1992

CyanoNews (Vol. 8, No. 1, February 1992)

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CYANONEWS

Volume 8 Number 1 February 1992

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)

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.

BULLETIN BOARD*BULLETIN BOARD*BULLETIN BOARD*BULLETIN BOARD*BULLETIN BOARD

The AFRC Robert Hill SYMPOSIUM ON PHOTOSYNTHESIS will be held at Imperial College, London March 30 to April 1, 1992. General Topics for discussion include: membrane complexes, light induced damage to PS II, photoinhibition and environmental constraints, and carbon regulation. Deadline for registration is March 6. Contact:

Jim Barber, Biochemistry Department, Imperial College of Science, Technology and Medicine, London SW7 2AY, U.K. (Tel) 071 581 1316 (FAX) 071 581 1317 (EMail) Umbc024@Vaxa.Cc.Imperial.Ac.Uk

An FESPP Workshop on ENVIRONMENTAL FACTORS AFFECTING PHOTOSYSTEM II will be held in Szeged, Hungary, July 5-8, 1992. The workshop will emphasize structural-functional responses to various environmental factors and stress conditions. The conference fee (US$ 220 or DM 350) includes full board and meals. A limited number of fellowships are available to young scientists. Contact:

Gabor Horvath, Institute of Plant Physiology, Biological Research Center, P.O. Box 521, Szeged, Hungary, H-6701. (Tel) 36-62-23022 ext.169 (FAX) 36-62-13726 or 36-62-23600 (E-Mail) H1520dro@Ella.Hu

There will be an INDUSTRIAL PHYSIOLOGY session at this year's annual meeting of the AMERICAN PHYCOLOGICAL SOCIETY in Honolulu, Hawaii, the first week of August, 1992. Five or six talks are being planned on (more or less) how to make money with algae. JOHN BENEMANN will chair the session.

The 1993 CYANOBACTERIAL WORKSHOP will take place at the Asilomar Conference Center in Pacific Grove, California. The Workshop will run from Sunday (May 30) to Tuesday (June 2), coinciding with the Memorial Day weekend. Unfortunately, it was not possible to get dates in July. Detailed information regarding the beautiful Asilomar Conference Center, registration, travel, accommodations, etc. will follow in a later newsletter. Contact:

Arthur Grossman or Michael Schaefer, Carnegie Institution, Department of Plant Biology, 290 Panama Street, Stanford, CA 94305-1297 (Tel) 415-325-1521 (FAX) 415-325-6857 (EMail) Schaefer@Popserver.Stanford.Edu

PILL-SOON SONG needs 10 kg of SPIRULINA algae from Texcoco in Mexico. Unfortunately, their minimum shipment is 100 kg, which costs over a thousand dollars. If you would like to purchase any of the extra algae at $20.00 per kg, please let him know. Contact:

Pill-Soon Song, Department of Chemistry, University of Nebraska, Lincoln, Nebraska USA. (FAX) 402-472-2044 (E-Mail) Pandp@Unl.Edu or Pandp@Unlinfo.Unl.Edu

There will be a special issue of the Journal of Luminescence devoted to LUMINESCENCE STUDIES OF PHOTOSYNTHESIS. The issue will appear in volume 51 or 52 and should be published in March or April 1992. Papers in the issue report studies of fluorescence properties of carotenoids, phycobiliproteins, and chlorophyll.

The PROCEEDINGS OF THE FIFTH INTERNATIONAL SYMPOSIUM ON NITROGEN FIXATION WITH NON-LEGUMES (Florence, Sept 1990) are now available from the publisher. The book collects contributions of 65 lectures and 87 posters (of which 20 lectures and 23 posters concern cyanobacteria). A wide range of topics are covered, of both theoretical and practical interest, with particular emphasis on the *Anabaena-Azolla* symbiosis. Contact:

Kluwer Academic Publishers, P.O. Box 17, 3300 AA Dordrecht, THE NETHERLANDS

The Internationale Revue der gesamten Hydrobiologie has published a special issue, Volume 76 Number 1 (1991), devoted to INTERACTIONS BETWEEN ZOOPLANKTON AND CYANOBACTERIA.

POST-DOC POSITION AVAILABLE

CONTACT: Larry Orr, Program Coordinator, Center for the Study of Early Events on Photosynthesis, Arizona State University, Tempe AZ 85287-1604, USA; (Tel) 602-965-1963 (FAX) 602-965-2747 (E-Mail) Photosyn@Asucps.Bitnet or Photosyn@Asucp1.La.Asu.Edu

RESEARCH: Mutational analysis of photosynthetic reaction centers from algae, cyanobacteria, and green and purple bacteria; design and synthesis of biomimetic systems; photosystem analysis by optical, X-ray, and EPR spectroscopy.

SUBMIT: A curriculum vitae and an application letter detailing research interests.

POSITIONS SOUGHT

APPLICANT: Ajay K. Vachhani, B.N.F., Molecular Biology and Agriculture Division, Bhabha Atomic Research Centre, Trombay, Bombay, 400 085 INDIA. (FAX) 91 22 6400128 (E-Mail) Nan@Tifrvax.Bitnet

EDUCATION: Ph.D. 1991, U. Bombay. Thesis - "Studies on the molecular genetics of nitrogen fixing cyanobacteria".

ACHIEVEMENTS: Development of a shuttle vector system for the filamentous nonheterocystous cyanobacterium Plectonema boryanum. Two research papers are being prepared for submission.

EXPERIENCE: Six years experience in standard molecular biological techniques, including DNA sequencing.

APPLICANT: Igor Brown, Head - Cyanobacterial Biology Research Laboratory, Odessa State University, Petr Velikiy Str.2, Odessa 270100 Ukraine. (Tel-lab) 007-0482-68-77-93 (Tel-home) 007-0482-23-20-80 (FAX) 007-0482-23-82-88 (E-Mail) Telinf@Node.Ias.Msk.Su [CV available from I.B. by E-Mail or from Jeff Elhai (see last page of newsletter)]

EDUCATION: Ph.D. 1981, Moscow State University, Moscow, Biochemistry. Thesis Advisor: V.P. Skulachev. Thesis Title: "Stabilization of bacteria energetic by sodium and potassium gradient".

RESEARCH GOALS: The study of the role of sodium in coupling photosynthetic processes. In particular, the identification of enzymes operating as the primary Na^+-pumps in cyanobacterial membranes and the determination of the chain of events linking light absorption to the generation of $\Delta\mu_{Na}$ in halo- and alkalo-tolerant cyanobacteria.

SELECTED REFERENCES: Brown et al (1983) Eur J Biochem 134:345-349; Brown et al (1990) Arch Microbiol 153:409-411; Brown et al (1990) FEBS Lett 270:203-206

REQUIREMENTS: Research position ideally for 3 - 5 years.

START: As soon as possible.

NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEWS*NEW

JOHN WRIGHT and Chris Linton have chemically identified the compound found in volatile products from *Bacillus* spp. primarily responsible for the previously described lysis of cyanobacteria by these bacteria [FEMS Microbiol Lett (1985) 30:263-267]. Isoamyl alcohol (3-methyl-1-butanol) is the prime anti- cyanobacterial compound in a complex mixture of volatile products of peptone catabolism [Lett Appl Microbiol (1991) 13:130-132]. They have also described the sequence of cytotoxic events leading to cell lysis.

PLASMID UPDATES

YUPING CAI announces a second generation of vectors to facilitate site-directed mutagenesis in *Anabaena* PCC 7120. Three plasmids, pRL271 (Cm^r, Em^r), pRL277 (Sm^r/Sp^r), and pRL278 (Km^r/Nm^r), take advantage of the conditional lethality of *sacB*-encoded levansucrase, permitting selection for double recombinants [Cai & Wolk (1990) J Bacteriol 172:3138-3145]. The three plasmids, identical except for their antibiotic resistance determinants, contain *sacB*, *oriV* from pMB1/pBR322 (functional for replication in *E. coli* but not *Anabaena*), *oriT* from pMB1/pBR322 (required for conjugal transfer), and a cloning region with many unique restriction sites. For the moment, a detailed description of components of these plasmids are published only in his Ph.D. thesis, excerpts from which (and of course the plasmids!) are available upon request.

JEFF ELHAI has constructed a new helper plasmid, pRL623, to aid in the conjugation of DNA into cyanobacteria possessing AvaIII restriction activity. The efficiency of transfer of DNA into *Anabaena* PCC 7120 is significantly impaired by the presence on the DNA of unprotected restriction sites for AvaI and AvaII [Elhai & Wolk (1988) Methods Enzymol 167:747-754]. Premethylation in E.coli with the helper plasmid pRL528 solves this problem. *Anabaena* PCC 7120 also contains an isoschizomer of AvaIII, however, and so a methylase that protects against restriction by the enzyme was added to pRL528 to form pRL623. The new plasmid now protects against all three known restriction activities of the strain.

MECHANISM OF NEUROTOXIN FROM OSCILLATORIA STUDIED

OLAV SKULBERG and coworkers (G Lilleheil, RA Andersen, and J Alexander) have shared some recent results concerning the mechanism of action of homoanatoxin, a neurotoxin produced by a strain of *Oscillatoria formosa*, a cosmopolitan freshwater species. The toxin is a secondary amine alkaloid, recognized as methylene-anatoxin-a, which has been shown to be toxic to mice after intraperitoneal injection (LD_{50} = 250 μg/kg) and to block neuromuscular transmission in an isolated phrenic nerve-hemidiphragm preparation of rat at a concentration of about 3.75 ug/ml organ bath fluid [Skulberg et al (1991), Environ Toxicol Chem (in press)]. A water extract of freeze-dried algal material containing the toxic principle (at about 1%, dry weight) was used to study the mechanism of action of the toxin.

The extract did not affect the initiation, propagation, or amplitude of electrically-induced compound action potentials recorded from the main phrenic nerve trunk. Furthermore, both single twitch and tetanic muscle contractions could still be elicited by direct electrical stimulation of the muscle even after the toxin had abolished nerve-initiated contractions. The amplitudes of the directly elicited muscle responses gradually declined after exposure to the extract. The observed effects of the homoanatoxin extract on neuromuscular transmission was only partly reversible, the degree of reversibility depending on the duration of exposure to toxin. The effects of the extract and curare were additive.

The results can be explained in two ways. First, the main action of homoanatoxin on neuromuscular transmission may be to prevent the muscle from responding to the transmitter acetylcholine (ACh), perhaps (like its structural analog anatoxin-a) by blocking ACh receptors. Alternatively, the toxin may interfere with presynaptic processes leading to decreased liberation of Ach. An additional direct effect on voltage-initiated processes of the muscle is also possible. Further studies aimed at disclosing effects of homoanatoxin on membrane potential, end-plate potentials, and mini-end-plate potentials in single muscle fibers are in progress.

SYNECHOCOCCUS PCC 7942 EXHIBITS CIRCADIAN RHYTHM

SUSAN GOLDEN tells us the remarkable news that we have had a transformable cyanobacterium capable of circadian rhythm in our midsts for years. She and Carl Strayer, in collaboration with Carl Johnson and Takao Kondo, used a *psbAI::luxAB* transcriptional fusion to measure the expression of *psbAI* in a culture of *Synechococcus* PCC 7942 (also known as R2) that had been entrained to a 12 hr light/12 hr dark cycle. The bioluminescence activity of luciferase (encoded by *luxAB*) rose and fell with a period of approximately 24 hr, even after the entrained culture was shifted to continuous light. Reversing the light and dark periods resulted in shifting the peak time of expression by 12 hours. Preliminary experiments at various temperatures indicate that the period is nearly invariant, which suggests that the rhythm is temperature-compensated — a salient feature of circadian clocks. Analysis of additional gene-*luxAB* fusions, in progress, may soon tell us whether *psbAI* is peculiar in its rhythmicity of expression or instead the transcription machinery itself is responding to a clock.

NEW PROPOSED NOMENCLATURE FOR HETEROCYST DIFFERENTIATION

The diversity of phenotypes observed when studying transposon-induced mutants of *Anabaena* PCC 7120 prompted ANNELIESE ERNST to seek order amidst the chaos. As a result, she has defined certain characteristics that are useful in describing mutants selected for their inability to grow on molecular nitrogen. Since they may prove to be of general utility, she passes them on to us for our consideration:

Fox$^-$	Unable to fix nitrogen in presence of oxygen
Fix$^-$	Unable to fix nitrogen, whether under aerobic or anaerobic conditions
Het$^-$	Unable to form either heterocysts or proheterocysts discernible by bright-field microscopy
Hen$^-$	Immature or aberrant heterocyst envelope or pore structure
Hgl$^-$	Defective in synthesis of heterocyst glycolipids
Hep$^-$	Defective in synthesis of heterocyst polysaccharides
Dab$^-$	Unable to strongly oxidize diaminobenzidine

YUPING CAI, inspired by this new phenotypic order, suggests corresponding changes in genotype designation. He points out that the increased activity in obtaining mutants defective in heterocyst differentiation may lead to a scarcity of available letters to place after the gene designation *het*. He proposes that usage of "*het*" be confined to those genes required for any obvious differentiation towards heterocysts. Genes leading to failure to differentiate by reason of severe fragmentation of the filaments upon removal of fixed nitrogen [Buikema & Haselkorn (1991) J Bacteriol 173:1879-1885] are to be considered a special class. Yuping suggests the designation "*fra*" for genes conferring this phenotype. According to this definition, *hetR* [Buikema & Haselkorn (1991) Genes & Develop 5:321-330], which is required for initiation of heterocyst differentiation is properly named, but *hetA* [Holland & Wolk (1990) J Bacteriol 172:3131-3137] and *hetB* [Bancroft et al (1989) J Bacteriol 171:5940-5948], which are required for proper formation of the heterocyst envelope, are not. He and others in Peter Wolk's lab have named five genes, *hetC*, *hetN*, *hetP*, *fraA*, and *fraB* in accordance with the proposed convention.

Any discussion on these matters is welcome, with the goal of achieving a coherent nomenclature without duplications.

CYANOBACTERIAL DNA POLYMERASES PURIFIED, CHARACTERIZED

NV NESTEROVA tells us of the isolation and partial purification of two DNA- polymerases from *Plectonema boryanum* Gom CALU 465 by herself, MI Mendzul, and SN Sukhanov. Lysates from cells broken with KCl and triton X-100 were precipitated with polyethyleneglycol (MW 6000) and subjected to chromatography on DEAE cellulose DE-52 and hydroxyapatite. Electrophoresis in a non- denaturing polyacrylamide gel permitted separation of two proteins with high DNA-polymerase activity, with molecular weights of 120 and 200-300 kDal. The two forms have similar activity profiles with regard to pH (8.5-9.0) and temperature (42-43 °C) but differ in their requirements for monovalent and divalent catians and their sensitivity to afidicoline, nalidixic acid, and spermidine. Manganous ions in the range of 35 mM to 60 mM specifically caused the 70- to 90-fold superactivation of both forms of DNA polymerase.

HEPATOTOXINS ANALYZED FROM COLLECTED CYANOBACTERIA

VLADIMIR THERNAJENKO brings us up to date on his work on toxic cyanobacteria. He has collected and purified cyanobacterial strains, including hepatotoxic *Microcystis aeruginosa*, collected from several bodies of water: Kiev reservoir, Ladoga lake, Razliv lake, the Gulf of Finland, Kursh Gulf, and a few small lakes in the St. Petersburg area. In some cases, hepatotoxic material from blooms was also collected. No neurotoxic blooms were discovered. Toxins were purified (in collaboration with Wayne Carmichael) from the hepatoxic material and strains cultured in the laboratory, and it was found that each sample contained one to five distinct hepatotoxins. His most pressing concern now is to develop a monitoring system for cyanotoxicity based on analytical HPLC and immunological detection.

EASTERN PHOTOSYNTHETIC WISDOM BROUGHT TO LIGHT

ALEXANDER PINEVICH sends greetings from the former Soviet Union (Russia), former Leningrad (St. Petersburg), and former Leningrad University (St. Petersburg University) with a summary of recent work on former blue-green algae (cyanobacteria) that has appeared thus far only in Russian language publications. He reports:

1. A demonstration that thylakoids are compartmentalized in specialized phases, being both the result and the cause of the ΔpH [Pinevich & Topchieva (1991). Microbiol 60:512-517; Pinevich & Protasov (1991). Proc Leningrad U Ser Biol 1:77-84].
2. Indications that the cyanobacterial PSI antenna has an analagous structure as that of anoxy-phototrophs, having a core and peripheral part [Pinevich & Koshina (1991). Proc Leningrad U Ser Biol 3:84-95].
3. A detailed hypothesis regarding the evolution of the light-harvesting antennae and argues for a revised phylogeny for oxygenic phototrophs, including cyanobacteria, prochlorobacteria, cyanelles, and rhodo-chloro- chromoplasts [Pinevich (1991). Cytol 33:3-21].
4. The isolation and preliminary description of a unicellular mutant of the filamentous cyanobacterium, *Anabaena* PCC 7118, with comments on the evolution of cyanobacterial morphotype [Khudyakov & Pinevich (1991). Microbiol in press].
5. A demonstration by means of DAPI epifluorescence that DNA may be associated with cyanobacterial carboxysomes in vivo [Pinevich & Grigoryeva, in preparation].
6. An analysis of a couple of cyanobacterial strains extremely rich in phycoerythrin [Pinevich et al, in preparation].

PERSPECTIVE:
THE PHYLOGENETIC RELATIONSHIP BETWEEN PROCHLOROPHYTES AND CHLOROPLASTS

With the discovery of *Prochloron didemni,* an oxygenic, phototrophic prokaryote containing chlorophylls a and b, it was generally thought that the "missing link" between green chloroplasts and prokaryotes had been found [Lewin & Withers (1975) Nature 261:697-698; Raven (1970) Science 169:641-646]. Comparison of an RNaseT1-generated 16S rRNA oligonucleotide catalogue from this organism with those of cyanobacteria and green chloroplasts did not support this hypothesis, however, although interpretation of the data was a matter of some debate [Seewaldt & Stackebrandt (1982) Nature 295:618-620; Van Valen (1982) Nature 298:493-494; Bremer & Bremer (1989) J Evol Biol 2:13-30]. Further study of this organism was hampered by its intractability to cultivation under laboratory conditions and to date it is still found only as an exosymbiont of didemnid ascidians. Thus, the discovery of a second prochlorophyte, *Prochlorothrix hollandica,* that is easily cultured in vitro raised expectations for a resolution to the question of the relationship between prokaryotes of this phenotype and green chloroplasts [Burger-Wiersma et al. (1986) Nature 320:262-264]. Such was not the case.

In contiguous papers, Turner et al. [(1989) Nature 337:380-382] and Morden and Golden [(1989) Nature 337:382-385, 339:400] presented analyses of molecular sequence data for different gene products of *P. hollandica* and arrived at contradictory conclusions. Turner et al. used a weighted least squares distance matrix method to analyze partial sequences of 16S rRNAs and concluded that although both *P. hollandica* and green chloroplasts fall within the cyanobacterial line of descent, they do not form a monophyletic group. Morden and Golden used a maximum parsimony analysis of protein sequences deduced from gene sequence data for the *psbA* locus that encodes the D1 protein of photosystem II. Depending on the weight assigned to a gap seven amino acids in length shared by green chloroplasts and *P. hollandica,* their results either support those of Turner et al. or lead to the opposite conclusion. A maximum likelihood analysis [Kishino et al. (1990) J Mol Evol 31:151-160] of the same data used by Morden and Golden was in concordance with the results of Turner et al., but again the weight assigned to the gap was pivotal.

Subsequently, Morden and Golden published maximum parsimony analyses of deduced protein sequences derived from gene sequence data for the large and small subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase [Morden & Golden (1991) J Mol Evol 32:379-395]. In this study, phylogenetic tree inferences indicated that *P. hollandica* is not a close relative of the green chloroplasts to the exclusion of the cyanobacteria.

At a recent colloquium held at Bodega Bay, California (Symbiogenesis, Prochlorophytes, and the Origins of Plastids, 5-7 September 1991) evidence was presented from two laboratories that indicates that not only are prochlorophytes and green chloroplasts not monophyletic, but the prochlorophytes themselves do not form a distinct clade. Ena Urbach et al used distance matrix and maximum parsimony methods to analyze the phylogenetic relationships inferred from 16S rRNA sequence data for *P. hollandica, Prochloron* sp., and the most recently discovered prochlorophyte, *Prochlorococcus marinus* [Chisholm et al (1988) Nature 334:340-343]. Brian Palenik

and Robert Haselkorn conducted similar studies on partial sequence data for the *rpoC1* gene that encodes a subunit of DNA-dependent RNA polymerase homologous to the γ subunit of cyanobacterial RNA polymerases. The work of these groups is to be presented in a forthcoming issue of Nature.

At present, then, the majority of the molecular sequence comparisons do not support a close phylogenetic relationship between green chloroplasts and prochlorophytes. The acquisition of chlorophyll b appears to have evolved independently at least three times and/or to have been mediated by lateral genetic transfer.

- Sean Turner

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