# CYANONEWS

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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.

SUBSCRIPTIONS - $10 or equivalent/year. (See address label for expiration date). No charge for electronic version.

CONTRIBUTIONS - Expected every couple of years: a new result, an upcoming meeting or a summary of a past meeting, a post-doctoral opening, a new publication, a request for strains, a change of life... something. See last page for addresses you can send news to.

HOW TO FIND OUT MORE ABOUT SOMETHING YOU READ HERE - Each news item contains, prominently displayed, the name of a contact person. A Directory of Cyanobacteriologists is distributed every two years or on request.

INSTRUCTIONS TO AUTHORS - Send news.





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## Matters Arising

Dalibor Stys seeks to complement his physico-chemical expertise and resources with genetically- or theoretically-minded COLLABORATORS to study the influence of individual proteins from thylakoid proteins on ion and temperature induced CHANGES IN PHOTOSYSTEM SEGREGATION AND STACKING. He wants to use thylakoid membranes for studies on specific and unspecific lipid-protein and protein-protein interactions as well as formation of membrane domains and ion-induced interactions between membrane lamellae.

He has access to and experience with many spectroscopical techniques and is looking for collaboration with any group that will supply him with mutants with defined modifications of thylakoid membranes.

CONTACT: Dalibor Stys, Plant Cell Biology, Box 7007, 220 07 Lund, Sweden. TEL: 46-46-222-3318, FAX: 46-46-222-4009, E-MAIL: Placebio-Dali@Macpost.Lu.Se or Dalibor.Stys@Placebio.Lu.Se

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L.V. Venkataraman offers to provide *SPIRULINA* TECHNOLOGY, on all aspects regarding setting up plants of various capacities, including information on product formulations.

CONTACT: L.V. Venkataraman, "Sudarshana", #236, 8th Cross, Gokulam 3rd Stage, Mysore 570 002 INDIA. TEL: 821-510006, FAX: 821-512539, TELEX: 0846-320 POLY IN

CRC Press has just published a new monograph entitled *TOXIC MICROCYSTIS*, edited by M.F. Watanabe, K.I. Harada, W.W. Carmichael, and H. Fujiki. It includes chapters on the ecology of *Microcystis*, and the chemistry and biologically effects of its toxins. The book is 400 pages long and costs US$189.95 (within U.S.A.) and US$228 (outside U.S.A.)

CONTACT: CRC Press, 2000 Corporate Blvd., N.W., Boca Raton, FL 33431-9868 U.S.A. TEL: 800-272-7737 (within U.S.A.) 407-994-0555 (outside U.S.A.) FAX: 800-374-3401 (within U.S.A.)

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Beverley Green is writing a REVIEW ON PIGMENT-PROTEINS for Annual Review of Plant Physiology, to be published in 1996 and would be grateful reprints of articles she may not have caught. She would also appreciate comments or questions on controversial points or any other aspect. The review covers cyanobacterial as well as plant proteins, structure determination, macromolecular organization, and molecular evolution.

CONTACT: Beverley R. Green, Botany Dept., University of B.C., Vancouver, B.C. V6T 1Z4 CANADA. TEL: 604-822-2349, FAX: 604-822-6089, E-MAIL: Beverley.Green@Mtsg.Ubc.Ca

## Meetings

The 15TH NORTH AMERICAN SYMBIOTIC NITROGEN FIXATION CONFERENCE will be held 13-18 August 1995 at North Carolina State University, Raleigh.

CONTACT: Gerald Elkan, Department of Microbiology, North Carolina State University, Box 7615, Raleigh, NC 27695-7615 U.S.A., TEL: 919-515-3945

A forum on the BIOTECHNOLOGY OF ALGAE will be held in Lake San Marcos Resort, San Diego, California (U.S.A.) 20 Sept 1995 in conjunction with the International Symposium on Plant DNA Preservation (17-20 Sept 1995).

CONTACT: E-MAIL: jonthn@aol.com

The FIRST INTERNATIONAL CONGRESS ON TOXIC CYANOBACTERIA 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 INTERNATIONAL ASSOCIATION OF APPLIED ALGOLOGY will hold its Congress in South Africa from 16-19 April 1996. Topics include algal production systems, photosynthesis and physiology, waste water treatment, and commercial ventures. Registration by the deadline of 30 Nov 1995 is US$200.

CONTACT: Johan Grobbelaar, Bloemfontein, Department of Botany and Genetics, University of the OFS, Bloemfontein 9300, SOUTH AFRICA. TEL: 27-51-4012514, FAX: 27-51-488772, E-MAIL: pjg@Rs.Uovs.Ac.Za

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: C.A. Rebeiz, Laboratory of Plant Pigment Biochemistry and Photobiology, 240 A, PABL, 1201 West Gregory Avenue, University of Illinois, Urbana IL 61801 U.S.A. TEL: 217-333-1968, E-MAIL: Tino@Vmd.Cso.Uiuc.Edu
RESEARCH: Either: (1) Study of apoprotein-chlorophyll interaction during the biosynthesis and assembly of functional light harvesting Chl a/b protein (LHC II) in higher plants, or (2) Cloning the [4-vinyl]chlorophyllide a reductase (4VCR) gene [Biochemistry 31:8460-8464 (1992)], an enzyme responsible for the heterogeneity of chlorophyll biosynthesis in plants [Ciba Foundation symposium 180, p177-193 (1994)].
REQUIREMENTS: Some expertise in one or more of the following: porphyrin biochemistry, protein isolation, purification and characterization, or plant molecular biological techniques. For the first position, experience in subcellular organelle isolation, purification and characterization would be helpful
AVAILABLE: Oct 1995
SEND: CV and three letters of recommendation

POSITION OFFERED: Post-Doc
CONTACT: Terry Bricker, Dept. of Microbiology, Louisiana State University, Baton Rouge LA 70803, U.S.A. E-MAIL: Btbric@Lsuvm.Sncc.Lsu.Edu
RESEARCH: Structure and function relationships in photosynthesis
SEND: CV and three letters of recommendation

POSITION OFFERED: Post-Doc
CONTACT: P. Sebban, Photosynthese Bacterienne, Bat. 24, Centre de Genetique Moleculaire, CNRS, 91198, Gif FRANCE. TEL: 33-1-69-82-38-26 FAX: 33-1-69-82-35-62 E-MAIL: Sebban@Citi2.Fr
RESEARCH: Electrostatic effects and proton conduction in bacterial reaction center membrane proteins.
REQUIREMENTS: Well-organized and flexible candidate able to pursue a multidisciplinary approach. Desirable but not definitely needed is experience in biochemistry and spectroscopy and knowledge of molecular biology and genetics.
AVAILABLE: From 1 Oct 1995 for three years
SEND: CV and statement of research interests

POSITION OFFERED: Post-Doc
CONTACT: David Kramer, Institute of Biological Chemistry, Washington State University, Pullman WA U.S.A. TEL: 217-244-8913 or 217-333-7407, E-MAIL: Kramer@Nemo.Life.Uiuc.Edu
RESEARCH: Characterization of photosynthetic electron transfer reactions in intact higher plants and in evolutionarily interesting algal and bacterial species.
REQUIREMENTS: U.S. citizenship or residence status. Experience desirable in one or more of following: isolation of membrane protein complexes, optical or electronics instrumentation, EPR spectroscopy, knowledge of photosynthetic or respiratory electron transfer reactions.
AVAILABLE: 1 Sept 1995

POSITION OFFERED: Post-Doc
CONTACT: H.Y. Yamamoto, University of Hawaii, 3050 Maile Way, Gilmore 202 B, Honolulu, HI 96822 U.S.A. E-MAIL: Yamamoto@Uhunix.Uhcc.Hawaii.Edu
RESEARCH: Molecular biology and physiological function of violaxanthin de-epoxidase, a key enzyme in the xanthophyll-dependent non-radiative energy dissipation of excess energy to down-regulate PSII photochemical efficiency.
REQUIREMENTS: self-motivated individual with a strong background in molecular biology and publication record sought. Knowledge and interest in photosynthesis highly desirable. Ph.D. in plant physiology, biochemistry, molecular biology, or related discipline required.
SEND: CV and names of three references

## Positions Sought

POSITION SOUGHT: Visiting professor/scientist (for 2-3 weeks only).
CONTACT: L.V. Venkataraman, "Sudarshana", #236, 8th Cross, Gokulam 3rd Stage, Mysore 570 002 INDIA. TEL: 821-510006, FAX: 821-512539, TELEX: 0846-320 POLY IN
RESEARCH EXPERIENCE: 25 years, basic applied aspects of *Spirulina* effluent treatment, integrated systems, biotransformations, bioenergy production. Over 200 publications.

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L.V. VENKATARAMAN has taken an early retirement from the Central Food Technological Research Institute in Mysore, India. He is keeping his academic research alive, continuing to guide research students and consulting on Spirulina biotechnology in India and abroad (see ANNOUNCEMENTS).

## David Laudenbach

We announce with great sadness that David Laudenbach died during surgery on Thursday June 15, 1995 at the age of 35. It is always sad to lose a colleague, and especially sad to lose a colleague who is so young and such an integral part of the cyanobacterial community.

David received his MSc and PhD at the University of Toronto. His research there focussed on the molecular genetic responses of *Synechococcus* PCC 7942 to iron deficiency. He isolated the gene for flavodoxin and showed that it was the second open reading frame of a dicistronic message whose transcription was tightly regulated by iron. He demonstrated that the first open reading frame encoded a protein with high homology to CP43, which he correctly guessed to be the iron-stress-induced, PS II, chlorophyll-binding protein that had been previously discovered in Lou Sherman's laboratory. He also cloned the gene for ferredoxin and showed that its expression was not affected by the concentration of iron in the growth medium. Before graduating David isolated and created mutants for the genes encoding iron superoxide dismutase and cytochrome $c_{553}$. The productivity of his graduate years set a pattern that would continue throughout the remainder of his career.

David left Toronto to do postdoctoral research at the Carnegie Institution for Plant Science, Stanford University. His project concerned the acclimation of *Synechococcus* to sulfur deficiency. David was able to functionally define systems involved in sulfate transport and sequence the genes encoding the components of these systems. He also defined a novel sulfur limitation induced gene, designated *rhd*, that may be involved in the utilization of certain thiol compounds during sulfur-limited growth. Finally, Dave discovered the regulatory gene *cysR* and postulated its involvement in controlling the utilization of thiocyanate during sulfur limitation. This work was extended to some of the highly productive projects that Dave developed independently as an Assistant Professor at the University of Western Ontario.

David was not the type of scientist that could be satisfied with one project and his curiosity always got the better of him. For example, while at Carnegie he started up collaborations with Dave Fork and Steve Herbert on the acclimation of *Synechococcus* to oxidative stress and its affect on the photosynthetic apparatus. His constant probing and 'playing' in the laboratory provoked both new ideas and the development of new projects. David was a talented and unique scientist.

David is survived by his wife Lori and two children Adam (5) and Theresa (3). A fund has been established for Adam and Theresa. If you wish to contribute please make cheques payable to "Lori Laudenbach in trust" and mail them to CIBC, 228 Oxford Street East, London, Ontario N6A 1T7, Canada. Enclose a letter stating who is making the contribution, including the names and addresses of all for group contributions, and that the contribution is to be directed to the trust fund for Adam and Theresa Laudenbach.

Arthur Grossman & Neil Straus

### Allen Acclaimed by National Society

Mary Mennes Allen was honored at the 1995 meeting of the American Society for Microbiology with the Carski Foundation Distinguished Teaching Award, in recognition of her career in inspiring undergraduates towards a career in science. Needless to say, a useful tool in her inspirational efforts has been her ongoing research into the function of cyanophycin in cyanobacteria.

### Controlled Expression of Foreign Genes in Chromosomes of *Synechococcus*

A few years ago workers at the University of Utrecht described a novel method to facilitate the stable insertion of foreign genes into an innocuous locus of the chromosome. The constructed chromosomal location, called PIM (for platform for integration in *metF*), consists of a promoterless *bla* gene and *oriV*, both from pBR322, a plasmid commonly used in molecular biology. Insertion of genes of interest into that site could be achieved, with selection for ampicillin (encoded by *bla*), upon recombination between the platform and their pBR322-derived vector. Dirk Geerts now tells us that he and others in Utrecht have extended the technique to permit inducible, high-level expression of genes placed in the platform. The original method has further been modified to greatly reduce the aberrant recombinational events that had plagued the technique in the past.

Their new vector, pTrcIS, provides a strong *trc* promoter whose expression is well controlled by the lactose repressor, encoded by the $lacI^q$ gene also on the plasmid. Downstream from this promoter is the *lac* operon ribosome binding site and a polylinker to facilitate transcriptional or translational fusions of inserted genes. Of particular utility is an NcoI site for the insertion of the 5'end of a gene directly to the ATG start codon. Flanking this region are a complete version of *bla* and *oriV*, required for integration into the platform. The Utrecht group also place *aadA*, determining resistance to streptomycin and spectinomycin.

They found, using *petE* (encoding the precursor to plastocyanin) from *Anabaena* PCC 7937 (*Anabaena variabilis*), and *uidA* (encoding ß-glucuronidase, GUS) from *E. coli*, that double recombination events placing the foreign gene into the platform occurred with a very low incidence of false positives when streptomycin was used as the selective agent. When ampicillin alone was used, the number of colonies recovered was much higher but the majority of recombinants were not true double recombinants, and the frequency with which the foreign gene was expressed in the recombinant varied from 0 to 100%, depending on the insert. Selection for streptomycin evidently ensures that virtually all of the colonies resulting from transformation of *Synechococcus* have the desired phenotype.

Expression of the foreign gene could be controlled within a wide dynamic range by the addition of graded amounts of the *lac* inducer IPTG, with full repression in the absence of the inducer. The highest level of induction was 36-fold, as judged by expression of GUS, or 100-fold, as judged by expression of *petE*. The level of expression by the $P_{trc}$-*uidA* fusion is almost 4-fold higher than that achieved by the strongest the cyanobacterial promoter ($P_{petF}$) tested.

The work has recently been fully described [Geerts et al (1995) Microbiol-UK 141:831-841].

### Excretion of Ammonia from Immobilized *Anabaena* Explained

Symbiotic cyanobacteria commonly release fixed nitrogen resulting from $N_2$ fixation to their hosts. It has long been a hope of some that cyanobacteria could be induced to excrete ammonia on a large-scale industrial setting. In 1987, Shi Ding-Ji and others [Planta 172:298-308] reported that ammonia excretion occurred simply by $N_2$-fixing cyanobacteria immobilized within polyurethane foams. Why this should be the case has been a mystery, but Shi describes to us how Duan Xue-Yan and others at Capital Normal University and Academia Sinica in Beijing have brought us one step closer to its solution.

The Beijing group found that *Anabaena* sp. strain 2B (isolated as an epiphyte of *Azolla caroliniana*) immobilized within polyurethane showed higher (1- to 2-fold) glutamine synthetase (GS) activity than a free-living culture over the several days following initiation of the experiment. Over the longer term, however, GS activity in immobilized *Anabaena* drops 10-20% below that of the free-living culture. This period of low GS activity roughly corresponds to the period of high production of ammonia by immobilized *Anabaena* reported earlier.

Hybridization of mRNA isolated from free and long immobilized cultures to a probe specific for GS mRNA indicate that the drop in GS activity is due to a corresponding decrease in GS message. In order to achieve this result, the group had to improve upon existing methods to extract RNA from immobilized cyanobacteria. Their modification permits efficient isolation, as judged by comparison of stable rRNA from immobilized and free cultures.

Details of the work have been published [Duan et al (1994) Chinese J Bot 6:102-106 (English)].

## X INTERNATIONAL CONGRESS ON NITROGEN FIXATION - Meeting Report

The 10th International Congress on Nitrogen Fixation took place 28 May to 3 June of this year in St. Petersburg, Russia. While the majority of presentations concerned themselves with the doings of heterotrophic bacteria, there were a few cyanobacterial nuggets, some of which are reported below.

Bernd Masepohl (Bonn) reported the identification of a NOVEL REPEATED DNA ELEMENT in *Anabaena* PCC 7120 with so far unknown function. This long tandemly repeated repetitive (LTRR) sequence is 37 bp long and contains an inverted repeat sequence. An LTRR-specific probe hybridized to numerous DNA regions in *Anabaena* PCC 7120 and many other cyanobacteria.

In addition he described the construction of a mutant derivative of *Anabaena* PCC 7120 defective in the FERREDOXIN-encoding gene, *fdxH*. The mutant exhibited much reduced nitrogenase activity, confirming that this [2Fe-2S] heterocyst ferredoxin (see B. Schrautemeier, below) is the principal electron donor to nitrogenase, but may also partly be replaced by (an) alternate donor(s).

The mechanism of cyanobacterial NITROGENASE REGULATION OR MODIFICATION into the inactive form of the Fe-protein is still an enigma, according to John Gallon (Swansea). ADP-ribosylation is evidently not involved, in contrast to the importance of such a modification in the case of glutamine synthase, as recently reported by Noel Carr and Nick Mann.

The mechanism of OXYGEN SENSING is now better understood in *Azotobacter vinelandii* (if we may slip in a noncyanobacterium). Ray Dixon (Sussex) described the characterization of the oxygen-sensor protein NifL. It contains FAD with FMN as minor component and controls the catalytic activity of NifA in the active ADP-bound form.

The regulatory protein OxyR plays a role under OXIDATIVE STRESS in *E. coli* and *Salmonella typhimurium*. Karin Jäger (Hannover) found an OxyR-like protein in *Anabaena variabilis* and *Anabaena* PCC 7120 by using a specific antibody against the *E. coli* protein. Southern blot analyses with the E.coli gene probe reveled no signal with cyanobacterial genomic DNAs.

Bernhard Schrautemeier (Bonn) reported on DUAL MO-NIF SYSTEMS expressed from separate nif gene clusters (nif1, nif2) of *Anabaena variabilis* ATCC 29413 that also were independently discovered by Teresa Thiel and coworkers in St. Louis. Teresa, using a *lacZ* reporter system and a fluorescent substrate, conclusively demonstrated localized expression of *nif1* (only in heterocysts) versus *nif2* (in all vegetative cells). Bernhard's approach emphasizes the time component/differential kinetics of oxygen-controlled *nif2*- versus developmentally-controlled *nif1*-expression after nitrogen deprivation: Nif2 is expressed only under strictly anaerobic conditions as early as 1-2 hours after nitrogen stepdown -- long before the appearance of proheterocysts. In contrast, Nif1 is expressed only after (pro)heterocysts have appeared, i. e. not earlier than about 10 hours after nitrogen depletion, irrespective of anaerobic or aerobic growth conditions. By using a standardized comparative induction assay monitoring nitrogenase activity during the 20 hours following nitrogen deprivation, he additionally demonstrated that *Anabaena* PCC 7120 has no characteristics of a functional Nif2 system.

Examining the region upstream from each *nifHDK* cluster, Bernhard hit upon different genes encoding ferredoxins: *fdxH1* and *fdxH2* for the *nif1* and *nif2* clusters, respectively. It is interesting that FdxH1 is oxygen-tolerant in vitro, while FdxH2 is rapidly inactivated by oxygen. Both are equally effective in donating electrons to nitrogenase isolated from heterocysts. The *fdxH2* gene, but not *fdxH1*, is followed by a gene, *fdxB*, encoding a second ferredoxin of unknown function, as also present downstream of *fdxH* from the nonheterocystous filamentous Plectonema PCC 73110. Hence the Nif2 system is homologous to the single, environmentally regulated Mo-Nif system expressed in all cells of nonheterocystous filamentous species [Smoker et al (1990) Meth Molec Cell Biol 2:59-65].

Many additional questions now arise from the work of Bernhard, Teresa, and coworkers. In particular: (a) How do *nif1* and *nif2* differ in their regulatory mechanisms yet intersect in their dependence on nitrogen deprivation? (b) What is the distribution of the two systems amongst nitrogen-fixing cyanobacteria? (c) What is the benefit of two coexisting *nif* systems?

-- Karin Jäger

## III EUROPEAN WORKSHOP ON THE MOLECULAR BIOLOGY OF CYANOBACTERIA - Meeting Report

### Bioenergetics and Physiology

Several presentations related to the structure and function of FERREDOXIN-DEPENDENT ENZYMES. Data from Herbert Böhme's group in Bonn suggests a common but not identical ferredoxin binding domain on the ferredoxin-dependent enzymes nitrate reductase, nitrite reductase and ferredoxin NADP-reductase (FNR). It is noteworthy that the same mutations in specific amino acids of ferredoxin that stimulated the reversed flow from NADPH-reduced FNR to oxidized ferredoxin also severely inhibited electron transport from reduced ferredoxin to FNR. Carlos Gómez-Moreno (Zaragoza) presented rapid kinetic characterizations of ferredoxin, flavodoxin and FNR mutants in which amino acids involved in the interaction between these proteins had been modified. Two others from Zaragoza, Maria Fillat and Marisa Peleato, described, respectively: (1) the overexpression in a protease-deficient *E. coli* of the 49-kDa form of FNR (the complete product of *petH*) from *Anabaena* and (2) the characterization of different FNR-phycobiliprotein complexes of Anabaena, isolated from vegetative cells and heterocysts.

CAROTENOID BIOSYNTHESIS was the focus of presentations by Gerhard Sandmann (Frankfurt) and Blanca Fernández (Sevilla). Sandmann reported the cloning of the zeta-carotene desaturase gene from *Anabaena* PCC 7120 by heterologous complementation. Fernández, using the *cat* gene as a reporter, showed stimulation of the expression of the phytoene desaturase (*crtP*) promoter at high light

intensities, a result in agreement with the photoprotective role ascribed to carotenes.

The molecular bases of the ADAPTIVE RESPONSES of cyanobacteria to changes in light conditions were addressed by a few presentations. Jean Houmard (Paris) reported that whereas changes in the photosynthetic photon flux density exerts a major influence on the differential expression of genes within the *psbA* and psbD multigene families, changes in light wavelength also result in profound modifications of the light harvesting apparatus, in those strains that exhibit chromatic adaptation. In *Calothrix* PCC 7601, different phosphorylated DNA-binding proteins, namely RcaA and B (which specifically bind to the promoter region of the phycoerythrin operon) and RcaD (which binds to the phycocyanin-2 operon), are expressed under green-light and red-light, respectively. However, no difference was found in the subunit composition of the RNA polymerase isolated from cells grown under the two conditions.

Insights into the LIGHT REGULATION of the *PSBA* GENE FAMILY (encoding D1 protein) in *Synechocystis* PCC 6803 were provided by Christer Jansson (Stockholm) and David Campbell (Umeå). Jansson found that *psbA2* and *psbA3*, two gene copies differing in their promoter elements, were light-regulated and transcribed at vastly different levels (30-fold higher for *psbA2*). Inactivation of *psbA2* by in vitro mutagenesis led to an 8-fold up regulation of *psbA3* gene transcription. Site-specific mutagenesis permitted the identification of putative Mn-binding amino acids within D1 and sequences involved in the light-triggered proteolytic degradation of this protein. Campbell reported that *Synechococcus* responds to excitation stress by replacing the constitutive form of the D1 protein (D1:1) by another form, D1:2, that confers increased resistance to photoinhibitory damage and a higher photochemical efficiency of PS II. This D1 exchange is a response to excess excitation of photosynthetic electron transport, and not a specific response to light intensity per se.

Aaron Kaplan (Jerusalem) described novel HIGH-$CO_2$-REQUIRING MUTANTS of *Synechococcus* PCC 7942. These mutants were obtained upon integration of plasmids containing DNA internal to the $CO_2$-related operons, selected from a library composed by short genomic fragments. The mutant-forming plasmids were retrieved and their genomic regions used as probes of wild-type transcripts and genomic DNA. By this means, Kaplan's group showed that the different regions are transcribed and do not lie in close proximity to each other in the chromosome. The study by fusion experiments of the promoter regions of genes modulated by changing $CO_2$ concentration indicated the presence of enhancing and repressing elements. Conserved sequences were found in the promoter region of several $CO_2$-responding genes. Francoise Joset's group (Marseille) described the characterization of a gene from *Synechocystis* PCC 6803, *hatR*, involved in a high affinity system of $HCO_3^-$ uptake and the identification of proteins showing different levels of synthesis in response to changes in the levels of inorganic carbon.

Aurelio Serrano (Sevilla) cloned the NAD(P)-dependent GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (G3P DHase) from *Synechocystis* PCC 6803, by complementation of an *E. coli gap*$^-$ mutant with a genomic library. The enzyme restored the glycolytic pathway in E.coli, and thus may be presumed able to function in that capacity in *Synechocystis* as well. Since G3P DHase is already known to be essential in the reductive pentose phosphate pathway, the enzyme may therefore play both anabolic and catabolic roles in *Synechocystis*. The sequence of the complementing gene predicted a protein very similar (70-80% identity) to G3P DHase from chloroplasts of higher plants. Southern blots using as probes the cloned G3P DHase genes of *Synechocystis* and *E. coli* indicated that two genes, one corresponding to each type, are present in the *Synechocystis* genome. However, since immunological and biochemical data are consistent with the presence in *Synechocystis*, of only an NAD(P)-dependent enzyme, Serrano suggested that the *E. coli*-like gene may be a pseudogene or a gene not expressed under normal culture conditions.

Two presentations had important implications regarding cyanobacterial RESPIRATION. Georg Schmetterer (of Vienna) obtained *cox*$^-$ mutants of *Synechocystis* PCC 6803 in which the three genes coding for the terminal oxidase of aa3 type were inactivated. Surprisingly, although no cytochrome *c* oxidation by membranes of the mutants was observed, the intact cells respire almost normally. Schmetterer explained these results by postulating the existence of An "alternative terminal oxidase", sensitive to KCN, that reduces $O_2$ in the dark with NAD(P)H. Gunther Peschek (Vienna) presented results indicating that the cyanobacterial cytochrome *c* oxidase might be subject to adenylate regulation. A putative mitochondria-like subunit IV gene (*ctaIV*) was identified in *Synechocystis* exhibiting consensus sequences of adenylate-binding enzymes.

Norio Murata (Okazaki) described very interesting results on the GENETIC MANIPULATION OF MEMBRANE LIPIDS in cyanobacteria. His group was able both to decrease the degree of unsaturation of fatty acids in *Synechocystis* PCC 6803 (by inactivating the corresponding desaturase genes) and to increase the degree of unsaturation of fatty acids in membranes of *Synechococcus* PCC 7942 (by transformation with foreign cyanobacterial desaturase genes). These changes did not affect rates of photosynthesis and photosynthetic electron transport and only scarcely affected heat stability of oxygen evolution. However, a lower degree of unsaturation enhanced photoinhibition at low temperatures and a higher degree of unsaturation accelerated recovery from photoinhibition. These results may help to elucidate the mechanisms of photoprotection of photosynthetic organisms at low temperatures.

-- Aurelio Serrano

**Nitrogen Regulation/Metabolism and $N_2$ Fixation**

Antonia Herrero and Ignacio Luque (both of Sevilla) reported results concerning the mechanism of GENE REPRESSION BY AMMONIA in *Synechococcus* PCC 7942. They found that mutant strains that express NtcA to a high and constitutive level still require the absence of ammonium to express NtcA-regulated genes (e.g. *nir* operon, *glnA*). They suggested that a coactivator may be required for the expression of nitrogen-regulated genes or that NtcA protein

is posttranscriptionally interconverted between an active and an inactive form in response to the nitrogen status. Antonia also proposed that the level of NtcA might contribute to the differential regulation of some genes through weak binding of the protein to sites deviating from the known NtcA consensus binding site.

José Frias (Sevilla) discussed the GENES OF NITRATE assimilation: their regulation and function. The *nir-nrtABCD-narB* gene cluster (the *nir* operon) of *Anabaena* PCC 7120 was cloned and analyzed. Northern analysis showed that the *nir* operon is transcribed in the absence of ammonium with or without nitrate or nitrite in the medium, this despite the fact that high levels of nitrate and nitrite reductase activities occur only in the former case. A 460 bp leader sequence between the Ntc-regulated promoter and the first codon of *nir* seems to lack any function: when removed no change in phenotype was observed. Insertional inactivation of *nrtA* resulted in mutants that were unable to transport nitrate at low external concentration (0.1 mM), but at high concentration (18 mM) nitrate was taken up at a slow rate and reduced to ammonium. High activities of the nitrate assimilation enzymes were observed at either level of nitrate concentrations but neither could repress heterocyst formation.

Paco Navarro (Sevilla) found two different genes, *gltS* and *gltB*, in *Synechocystis* PCC 6803, that encode ferredoxin-dependent GLUTAMATE SYNTHASES (GOGAT). Inactivation of either one did not significantly impair growth (concomitant inactivation of both has not yet been tried). While *gltS* was present in many other cyanobacteria tested, *gltB* was additionally found only in *Pseudoanabaena* PCC 6903. Both enzymes expressed in *E. coli* accept electrons from PetF-type ferredoxins, but flavodoxin was inactive. Interestingly, GltB was equally active with heterocyst ferredoxin (FdxH). Figueroa (Sevilla) cloned *gltS* from *Anabaena* PCC 7120. GltS activity was highest in crude extracts of cells using $N_2$ as the nitrogen source, as opposed to nitrate or ammonium, hinting at a role for this GOGAT in heterocyst metabolism.

Reyes and Florencio (Sevilla) reported that the REDOX STATE controls the transcription of *glnA*, encoding GLUTAMINE SYNTHETASE (GS). Transcript abundance was high when *Synechocystis* PCC 6803 was grown in the light or in the dark with glucose and low in the dark without glucose or when DCMU (a PSII-inhibitor) or DBMIB (a cytochrome $b_6/f$ complex-inhibitor) was added. N-starvation provoked a delay in decrease of *glnA* transcripts suggesting a connection between nitrogen and redox controls of transcript levels. Crespo (Sevilla) found that redox control seems also to govern inactivation of glutamine synthetase (GSI) in vivo. In this case, however, the addition of DBMIB, leading to a reduced plastoquinone (PQ) pool, was not inhibitory. This result suggests that the redox state of PQ or a component of the $b_6/f$-complex is a signal for modification.

The same group also characterized a SECOND TYPE OF GLUTAMINE SYNTHETASE from *Synechocystis* PCC 6803, encoded by *glnN*, similar to the GSIII-type enzymes found in the *Bacteriodaceae*. GlnN has a larger subunit size (75kD) than the 50kD product of *glnA* and, unlike the dodecameric GlnA, probably exists in its native state as a hexamer. According to Western blot analysis, GSIII is more abundant in PCC 6803 and other non nitrogen-fixing cyanobacteria when they are starved for nitrogen. GSIII is lacking, however, in $N_2$-fixing *Anabaena* PCC 7120.

Nicole Tandeau de Marsac and coworkers (Paris) described $P_{II}$ PROTEIN as the central node for the coordination of nitrogen and carbon assimilation in cyanobacteria. $P_{II}$ is a protein whose homologue in enteric bacteria is involved in regulation of GS activity and Ntr-regulated gene expression. She reported that a $P_{II}$-deficient mutant of *Synechococcus* PCC 7942 can take up nitrate even in the normally inhibitory presence of ammonium. The mutant has also lost the ability to adapt rapidly to changes in light, nitrogen, and carbon supplies. $P_{II}$ thus functions to integrate nutritional stimuli and to reestablish a proper C/N-ratio for balanced cell growth. In *Calothrix* PCC 7504, $P_{II}$ is found to be unmodified during the hormogonial stage of growth, whereas $P_{II}$ modification is most pronounced under conditions of heterocyst differentiation. Thus, in filamentous strains $P_{II}$ may be additionally involved in cell differentiation processes.

Karl Forchhammer (Munich) devised an in vitro test for PHOSPHORYLATION OF $P_{II}$ that clearly demonstrated that 2-ketoglutarate is sufficient to activate $P_{II}$ kinase from *Synechococcus* PCC 7942. No other compound tested (e.g., glutamine or other amino acids) could substitute or counteract the stimulation by 2-ketoglutarate. The latter may serve as an intracellular signal to monitor the balance of assimilated carbon and nitrogen that is sensed by $P_{II}$ kinase and transmitted to $P_{II}$ by protein serine phosphorylation.

Lucas Stal (Amsterdam) made an interesting observation related to the CAPABILITY OF NONHETEROCYSTOUS CYANOBACTERIA TO FIX NITROGEN predominantly in the light. He noted that a Cyanothece strain is impaired in nitrogen fixation when grown in batch cultures, where they produce sulfated extracellular polysaccharides and thus rapidly deplete the medium of sulfate. In continuous cultures with a continuous supply of sulfate nitrogenase activity was high and confined predominantly to the light. The same was true when sulfate was added to a sulfate-depleted batch culture. This intriguing observation leaves us once more perplexed (as with the case of *Trichodesmium*): how do they do it without heterocysts?

-- Bernhard Schrautemeier

**Ecology**

One perennial problem for ecologists is that of IDENTIFYING the ORGANISMS present in natural populations. Strain identification is also a problem for those of us working on newly isolated strains. A variety of molecular techniques are available for strain identification and discrimination; the results presented on three posters (Anneliese Ernst, Konstanz; Gary Barker, Bristol; Suzzanne McColl, Liverpool) lend yet more evidence to what has been long suspected, namely that natural populations of cyanobacteria consist of many distinct clones.

The biology of GAS VACUOLATE CYANOBACTERIA was covered in two presentations. The advantages accruing from gas vesicle production by *Aphanizomenon* in the Baltic Sea is being quantified by Walsby and co-workers (Bristol); such colonial forms can gain a 3-fold photosynthetic advantage over their non-buoyant competitors by rapidly moving back towards surface, and light, after mixing-events. The genes involved in producing gas vesicles and the interactions between the gene products were described by Hayes et al. Once thought to be a simple structure formed by self assembly of a single type of protein, it is now clear that it takes the concerted action of at least six different gene products to assemble these structures.

Elke Dittmann et al. (Berlin) described progress toward the complete characterization of the genes encoding PEPTIDE SYNTHETASES of cyanobacteria. These incredibly complex enzymes are responsible for the synthesis of the cyclic peptide toxins. The group in Berlin have partially characterized a gene from *Microcystis aeruginosa* using conserved domains from peptide synthetases to provide probes. This approach is similar to that described by Leo Rouhiainen et al. (Helsinki) in Urbino for the genes from *Nodularia.* With the genes available from a number of organisms it should now be possible to study the biological role of these toxic compounds.

Molecular mechanisms of SALT TOLERANCE were described in two presentations (Martin Hagemann and Ellen Zuther, Rostok). A total of 18 salt sensitive mutants of *Synechocystis* PCC 6803 were produced by random cartridge mutagenesis; 9 of these were unable to synthesize glucosylglycerol. One of the genes identified, *stpA*, had been previously characterized (Francoise Joset, Marseille). Three other ORFs have been characterized in Rostock; the role of theses genes has yet to be confirmed but glucosylglycerol transport and positive regulation of glucosylglycerol synthesis seem likely candidates.

Nigel Robinson (Newcastle) gave a lucid summary of work carried out in his laboratory on the regulation of expression of the METALLOTHIONINE-ENCODING GENE *smtA* from *Synechococcus* PCC 7942. SmtB is a repressor of *smtA* expression that dissociates from the *smtA* promoter in the presence of $Zn^{2+}$. Upstream of *smtB* is *smtZ*, a gene that encodes a protein the C-terminal end of which has the features of a zinc-finger: SmtZ may up-regulate *smtA* expression in the presence of $Zn^{2+}$. Upstream again is *dnaG*, encoding primase which could be a zinc metalloprotein. An octameric palindrome HIP1 (5'-GCGATCGC-3') is involved in the deletion of *smtB* in cells selected for zinc tolerance. This sequence occurs at much higher than expected frequencies in many cyanobacteria (but not in any of the marine isolates investigated); is it involved in genome plasticity? We will have to wait for the answer to that question.

All of the presentations at the meeting were excellent and I wish I had the time to write about them all (in particular Nick Mann, Warwick, gave a first class talk of extreme ecological relevance, but mine came straight afterwards so I missed most of what he saying) but at least you now have the gist of some of the topics covered.

- Paul Hayes

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# *Late Breaking News!*

## BULLETIN BOARD*BULLETIN BOARD*BULLETIN BOARD*BULLETIN BOARD*BULLETIN BOARD*BULLETI

POSITIONS OFFERED: Two post-doc openings

CONTACT: Sabeeha Merchant, Department of Chemistry and Biochemistry, UCLA, 405 Hilgard Avenue, Los Angeles, CA 90095-1569. TEL: 310-825-8300, FAX: 310-206-1035, E-MAIL: Merchant@Chem.Ucla.Edu

RESEARCH (Position 1): Copper-responsive gene expression in the context of adaptation to copper-deficiency and the assembly of the photosynthetic apparatus [EMBO J (1991) 10:1383; EMBO J (1995) 14:857; Plant Cell 7:623]

REQUIREMENTS (Position 1): Formal education and research experience in biochemistry, molecular biology, or genetics.

RESEARCH (Position 2): Cytochrome biogenesis with an emphasis on the isolation and functional analysis of genes involved in the specification of cofactor (heme) -apoprotein assembly [EMBO J (1992) 11:2789; J Biol Chem 269:5824; Mol Gen Genet (1995) 246:156].

REQUIREMENTS (Position 2): Research experience in biochemistry, molecular biology or genetics.

SEND: CV, publication list, relevant reprints, and letters of recommendation

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MASAHIRO ISHIURA, TAKAO KONDO, and the rest of the circadian rhythm team formerly of National Institute for Basic Biology in Okazaki has moved to Nagoya University, where they will continue studying cyanobacterial clocks.

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