliprotein lyases. An increasing number of lyases has been identified in cyanobacteria but only few examples of the three known lyase types have been characterized in detail so far. Heterodimeric E/F-type lyases catalyze chromophore addition to the cysteine α-84 of phycocyanins and phycoerythrocyanins and can have an additional isomerase activity. T-type lyases serve the cysteine ß-155 binding site in phycocyanins and phycoerythrocyanins. The S/U-type in contrast acts as a more universal lyase and attaches bilins to cysteine α-84 of allophycocyanins and phycoerythrins and cysteine β-84 of all phycobiliproteins. In contrast to the majority of cyanobacteria, the unicellular marine cyanobacterium *Prochlorococcus marinus* MED4 uses an intrinsic divinyl-chlorophyll-dependent light-harvesting system for photosynthesis. Despite the absence of phycobilisomes this high light adapted strain possesses β-phycoerythrin (CpeB), a putative S-type lyase (CpeS) and enzymes for the biosynthesis of phycoerythrobilin (PEB) and phycocyanobilin (PCB). Here we spectroscopically characterize the chromophore binding properties of CpeB and CpeS. Both PEB and PCB assemble spontaneously to CpeB forming fluorescent complexes. The putative lyase CpeS covalently binds PEB as shown by affinity chromatography whereas PCB is attached non-covalently. Furthermore we present strong evidence for the chromophore transfer from the putative lyase CpeS to the phycobiliprotein CpeB. Spontaneous and lyase catalyzed chromophore attachment is compared via the spectroscopic features of the reaction products.

P31. ASSEMBLY OF A BACTERIAL MEMBRANE ORGANELLE

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Cells require a source of energy, usually provided by membrane-protein complexes borne on infoldings of membranes elaborated within organelles such as mitochondria and chloroplasts, and within some bacteria. In order to study the biogenesis of these essential energy-yielding membranes we used the well characterised prokaryote *Rhodobacter sphaeroides* to establish the relationship between 'nascent' (UPB) and 'mature' (ICM) intracytoplasmic photosynthetic membranes. Both types of membrane were purified and their morphology, protein organisation, and function compared at the single membrane level using electron, atomic force and fluorescence microscopy. The proteomes of the early and mature, budded membranes reveal fundamental differences, with the more complex UPB proteome possessing many biosynthetic functions and the mature ICM devoted to its core photosynthetic function. Gentle lysis of cells containing histidine-tagged ICM, in the presence of a surface containing Ni-NTA and subsequent AFM of this surface, demonstrates the existence of at least some 'free-living' ICM vesicles within the bacterial cytoplasm. Thus, these vesicles, which possess the machinery for converting light energy into ATP, can be regarded as bacterial membrane organelles.

P32. SUBUNIT INTERACTIONS AND MECHANISM OF THE *RHODOBACTER CAPSULATUS* MAGNESIUM CHELATASE COMPLEX: INSIGHTS FROM ENZYME KINETIC STUDIES

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The biosynthetic pathway for (bacterio)chlorophyll is initiated by magnesium chelatase (BchI, BchD, BchH). This first step involves insertion of magnesium into protoporphyrin IX, a process that requires ATP hydrolysis. The next step in (bacterio)chlorophyll synthesis is the SAM dependent methylation of magnesium protoporphyrin catalysed by BchM. Structural information shows the BchI and BchD subunits form a double-hexameric enzyme complex while BchH binds protoporphyrin IX and can be purified as BchH-protoporphyrin. Utilizing the *Rhodobacter capsulatus* magnesium chelatase subunits, data from continuous magnesium chelatase assays can be analyzed such that the BchD subunit is treated as the enzyme with both BchI and BchH-protoporphyrin IX as substrates. Michaelis-Menten type kinetics was observed with the BchI subunit whereas the BchH subunit exhibited sigmoidal kinetics with positive cooperativity (Hill coefficient of 1.85). The BchI:BchD complex had intrinsic ATPase activity and addition of BchH caused a large increase in ATPase activity. The stimulation of ATPase activity by BchH was concentration dependent and yielded sigmoidal kinetics which indicates that there is more than one binding site for the BchH subunit on the BchI:BchD complex. The increased ATPase activity initiated by the BchH subunit continued despite cessation of magnesium chelation activity and this indicates one or more secondary roles for ATP hydrolysis, and possibly an as-yet unknown switch required to terminate ATPase activity. One of the secondary roles for BchH stimulated ATP hydrolysis by a BchI:BchD complex is in the correct binding of protoporphyrin to BchH to form BchH-protoporphyrin which is subsequently capable of participating in magnesium chelation activity. These data suggest that ATP hydrolysis by the BchI:BchD complex causes a series of conformational changes in BchH to effect substrate binding, magnesium chelation and product release. Testing BchM as a potential moderator of ATP hydrolysis revealed that this subunit interacts with BchH in a 1:1 ratio and kinetic data suggest channeling between the magnesium chelatase and BchM.

P33. LIFETIMES OF CHLOROPHYLL AND CHLOROPHYLL-BINDING PROTEINS IN THE CYANOBACTERIUM *SYNECHOCYSTIS* sp. PCC 6803

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There is a vast disparity between the lifetime of chlorophyll (about a week in *Synechocystis*) [1] and chlorophyll-binding proteins such as the PsbA (D1) protein (on the order of an hour). This disparity raises the question how assembly of photosynthetic complexes is orchestrated as accumulation of free chlorophyll in the cell would be harmful in the light and in the presence of oxygen and free polypeptides may not be stable in the membrane. In order to gain insight in the lifetimes of photosystem II (PSII) chlorophyll and proteins, we developed a combined stable-isotope labeling (15N) and mass spectrometry method to be able to follow both old and new pigments and proteins. Photosystem I-less *Synechocystis* cells were provided with 15N-ammonium nitrate at a specific time, and cells were harvested at specific intervals. PSII complexes, carrying a His tag on the CP47 subunit, were isolated from a cell extract, and the ratio of labeled and unlabeled protein and chlorophyll was determined. The PsbA (D1), PsbB (CP47), PsbC (CP43), PsbD (D2), PsbE and PsbF (cytochrome b-559), PsbH, PsbO, and Psb27 proteins were identified in the complex by mass spectrometry. The half-lives of PSII proteins ranged from 3 hours (D1) to 30 hours (PsbE and PsbO), but interestingly during the first nine hours of labeling the amount of unlabeled PsbE and PsbO protein present in PSII complexes increased by ~25% suggestive of a considerable pool of unincorporated polypeptide that was present at the time of labeling. A similar phenomenon was observed with chlorophyll (~35% increase). To determine the role that SCPs (small Cab-like proteins, which are associated with damaged PSII [2]) may play in this process, similar labeling was carried out with the PSI-less/SCP-less strain. In this strain the half-life of PSII chlorophyll was about 4-fold shorter than in the PSI-less mutant (as expected [1,2]) but interestingly in the PSI-less/SCP-less mutant no increase in unlabeled protein or chlorophyll was observed after labeled ammonium acetate was added. These results suggest that: (1) *Synechocystis* cells have reservoirs of a significant amount of chlorophyll and/or its precursors. (2) There is a pool of selected PSII polypeptides in the thylakoid membrane that are not incorporated into a PSII complex. And (3) SCPs may function both as reservoirs of chlorophyll precursors and chlorophyll in *Synechocystis* cells and in stabilizing non-incorporated PSII proteins.

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P34. TRACKING STRUCTURAL CHANGES WITHIN THE BILIN CHROMOPHORE DURING LIGHT SENSING IN PHYTOCHROME USING FEMTOSECOND STIMULATED RAMAN SPECTROSCOPY

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Photochemical interconversion between the red (Pr) and far-red (Pfr) absorbing form of the photosensory protein phytochrome initiates signal transduction transduction pathways in bacteria and higher plants that affect growth and development. The Pr-to-Pfr photoreaction is driven by an ultrafast bond isomerization at the C15=C16 methine-bridge within the bilin chromophore. Using femtosecond stimulated Raman spectroscopy (FSRS), we track the ultrafast nuclear dynamics during the isomerization by recording transient vibrational structural changes of the chromophore after excitation of Pr. The enhanced intensity of the C15-H hydrogen out-of-plane (HOOP) mode, and the appearance of red-shifted C=C stretch and N-H in-plane rock by 500 fs reveal the initial distortion of the C15=C16 bond to occur during the excited-state. In cyanobacterial phytochrome Cph1, the HOOP and skeletal modes evolve within 3 ps to a Lumi-R-like pattern in the excited-state, during which time 85% of the excited population relaxes back to Pr. The remaining 15% relaxes to the ground state Lumi-R photoproduct in 30 ps, revealing that the 15% photochemical quantum yield is determined within 3 ps after excitation [Dasgupta et al 2009 *Proc. Natl. Acad. Sci.* 106, 1784-1789]. Detailed vibronic analysis of the Pr absorption spectrum using resonance Raman intensities support 1) a homogenous photoexcitable Pr ground state population and 2) a moderate, but similar excited state displacement along the C=C stretch and the HOOP mode, emphasizing evolution on a multimodal nuclear coordinate during the ~150 fs Franck-Condon dynamics. To test these conclusions, more recent FSRS analysis of plant and cyanobacterial phytochromes substituted with isotope-labeled phycocyanobilin will be presented.

P35. SUBSTRATE RADICAL INTERMEDIATES IN CYANOBACTERIAL BILIN REDUCTASES

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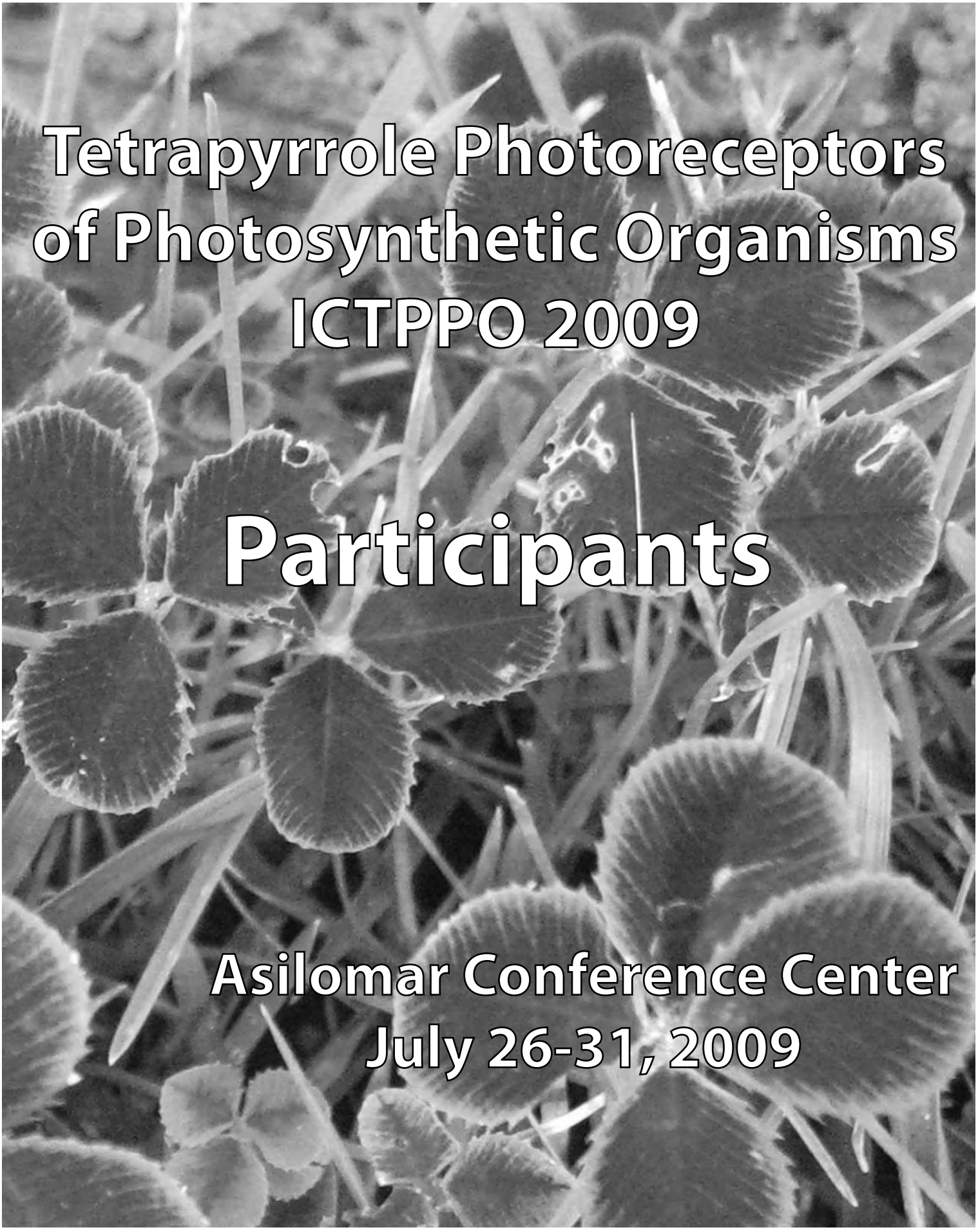
Phycocyanobilin:ferredoxin oxidoreductase (PcyA) is a member of a group of cyanobacterial bilin reductases that regioselectively reduce one or two double bonds of biliverdin IXα to produce linear tetrapyrrole chromophores that are used to capture light for photosynthesis and to detect light in phytochrome photoreceptors. In these bilin reductases, the reductions proceed via substate-centered radical intermediates and involve successive steps of electron and proton transfers. The enzymes are highly unusual as they directly deliver the electrons from an extrinsic reductant to the substrate without the help of any cofactors. We present recent results from high-field EPR, ENDOR and ESEEM of the trapped radical intermediate in wild-type and mutants of PcyA from *Synechocystis* sp. PCC6803 together with density functional theory calculations that help to identify the exact nature of the radical intermediates and thus enable us to clarify the reaction mechanism.



Tetrapyrrole Photoreceptors of Photosynthetic Organisms ICTPPO 2009

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