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CYANONEWS

Volume 8 Number 2 July 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.

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EDITORIAL OFFICES OF CYANONEWS TO MOVE! The office staff of CyanoNews has found it necessary to relocate from Michigan, and rather than train new help, the international headquarters of the newsletter will make the move as well. Address all further correspondence to:

CyanoNews, Department of Biological Sciences, Florida International University, University Park Campus, Miami FL 33199 USA. (Tel) 305-348-2201, (Fax) 305-348-3094, (E-mail) Cyano@Msu.Bitnet

The FIRST LATIN AMERICAN BIODETERIORATION SYMPOSIUM will be held in Campos do Jordao-sp-Brasil, August 30 to September 03, 1992. The scientific program is divided into three main areas: biodeterioration of industrial process and products; biodeterioration of cultural property; and biodegradation of solid and liquid wastes including bioremediation and bioleaching. Contact:

Mrs. Rosely and/or Mrs. Lena. (Tel) (0055192)427022, (Fax) (0055192)427827.

The 1993 WORKSHOP ON THE MOLECULAR BIOLOGY OF CYANOBACTERIA will be held May 30 to June 2, 1993 at the Asilomar Conference Center, Pacific Grove, CA. The organizers ask that anyone interested in attending the conference NOTIFY THEM BY AUGUST 31, 1992. This is so that they can obtain an estimate of the number of people who will attend, which they will use in applying for external funding. They ask also that you indicate whether you would like to present results orally or as a poster, and if so, the subject of the presentation (a title if possible). A second circular containing registration information, abstract forms, etc. will be sent out this fall. Abstracts will be due by Jan. 31 to allow enough time to put together a coherent program. Contact:

Arthur Grossman and Michael Schaefer, Carnegie Institution of Washington, Department of Plant Biology, 290 Panama Street, Stanford, CA 94305 U.S.A. (Fax) 415-325-1521, (E-mail) W5.C38@Stanford.Bitnet

Michael Schaefer, coorganizer of the 1993 Workshop on the Molecular Biology of Cyanobacteria (see Meeting Announcement, above) would like to apologize to the cyanobacteriological community for any hysteria that may have resulted from recent announcements in the ASPP Newsletter and the Plant Molecular Biology Reporter, placing the Workshop in 1992 rather than in 1993. However, he also wants to make clear that his news release went out with an unmistakable "1993", as evidenced by the fact that the ASM Newsletter got it right. Please address all claims on nonrefundable airplane tickets to ASPP and ISPMB, not to Michael.

The 6TH INTERNATIONAL CONFERENCE ON APPLIED ALGOLOGY will take place in Trebon, Czechoslovakia, 6-11 Sept 1993. The meeting will attempt to bridge the gap between basic research and practical application of microalgae, cyanobacteria, and anoxygenic photosynthetic bacteria. Subjects include: photosynthesis and stress, design of photoreactors, hydrogen production, and waste treatment. Contact:

Conference on Applied Algology c/o Jiri Doucha - Institute of Microbiology, Opatovicki Mlyn, 37901 Trebon - Czechoslovakia. Phone:+42-333-2421 or -3080. Fax +42-333-2268.

The International Rice Research Institute (IRRI) has come out with a pamphlet entitled *BIOFERTILIZER GERMPLASM COLLECTIONS AT IRRI*, by I. Watanabe, P.A. Roger, J.K. Ladha, and C. Van Hove. After a brief introduction outlining the goals and history of the collections at IRRI, the 66 page pamphlet proceeds to describe collections of Azolla, aquatic legumes and rhizobia, free-living N_2-fixing bacteria, and cyanobacteria. This pamphlet complements an earlier one, *The Blue-green Algae Collection at IRRI* (1991), which gives much greater detail about each strain. Contact:

International Rice Research Institute, P.O. Box 933, Manila 1099, Philippines.

ERIK SÖDERBÄCK has completed and published his Ph.D. dissertation in the Department of Botany, Stockholm University. The dissertation, entitled *Developmental Patterns in the Nostoc-Gunnera Symbiosis*, is accompanied by six papers concerning the nitrogen and carbon exchange between the cyanobacterium and its host.

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DUANE MOSER has finished up his Masters degree in Toivo Kallas' laboratory and is currently pursuing his Ph.D. with Ken Nealson. His new address:

Center for Great Lakes Studies, University of Wisconsin-Milwaukee, Milwaukee, WI 53204 USA.

JACCO KROMKAMP has moved from Tony Walsby's laboratory to the Netherlands Institute of Ecology. He is now working on the regulation of photosynthesis in an ecological context, particularly the kinetics and mechanisms of photoadaptation during light-shade transition and vertical mixing. His new address:

Netherlands Institute of Ecology, Centre for Estuarine and Coastal Ecology (NIOO-CEMO is OK), Vierstraat 28, NL-4401 EA Yerseke, THE NETHERLANDS. (Tel) 31-1131-1920, (Fax) 31-1131-3616, (E-mail) Surf230@Kub.NL

JEFF ELHAI, having completed possibly the longest post-doc on record in the laboratory of Peter Wolk, has retired to Miami, Florida (visitors welcome). He will continue work on differentiation in *Anabaena* and begin work on plant-cyanobacterial associations, whenever he is not dispatching his teaching duties as the sole faculty member of the Subdepartment of Microbial Genetics. His new address (after August 11):

Department of Biological Sciences, Florida International University, University Park Campus, Miami FL 33199 USA. (Tel) 305-348-2201, (Fax) 305-348-3094, (E-mail) Cyano@Msu.Bitnet

JOHN ALLEN has moved from Oslo to take a position at Lund University. His new address:

Plant Cell Biology, Lund University, Box 7007, S-220 07 Lund, SWEDEN. (Tel) +46 46 107788 (Fax) +46 46 104113, (E-mail) John.Allen@Placebio.Lu.Se

SPOTLIGHT ON RESEARCH INSTITUTES

Some time ago, there was a feature in CyanoNews entitled Spotlight on Industry, highlighting the activities of companies that had major interests in cyanobacteria. That space is still available for any company that wishes to describe itself, but this issue we hear from a research institute with a specialized mission of great interest to those interested in making practical use of cyanobacteria.

The INSTITUT FÜR LEBENSMITTEL- UND UMWELTFORSCHUNG (Institute for Food and Environmental Research) in Bergholz-Rehbrücke, Germany studies means by which microalgae, particularly cyanobacteria, can be exploited in the service of the environment and for the production of compounds of interest in pharmacology, cosmetics, and nutrition. Special emphasis is placed on three areas of research and development. First, the institute seeks to optimize parameters for the mass cultivation of phototrophic microorganisms in closed bioreactors. Second, it develops protocols for extracting useful substances from microalgae, such as phycobiliproteins, polysaccharides, lipoproteins, and polyunsaturated fatty acids, so as to preserve the activity of the substance or enzyme. Third, it studies how microalgae can be used in bioremediation, to remove heavy metal contamination from water and to reclaim lost land.

MUTANT *SPIRULINA* PRODUCES MORE PROTEIN

IGOR BROWN tells us that five long years of work on mutants of *Spirulina* has borne fruit. His laboratory now has several stable mutants of *Spirulina platensis* that may be of practical use. One mutant consists of 80% protein and a second produces c-phycocyanin at twice the levels of the wild-type strain. Even taking into account the slower rate of growth of these mutants, they permit a given harvest of protein in 1.5 to 2-times more quickly than required by the wild-type strain.

RAPID PLASMID DNA EXTRACTION BY ELECTROPORATION AND LEAKAGE

TOIVO KALLAS and Duane Moser report that electroporation provides a simple and efficient method to extract plasmid DNA from *Nostoc* PCC 7121. Voltages above 12 kV/cm cause increasingly copious release into the medium of phycobilins and nucleic acids from a *Nostoc* strain carrying pRL25. These crude extracts could transform *E. coli* to kanamycin resistance and transformants were shown to contain pRL25.

A significant amount of nucleic acids was not released by electroporation of the marine cyanobacterium *Synechococcus* PCC 7002 carrying pAQE19. However, leakage of plasmid DNA occurred from washed cells, even in the absence of applied voltage, as detected by transformation of *E. coli* and analysis of plasmids recovered from *E. coli*. Electroporation or low ionic strength washes follwed by transformation of *E. coli* with the supernatants may provide simple means for recovery and analysis of recombinant plasmids from cyanobacteria.

DEBILITATING MUTATION FOUND IN COMMONLY USED NEOMYCIN/KANAMYCIN GENE

A warning from Jim Wallis, University of California at Davis, prompted JEFF ELHAI to check the pedigrees and restriction patterns of plasmid vectors and cassettes used by many laboratories in their favorite blue-green. Jim had noted the finding of Yenofsky et al. [Proc Natl Acad Sci USA (1990) 87:3435-3439] that some common plasmid vectors conferring resistance to kanamycin/neomycin carry a defective *npt* gene. A point mutation decreases the activity of neomycin phosphotransferase, resulting in lower levels of antibiotic resistance. The presence of the mutation is easily ascertained, since it destroys a XhoII/BstYI site, leading to an alteration in the banding pattern. Jim observed that pRL25, an *Anabaena/E.coli* shuttle vector, has this mutation. In fact, as determined by Jeff, this mutation is found in all *Anabaena/E.coli* shuttle vectors with the prefix "pRL" (including published vectors pRL25C and pRL488), in many cloning vectors (including published vectors pRL447 and pRL498), and in Km/Nm cassettes C.K1 and C.K3. Despite this mutation, defective *npt* preceded by P_{psbA} (as in C.K3) gives very strong selection in *Anabaena*.

MEMBRANE POTENTIAL MEASURED DIRECTLY, ROLE OF SODIUM STUDIED

IGOR BROWN, along with V.D. Tarenko, I.V. Timofeyev, and E.S. Timofeyeva, has succeeded in penetrating the cytoplasmic membrane of a cyanobacterium with a microelectrode to measure directly the potential across the membrane. They used the rather large halo- and alkali-tolerant cyanobacterium *Lyngbya convervoides*. In alkaline medium (pH greater than 9.0), the inside of the cell was electronegative relative to the outside in 75% of the cases measured. Na^+ stimulated the rate of generation of light-induced transmembrane potential and overcame the inhibition of this generation by protonophore uncouplers. K^+, Cl^-, and mannitol had no similar effect. The addition of monensin, a sodium/proton antiporter, increased the sensitivity of the transmembrane potential to uncouplers. They concluded that an intracellular microelectrode is capable of directly measuring the potential across a cyanobacterial cytoplasmic membrane. Furthermore, *L. confervoides* appears to possess a primary sodium pump in its cytoplasmic membrane.

Brown also tells us of studies intended to understand the role of sodium in the development of membrane potential in cyanobacteria. He, G.P. Gorbik, and O.Yu. Mirochnik found that light-induced, Na^+-dependent proton uptake from alkaline medium by *Synechocystis* PCC 6803 is electrogenic in nature and not related to Na^+/H^+ antiporter activity. This process is energized only by noncyclic photosynthetic electron transport. However, in the marine cyanobacterium *Oscillatoria brevis*, cyclic photosynthetic tranport is also effective. *Synechocystis* is protected by 100 mM NaCl against the lethal effects of the uncoupler pentachlorophenol. Brown and coworkers made use of this fact in an interesting way, obtaining mutants, by ampicillin enrichment, that were no longer able withstand incubation with uncoupler, despite the presence of 100 mM NaCl. Salt alone did not effect growth. This work will appear in Biokhimiya.

ESSENTIAL GENE CLONED, CODES FOR PROTEIN-FOLDING ENZYME

Peptidyl-prolyl cis-trans isomerase (PPIase, EC 5.2.1.8) catalyzes in vitro the cis-trans isomerization of the peptidyl-prolyl peptide bond in oligopeptides and accelerates slow, rate-limiting steps in the folding of several proteins. AARON KAPLAN tells us that Miriam Hassidim, Judy Hurwitz, and Rakefet Schwarz in his laboratory have identified a gene from *Synechococcus* PCC 7942 encoding PPIase (located 2.6 kb downstream of *rbcLS*), sequenced it (EMBL GenBank accession number X65028), and shown it to be transcribed. Insertion into the gene of a DNA fragment conferring kanamycin resistance and subsequent transfer of this construction to *Synechococcus* resulted in merodiploids containing both the wild type and the modified genomic region. They were not able to isolate a kanamycin resistant mutant in which all the genomic wild type copies were substituted, suggesting that such replacement may be lethal.

OPEN-AIR AND IMPROVED SEMI-CLOSED MASS CULTIVATION SYSTEMS

ROGERIO LACAZ-RUIZ, along with E.N. Mos, C.G. Lima, and M.A.M. Ribeiro, is seeking to develop an outdoor system of cultivating *Spirulina maxima* for production of biomass during the winter season. They evaluated biomass production, protein content, and amino acids profile as affected by various environmental conditions, such as ambient temperature, culture temperature, relative humidity, and rainfall. Maximum yield was 8.08 g/(m^2·day), with best production from 8:00 AM to 1:00 PM. The work is described in a paper appearing in Brasil J Vet Res Anim Sci, Vol 27.

OTTO PULZ reports on recent work aimed at increasing the yield of large scale cultivation of microalgae. Microalgae are commonly cultivated in open raceway systems, with water depths of 15 cm or more. Under these conditions, light limitation permit a concentration of biomass of no more than 1 g/l. The open system has additional disadvantages, particularly the loss of expensive CO_2 to the atmosphare and the risk of contamination. Pulz and coworkers devised a large scale open system in which the CO_2-enriched algal suspension is spread by a distributing nozzle to an inclined assimilation surface. The resulting thin layer (5 to 10 mm) permitted concentration of biomass to levels between 4 and 9 g/l. The loss of CO_2 remains a serious problem, however.

Cultivation of microalgae in a closed system eliminates the problems of CO_2 loss and contamination but introduces the problem of oxygen accumulation. Pulz and coworkers turned to a semi-closed system, in which oxygen accumulation was eliminated by means of a dipping jet in the cooler. The system consists of thin, tubular plates, stacked vertically with a spacing of 20 cm. Using this system they were able to achieve a daily harvest of 175 g/m^2 ground area with five parallel plates.

HIGH-CO_2-REQUIRING MUTANTS SHED LIGHT ON CO_2 UTILIZATION

The molecular basis of the CO_2-concentrating mechanism of cyanobacteria [Kaplan et al. (1991) Plant Physiol 97:851-855] is being analyzed using high-CO_2-requiring mutants. AARON KAPLAN relates how members of his laboratory have obtained several such mutants in *Synechococcus* PCC 7942 mapping near *rbcLS* (encoding rubisco). The close proximity of these mutations indicates the presence of a gene cluster involved in the ability of the organism to adapt to changes in ambient CO_2.

Two high-CO_2-requiring mutants, E1 and EK6, characterized by Rakefet Schwarz, Judy Hurwitz, and Miriam Hassidim, displayed aberrant carboxysomes. EK6 was constructed by inserting *npt* (determining resistance to kanamycin) close to the 3' end of *rbcS* (encoding the small subunit of rubisco), thus extending the small subunit by 24 amino acids. The modification resulted, as expected, in a larger small subunit: 17 kDal, as compared to 14 kDal in the wild type strain. The resulting defect in carboxysome structure indicates a possible role for the small subunit in the structural organization of the carboxysomes. In vitro-activated rubisco from E1, EK6, and wild type *Synechococcus* all had similar kinetic parameters. In situ, however, rubisco appeared to be in a low state of activation in the two mutant strains pretreated with low CO_2, as judged by the appearance of a lag phase when carboxylation was monitored in permeabilized cells supplied with saturating CO_2 and ribulose 1,5-bisphosphate. Pretreatment of the cells with high CO_2 virtually abolished the lag. In support of a low state of activation of rubisco in E1 and EK6, the internal pool of ribulose 1,5-bisphosphate was found following low CO_2 treatment to be much higher in mutant cells than in wild type cells. The level was reduced in mutant cells pretreated with high CO_2. These findings can explain why the high-CO_2-requiring mutants that possess aberrant carboxysomes fail to grow on low CO_2: the inactivated state of rubisco under conditions of low CO_2 results in an apparent 100-fold lower photosynthetic affinity for extracellular C_i -- too low to permit growth of the mutants in the presence of low CO_2.

Two additional high-CO_2-requiring mutants, D4 and R14, differ from other such mutants in exhibiting normal apparent photosynthetic affinity for inorganic carbon. They were obtained by deletion or inactivation of an open reading frame (ORF) immediately downstream of *rbcLS*. Sequence analysis and metabolic complementation of the mutants by inosine 5'-monophosphate identified this ORF (EMBL GenBank accession number M91187) as the cyanobacterial equivalent of *purK*, the eubacterial gene encoding subunit II of phosphoribosyl aminoimidazole carboxylase in the purine biosynthetic pathway. Exposure of high-CO_2-grown *Synechococcus* to low CO_2 conditions led to the induction of transcription of *purK*, suggesting that purine biosynthesis may be involved in the process of adaptation of cyanobacteria to changing ambient CO_2 concentration.

Eduardo Marco, Nir Ohad, Judy Hurwitz and Rakefet Schwarz have found a mutant, N5, that is defective in the ability to accumulate C_i internally and therefore exhibits a very low apparent photosynthetic affinity for C_i but a similar V_{max} to that of the wild type. The mutant was constructed by inactivation of an ORF (521 amino acids, EMBL GenBank accession number X65027) located 12 Kbp upstream of *rbcLS* and on the opposite strand. The ORF is highly homologous to *ndhB*, encoding subunit II of NADH dehydrogenase in *Synechocystis* PCC 6803 [Ogawa (1991) Proc Natl Acad Sci 88:4275-4279]. Northern analysis showed that transcript abundance was somewhat higher in wild type cells exposed to low CO_2. Mutant and wild type cells exposed to 5% CO_2 in air exhibited similar photosynthetic electron transfer capability, as measured by fluorescence and thermoluminescence. On the other hand, a significant decrease in variable fluorescence and a shift from a Q_B to a Q_A signal were observed when the mutant cells were exposed to low CO_2 under continuous light. These results may indicate that subunit II of NADH dehydrogenase is essential for the functional operation of photosynthetic electron transport in *Synechococcus* under low but not under high levels of CO_2. The means by which high levels of CO_2 rescues the mutant is not yet understood.

MEETING REPORT - Second European Workshop on the Molecular Biology of Cyanobacteria

The Second European Workshop on the Molecular Biology of Cyanobacteria organized by Paul Hayes, Nick Mann, and Tony Walsby, was held in Bristol, U.K., April 4 - 7. It was arranged so that overview lectures covering broad aspects of cyanobacterial biology, ranging from taxonomy and evolution to differentiation and gene regulation, were interspersed amongst more specific 15 min talks and poster watching. Of course, The meatiest part of the workshop may well have been the informal discussions, but these must remain unreported for fear of generating paranoia amongst conversationalists at future meetings.

Regulation of Gene Expression

How environmental signals are transduced to the sites of gene regulation was discussed in several papers, including that of Jean Houmard, Nicole Tandeau de Marsac, and André Sobczyk, related to chromatic adaptation by *Calothrix* PCC 7601. *Calothrix* growing under green light expresses the *cpeBA* operon (leading to increased phycoerythrin), while under red light the *cpc2* operon is induced (leading to increased phycocyanin). The Paris group isolated two proteins, RcaA and RcaB (Regulator for Complementary chromatic Adaptation) that can bind at the promoter region of the *cpeBA* operon. One of the two proteins cannot bind DNA unless it is phosphorylated. The appearance of these DNA-binding proteins in the cell only under green light is consistent with the hypothesis that they serve as positive transcriptional factors mediating the transduction of the light signal during chromatic adaptation.

Light intensity as well as quality affects gene expression, and Abdalla Mohamed, Jan Eriksson, Haile Ghebremedhin, and Christer Jansson presented work concerning the regulation of *psbA*, a light-regulated gene, in *Synechocystis* PCC 6803. There are three *psbA* genes in this organism. 97% of the *psbA* transcripts originate from *psbA2* and 3% from *psbA3*. Transcription from *psbA1* was not detected. *psbA1* also differs from the others in its sequence: *psbA2* and *psbA3* are almost identical, but *psbA1* shows substantial deviation. The two expressed genes are also very similar in their promoter regions and differ markedly from the *psbA1* promoter.

Metals evidently regulate the expression of genes in cyanobacteria coding for proteins that require them for activity or proteins that protect the cell against metal toxicity. Arnaud Bovy, Geert de Vrieze, Mies Borrias, and Peter Weisbeek described how genes (from *Anabaena* PCC 7937) encoding plastocyanin (PC), a copper protein, and ferridoxin (FD), an iron-sulfur protein, are regulated by the availability of copper and iron, respectively. They found that the stability of the FD transcript is 8- to 10-fold higher in iron-grown cells as compared to iron-deprived cells, while promoter activity is constitutive. In contrast, transcriptional initiation rather than message stability appears to play the major role in controlling copper-dependent expression.

Increased levels of one of a number of trace metals leads to increased abundance of *smtA* transcripts, encoding metallothionein from *Synechococcus* PCC 7942. Nigel Robinson, Jennifer Turner, James Huckle, Amit Gupta, Andrew Morby, and Brian Whitton insertionally inactivated *smtA* and found that the resulting mutant strain

exhibited increased sensitivity to cadmium but grew as well as the wild type in medium without heavy metals. They also described fascinating DNA amplification and rearrangment events that appear to play a role in the expression of *smtA*. One event is the excision of an adjacent, divergent gene called *smtB* at an 8-bp palindrome (GCGATCGC) that occurs at the borders of the excision. The sequence of *smtB* suggests that its gene product is a DNA-binding protein. The palindromic sequence appears in Synechococcal sequences at a surprisingly high frequency, about once every 500 bp.

Respiration and Photosynthesis

The flexibility of cyanobacterial respiratory and photosynthetic electron transport activities was demonstrated in several interesting reports, including a study by Georg Schmetterer and Daniel Alge. They reported the isolation and sequence of the *coxBAC* locus of *Synechocystis* PCC 6803, encoding subunits II, I, and III, respectively, of the aa_3-type cytochrome *c* oxidase. Mutants inactivated in either *coxA* or *coxC* were constructed, and their membranes showed no cytochrome *c* oxidase activity. Nonetheless, the mutants showed normal rates of oxygen consumption in the dark and normal sensitivity to respiratory inhibitors. Evidently, cytochrome *c* oxidase is not the major terminal oxidase of the respiratory chain in *Synechocystis* PCC 6803.

Etana Padan described progress in identifying the redox carriers in anoxygenic photosynthesis by *Oscillatoria limnetica*. She with Boaz Arieli and Yosepha Shahak found a novel sulfide-quinone reductase (SQR) that is induced when *Oscillatoria* is shifted from oxygenic to anoxygenic photosynthesis. SQR is a membrane protein that can be isolated in active form from induced thylakoids and is found also in the membranes of the green bacterium *Chlorobium*. The study of light-dependent electron transport from Na_2S to $NADP^+$ in a cell-free system revealed that electron transfer from sulfide to PS I occurs via the cytochrome b_6f complex and is coupled to a directional movement of protons.

PS I and PS II are laterally separated in *Prochlorothrix hollandica*. Hans Matthijs, on behalf of himself, Georg van der Staay, and Luuc Mur, presented a picture of how antenna are organized in the thylakoids of *Prochlorothrix*. He showed how chlorophyll *a*/*b*-binding antenna are associated with PS II, as in chloroplasts, but PS I appears to lack its own LHC I (light harvesting complex)-type of antenna. Low light intensity induced additional synthesis of chlorophyll *a*/*b*-binding antenna, as in higher plants, but also resulted in an increased PS I/PS II ratio, as in cyanobacteria.

The significance of protein phosphorylation of thylakoid proteins was emphasized by Nigel Silman, Nick Mann, and Noel Carr. They presented the interesting observation concerning a protein kinase from the thylakoid membranes of *Synechocystis* PCC 6803 that requires illumination under State 2 light (580 nm) for the specific phosphorylation of a polypeptide with a molecular weight of 18 kdal. They found that the activity of this kinase could be stimulated by the addition of ribulose 5-phosphate, and this treatment removes the requirement for light.

Protein Targeting and Localization

Several interesting questions were raised concerning protein sorting in cyanobacteria. Dirk Geerts, Léon van Tegelen, Núria Rodríguez Ciurana, Job Dekker, Mies Borrias, and Peter Weisbeek showed that signal sequences on plastocyanin (PC) from *Anabaena* PCC 7942 were sufficient to accurately (if not efficiently) direct transport in an in vitro plant system. PC synthesized in *Synechococcus* PCC 7942 from genes of both *Anabaena* and *Arabadopsis* was targeted to the thylakoids (as expected) as well as to the periplasm.

Günter Peschek, Harald Kraushaar, Christian Obinger, Helmut Niederhauser, and Silvia Hager showed that cytochrome *c* oxidase, encoded by a single operon, is targetted in *Synechocystis* PCC 6803 to both the plasma and thylakoid membranes. They proposed that environmentally responsive proteases might unmask one of two potential leader sequences on the cytochrome *c* oxidase peptides, so as to influence the ratio of the protein in the two compartments.

Chaparonins have been implicated in the folding and assembly of proteins. Karin Jäger and Birgitta Bergman told of their work concerning the localization of chaparonin-60 in cyanobacteria. Immunogold localization using anti-sera against chaperonin-60 of *E. coli* detected the protein predominantly in polyhedral carboxysomes of vegetative cells and in carboxysome-deficient heterocysts of *Anabaena* PCC 7120. They proposed that chaparonin-60 participates in the assembly of Rubisco and possibly heterocyst-specific proteins.

Salt and Nutrient Stress

Another popular topic was the response of cyanobacteria to salt and nutrient stress. David Scanlan presented a novel technique that he along with Helen Chadd, Nick Mann, and Noel Carr developed to identify

microelement deficiencies in natural marine populations. The marine cyanobacterium *Synechococcus* WH7803 was grown under limitation for P or Fe^{+3} in the presence of ^{35}S-methionine to identify polypeptides specifically induced or repressed by the limitation. Antibodies raised against such peptides may be used as probes to assess nutrient stress.

Different groups approached the mechanism of salt tolerance from different directions. Françoise Joset related how Boyomo Onana and Robert Jeanjean in her laboratory isolated a mutant of *Synechocystis* PCC 6803 that can no longer grow on more than 0.1 M NaCl. The strain contains two relevant mutations. One is required for normal respiration, chemoheterotrophic growth, and salt tolerance. The other is required to stimulate cytochrome oxidase and PS I activities in response to high salt. Ellen Zuther and Martin Hagemann succeeded in isolating salt-sensitive mutants of *Synechocystis* PCC 6803 by transforming the strain with chromosomal DNA randomly ligated to a kanamycin resistance gene from Tn903.

Ofra Matan and Elisha Tel-Or tried instead to increase the salt tolerance of a freshwater strain, *Synechococcus* PCC 7942, by giving the strain the capacity to synthesize the osmoregulant glycinebetaine. Two genes, *betA* and *betB*, encoding enzymes responsible for the conversion of choline to glycinebetaine, were introduced from *E. coli* into *Synechococcus* by transformation. The resulting strain synthesized glycinebetaine, so long as both high salt and choline were present, but did not gain higher salt tolerance.

- Ulrike Holzmüller and Aaron Kaplan

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