

CyanoNews

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# CYANO NEWS

Volume 4 Number 3

December 1988

CYANO NEWS - 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).

SUBSCRIPTION RATE - one communication every two years or so (your address label shows the date of your last communication). A communication might be a new result, news of an interesting meeting, a post-doctoral opening, a request for strains, a new article, even confirmation of your address!

WHERE TO SEND CONTRIBUTIONS - See the last page.

HOW TO GET ON THE MAILING LIST - See the last page.

HOW TO FIND OUT MORE ABOUT SOMETHING YOU READ HERE - The name of the correspondent for each item in this newsletter is capitalized, so you know who to write to for more information. The correspondent's address appears at the end of the newsletter.

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You will receive an updated DIRECTORY OF CYANOBACTERIOLOGISTS with the next newsletter. If your address, telephone number, or research interests have changed please contact JEFF ELHAI. If you have a computer address that you would like to include, please send that in too.

The Academy of Natural Sciences will convene an International Symposium on Cyanobacterial Research, focused on RECENT ADVANCES, RESEARCH NEEDS, AND MANAGEMENT IMPLICATIONS. It will be held 16-20 April 1989, at Stroud Water Research Center, about an hour from Philadelphia, Pennsylvania, U.S.A. The list of topics covers basic and applied research on physiology, ecology, succession, and management of cyanobacteria. The goal of the symposium is to encourage discussion and speculation in these areas. Manuscripts from each presentation as well as discussions and session summaries will be published in a peer-reviewed proceedings volume. Interested individuals should submit short abstracts (not exceeding 250 words) on any topic to: Kevin Sellner, The Academy of Natural Sciences, Benedict Estuarine Research Laboratory, Benedict, MD 20612, USA. (Tel): 301-274-3134. Telemail (OMNET): BENEDICT.LAB. Abstracts (plus two copies) should be received by 15 December 1988. Completed manuscripts will be due prior to the meeting.

The THIRD INTERNATIONAL WORKSHOP ON THE MOLECULAR BIOLOGY OF CYANOBACTERIA will be held in Toronto July 27-29, 1989, just preceding the joint meeting of the American Society of Plant Physiologists and the Canadian Society of Plant Physiologists, July 30 - August 3 (also in Toronto). The registration fee has tentatively been set as \$50 (Canadian) or \$40 for students, and housing, at the University of Toronto, will be \$25 per night, including breakfasts. Attempts will be made to rebate a portion of student expenses, the proportion depending on what outside funding the organizers can line up. Contact: John Coleman, Dept. of Botany, University of Toronto, Toronto, CANADA M5S 1A1. (John is already sending registration forms to everyone on the CyanoNews mailing list)

RECENT ADVANCES IN ALGAL BIOTECHNOLOGY will be the theme of the 5th International Conference of the Society of Applied Algology, to be held January 28 to February 2, 1990, at the Dead Sea, Israel. Topics will include:

- Technology and physiology of biomass production
- Products from algae and their uses
- Genetics and cell biology
- Environmental limitations and growth physiology

For further information, contact the conference secretariat at: Algology Conference, Melia-Te'um, POB 8388, 91 082 Jerusalem, Israel. Tel: (02) 667402, 637572. Telex: 25628 TEUMJ IL

In the panel discussion printed in the last newsletter, several panelists mentioned traits that would be desirable in the perfect strain of *Spirulina*. JACQUE FEUILLADE comments that our panel might well have looked beyond *Spirulina*. He notes that *Oscillatoria rubescens* can grow optimally at 20°C (as requested by one panelist) and is very closely related to *Spirulina*. This strain is adapted to temperate climate and perhaps easier to harvest than *Spirulina*.

#### PLEA FOR CONSISTENT STRAIN DESIGNATIONS

ROSI RIPPKA and MIKE HERDMAN pass on to us their observation that strain numbers given to cyanobacterial isolates seem to have been causing some confusion. Culture collections normally describe each of their strains with the following information: genus, species (if a specific name has been appended, acronym of the culture collection, strain number. For example:

*Gloeobacter violaceus* PCC 7421

*Gloeobacter violaceus* ATCC 29082

PCC is the acronym for the Pasteur Culture Collection of Cyanobacteria, and ATCC is the acronym for the American Type Culture Collection. In the numbering system of the PCC, the first two of the four or five digits given to a strain indicate the year in which the strain entered the collection (in an axenic state). The remaining digits are given serially to new isolates (e.g. PCC 73102 was the 102nd axenic isolate in 1973 -- a good year!)

The confusion arises when authors employ the wrong acronym or omit it, perhaps because the strain collection does not use an acronym in its strain designations. For example (and we hope that the authors will not be offended by this citation -- it just happens to be the most recent example), Schneider et al. (J. Bacteriol. 170:4136) employed "*Nostoc* MAC PCC 7911". PCC 7911 does not exist (it used to designate an isolate of *Pseudanabaena*, but the strain was lost). Our correspondents hypothesize that "7911" is the number appended to *Nostoc* MAC in Jack Meeks' culture collection. *Nostoc* MAC is carried in the PCC, but under the designation PCC 8009. Attempts to guess the strain collection from the number are confounded by the fact that the same numbering system in use by the PCC is currently employed in several laboratories (those of Jack Meeks and John Waterbury, for example). Therefore, if a culture collection acronym is lacking or omitted by accident in a publication, the readers will encounter difficulties and might address a request for the strains described to the wrong collection.

Our correspondents close by noting that such problems could be avoided if (1) all culture collections (even minor ones!) have an acronym, and (2) we all diligently use them.

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#### COPING WITH SULFUR DEPRIVATION: THE FATE OF A THIRD PHYCOCYANIN

Three phycyanin operons have been characterized in *Calothrix* sp. PCC 7601. NICOLE TANDEAU DE MARSAC and coworkers (D. Mazel, P. Marlière, and J. Houmard) report that the three are each regulated in a distinctive fashion. Regulation of the third operon represents a novel response to environmental stress. The first operon (*cpc1*) encodes the  $\beta$  and  $\alpha$  subunits (*cpcB1* and *cpcA1*, respectively) of the "constitutive" phycocyanin (PC1) (Mazel et al. (1988), Mol. Gen. Genet. 291:296). The second operon (*cpc2*) encodes the  $\beta$  and  $\alpha$  subunits (*cpcB2* and *cpcA2*, respectively) of the "inducible" phycocyanin (PC2) and its associated linker polypeptides L<sub>P</sub>30.5, L<sub>P</sub>32.4 and L<sub>P</sub>9.7 (*cpcH2*, *cpcI2*, and *cpcD2*, respectively). These genes are transcribed only in cells grown under red light (Tandeau de Marsac et al. (1988), Photosynth. Res. 18:99). The third operon (*cpc3*) has the same physical organization as the *cpc2* operon, it encodes the  $\beta$  and  $\alpha$  subunits (*cpcB3* and *cpcA3*, respectively) of a third phycocyanin (PC3) and its associated linker polypeptides L<sub>P</sub>30.8, L<sub>P</sub>31.6, and L<sub>P</sub>8.1 (*cpcH3*, *cpcI3*, and *cpcD3*, respectively). Analysis of the deduced amino acid sequences of the genes of the *cpc3* operon revealed that the PC3 subunits and the associated linker polypeptides lack sulfur-containing amino acids except in positions where chromophores bind. Under sulfur limitation, the expression of the *cpc3* operon is specifically switched on, while that of the *cpc1*, *cpc2*, and *cpe* (encoding the  $\beta$  and  $\alpha$  subunits of phycoerythrin) operons is switched off. Since phycocyanins represent approximately 35% of the total cell protein, this type of adaptation is a substantial economy for the cells and allows them to survive in extreme growth conditions. N.T. knows of no other example of this type of adaptation.

## MECHANISM OF SALT TOLERANCE IN NITROGEN-FIXING CYANOBACTERIA

SHREE KUMAR APTE describes a recent test of the hypothesis that  $\text{Na}^+$  exclusion forms the basis for cyanobacterial salt tolerance. This model, originally proposed by Apte and Thomas (Eur.J.Biochem. 154:395-401, 1986), was experimentally tested by growing salt sensitive and salt tolerant strains of *Anabaena* under conditions that modified  $\text{Na}^+$  influx. Treatments that inhibited  $\text{Na}^+$  influx (e.g., alkaline external pH, external  $\text{K}^+$  in excess of 25 mM, presence of nitrate or ammonium in the growth medium) remarkably enhanced the salt tolerance of both the salt-tolerant strain (*Anabaena torulosa*) and the salt-sensitive strain (*Anabaena* sp. strain L-31). These experiments established a perfect negative correlation between  $\text{Na}^+$  influx and salt tolerance (measured as growth and nitrogenase activity) and identified reduction of  $\text{Na}^+$  influx as a major mechanism in alleviating salt stress in nitrogen-fixing cyanobacteria.

## CYANOBACTERIA FROM INDIAN WATERS CHARACTERIZED

G. SUBRAMANIAN surveyed the cyanobacterial flora of the southern east coast of India, including a portion of the Bay of Bengal, Palk Strait, Palk Bay, and Gulf of Mannar. The cyanobacteria were observed predominantly along the shores, back waters, and salt pans. The Gulf of Mannar, with its rocky shore, was the richest among the different zones. A total of 114 species of 33 genera belonging to 14 families was recorded. Of these, 47 species have originally been recorded from fresh water sources [Desikachary, 1959]. Under the hypersaline conditions of the salt pans, heterocystous forms were totally absent. 35 strains of 19 species belonging to 11 genera have been isolated and purified. 15 of these so far have been made axenic. One of these, *Phormidium valderianum* BDU 3501 has been partially characterized. It is resistant to four antibiotics at low concentrations (less than 100 ug/ml) and susceptible to two antibiotics. It not only shows resistance but also an induction of growth with increasing concentrations of ampicillin. It has a very wide range of salinity tolerance: from near fresh water conditions to 90 ppt NaCl.

## DESICCATION-TOLERANCE IN NOSTOC COMMUNE

MALCOLM POTTS tells us about molecular studies aimed at understanding the mechanisms by which *Nostoc commune* tolerates desiccation in the field. The thesis work of Wen-Qin Xie in his laboratory includes the cloning and sequencing of the *rpoBC1C2* genes of *N. commune* UTEX 584. These genes encode, respectively, the  $\beta$ ,  $\gamma$ , and  $\beta'$  subunits of the cyanobacterial DNA-dependent RNA polymerase [Xie, Jäger, and Potts, submitted]. *rpoC1* and *rpoC2* are linked, and each carries different conserved domains found in the single *rpoC* gene of *E. coli*. The structural organization of cyanobacterial *rpoBC1C2* genes has been conserved within the chloroplast genomes of higher and lower plants. A rapid method for the construction, isolation, and sequencing of deletion clones was used to sequence the *rpo* operon [Xie and Potts, Gene Analysis Techniques, in press].

Probing of the genomic DNA from field materials of *N. commune* HUN with *rpoBC1C2* revealed that the *rpo* genes were present in the hypermethylated fraction II DNA and absent in the hypomethylated fraction I DNA [Jäger and Potts, Gene, in press].

Nitrogenase Fe-protein remains undegraded in colonies of *N. commune* after more than seven years of desiccation. Fe-protein-specific antibody detected one major and one minor band in Western blots, and immunolabelling detected Fe protein on heterocyst ribosomes within 30 min of the rewetting of material desiccated previously for two years [Peat, Powell, and Potts, Protoplasma, in press]. At least for *N. commune* UTEX 584, the multiple Fe-protein bands may reflect the presence of multiple (three) *nifH*-like sequences in the genome [DeFrancesco and Potts, J. Bacteriol. 170:3297-3300, 1988].

## METHOD FOR PRODUCING RANDOM INSERTIONAL MUTATIONS

Franck Chauvat describes a method used in his laboratory to produce random mutants of *Synechocystis* PCC 6803 defective in photosynthesis. A kanamycin-resistance cartridge was ligated to restriction fragments of genomic DNA from *Synechocystis* and the resulting ligation mixture was used to transform the cyanobacterium. Photoheterotrophic mutants were found harboring deletions of up to 50 kb. Experiments in which one mutant was transformed with DNA from another yielded a genetic-physical map of a region exceeding 60 kb that contains *psbB*, *psbC*, and *psbD-1*.

## FORUM ON CYANOBACTERIAL DOMINANCE

Warwick Vincent and his colleagues at the Department of Scientific and Industrial Research sponsored an international Forum on Cyanobacterial Dominance, held February-March 1988 at their Taupo Research Laboratory, New Zealand. The aim of the forum was to investigate the nature of controls on cyanobacterial growth. There is growing concern about the increasing occurrence of cyanobacterial blooms in New Zealand. Although a number of lakes were investigated, the main experiments involved Rotongaio and Okaro, lakes formed in two explosion craters. Rotongaio is very rich in phosphorus and carries a much higher biomass, which, at the time of the forum, consisted mainly of a nitrogen-fixing, filamentous cyanobacterium, a new species of *Anabaena*. Lake Okaro had a more diverse phytoplankton population, including two forms of *Microcystis*.

Three sets of programmes were carried out. The first programme examined the mixed layer over diurnal cycles and tried to find whether cyanobacteria have adaptational mechanisms that allow them to out-compete other algal groups. The second programme tested the hypothesis that zooplankton grazing promotes cyanobacterial growth. The third programme examined the physiological and growth response at longer time scales (i.e. generation times) and addressed the question of whether iron availability and the ratio of nitrogen to phosphorus can favor cyanobacterial growth.

Before the experimental programmes were started, the participants reviewed the literature concerning cyanobacterial performance. These reviews, together with the abstracts of the experimental work are published in a special volume devoted to the forum in the New Zealand Journal of Marine and Freshwater Research, vol. 21. The research papers will appear shortly in a special issue of Archives für Hydrobiologie.

(Contributed by JACCO KROMKAMP)

## VI INTERNATIONAL SYMPOSIUM ON PHOTOSYNTHETIC PROKARYOTES

Every three years, a large fraction of the world's cyanobacterial wisdom congregates as part of an International Symposium on Photosynthetic Prokaryotes. This year, the sixth edition was hosted in the Netherlands by Luuc Mur and others at the University of Amsterdam, 8 - 13 August. A sampling of some of the interesting reports is provided below. Needless to say, this sampling does not include many important talks and posters -- even whole areas are omitted. There was too much to take in even while there, certainly too much to summarize. For reason of space, only those actually presenting the papers are named. If you would like to contact one of the authors but don't know the address, you may send your message to the newsletter and it will be forwarded.

Two important pieces of news fit into none of the categories listed below. First, the VII International Symposium is set for 1991 in Amherst, Massachusetts, U.S.A. Second, the Israeli team of Shimshon Belkin, Mickey Gurvitz, Leah Kami, and Elisha Tel-Or fended off spirited challenges from Wales, Italy, and U.S.A. in the symposium's official contest: *sjoelbakken* (something like throwing hockey pucks at mouseholes). The victors won the right to display the historic blue-green-purple plaque, symbolic of photosynthetic excellence, until the next symposium.

## Prochlorophytes

The prochlorophyte *Prochlorothrix hollandica* is a chlorophyll a/b containing prokaryote that shows similarity on one hand to cyanobacteria and on the other to green plant chloroplasts. As can be expected, the reports on this organism focussed on these comparisons.

The cytological characteristics of the organism generally resemble those of cyanobacteria. Uwe Jürgens reported that the peptidoglycan-polysaccharide complex of *P. hollandica* was similar in structure and function to that of cyanobacteria and Gram-positive bacteria. From the presence of RuBisCO in the polyhedral bodies of *P. hollandica*, Anna Hawthornthwaite inferred that these structures are analogous and functionally equivalent to the carboxysomes of other autotrophic prokaryotes. A complete immunological identity was demonstrated between RuBisCO from *P. hollandica* and that from the cyanobacterium

Chlorogloeopsis fritschii. J.K. Volkmann concluded that the lipid composition of the prochlorophyte was quite similar to that of cyanobacteria, based on the presence of hopanoids, the low abundance of triacylglycerols, and the absence of sterols.

The overall structure and function of the thylakoid membranes in P. hollandica exhibit some similarities to chloroplast thylakoids, but also several distinct differences. As with chloroplast thylakoid membranes, those of P. hollandica show stacking (poor) and lateral heterogeneity [Hans Matthijs]. It is already known [Bullerjahn et al., Eur. J. Biochem. (1987), 168:295-300] that the chlorophyll protein complexes of P. hollandica can be assigned a role in PSI, PSII, or antenna complexes. However, no immunological cross-reaction of the LHC-like chlorophyll a/b protein complex to chloroplast LHC-II has been observed. Circular dichroism spectra showed that the a/b complex differs from analogous complexes in chloroplasts [Hans Matthijs]. Both G. van der Staay and Anton Post reported phosphorylation of thylakoid proteins, and this was probably related to state transitions. Phosphorylation takes place in the light as well as in the dark, as observed in Prochloron didemi but not in most chloroplasts. Post found a difference in molecular mass between phosphorylated and non-phosphorylated complex proteins and concluded that the LHC-like a/b complex is not involved in reversible phosphorylation.

Sue Golden reported that the nucleotide sequence of the two *psbA* genes from P. hollandica have features that classify them as "chloroplast-like" rather than "cyanobacterial-like". On the other hand, S. Turner concluded from the nucleotide sequence of the 16S rRNA that P. hollandica is a definite, but deeply branching member of the cyanobacterial line of descent, and not closely related to chloroplasts.

-- contributed by TINEKE BURGER-WIERSMA

### Taxonomy

At the previous symposium in Grindelwald (1985), John Waterbury stated that a unified taxonomy of Cyanobacteria was nearing realization. We are still far away from this goal. Some of the main problems were addressed in a mini-symposium chaired by Rosi Rippka, particularly the problems of type-species and of the morphological instability of axenic cultures.

Lucien Hoffmann presented results of a study on a hitherto poorly studied group, the false-branching heterocystous cyanobacteria. Studying herbarium material and cultures, he found in general a good agreement between the botanical and microbiological approaches, but concluded that culture studies help to refine the taxonomic treatment, due to the larger variety of characters that can be studied. Annick Wilmotte in her study of seven Oscillatoriaceae strains compared the results obtained from a morphological study with those gained by molecular methods. DNA-DNA hybridizations, realized for one of the clusters defined by morphology, confirmed the homogeneity of this group. The results of partial 16S rRNA sequencing proved to be useful in revealing the degree of relatedness between the clusters thus defined.

-- contributed by LUCIEN HOFFMANN

### Symbiotic Cyanobacteria

About a dozen papers dealt with nitrogen-fixing cyanobacteria in or isolated from cyanobacterial symbioses.

Azolla: An ultrastructural characterization of some Azolla symbionts demonstrated the germination of akinetes and the existence of an inner envelope appressing the symbionts to the cavity walls [Sandy Nierzwicki-Bauer]. Immunocytochemical analysis emphasized that the Fe-protein of nitrogenase appears in only heterocysts and at a late stage of the heterocyst differentiation process [Bergitta Bergman]. Also, the 32kD protein of PSII was detected by immunogold labeling. Vegetative cells, heterocysts, and akinetes all contained the protein [Ellen Braun-Howland]. However, heterocysts of the Nostoc MAC isolate apparently lack mRNA for the *psbA* gene (which encodes the 32kD protein) [Mark Alley]. A comparison of the morphology and electrophoretic enzyme patterns of ten presumptive Azolla isolates resulted in one strain being identified as an epiphyte, two as Nostoc, and six as Anabaena strains [Bill Zimmerman]. The addition of fructose and a soluble extract of Azolla stimulated growth, differentiation, and nitrogen fixation by the isolate Anabaena azollae [Elisha Tel-Or].

Liverworts: The cellular and subcellular occurrence and distribution of the Fe-protein, RuBisCo, and phycoerythrin in Nostoc-Anthoceros was similar to that of a free-living isolate, but the level of glutamine synthetase in heterocysts of the symbiont was specifically reduced [Bergitta Bergman]. The removal of glucose from the Nostoc LBG1 isolate from Anthoceros elicits massive cell division, culminating in the formation of motile hormogonia, with no change in the rate of DNA replication [Dave Adams].

Cycads: Comparison of DNA restriction fragment length polymorphisms of five cycad species demonstrated that several cyanobacteria form symbioses with coralloid roots of cycads [Peter Lindblad]. No difference was found in the growth and physiological characteristics between hydrogen-consuming and hydrogen-producing cultures of a photoheterotrophically-grown cycad isolate, Nostoc Cc [Mario Tredici].

-- contributed by BERGITTA BERGMAN and LUCIEN HOFFMANN

## Ecology

Several talks and posters focused on anoxygenic photosynthesis. Dick Castenholz described how Oscillatoria boryana maintains substantial rates of photosynthesis over a wide range of sulfide concentration by rapidly shifting between anoxygenic and oxygenic modes. Similar photosynthetic versatility was reported for Microcoleus chthonoplastes by R. de Wit.

The mechanism of buoyancy regulation and other factors that affect the dominance of cyanobacteria in lakes received the attention of several papers. The influence of the gas vesicle proteins GVPa and GVPc in determining the cylinder diameter and the critical pressure of gas vesicles was described by Tony Walsby and Paul Hayes. Two papers [Bas Ibelings; Jacco Kromkamp] reported that the change in the "ballast" (polysaccharides) content is the principle mechanism of buoyancy regulation in diurnal cycle.

Geoffrey Codd presented interesting results concerning cyanobacterial toxin production, giving evidence against the involvement of extrachromosomal DNA in the synthesis of peptide toxins.

Concerning the potential use of cyanobacteria as biofertilizers, Eduardo Fernandez Valiente reported the abundance of heterocystous cyanobacteria in rice fields in Valencia (Spain) with a high input of fertilizers.

-- contributed by EDUARDO FERNANDEZ VALIENTE

## Metabolism

The metabolism section of the conference produced many novel and exciting results. It is remarkable the extent to which molecular genetic techniques now complement metabolic studies, and many of the papers presented in the metabolism section reflected this.

A discussion section devoted to posters on metabolism centred around three issues: nitrogen metabolism, autotrophic and non-autotrophic carbon metabolism. A fascinating poster relating to the basis of obligate photoautotrophy was presented by Rosi Rippka. A spontaneous mutant of the obligate photoautotroph Anabaena PCC 7120 was isolated that had acquired the status of an obligate chemo- or photoheterotroph. The mutant has interesting properties, including greatly depressed pigment levels when growing on dinitrogen (the cells are not nitrogen-limited, since the growth rate under these conditions is similar to growth rates with nitrate or ammonia). Growth of the mutant is not attributable to the acquisition of a glucose transport system but seems to be related to a mutation in a regulatory gene that leads to functions in vegetative cells normally expressed only in heterocysts.

The role of the enigmatic reversible hydrogenase of cyanobacteria has been clarified, at least for two species, by John van der Oost (working with Cyanothece) and Lucas Stal (working with Oscillatoria limosa from North Sea mats). Under anaerobic conditions in the dark, the enzyme functions in fermentative metabolism to remove excess reducing equivalents, as it does in fermentative bacteria. Such work impinges on the ecology of cyanobacterial mats, and it was noted [Marlies Villbrandt] that the pattern of nitrogen fixation by Oscillatoria in such mats demonstrates the extraordinarily fine oxygen balance in the mats of this respiration-dependent but oxygen-labile process: nitrogen fixation occurs over a short time period at dawn and dusk.

Several presentations were concerned with the response of cyanobacteria (particularly *Synechococcus* PCC 6301) to sulfur deprivation: (1) induction of three cytoplasmic membrane proteins associated with sulfate uptake [Laura Green], (2) expression of a third gene for a phycocyanin molecule possessing less sulfur [Didier Mazel; see News in this issue], (3) activation of a membrane-bound protease with specificity for C-phycocyanin [Ahlert Schmidt], and (4) excretion of nitrite due to the loss of nitrate reductase [Elisabeth Krämer].

-- contributed by GEOFFREY SMITH

### Nitrogen Fixation

- Anabaena variabilis* expresses an alternative V-nitrogenase when grown in an Mo-deficient, V-supplemented medium. The enzyme is characterized by enhanced H<sub>2</sub>-formation and C<sub>2</sub>H<sub>6</sub>-production during the reduction of C<sub>2</sub>H<sub>2</sub> to C<sub>2</sub>H<sub>4</sub>. [T. Kentemich]
- The Fe-protein of nitrogenase of *Anabaena variabilis* (ATCC 29413) is modified when the culture is exposed to ammonia (at alkaline pH), O<sub>2</sub>, and darkness. The modification is observed with Western blots using antibody prepared against the purified Fe-protein of *A. variabilis*. [Sabine Reich]
- Revertants of the fix<sup>-</sup>-strain *Nostoc* MAC (PCC 8009) have been screened for the arrangement of *nifHDK*. One fix<sup>+</sup>-revertant shows a contiguous *nifHDK* arrangement, while all others characterized so far have *nifHDK* interrupted by an insert. This is the third report of a contiguous *nifHDK* in a cyanobacterium. [Jack Meeks]
- Immunofluorescence labelling of nitrogenase was used to show the presence of nitrogenase in previously fixed and permeabilized natural cell assemblages. [Hans Paerl]
- Nickel is required during adaptation of NO<sub>3</sub><sup>-</sup>-grown cultures of heterocystous cyanobacteria to diazotrophic conditions. The Ni-dependent reaction has not been identified, but some evidence points to its participation in the metabolism of cyanophycin. Thus far, nickel is known to be a cofactor only in hydrogenases and ureases. [Geoffrey Smith]
- The soluble hydrogenase from *Anabaena cylindrica* has been purified and characterized as a dimeric enzyme. The reaction centre of H<sub>2</sub> is located on the Ni-containing subunit, while the dyes used in the hydrogenase assays react with the other subunit [Geoffrey Smith].

-- contributed by ANNELIESE ERNST

### Bioenergetics

There was a tour de force of PSII in cyanobacteria, using the techniques of molecular genetics to demonstrate the participation of identifiable proteins in electron transfer and in distinct steps of energy transfer from the phycobilisome to the reaction center [Larry Bogorad, Sue Golden, Himadri Pakrasi, Sergei Shestakov, and Wim Vermaas]. A calmodulin-like binding site for calcium in PSII was described (by M.C.W. Evans), which promises new insights into the chemical identity of a region still untouched by genetics. Redox carriers that deliver electrons to PSI or the bacterial reaction center were discussed in detailed studies of donor-acceptor interactions [David Knaff and M.A. Cusanovich] and in the fascinating context of environmentally regulated gene expression of plastocyanin or cytochrome c<sub>553</sub> in cyanobacteria [Peter Weisbeek]. The transfer of electrons from PSI to nitrogenase in the heterocyst requires a specific ferredoxin [Herbert Böhme], and attention was drawn to yet another ferredoxin species with an extraordinary hydrophathy characteristic [Lyndon Rogers].

A role for cytochrome aa<sub>3</sub> oxidase in cyanobacterial respiration gains greatly in credence from work on its location and partial purification [Günter Peschek]. The study of another intriguing respiratory chain in *Rhodobacter capsulatus*, with trimethylamine-N-oxide and dimethylsulfoxide as electron acceptors, is shedding light on very interesting cytochrome and pteridine constituents [A.G. McEwan].

There seemed to be hints that new similarities among electron transfer processes of prokaryotes will be forthcoming.

-- contributed by DAVID KROGMANN



## Genetics

### Anabaena PCC 7120:

- Ian Bancroft reported on an almost completed physical map of the genome, estimated to be 6.3 million base pairs in size. He described a new method of pulsed field gel electrophoresis, which permitted running large gels (36cm x 36cm) with little or no distortion. At present the map consists of AvrII, SalI, and PstI sites.
- Terry Thiel generated considerable excitement with her success in moving DNA into Anabaena using electroporation. Among the important factors required for success were: a concentrated cell suspension (up to  $10^9$  cells/ml); a lot of DNA (at least 1  $\mu$ g/ml); and diluting the cells with cold medium immediately after electroporation. A caveat was raised in discussion, however, that electroporation may be mutagenic in Calothrix.
- Herbert Böhme cloned the gene (fdxH) encoding a heterocyst-specific ferredoxin from Anabaena PCC 7120. The gene, now sequenced, is located approximately 7 kb downstream from nifK. Two transcripts were detected by Northern analysis, both observed only at a late stage of heterocyst differentiation. The promoter sequence for one of the transcripts does not resemble any other known promoter. The gene was expressed and the holoprotein was assembled correctly in E. coli.

### Calothrix PCC 7601 and PCC 7504

- Didier Mazel presented an elegant description of phycocyanin gene control under conditions of sulphur deprivation [see News, this issue].
- Nicole Tandeau de Marsac spoke about differentiation in Calothrix. Homogonia differentiation occurs in 100% of the cells of Calothrix PCC 7601 if the culture is grown in green light, transferred to fresh medium, and grown further under red light. Under aerobic conditions, red light promotes homogonia differentiation, whereas green light promotes heterocyst differentiation. In Calothrix PCC 7601, homogonia and heterocysts were never observed in the same filament. In Calothrix PCC 7504, both were observed but at opposite ends of the filament. Thus, homogonia and heterocysts appear to be mutually exclusive end products of differentiation.

### Synechococcus PCC 7942 (Anacystis nidulans R2)

- Mies Borrias described the construction of a "platform" within Anacystis that provides a simple and effective method for studying gene expression. Any gene of interest that has been cloned into pBR322 can be placed by homologous recombination into the platform.
- Peter Weisbeek has studied the genes encoding electron transport proteins. Attempts at inactivating the gene encoding ferredoxin were unsuccessful, suggesting that this gene is essential. The gene encoding plastocyanin from Anabaena variabilis ATCC 29413 was isolated and transformed into Anacystis. Expression of the gene was observed at a very low level compared with that in Anabaena, and no regulation by copper was observed. There may indeed be important differences in promoter recognition and control amongst different cyanobacteria.
- Susan Golden reported on psbA::lacZ fusions, integrated into the chromosome as single recombinants to preserve psbA function. Under high light regimes, psbAI is not expressed, but it is induced 600-fold under low light. In contrast, psbAII and psbAIII are poorly expressed under low light and are induced 9-fold and 14-fold, respectively, in high light.

### Synechocystis PCC 6803

- Lawrence Bogorad described the isolation of three Photosystem II mutants. The essential lesion of one was determined to be a 202 bp deletion in the psbC gene. Two other mutants had lesions in genes encoding the 47 kD protein. None of the three contained intact reaction centres.
- Sergei Shestakov told of eight complementation groups, found by cross-transformation analysis of mutants that fail to grow photoheterotrophically. Several mutants could be complemented by specific fragments of DNA cloned from Synechocystis PCC 6803. Some of these cloned fragments cross-hybridized with plant nuclear DNA. One complementing fragment cross-hybridized with a large unidentified open reading frame from the tobacco chloroplast (orf1708).

--Himadri Pakrasi has cloned the genes encoding *cytb<sub>559</sub>* (*psbE* and *psbF*, coding for the  $\alpha$ - and  $\beta$ -subunits, respectively). These genes form part of an operon: *psbE-psbF-psbI-psbJ*. Deletion of the entire operon gave rise to strains in which Photosystem II and *cytb<sub>559</sub>* were absent. Alteration of a tyrosine residue to aspartate in the  $\alpha$ -subunit *cytb<sub>559</sub>* yielded a strain that cannot grow in the absence of glucose, indicating that *cytb<sub>559</sub>* is an essential part of photosystem II.

#### Synechococcus PCC 7002 (Agmenellum PR6)

--Don Bryant reported on the analysis of many of the genes involved in photosynthesis in *Synechococcus* PCC 7002 (and also in *Nostoc* MAC PCC 8009). The Photosystem I genes, *psaA*, *psaB*, and *psaC*, have been cloned and sequenced in *Synechococcus*. *psaC* has also been cloned and sequenced from *Nostoc*. All three genes show considerable similarity with the corresponding genes from plant chloroplasts. Six phycocyanin genes have been cloned and sequenced. Mutations in two linker genes, *cpcC* and *cpcD* gave incomplete phycobilisomes. Five allophycocyanin genes have been characterised. The *apcC* gene is non-essential but appears to stabilise the phycobilisome core. Mutations in *apcD* had no effect on phycobilisome assembly. The *apcE* gene product is essential for phycobilisome assembly. It contains three repeating amino acid elements that may play a role in linker function.

-- contributed by MARTIN MULLIGAN

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