

CYANO NEWS

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CYANO NEWS is 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. If you have a new result, if you know of an interesting meeting, if you have a post-doctoral opening, if you want strains, if you've published/submitted an article, if you have an insight or speculation into the cyanobacterial world... why not tell us about it? It's news to us. Please send all contributions to one of the addresses listed on the last page. DEADLINE for the next issue is DECEMBER 1, 1987.

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.

NEWS*

P.B.NANDKAR, who is studying ecophysiological aspects of cyanobacteria, would like to know if anyone could supply him with LPP-1, N-2 and A-1 viruses and associated cyanobacteria.

FRANCISCO AYALA describes research being conducted on Spirulina production in the arid region of Antofagasta (northern Chile). The main objective is to optimize yield in outdoor basins (currently 400 m²). He is interested in exchanging technical information with colleagues working on Spirulina production and halotolerant strains that grow in regions with high irradiance.

TAXONOMY OF CYANOBACTERIAL SYMBIONTS

BILL ZIMMERMAN and Thomas Lumpkin at Washington State University in collaboration with Iwao Watanabe of the International Rice Research Institute in the Philippines have begun this year to intensively study the biochemical taxonomy of accessions of the Azolla-Anabaena symbiosis. Their approach is to analyze isolates for isozymes, total proteins, and DNA restriction fragment polymorphisms. The primary focus is on intact associations, but cultures of Anabaena-free Azolla and independent Anabaena azollae are also included in the analysis. If anyone has new isolates of Azolla-Anabaena they would certainly appreciate getting them. In fact, they would be overjoyed to receive cultured cyanobacteria from any symbiosis (e.g., Nostoc from Cycas or Gunnera).

CHLOROPHYLL-PROTEIN COMPLEXES FROM PROCHLOROTHRIX

GEORGE BULLERJAHN reports on recently completed work on the composition of the thylakoid membrane from Prochlorothrix hollandica (after apologizing that he had turned his attention from cyanobacteria to this prochlorophyte). Five chlorophyll-protein complexes, CP1-CP5, were resolved and analyzed by spectroscopic and immunological methods. CP1 contains the PSI reaction center. CP4 contains the major chlorophyll-a-binding proteins of the PSII core. CP2, CP3, and CP5 contain functionally bound Chl a and b but are immunologically distinct from the LHC-II complex of higher plant thylakoids. CP5, however, did weakly cross-react with an antibody prepared against a Chl-a-protein from Anacystis. A full report of this work will soon appear in European Journal of Biochemistry.

WHAT WOULD YOU DO WITH THOUSANDS OF SMALL CYANOBACTERIAL BLOOMS?

GREGORY PATTERSON was awarded the National Cancer Institute project to discover novel antitumor drugs from cultured cyanobacteria. The goal of the project is to acquire several thousand samples and to test extracts from these samples for selective toxicity to various human slow-growing tumor cell lines. The samples will also be screened for anti-AIDS activity at the National Institutes of Health. Obviously, this project will enormously expand the number of identified cyanobacterial strains and promises to be a major benefit to the cyanobacterial research community. Needless to say, Patterson would gladly receive axenic and/or unialgal cultures of cyanobacteria for mass culture.

Two summer meetings devoted or partially devoted to cyanobacteria have already taken place by press time. Approximately 90 researchers gathered on the beautiful blue-green shores of Lake Mendota at the University of Wisconsin June 8-10 to discuss the Molecular Biology of Photosynthetic Prokaryotes. About one-fourth of the papers presented directly concerned cyanobacteria. Special thanks are due to Paul Ludden and his staff for putting together a well-run and enjoyable meeting.

Somewhat over a hundred cyanobacteriologists met in St. Louis a month later (July 17-19) for the Second Workshop on the Molecular Biology of Cyanobacteria. Graduate students, post-docs, and principle investigators alike presented their results in a series of sessions. In a single room, unicellular cyanobacteriologists heard the woes of their filamentous counterparts, and the nitrogen fixers reacquainted themselves with the mysteries of photosynthesis and the wonderful techniques being used to unravel them. A special round of applause was given to the organizing committee of Lou Sherman, Himadri Pakrasi, and Terry Thiel and to the program committee of Don Bryant and Arthur Grossman.

A short business meeting was held at the end of the program, at which time it was tentatively decided to reconvene July 29-August 2, 1989 in Toronto, again overlapping with the meeting of the American Society for Plant Physiology. Peter Wolk pointed out that it is not too early to start planning -- Eastern European cyanobacteriologists might be able to attend next time if they know far enough in advance. Finally, John Pierce wished to announce that he had a very good time.

Several participants reported on portions of these two meetings. Reports not found below, concerning recombination, stress response, and miscellaneous topics, will be presented next issue of the newsletter.

PHYCOBILISOMES - ASSEMBLY AND REGULATION OF BIOSYNTHESIS

The techniques of modern molecular biology, now being applied to a variety of cyanobacterial species, are yielding interesting data regarding both the regulation of genes encoding phycobiliprotein components as well as the biosynthesis and assembly of this light-harvesting complex. Genes encoding phycobilisome polypeptides have now been isolated from several species: *Anacystis/Synechococcus*, *Aphanacapsa/Synechocystis*, *Nostoc*, *Fremyella/Calothrix*, *Anabaena*, and *Pseudanabaena*. Data presented at the St. Louis meeting by members of several labs (Don Bryant, Pam Conley, Jim Dubbs, Arthur Grossman, Nicole Tandeau de Marsac, and Barbara Zelinskas) illustrated that many similarities exist between gene regulation in cyanobacteria and other prokaryotes. The following themes emerged:

- Genes encoding phycobilisome components are clustered together on the genome and are often cotranscribed. There are often multiple transcripts from a given gene cluster.
- Hairpin structures resembling prokaryotic rho-independent transcription termination sequences are found at the 3'-ends of several transcripts encoding phycobiliproteins (and gas vacuole proteins). In many transcripts, regions with the potential to form secondary structure occur within and between genes. These may be important in determining transcriptional attenuation, processing, stability.
- There is no definitive phycobiliprotein promoter sequence. In some but not all organisms, phycobiliprotein genes are preceded by *E.coli*-like "-10" and "-35" sequences.

Nicole Tandeau de Marsac reported two interesting spontaneous mutants of *Calothrix/Fremyella* deficient in phycocyanin (PC) and phycoerythrin (PE). They are apparently the result of insertion within a cryptic open reading frame, located downstream from the constitutive phycocyanin gene cluster, of a sequence that has structural characteristics of prokaryotic transposable elements. PE transcripts in the mutants are missing, but PC transcripts are present but not translated at wild-type levels, suggesting some form of translational repression.

Questions concerning phycobilisome assembly and biosynthesis are being addressed in several labs by using techniques of gene inactivation and UV mutagenesis:

- A UV-generated mutant of *Synechocystis* 6701 that cannot assemble PC hexamers from trimers (and has decreased levels of PC and PE) was described by Lamont Anderson. It was found to have a single nucleotide substitution that converts a proline residue at the fifth position in the PC- α subunit to leucine, suggesting that this residue may be important for the assembly of PC hexamers.

- Vicki Stirewalt and Jian-Hui Zhou characterized strains of *Synechococcus* 7002 in which genes encoding α -AP or the small core linker protein (ApcC) had been interrupted by antibiotic resistance markers. Strains lacking α -AP grow very slowly, but curiously, *apcC*⁻ strains appear to have normal phycobilisomes, suggesting the 9kDa linker is not critical for proper assembly of the core or for energy transfer.
 - Don Bryant told of a previously unidentified but highly conserved open reading frame (*cpcE*) that has been found in three cyanobacterial species to be associated with PC genes. The product of this gene has now been implicated in the attachment of bilin chromophores to α -PC subunits.
- In addition, the phycobilisome anchor and some rod linker proteins from *Anacystis nidulans* R2 were found by Harold Riethman to be ConA-reactive glycoproteins, a rare occurrence in prokaryotic organisms. Perhaps this glycosylation is important for the interaction of the linkers with the phycobiliprotein subunits.

(contributed by Pam Conley)

PHOTOSYSTEM II PROTEINS - STRUCTURE AND FUNCTION

Several talks and posters at both meetings focused on genetic approaches to elucidate the functions of the core proteins in Photosystem II:

- psbA (encoding D1): Jeff Gingrich described the molecular lesions in several new diuron- or atrazine-resistant mutants of *Synechococcus* 7002. There are three copies of *psbA*, as in *Synechococcus* R2. Jacqueline Vrba compared the sequences of the four copies of *psbA* in *Anabaena* 7120.
- psbD (encoding D2): Sue Golden and Jeff Gingrich cloned and sequenced the two *psbD* genes from *Synechococcus* R2 and 7002 respectively. As in chloroplasts and *Synechocystis* 6803, one of the two *psbD* genes overlaps the single *psbC* gene. Wim Vermaas reported that site-specific mutation at either his-197 or his-214 resulted in the loss of PSII.
- psbB and C (encoding CP47 and CP43): The disruption of either gene of *Synechocystis* 6803 led to the inactivation of PSII activity, as related by Wim Vermaas. He and Shelly Carpenter also described the construction of chimeric proteins composed of regions of CP47 or CP43 from spinach and 6803. Some of the chimeras were active, some not.
- Extrinsic 33kd protein: The gene for this protein (which Lou Sherman's group has named *woxA*) has been isolated from *Synechocystis* 6803 (described by Judy Philbrick) and from *Synechococcus* R2 (described by K.J. Reddy). The nucleotide sequences of both are relatively poorly conserved compared to the higher plant gene -- less than 50% similarity. *Synechocystis* mutants lacking a functional gene do not evolve oxygen.
- Non-directed mutagenesis: Valdis Dzelzkalns has sought nitrosoguanidine-induced mutants of *Synechocystis* 6803 that are defective in PSII activity. Three of the mutants were further characterized and found to be unlinked. One of them has been shown to be a deletion in *psbD*.

Judy Brusslan and Sue Golden both presented interesting results concerning the expression of the three *psbA* genes in *Synechococcus* R2. *psbAI* (copy one) message is predominant in wild-type cells grown without herbicide. The addition of diuron induces *psbAII* and *psbAIII* 2-7 and 5-15 fold, respectively, without affecting *psbAI*. Translation of *psbAIII* (as judged by a *lacZ*-translational fusion) is higher in a *psbAI*⁻ background and also is increased by high light intensity. A comparison of PSII function and transcript levels suggests that there is posttranscriptional regulation of *psbA* gene expression.

(contributed by Jeff Gingrich and Florence Gleason)

PHOTOSYNTHETIC ELECTRON TRANSPORT

Talks and posters concerning proteins involved in photosynthetic electron transport attempted to define the organization of genes, draw evolutionary relationships amongst similar proteins, or in some cases, find for a protein some physiological role. Dave Krogmann and his collaborators have exploited homogeneous, natural blooms of *Microcystis aeruginosa* to isolate and determine the amino acid sequences

of cytochromes c553 (a soluble electron carrier) and c550 (a protein of unknown function). These sequences show two regions of homology and suggest that c550 evolved from a restructuring of fragments of a c553 gene. The nucleotide sequence of c553 from *Anacystis nidulans* R2 (reported by Cathy McDowell) shows a 24 residue N-terminal extension, which might function as a transit sequence.

Toivo Kallas described genes encoding the *Nostoc* PCC 7906 thylakoid b6-f complex. The genes for Rieske FeS protein (nuclear encoded in plants) and cytochrome f are cotranscribed as are those for b6 and subunit IV. A repetitive DNA sequence (>50 genomic copies), which lies between the FeS and f genes and downstream of SUIV, has been involved in a recent rearrangement and possibly in the evolutionary reordering of the b6-f genes.

The genes encoding ATP synthetase have been closely scrutinized by two groups:

- Don Bryant reported an arrangement of ATP synthetase genes for the cyanellar DNA of *Cyanophora paradoxa* intermediate to that seen in cyanobacteria and plants. The atpD (delta) gene (nuclear in plants) is encoded by cyanellar DNA, and the atpF (b) gene has no introns as in cyanobacteria. However, the atpC (gamma) gene is not close to atpA (alpha) -- rather, as in plants, it appears to be in the nucleus.
- Stephanie Curtis described the gene organization of these genes in *Anabaena* 7120. As with *Anacystis*, the genes are organized as two clusters: a small cluster encoding the beta and epsilon subunits and a large cluster encoding subunits in the order a:c:b':b:delta:alpha:gamma. Unlike *Anacystis*, *Anabaena* has overlapping genes for b and delta and does not have a ferridoxin gene downstream from the gene for the gamma subunit. The region upstream from atpB distinguishes *Anabaena* from *E.coli*, in that there is no gene there encoding the gamma subunit, and from *Cyanophora* and higher plant chloroplasts, in that there is no gene there encoding the large subunit of rubisco. Thus *Anabaena* is unique (as we always sensed).

Two groups told of their efforts to clone thioredoxin genes from cyanobacteria:

- Eric Muller isolated and sequenced from *A. nidulans* R2 the gene encoding thioredoxin m, a reductive, regulatory protein involved in the light-dependent activation or deactivation of many key enzymes. In repeated attempts, the wild-type gene could not be replaced with a gene disrupted with a kanamycin-resistance cassette. Rather, tandem addition of the disrupted copy to the wild-type region occurred, suggesting an indispensable function for thioredoxin m in this organism.
- Florence Gleason described the isolation of a thioredoxin gene from *Anabaena* 7119, identified by hybridization to the *E.coli* thioredoxin gene and by complementation of a thioredoxin-deficient strain of *E.coli*. Hybrid thioredoxin genes composed of parts from *Anabaena* and *E.coli* genes were constructed and found to be functional. However, the *Anabaena-E.coli* (N to C terminal) thioredoxin was superior to the opposite construct, indicating that crucial protein interaction domains occur in the C terminal portion of the molecule. The unusual thioredoxin gene from *Anabaena* 7120 (described by J. Alam and S. Curtis) could not complement thioredoxin-deficient *E.coli*, even though the gene was expressed. This suggests that this thioredoxin has some function unique to cyanobacteria.

(contributed by Florence Gleason and Toivo Kallas)

CYANOBACTERIAL CLONING VECTORS AND TRANSCRIPTIONAL REPORTERS

A few labs are constructing new cyanobacterial vectors or trying to understand old ones. Steven Gendel described the use of transposon mutagenesis to localize the replication functions of the small plasmid in *Anacystis nidulans*. Transposons inserted into the apparent replication region of a hybrid vector were deleted during transformation of *Anacystis*, suggesting that both structural and sequence features of this region are important for function in *Anacystis*. John Cobley has developed a vector and gene transfer system for the chromatically adapting species *Fremyella diplosiphon*. He has constructed a vector that can be transferred by conjugation from *E. coli* to *Fremyella*, using part of an endogenous *Fremyella* plasmid to provide replication functions. This hybrid vector encodes resistance to both chloramphenicol and aminoglycosides (e.g. neomycin). In Soo Bae, Betsy Read, and Bill Snyder are in the

process of constructing expression vectors for use in *Synechococcus* R2 and *Synechocystis* 6803. They make use of regulatable promoters -- from a gene activated by iron deficiency and from phage λ .

Other labs have made specialized vectors to monitor transcriptional activity. Jeff Elhai described efforts to use the luciferase gene as a reporter system in *Anabaena*. Preliminary results show that luminescence can be easily detected in single cells carrying gene fusions that put the luciferase gene under the control of cyanobacterial promoters. Fusions with the Rubisco promoter showed high levels of activity (bright cells). Jeff is working to develop the system to the point of being able to clearly detect differential activity of gene fusions during differentiation. Stephanie Curtis is using a similar promoter-probe vector, with the CAT (chloramphenicol acetyl transferase) gene replacing luciferase, to dissect promoters from *Anabaena*. Her collection of promoters display an *E. coli*-like -10 but no -35 consensus sequence. D.J. Scanlon described the construction and of plasmids carrying promoterless beta-galactosidase or neomycin phosphotransferase genes that can be transferred by conjugation into filamentous cyanobacteria.

(contributed by Steve Gendel)

DNA REARRANGEMENTS NEAR NITROGEN-FIXATION GENES

Nif gene rearrangements was a hot topic at both meetings. Most of the discussion was aimed at understanding the two DNA rearrangements that take place late in heterocyst differentiation in *Anabaena* 7120 (Nature 314:419-423 [1985]; Nature 327:526-529 [1987]). One rearrangement precisely deletes 11 kb of DNA, bounded by 11 bp direct repeats, from within the *nifD* gene. The excision requires an excisase encoded by a gene (*xisA*) internal to the deleted fragment (Cell 44:905-911 [1986]). Bill Buikema reported the over-expression of the *xisA* gene product in *E. coli*, achieved by removing the 11 bp direct upstream from *xisA*. This work is a prelude to the development of an *in vitro* system to assay for excisase activity. Peter Lammers and Casilda Provencio described their attempt to mimic an *xisA* mutation in *Anabaena*, driving anti-sense *mic(xisA)* RNA from the glutamine synthetase gene promoters. Unfortunately, initial Southern hybridization experiments demonstrate that exconjugants containing the *mic(xisA)* construction have normal steady-state levels of the *nifD* rearrangement.

A second rearrangement deletes 55 kb of DNA with one break point near the *nifS* gene and the other near *rbcl*. Martin Mulligan gave evidence that a ferredoxin gene and *nifB* are brought close to *nifS* after the second rearrangement. These genes, as well as an unidentified open reading frame, may be part of an operon (5'-*nifB*-Fd-*nifS*-ORF-3'), however, transcriptional start sites have yet to be assigned. Claudio Carrasco and Jim Golden reported a physical map of the 55 kb element. Interestingly, *Anabaena* 29413 DNA lacks homology to this element and its *rbcl* is close to *nifS* (true in 7120 only after the rearrangement). These results suggest that *Anabaena* 29413 does not have a homologue of the 55 kb element anywhere in its genome.

The nitrogen-fixing, non-heterocystous cyanobacterium *Plectonema boryanum* also apparently experience a *nif*-linked rearrangement under nitrogen-fixing conditions, as reported by Jamesina Smoker and Susan Barnum. They have excluded alternate interpretations such as changes in the state of methylation. DNA clones that may contain the rearranged *nif* region have recently been isolated and may hold the key to a definitive description of the phenomenon.

A major unsolved question remains: What selective advantage does the presence of mobile genetic elements near or within *nif* genes confer on their hosts. Jack Meeks told of the isolation of two revertants of *Nostoc* MAC capable of aerobic nitrogen fixation. One revertant contained the *nifD* excision element but the other did not. No phenotypic differences between the strains have been detected. Jack also looked for this element in symbiotic cyanobacteria. The major symbiont of *Azolla* does not contain the *nifD* excision but instead has a contiguous *nifHDK* operon. In contrast, *Nostoc* sp. 7801, grown free-living or in association with the bryophyte *Anthoceros*, has an interrupted *nifHDK* region, although the contiguous operon can also be detected at a lower hybridization intensity. At least in one case, therefore,

the nifD excision is not required for heterocyst development, just as the nifS rearrangement appears to be dispensable in *Anabaena* 29413. Why then do they persist? One possible explanation is that the regulation of excision events is so tightly coupled to differentiation of the non-dividing, terminally differentiated heterocyst that excision events in dividing vegetative cells never occur. Against this, Meeks reported that DNA from *Nostoc* (MAC) cultures growing on ammonia contains easily detectable but non-stoichiometric rearrangement bands. Despite the apparent "looping out" of the excision, the element has persisted through many years of laboratory culturing, suggesting some selective pressure against loss of the excision. The possibility that the rearrangements are reversible cannot be ruled out.

(contributed by Peter Lammers)

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