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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 for a hard copy. Free by E-Mail.

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 -Contact the person whose name is capitalized in the news item. Addresses are given at the end of the issue. Also, a Directory of Cyanobacteriologists is distributed every two years.

INSTRUCTIONS TO AUTHORS - Send news.

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The 10th AUSTRALIAN NITROGEN FIXATION CONFERENCE, to be held 7-10 Sep 1993 in Brisbane, will include symposia centered around several themes, some of which may be: non-symbiotic N2 fixation, microbe-plant genetic interactions, the role of molecular genetics in microbial ecology, nitrogen fixation in agricultural production systems, and metabolic pathways associated with nitrogen fixation.

Contact: H. Van Bushby, CSIRO Div of Tropical Crops & Pastures, 306 Carmody Road, Santa Lucia Qld 4067, Australia. (Fax) 61-7-3713946. Or phone P. Dart (61-7-3652867) or Lex Diatloff (61-7-8779311).

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Umea, Sweden, will be the site of the EUCHEM-conference on ULTRAFAST PROCESSES IN CHEMISTRY AND BIOLOGY, 13-16 June, 1994. Amongst the topics to be addressed are energy transfer and electron transfer in photosynthesis.

Contact: The Swedish National Committee for Chemistry, Wallingatan 26 B, S-111 24 Stockholm. (Tel) 46-8-115280, (Fax) 46-8-106678.

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The 8th INTERNATIONAL SYMPOSIUM ON PHOTOTROPHIC PROKARYOTES is scheduled for 10-15 Sep 1994 in Urbino, Italy. In addition to themes common to previous symposia, e.g., biogenesis, regulation, structure, and function of the photosynthetic apparatus, metabolism, ecology, and taxonomy, the organizers have added a new theme: basic and applied biotechnological processes

Contact: Stefano Ventura, CNR - Centro di Studio dei Microrganismi Autotrofi, piazzale delle Cascine 27, I-50144 Firenze Italy. (Tel) 39-55-350542, (Fax) 39-55-330431, (E-mail) Ventura@csma.fi.cnr.it

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The 6th AFRICAN ASSOCIATION FOR BIOLOGICAL NITROGEN FIXATION (AABNF) CONFERENCE will be held in Harare, Zimbabwe, 12-17 September 1994, focusing on the agronomic, socio-economic and environmental benefits of biological nitrogen-fixing systems in Africa.

Contact: The Secretary, AABNF, Department of Soil Science, University of Zimbabwe, Box MP 167, Mount Pleasant, Harare, Zimbabwe. (Tel) 263-4-303211 ext 1412. (Fax) 263-4-732828. (Telex) 26580 UNIVZ ZW. (Email) rcrust@zimbix.uz.zw (or) smoyo@zimbix.uz.zw

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The 8th EUROPEAN BIOENERGETICS CONFERENCE will be held in Valencia, Spain, 12-17 Sep 1994 and will emphasize several topics, including the structure of energy-transfer proteins, electron-transport systems, light-harvesting systems, reaction centers, and sodium bioenergetics and salt tolerance. Those wishing to receive the second announcement should send their complete postal address, telefax number and areas of interest.

Contact: Eduardo Rial, EBEC 94, Centro de Investigaciones Biologicas, Velazquez 144, 28006 Madrid, Spain. (Fax) 34-1-5627518, (Email) ciber12@cc.Csic.Es

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Next year's Gordon Conference on BIOPHYSICAL ASPECTS OF PHOTOSYNTHESIS is tentatively scheduled for August 7-12, 1994. Suggestions regarding the program (topics, speakers, format, etc.) are welcome. Also, alternative dates for the meeting will be considered. Send your comments within the next two months.

Contact: Marion Thurnauer, Argonne National Laboratory, 9700 S. Cass Avenue, Argonne IL 60439 U.S.A. (Tel) 708-252-3545, (Fax) 708-252-9289, (E-Mail) thurnauer@anlchm.chm.anl.gov or thurnaue@anlchm.bitnet

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There will be a TRAINING COURSE on FIELD AND LABORATORY TECHNIQUES FOR SOUTH-EAST ASIA AND THE PACIFIC, entitled "Photosynthesis and Productivity in a Changing Environment", 5-26 Jan 1994 in Bangkok, Thailand. The course will cover a wide range of topics, including field photosynthesis measurements, carbon and nitrogen assimilation, algal systems, and biofuels and energy balance. Applications must arrive by 31st August, 1993 and should include: (a) a detailed curriculum vitae, (b) a brief description of present or proposed work and its relevance to the course program, and (c) a letter of support from an employer or supervisor.

Contact (applicants from Thailand): Morakot Tanticharoen, School of Bioresources and Technology, King Mongkut's Institute of Technology Thonburi, Bangmod, Rasburana, Bangkok 10140. (Tel) 66-2-4270039 ext. 7000, (Fax) 66-2-4279062, 4278077, (Telex) 72383 KMITT TH.

Contact (applicants from other south-east Asian and Pacific countries: David O. Hall, Division of Life Sciences, King's College London, University of London, Campden Hill Road, London W8 7AH, UK. (Tel) 44-71-3334317, (Fax) 44-71-9377783, (Telex) 8954102 BBSLON G.

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JIRI KIVIRANTA has completed and published his Ph.D. dissertation, entitled Toxins of Cyanobacteria (Blue-Green Algae) -- a Biological and Chemical Study. One main focus of his work was screening for cyanobacteria toxic to mosquito larvae. The toxin most effective against mosquitos came from a hepatotoxic fraction extracted from Microcystis aeruginosa strain 205. It was analyzed and determined to be [Dha7]microcystin-RR.

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Judy Acreman has sent in a description of the UNIVERSITY OF TORONTO CULTURE COLLECTION (UTCC), of which she is curator. The UTCC, officially opened in 1987, makes available cyanobacterial cultures from a collection of about 250 isolates of freshwater algae and cyanobacteria. One of the main goals of the UTCC is to develop a collection of these organisms that is representative of species native to Canada. Of particular interest are isolates from areas under environmental stress, for example, acid-stressed lakes, metal-polluted sites and organically polluted sites. There are currently 40 isolates of 24 species of cyanobacteria in the collection, and more will be added as new isolations proceed. Deposits of research cultures are welcome, particularly those isolated from locations in Canada. To be considered for deposit, the cultures should be positively identified at least to genus and if not axenic, at least have only bacteria present as contaminants.

In addition to distributing cultures, the UTCC also offers the following services: a) custom-isolation of algae and cyanobacteria, b) training in methods of isolation and culture of these organisms, c) safe-deposit of cultures on a confidential basis, d) preparation of media.

To obtain a catalogue of species in culture or for further information contact:

Judy Acreman, UTCC, Botany Department, University of Toronto, Toronto, Ontario, Canada M5S 3B2. (Tel) 416-978-3641, (Fax) 416-978-5878, (E-mail) JAcreman@botany.Utoronto.Ca

POSITIONS AVAILABLE

POSITION: Postdoc

CONTACT: Steve Theg, Section of Plant Biology, University of California, Davis CA 95616 U.S.A. (Tel) 916-752-0624, (E-mail) SMTheg@UCDavis.Edu

START: After July 1, 1993

RESEARCH: Study the assembly and function of the three extrinsic subunits of the photosynthetic oxygen evolving complex in chloroplasts. The long range goals of the project are to identify specific amino acids involved in holding the complex together and in imparting its biological activity. The experimental approaches will lean heavily on techniques for random and site-directed mutagenesis, in vitro chloroplast protein import, in vitro protein reconstitution, and analysis of photosynthetic capacities by various biophysical means.

REQUIREMENTS: Ideally, an intimate understanding of photosystem II reactions, both theoretical and practical knowledge of molecular biology (cloning, sequencing, etc.), experience in biophysical techniques applied to chloroplast bioenergetics (i.e., fluorescence, spectroscopy, etc.), and familiarity with the field of protein trafficking. Persons meeting some, but not all, of these criteria are still encouraged to apply; clear thinking, desire, and solid scientific training will go a long way in filling in the gaps.

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POSITION: Post-doc

CONTACT: Peter Brzezinski, Department of Biochemistry and Biophysics,

Chalmers University of Technology, S-412 96 Goteborg, Sweden.
(Tel) 46-31-7722807, (Fax) 46-31-7722813,
(E-mail) Peter@Biofy2.Bcbp.Chalmers.Se

RESEARCH: Use various biophysical and genetic techniques to study bacterial and plant photosynthesis. Current work in the project associated with the position focuses on reconstitution of PSII in phospholipid vesicles and time-resolved measurements of light-induced electrogenic events associated with electron and proton transfer. There is great flexibility in the design of the project.

SEND: A brief description of research interests, CV, and the names and addresses of at least two references.

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POSITION: Post-doc

CONTACT: Terry Bricker, Dept. of Botany, Louisiana State University, Baton Rouge LA 70803 U.S.A.

RESEARCH: Molecular biochemistry of photosynthesis. One position involves the production and characterization of mutants in the CPa-1 protein of PS II [Putnam-Evans & Bricker (92) Biochem 31:11482]. Another involves the study of protein-protein interactions in PS II [Frankel & Bricker (92) Biochem 31:11059].

SUPPORT: Initially for 1 year.

START: Anticipated starting date Sept. 1 1993.

SEND: CV

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Shanghao Li (1917-1993)

Shanghao Li passed away suddenly on February 24, 1993 at the Institute of Hydrobiology of the Chinese Academy of Science in Wuhan. He will be missed in the scientific communities both in China and abroad as well as by those of us who knew him directly or indirectly.

Shanghao Li was born in the Guangzou Province in China. He went to Zhongshan University to study biology and joined the Institute of Hydrobiology in the early 1950's. His early research focused on limnology and the systematics of algae, especially diatoms and green algae. From the mid-1970's, he studied the physiological ecology of cyanobacteria. He introduced the method of applying on a grand scale Anabaena as a fertilizer in rice fields, the success of which was immediately recognized by his colleagues and by local farmers. The culture collection of algae at the Institute, including cyanobacteria in his own laboratory, is among the best in Asia.

Shanghao Li was the author of numerous research papers and reviews and was the editor of a number of books. He was often an invited speaker at international conferences and also hosted many foreign colleagues in China. He was elected a member of the Chinese Academy of Science in 1980, and in 1981 he was appointed Director of the Institute of Hydrobiology. He served on several editorial boards of journals and publications (including CyanoNews) and on organizing committees of conferences.

To those of us who knew him personally, Professor Li was an outstanding scientist, a patient teacher, a giving friend, and a man of family. He always found time for his students, his friends, and his family. We students learned from his teaching to appreciate the good things in science and to gain confidence in our research. He was ready to help but seldom mentioned any unfairness to himself.

There were quite a few political movements in China against the educated, and Professor Li survived each of them, as a cyanobacterium resists adverse conditions. After 10 years of the Cultural Revolution, he was among the first to remake contact with the world. He even participated in some political processes to persuade the government to maintain an open door policy. Many of his students went abroad to study various aspects of cyanobacteria. While respecting their opinions, Professor Li always reminded his students that there were opportunities in China and that China needed well-trained scientists, not only for its scientific progress but also for its political progress.

Shanghao Li was also talented in music and Chinese art and literature. He enjoyed playing piano with his wife and loved walking with his grandson at sunset along the coast of the beautiful East Lake, where the Institute of Hydrobiology is located. His life exemplified all that is good in science and in human beings, an he will be remembered that way.

Professor Li, we all miss you!

-- Jindong Zhao

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FREMYELLA,NOSTOC PLASMIDS SHARE COMMON FEATURES

MIKE SCHAEFER, recently arrived in his new position in Missouri, reports a curious finding. He sequenced the origin of replication from pFdAI (a shuttle vector used in Fremyella) and found it to be very similar to that of plasmid pDU1, the plasmid from Nostoc PCC 7524 that is the basis for many shuttle vectors used in Anabaena PCC 7120 and other strains. The similarity includes an open reading frame of unknown function and regions of dyad symmetry. Conceivably, the shuttle vectors developed for Anabaena may work in Fremyella, and Fremyella vectors may work in Anabaenas and Nostocs. Has anyone ever tried?

DUNE CYANOBACTERIA DESCRIBED, AVAILABLE

BOB WEBB, another recent arrivee at a new job, this one in Texas, has isolated some new cyanobacteria from the gypsum dunes at White Sands, New Mexico. He has two unicellular strains, three filamentous strains, and one that grows in very short filaments, about four cells within a sheath. The unicellular strains and two of the filamentous strains grow fairly well on plates without nitrate. He invites anyone with a possible interest in these strains to let him know: they are available.

SPIRULINA GROWN ON CITRUS INDUSTRY EFFLUENT

ROGERIO LACAZ-RUIZ has given us a progress report on how he, M.E. Kornfeld, and M.A. Zanetti in Sao Paulo, Brazil, have used waste water from the citrus industry to grow Spirulina platensis. The waste water was supplemented with a wood ash alkaline solution, nitrogen, and phosphorus. They have received a patent for this growth medium. Spirulina accumulated in this growth medium at the rate of 0.5 mg/ml during a five day growth period, with little contamination by green algae. The crude protein content of the biomass was 52% by dry weight. The medium costs US$1.37/liter, 4.2% less than CFTRI, an alternative synthetic medium.

UNIQUE RUBISCO ACTIVASE IN HETEROCYSTOUS CYANOS

BOB TABITA let us know that his laboratory has identified in Anabaena sp. strain CA three open reading frames downstream from rbcS, encoding the small subunit of Rubisco. One, labeled rca, encodes Rubisco activase and lies about 2 kb downstream from rbcS. The other two have no recognizable function and lie between rbcS and rca. Interestingly, rca from Anabaena CA does not show clear hybridization to DNA from unicellular or nonheterocystous filamentous cyanobacteria. There is very strong hybridization, however, to DNA from other strains of Anabaena and Nostoc. The work has recently been published [Plant Mol Biol 21:753-764].

RED CYANOBACTERIA INFEST CORAL REEFS

Recent times have seen a steady decline in the health of coral reef ecosystems, and much of this decline may be attributable to coral diseases associated with cyanobacteria. Two of the most common of the known diseases affecting coral are black band disease and white band disease. In both cases, a band of activity sweeps across the coral surface, destroying coral tissue. Black band consists of a consortium of bacteria, in many ways analogous to a microbial mat community. The color is due to phycoerythrin from the dominant species, Phormidium corallyticum. Much less is known about white band disease.

First black, then white... now LAURIE RICHARDSON tells us she has found a new plague on corals: red band disease. In many ways red band is similar to black band. The most obvious differences are that the band is brick red in color and is dominated by a species of the genus Oscillatoria. Unlike black band, however, red band progresses only in the light and at a much slower rate of 1 mm per daylight period. It is not clear what controls movement of the band. In the case of black band, control by light has been excluded, raising the possibility that chemotaxis is involved.

In passing, it should be noted that red band disease has never been observed outside the Bahamas. Scientists wishing to study the disease must therefore be willing to spend a significant period in these tropical islands, a point to consider when choosing an experimental system.

GENES ENCODING EUKARYOTIC-TYPE RNA-BINDING PROTEINS, FOUND IN CYANOBACTERIA

Those of us who study cyanobacteria often have an interest in eukaryotes greater than that of your average bacteriologist. This situation arises in part because at least one ancient member of our chosen class of organisms snuck into a eukaryote a billion or so years ago, forcing us to comprehend the behavior of the nucleus in order to appreciate the condition of these chloroplast descendants. It was ironic, then, to hear two years ago [Kathe et al (1990) Science 250:1566-1570; Kuhsel et al (1990) Science 250:1570-1573] that cyanobacteria possess a gene with an intron, that eukaryotic device previously unknown to bacterial genomes. MARTIN MULLIGAN now comes with the news that the connection with eukaryotes appears stronger than we thought. He tells us that heterocyst-forming cyanobacteria have multiple genes encoding proteins that are similar to the RNP family of eukaryotic RNA-binding proteins, a family that includes snRNP proteins (responsible for the excision of introns) and certain regulatory proteins. Previous to this account, such genes had not been discovered outside of the eukaryotes, unless one counts chloroplasts as exceptions.

Three genes from two strains of cyanobacteria (Anabaena and Chlorogloeopsis) have been sequenced. All three putative gene products contain a single RNA Recognition Motif (RRM) that includes the highly conserved RNP1 and RNP2 regions and all three have a short glycine-rich carboxy-terminal tail. RNA-binding protein genes are abundant in heterocyst-forming filamentous cyanobacteria but are not abundant in non-heterocyst-forming filamentous or unicellular cyanobacteria, raising the possibility that the cyanobacterial proteins may play a role in gene expression during heterocyst differentiation. Although the exact function of the cyanobacterial gene products is not yet known, their similarity to eukaryotic proteins suggests that they may play a role in RNA processing - either in splicing reactions or in processing the 3' end of nascent cyanobacterial mRNA. The unexpected presence of these genes in cyanobacteria has some intriguing implications for the evolution of RNA binding proteins and RNA processing.

NOVEL METHOD TO SEPARATE ENANTIOMERS: APPLICATION TO HOMOANATOXIN-A

Homoanatoxin-a is the neurotoxic compound produced by Oscillatoria formosa. A new gas chromatographic technique allows the enantiomer-specific separation of the bicyclic secondary amine, reports OLAV SKULBERG, who recently developed the procedure in collaboration with John-Erik Haugen and Michael Oehme (Norwegian Institute for Air Research), Markus Mueller (Swiss Federal Research Station), and Timothy Gallagher (University of Bristol).

Separation of cyanophyte neurotoxins into their enantiomers is of considerable interest. The chemical synthesis of enantiomeric substances gives a racemate (a precise 1:1 mixture of both enantiomers), while biogenic formation normally results in a single enantiomer. In many cases, only one of the enantiomers shows relevant bioactivity. The other is inactive or even antagonistic. A simple technique to separate enantiomeric neurotoxins permits:

1. confirmation of the enantiomeric purity of synthetically produced neurotoxins that have been separated into single enantiomers by classical techniques, such as the formation of diasteriomers.
2. Identification of the enantiomers formed by cyanophytes, and evaluation of the enantiomer-specificity of the biosynthesis.

Recently, new routine gas chromatographic methods have been developed that allow the trace level separation of enantiomers on special tailor-made enantiomeric stationary phases. Such phases consist of a chiral modifier, for example, a modified cyclodextrin dissolved in a methyl-phenyl-polysiloxane. This technique permits the separation of the chiral neurotoxin homoanatoxin-a into its enantiomers.

Homoanatoxin-a, from extracts of Oscillatoria formosa (NIVA-CYA 92) and as a synthetic racemic mixture, was transformed into the heptafluorobutyryl derivative by acylation to obtain a thermally more stable and less polar compound suitable for gas chromatography. Compounds were detected by negative ion chemical ionization mass spectrometry (NICI). Mass m/z 315 [M-3HF]- was used to monitor the compounds in the NICI mode. Figure 1 shows that the racemic mixture can be completely separated on a glass capillary column as short as 12 m (0.32 mm i.d.) coated with 20% of a modified beta-cyclodextrin dissolved in 85% methyl- 15% phenylpolysiloxane. It is evident that the water extract from the culture of NIVA-CYA 92 contained only one enantiomer. At the moment, the exact enantiomer conformation of each signal is unknown.

The method presented has the following advantages:

* Complete separation of enantiomers within 15 minutes
* Stationary phase is compatible with selective detection using an electron capture detector or NICI mass spectrometry
* Selectivity and detection limits allow the quantification and enantiomeric separation of subpicogram amounts, corresponding to sub-parts per billion in water samples

[Figure 1 omitted in electronic version]

COMMENTARY: AT-BIAS AND PHYLOGENY OF PROCHLOROPHYTES

In Cyanonews Vol. 8, No. 1, Sean Turner outlined current evidence that no known prochlorophyte is specifically related to the ancestor of the green chloroplast and that none of the known prochlorophytes are related to each other. These conclusions are supported by sequence data from 16S rRNA and genes encoding RuBp carboxylase, ATP synthase, and DNA-dependent RNA polymerase [for references see Cyanonews Vol. 8, No. 1, and Origins of Plastids, R.A. Lewin editor, Chapman & Hall, 1993]. In Cyanonews Vol. 9, No. 1, Chris Howe questioned these results. His argument was that the high AT-bias of the chloroplast may artificially group it farther apart from prochlorophytes with less AT-biased genomes or that unrelated organisms with convergent %GC content may be grouped together.

This criticism ignores two points. The first is that most of the AT-bias in genes is found in the third position of the codon. The analyses of RNA polymerase sequence data, for example, did not use the third codon position for this reason and for the reason that the organisms are too diverged for this position to contain useful phylogenetic information. The %GC of the first two codon positions alone of the RNA polymerase fragments of Prochloron, Prochlorothrix, Prochlorococcus, and maize are quite similar so that we expect much of the %GC-bias to have been removed.

As to the second point: What is the %GC of the genomes of different prochlorophytes? The work of Herdman suggests that the genome of Prochloron is 40.5% GC [Arch Microbiol (1981) 129:314-6]. The work of Burger-Wiersma, et al. suggests that the genome of Prochlorothrix is 53% GC [Int J Systematic Bacteriol (1989) 39:250-257]. We don't know the %GC of Prochlorococcus. Since RNA polymerase is a highly expressed protein we would expect its codon usage and %GC to reflect that of the organism. The %GC based on RNA polymerase sequences does seem to match that of whole cyanobacterial genomes where each are known (data not shown). Based on RNA polymerase gene sequences, the %GC of Prochloron, Prochlorothrix, Prochlorococcus, and maize chloroplast can be estimated as 42%, 56%, 41-35% (two strains), and 38%, respectively. Prochlorococcus, in particular, clearly has a high AT-bias -- low %GC. The third codon position alone is 32% GC and 20% GC, respectively, for two strains of Prochlorococcus in culture, compared to 27% GC for maize chloroplast (known to be low %GC) and 79% GC for WH8103, a marine Group A Synechococcus (known to be high %GC).

Despite the apparent high AT-bias of Prochlorococcus, it still groups closely with marine Group A Synechococcus and shares with them an amino acid insertion in RNA polymerase found in no other known cyanobacteria. Even with an AT-bias similar to that of the maize chloroplast, Prochlorococcus does not group with the green chloroplast lineage. This result suggests that AT-bias in RNA polymerase gene sequences in general has not been strong enough to affect the major features of trees derived from those sequences.

Although one should always exercise caution in phylogenetic inferences, sequence data from several molecules providing similar phylogenetic trees supports the conclusion that the prochlorophytes are a polyphyletic group and that none of the known prochlorophytes is related to the chloroplast lineage (though one so related could still be discovered). Chlorophyll b synthesis thus seems to be an ability either: (1) of ancient origin that has been lost or has become cryptic in multiple cyanobacterial lineages, (2) that has migrated by horizontal gene transfer, (3) that has arisen on multiple occasions by independent mutations, or (4) any combination of the above.

- Brian Palenik

MEETING REPORT*MEETING REPORT*MEETING REPORT*MEETING REPORT*MEETING REPORT

The 1993 Cyanobacterial Workshop was held May 30 - June 2 at Asilomar Conference Center, Pacific Grove California. The summaries below represent only a slice of the hundred or so talks and posters contributed, not to mention the scientific exchanges that took place against the roar of the Pacific Ocean. The meeting was organized by Arthur Grossman (Stanford University) and Mike Schaefer (University of Missouri-Kansas City) who somehow managed to coordinate matters despite their separation by a couple of thousand miles. As evidence that the coordination succeeded and was appreciated by those in attendance, it was decided to model the next meeting after this last (imitation being the sincerest form of flattery). The next workshop will be held in 1995, also at Asilomar, organized by Don Bryant (Pennsylvania State University) and Neil Straus (University of Toronto), who have no excuses, since they're only about 400 miles apart.

Photosynthesis

Photosystem I (PS I): Don Bryant (Pennsylvania State University) reported progress in studying the PS I complex from Synechococcus PCC 7002. In this cyanobacterium, PS I is comprised of eleven polypeptides namely, PsaA, PsaB, PsaC, PsaD, PsaE, PsaF, PsaI, PsaJ, PsaK, PsaL and PsaM. The genes encoding all these polypeptides except PsaI, PsaM and PsaN have been cloned and characterized. A combination of interposon mutagenesis and overproduction of some of the above polypeptides in Escherichia coli was used to reveal information about PS I. PsaD is required for stabilization and correct orientation of PsaC on the PS I complex, and PsaE polypeptide is required for cyclic electron transport. Wendy Schluchter (Pennsylvania State University) told us that PsaK and PsaL mutants grow in DCMU but their growth rates are slower in low light. PsaL- mutant do not form trimeric PS I complexes. Furthermore, this mutant exhibits altered state transitions: energy transfer from the phycobilisome to PS I is impaired. Vim Vermaas (Arizona State University) reported that a PS I- mutant of Synechocystis PCC 6803 grows at 5 uE/m2×ªs of light if adapted properly and supplied with sugar. PS II in this strain shows normal function, and electrons from plastoquinone go to cytochrome oxidase instead.

Photosystem II (PS II): Himadri Pakrasi (Washington University, St. Louis) reported complementation of SK18, a mutant of Synechocystis PCC 6803 that does not have a functional PS II. The open reading frame (ORF) that was complemented in the mutant shows similarity to a gene from E. coli, prcA, that encodes a carboxy-terminal processing protease. Interposon mutagenesis of this ORF in Synechocystis results in a larger D1 protein. Therefore, he and his colleagues propose that this ORF (designated ctpA) encodes the carboxy-terminal processing protease for the D1 protein.

Herbicide resistance: Sergei Shestakov (Moscow State University) reported complementation of Synechocystis mutants that are resistant to the phenolic herbicide, dinoseb, and the carotenoid biosynthesis inhibitor, difunone. Gene inactivation experiments showed that the molecular basis of dinoseb resistance is associated with the absence of the product of a gene designated drgA. DrgA does not show homology with any known proteins. It was proposed that drgA encodes a protein that is involved in the conversion of dinoseb and metronidazole to highly toxic agents, perhaps through a ferredoxin-dependent pathway. Difunone resistance results from either a 3 base pair deletion or a duplication within the dfrA gene. dfrA encodes a product of 74.5 kDa. The carboxy-terminus shows a helix-turn-helix domain and is homologous to phoR from B. subtilis, which acts as transcriptional regulator.

Light regulation of psbA gene expression: Susan Golden (Texas A&M University) gave an update on the differential expression of the psbA gene family in

Synechococcus PCC 7942. Genes psbAII and psbAIII are expressed at very low levels when cells are grown at low light, but rapidly increase their expression upon a shift to high light. The levels of psbAI message are high in low light, but they drop dramatically within a few minutes upon a shift to high light. After prolonged incubation in high light, the expression of the psbAI message increased again to the same levels as is in low light. After six hours in high light, the total psbA message is four times higher than before the increase in light intensity. Systematic analysis of the control regions of psbAII and psbAIII genes showed that three elements are present upstream of the of each gene: a basal constitutive promoter, a negative element upstream of the promoter, and a light-responsive element downstream of the transcription site. The light-responsive elements increase expression from the native promoter or a heterologous promoter in a position- and orientation-independent manner indicating enhancer activity. However, the ability to confer light-responsive expression is orientation-dependent. In addition, low fluence blue light was shown to elicit the same changes in psbA expression that are induced by exposure to high light. A pulse of red light after exposure to blue light significantly attenuates the blue-light-mediated increase in psbAII and psbAIII messages.

(Contributed by Nikos Tsinoremas)

Phycobilisomes

Walter Sidler (E.T.H., Zuerich) described how he and coworkers have successfully reconstituted the rod core complex, (alpha-beta)6PC×ªLRC29.5×ª (alpha-beta)3AP×ªLC8.9 from Mastigocladus laminosus. This reconstitution required using linker polypeptide LRC29.5 which had been overexpressed in E. coli. The authors suggested that this requirement might result from proteolysis of LRC29.5 in preparations from M. laminosus. Hopefully this reconstituted complex will form beautiful crystals suitable for X-ray crystallography! The core-membrane linker (LCM) functions to organize the allophycocyanin trimers within the core complex and thus determines the overall shape of the phycobilisome. Core complexes with two cylinders (Synechococcus PCC 6301) and three cylinders (Synechococcus PCC 7002) have been previously characterized.

Axel Ducret (E.T.H., Zuerich) showed electron micrographs of core complexes reconstituted from Anabaena PCC 7120. In his interpretation of the data, a core complex consists of three cylinders plus an additional allophycocyanin (AP) complex attached to each side of the top cylinder (i.e. a three-and-two-halves-cylinder core complex). I wonder if a five cylinder phycobilisome might occur in another species.

Samuel Beale (Brown University) reported evidence, derived from work on

Cyanidium caldarium ("cyanobacterium", honoris causa?), that supports an interesting pathway of phycobilin synthesis. If this pathway is present generally in cyanobacteria, then it should be possible to find phycoerythrobilin in all cyanobacteria regardless of whether phycoerythrin is synthesized. The pathway begins with the conversion of protoheme to biliverdin IXalpha by heme oxygenase. Two enzymes are required to convert biliverdin IXalpha to (3Z)-phycoerythrobilin. 15,16-dihydrobiliverdin IXalpha is the intermediate in this conversion. The two enzymes each catalyse a two-electron reduction, and require NADPH and ferredoxin. The isomerization of (3Z)-phycoerythrobilin to (3Z)-phycocyanobilin requires a specific isomerase. Z-to-E isomerizations of these latter two bilins is enzymatic and requires glutathione.

Craig Fairchild (U.C. Berkeley) described his work two proteins, CpcE and CpcF, required for the proper attachment of phycocyanobilin attachment to the alpha subunit of phycocyanin (PC). These polypeptides were purified from overexpressing E. coli and shown to associate with each other. The complex (CpcEF) catalyses not only bilin attachment but also the transfer of bilin from one alpha subunit to another. CpcEF also associates with PC and quenches fluorescence emission.

The last three speakers described work on chromatic adaptation in Fremyella diplosiphon. Michael Schaefer (University of Missouri, Kansas City) described two mutants which fail to respond to red light. He and coworkers at the Carnegie Institution, Stanford have complemented these mutants using a mobilizable plasmid library. The complementing gene, designated rcaC, shows strong identity to phoP, a regulatory protein involved in phosphate metabolism in Bacillus subtilis. The exact role of rcaC in chromatic adaptation is presently unknown.

Nancy Federspiel and colleagues (University of Idaho, Moscow) reported progress on in vivo and in vitro footprinting of the promoter of the cpeBA operon (cpeBA encodes the beta and alpha subunits of phycoerythrin). Using dimethyl sulfate, they identified two G residues within the promoter that are protected in DNA isolated from cultures grown in either red or green light. They concluded, therefore, that the protecting factor is bound to the promoter, independent of light quality. This conclusion differs from that drawn by Nicole Tandeau de Marsac (Institut Pasteur) and coworkers, working with the similar strain, Calothrix PCC 7601. They found that the same residues were protected only in green light.

John Cobley (University of San Francisco) presented the recent work from his laboratory concerning a mutant, F. diplosiphon SF48, that fails in green light to assemble phycoerythrin into the phycobilisome. Using a mobilizable cosmid library it was possible to complement this mutant and thereby clone the complementing gene, which has been named cpeF. cpeF has more than 30% sequence identity to both mpeV and mpeU, genes from Synechococcus WH8020. CpeF most probably attaches a phycoerythrobilin to a specific cysteine in phycoerythrin. It will be particularly interesting to see if the expression of cpeF in F. diplosiphon is dependent on green light.

(Contributed by John Cobley)

Nitrogen Metabolism and Heterocyst Differentiation

Nitrogen Metabolism:

In Synechococcus PCC 7942, the genes involved in nitrate assimilation are organized into a cluster, nirA-nrtABCD-narB, and expressed as an operon. Tetsuo Omata (Nagoya University) reported the presence of two ammonium-repressible genes in the region upstream of nirA. One open reading frame (orf349) is required for the expression of maximal activity of nirA. A mutant affected in the other (orf309) exhibits normal levels of nitrate reductase and nitrite reductase activities but nonetheless grows slowly with nitrate or nitrite as the nitrogen source. The predicted protein sequence encoded by orf309 is similar to that of transcriptional regulators of the LysR family.

Enrique Flores (Universidad de Sevilla) talked about a second regulatory protein, NtcA, which is involved in transcriptional activation of ammonium repressible genes. NtcA is found in a variety of unicellular, filamentous, and heterocystous cyanobacteria. There is considerable sequence similarity in the three ntcA genes that have been sequenced (from Synechococcus PCC 7942, Synechocystis PCC 6803 and Anabaena PCC 7120), particularly in a conserved helix-turn-helix motif. Footprinting studies indicate a consensus binding sequence of GTA..N8..TACA, found with only minor variations upstream from a number of nitrogen-regulated genes from different cyanobacteria. These genes include nirA, glnA, hetA, hetR, patA, and ntcA itself.

T.S. Ramasubramanian (Texas A&M University) reported on the characterization of a gene (bifA) of Anabaena PCC 7120 that is evidently identical to ntcA (although the identification of the two genes were by wholly different approaches). Analysis of the binding of BifA to glnA, xisA, and rbcL upstream sequences yielded a consensus recognition sequence of TGT..N9-10..ACA, very close to the sequence obtained by the Seville group. BifA is present in both vegetative cells and heterocysts. A mutant in which bifA had been insertionally inactivated failed to grow on N2 or nitrate.

Cyanothece BH68, a unicellular cyanobacterium showcased by Milagros Colon-Lopez (Purdue University), exhibits the ability to fix nitrogen in the presence of oxygen. When grown with an alternating light/dark cycle, N2-

fixation is restricted to the dark period and reaches peak activity at a time coinciding with maximal respiratory activity. In a like fashion, photosynthetic O2 evolution is confined to the light period, peaking 4-6 h after the dark/light transition. The periodicity of N2-fixation is retained when growth is shifted to continuous light. Richard Bradley (State University of New York, Binghamton) suggested that Cyanothece may employ covalent modification of nitrogenase as a form of post-translational control to regulate nitrogenase activity. Western blot experiments using antibodies directed against the Fe-subunit of nitrogenase revealed a band migrating at 38-kDa under conditions of aerobic nitrogenase activity and one at 40 Kda under conditions in which nitrogenase activity was absent.

Heterocyst differentiation:

A number of talks and posters addressed the question of heterocyst differentiation and patterned development. Francisco Leganes (Michigan State University) sought a connection between the two developmental processes of heterocyst and akinete differentiation. He isolated a number of mutants of Nostoc ellipsosporum that are defective in the differentiation of both cell types (and hence cannot fix nitrogen in the presence of air). The two processes appear therefore to be related by a common mechanism.

Several groups have found genes turned on early in the response of Anabaena PCC 7120 to nitrogen deprivation. Genes involved in nitrate assimilation seem to be among the earliest induced, reported Yuping Cai (Michigan State University). Bill Buikema (University of Chicago) passed on news of a gene (pknA) encoding a eukaryotic-type serine/threonine protein kinase, isolated from Anabaena by PCR. Transcripts of pknA begin to accumulate 2.5 hr after nitrogen stepdown. Stephanie Curtis (North Carolina State University) described the isolation of gnd, encoding 6-phosphogluconate dehydrogenase. The gene has multiple transcripts, at least one of which becomes more abundant at about 6 h after removal of fixed nitrogen from the medium.

HetR is a gene required for heterocyst formation, which, we were told by Yuping Cai, is essential for the expression of several genes induced during the course of differentiation. Most remarkably, extra copies of hetR in wild type Anabaena produces multiple heterocysts. Francisca Fernandez-Pinas (Michigan State University) showed that extra copies of a newly discovered gene, hetP, also produces multiple heterocysts. Mutation in hetP appears like hetR- strains: no fragmentation and little if any sign of differentiation.

Other genes were described that are involved in unusual patterns of heterocysts. Bill Buikema described patB, a gene induced 3 h after nitrogen stepdown and whose predicted product has a DNA-binding motif in its carboxy terminus. A mutation in patB shows increased heterocyst frequency. Jim Golden

(Texas A&M University) told us about a mutant strain PFM1 that has a patB--like phenotype. A cosmid clone 8E11 that suppresses this phenotype was identified and was found to suppress heterocyst development in wild type Anabaena. A small fragment from 8E11 containing a 1200-bp ORF was sufficient in multicopy to mimic the effect of the entire cosmid, but weirdly enough, fragments containing sequences adjacent to the 1200 bp ORF on a high copy shuttle vector had the opposite effect: it induced the formation of heterocysts, even in nitrate-containing medium.

Todd Black (Michigan State University) showed work that support the idea that lipid biosynthesis may be involved in heterocyst differentiation. A Het-mutant was obtained by transposon mutagenesis, and sequences flanking the transposon insertion were found to define an ORF that resembles beta-ketoacyl reductases, involved in the synthesis of fatty acids, polyketides, and several other compounds. Upstream from the first ORF was a second, whose predicted product has domains also found in polyketide synthetases. Downstream, and on the opposite strand, is an ORF that shows homology to a gene from Bacillus subtilis encoding a regulatory gene. Reminiscent of the Texas A&M group's results with PFM1 (above), extra copies of the first ORF in Anabaena results in a Het- phenotype, and extra copies of the 3' ORF stimulates double heterocyst formation. Extra copies of both ORFs together yielded a normal phenotype.

Two DNA rearrangements are known to occur during heterocyst differentiation in Anabaena PCC 7120. A third DNA rearrangement, which involves the excision of about 11.5 kb of DNA, was reported by two groups. Andrey Matveyev (Stockholm University) analyzed restriction patterns of vegetative cell and heterocyst DNA by pulsed-field gel electrophoresis. Jim Golden told how the same rearrangement was found in his laboratory during the mapping of a cosmid isolated through the complementation of PFM1 (above).

(Contributed by TS Ramasubramanian and Nick Mann)

Redundancy and Response to Environmental Stress

One major theme of the 1993 Cyanobacterial Workshop was how cyanobacteria respond to environmental stresses. It was clear from the workshop that we are reaching a better appreciation of the cyanobacterial machinery for sensing and responding to particular stresses. For example, Jackie Collier (Stanford University) described a genetic approach to understanding how cyanobacteria break down phycobilisomes in response to nitrogen- or sulfur-deprivation and described a newly isolated gene, designated nblA, which is required for this process.

Within the general theme of stress responses, however, it kept coming up that many cyanobacteria are not necessarily optimized for maximum growth under optimal conditions. Many have instead opted for "redundancy" as a way of insuring survival under many conditions. This was demonstrated at the meeting by the reports of many proteins which apparently duplicate the functions of other proteins for reasons that are not clear. For example, Javier Florencio (Universidad de Sevilla) described how Synechocystis PCC 6803 has not one, but two genes encoding glutamine synthetase (GS). The second one, referred to as glnT, exhibits low homologies (about 10%) with other cyanobacterial GS's and instead is more similar to the GS of Bacteroides fragilis. Filamentous nitrogen-fixing cyanobacteria appear to lack a homolog of the gene. Unlike glnA (encoding the conventional GS) glnT is induced by removal of nitrate from the medium. Although this finding may provide a clue as to the function of the second GS, the question remains: Why have two GS proteins?

Several other examples of redundancy were presented. Lou Sherman (Purdue University) demonstrated the presence of a gene (isiA) encoding an alternative to the PS II protein CP43. The gene is induced under iron limitation and expresses a protein with a shorter hydrophilic loop relative to CP43. Golden (S) described the regulation of the three copies of psbA in Synechococcus PCC 7942 under different light intensities and qualities. Georg Schmetterer (Universitaet Wien) provided evidence for an alternative oxidase pathway in Synechocystis PCC 6803. Terry Thiel (University of Missouri, St. Louis) offered one of the best examples of "redundancy" in showing that Anabaena variabilis ATCC 29413 has not only the canonical nif cluster of nitrogen fixation genes, but also a set, nif2, for molybdenum-dependent nitrogen fixation under anaerobic conditions, and a set, vnf, for vanadium-dependent nitrogen fixation when molybdenum is not available. The presentation of Martin Mulligan (Memorial University of Newfoundland) on the recently discovered RNA binding proteins suggests that they are also found in multiple copies.

Bianca Brahamsha (Scripps Institution of Oceanography) and Laurie Caslake (Pennsylvania State University) presented evidence showing that Anabaena PCC 7120 and Synechococcus PCC 7002 both have multiple RNA polymerase sigma factors in addition to the housekeeping sigma factor, SigA. The known alternative sigma factors of PCC 7120 (SigB and SigC) are induced under nitrogen stress, and removal of nitrogen also appears to differentially regulate the genes for alternative sigma factors from PCC 7002.

Aside from the obvious case of a vanadium-nitrogenase and some suggestions of the importance of relative protein stability, we really don't know much about the selective advantage of having these protein families. Perhaps redundancy itself is of value or perhaps more of the reasons for these duplicated functions will be presented at the next Cyanobacterial workshop.

(Contributed by Brian Palenik)

Protein Phosphorylation (and other matters)

Several presentations included evidence for the role of two component sensory systems and protein phosphorylation in modulating the metabolism of cells in response to a range of environmental transients.

Shivanthi Anandan (Texas A&M University) described a cloning strategy that was aimed at isolating genes involved in a signal transduction pathway that might regulate light responsive gene expression in Synechococcus sp. PCC 7942. Regions conserved amongst response regulator sequences were used to pull out two genes, which exhibited limited similarity to the bacterial response regulators OmpR and PhoB. Experiments with a mutant carrying an inactivated form of one of these genes suggested that it might be involved in the sensing of low- to high-light transitions. David Laudenbach (University of Western Ontario) reported, as part of a talk on the acclimation of Synechococcus sp. PCC 7942 to sulfur stress, that genes encoding a two component sensory system resided on the large 50-kb endogenous plasmid. [Bianca Brahamsha, below, discusses N.M.'s own presentation, which is certainly pertinent to the discussion here -- ed.]

Sergey Shestakov (Moscow State University) described the characterization of a herbicide (difunon) resistance gene from Synechocystis sp. PCC 6803, which on sequence analysis turned out to be homologous to the phoR (histidine protein kinase) gene of Bacillus subtilis. Michael Schaefer (University of Missouri, Kansas City) related how a genomic fragment from Fremyella diplosiphon encodes two histidine protein kinase genes as well as a eukaryotic-type serine/threonine kinase. This genomic fragment complemented a mutant of the blue mutant class that is defective in chromatic adaptation. In keeping with this theme of protein phosphorylation, Martin Hagemann (Universitaet Rostock) has found that this form of covalent modification occurs during the response of Synechocystis sp. PCC 6803 to salt stress.

Not in keeping with this theme, but a very interesting talk nonetheless, Georg Schmetterer (Universitaet Wien) presented evidence for a branched pathway of terminal respiratory electron transport. Having cloned and sequenced the genes coxABC coding for the three subunits of cytochrome c oxidase from Synechocystis sp. PCC 6803, a mutant carrying an interrupted coxA gene was constructed. No trace of cytochrome c oxidase activity could be detected in either thylakoid or cytoplasmic membranes from the mutant, but when O_2 uptake was measured in the dark, the mutant was found to respire almost normally, suggesting that Synechocystis sp PCC 6803 contains one or more additional respiratory terminal oxidases that are cyanide-sensitive. An interesting phenotype of the mutant is that it cannot grow chemoheterotrophically, even with the brief pulses of light that permits such growth in the wild-type strain.

(Contributed by Nick Mann)

Miscellaneous Topics

Carl Johnson (University of Tennessee) reported on work he and several colleagues have done on circadian rhythms in Synechococcus PCC 7942. Using lux fusions to the psbAI promoter, they monitored bioluminescence following entrainment of the culture to light and dark cycles and found that expression of the psbAI-lux fusion exhibited the criteria of circadian rhythms, namely, persistence in constant conditions, phase resetting by light/dark signals, and temperature compensation of the period. Furthermore, he described an amazing apparatus that is capable of monitoring the bioluminescence of isolated colonies on plates. Using this device to screen mutagenized colonies, they have isolated three mutants: one that is completely arrhythmic, a long period mutant, and a short period mutant. Once again, prokaryotes provide a genetically manipulatable system to model a behavior more associated with eukaryotes, and we look forward to future developments in the molecular and genetic characterization of the clock.

For those of you who have marvelled at how a dried-out colony of Anabaena PCC 7120 forgotten on an old dried out BG11 plate comes back to life when placed in liquid, Pete Lammers (New Mexico State University) may have part of the answer. Using antibodies against a consensus peptide found in dehydrins, a family of desiccation proteins that accumulate in plants in response to dehydration stress, his laboratory identified a 40-kd polypeptide in Anabaena PCC 7120. This 40-kd polypeptide, which they call cyanodehydrin, is induced by osmotic stress (sucrose, sorbitol, PEG). They have also found putative cyanodehydrins in two other filamentous cyanobacteria: Calothrix PCC 7601 and Nostoc PCC 7911. Although plant dehydrins accumulate in response to dehydration caused by a variety of stresses, their function has not been determined. It will now be possible to address such functional questions in the genetically manipulatable Anabaena PCC 7120.

Marine group A Synechococcus were represented in two posters and a talk. These phycoerythrin-containing unicellular cyanobacteria are abundant in the oligotrophic open ocean and are thought to be responsible for 5 to 25% of primary production. Their adaptive responses to nutrient limitation and other stresses are of interest. John Rueter and others at Portland State University are studying the interrelationship of iron-, light-, and nitrogen-limitation in Synechococcus WH7803 grown in continuous culture. Nicholas Mann (University of Warwick) described the response of Synechococcus WH7803 to phosphate limitation. He and coworkers have isolated a gene, pstS, which is induced by phosphate limitation. Its product is localized to the cell envelope, and it shows 35% identity to the inducible periplasmic phosphate binding protein of E. coli. The have also cloned from WH7803 genes encoding homologs to proteins, PhoR and PhoB, that regulate the response of E. coli to phosphate deprivation by means of a two component sensory system.

Brian Palenik described the use of RNA polymerase sequence data (that derived from a conserved portion of the cyanobacterial rpoC1 gene) to study the evolution and ecology of marine Synechococcus and Prochlorococcus sp. He also urged anyone interested in the evolution of cyanobacteria to include Gloeobacter in her/his analyses, as members of the genus appear by both RNA polymerase and 16S rRNA sequence data to be representatives of the oldest known cyanobacterial lineage.

(Contributed by Bianca Brahamsha)

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