

Volume 11 Number 1

February 1995

- CYANONEWS a newsletter intended to provide cyanobacteriologists with a forum for rapid informal communication, unavailable through journals. Everything you read in this newsletter is contributed by readers like yourself. Published occasionally, about three times per year.
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NEWS

- * Chromophore spectra made plain
- * Startling glycolipid genes in hetR region
- * Plastocyanin works in normally PC-less strain
- * Calcium powers cyanobacterial membranes
- * New extrinsic PSII protein
- * Meeting report: Phototrophic Prokaryotes

ANNOUNCEMENTS

- * Daniel Arnon (1910-1994)
- * Meetings
- * Positions sought, Positions available
- LATEST REFERENCES

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Matters Arising

That mammoth project, *THE MOLECULAR BIOLOGY OF CYANOBACTERIA*, edited by Don Bryant, has finally been published, all 28 chapters and 916 pages. The book is divided into four parts: (1) molecular evolution and taxonomy, (2) structural and functional aspects of the photosynthetic apparatus, and (3) biochemical processes, and (4) gene regulation and the phenomena they regulate. It is available at US \$355 (hardbound) or US \$190 (paperback).

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Dept., P.O. Box 322, 3300 AH Dordrecht, The Netherlands.

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E-MAIL: Services@Wkap.Nl

Gisela Hoschek is offering a novel service. She is recently retired from laboratory work (molecular biology, biochemistry, molecular genetics) but wants to stay connected and active. Her idea is to offer her services to HELP TRANSLATE OR EDIT research papers or grant applications in English written by authors who are not native speakers. She is fluent in German and passable in French.

CONTACT: Gisela Hoschek, 1124 Nardo Road, Encinitas, CA 92024 USA. TEL: 619-944-4233, E-MAIL: Hoschek@Jeeves.Ucsd.Edu As seems to happen once every year or two, Peter Wolk is wondering whether any cyanobacteriologist might care to accompany him on a BACKPACKING TRIP in the U.S. Rockies. The trip will occur sometime this summer. CONTACT: Peter Wolk, 22333cpw@msu.edu or 517-353-2049.

P.K. Singh hopes to use the newsletter as a vehicle for disseminating information about the various COLLECTIONS OF CYANOBACTERIA held throughout the world. As a start, he has described the objectives of the National Facility for Blue-Green Algal Collection (in New Delhi, India) of which he is the Project Director. Its objectives are:

- 1.To act as a national center for cultures of cyanobacteria;
- 2.To conduct research on cyanobacteria, especially the occurrence and distribution of N₂-fixing strains, their isolation, maintenance, and preservation, but also cyanobacterial physiology and genetics;
- 3.To provide advice to farmers in the use of cyanobacterial biofertilizers;
- 4.To organize seminars, conferences, and training sessions;
- 5.To act as a strong center for national research and development;
- 6.To provide advice on policy matters.
- CONTACT: National Facility for Blue-Green Algal Collection (Auditorium Complex), Indian Agricultural Research Institute, New Delhi-110012, INDIA. TEL: 91-011-578-8431, FAX: 91-011-575-2006, E-MAIL: Guest%Bic-iari@Dbt.Ernet.in

CONTACT (North America): Kluwer Academic Publishers Group, Order Dept. P.O. Box 358, Accord Station, Hingham MA 02018-0358 USA. TEL: 617-871-6600, FAX: 617-871-6528,

The 12th Annual Eastern Regional Photosynthesis Conference is scheduled for 24-26 March, 1995 at Marine Biological Laboratory, Woods Hole, MA, USA. Undergraduates, graduate students, and post-doctoral fellows are especially encouraged to deliver oral presentations. A single fee covering registration, accommodations for two nights, and meals starts at US\$162. Checks should be made payable to City College Bursar c/o Regional Photosynthesis Conference.

CONTACT: Marilyn Gunner, Dept. of Physics, City College of New York, 138th St. and Convent Ave, New York, NY 10031 USA.

TEL: 212-650-5501. FAX: 212-650-550312,

E-MAIL: Gunner@Sci.Ccny.Cuny.Edu

The Third European Workshop on the Molecular Biology of Cyanobacteria is scheduled for 11-14 May, 1995 in Sevilla, Spain. The registration fee will be 25,000 Spanish pesetas, with hotel lodging starting at 18,500 pesetas per person. Some fellowships are available. The deadline for receipt of payment and registration is Feb 28, 1995. These fees may be paid by bank transfer to: Cyanobacterial Workshop, account no. 43-475-527621, Banco Herrero, Calle Rioja, no. 7, E-41001 Sevilla, Spain (remembering to state your name on the bank transfer for identification).

CONTACT: Enrique Flores, Instituto de Bioquimica Vegetal y Fotosintesis Universidad de Sevilla-CSIC Facultad de Biologia Apartado 1113, E-41080 Sevilla Spain. FAX: +34-5-462 01 54, EMAIL:cyano@cica.es

The VTH CYANOBACTERIAL MOLECULAR BIOLOGY WORKSHOP will again be on the Pacific Ocean in Asilomar, California U.S.A., 21-25 July, 1995. Registration is U.S. \$50 and housing and meals begin at U.S.\$225. Deadline for abstracts is May 1. Deadline for registration is June 9.

CONTACT: Don Bryant, S-234 Frear Bldg., Dept. of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802. TEL: 814-865-1992, E-MAIL: DAB14@Psuvm.Psu.Edu The FIRST INTERNATIONAL CONGRESS ON TOXIC CYANOBACTERIA (BLUE-GREEN ALGAE) is the new descendent of the formerly biannual Nordic Symposia on Toxin-producing Algae. The Congress will be held on the Danish island of Bornholm in the Baltic on 20-24 August 1995. It is planned that the proceedings will be published.

CONTACT: Peter Henriksen, Dept. of Phycology, Botanical Institute, Ö. Farimagsgade 2 D, DK-1353 Copenhagen K, DENMARK. TEL: 45-35-32-22-90 or 45-35-32-22-99, FAX: 45-35-32-23-21, E-MAIL: PHenriks@Bot.Ku.Dk

The 13th International Symposium on Cyanophyte Research will take place in Rome 27 Aug to 3 Sep 1995. The Symposium will focus on taxonomy, extreme environments, biodiversity, cyanobacterial associations with other organisms, and ecophysiology. Registration is 200,000 lira. Meals and hotel accommodations start at 900,000 lira for the nine day symposium.

CONTACT: Patrizia Albertano, Department of Biology, University of Rome `Tor Vergata', via della Ricerca scientifica, 00133 Rome Italy. TEL: 39-6-72594345, FAX: 39-6-2023500,

E-MAIL: Albertano@Tovvx1.Ccd.Utovrm.It

The European Society for Photobiology will hold its 6th Congress in Cambridge (Churchill College) from 2nd to 9th September 1995. The congress will have special session on "Carotenoids in Photosynthesis and Medicine" and "Application of protein engineering for the study of light reactions of oxygenic photosynthesis"

CONTACT: Paul Heelis, Faculty of Science, Health and Medical Studies, The North East Wales Institute, Plas Coch, Mold Road, Wrexham, Clwyd, LLI 2AW,UK. FAX: 44 (0) 1978 290008, E-MAIL: Heelisp@Newi.Ac.Uk

Positions Offered

POSITION OFFERED: Post-Doc

CONTACT: Bruce Greenberg, Dept. of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, CANADA. TEL: 519-888-4567 x3209, FAX: 519-746-0614, E-MAIL: Greenber@Biology.Watstar.UWaterloo.CA

RESEARCH: (1) UV-B impact on plants, (2) Photoinduced toxicity of priority pollutants to plants. Both projects will be carried out at the biochemical and cellular levels, with effects on photosynthesis a major interest.

SEND: CV and names of three references.

POSITION OFFERED: Post-Doc

CONTACT: James Yungel, NASA Goddard Space Flight Center's Wallops Flight Facility in the Airborne Oceanographic Lidar (AOL) program. E-MAIL: Hoge@Osb1.Wff.Nasa.Gov or Yungel@Wff.Nasa.Wff

RESEARCH: Investigate optical properties of phycourobilin and phycoerythrobilin pigments contained in marine phytoplankton as it relates to remote detection of these pigments from laser excited fluorescence spectra and passive ocean color spectra.

SEND: Brief description of experience in related research.

POSITION OFFERED: Post-Doc

CONTACT: H.Y. Yamamoto, HITAHR, University of Hawaii, 3050 Maile Way, Gilmore 202 B, Honolulu, Hawaii 96822, USA

- RESEARCH: Biochemistry and mechanism of down regulation of PSII photochemical efficiency by xanthophyll-dependent non-photochemical chlorophyll fluorescence quenching.
- **REQUIREMENTS:** Research experience using isolated chloroplast systems. Experience or knowledge of chlorophyll fluorescence, xanthophyll cvcle. carbon-fixation, spectrophotometric methods and pigment-protein separations is highly desirable. Candidate should be self-motivated, able to work independently and accomplishment orientated.
- SUPPORT: One year with possible extension for another year.
- SEND: CV, letter of application, two confidential letters of recommendation.

Positions Sought

POSITION SOUGHT: Post-doc or sabbatical replacement

- CONTACT: S.A. Kulasooriya, Dept. of Botany, University of Peradeniya, Peradeniya, SRI LANKA. FAX: 94-8-32343
- TEACHING EXPERIENCE: 28 years at University of Peradeniya. Lectures, laboratory classes, field classes in Introductory Botany, Plant Diversity, Mycology, Microbiology, Soil Biology, Biological Nitrogen Fixation. Soil Fertility.

RESEARCH EXPERIENCE (abridged):

Ph.D., University of London (1971), G.E. Fogg and Peter Fay, advisors on "Heterocyst Differentiation and Nitrogen Fixation in Blue-green Algae". See Proc Royal Soc Lond (1972) 181:31-52. Nitrogen fixation in rice fields. See Ann Bot (1981) 47:31-52. **POSITION OFFERED: Post-Doc**

- CONTACT (before March 31): Carl Johnson c/o Susan Golden, Dept. of Biology, Texas A&M University, College Station, TX 77843 U.S.A. E-MAIL: JohnsonC@Bio.Tamu.Edu
- CONTACT (after March 31): Carl Johnson, Dept. of Biology, Box 1812-B, Vanderbilt University, Nashville, TN 37235 U.S.A. E-MAIL: JohnsonC@Vuctrvax.Bitnet
- RESEARCH: Study molecular basis of circadian rhythmicity in cyanobacteria, using reporter strain to isolate, clone, and identify genes involved in the circadian clockwork [see Science 266:1233-1236 and Proc Natl Acad Science USA 90:5672-5676].
- **REQUIREMENTS:** Training in current molecular genetic techniques and strong interest in circadian rhythms.
- SEND: CV, summary of doctoral dissertation and current research interests, two letters of recommendation.

- Sabbatical leave at International Rice Research Institute (IRRI), examining rice field cyanobacteria, especially epiphytic strains. See Blue-green Algae and Rice, by PA Roger and SA Kulasooriya (1980), IRRI, Los Baños, and Soil Sci Plant Nutr (1981) 27:19-27.
- Nitrogen fixation by Azolla-cyanobacterial symbiosis Biological nitrogen fixation and maintenance of soil fertility in sustainable ecosystems. See Plant Soil (1991) 135:297-302, Biol Fert Soil (1992)14:37-42.

SERVICE (abridged):

Visiting consultant FAO/IAEA Division of Soil Fertility, Irrigation and Crop Production, Vienna, Austria

UNDP Advisory Board on Nitrogen Fixation, IRRI Chairman, FAO Expert Consultation on Bio and Organic Fertilizers, Bangkok.

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NANCY FEDERSPIEL has moved ... You know all this? You heard long ago that she left U. Idaho for a biotech outfit in California? Well, she's moved again. This time to the Genome Center, with Ron Davis' group, at Stanford University. She hopes someday to return to cyanos.

Genome Center, Dept. of Biochemistry, Stanford University, Stanford CA 94305. E-MAIL: NFeder@Genome.Stanford.Edu

P.K. SINGH has left the Indian Institute of Sugarcane Research to take a position as Project Director of the National Facility for Blue-Green Algal Collection [See announcement, this issue].

National Facility for Blue-Green Algal Collection (Auditorium Complex), Indian Agricultural Research Institute, New Delhi-110012, INDIA. TEL: 91-011-578-8431, FAX: 91-011-575-2006, E-MAIL: Guest%Bic-iari@Dbt.Ernet.in

ZHAO JINDONG, after leaving Don Bryant's lab at Pennsylvania State University, and resting for a while at Applied Biosystems in California, now finds himself in a faculty position at Beijing University, still interested in photosynthetic electron transport.

Dept. of Plant Molecular Biology, College of Life Sciences, Beijing University, Beijing 100871, P.R.CHINA. TEL: 86-1-250-1855, FAX: 86-1-258-5864

GOVINDJEE hasn't moved but electrons will have to travel a different path to reach him.

E-MAIL: Govindje@aries.scs.uiuc.edu (note only one "e" at end) or (preferably) Gov@Uiuc.Edu. E-mail with enclosures can be sent to Govindjee@Powershare.Life.Uiuc.Edu. FAX: 217-244-7246 (office), and 217-337-6196 (home).

Daniel I. Arnon died suddenly of cardiac arrest at the age of 84 on Tuesday, December 20, 1994 in Berkeley, California. At the time of his death, Arnon was an emeritus faculty member at the University of California at Berkeley, where he had spent his entire academic career carrying out his pioneering work on the biochemistry of photosynthesis. With his passing, an era in the field of photosynthesis has ended.

In a series of historical papers in the mid-1950's, Arnon's Berkeley group, which included M. B. Allen and F. R. Whatley, discovered that chloroplasts were capable of synthesizing ATP in the light, in a process Arnon called 'photosynthetic phosphorylation" (photophosphorylation) to distinguish it from oxidative phosphorylation. The first type of chloroplast phosphorylation discovered, denoted "cyclic phosphorylation," produced only ATP in the light. This discovery was followed by another revolutionary finding--that both ATP and NADPH could be produced photochemically and that their production was linked to the evolution of oxygen in a series of reactions called "non-cyclic photophosphorylation.". As part of this series of studies, isolated chloroplasts were found to be able to carry out complete photosynthesis in the light, a finding that proved that "cell-free" photosynthesis was possible .In an extensive series of papers with M. Losada and A. Trebst that followed this work, the Arnon group then extended these early observations by showing that the photophosphorylation reactions could generate the ATP and NADPH required for CO₂ assimilation. This was the first demonstration that complete photosynthesis, the process central to life on our planet, could be experimentally obtained outside a living cell. As a result of this work, the field of photosynthesis had been permanently changed: for the first time it was realized that the chloroplast had the complete capacity to carry out the reactions of photosynthesis, whereby light-energy is converted into organic compounds.

The discovery of non-cyclic and cyclic phosphorylation led Arnon to consider the mechanism of these processes. This resulted in K. Tagawa and Arnon identifying and characterizing the iron-sulfur protein, chloroplast ferredoxin in the early 1960s. Several other laboratories had been working with this protein under other names but the Berkeley work clarified the role of this protein in both the cyclic and non-cyclic pathways. Through their study of ferredoxin-NADP⁺ reductase, the enzyme actually involved in NADPH formation, M. Shin and Arnon were able to define the mechanism of NADP⁺ reduction, and in further work, to present their view that ferredoxin serves as the natural catalyst of the cyclic pathway. In characterizing chloroplast ferredoxin, Tagawa and Arnon had noted that this carrier had a midpoint redox potential more electronegative than the NADPH/NADP⁺ couple, raising the possibility that CO_2 fixation might occur directly through the input of electrons from reduced ferredoxin without utilizing the reduced pyridine nucleotide system. This realization stimulated studies on photosynthetic bacteria which led to the discovery of the reductive carboxylic acid cycle for CO_2 fixation in work with Mike Evans and Bob Buchanan in the mid-1960s. This pathway is independent of the pathway previously demonstrated in higher plants by Melvin Calvin, J. Bassham and A. Benson, also on the UC Berkeley campus.

The 1970s led Arnon and his group to consider mechanisms of electron transfer in chloroplast photosynthesis. Based on his belief of the role of non-cyclic and cyclic phosphorylation in chloroplasts, Arnon proposed a mechanism for electron transfer the ran against the main current in the photosynthetic field: that Photosystem I was involved only in the cyclic pathway and only Photosystem II was linked to the non-cyclic pathway. This view, held in modified form until his death, differed from the widely accepted Z-scheme for electron transport in chloroplasts in which the two photosystems cooperate in transferring electrons from water to NADP⁺. Up to the time of his death, Arnon was still regularly coming to his office in the Department of Plant Biology, writing extensively on his views on photosynthesis.

Arnon was recognized professionally both nationally and internationally. He was a member of the U. S. National Academy of Sciences and academies in Sweden, France and Germany. He was a Guggenheim Fellow with David Keilin in Cambridge, England and Hugo Theorell at the Karolinska Institute in Stockholm, Sweden and a Fulbright Scholar with Otto Warburg in Berlin. In 1973 he was awarded the National Medal of Science for "his fundamental research into the mechanism of green plant utilization of light to produce chemical energy and oxygen and for contributions to our understanding of plant nutrition." In recent years we have seen the loss of major figures in our field: Robin Hill, Bessel Kok and Warren Butler. Equally as significant is the passing of Dan Arnon. His contributions over an almost 50 year period on this field are inestimable.

Richard Malkin, Dept. of Plant Biology, University of California, Berkeley

(Editor's note: While all of us are indebted to Daniel Arnon for changing the way we think about photosynthesis, some may not be aware that he also deserves our remembrance every time we grow a strain in A&A (Allen & Arnon) medium).

Theory Developed for Analysis of Polarization Spectra

In a previous issue of CyanoNews [Volume 10, Number 1, February 1994] ANDREY DEMIDOV reported on his work developing a new theory for analyzing the polarization spectra of complex molecular systems with energy transfer between molecules (chromophores). Molecules can have strongly overlapping spectra. Demidov succeeded in deriving analytical formulae for determination of the polarization spectra (at steady-state) and depolarization kinetics (δ - pulse excitation) in cases of double- and triple-chromophore complexes. The formulae were tested by applying them to C-phycocyanin subunits and monomers, and good agreement with experimental data by Mimuro et al. [Biochim Biophys Acta (1986) 848:155] were found. The results of these investigations have been published (see his references below under PHYCOBILISOMES).

Demidov is now considering the case of higher aggregates - trimers, hexamers, etc. His preliminary results are promising and can be used for quantitative deconvolution of absorption and fluorescence spectra of allophycocyanin trimers. Such deconvolution will provide individual spectra of alpha and beta chromophores. Deconvolution involves absorption, fluorescence, and polarization spectra, i.e. it is based on the spectroscopic approach.

He is looking for someone who may be interested in cooperation on this matter. Cooperation can be in the form of work (preferably) in some particular laboratory having necessary facilities and APC supplies, or in the form of supplying the experimental data.

Demidov can be reached c/o D Andrews, University of East Anglia, School of Chemistry, Norwich, Norfolk NR4 7TJ UK, or by E-mail at A.Demidov@Uea.Ac.Uk

Plastocyanin Promotes e Transport in PC-less Strain

Plastocyanin, a copper-bearing protein, is used by plants and most green algae to donate electrons to Photosystem I. The protein is relatively rare in cyanobacteria, which use cytochrome c_{553} instead as the PSI donor. *Synechococcus* PCC 7942 is an example of a cyanobacterium that evidently lacks plastocyanin and the gene, *petE*, that encodes it. DIRK GEERTS and others at Utrecht, in collaboration with Hendrik Schubert and Hans Matthijs, took a *petE* gene from a cyanobacterium, *Anabaena* PCC 7937, that <u>does</u> use plastocyanin and expressed it in *Synechococcus*, wondering whether the strain would make any sense out of it at all. To their surprise, the foreign protein was readily accepted by PSI, and electron flow through PSI was markedly enhanced.

The *petE* gene from *Anabaena* was expressed from an inducible *E. coli* promoter, P_{trc} , thereby disconnecting transcription of the gene from its normal regulation by the availability of copper. The gene product was efficiently processed in *Synechococcus* and properly targeted to the thylakoid lumen. Isolated thylakoid membranes from strains expressing *petE* showed up to 2.5-fold higher rates of electron transport than native membranes, and a similar enhancement was evident in whole cells.

The activity of exogenous plastocyanin in *Synechococcus* may have interesting implications regarding the mechanism by which electrons are distributed between photosynthesis and respiration. Plastocyanin nearly abolished the competition between the two systems. These and other results are described in a recently published paper [Geerts et al. (1994) J Biol Chem 269:28068-28075].

Surprise Glycolipid Biosynthetic Genes in het Region

The region of the *Anabaena* PCC 7120 chromosome near *hetN* has already provided a few surprises for those interested in heterocyst differentiation, for example genes that in multicopy induce unscheduled heterocyst differentiation [Black et al (1994) J Bacteriol 176:2282-2292]. CHRIS BAUER tells us that the region still has a few eye-openers left. Chris also noted that a cosmid containing the *hetN* region affected heterocyst differentiation, but what really caught his attention were three genes upstream from *hetN*: *hglB*, *hglC*, and *hglD* (for *h*eterocyst glycolipid).

hglB has already been partially characterized. It contains domains for acyl-carrier protein and NAD(P)H β -ketoacyl reductase similar to those found in polyketide or fatty acid synthases. According to Chris' sequence, hglC contains acyl/malonyl ACP transferase and β -keto-synthase domains, and *hglD* (only partially sequenced) also contains a β -keto-synthase domain. Inactivation of any of the three genes gave rise to a Fix⁻ phenotype: regularly spaced, ultrastructurally normal heterocysts (as judged by light microscopy) incapable of nitrogen fixation. All three genes, and *hetN* as well, are transcribed from 6 to 12 hours after nitrogen limitation, as seen by Northern time-course blots.

These observations prompted a thin layer chromatography experiment to check for the presence of heterocyst-specific glycolipid in mutants defective in hglB, hglC, or hglD. It turns out that all three mutants fail to produce the lipid, and Chris now believes that the products of these genes are involved in glycolipid biosynthesis.

Calcium Fluxes Can Energize Cyanobacteria

Cyanobacteria have been isolated from fresh, brackish, salty, and extremely saline waters, and many highly mineralized bodies of water are more greatly enriched with Ca^{2+} or Mg^{2+} than with Na⁺ [Hammer UT (1986) *Saline like ecosystems of the world*. W. Junk, Dordrecht, p. 126]. IGOR BROWN has been inspired by two ideas regarding the earth's early environment to propose that cyanobacteria living in calcium-enriched waters may use calcium fluxes to couple bioenergetic reactions.

Ancient bodies of water are postulated to have been alkaline with Ca²⁺ as the major cation [Ronov (1964) Geochem [Moscow] 8:715], and cyanobacteria are thought to have been amongst their first inhabitants [Zarvazin (1993) Microbiol [Moscow] 62:789]. If these two ideas were true, reasoned Brown, then saturation by Ca²⁺ and Mg²⁺ would have prevented the generation of effective levels of $\Delta\mu_{H+}$ or $\Delta\mu_{Na+}$. On this basis, they have proposed that Ca²⁺ may under some conditions play the role of coupling cation [Brown II (1994) Biochem [Moscow] 8:715]. *Gloeobacter violaceus* living on limestone [Rippka (1974) Arch Microbiol 100:419] was studied to test this hypothesis.

Brown and Galina Gorbik previously showed that Na⁺ can support electrogenic import of extracellular H⁺ by *G. violaceus* cells from alkaline medium [Gorbik GP, Brown II (1994) Int Symp Photosynth Prokar. Abstracts. Urbino, Italy. 1994. p.25]. Now they report that Ca^{2+} is able to substitute for Na⁺ to support growth of the strain under alkaline conditions as well as electrogenic import of extracellular protons. The optimal Ca^{2+} concentration for these phenomena is about 9 mM. G. violaceus is not able to grow under alkaline conditions (pH >= 9) if any BG-11 medium salt containing Na⁺ or Ca^{2+} is replaced by a similar K⁺-containing salt. Growth of the strain is normal under alkaline conditions in standard BG-11 (approximately 20 mM Na⁺).

On the basis of this and other data [Geisler M et al (1993) J Mol Biol 234:1284], Brown and Gorbik suggest that $\Delta \mu_{Ca}$ may provide energetic coupling in cyanobacterial membranes.

New Extrinsic Proteins Found in Photosystem II

SHEN JIAN-REN reports that his group has found two new extrinsic proteins in purified cyanobacterial photosystem II particles, namely, cytochrome c_{550} and a 12 kDa protein. They have demonstrated that these two extrinsic proteins are required to maintain maximal activity of oxygen evolution in cyanobacterial photosystem II.

VIII INTERNATIONAL SYMPOSIUM ON PHOTOTROPHIC PROKARYOTES - Meeting Report

The triennial International Symposium on Phototrophic Prokaryotes called to the ancient Italian village of Urbino the usual collection of red, green, and blue-green aficionados. The Symposium exhibited the most vigorous competition (dance contest) and youngest convener (Luca Zannoni, age 8) in recent memory but also boasted a variety of interesting talks and poster. The perspectives below are intended to provide a flavor of the meeting; a complete summary of all the varied high points could not possibly fit into a single newsletter. If you want a fuller account of progress in cyanobacteriology, you may have to wait until the IX International Symposium, to be held in 1997 in Vienna, Austria.

Antennae and Reaction Centers

Much of interest was presented concerning antenna systems. Rowe and Griffiths have isolated a gene encoding a protochlorophyllide reductase from *Phormidium laminosum*. It will be interesting to see if this enzyme functions as the light-induced reductase in cyanobacteria. Going slightly further afield, Partensky and LaRoche showed that the N-terminus of the apoprotein of the light-harvesting apparatus from the prochlorophyte *Prochlorococcus* is 82% similar to the CP43' protein (encoded by *isiA*). The authors speculated that *Prochlorococcus* could have descended from cyanobacterial ancestors and had replaced the phycobilisome with a CP43'-like antenna which might have given them an advantage for growth in iron-depleted oceanic areas.

For the noncyanobacterially inclined, Judy Shiozawa and Reiner Feick rekindled the chlorosome debate by presenting evidence that proteins play an important role in chlorosome structure in *Chloroflexus aurantiacus*. Digestion of purified chlorosomes by proteases produced distinct changes in the shape of the chlorosomes. Katsiou and Tadros have cloned a new LH II gene family from *Rhodopseudomonas palustris* which they have named $\alpha \beta_e$ (bringing the total number to five). The β_e subunit is the same as the β_b subunit, but the α_e is very different from the other α subunits. This may allow the formation of complexes with different absorbance characteristics. Indeed, more than one LH II can be detected in the ICM in vivo by spectroscopic analyses.

An interesting development from Kjaer, Golbeck, and Scheller on green- sulfur bacterial reaction centers was presented. They have isolated reaction centers from Chlorobium vibrioforme with two intact Fe-S centers which resemble the higher plant F_A and F_B clusters. The isolated reaction center complex contains 6 polypeptides and supported photoreduction of NADP⁺ when ferredoxin and FNR were added. Returning to cyanobacteria, Muhlenhoff, Bryant, Zhao, and Setif presented data on a cyanobacterial PS I complex that had been cross-linked with flavodoxin. Flavodoxin was covalently linked to the PsaC and PsaD proteins and required the PsaE protein in order to bind in the proper orientation. This cross-linked complex could not support electron transport to FNR. However, flavodoxin could be functionally photoreduced from the semi-quinone to the fully reduced form. Wim Vermaas presented comparisons between heliobacterial and cyanobacterial reaction center complexes: an evolutionary model in which PS I and II of cyanobacteria evolved from a homodimeric reaction center, which probably resembled the heliobacterial reaction center.

-- Wendy Schluchter

Much work on microbial mat ecology has examined how nutrient availability may influence species composition and distribution. In these complex photosynthetic systems nutrient availability may only play part of the story. Light as an ecological factor had been largely unstudied. Dick Castenholz presented results of a recent examination of how UV and visible light may influence survival strategies of various cyanobacterial groups. This work along with similar studies of deeper-lying Chloroflexus species (presented by Beverly Pierson) is providing interesting new insights into the structure of mat communities. It will be interesting in the future to see how field-based studies such as these will dovetail with pure-culture biochemical work on, for example, complementary chromatic adaptation. The role of sulfide and its metabolism by various cyanobacterial species in the laboratory is combined with ongoing detailed field work in the work of Rethmeier et al., to likewise generate a comprehensive picture of the role of sulfide in affecting cyanobacterial photosynthetic activity and distribution.

Newly described species were the focus of several nice presentations. A few that particularly caught my eye were: (1) a beautifully detailed study of a red strain of *Spirulina* subsalsa from a freshwater lake (by Luisa Tomaselli et al.) (2) the description of *a Leptolyngbya* spp. with an eye-spot type structure by Patrizia Albertano and Maria Grilli Caiola, and (3) an intriguing *Phormidium* sp. responsible for killing corals in the Florida Keys which is currently being well-documented in situ utilizing microsensors for making oxygen, sulfide and pH measurements in the field by Laurie Richardson

New advances in taxonomy are being provided by the use of molecular techniques. Research in this area, utilizing these new and still developing technologies, is providing something of an explosion of new information. Annick Wilmotte and Michael Herdman both presented good overviews of what we can and cannot get out of the various phylogenetic tree schemes available along with some important interpretation caveats. These techniques are finally offering us the tools needed to clarify the confusing taxonomic picture of the cyanobacteria that we all know and love.

These taxonomic findings may also have large ecological implications. One such example was new data on the group *Microcoleus chthonoplastes* which is suggesting that this group maintains a high degree of genotypic and phenotypic homology world-wide with important implications for a high degree of specificity for their particular niche habitat.

This meeting also provided a chance for many of us ecologically-inclined souls to hear and see presentations on a somewhat bewildering array of biochemical and molecular topics. In particular the series on reaction centers were particularly interesting even if they sent the majority of ecologists out looking for molecular-speak glossaries. Edification is good for the soul. Especially in surroundings like the Toscany valley. In conclusion, many thanks to the organizers. It was a great meeting!

-- Lee Prufert-Bebout

Light Receptor and Signal Cascade in Cyanobacteria?

The mechanisms by which cyanobacteria recognize changes in light quality and intensity is thus far incompletely understood. Studies aimed at understanding the signal transduction pathway linking light to light-regulated behavior may proceed profitably down two parallel tracks: analysis of light-regulated genes and comparative studies of photoreceptors.

Susan Golden described the transcriptional regulation of the *psbA* genes of *Synechococcus* PCC 7942, encoding the D1 proteins of photosystem II. It is very interesting that the transcription of a *psbA* gene is regulated by blue light signal. The cis element for the blue light response has been provisionally identified using many mutants carrying deletions in the 5' upstream region of the *psbA* genes. The blue light signal is very likely to be recognized by a specific photoreceptor or photoreceptors in cyanobacterial cells. A mutant defective in the blue light response might provide a clue as to the nature of the cyanobacterial blue light receptor. Franck Chauvat identified sequence elements common to light-regulated promoters of *Synechocystis* PCC 6803. The mechanisms of signal transduction of light will be gradually solved by the isolation of light-regulated genes and genetic studies of mutants defective in the regulation of their expression by light. Meanwhile, a direct approach to defining the associated photoreceptors have been producing interesting insights. W.D. Hoff presented a photoreceptor of halophilic purple bacteria. They purified the photoactive yellow protein (PYP) and determined the molecular structure of PYP. A photocycle of PYP is similar to that of rhodopsin. However, the chromophore of PYP is not a retinal. The chromophore is surrounded by the apoprotein, and PYP associates with the membrane. The structure of PYP is very different from that of rhodopsin.

Hans Matthijs suggested that a rhodopsin-like receptor acts as a sensor for complementary chromatic adaptation of the cyanobacterium, *Fremyella diplosiphon (Calothrix)*. They showed that the cyanobacterium contains a retinal. It should be proven whether a photoreceptor homologous to PYP can be found in cyanobacteria or whether cyanobacteria generally contain a retinal. However, it is likely that cyanobacteria contain a photoreceptor similar to the well-known photoreceptors.

-- Toshio Sakamoto

Cyanobacterial Development

closer discovering We are coming to how heterocyst-forming cyanobacteria determine the frequency and spacing of heterocysts along a filament. The results from Jeff Elhai's poster imply that the determining factor for the initiation of heterocyst development may reside in individual cells and not signals from adjacent cells in a filament. He found that long filaments of Anabaena containing luxAB driven by the *hetR* promoter and filaments sonicated to single cells both expressed the same level of luciferase activity upon induction by nitrogen deprivation. Consistent with this result, an EM survey showed that a single-cell mutant of the same strain responds quite inhomogeneously to nitrogen starvation: only about 5% of its cells produce heterocyst-specific polysaccharide.

From the results of Peter Rowell et al. one can begin to build a model to explain Elhai's results. They found that formazan deposition in A. cylindrica filaments incubated with MTT was localized to cell poles and distributed in a pattern along a filament at potential sites of heterocyst development. Thus an apparent differential accumulation of polarly localized respiratory electron transport, possibly laid down as a function of pole age, may have a role in determining which cells are destined to become heterocysts. At sites where intercalary heterocysts will form, two vegetative cells with nearly equal amounts of formazan deposition are found; these two cells must somehow communicate to decide which will generate the daughter cell that differentiates into a heterocyst. Jack Meeks noted that in many Fox mutants of Nostoc ATCC 29133 intercalary heterocyst doublets are common, possibly because of a defect in this cell to cell communication.

Of course akinetes exhibit polarity as well. Sili and Vincenzini presented some beautiful micrographs showing polar germination of *Cyanospira* akinetes.

Bill Buikema and Bob Haselkorn reported obtaining up to 15% heterocysts along a filament by overexpressing *hetR* transcription in *Anabaena* PCC 7120 containing a plasmid with *hetR* behind the copper inducible *petE* promoter. It will

be interesting to see if HetR is a point of control for increasing the frequency of heterocysts in symbiotic systems. Bill Buikema is investigating the green fluorescent protein (GFP) as an alternative reporter to *luxAB* in cyanobacteria. Expression of GFP is easy to detect and has advantages over *luxAB* in not requiring a substrate or oxygen for activity but, unlike *luxAB*, it is apparently toxic at high concentrations. As was repeatedly stressed, *luxAB* can serve as a useful reporter even without a \$140,000 photometer.

Several different sized transcripts per gene, whether due to multiple promoters or processing of larger transcripts, appears to be a common theme in cyanobacteria. For example, Mike Summers reported 16 different transcripts within the 4 region, encoding glucose-6-phosphate gene zwf dehydrogenase. As the case with most cyanobacterial transcripts, it is unknown how the levels of the specific mRNAs are regulated. In a poster, Martin Mulligan et al. showed the ubiquity of RNA-binding proteins in cyanobacteria. These proteins are known to have a variety of functions in eukaryotes (including in chloroplasts, where post-transcriptional regulation predominate).

Regarding hormogonia differentiation, I reported that in Nostoc ATCC 29133, just 2 bases upstream from a gene involved in decreasing sensitivity to a hormogonia inducing factor, there is a gene with homology to the same family of NAD(P)H-oxidoreductases that includes hetN. Perhaps proteins of this family are of use in the production or modification of signals affecting cyanobacterial differentiation. In two posters from Dave Adams' lab, S. Babic reported the isolation and initial characterization of mutants in hormogonium formation and H. Doherty reported cloning ftsZ from A. 7120; future work will examine whether ftsZ expression is regulated early in hormogonium development. Doug Campbell has found that hormogonium differentiation in Calothrix is favored by signals that inhibit heterocyst formation and vice versa. The role, if any, of the P_{II} protein a hormogonia/heterocyst (encoded bv glnB) in differentiation-signaling pathway is still under investigation.

--Mike Cohen

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