BIOSYNTHESIS OF THE PHOTOSYNTHETIC APPARATUS: MOLECULAR BIOLOGY, DEVELOPMENT AND REGULATION

Richard Hallick, L. Andrew Staehelin and J. Philip Thornber, Organizers
April 21 — April 27

Plenary Sessions

April 22:
Genes for Components of the Photosynthetic Membrane . . . . . 292—293
Organization and Expression of the Chloroplast Genome . . . . . 293—295

April 23:
Transcriptional and Translational Regulation of Chloroplast Gene Expression . . . . . 295—296
Environment and Gene Expression . . . . . 297—298

April 24:
Organization, Function and Dynamics of Photosynthetic Membranes . . . . 298—300
Agricultural Applications of Research on the Photosynthetic Apparatus . . . . . 300—301

April 25:
Biosynthesis and Assembly of Chloroplast Components . . . . . 302—303

April 26:
Molecular Organization and Assembly of Bacterial Photosynthetic Membranes . . . . . 304—305
Transformation of Photosynthetic Systems . . . . . 305—306

Poster Sessions

April 22 and April 23:
Molecular Biology and Biochemistry of the Photosynthetic Apparatus
Poster Abstracts 1311 — 1338 . . . . . 307—316

April 24 and April 25:
Structure and Function of Photosynthetic Membranes
Poster Abstracts 1339 — 1371 . . . . . 316—326

Genes for Components of the Photosynthetic Membrane

1282 *Isolation, Characterization and Expression of Genes Involved in Photosynthesis.* *Broglie, R., Coruzzi, G., Lamppa, G., Keith, B., and Chua, N.-H. Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, NY, NY 10021*

Chloroplast biogenesis requires the coordinate expression of both the nuclear and chloroplast genomes. While the chloroplast genome has been shown to encode several polypeptides, structural genes for many chloroplast proteins are localized in the nucleus. These proteins are synthesized on cytoplasmic ribosomes as precursors containing an amino terminal transit peptide. The transit peptide presumably facilitates post-translational entry of the precursors into chloroplasts. In higher plants, the most abundant nuclear gene product destined for the chloroplast is the small subunit (S) of ribulose bisphosphate carboxylase (RUBISCO). To study the regulation of the nuclear genes encoding S and to elucidate the amino acid sequence of the transit peptide we have isolated a cDNA clone (pW9) encoding the wheat RUBISCO small subunit. pW9 was characterized by hybrid selection, immunoprecipitation, and DNA sequence analysis. This clone encodes 128 amino acid residues of the mature S and a full length transit peptide of 47 amino acids. Comparison of the amino acid sequence of the wheat small subunit precursor and that of the pea small subunit precursor (1,2) reveals several interesting features. The two transit peptides show homologies at the cleavage site (Cys-Met) as well as at the flanking sequences, and there is a striking conservation of positively-charged amino acids. The wheat and pea small subunit precursors are taken up interchangeably into either chloroplast type, in vitro. pW9 and pSS15, a cDNA clone encoding the pea small subunit (1) were used as hybridization probes to study the organization of nuclear genes encoding these proteins. Southern blot analysis reveals 5-6 EcoRI fragments containing small subunit genes in wheat and pea nuclear DNA, indicating the existence in both plants of a small multigene subunit family. Nuclear DNA sequences encoding the small subunit were cloned into lambda DNA and further subcloned into pBR322 or pBR325. R-looping and DNA sequence analysis of a wheat genomic clone (pWS 1.8) reveals an intron of 289 bases which interrupts the gene between the 2nd and 3rd amino acids of the mature protein. The concensus sequence AG-GT is present at the exon-intron junctions. The 5' non-coding region of the gene contains a TATATATA box and a CCAT box both of which are putative regulatory sequences for eukaryotic genes. In pea, 7 different genomic clones were isolated which contain several different EcoRI fragments encoding the small subunit gene. In dot blot experiments, only two of these clones hybridized specifically to a 3' probe derived from the non-coding region of pSS15 under the most stringent salt wash conditions, suggesting that one or both of these genes are expressed in pea leaves. The characterization of these clones is presently in progress.

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Supported by NIH Grant GM25114 and Monsanto Company.

1283 IDENTIFICATION AND CHARACTERIZATION OF CHLOROPLAST GENES FOR THYLAKOID MEMBRANE PROTEINS, W. Bottomley, G. Zurawski and P.R. Whitfeld, CSIRO, Division of Plant Industry, PO Box 1600, Canberra City, A.C.T. 2601 (Australia).

Chloroplast thylakoids are highly organized membrane systems which contain the components of the photosynthetic electron transport system. Many of these components are organized into isolatable complexes such as photosystems I & II, ATP synthase and the cytochrome b_6/f complex. Of the components so far studied, it appears that the genes coding for the proteins of these complexes are distributed between the nucleus and the chloroplast.

The thylakoid protein genes which have so far been reported to be contained in the chloroplast genome include five subunits of ATP synthase[1], three polypeptides of the cytochrome b_6/f complex[2], the chlorophyll-binding protein of photosystem I & II[2] and a herbicide-binding polypeptide associated with photosystem II[3]. The arrangement of the genes on the genome do not favour an organization similar to the bacterial operon model. For instance, while the β & ε subunits of ATP synthase are either adjacent or overlapping, the α subunit is remote. Because of this dispersal of subunits around the genome it seems likely that some form of control of expression is contained in the DNA sequences either within or near the genes.

Sequence data available on the α, β & ε subunits of ATP synthase the herbicide binding protein and other possible thylakoid proteins from a number of plants do not reveal any obvious controlling sequences. However, examination of sequences near the 5' and 3' end of the mRNA suggest that the control of expression is procaryotic in nature.

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1284 SYNTHESIS OF THE CHLOROPLAST ATP SYNTHASE AND CYTOCHROME b-f COMPLEX, John C. Gray, Andrew L. Phillips, Christopher J. Howe, David L. Willey and Alison K. Huttly, Botany School, University of Cambridge, Cambridge CB2 3EA, U.K.

The synthesis of components of the ATP synthase and cytochrome b-f complex has been studied using protein synthesis by isolated pea chloroplasts and transcription and translation of fragments of wheat and pea chloroplast DNA in a cell-free extract from E. coli. Isolated pea chloroplasts incorporated labelled amino acids into three components of the cytochrome b-f complex of 38kD (cytochrome f), 19.5kD (cytochrome b-563) and 15.2kD. Assembly of newly-synthesised 19.5kD polypeptide into the complex required the addition of 5mM Mg-ATP to the medium, although assembly of the 38kD and 15.2kD polypeptides did not require added Mg-ATP. The genes for cytochrome f and the 15.2kD polypeptide have been located in pea chloroplast DNA and the nucleotide sequences of the genes have been determined. The gene for cytochrome f specifies an amino-terminal amino acid sequence which does not appear in the mature protein. This may be involved in directing cytochrome f to the thylakoid membrane.
The genes for the α,β and ε subunits of CF_1 and the proton-translocating subunit (subunit III) of CF_o have been located in wheat and pea chloroplast DNA. In both species the genes for these subunits are arranged in two clusters with β close to ε and α close to the proton-translocating subunit gene. In wheat the clusters are 20kbp apart whereas in pea the clusters are 50kbp apart. In both species the genes for β and ε are close to, and transcribed divergently from the gene for the large subunit of ribulose bisphosphate carboxylase. The genes for α and the proton-translocating subunit are transcribed from the same strand. The nucleotide sequence of the wheat gene for the proton-translocating subunit has been determined and indicates an amino acid sequence which is identical to the known sequence of the spinach subunit.

Organization and Expression of the Chloroplast Genome

1285 CHLOROPLAST GENES AND TRANSFORMATION IN CHLAMYDOMONAS REINHARDII, J.-D. Rochaix, M. Dron, M. Rahire, M. Schneider, J.-M. Boissel and J. van Dillewijn, Departments of Molecular and Plant Biology, University of Geneva, 1211 Geneva 4, Switzerland.

Several genes have been identified on the circular chloroplast genome of the green unicellular alga Chlamydomonas reinhardii. They include the genes coding for ribosomal RNA, for several tRNAs, for the large subunit of ribulose bisphosphate carboxylase (LS) and for several thylakoid polypeptides. A DNA sequence analysis of several regulatory sequences of chloroplast genes and of their coding regions has shown that the 5' upstream regions contain sequences which resemble bacterial translational and transcriptional signals. These regions also include several repeats whose function is not clear. The 3' untranslated regions display stem-loop structures which are reminiscent of bacterial terminators. There is a limited codon usage in the coding regions.

A sequence comparison of the LS genes from wild-type and from a uniparental mutant isolated by Spreitzer and Mets (1) has revealed a single nucleotide change. The mutation replaces a gly residue with asp in the first active domain of the protein. This result provides a first correlation site between the genetic and physical chloroplast DNA maps of C. reinhardii.

We have recently developed a transformation system in C. reinhardii which should greatly help in elucidating the function and regulation of genes coding for chloroplast polypeptides. A cell wall deficient arginine auxotroph was complemented by transformation with a plasmid containing the yeast arg4 locus (2). We have constructed transformation vectors by inserting nuclear and chloroplast DNA fragments into a plasmid containing the yeast arg4 locus as selective marker. After transformation of C. reinhardii and yeast with pools of these hybrid plasmids, several autonomously replicating plasmids have been recovered which act as shuttle vectors between E.coli and C. reinhardii and E.coli and yeast. Some of these plasmids appear to be maintained in C. reinhardii for at least 70 generations. Among the plasmids replicating in yeast some contain restriction fragments which have been mapped on the chloroplast genome.

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1286 EVOLUTIONARY ASPECTS OF CHLOROPLAST GENOME ORGANIZATION AND EXPRESSION
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Chloroplast DNA rearrangement and recombination processes are being studied in the context of the overall evolutionary stability of the chloroplast genome. A remarkable feature of the chloroplast genome is that a large, 10-25 kb inverted repeat sequence has been retained in its basic structure for over 500 million years of plant evolution. We have previously shown (1) that major sequence rearrangements, specifically inversions and transpositions, are extremely rare in those angiosperm chloroplast genomes which possess the large inverted repeat and increase markedly in frequency in the single group of angiosperms, represented by the legume species pea and broad bean, whose genomes have lost one entire segment of the inverted repeat. We have now extended our analysis to include a number of other legume chloroplast DNAs that lack the inverted repeat and can draw three major conclusions: 1). The loss of the inverted repeat occurred prior to all the other rearrangements observed in legumes such as pea and broad bean. 2) All characterized chloroplast DNA rearrangements are inversions. 3) There is a broad range of rearrangement frequencies in non-inverted repeat-containing chloroplast DNAs.

We have recently shown (2) that chloroplast DNA from the common bean, Phaseolus vulgaris, consists of two equimolar populations of molecules differing only in the relative polarity of their single copy sequences. The same phenomenon has now been observed in a number of diverse species and almost certainly results from frequent intramolecular recombination between the two segments of the inverted repeat. We are now testing the hypothesis that when one segment of the inverted repeat is missing, this specialized, probably site-specific, recombination system mediates an increased frequency of inversions throughout the entire chloroplast genome.

In spite of the striking overall stability of most chloroplast genomes both in structure and in sequence, a remarkable degree of plasticity exists in the manner in which specific chloroplast genes are transcribed and their transcripts subsequently processed. For example, in most plants the gene for the large subunit (LS) of ribulose bisphosphate carboxylase is transcribed into a single RNA species of 1.6 kb which is colinear with the gene, whereas in mung bean and related legumes the 1.6 kb transcript represents only a minor fraction of LS transcripts, and instead, two larger LS transcripts of approximately 2.4 and 2.6 kb predominate (3). We will present fine structure mapping and sequencing data which provide a molecular basis for these additional high molecular weight mung bean LS RNAs, and will analyze transcriptional variation from a number of other chloroplast genes in order to develop a general picture of the evolution of chloroplast transcriptional units.

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1287 MUTATIONS AND GENE CONSTRUCTIONS ALTERING THE EXPRESSION AND FUNCTION OF CHLOROPLAST GENES, Lee McIntosh, MSU-DOE Plant Research Laboratory/Biochemistry Department, Michigan State University, East Lansing, Michigan 48824

There are few well-characterized chloroplast gene mutations and none available for which the mechanism is understood at the molecular level. Possibly the best candidate for study at this time is the chloroplast encoded 32 Kilodalton thylakoid membrane polypeptide. This photosystem II protein is rapidly labeled in the light and is the major membrane-bound polypeptide produced by isolated chloroplasts. Recently, it has also been identified as the triazine receptor protein (1). We have now sequenced the 32 Kd gene from Zea mays and are finishing the gene sequences for two biotypes of Amaranthus hybridus. The 32 Kd gene encodes a protein of 317 amino acids and contains no lysine. The molecular weight of 34.8 Kd predicted from the DNA sequence closely agrees with earlier molecular weight estimates of 34-34.5 Kd. Work on Amaranthus hybridus has shown that there is a maternally inherited biotype which is resistant to triazine herbicides (2). This predicts a possible mutation within the 32 Kd gene. We are currently finishing the 32 Kd gene sequence from both sensitive and resistant biotypes and will discuss the possible binding site for azido-atrazine on the 32 Kd polypeptide.

Another method of investigating the affect of chloroplast gene structure on experession is to maximally express these genes on plasmids in E. coli. We have recently constructed a series of deletions in the area directly preceding the maize gene encoding the large subunit of ribulosebisphosphate carboxylase. These experiments resulted in a series of clones which vary in the amount of large subunit produced from no expression until it is the major protein in E. coli. We are now trying to correlate these different levels of expression with specific chloroplast gene features.

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1288 CORRELATION OF THE PHYSICAL AND GENETIC MAPS OF *CHLAMYDOMONAS* CHLOROPLAST DNA, Laurens Mets, Department of Biology, University of Chicago, Chicago, IL 60637

We have developed two genetic methods in *Chlamydomonas* which could be used for mapping physical sites on chloroplast DNA (e.g. restriction site polymorphisms or deletions) relative to genetic markers. The common feature of these two methods is that they require scoring relatively few progeny to obtain reliable estimates of apparent recombination frequencies and therefore allow mapping of markers which are laborious to score. The first method, "paternal marker selection," is a streamlined modification of zygote clone analysis. It has been used to show linkage between a ribulose bisphosphate carboxylase large subunit gene marker (mutant 10-6C) and markers for resistances to erythromycin, streptomycin, and diuron. It has long been presumed that these latter markers are carried on chloroplast DNA, but their linkage to mutant 10-6C provides the first direct evidence. The second method, "selected recombinant analysis," focuses on progeny cells with recombinant phenotypes in order to obtain finer genetic maps. It reveals that two independent phenomena -- parental bias and allelic bias -- strongly influence apparent recombination frequencies and obscure linkage relationships. A molecular model for the allelic bias suggests that a large proportion of progeny cells with recombinant phenotypes are actually genetically heteroplasmic. Further analysis has confirmed predictions of this model. The data allow us to determine that erythromycin resistance, streptomycin sensitivity, and diruon sensitivity are the dominant alleles of these markers.

The presence of a large inverted repeat in chloroplast DNA implies that the relationship between recombination frequencies and physical distances along the DNA molecule will be complex. A detailed theoretical model of the expected recombination process serves as a guide in attempts to correlate the genetic data with the DNA structure.

Transcriptional and Translational Regulation of Chloroplast Gene Expression

1289 ORGANIZATION AND EXPRESSION OF *Euglena* CHLOROPLAST TRANSCRIPTION UNITS, R. B. Hallick, M. J. Hollingsworth, G. D. Karabin, J. A. Nickoloff, C. Passavant, and G. L. Stiegler, Department of Chemistry, University of Colorado, Boulder, Colorado 80309

We have been involved in a characterization of the chloroplast (ct) genes of the unicellular photosynthetic eucaryote, *Euglena gracilis*. Our approach is to first identify genes via membrane filter hybridization, using as probes either purified RNAs, restriction fragments internal to previously characterized genes of chloroplast or bacterial origin, or chemically synthesized oligodeoxynucleotides of defined sequence. Previous work on the physical mapping and cloning of this DNA, and the tandem rRNA operons has been reviewed (1). Second, the arrangement of genes and gene clusters is determined by nucleotide sequence analysis. Third, the temporal regulation and processing of ct RNAs during light induced ct development is defined using gene specific probes and Northern hybridizations. Ct tRNA genes of *Euglena* are in many cases arranged as closely spaced, multigene clusters. We have sequenced four of these, containing 17 different genes. Some of these may be initially transcribed into multicistronic pre-tRNAs. Several protein coding loci have been identified, including genes for the large subunit of RuBP-carboxylase (2), α and β subunits of CF_1-ATPase, the 32 kd polypeptide of photosystem II (32 kd), and EF-Tu. From nucleotide sequence analysis we find that the LS gene is interrupted by at least three introns. The 1.4 kbp of exon sequences are dispersed over 2.5-3.5 kbp, or more. This is the first example of a split chloroplast protein gene. Genes for several chloroplast proteins are transcriptionally induced during light dependent ct development, including the LS, 32 kd, and β subunit of CF_1 genes. In addition, multiple high MW precursor mRNAs are detected, which are processed to mature RNAs via several intermediate species. (Supported by NIH Grants GM21351 and GM28463.)

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1290 SELECTIVE IN VITRO TRANSCRIPTION OF CHLOROPLAST GENES

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We are interested in the transcription and regulation of chloroplast (ct) genes in the unicellular, photosynthetic eukaryote Euglena gracilis, and also in higher plant chloroplasts. The isolation of a nucleoprotein complex from Euglena chloroplasts active in selective in vitro transcription of the rRNA operon has been previously described (1). The DNA-dependent RNA polymerase responsible for rRNA transcription remains tightly bound to the DNA template. The nucleoprotein complex can be treated with restriction enzymes, giving rise to specific run-off transcripts that allow a detailed analysis of 5' promoting sequences. In contrast, transcriptionally active extracts can be obtained from isolated, intact Euglena or spinach chloroplasts that give distinct in vitro transcription products when appropriate templates are added. A Euglena $tRNA^{Val}$-$tRNA^{Asn}$-$tRNA^{Arg}$-$tRNA^{Phe}$ gene cluster can be transcribed in a Euglena, as well as in a spinach, ct transcription extract. The in vitro transcription products are processed into at least three of the four mature tRNAs as analyzed by comparative fingerprints, using the same mature ct tRNAs transcribed in a eukaryotic cell free system (2). We have extended our work to characterize the transcriptional properties and processing of a spinach $tRNA^{Val}$, containing a 490 bp intron. Other Euglena tRNA gene clusters and spinach tRNA genes are exchangeable and correctly transcribed in both systems. Transcription is specific in that eukaryotic or prokaryotic tRNA genes are not transcribed by ct RNA polymerase. A 'consensus sequence' 5' to all transcribed tRNA genes or tRNA gene clusters might serve as a putative promoter for transcription initiation. Furthermore, cutting internal sites in tRNA genes with restriction enzymes prior to transcription will abolish the expression of the tRNA coding loci, indicating that internal sequences are crucial for transcription or processing of the transcripts. The rRNA transcription system differs from the soluble RNA polymerase activity in their template specificity, salt and heparin sensitivity. Both activities also differ in their drug sensitivity when compared to eukaryotic and prokaryotic RNA polymerases. A 3' CCA adding activity has been isolated and characterized from the ct transcription extract as well. The enzyme adds the 3' CCA to the in vitro and in vivo transcribed and processed ct tRNAs. (Supported by DFG Grant I3-Gr73311-1 to W. G., NIH Grant GM28463 to R. B. H., and NIGMS Grant GM19199 to D. M. P.).

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1291 LIGHT REGULATION OF NUCLEAR-CODED GENES FOR CHLOROPLAST PROTEINS: RIBULOSE 1,5-BISPHOSPHATE CARBOXYLASE SMALL SUBUNIT AND CHLOROPHYLL a/b PROTEIN,

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Light is involved in the development of the photosynthetic apparatus of higher plants at several different levels. Previous studies have demonstrated that phytochrome action can regulate the levels of translatable mRNAs for the small subunit of ribulose 1,5-bisphosphate carboxylase (SSU) and for the light-harvesting chlorophyll a/b-apoprotein (chl a/b-protein) in Lemna gibba (1,2) and for the chl a/b-protein and the NADPH-protochlorophyllide-oxidoreductase in barley (3,4). In order to examine the molecular details of this light regulation, we have cloned hybridization probes for both proteins.

A set of cDNA clones was constructed from poly(A) RNA in the Pst site of the plasmid pBr 322. Genomic clones were constructed from Lemna nuclear DNA which had been partially digested with EcoRl and inserted into the lambda vector Charon 4. Both libraries were screened with cDNA probes made from RNA which had been size selected and assayed by in vitro translation. Probes for both RNAs have been identified and partially characterized. Genes for both proteins occur as multigene families in Lemna. One of the genomic clones of 10.7 kb contains 2 SSU genes separated by about 2.6 kb.

A cDNA clone for the SSU and a subclone of a genomic clone encoding the chl a/b-protein have been used to investigate phytochrome action on the sequence content of the RNAs encoding the two proteins. Hybridization of the labeled probes to Northern blots of total RNA from plants given various regimes of illumination demonstrates that the amounts of the two sequences decline in complete darkness and that one minute of red illumination can rapidly cause an increase in the amounts of both sequences. These increases can be prevented by illumination with far-red light given immediately after the red. Thus phytochrome action must affect either transcription of these genes or the stability of the new transcripts.

Further support for transcriptional control by phytochrome action comes from studies of the transcripts produced by isolated Lemna nuclei. These isolated nuclei incorporate ^{32}P-UTP into RNA in vitro, and this incorporation is sensitive to low concentrations of α-aminitin. Hybridization of the labeled RNA transcripts to each of the cloned probes shows that phytochrome action in the intact plants results in differences in the nuclear transcripts in vitro.

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Environment and Gene Expression

1292 STRUCTURAL CHARACTERIZATION OF THE RAPIDLY-METABOLIZED 32000 DALTON THYLAKOID PROTEIN, Marvin Edelman, Jonathan B. Marder, Robert Fluhr and Autar K. Mattoo, Department of Plant Genetics, Weizmann Institute of Science, Rehovot 76100, ISRAEL

Our studies have concentrated on a 32 kd protein partially surface exposed at the photosynthetic membranes of the aquatic angiosperm, **Spirodela oligorrhiza**. In the light, this is one of the major products of chloroplast protein synthesis. However, the protein fails to accumulate due to rapid turnover. Both synthesis and degradation are sensitive to light intensity, but differential sensitivity to inhibitors suggests different mechanisms. The 32 kd protein is suggested to have an important function in photosynthetic electron transport, and may be a target molecule for certain herbicides [1]. The DNA sequences of the 32 kd gene from spinach and tobacco predict identical amino acid sequences [2]. Our preliminary DNA sequence results from Spirodela suggest that the primary structure of the protein will be at least highly similar. We have previously used various proteolytic fingerprinting techniques both on the membrane and on isolated 32 kd polypeptide to show that the protein has a number of structural properties which are conserved in a wide range of different plant species [3]. We are currently working to relate these properties to the predicted primary structure.

During electrophoresis in the presence of small amounts of papain, the 32 kd protein undergoes initial partial proteolysis into unequal pieces of approximately 12 and 20 kd. By performing papain cleavage on 32 kd protein labelled with several different tritiated amino acids, we are able to show the distribution of polar and non-polar residues between the two fragments. The results suggest that the 12 kd piece is substantially more hydrophylic than the 20 kd piece. This is confirmed by proteolysis of the 32 kd protein **in situ** on the membrane; both trypsin and chymotrypsin remove all or most of the 12 kd portion leaving the 20 kd intact on the membrane.

The 32 kd protein is encoded and translated within the chloroplast as a 33.5 kd precursor polypeptide. Processing to the 32 kd form occurs **in vivo** on the membrane and commences only after completion of the 33.5 kd polypeptide chain [4]. Partial proteolysis of the precursor with papain yields fragments of 20 and 13.5 kd. Thus the precursor has an extra short sequence associated with what becomes the hydrophylic end in the mature protein.

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1293 *THE LIGHT-DEPENDENT CONTROL OF PLASTID DEVELOPMENT IN BARLEY (HORDEUM VULGARE).*

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The light-induced greening of etiolated barley plants is used as a model to study the light-dependent control of plastid development. Upon illumination a rapid transformation of etioplasts to chloroplasts is induced. The effect of illumination does not only include the light-dependent chlorophyll synthesis but also the appearance or decline of specific proteins within the plastid membrane fractions. So far two of these proteins were studied in more detail. The light-harvesting chlorophyll a/b protein (LHCP) is one of the major protein constituents of the thylakoid membrane of chloroplasts. However, this protein is not detectable among the membrane polypeptides of etioplasts. Illumination of dark-grown barley plants induces a massive insertion of the LHCP. The appearance of the protein is controlled by the cooperation of at least two distinct photoreceptors: Protochlorophyllide and phytochrome. In dark-grown barley plants not only the LHCP but also its mRNA is not detectable. The light-dependent appearance of mRNA activity for the LHCP is under the control of phytochrome (P_{fr}). Even though the appearance of mRNA activity is induced via P_{fr} by a single red light pulse, the assembly of the complete LHCP takes place only under continuous illumination which allows chlorophyll synthesis. The second protein analyzed so far is the NADPH-protochlorophyllide-oxidoreductase. This enzyme catalyzes the light-dependent reduction of protochlorophyllide to chlorophyllide and thus, controls one of the first detectable light-dependent reactions during the greening period. It is generally assumed that this enzyme is responsible for the overall chlorophyll synthesis and accumulation during the greening period. In contrast to this hypothesis we found a rapid decline of the enzyme during illumination. In addition to the decrease of the enzyme protein the translatable mRNA coding for the enzyme also declines rapidly under the influence of light. Also this effect is mediated by phytochrome. Using cloned cDNA as hybridization probes we have demonstrated that the light-induced changes of the two translatable mRNAs for the NADPH-protochlorophyllide oxidoreductase and the LHCP are both paralleled by corresponding changes in the steady state concentration of the mRNA sequences. Thus, one mechanism by which phytochrome might exert its effect an the translatable mRNAs can be excluded, it does not appear to be the activation or inactivation of preexistant mRNA-sequences.

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1294 EFFECT OF LIGHT ON PHOTOSYNTHETIC MEMBRANE STRUCTURE, ORGANIZATION AND FUNCTION, Anastasios Melis, Division of Molecular Plant Biology, University of California, Berkeley, CA 94720

The light environment during plant growth determines the structural and functional properties of higher plant chloroplasts, thus revealing a dynamically regulated developmental system. The present work deals with the effect of the light environment during plant growth on the regulation of photosystem stoichiometries and on the chlorophyll-protein complex biosynthesis. Photosystem organizational changes are correlated with changes in the chloroplast ultrastructure. Chloroplasts were isolated from sun-adapted and shade-adapted species and also from plants growing in the laboratory under various regimes of light quality and light duration. The stoichiometric concentrations of photosystems I and II were determined from the amplitude of the absorbance difference spectra in the red (ΔA_{700}) and in the ultraviolet (ΔA_{320}) regions, respectively. The effective light-harvesting antenna size and its chlorophyll composition were determined separately for PSI and for PSII from the rate constant of the respective photoactivity under limiting excitation conditions. Electron microscopy provided information on the light-induced morphological changes in the chloroplast ultrastructure. Chloroplasts from sun-adapted species showed thin grana stacks and an extensive intergrana membrane (stroma thylakoid) system. They had a PSII/PSI reaction center ratio of about 1.8, considerably greater than unity. Shade-adapted species showed large grana, often extending across the entire chloroplast body. They had a PSII/PSI reaction center ratio significantly greater than 1.8, in some cases approaching 4.0. Determinations of the effective light-harvesting antenna size of PSI and PSII revealed that the number of chlorophyll molecules per reaction center was about the same in sun and shade-adapted plants. Thus, the main strategy in the higher plant sun and shade adaptation appears to be a change in the relative amounts of the two photosystems (chlorophyll-protein plus reaction center complex) and a concomitant change in the chloroplast ultrastructure, rather than simply a change in the size of the light-harvesting antenna associated with PSI and PSII. The structural and functional properties of sun and shade-adapted chloroplasts were simulated in the laboratory with plants grown under blue-enriched and far red-enriched illumination, implicating the possible regulatory role of a blue light receptor and/or of phytochrome in chloroplast biosynthesis. Plants growing under illumination of short duration failed to synthesize the Chl ab light-harvesting complex and lacked grana. They did possess, however, fully functional PSII and PSI reaction center complexes with a small chlorophyll a light-harvesting antenna. We conclude that the biosynthesis of PSII and PSI reaction centers may proceed independently of the biosynthesis of the Chl ab LHC. Light quality and light duration variations during plant growth may have a differential effect on the expression of photosynthetic genes in the nuclear and chloroplast genomes of higher plants.

Organization, Function and Dynamics of Photosynthetic Membranes

1295 THE PHOTOSYNTHETIC LIGHT REACTIONS AND THEIR ORGANIZATION IN CHLOROPLAST MEMBRANES, Kenneth Sauer and Kerry K. Karukstis, Department of Chemistry and Chemical Biodynamics Laboratory, University of California, Berkeley, CA 94720

We have studied chlorophyll fluorescence decay kinetics to monitor the path and time course of excitation as it passes through the light-harvesting antenna pigments of higher plant chloroplasts to reach the reaction centers of Photosystems I and 2 (PS 1 and PS 2). The extent of excitation transfer is a function of membrane organization and is influenced by divalent ions (Mg^{2+}), illumination wavelength and the phosphorylation of membrane proteins. Two types of PS 2 centers are present: α centers exhibit excitation transfer between adjacent antenna domains; β centers have isolated antenna domains and exhibit a lower photochemical efficiency.

(1) Three resolved fluorescence decay components are seen in broken chloroplasts.[1] The two faster decays (50-100 ps and 400-700 ps) are largely insensitive to the photochemical state of the reaction center, whereas the slowest (800-2000 ps) component has an amplitude that increases up to 30-fold as the reaction centers become progressively saturated by high illumination intensity or blocked by inhibitors or chemical reduction.

(2) The variable yield (amplitude) component of fluorescence exhibits a lifetime that is essentially independent of the state of the PS 2 reaction centers for thylakoids in the absence of added Mg^{2+}, but the lifetime doubles (800→1600 ps) upon closing the centers in thylakoids in the presence of 5 mM Mg^{2+}. This is interpreted to reflect the effectiveness of Mg^{2+} to promote excitation transfer among adjacent antenna domains.[2]

(3) Chemical redox titrations show two one-electron midpoint potentials at $E_{m,8.0}$ = 120 mV (Q_H) and -350 mV (Q_L),[3] which are close to those previously reported for the electron acceptors of β and α centers, respectively.

(4) Subchloroplast fragments that retain PS 2 and the ability to evolve oxygen, but show no detectable PS 1 activity,[3] exhibit fluorescence induction curves characteristic of α centers, with a small contribution from β centers. Redox titrations indicate that Q_H is the predominant acceptor present. These results argue against the association of α centers with Q_L only.

(5) Changes in the lifetimes of the intermediate and slow decay components reflect the extent

of migration of excitation among nearby photosynthetic pigment domains as well as the effectiveness of quenchers that deactivate the electronic excitation.

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1296 SUPRAMOLECULAR ORGANIZATION OF PHOTOSYNTHETIC MEMBRANES, L. Andrew Staehelin, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309.

Chloroplast membranes (thylakoids) of higher plants and green algae are spatially differentiated into unstacked (stroma) and stacked (grana) membrane regions, the functional significance of which has remained an enigma. Biochemical and structural studies have revealed that grana and stroma thylakoids differ in their composition. Highly enriched fractions of grana and stroma membranes can be obtained by mechanical disruption of the thylakoids followed by separation of the membranes on a sucrose gradient, or by means of an aqueous polymer two-phase separation system. Analysis of such fractions has revealed that, with the possible exception of the cytochrome f/b_6 complexes, all other intrinsic macromolecular complexes are nonrandomly distributed between grana and stroma thylakoids. Thus approx. 85% of PSII complexes and 70-90% of the chl a/b light harvesting complexes (chl a/b LHC) are found in the stacked membrane regions, while the nonappressed stroma membranes contain >85% of PSI and 100% of the ATPase. Freeze-fracture electron microscopy has confirmed and extended these observations and has led to the following tentative relationship between functional and structural membrane units: PSII reaction center complexes and 8 nm EF particles; PSII-tightly bound chl a/b LHC units and approx. 10.5, approx. 13 and approx. 16 nm EF particles; PSI-light harvesting antennae and 10-11 nm PF particles; cytochrome f/b_6 complexes and 8-9 nm PF particles; hydrophobic segment of the coupling factor and 9-10 nm particles; "free" chl a/b LHC units and 8-9 nm particles. Adhesion between grana thylakoids appears to be mediated largely by the "free" chl a/b LHC particles, and can best be modeled by assuming a combination of van der Waals attraction forces and electrostatic repulsive forces between the negatively charged membranes. Regulation of energy distribution between PSII and PSI has recently been shown to be correlated with the phosphorylation/dephosphorylation of the chl a/b LHC. Our current results reveal that in dark control (dephosphorylated) membranes, most of the LHC is found in the grana regions. Light-induced phosphorylation of the chl a/b LHC, which increases electrostatic repulsion between membranes, leads to a reduction of thylakoid stacking from 69% to 52% and to a net migration of chl a/b LHC from the PSII-rich grana membranes to PSI-rich stroma membranes, thereby allowing more of the trapped energy to be delivered to PSI. Upon dephosphorylation in the dark, the chl a/b LHC particles resegregate into the stacked membrane regions. These and other results demonstrate that phosphorylation of molecules involved in membrane adhesion can a) regulate the amount of membrane adhesion, b) control the spatial arrangement of these molecules, and c) thereby regulate membrane function. Supported by grant GM 22912.

1297 REGULATION OF INTERACTIONS AMONG PIGMENT-PROTEINS IN THYLAKOIDS, Charles J. Arntzen, MSU-DOE Plant Research Laboratory, East Lansing, MI 48824

Plants have evolved two mechanisms to adapt to variable light conditions in the environment. Long-term acclimation to low light (shade) involves the synthesis and accumulation of increased levels of antennae chlorophyll-proteins. The necessity for electron transport via two reactions in series requires that these pigments distribute excitation energy to both photosystem (PS) I and II; control over this distribution is accomplished via a second, short-term acclimation process utilizing the reversible phosphorylation of the light-harvesting complex (LHC) which primarily serves photosystem II. This second adaptive process adjusts for transient variations in light quality which occur within the plant canopies, as well as for selective cellular demands for increased ATP which can be generated via cyclic electron flow. The mechanism by which protein phosphorylation leads to an alteration in the distribution of absorbed excitation energy to PS I and II has now been elucidated. It is accomplished via the lateral movement of the LHC between the grana (enriched in PS II) and stroma lamellae (enriched in PS I). The data which lead to this conclusion, as well as a discussion of the importance of protein phosphorylation in optimizing the quantum efficiency of photosynthesis will be discussed.

Biosynthesis of the Photosynthetic Apparatus: Molecular Biology, Development and Regulation

1298 *DEVELOPMENT OF PHOTOCHEMICAL COMPETENCE IN HIGHER PLANTS.* Neil R. Baker[1], Andrew N. Webber[1], Michael Bradbury[1], John P. Markwell[2], Maxine G. Baker[1] and J. Philip Thornber[3]: [1]Department of Biology, University of Essex, Colchester CO4 3SQ, U.K.; [2]Department of Agricultural Biochemistry, University of Nebraska, Lincoln, NE 68583; [3]Department of Biology and Molecular Biology Institute, UCLA, CA 90024.

The development of energetic interactions between components of the photochemical apparatus during chloroplast biogenesis in wheat leaves grown under a natural diurnal light regime will be examined. Leaves of monocotyledonous plants, such as wheat, have a basal intercalary meristem and exhibit a progressive sequence of plastid differentiation from the base to the tip. Photochemically active PS1 and PS2 reaction centres and light-induced O_2 evolution were detected in the earliest, experimentally manageable stages of leaf development (the basal 0.5 cm segment of a 4 day old leaf). Although this tissue could photooxidise water and sustain a steady-state rate of electron flow to a terminal electron acceptor, which was not CO_2, for 4 min, the light-harvesting apparatus was poorly developed. Analyses of PS1 and PS2 fluorescence kinetics at 77°K demonstrate that excitation energy spillover from PS2 to PS1 occurs at this early stage, when the chl a/b is greater than 20, and then progressively increases in magnitude with chlorophyll synthesis. At the later stages of greening accumulation of large amounts of the light-harvesting chl a/b-protein (LHCP) decreases the efficiency of energy transfer from PS2 to PS1; the LHCP effectively competes with PS1 for available excitation energy within PS2 matrices. This phenomenon is similar to that observed in greening etiolated leaves. These fluorescence kinetic measurements at 77°K provide information solely on the energetic interactions of the chl matrices in the 'frozen' or 'static' membrane. Information on the development of 'dynamic' regulation of excitation energy distribution was obtained by studying the State I - State II transition (a reversible enhancement of PS1 at the expense of PS2) during the course of the fluorescence induction curve of greening tissue at 20°C. A State I - State II transition could not be detected shortly after the onset of chlorophyll synthesis, but developed rapidly and was almost maximal by the end of the lag phase of chlorophyll synthesis. The ability to perform a State I - State II transition did not appear to correlate with the accumulation of LHCP; a finding substantiated from studies of a chl b-less barley mutant. The enhancement of PS1 fluorescence during PS2 fluorescence quenching in the intact leaf may be attributable to either (i) a direct regulation of the rate of electron flow between PS2 and PS1 and/or (ii) an enhancement of the transfer of excitation energy from LHCP and PS2 to PS1, which is thought to occur as a result of thylakoid protein phosphorylation by a membrane-bound kinase. The development of the State I - State II transition *in vivo* did not appear to correlate with either changes in the total thylakoid protein kinase activity (expressed per unit of membrane protein), the specific phosphorylation of the LHCP or the capacity for ATP-induced PS2 fluorescence quenching by isolated thylakoids.

Agricultural Applications of Research on the Photosynthetic Apparatus

1299 HERBICIDE RECEPTOR PROTEINS OF CHLOROPLAST MEMBRANES, Katherine E. Steinback, Advanced Genetic Sciences, Inc., P.O. Box 3266, Berkeley, CA 94703.

A large percentage of commercial herbicides inhibit photosynthetic electron transport processes localized within the chloroplast thylakoid membrane; for several chemical classes of herbicides this inhibition is the primary mechanism of action. Herbicide-induced inhibition of Photosystem II (PS II) electron transport results from non-covalent, reversible binding of one herbicide molecule per PS II complex. The discovery of weed species showing extreme resistance to certain chemical classes of herbicides, notably the triazines, has led to the concept that the herbicide resistance trait can be transferred and/or induced in crop plants. An understanding of the molecular nature of the binding sites for inhibition of photosynthetic electron transport has direct bearing on this possibility as well as on rational chemical design of herbicides directed against PS II.

Research conducted in a number of laboratories has led to the concept that several herbicide classes, including ureas, amides, triazines, uracils and nitrophenols, display competitive binding properties, indicating that they share overlapping domains of interaction at a common binding site within the PS II complex. Furthermore, exogenously added plastoquinone analogs have recently been found to displace bound herbicides. These observations suggest that herbicides exert their inhibitory action by displacement of a native plastoquinone molecule (acting as the second electron carrier on the reducing side of PS II) from its binding site.

Within the PS II complex, several polypeptides are required for high affinity interaction of the herbicide with its binding site; these include a polypeptide of 32-34 kilodaltons and polypeptides of 42-47 kilodaltons. These polypeptides co-purify in isolated, photochemically active PS II reaction center preparations. Several lines of evidence suggest that the principle determinant of triazine herbicide binding sites is the polypeptide species of 32-34 kilodaltons; this polypeptide has been shown to be rapidly synthesized and turned over in green plants at all stages of development. That it serves as the site of triazine binding further suggests that this polypeptide may serve as the principle binding domain for native plastoquinone molecules participating as the second electron carriers of PS II (termed "B"). It is interesting to speculate that the rapid turnover of this membrane component is a consequence of its specialized function at the reducing site of PS II in that this protein is in proximity to its bound semiquinone anion during the course of light-dependent electron transport.

1300 HERBICIDE TARGET SITES, MODE OF ACTION AND DETOXIFICATION: CHLOROACETANILIDES AND GLYPHOSATE, Ernest G. Jaworski, Thomas J. Mozer, Stephen G. Rogers and David C. Tiemeier, Monsanto Corporate Research Laboratories, St. Louis, MO 63167

Chloroacetamides represent a unique class of selective herbicides which are widely used on all major agronomic crops. While their mode of action is not yet understood, considerable research has been done on their metabolism and detoxification (1). Information relating to a specific mechanism for their detoxification will be presented (2).

Glyphosate, N-phosphonomethylglycine, is probably one of the most interesting herbicides to be discovered in the past 25 years. This non-selective broad-spectrum postemergence weed killer may have several modes of action (3). A major pathway for its inhibitory effect was suggested as early as 1972 (4). It was hypothesized that the aromatic biosynthesis pathway was being blocked. Subsequent studies have implicated, specifically, the inhibition of EPSP synthase, an enzyme involved in the conversion of shikimate-3-phosphate to 5-enol-pyruvylshikimate-3-phosphate (5,6). Molecular genetic studies with cloned EPSP synthase have added credence to the hypothesis cited above and representative supporting data will be presented.

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1301 TRIAZINE AND OTHER PHOTOSYSTEM II HERBICIDES, H. M. LeBaron, Biochemistry Department, CIBA-GEIGY Corporation, Greensboro, NC 27409

The herbicidal properties of the phenylureas and s-triazines, which were discovered in the 1950's, were found to be due to an inhibition of the Hill reaction. Their primary mechanism of action is a blocking of electron transport between the primary electron acceptor Q and plastoquinone (PQ) in Photosystem II.

Isolated chloroplasts from both tolerant and susceptible plant species were found to be equally sensitive to these herbicides. Differential tolerance of intact plants was associated with differences in rates of herbicide metabolism, uptake and translocation.

A triazine resistant biotype among a previously susceptible population of weeds was first reported in 1970[1]. The distribution and frequency of this phenomenon have increased greatly since that time. Triazine resistance has now been confirmed in 31 weed species from 19 genera, mostly in northern U.S., Canada and northern Europe. In 1975, Radosevich discovered that photosynthesis by isolated chloroplasts from resistant biotypes was not inhibited by atrazine, leading to much research on the nature and mechanism of selectivity.

Triazine resistance is due to a change in the herbicide binding site of the chloroplast membrane, based on a minor modification of the protein in the PSII complex. The weed biotypes show cross resistance to all s-triazine herbicides, but range in sensitivity to diuron and other PSII herbicides from resistant to moderately susceptible, indicating that they have closely related, but not identical, binding sites. This chloroplast resistance is inherited through the female parent and is controlled by cytoplasmic DNA.

The triazines represent a major class of herbicides, with a worldwide use of almost 200 million pounds today. Nevertheless, herbicide resistance has not and should not become a major economic problem. We are fortunate to have a wide range of herbicides with different modes of action which can effectively control resistant biotypes. Since no single herbicide controls all weed species throughout the season, combinations of herbicides, tillage and/or other means of control had become common practice before resistance developed.

On the other hand, herbicide resistant weeds could be of great value to us in several ways. Triazine resistant biotypes will continue to be useful tools in plant physiological studies, such as photosynthesis, herbicide binding, and chloroplast structure. The extensive research to develop herbicide resistant plants via genetic engineering and conventional breeding should lead to important breakthroughs. Further details of this research, as well as the role of the 32 kilodalton polypeptide and of lipid metabolism and distribution in chloroplast membranes, will be discussed.

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Biosynthesis and Assembly of Chloroplast Components

1302 THE NATURE AND FUNCTION OF THE CHLOROPLAST FAS SYSTEM, Paul K. Stumpf, Department of Biochemistry and Biophysics, University of California, Davis, CA 95616

The chloroplast is a unique organelle in terms of fatty acid synthesis. It is the sole site for the synthesis of the C_{16} and C_{18} fatty acid in the leaf cell (1); it is the sole site for the introduction of the $C_{9,10}$ double bond system (2); it transports oleic acid to the cytosolic component of the leaf cell for further modifications; it synthesizes all of its complex lipids (3); it depends on the cytosolic compartment for the generation of its prime substrate, namely free acetate (4); by the mechanism of photophosphorylation, NADPH, molecular oxygen and ATP - required components for fatty acid synthesis and desaturation - are generated *in situ*.

Of further interest, the molecular structure of the fatty acid synthetase (FAS) in the chloroplast has recently been shown to be procaryotic in nature, i.e. very similar to a similar system described for the *E. coli* cell (5) rather than eucaryotic in properties. Unlike the eucaryotic system found in animal, yeast and fungi cells which is a polyfunctional polypeptide with homo- or heterodimeric properties (6), the chloroplast FAS system consists of 6 to 7 individual enzyme proteins that can be separated and purified. The β-ketoacyl ACP condensation reaction is controlled by two enzymes, the first responsible for the synthesis of the $C_2 \rightarrow C_{16}$ fatty acids and the second responsible for the conversion of the C_{16} to C_{18} fatty acid (7).

(Supported in part by an NIH Grant #29415-01.)

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1303 THE ROLE OF THE ENVELOPE MEMBRANES IN CHLOROPLAST BIOGENESIS, Kenneth Keegstra, Kenneth Cline, Jaen Andrews, Margaret Werner-Washburne, Department of Botany, University of Wisconsin, Madison, WI 53706.

The chloroplast envelope mediates at least two important processes during chloroplast development. The envelope is responsible for the synthesis of galactolipids (1), the major lipids in plastid membranes. It also functions in the uptake of cytoplasmically-synthesized plastid proteins (2). The envelope consists of a pair of bilayer membranes which differ dramatically in lipid and polypeptide composition (3) as well as enzyme activities (4). Each of these two membranes play a role in the sythesis of galactolipids. The early steps occur in the inner membrane while the final galactosylation steps occur in the outer (4). These rather unexpected findings suggest that during galactolipid synthesis there may be movement of intermediates and products between the two membranes. Recent observations made by freeze-fracture electron microscopy reveal the presence of contact sites between the two membranes. The possible role of these contact sites in galactolipid biosynthesis as well as protein uptake will be discussed.

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Biosynthesis of the Photosynthetic Apparatus: Molecular Biology, Development and Regulation

1304 DEVELOPMENT AND ORGANIZATION OF THE PHOTOSYSTEM II COMPLEX, Itzhak Ohad, Department of Biological Chemistry, The Hebrew University of Jerusalem Jerusalem, Israel

Photosystem II units in Chlamydomonas can be considered as supermolecular complexes consisting of the water splitting complex, the reaction center II, including the core antenna and Q-B complex, containing the "32 KD" polypeptide and the light harvesting chlorophyll a,b-protein complex. At least 6 polypeptides participating in the formation of these complexes are phosphoproteins (1). In addition, a membrane bound kinase and phosphoprotein phosphatase appear to be associated with the photosystem II unit and are responsible for the reversible phosphorylation of these polypeptides. A membrane bound protease might be involved in the light induced turnover of the "32 KD" polypeptide. The synthesis and assembly of these various PS II unit components is an independent stepwise process. Membranes containing PS II units deficient in different polypeptides can be formed in vivo in conditional algal mutants by alternate exposure to light-dark regimes and reversible inhibition of protein synthesis in the chloroplast or cytoplasm (2) or by exposing such cells to high light intensity (photoinhibition) (3). Drastic pleotropic effects are caused by such alterations of the PS II unit polypeptides concerning the phosphorylation pattern (4), functional assembly of its subcomplexes or formation of the herbicide binding site (5). The maturation of the PS II units during formation of photosynthetic membranes including the development of a normal phosphorylation pattern and segregation of PS II units into grana appears to be correlated with the formation of the light-harvesting antenna of photosystem I which is a late event in the developmental process.

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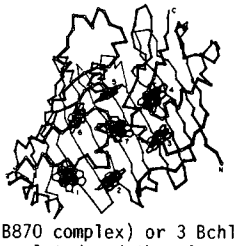
1305 DIFFERENTIAL REGULATION OF THE ACCUMULATION OF THE LIGHT-HARVESTING CHLOROPHYLL a/b COMPLEX AND RIBULOSE BIS-PHOSPHATE CARBOXYLASE/OXYGENASE IN GREENING PEA LEAVES, John Bennett, Gareth I. Jenkins and Martin R. Hartley, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom.

Chloroplast development involves the nucleus, cytoplasm and chloroplast of plant cells. This may be illustrated with respect to the major protein of the soluble phase of the chloroplast (the CO_2 fixing enzyme, ribulose 1,5-bisphosphate carboxylase/oxygenase) and the major protein of the thylakoid membranes (the light-harvesting chlorophyll a/b complex, LHC). The small subunit (SSU) of the carboxylase and the apo-protein (LHCP) of LHC are encoded in nuclear DNA, translated on cytoplasmic ribosomes and transported into the chloroplast in precursor form. Inside the chloroplast, the SSU combines with the large subunit (LSU) of the carboxylase which is encoded in chloroplast DNA and synthesized on chloroplast ribosomes. The LHCP enters the chloroplast, binds to the thylakoid membrane and becomes ligated with chlorophyll a and chlorophyll b, which are both synthesized within the organelle. We have studied the extent to which the biosynthetic events in the nucleo-cytoplasmic compartments are co-ordinated with those inside the chloroplast during the greening of pea leaves. We have examined the levels of LSU, SSU and LHCP using a highly specific radioimmune assay. The proteins are separated electrophoretically and then transferred to nitrocellulose filters, where they are probed with specific antibodies. Antibody-antigen complexes are located using (I^{125})-protein A. The steady state levels of the corresponding mRNAs have been determined using specific cloned cDNA probes. In the case of the SSU, the mRNA and protein levels are near the limit of detection in dark-grown plants but appear to increase in parallel during greening under continuous white light, with a lag of 12-24 h. The LSU and its mRNA show similar but not identical characteristics. In contrast, the LHCP mRNA is readily detectable in dark-grown plants but accumulates further under illumination. However, the LHCP itself is undetectable in dark-grown plants and appears only under continuous illumination with a lag of 3-6 h. The appearance of SSU and its mRNA is under simple phytochrome control but the photocontrols on LSU and LHCP mRNAs are yet to be fully characterized. We conclude that the syntheses of LSU and SSU are regulated largely, if not entirely, at the mRNA level and that co-ordination is close but not absolute. In the case of LHC, post-translational control is particularly important in the regulation of the accumulation of the protein in the thylakoid membrane. Continuous chlorophyll synthesis is required for the stabilization of the LHC, especially in young tissue.

Molecular Organization and Assembly of Bacterial Photosynthetic Membranes

1306 PHOTOCHEMICAL REACTION CENTERS AND LIGHT-HARVESTING ANTENNAE OF PHOTOSYNTHETIC BACTERIA, J. Philip Thornber, Richard J. Cogdell, Beverly K. Pierson, Richard E.B. Seftor, Department of Biology, University of California, Los Angeles, CA 90024

The lecture will provide plant-oriented researchers with a comparative, state-of-the-art description of the isolation and characterization of chlorophyll-proteins (CPs) of photosynthetic bacteria. CP research is more advanced for bacteria than for plants. For example, unlike plants, photochemical reaction centers (RCs) of many purple bacteria have been purified away from all extraneous material. The smallest isolated unit, which still performs the primary photochemical event exactly as occurs *in vivo*, contains 4 bacteriochlorophyll (Bchl), 2 bacteriopheophytin (Bph), one carotenoid, 1-2 quinone molecules, an Fe atom and 3 polypeptides (M_r=20-30,000) in a 1:1:1 ratio. RCs isolated from Bchl *b*-containing organisms, *R. viridis* and *T. pfennigii*, present certain advantages: the RC of *R. viridis* is the first photochemically active component to be crystallized [1] and both RCs have spectral and subunit compositions different from Bchl *a*-RCs. Further studies on the Bchl *b*-RCs will therefore lead to greater clarification of the mechanism of the primary event, in particular to the first unequivocal 3-D picture of the arrangement of Bchl and Bph in the RC. We have recently isolated the RC (M_r=52,000) from *Chloroflexus aurantiacus*, a thermophilic green bacterium which is thought to be a more primitive evolutionary form of all known photosynthetic organisms. Although analyses reveal this component is more similar to the purple than to the green bacterial (and plant) RCs, it has its own specific characteristics: 2 polypeptides (M_r=28 and 30,000) and a Bchl/Bph ratio = 3/3.



The best described of all CPs is a minor component of the antenna of green bacteria. As anticipated, the 7 Bchl molecules in each of the three subunits of this water-soluble Bchl-*a* protein are oriented at diverse angles within a 42,000 M_r protein sheath (Fig. 1). Will this bag-like structure be typical of other CPs? Improved procedures have yielded the antenna complexes of Bchl *a*-containing purple bacteria in pure form: a B870 (Bchl absorbing at 870 nm)-protein from all species and B800-850-protein from some bacteria. Of all detergent-soluble CPs, these two have perhaps the simplest composition, but, as yet have not been crystallized. Each complex is probably composed of two M_r ~ 6,000 polypeptides associated with one carotenoid and 2 Bchl (B870 complex) or 3 Bchl (B800-850 complex) molecules [2]. Some of these apoproteins are phosphorylated and the significance of this and their amino acid sequence will be discussed.

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1307 BIOSYNTHESIS OF THE PHOTOSYNTHETIC MEMBRANES OF *RHODOPSEUDOMONAS SPHAEROIDES*, Samuel Kaplan, Brian D. Cain, Timothy J. Donohue, William D. Shepherd and Grace S. L. Yen, Department of Microbiology, University of Illinois, Urbana, IL 61801

Previous studies on the biosynthesis of the photosynthetic membrane system (Intracytoplasmic Membrane, ICM) of *Rp. sphaeroides* has revealed that in steady-state phototrophic cells, new protein (incuding cytochromes and photochemically active proteins) and pigments are continuously synthesized and incorporated into preexisting ICM throughout the division cycle, whereas new phospholipid although continuouisly synthesized at the whole cell level is only incorporated into the ICM at the time of cell division (1). This observed uncoupling of macromolecule incorporation can be monitored by changes in the physical state of ICM lipids throughout the cell cycle (2) and by treatment of cells with cerulenin. The mechanism by which phospholipids are transferred from outside the ICM to the ICM remains to be determined (3). More recent studies have employed our finding (4) of a unique phospholipid molecule (N-acylphosphatidylserine) in the membranes of *Rp. sphaeroides* as a means of demonstrating the functional separation of phospholipid between the ICM and cytoplasmic membrane (CM) despite the physical continuity of these two membrane systems (5). Other investigations involving freeze-fracture studies of the isolated ICM lead to the conclusion that certain new proteins (fixed photosynthetic units) are incorporated into preexisting ICM as discrete units and reveal a cyclical change in packing density, but not size, of these units as the cell proceeds through the cell cycle. The packing density may also be independent of light intensity. Results will also be presented which suggest that when cells are returned to chemoheterotrophic conditions following photoheterotrophic growth (6), the ICM incorporates new protein at a rate slower than growth and assumes the characteristics of a respiratory membrane system. The implications of these findings on ICM synthesis and development will be discussed.

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1308 CORE SUBSTRUCTURE IN CYANOBACTERIAL PHYCOBILISOMES, Alexander N. Glazer, Jeffrey C. Gingrich, and Daniel J. Lundell, Department of Microbiology and Immunology, University of California, Berkeley, CA 94720.

Cyanobacterial phycobilisomes consist of two structural domains--rods and a core. The rod structure in all instances consists of stacks of phycocyanin discs (and phycoerythrin, or phycoerythrocyanin, if present) in association with linker polypeptides of 27-33 kilodaltons.[1] The core substructure is of two basic types--those with two (Synechococcus 6301) and those with three cylindrical elements (the majority of others). The two part core has been extensively examined.[2] Here we compare it with the three part core from Synechocystis 6701 mutant CM 25 (lacking phycoerythrin). A partial polypeptide composition of the core was determined by densitometric scanning of SDS gels; core components were isolated by sucrose density gradient centrifugation and chromatography on DEAE-cellulose and were characterized as to spectra and composition. There are 8 and 12 trimeric units in the core of Synechococcus 6301 and Synechocystis 6701 phycobilisomes, respectively. However, the copy numbers of complexes A and B are different (Table 1). The same types of complexes were present in each phycobilisome. Of note is the presence of two new biliproteins in Synechocystis 6701, of 99 and 18.5 kilodaltons, analogous to the 75 and 18.3 kilodalton polypeptides in Synechococcus 6301, and two potential terminal energy acceptor complexes, C and D. The implications are that the two cylindrical elements in the Synechococcus 6301 core are preserved in the Synechocystis 6701 core. These two cylinders rest on the thylakoid membrane and contain the terminal energy acceptors. The third cylindrical element in the Synechocystis 6701 core then would consist of only complexes A and B, neither of which is a terminal acceptor, and would not be in contact with the membrane.

TABLE I Phycobilisome Core Components

Synechococcus 6301 Complex	Copy #	λ^{f}_{max} [a]	Synechocystis 6701 Complex	Copy #	λ^{f}_{max}
A $(\alpha^{AP}\beta^{AP})_3$	2	660	$(\alpha^{AP}\beta^{AP})_3$	4	657
B $(\alpha^{AP}\beta^{AP})_3 \cdot 10.5K$	2	662	$(\alpha^{AP}\beta^{AP})_3 \cdot 10K$	4	660
C $(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP}) \cdot 10.5K$	2	678	$(\alpha_1^{APB}\alpha_2^{AP}\beta_3^{AP}) \cdot 10K$	2	678
D $(\alpha^{AP}\beta^{AP})_2 \cdot 18.3K \cdot 75K$	2	678	$(\alpha^{AP}\beta^{AP})_2 \cdot 18.5K \cdot 99K$	2	678

[a] AP, allophycocyanin; APB, allophycocyanin B; λ^{f}_{max}, fluorescence emission maximum.

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Transformation of Photosynthetic Systems

1309 THE REGULATION BY OXYGEN OF GENES FOR THE PHOTOSYNTHETIC APPARATUS OF RHODOPSEUDOMONAS CAPSULATA, Barry L. Marrs, Alan J. Biel and W. Gregg Clark, Department of Biochemistry, Saint Louis University School of Medicine, Saint Louis, MO 63104

Synthesis of the photosynthetic apparatus of Rhodopseudomonas capsulata and related bacteria is regulated by oxygen tension. Atmospheric pO_2 strongly inhibits the formation of photosynthetic membranes, including light-harvesting and reaction center pigment-binding proteins and bacteriochlorophyll biosyntheses. Carotenoid synthesis is depressed to a lesser extent. Photosynthetic membrane synthesis proceeds when pO_2 falls, whether or not light is present. At 0.1 atmospheric pO_2 for example, chromatophore formation is extensive, while the rates of respiration and growth remain the same as under atmospheric pO_2. The cloning and mapping of a region of the Rhodopseudomonas capsulata chromosome coding for the components unique to photosynthetic membranes has enabled analyses of the patterns of transcription of bacteriochlorophyll and carotenoid biosynthetic genes and pigment-binding protein genes. Two methods of transcriptional analysis were employed. We measured the change in transcription of the photosynthesis region during a transition from high to low pO_2 by Southern blotting. RNA sampled at various times during transition was labeled in vitro and hybridized to restriction fragments bearing the genes of interest. Three different responses to O_2 were identified. Regions coding for carotenoid biosynthetic enzymes showed no change in transcription in response to O_2, while transcripts hybridizing to regions containing bacteriochlorophyll biosynthetic genes showed a modest increase which leveled off after a few minutes. A dramatic increase in transcription, which did not level off during the course of the experiment (16 min), was shown by the DNA fragment that contains the rxcA gene. Mutations in rxcA result in the loss of reaction center and light-harvesting complex I peptides, so this may be the region that carries the structural genes for pigment-binding proteins, which are needed in stoichiometric rather than catalytic amounts. Our second approach was to isolate mutants of R. capsulata in which the Mud(lac) phage was inserted in the photosynthesis region of the chromosome, placing ß-galactosidase synthesis under the control of various local promoters. Insertions in the bchH or bchG genes, which code for the first and last steps of the bacteriochlorophyll-specific biosynthetic pathway, showed only a two-fold increase in ß-galactosidase specific activity for cells grown under low pO_2 compared to high. Bacteriochlorophyll precursors in both of these mutants are fifty times more concentrated at low pO_2

than at high, so an early step in the common tetropyrrole pathway is implicated as important in regulation. Carotenoid accumulation does not respond to pO_2 in these bacteriochlorophyll-less mutants. Mud(lac) insertions at other sites are currently under investigation. The results of the two methods are in excellent agreement, and suggest that regulation of transcription by pO_2 is restricted to a few key steps in photosynthetic membrane formation.

1310 TRANSFORMATION AND CLONING VECTORS OF THE CYANOBACTERIUM ANACYSTIS NIDULANS. Louis A. Sherman, Susan S. Golden and Carolyn Vann, Division of Biological Sciences, University of Missouri, Columbia, MO 65211.

The unicellular cyanobacterium, Anacystis nidulans R2, provides an excellent system for the cloning and analysis of genes coding for photosynthetic proteins. This organism performs oxygenic photosynthesis similar to green plants, but retains many important bacterial characteristics. These characteristics include the ability to transform cells with exogenous DNA, the existence of indigenous plasmids, and the relatively simple production and analysis of mutants. Because of the importance of transformation to our goals, we have studied this process in some detail. We have determined that non-photosynthesizing cells are capable of transformation and are transformed better than photosynthesizing cells. Using both chromosomal markers ($DCMU^{res}$) and plasmid markers (Ap^{res} and Cm^{res}), we have shown that: (a) there is no specific relationship between growth phase and cell competence--cells at any growth stage transform well; (b) the highest transformation frequencies are obtained at low input DNA concentrations, and for long incubation periods. At 25 ng plasmid DNA/10^8 cells, we obtain values as high as 5×10^{-6} transformants/total molecules of input DNA; (c) transformation in the dark results in at least a two-fold increase in transformants; and (d) treatment of cells with photosynthetic inhibitors such as DCMU, CCCP, valinomycin, and nigericin still permits high levels of transformation.

We have produced a series of hybrid cloning vectors that can replicate in both A. nidulans and Escherichia coli. This was accomplished by in vivo and in vitro manipulation of the A. nidulans indigenous plasmids and E. coli plasmids such as pBR328. One type of hybrid (e.g. pSG111) consists of pBR328 and the indigenous 5.3 Mdal plasmid, resulting in an 8.3 Mdal chimera which confers Ap^{res} and Cm^{res} to both parental organisms. We have also cloned the 33 Mdal A. nidulans plasmid into pBR328 (pLM101), although this hybrid is somewhat unstable upon transformation. Cosmid vectors have been constructed from the pSG series by inserting DNA-containing lambda cos into specific restriction endonuclease sites. We have tested the ability of some of these chimeras to act as vectors by cloning the gene cluster containing rRNA and tRNA genes (pAN4) into the hybrids. Using DNA from one of our DCMU-resistant mutants, we are cloning the genes responsible for this herbicide resistance. We have also utilized heterologous probes to detect specific photosynthesis genes. Using the E. coli unc genes, we have located at least three of the genes in A. nidulans that code for the H^+-translocating ATPase subunits. We are currently trying to determine if these genes are arranged in an operon similar to the E. coli gene cluster. This type of analysis has also enabled us to determine that certain photosynthesis genes (e.g., the large subunit of RuBP carboxylase) are coded by the indigenous plasmids. This work was supported by grants from the Competitive Grants Research Office of USDA and the DOE.

Molecular Biology and Biochemistry of the Photosynthetic Apparatus

1311 ORGANIZATION AND EXPRESSION OF THE NUCLEAR GENES WHICH ENCODE THE MAJOR CHLOROPHYLL A/B BINDING PRECURSOR POYLPEPTIDE IN PETUNIA. Pamela Dunsmuir and John Bedbrook*, CSIRO Division of Plant Industry, Canberra City Australia. *Present Address-Advanced Genetic Sciences, Inc., Berkeley California U.S.A.

Analysis of cDNA clones from Petunia leaf poly A^+ RNA indicates that there are at least five different genes for the major chlorophyll a/b binding precursor polypeptide which are transcribed. The cDNA clones show up to 11% nucleotide sequence divergence in the protein coding regions, and amuch greater level of divergence (greater than 60%) between the 3' untranslated regions of the different clones.

Characterization of Petunia genomic DNA using Southern hybridizations, and also through cloning in Charon 28 shows that there are at least sixteen nuclear genes for the major chlorophyll a/b binding precursor polypeptide. Further, the genes can be grouped into small multigene families containing between two and five closely related genes. At least one gene from each family is expressed in leaf tissue, and genes from the same gene family are closely linked in the genome for several of the gene families.

We are examining the sequences flanking the different genes as well as characterizing developmental and environmental effects on the expression of the different genes so that we may understand the factors which regulate the levels of these distinct chlorophyll a/b binding poplpeptides in the thylakoid membrane.

1312 CONSTRUCTION, MAPPING AND EXPRESSION OF CLONE LIBRARY OF N. OTOPHORA CHLOROPLAST DNA, Yu S. Zhu, Paul S. Lovett, Elizabeth J. Duvall, Shain-dow Kung, Department of Biological Sciences, University of Maryland Baltimore County, Maryland

A total of 27 out of 28 BamHI fragments of N. otophora ct-DNA have been cloned into pBR322 and transformed into HB101. In order to position these clones, a restriction map of BamHI and SmaI of N. otophora was constructed. The genes for 16S, 23S and 5S rRNA, and the LS of RuPBCase were located and marked on the map relative to the inverted repeat regions and the large and the small single copy.

Attempts to employ an in vitro plasmid-directed protein synthesis system in Maxicells to express the cloned fragments met with some success. pBR322 carrying ct-DNA fragments Bam7, 12 and 16 produced 3 ct-DNA coded polypeptides (MW 43, 29 and 13.5 kD). The LS gene, however, was not expressed due to internal cleavage of the gene by BamHI.

It was further demonstrated that unique restriction fragments generated by SmaI, SalI, HindIII or EcoRI contain intact LS genes suitable for expression studies. Fragments Sal 6 (14Kb) and Hind 2 (11 Kb), containing intact LS genes, were cloned into CSR 603 as PRCZ 1 and PRCZ 2, using pBR325 and pBR322 as the respective vectors. The LS genes in these clones were expressed, apparently using their own promoters in E. coli, as determined by an in situ immunoassay, Ouchterlony double diffusion and immunoelectrophoresis.

1313 BOTH THE CHLOROPLAST AND NUCLEAR GENOMES OF *CHLAMYDOMONAS REINHARDI* SHARE HOMOLOGY WITH *E. COLI* GENES FOR TRANSLATIONAL AND TRANSCRIPTIONAL COMPONENTS, John C. Watson and Stefan J. Surzycki, Department of Biology, Indiana University, Bloomington, IN 47405

Extensive sequence homology can be detected between *E. coli* genes encoding proteins of the translational and transcriptional machineries and both the chloroplast and nuclear genomes of *C. reinhardi*. Using appropriate heterologous DNA:DNA hybridization conditions, we previously found that ^{32}P-chloroplast (cp) DNA hybridizes to *E. coli* elongation factor (EF) Tu genes. With *E. coli* EF-Tu gene probes we mapped this gene on the chloroplast genome and determined the transcriptional polarity (*Proc Natl Acad Sci* 79: 2264). Here we report that cpDNA probes hybridize to either the *E. coli* r-protein L22 or S19 gene of the S10 operon, and at least one of three α operon r-protein genes (for S4, S11 and S13). Further, cpDNA probes hybridize to *E. coli* genes for the β and β' subunits of RNA polymerase. The regions homologous to r-protein and RNA polymerase genes were located on the cpDNA physical map by probing gel blots of cpDNA with labeled fragments carrying these *E. coli* genes. The S10 operon probe hybridizes to a region (fragment *Eco*12) between the EF-Tu gene and the rDNA-containing inverted repeat. The α operon probe hybridized to a region on the opposite side of the genome from the EF-Tu and S10 regions (fragment *Eco*13). Both the β and β' RNA polymerase gene probes reacted with a 3.85 kb region near one end of fragment *Eco*2. Probing *Eco*RI digests of nuclear DNA with *E. coli* gene probes revealed fragments homologous to the EF-G gene and the *spc* r-protein operon. Surprisingly, sequences homologous to the β subunit of RNA polymerase gene are present not only in cpDNA but in nuclear DNA as well.

1314 HOMOLOGY OF ANACYSTIS NIDULANS CHROMOSOMAL AND PLASMID DNA TO HETEROLOGOUS PROBES, Carolyn Vann, Gary H. Perrot and Louis A. Sherman, Division of Biological Sciences, University of Missouri, Columbia, MO 65211.

Chromosomal and plasmid DNA isolated from the cyanobacterium Anacystis nidulans R2 has been examined for homology to heterologous probes. Several probes carrying various subunits of the eight polypeptides of the proton-translocating ATPase operon (unc) of Escherichia coli (1) have shown homology to Anacystis chromosomal DNA. Probing with pRPG54, which contains the entire unc operon (6.5 Kbp), results in hybridization to a single 8 Kbp Hind III fragment of Anacystis. With probes containing shorter pieces of the unc operon, we have observed hybridization to at least 3 subunits: b or δ , α or γ and β or ε . Two other probes show homology to both Anacystis chromosomal and plasmid DNA. One of these is a probe from the cyanobacterium Anabaena 7120, pAn602 (2), which contains approximately 85% of the gene for the large subunit of RuBP carboxylase as well as additional flanking DNA. The other probe is pCR34.1 (3), which contains an internal fragment of the carboxylase large subunit gene from Chlamydomonas reinhardtii chloroplast DNA. Chromosomal DNA has been restricted with Eco RI and cloned into the lambda vector Charon 30 to construct a library of Anacystis genes. Completion of the library now permits DNA sequences showing homology to heterologous probes to be further characterized.

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1315 THE SMALL SUBUNIT OF RIBULOSE BISPHOSPHATE CARBOXYLASE MAY BE A CHLOROPLAST DNA ENCODED PROTEIN IN THE CHROMOPHYTIC ALGA, OLISTHODISCUS LUTEUS, Michael Reith and Rose Ann Cattolico, Univ. of Washington, Seattle, WA 98195

In all higher plants and green algae investigated, the large subunit of ribulose bisphosphate carboxylase (RuBPCase) is encoded on the chloroplast DNA while the gene for the small subunit is encoded in the nucleus. In the Chromophytic alga (chlorophyll a/c line), Olisthodiscus luteus, both of these genes may be present on the chloroplast DNA. Olisthodiscus cells were labelled with ^{14}C-sodium bicarbonate in the presence of several protein synthesis inhibitors. When inhibitors of cytoplasmic protein synthesis (cycloheximide or anisomycin) were added, both subunits of RuBPCase became labelled. In the presence of chloramphenicol or streptomycin (inhibitors of chloroplast protein synthesis) neither of these proteins was synthesized. Hybrid selection experiments appear to support these results. Cloned DNA representing approx. 65% of the total chloroplast genome was denatured and fixed to nitrocellulose filters. The filters were hybridized to total cellular RNA, washed extensively and the RNA eluted. The RNA was translated in a wheat germ in vitro translation system and the products displayed on SDS gels. Among the selected proteins were two with molecular weights corresponding to those of the large and small subunits of RuBPCase (58,000 and 16,500 d, respectively). Antibodies to the two subunits are being produced and will be used to verify these results. Mapping studies are also in progress to locate these genes on Olisthodiscus chloroplast DNA.

1316 *In vitro Transcription of Spinach Chloroplast Genes With Chloroplast and E. coli RNA Polymerases.* *Emil M. Orozco, Jr., John E. Mullet and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, New York, NY 10021*

The 5'-termini of the genes for the beta subunit of the chloroplast ATPase and the large subunit (LS) of ribulose bisphosphate carboxylase are within a 1040 base pair MspI-PstI DNA fragment. This DNA was ligated to both pUC8 and pUC9 DNAs to produce two recombinant DNAs containing the insert in either orientation. In vitro transcription experiments were performed with these recombinant DNAs as templates and the 5'-termini of the products were analyzed by S1 mapping techniques. The in vitro transcripts of the beta gene produced by chloroplast RNA polymerase have primarily two 5'-termini, one of which corresponds to the 5'-terminus of a major in vivo mRNA. The in vitro transcripts of the LS gene produced by the chloroplast RNA polymerase have predominantly the same 5'-end as the in vivo LS mRNA. These results were obtained irrespective of the orientation of the chloroplast DNA insert in the recombinants. E. coli RNA polymerase also initiates transcription of the beta and LS genes at specific sites. However, the transcriptional initiation of these genes with E. coli RNA polymerase is dependent on their orientation with respect to the vector DNA background. We are currently using the chloroplast in vitro transcription system to determine the nucleotide sequences necessary for accurate transcriptional initiation of chloroplast genes. *(Supported by NIH GM30726 and Monsanto Company)*

1317 AUTOREGULATION OF TRANSLATABLE PHYTOCHROME mRNA LEVELS. Peter H. Quail, James T. Colbert and Howard P. Hershey, Botany Dept., University of Wisconsin, Madison, WI, 53706.

Translatable phytochrome mRNA represents ~10^{-2}% of the total poly(A) RNA present in etiolated Avena seedlings as judged by incorporation of radioactivity into the immunoprecipitable apoprotein in a cell-free translation system. Poly(A) RNA from green tissue, in contrast, contains no detectable translatable phytochrome mRNA. Irradiation of etiolated seedlings with 5 s red light induces a decline in this mRNA that is detectable within 15-30 min, shows a 50% reduction within 50-60 min and results in a >20-fold reduction within 2 h. The effect of the red light pulse is reversed by an immediately subsequent far-red pulse indicating that phytochrome exerts autoregulatory control over its own translatable mRNA level. Red-light dose response curves show that the system is sensitive to very low light levels. Conversion of <1% of the total cellular phytochrome to the active Pfr form is sufficient to induce ~60% of the maximum response and 20% Pfr saturates the response. The change in translatable phytochrome mRNA levels is ironically one of the most rapid phytochrome-induced alterations in any cellular mRNA yet recorded. Thus autoregulation of phytochrome mRNA presents an attractive opportunity to examine the early sequence of events in phytochrome control of gene expression. Phytochrome cDNA probes will be used to determine, by northern blot analysis, whether the changes in translatable mRNA are the result of changes in phytochrome mRNA mass and the results will be reported.

1318 PLASTOME MUTATION IS A POST-TRANSCRIPTIONAL DEFECT, Barbara B.Sears and Reinhold G. Herrmann, University of Düsseldorf, Federal Republic of Germany

Non-Mendelian mutations affecting the photosynthetic machinery are either pleiotropic or may be traced to specific mutational defects. In our analysis of a plastome (plastid genome) mutation of Oenothera hookeri, we have found a fused protein product of the β and ε subunits of CF_1. Other investigations have shown that these two genes are located next to each other on the cpDNA molecule and are transcribed together onto one messenger RNA (Zurawski et al., PNAS 79:6260). Our immunoanalysis and peptide mapping of proteins isolated from the mutant plants suggest that the two genes are at least partially fused. However in vitro translation of the mRNA results in β and ε polypeptides which are equivalent to those of wild-type. Furthermore, Northern analysis of the mRNAs reveals no differences, and DNA analysis shows that no major changes have occurred in the β-ε region. These data indicate that the mRNA and structural genes for β and ε are not altered in this mutant. We suggest that a defect in the translational machinery of the plastid is responsible for the mutant phenotype.

1319 NUCLEOTIDE SEQUENCE OF THE GENE FOR THE LARGE SUBUNIT OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE FROM THE CYANOBACTERIUM ANABAENA 7120, Stephanie E. Curtis and Robert Haselkorn, the University of Chicago

The nucleotide sequence of the gene for the large subunit of ribulose-1,5-bisphosphate carboxylase (rbc A) from the nitrogen-fixing cyanobacterium, Anabaena 7120, has been determined. The sequence of the rbc A coding region, the corresponding amino acid sequence and gene flanking regions are presented and compared with the nucleotide sequences of rbc A genes from Chlamydomonas, spinach and maize. The Anabaena rbc A coding region shows 70-75% homology with the corresponding regions from the plant genes. Most nucleotide changes occur in the third positions of codons, often giving no change at the amino acid level. With the exception of the ribosome binding (Shine-Dalgarno) site of the 5' flanking region, there is no significant homology between the 5' or 3' flanking regions of the Anabaena and plant rbc A genes. The Anabaena large subunit protein sequence, derived from translation of the rbc A nucleotide sequence, shows considerable homology (80-85%) with the proteins from plant sources. There are long stretches of unbroken homology, including regions identified as containing residues at the active site of the enzyme. Amino acid homology is poorest at the extreme amino termini of the large subunit proteins, with the Anabaena protein differing in length at this end by one additional amino acid. There is no length variation at the carboxy termini of the large subunit proteins. Analysis of codon utilization in the Anabaena rbc A gene reveals asymmetries also observed in two other sequenced Anabaena genes.

1320 DARK REPRESSION OF PLASTID DEVELOPMENT IN EUGLENA GRACILIS IS NOT ACCOMPANIED BY MAJOR CHANGES IN PLASTID DNA METHYLATION. E. Brownell*, L. Kaufman#, H. Lyman*#
*Deparment of Anatomical Sciences and #Cellular and Developmental Biology Program, S.U.N.Y. Stony Brook, Stony Brook, N.Y. 11794.
Dark grown cells of the phytoflagellate Euglena gracilis contain about 10 proplastids, each of which synthesizes protochlorophyll, plastid rRNA, and a few plastid-specific proteins. When exposed to light, these proplastids develop into fully functional chloroplasts. During the developmental transition from proplastid to chloroplast, many new cytoplasmic and plastid components are synthesized, including chlorophyll, carotenoids, and lipids. If these light grown cells are then returned to the dark, the chloroplasts will subsequently revert to the proplastid phenotype.

In this study, we asked whether dark repression of chloroplast development is accompanied by changes in plastid DNA methylation patterns. We have assayed proplastid and chloroplast DNAs with several restriction enzymes that are known to be sensitive to base modification. Our preliminary data suggest that there are no gross DNA methylation differences between these two developmental stages. We therefore conclude that: 1. plastid DNA may be modified at bases that we cannot assay by conventional restriction enzyme techniques; 2. dark repression may be related to changes in nuclear gene expression and/or modification; 3. the minor amount of modification that we do observe in plastid DNA may play an important regulatory role in a small subset of plastid genes.

1321 STABLE INTEGRATION OF FOREIGN DNA INTO A CYANOBACTERIAL CHROMOSOME, John Williams and Aladar Szalay, Boyce Thompson Institute for Plant Research, Ithaca, NY 14853
Synechococcus R2 is a unicellular cyanobacterium that posseses an oxygenic photosynthetic system like that of the chloroplast and also has a natural capacity to be transformed efficiently by DNA. We found that Synechococcus R2 is transformed to antibiotic resistance by chimeric DNA consisting of a fragment of Synechococcus R2 chromosomal DNA linked to antibiotic resistance genes from E. coli. The foreign genes must be linked to cyanobacterial DNA to be effective in transformation. Analysis of transformants by Southern hybridization indicates that chimeric DNA integrates into the recipient chromosome by homologous recombination. When integration occurs by addition (e.g., by a single reciprocal cross-over between a circular chimeric DNA molecule and recipient chromosome), the integrated foreign genes are flanked by a duplication of cyanobacterial DNA and consequently are unstable. Stable transformants are produced, however, when integration occurs by replacement of recipient DNA with homologous DNA containing the foreign insertion. Foreign DNA can integrate by replacement only when linked in a chimeric molecule so as to interrupt the continuity of the cyanobacterial DNA. The latter is not duplicated by the replacement mechanism and consequently the the foreign DNA is stable. As many as 1 cell in 300 is transformed, and stable transformants are produced about 100-fold more frequently than unstable ones. Foreign DNA 20,000 nucleotide base pairs in length can be integrated by replacement. Mutagenesis is associated with transformation, dependent on the location of the foreign insertion within the cyanobacterial DNA. These properties of transformation in Synechococcus R2 can be exploited to modify precisely and extensively the genome of this photosynthetic organism.

1322 LIGHT INDUCED DEVELOPMENT OF mRNAs IN *Euglena gracilis*, M. J. Hollingsworth and R. B. Hallick, University of Colorado, Boulder, CO 80309
The chloroplast DNA of *Euglena gracilis* codes for a number of protein genes. When grown on heterotrophic media in the dark, *Euglena* contain only undeveloped etioplasts. It is possible to follow their maturation to chloroplasts and the concomitant induction of mRNAs by exposing the dark grown *Euglena* to light. Whole cell RNAs from 0, 12, 24, 36, 48, 60, and 72 hour stages of light induced development have been purified from *Euglena*. Northern blots of these RNAs have been probed with radioactively labeled DNAs specific for the large subunit of ribulose bisphosphate carboxylase, the β subunit of CF_1-ATPase, the 32 kd polypeptide of Photosystem II, and the EF-Tu genes. In all cases, at least two signals were seen for each developmental stage. The higher molecular weight RNAs could be precursors to the lower molecular weight RNA. The EF-Tu probe was the only one that gave a constant signal from 0 to 60 hours. All the others showed a marked increase in signal as the exposure to light increased.

1323 ANALYSIS OF A *Euglena gracilis* tRNA GENE CLUSTER: LOCALIZATION BY OLIGODEOXYNUCLEOTIDE tRNA GENE PROBE HYBRIDIZATION AND SEQUENCE ANALYSIS, Gerald D. Karabin and Richard B. Hallick, University of Colorado, Boulder, Colorado 80309

The organization and the nucleotide sequence of the tRNA genes in the 2.0 kilobase pair (kbp) *Eco* RI restriction fragment *Eco* Q of *Euglena gracilis* Klebs, strain Z Pringsheim chloroplast DNA have been determined. This fragment, cloned in pBR325 to form the plasmid pEZC300, contains five tRNA genes. The tRNA gene locus in *Eco* Q has been mapped by Southern gel analysis using a chemically synthesized ^{32}P-labeled tetradecadeoxynucleotide tRNA gene probe. The DNA sequence of 870 bp from *Eco* Q containing the entire tRNA gene locus was determined. The DNA sequence of the tRNA genes was used to deduce the primary and secondary structures of the tRNAs. The organization of this tRNA gene cluster is $tRNA^{Gln}_{UUG}$-14 bp spacer-$tRNA^{Ser}_{GCU}$-174 bp spacer-$tRNA^{Met}_{CAU}$-12 bp spacer-$tRNA^{Gly}_{GCC}$-5 bp spacer-$tRNA^{Thr}_{UGU}$. The polarity of the $tRNA^{Gln}_{UUG}$ and $tRNA^{Thr}_{UGU}$ genes is of the opposite orientation with respect to the other three tRNA gene sequences, but of the same polarity as the rRNA genes located approximately 40 kbp downstream. The $tRNA^{Met}$ gene is a putative initiator tRNA. The five tRNA genes are separated and flanked by A-T rich spacer sequences. This gene arrangement is consistent with the model that *Euglena gracilis* chloroplast tRNA genes are transcribed into multicistronic tRNA precursors.

1324 USE OF SYNTHETIC OLIGONUCLEOTIDES FOR THE ISOLATION OF THE CLONED DNA SEQUENCE CORRESPONDING TO THE PHYCOCYANIN β-SUBUNIT OF *ANACYSTIS NIDULANS* R-2, Nicole Tandeau de Marsac*, Jean Houmard*, Marie-Christine Rebière*, Anne-Marie Castets*, Isabelle Saint-Girons†, Catherine Gouyette††, Annick Popinel††, Tam Huynh-Dinh††, and Jean Igolen††, *Unité de Physiologie Microbienne, †Unité de Biochimie Cellulaire, ††Unité de Chimie Organique, Dpt. B.G.M., Institut Pasteur, 28 rue du Docteur Roux, F-75724 Paris Cedex 15

A gene library from *Anacystis nidulans* R-2 has been recently constructed and successfully used to select a cloned gene involved in methionine biosynthesis (1). This strain is closely related to *Synechococcus* 6301 for which the amino acid sequence of the phycocyanin β-subunit (β-PC) has been determined. We have therefore used specific synthetic oligonucleotides as hybridization probes to look for the gene corresponding to β-PC in our gene library.

Our results showed that the recombinant cosmid pTH445 contained a DNA sequence complementary to one of the eight oligonucleotides synthesized. The cloned DNA from pTH445 has been further characterized by restriction analysis and nucleotide sequencing.

(1) Tandeau de Marsac, N., Borrias, W.E., Kuhlemeier, C.J., Castets, A.M., van Arkel, G.A. and van den Hondel, C.A.M.J.J. (1982) *Gene*, 20, 111-119.

1325 REGULATION OF LIGHT-HARVESTING CHLOROPHYLL-BINDING PROTEIN (LHCP) mRNA IN CHLAMYDOMONAS, Stephen H. Howell, Hurley S. Shepherd, Gerard Ledoigt, Patrice Imbault and Udo Johanningmeier, Biology Dept. C016, University of California San Diego, La Jolla, California 92093.

Light-harvesting chlorophyll a/b protein (LHCP) synthesis is highly regulated during the cell cycle in light-dark synchronized C. reinhardi cells. LHCPs are a family of cytoplasmically synthesized proteins which are imported into the chloroplast. LHCPs are derived from at least two precursor proteins (32 kd and 30 kd) that are synthesized in vitro and immunoprecipitated by antiserum against chlorophyll-protein complex II proteins. A DNA copy of the mRNA encoding a 32 kd LHCP precursor was cloned from CDNA synthesized from poly(A) RNA obtained from mid-light-phase synchronous cells. Using cloned cDNA (pHS16) as a hybridization probe, we found that a single 1.2 kb RNA complementary to pHS16 accumulates in a wave-like manner during the mid-light phase of the 12 hr light-12 hr dark cycle and correlates with the pattern of chlorophyll synthesis. Light, during the light phase in the light-dark cycle, is required for accumulation of this RNA.

Supported by a grant from the NSF.

1326 HIGH RATES OF PROTEIN SYNTHESIS BY ISOLATED INTACT CHLOROPLASTS, Leonard Fish, Raymond Deshaies and Andre T. Jagendorf, Plant Biology Section, Cornell University, Ithaca, NY 14853

Recent procedural improvements with intact chloroplasts isolated from young pea plants have led to rates of protein synthesis up to 100 times faster than any previously reported. Currently up to 100 nmoles of ^{3}H-leucine are incorporated/mg Chl in a 20 min incubation, with an apparent initial rate of 750 nmoles/mg Chl/hr. SDS gels of labeled thylakoid membranes show over 40 radioactive polypeptides, compare to the 9 to 15 reported previously. Calculations of doubling time show that these rates are equal to those for doubling of chlorophyll in the original pea plants; so *in vivo* rates have been achieved. Since leucine is 10 mol % of the proteins made, and 4 ATP are needed for each amino acid, protein synthesis under these conditions consumes ATP at 30 μmoles/mg Chl/hr. Slightly higher rates of leucine incorporation are found when ATP is supplied in the dark, as long as equimolar Mg^{++} is also added. This, together with the absence of any appreciable lag, shows that the enzymes for protein synthesis do not require light-activations the way Calvin cycle enzymes do. An inhibition by excess ATP can be traced in part to chelation of Mg^{++}; these chloroplasts leak Mg^{++} slowly and can lose up to 2/3 of their initial content in a 30 min incubation unless the external free Mg^{++} conc. is 0.75 mM.

Supported in part by a grant from USDA/SEA/CRGO Photosynthesis Program.

1327 PHOTOSYNTHESIS AND ACTIVATION OF RIBULOSE BISPHOSPHATE CARBOXYLASE IN WHEAT SEEDLINGS. REGULATION BY CO_2 AND O_2. John T. Perchorowicz and Richard G. Jensen. Departments of Plant Sciences and Biochemistry, University of Arizona, Tucson, Arizona 85721

Photosynthetic carbon assimilation in plants is regulated by the activity of ribulose 1,5-bisphosphate carboxylase/oxygenase. Increasing irradiances illuminating wheat seedlings increased net CO_2 fixation along with the activation of ribulose bisphosphate carboxylase. Although the carboxylase requires CO_2 to activate the enzyme, changes in external CO_2 between 100 and 1400 μl (l)$^{-1}$ did not cause changes in activation of the leaf carboxylase in the light. With these CO_2 levels and 21% O_2 or 1% or less O_2, the levels of ribulose bisphosphate were high and not limiting for CO_2 fixation. With high leaf ribulose bisphosphate the $K_{act}(CO_2)$ of the carboxylase must be lower in the dark, where ribulose bisphosphate is quite low in leaves. When illuminated in the absence of CO_2 or O_2, activation of the carboxylase dropped to zero while ribulose bisphosphate levels approached the binding site concentration of the carboxylase, probably by forming the inactive enzyme-ribulose bisphosphate complex.

The mechanism for changing activation of the ribulose bisphosphate carboxylase in the light involves not only Mg^{2+} and pH changes in the chloroplast stroma, but probably also ribulose bisphosphate concentration. In light when ribulose bisphosphate is greater than the binding site concentration of the carboxylase, Mg^{2+} and pH probably determine the ratio of inactive enzyme-ribulose bisphosphate to active enzyme-CO_2-Mg^{2+}-ribulose bisphosphate forms. Higher irradiances favor more optimal Mg^{2+} and pH and greater activation of the carboxylase leading to greater photosynthesis. Supported by the Science and Education Administration of the United States Department of Agriculsture under Grant 5901-0410-8-0114-0.

1328 LIGHT DEPENDENT ASSEMBLY OF RIBULOSE BISPHOSPHATE CARBOXYLASE. Harry Roy, Mark Bloom, and Patrice Milos. Rensselaer Polytechnic Institute, Troy, N.Y. 12181

Ribulose 1,5-bisphosphate carboxylase (RuBPCase) is composed of eight small subunits (14 kD) and eight large subunits (55 kD). Newly synthesized large subunits are associated with two complexes having sedimentation coefficients of 7S and 29S.

Assembly of RuBPCase occurs in isolated intact chloroplasts in the light, but not in the dark. When extracts of chloroplasts are treated with ATP and GTP, RuBPCase assembly is accelerated while the 29S large subunit complex is maintained. In the presence of Mg^{2+}, ATP brings about almost complete dissociation of the 29S complex, while GTP and a nonhydrolysable analog of ATP are without effect. These results indicate the existence of a complex set of reactions involving nucleotides, Mg^{2+}, and several putative intermediates in RuBPCase assembly. It is postulated that these reactions at least partly account for the light dependence of RuBPCase assembly. In particular, ATP and GTP promote the assembly of large subunits into RuBPCase.

This material is based upon work supported by the U.S. Department of Agriculture under agreement No. 59-2367-1-1-633-0. Any opinions, findings, and conclusions or recommendations expressed in this publication and those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

1329 CHLOROPLAST GENE DOSAGE REGULATES THE LEVEL OF RuBP CARBOXYLASE/OXYGENASE HOLOENZYME IN CHLAMYDOMONAS REINHARDII, William L. Ogren, Robert J. Spreitzer and Chris J. Chastain, U. S. Department of Agriculture and University of Illinois, Urbana, IL 61801

Five uniparentally-inherited Chlamydomonas reinhardii mutants have been recovered that lack ribulose-1,5-bisphosphate carboxylase and oxygenase activities. Each mutation results in a light-sensitive acetate-requiring phenotype and is genetically associated with every other mutation at the rcl-u-1 chloroplast locus. The rcl-u-1-18-5B mutation results in the loss of the chloroplast-encoded large subunit and nuclear-encoded small subunit. Photoautotrophic revertants of the 18-5B mutant, selected to explore the possibility of altering the carboxylase/oxygenase ratio and to investigate chloroplast genetics, were recovered spontaneously at a frequency of 6×10^{-6}. Five revertants from three independent experiments were maintained on minimal medium for further study. Each had a reduced level of holoenzyme protein (50% of wild-type) when grown in minimal medium in the light. The level was even lower in acetate medium in the dark (15% of wild-type). The revertants segregated acetate-requiring colonies in a non-mendelian, biparental pattern when crossed with wild-type mt- or when grown vegetatively in acetate medium in the dark. Since the acetate-requiring segregants can only survive on acetate medium in the dark, these results suggest that multiple mutant and revertant alleles of a single chloroplast gene are present in photoautotrophically-grown revertant cells. Thus, we conclude that a reduced number of functional large subunit genes results in a reduced level of holoenzyme protein. The specific activity of carboxylation and the carboxylase/oxygenase ratio of the revertant enzymes are normal. The heteroplasmic characteristic of the revertants is stable and heritable.

1330 SYNTHESIS OF PHOTOSYNTHETIC PROTEINS AND MESSAGES DURING MAIZE LEAF DEVELOPMENT, Timothy Nelson and William Taylor, Genetics Dept., Univ. of Calif., Berkeley, CA

The accumulation during maize leaf development of major thylakoid membrane complexes and soluble carbon fixation enzymes was followed by immunological measurement of marker proteins, generating a schedule of appearance for each enzyme or protein complex. A detailed schedule of the corresponding mRNA accumulation during development was generated both by immunoprecipitation of in vitro translation products of leaf RNA and by direct measurement of individual mRNAs with cloned cDNA probes for nuclear genes. Expression of specific chloroplast genes was followed with cloned chloroplast DNA probes. Proteins representative of electron transport and ATP generation appear early in development, photosystem and carbon fixation proteins appear next, followed by proteins enabling the generation of reducing power. The schedule of mRNA appearance differs in several cases from the schedule of protein appearance.

1331 ASSEMBLY OF PHOTOSYSTEM II: ASPECTS OF NUCLEAR REGULATION, Kenneth Leto and Lee McIntosh[1], Central Research and Development, DuPont Co., Wilmington, De. 19801 and [1]MSU/DOE Plant Research Lab, Michigan State University, E. Lansing, Mi. 48824

The green nuclear maize mutant hcf*-3 lacks PS-II reaction centers. Upon electrophoresis at 4C, hcf*-3 lamellae exhibit the loss of prominent stainable 47 and 32kD polypeptides. While CPa was present in both WT and hcf*-3 thylakoids, fluorescence visualization revealed two novel chla-protein complexes of differing carotenoid composition in WT membranes. The apoprotein of both novel complexes was the 47kD species as determined by proteolytic mapping. Both novel complexes were seen in a PS-II particle lacking CPa. The 47kD polypeptide is a probable component of the PS-II reaction center, while neither CPa nor it's apoprotein are required for PS-II activity.

Hcf*-3 thylakoids fail to accumulate the chloroplast-encoded 32-34kD lamellar polypeptide when attatched leaves are fed ^{35}S-methionine. Nevertheless, mRNA encoding this polypeptide is present in total hcf*-3 ctRNA as evidenced by Northern hybridization. Total hcf*-3 ctRNA directs the synthesis of a 34kD polypeptide in a cell-free translation system. Thylakoids prepared from intact chloroplasts fed a short pulse of ^{35}S-methionine in vitro accumulated label at 34kD. This suggests that the nucleus controls the stabilization/turnover, but not the initial integration or synthesis of the 32kD polypeptide in hcf*-3.

1332 ISOLATION OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE FROM THE MARINE CHROMOPHYTE OLISTHODISCUS LUTEUS, Scott Newman and Rose Ann Cattolico, Univ. of Wash. Seattle98195
Research on the enzyme RuBP carboxylase has been limited to bacteria, cyanobacteria, and Chlorophytic (chl. a,b) algae and higher plant representatives. In this study, we present data on the analysis of this important CO_2-fixing enzyme from a representative of the superphyla Chromophyta (chl. a,c containing plants).

Cells were disrupted using a French press and unbroken fragments were removed by centrifugation. The recovered supernatent was layered on a 1.2M sucrose pad and centrifuged at 105K x g for 2 hours. Virtually no activity remained in the supernatent. The enzyme obtained from the dense sucrose layer was precipitated with $(NH_4)_2SO_4$ (35% saturation). Further purification of RuBPCase was achieved by cenrifuging the redissolved pellet through a 10-40% linear sucrose gradient. Enzyme activity was monitored throughout the isolation by incorporation of $^{14}CO_2$ into an acid insoluble product, and enzyme purity determined by silver stain analysis of 12% PAG gels.

We have observed that the Olisthodiscus luteus holoenzyme is large (the Chlamydomonas holoenzyme does not enter a 1.0M sucrose step gradient) and this enzyme responds differently to $(NH_4)_2SO_4$ precipitation (most other RuBPCases require 55% $(NH_4)_2SO_4$ saturation to precipitate this protein). Studies are in progress to analyze the evolutionary relatedness of O.luteus RuBPCase to other plant species.

1333 GLYOXYLATE AND GLUTAMINE EFFECTS ON PHOTOSYNTHETIC CARBON METABOLISM IN CHLOROPLASTS AND CELLS OF SPINACH, Arthur L. Lawyer, Karen L. Cornwell, Sherry L. Gee, and James A. Bassham, Chevron Chem. Co., 940 Hensley St. Richmond, CA 94804 (A.L.L.) and Lab. Chem. Biodynamics, Lawrence Berkeley Lab., Univ. Ca., Berkeley, Ca 94720.

Addition of mM sodium glyoxylate to spinach chloroplasts was inhibitory to photosynthetic incorporation of $^{14}CO_2$ under conditions of both low (400 ppm) and high (9 mM) CO_2. Incorporation of ^{14}C into most metabolites decreased. Labeling of 6-P-gluconate and fructose-1,6-bis-P increased. This suggested that glyoxylate inhibited photosynthetic carbon metabolism indirectly by decreasing the reducing potential of chloroplasts through reduction of glyoxylate to glycolate. This hypothesis was supported by measuring the reduction of ^{14}C-glyoxylate by chloroplasts. Incubation of isolated mesophyll cells with glyoxylate had no effect on net photosynthetic CO_2 uptake but increased labeling was observed in 6-P-glyconate, a key indicator of decreased reducing potential. The possibility that glyoxylate was effecting photosynthetic metabolism by decreasing chloroplast pH cannot be excluded. Increased ribulose-1,5-bis-P and decreases in P-glyceric acid and glycolate upon addition of glyoxylate to chloroplasts suggested that ribulose-bis-P carboxylase and oxygenase might be affected either indirectly or directly by glyoxylate inhibition. Glyoxylate decreased labeling from $^{14}CO_2$ into glycolate and glycine by isolated mesophyll cells but had no effect on photosynthetic metabolism in chloroplast preparations but did increase $^{14}CO_2$ incorporation by 15% in isolated mesophyll cells under air levels of CO_2.

1334 IN VITRO EXPRESSION OF CHLOROPLAST GENES IN LYSATES OF HIGHER PLANT CHLOROPLAST, D.P. BOURQUE, D. ZAITLIN, J.D.J. BARD, UNIVERSITY OF ARIZONA, TUCSON, AZ 85721

An in vitro coupled transcription-translation system that responds to the addition of exogenous DNA has been prepared from the lysates of isolated chloroplasts. These lysates appear to be comparable to E. coli cell lysates in transcriptional and translational fidelity and efficiency, since similar protein products are obtained from in vitro transcription and translation of E. coli or chloroplast DNA in E. coli cell lysates and in tobacco and spinach chloroplast lysates. When tobacco chloroplast DNA is used as template in chloroplast lysates a single protein is immunoprecipitated from the translation products by antibody to ribulose 1, 5-bisphosphate carboxylase/oxygenase holoenzyme. Transformed plasmids containing fragments of chloroplast DNA is also transcribed and translated by chloroplast lysates.

The effects of Mg^{++} concentrations on transcription and translation and the kinetics of the reaction were examined in the chloroplast lysate system. Chloroplast lysates show the greatest response to exogenous DNA at 14mM Mg^{++}. A maximal level of ^3H-RNA cpm was attained 20 minutes after the initiation of the reaction. The addition of 0.333 units of E. coli polymerase to the reaction resulted in a 10 fold increase in labeled RNA compared to reaction mixtures lacking polymerase. A maximal level of labeled protein was attained by 30 minutes after the initiation of the transcription-translation reaction when protein synthesis was monitored at 15 minute intervals.

1335 BIOSYNTHESIS OF THE P700 CHLOROPHYLL A-PROTEIN. E. Vierling* and R.S. Alberte, Univ. of Chicago, Chicago, IL 60637. In higher plants formation of the thylakoid membranes and accumulation of the chlorophyll-proteins are light dependent processes. In order to gain insight into the mechanism of the light induced control of appearance of the P700 chlorophyll-a-protein, biosynthesis of the 62kD P700 chlorophyll a-apoprotein was studied. Antibodies generated against the 62kD apoprotein were employed to examine rates of protein synthesis and accumulation using "Western" blotting techniques or immunoprecipitation of in vivo labeled (^{3}H-Leu) leaf proteins. Dark grown barley seedlings neither contain, nor synthesize detectable levels of the 62kD polypeptide. However, newly synthesized apoprotein can be detected within the first 15 min of illumination. After 4 hr of illumination, P700 chlorophyll a-apoprotein synthesis accounts for up to 10% of the total cellular membrane protein synthesis. The rate of production of the 62kD polypeptide appears to be coordinated with chlorophyll synthesis during the first 24 hr of light induced chloroplast development. Apoprotein synthesis is not, however, directly coupled to chlorophyll synthesis; if seedlings are returned to darkness after a period of illumination, apoprotein synthesis continues although the relative rate of synthesis declines by 50% after 2 hr in the dark. Accumulation of the apoprotein also continues in the dark, but at a decreased rate. The mechanisms underlying these light regulated changes in the rate of P700 chlorophyll a-protein biosynthesis remain to be defined. Observations of a 74kD polypeptide which may represent a precursor polypeptide to the P700 chlorophyll a-apoprotein will also be discussed.
*Current address. Botany Dept., Univ. of Georgia, Athens, GA 30602.

1336 HIGH EFFICIENCY MUTAGENESIS OF THE PHOTOSYNTHETIC APPARATUS OF R. capsulata. Krisztina M. Zsebo, Doug Youvan and John E. Hearst, Laboratory of Chemical Biodynamics, University of California, Berkeley, CA 94720.

A genetic system has been established for mutagenizing the photosynthetic apparatus of Rhodopseudomonas capsulata utilizing a previously described R-prime plasmid[1] and a derivative of transposon Tn5 called Tn5.7 which codes for resistance to streptomycin, trimethoprim, and spectinomycin. The combination of the gene dosage effect of streptomycin and the self-deleting property of the R-prime in a rec A background[2] provides a system for high efficiency replacement of wild type alleles with mutagenized ones in R. capsulata The R-prime bearing the PSA genes is mutagenized in E. coli and then conjugated into R. capsulata. Transconjugants selected on 15$\frac{\mu g}{ml}$ streptomycin maintain the prime portion of the R-prime plasmid at high efficiency until reciprocal recombination with the chromosome occurs. The wild type allele is then deleted on the plasmid. The high efficiency replacement of wild type alleles with mutagenized ones is aided by the absence of a hot spot for transposition of Tn5.7 into the chromosome as with Tn7.[2]

The transposon Tn5•7 has the right and left arms of wild type Tn5 except for the last nucleotide in the Bgl II recognition site in the left arm. The internal Kan^R region of wild type Tn5 has been replaced with the antibiotic resistance from Tn7. Random Tn5.7 insertions into various photosynthetic genes have been isolated.
(1) Marrs, B. (1981) J. Bact. 146, 1003-1012; (2) Youvan, D. et al. J. Mol. Biol. in press

1337 GENE CONTROL OF PHYCOBILISOME PHOTOSYNTHETIC ADAPTATION. A. Lonneborg, S.R. Kalla, J.-E. Hallgren, G. Oquist and P. Gustafsson. Depts of Microbiology and Plant Physiology, Umea University, Umea, Sweden.

Cyanobacteria (blue-green algae) are prokaryotes but their mode of photosynthesis resembles, except in the light harvesting system, that of higher plants. One major component in the light harvesting system in cyanobacteria is the phycobilisome, a macromolecular aggregate of water-soluble chromoproteins. The phycobiliproteins in the cyanobacteria A. nidulans, which we have chosen for our studies, are C-phycocyanin and allophycocyanin.

We are interested in the light induced regulation of the phycobilisome as well as its biogenesis. Regulation of the composition can occur both as a variation in size and as the number of light antennas. To study the dynamic changes of the phycobilisome during adaptation we perform shifts between different light conditions. Another area of interest is to use hybrid-DNA technology to get more insight into the adaptation process at the genetic level. We have constructed a gene bank of A. nidulans DNA in the vector pBR322 in E. coli. By using a synthetic oligonucleotide complementory to the amino acid sequence of one phycocyanine subunit we have localized the corresponding gene on the chromosome of the bacteria. We are preparing to use isolated genes to study genome organization and structure and also as probes for m-RNA-production during adaptation. Another interesting aspect is to use isolated genes to study evolutionary aspects of the photosynthetic apparatus.

1338 POSSIBLE MECHANISMS OF PLASTID NUCLEO-CYTOPLASMIC INTERRELATIONSHIPS. Caroline R. Perry, J.W. Bradbeer. King's College London, SE24 9JF.
Two mutant lines of barley Hordeum vulgare L. "Albostrians" and "Saskatoon" are being used to explore the inter-relationships between plastid and nucleo-cytoplasmic systems. In both mutants a nuclear mutation is expressed as an irreversible mutation of the plastid DNA which is seen visually by a change in plastid phenotype.

The plastids segregate to give white-striped, and wholly white as well as green leaves. Albino leaves contain no plastid ribosomes and in consequence they lack polypeptide synthesis in the plastids, although the cytoplasmic ribosomes are present.

Immunoelectrophoresis has detected reduced synthesis of some plastid polypeptides encoded by the nuclear genome. It has been suggested that the plastid DNA of the mutant cells is defective and that it is unable to provide a regulatory molecule which is thought to function by derepressing transcription of the nuclear genes for plastid polypeptides. Studies involve comparisons of plastid DNA from wild type and mutant barleys. At present it has been established that the mutant plastid DNA contains an addition mutation which is thought to be located in the plastid ribosomal RNA coding region. The effect of this mutation on the control of transcription may lead to an insight into the mechanism of plastid nucleo-cytoplasmic inter-relationships.

Structure and Function of Photosynthetic Membranes

1339 STUDIES OF THE RECONSTITUTION OF CHLOROPLAST ELECTRON TRANSFER REACTIONS USING RESOLVED MEMBRANE COMPLEXES FROM THYLAKOIDS. Richard Malkin and Eric Lam, Division of Molecular Plant Biology and Department of Biophysics, U. of California, Berkeley, CA.
The reconstitution of chloroplast noncyclic electron transport from water to NADP has been achieved using three resolved membrane complexes isolated from spinach chloroplasts. A PSII preparation transfers electrons from water to plastoquinone; a cytochrome b-f complex transfers electrons from reduced plastoquinone to plastocyanin, and a PSI preparation transfers electrons from reduced plastocyanin to NADP. In the presence of the appropriate protein cofactors, NADP photoreduction shows an absolute dependence on the presence of all three complexes. The reconstituted system was found to be sensitive to low concentrations of known inhibitors of noncyclic electron transport. In the presence of PSII and the cytochrome complex, plastocyanin can be used as a terminal electron acceptor. In this case, O_2 evolution was stoichiometrically linked to plastocyanin reduction. Interaction between PSII and the cytochrome complex could be demonstrated on the basis of preincubation experiments with the complexes. A cation requirement for the overall reaction was observed even though partial reactions showed no such requirement. This suggests cations allow an interaction of the two complexes which facilitates electron transfer between them. It was also possible to reconstitute electron transfer reactions involving cytochromes of the cytochrome complex in the presence of either PSI or PSII and inhibitor sensitivity indicates these reactions occur by physiological pathways. The in vivo implication of these results in terms of membrane organization of complexes will be considered. (Supported by grants from NSF and NIH)

1340 THE APPEARANCE OF PHOTOSYNTHETIC PROTEINS IN DEVELOPING MAIZE LEAVES, Stephen P. Mayfield, William C. Taylor, University of Calif., Berkeley, Calif. 94720

The appearance of leaf proteins and mRNAs which collectivly reflect the ability to photosynthesize were directly measured in leaf sections of developing maize seedlings. The third leaf of ten day old maize seedlings was cut into successive sections from the base (youngest) to the tip (oldest) of the leaf. Water-soluble and lipid-soluble proteins were extracted, separated on polyacrylamide gels and electrophoretically transferred to cyanogen bromide paper. Blots of water-soluble proteins were then probed with antibodies raised against; RuBPCase, PEPCase, Pyruvate Pi Dikinase, Plastocyanin, and Fe-NADP Reductase. Blots of the lipid-soluble proteins were probed with antibodies raised against the LHCP of PS II, the P-700 protein of PS I, and the α subunit of CF_1. The data show that the proteins appear in a sequence that permits efficient accumulation of photosynthetic activities.

The appearance of mRNAs encoding RuBPCase large and small subunit, PEPCase, and the LHCP were also monitored in the leaf sections with cloned cDNA probes for each message. With few exceptions the pattern of protein accumulation follows that of mRNA accumulation.

Biosynthesis of the Photosynthetic Apparatus: Molecular Biology, Development and Regulation

1341 TENTOXIN STOPS THE TRANSPORT OF NUCLEAR-CODED PROTEINS INTO THE CHLOROPLAST, K. C. Vaughn and S. O. Duke, USDA, So. Weed Sci. Lab, P.O. Box 225, Stoneville, MS 38776,USA

Immunochemical procedures were used to establish that the fungal toxin, tentoxin, stops the transport of certain proteins into the chloroplast. Immunocytochemistry of polyphenol oxidase (PPO), a nuclear-coded protein, revealed that the thylakoids for control tissues were heavily labelled with either peroxidase- or ferritin-labelled antibodies. Similar results were obtained by using the cytochemical oxidation of DOPA to localize PPO. Although these same procedures, used on tentoxin-treated plants, showed that all the antibody label was associated with the plastid envelope, no cytochemical staining (DOPA-oxidase activity) was noted along the envelopes in tentoxin-treated plants. Immunoprecipitation of PPO and subsequent SDS-PAGE of extracts from both tentoxin-treated and untreated control plants revealed a single 37 kDa protein band. When gels were stained for DOPA-oxidase activity only the protein band of the control extract stained positively. These data suggest that tentoxin interrupts the incorporation of PPO (and probably other proteins) into the chloroplast and this interruption results in an inactive PPO molecule.

1342 REGULATION OF CYANOBACTERIAL PHYCOBILISOME STRUCTURE BY TEMPERATURE AND LIGHT QUALITY, Lamont K. Anderson, M. Carmen Rayner and Fredrick A. Eiserling, UCLA, Los Angeles, CA

When cultured in white light at 30°C (30W cells), Nostoc sp cyanobacteria produce phycobilisomes containing 4 non-chromophoric proteins in the 25-40 kdalton range. These proteins (the Group II proteins) associate with the phycobiliproteins phycoerythrin (PE) and phycocyanin (PC) to form the arms of the phycobilisome structure. The ratio of PE:PC in these phycobilisomes is app. 1.3:1. When shifted to green light at 30°C (30G), the cells undergo complementary chromatic adaptation, decreasing the PC content and increasing the PE content to yield a PE:PC ratio of 2:1 in isolated phycobilisomes. An identical change in the PE:PC ratio is elicited by transfer to 40°C in white light (40W). In both 30G and 40W cells, the change in PE:PC is accompanied by the disappearance of the 34.5kd Group II protein from the phycobilisome structure. Sucrose gradient analysis of phycobilisome dissociation products suggests that 30W cells make two types of arm structures: a PE-PC arm species (PE:PC=2:1) and a PC arm species that has little or no PE. The 34.5kd protein is found only with the PC arm complex. Dissociation analysis of phycobilisomes that lack 34.5kd protein (from 30G or 40W cells) reveals the absence of the PC species arm structures, suggesting that the 34.5kd protein is required for the formation of these arms. Since this Nostoc does not synthesize a second species of PC when adapting to the PC-enriched state, the means of effecting complementary chromatic adaptation at the structural level may involve the regulation of only one structural protein, the 34.5kd protein. A model demonstrating this concept is proposed. The potential for temperature and light quality to induce structural changes through the same mechanism offers a new approach to chromatic adaptation.

1343 DIFFERENTIAL PROTEIN INSERTION INTO DEVELOPING PHOTOSYNTHETIC MEMBRANES REGIONS OF *RHODOPSEUDOMONAS SPHAEROIDES*, Gordon S. Inamine, Patricia A. Reilly, Francis X. Steiner and Robert A. Niederman, Rutgers University, Piscataway, NJ 08854

Pulse-chase studies in *R. sphaeroides* have suggested that much of the B800-850 light-harvesting (LH) bacteriochlorophyll *a* (Bchl)-protein complex is inserted directly into intracytoplasmic photosynthetic membranes (ICM), whereas the B875 LH and reaction center (RC) complexes are preferentially inserted at ICM growth initiation sites. These sites are isolated with an upper pigmented band (UPB) which sediments more slowly than the ICM-derived chromatophores in sucrose gradients. When Bchl synthesis in the δ-aminolevulinate-requiring mutant H-5 was initiated by restoration of this porphyrin precursor to deprived cells, insertion of pigment-proteins was shown to be regulation in this site-specific manner. The basis for this differential polypeptide insertion was examined further with inhibitors of RNA and protein syntheses. The incorporation of L-[^{35}S]met into the UPB as a whole was less sensitive to rifampicin, chloramphenicol and kasugamycin than in chromatophores. This was also observed for apparent polypeptides of the Bchl-protein complexes of the UPB which were resolved by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (PAGE). These results suggest increased stability for the translation machinery at sites of ICM growth initiation. Endogenous mRNA-dependent protein synthesis in disrupted cells resulted in a two-fold higher specific radioactivity in the UPB than in chromatophores and preferential insertion of apparent LH polypeptides into the UPB. These labeling patterns are under further investigation with a two-dimensional PAGE procedure developed to resolve RC and LH polypeptides. (Supported by PHS grant GM26248 (R.A.N.) and a Busch Postdoctoral Fellowship (F.X.S.).)

1344 ON THE ASSEMBLY OF THYLAKOID MEMBRANES IN ETIOLATED AND IN GREENING PLASTIDS, Cornelius Lütz, Institute of Botany, University of Cologne, Gyrhofstr. 15, 5 Cologne 41, W. Germany

The inner structural system of etioplasts, the prolamellar/prothylakoid (PLB/PT)-complex is widely used for developmental studies on thylakoid formation during greening. Our analytical and ultrastructural studies using either the PLB/PT-complex or its separated components have shown: 1) the tubules forming the paracrystalline PLBs are highly enriched in lipids, but low in proteins or pigments. 2) Extracts of PLBs are able to rebuild PLB-similar tubular complexes in vitro. 3) Enzymatic degradation of the PLB/PT-structures shows great differences in response to the treatments and supports our ideas that PLBs are seen as dark artefacts of membrane development, formed by aggregation of lipids. But PTs are mainly involved in the first steps of greening, as will be shown by analysis of membrane proteins and of chlorophyll formation.

It is concluded, that the search for etiolated plant systems for experiments to understand membrane assembly of thylakoids, should take into consideration the developmental stage of prothylakoids. The appearance of well developed prolamellar bodies indicates prolonged darkness during growth, which is only rarely found in nature.

1345 CHLOROPHYLL-PROTEIN COMPLEXES IN MARINE PHYTOPLANKTON, Barbara A. Boczar and Barbara B. Prezelin, Dept. of Biological Sciences, University of California, Santa Barbara, CA 93106

Modified gel electrophoresis and detergent solubilization techniques have been employed to isolate four chlorophyll-protein complexes from the marine dinoflagellate, Glenodinium sp. (Boczar et al (1980) FEBS LETTS 120, 243-247). Little free pigment is generated by this approach, and, most interestingly, all chlorophyll c_2 is localized within one chlorophyll a-containing protein band. These techniques have been successfully used to isolate similar components from a variety of marine phytoplankton including Exuviella sp. (a dinoflagellate which has a high chlorophyll a/chlorophyll c ratio and reduced peridinin content), the red tide dinoflagellate, Gonyaulax polyedra, and a chlorophyll c_1-c_2-containing diatom, Nitzschia closterium.

The further characterization of the chlorophyll a-chlorophyll c-protein complex of Glenodinium sp. is presented here, including data on fluorescence excitation and emission characteristics, chromophore ratios, and peptide composition. A salt effect on the electrophoretic banding pattern has also been observed, is being presently studied, and resulting conclusions will be presented.

1346 SPATIAL DIFFERENTIATION IN PHOTOSYNTHETIC AND NON-PHOTOSYNTHETIC MEMBRANES OF RHODOPSEUDOMONAS PALUSTRIS. A.R. Varga and L.A. Staehelin. MCD Biology, Univ. Colorado Boulder, Co. 80309.

The cytoplasmic membrane (CM) and the photosynthetic intracytoplasmic membranes (ICM) of Rhodopseudomonas palustris are spatially differentiated into regions of extremely high intramembrane particle (IMP) density, 4400-9800/um^2 and areas of lower IMP density, 2700-5900/um^2. The high IMP-density areas are always seen in association with photosynthetic membrane stacks. This differentiation is also seen in those areas of the CM which adhere to the underlying ICM implying that the CM too, is specialized for photosynthesis in these regions. Changes in IMP size distribution in response to changes in light intensity during growth have been measured. We have found that as light levels are decreased from 8500 lux to 100 lux, the average particle diameter in the protoplasmic face (PF) of the stacked ICM and CM increases from 8.6 nm to 10.3 nm. There is also a distinct periodicity in the sizes of IMPs found in the stacked regions: 7.5, 10.0, 12.5 and 15.0, with the larger size peaks becoming more pronounced as light intensity decreases. This suggests that as light levels decrease, subunits of discrete size are added to a "core" particle. Such a model is presented here. Preliminary experiments using mild detergent solubilization of Rps. palustris membranes indicate that several pigment protein complexes can be resolved by polyacrylamide gel electrophoresis. Changes in the composition of these complexes which correlate to changes in light intensity and particle size are presently being analysed.

Supported by grant GM 22912.

1347 BILAYER AND NON-BILAYER STRUCTURES FORMED FROM GALACTOLIPIDS ISOLATED FROM PLANT PHOTOSYNTHETIC MEMBRANES, Sallie G. Sprague and L. Andrew Staehelin, MCD Biology, University of Colorado, Boulder, CO 80309

Mono- and digalactolipids (MG and DG , respectively) constitute roughly 50% and 25% of the non-pigment lipid in plant photosynthetic membranes. We plan to use plant galactolipid vesicles to lipid-enrich thylakoids and have characterized the structures formed by these two major membrane lipids. Lipids were isolated from spinach thylakoids by organic solvent extraction, and purified by silicic acid column chromatography. MG and DG were hydrated, individually and in-defined mixtures, by heating and/or sonication of lipid films in water, buffer or detergent. In the absence of detergent, MG was partially, but selectively, retained on the walls of the container. After solubilization in Triton and subsequent detergent removal (Biobeads adsorption), MG formed only amorphous aggregates. DG readily formed bilayer liposomes whose size depended, e.g., on the extent of sonication. DG liposomes accommodated up to 25% MG(w/w) before "lipidic particles" were observed in freeze-fractured material. Equal weight mixtures of MG and DG produced extensive tubular structures (7-9nm repeat) and hexagonal and square packed arrays of 13nm particles. Reverse phase evaporation of the galactolipids in Freon 11 transferred both MG and DG quantitatively to the aqueous phase. MG alone formed extensive hexagonal II-type tubes. DG again formed bilayer vesicles that could accommodate approximately 25% MG without formation of "lipidic particles." Glycerinated and non-glycerinated samples gave essentially the same structures. Supported by Grants NIH-GM22912 and NSF-PCM8118627.

1348 RECONSTITUTION OF CHLOROPHYLL-PROTEIN COMPLEXES ISOLATED FROM SDS POLYACRYLAMIDE GELS INTO LIPOSOMES, T.L. Goodman and L.A. Staehelin, Univ. of Colorado, Boulder, CO. 80309

Chlorophyll-protein complexes from thylakoid membranes can be visualized as green bands on polyacrylamide gels. The band pattern varies with the conditions used for membrane solubilization and electrophoresis. The nonionic detergent octyl glucoside (OG) has been shown to stabilize the chl a/b LHC oligomer chl a/b-P2**, as well as the putative PSI oligomer chl a-P1*. The structural and functional relationship of these bands to chlorophyll-protein complexes in vivo is unclear. To help resolve this question, we have developed a simple technique to isolate these complexes directly from gels and incorporate them into liposomes. Spinach thylakoids are solubilized in 0.88% OG plus 0.22% SDS to increase yield. The bands are cut from preparative gels and minced in a Virtis homogenizer in 30mM OG. Following centrifugation and concentration, the material is added to liposomes, dialyzed to remove detergent, and prepared for freeze-fracture electron microscopy. Initial experiments indicate that although the material isolated from both the chl a/b-P2** and chl a/b-P2 bands can be precipitated out of solution with $MgCl_2$, only chl a/b-P2** is capable of forming particles in liposomes. These particles have diameters of 8-10nm and form hexagonal aggregates similar to Triton solubilized chl a/b LHC purified on sucrose gradients. This supports the theory that chl a/b-P2** represents the in vivo form of the LHC and that chl a/b-P2 is a breakdown product. Both of the PSI related bands, chl a-P1 and its purported oligomer chl a-P1* form particles of approximately 10 and 12nm. Thus, our findings demonstrate that globular pigment-protein complexes up to 12.5nm in diameter can be separated as intact particles under nondenaturing conditions on suitably formulated polyacrylamide gels. Supported by GM 22912.

1349 IS TURNOVER OF THE 32,000-DALTON THYLAKOID PROTEIN A SIGNAL FOR ADAPTATION OF THE CHLOROPLAST TO TRIAZINE HERBICIDES AND TO LIGHT CONDITIONS? Autar K. Mattoo*, Judith B. St.John** and William P. Wergin***, *Department of Botany, University of Maryland at College Park and *Plant Hormone, **Weed Science and ***Plant Stress Laboratories, Agricultural Research Center, USDA, Beltsville, Md. 20705.

When Spirodela oligorrhiza is cultivated on sublethal doses of atrazine, the 32,000 dalton (32K) membrane protein of the chloroplast is synthesized actively but its breakdown is considerably decreased compared to control plants. Other changes include a higher linolenic to linoleic acid ratio in the thylakoid membrane lipids, less starch, more osmiophilic globules and a reduced stroma lamellar system. Also, the grana which were randomly oriented contained more numerous and elongated lamellae. These alterations in the lipid composition and ultrastructure of the chloroplast resemble those previously observed in triazine-resistant weed biotypes and in chloroplasts developed under minimum light. The metabolism of the 32K protein also slows under minimum light and in the presence of atrazine (Edelman et al. (1983) in: Chloroplast Biogenesis (ed. RJ Ellis), Cambridge University Press, U.K., in press). We propose that the turnover of the 32K membrane protein may be a signal for adaptation of plants to PS-II herbicides and to changing light conditions.

1350 WHAT IS THE STEP STIMULATED BY CYTOKININS IN THE BIOSYNTHESIS OF PHOTOSYNTHETIC MEMBRANE POLYPEPTIDES IN TOBACCO CELL SUSPENSION CULTURES? Claude Péaud-Lenoël, Jacques Barbet and Michèle Axelos, Département de Biologie Moléculaire et Cellulaire, Faculté des Sciences de Luminy, C 901, Marseille Cedex 9, 13288 FRANCE.

Besides light, cytokinins are known to stimulate the differentiation of photosynthetic membranes in tobacco cell suspension cultures. However, the cells are able to synthesize and organize most of their plastid polypeptides regardless of the presence of the hormone in the medium. The cytokinins specifically stimulate the appearance of chlorophylls and of a few essential membrane proteins which we consider as molecular markers of the cytokinin activity. Prominent amongst these are the polypeptides of the light harvesting-chlorophyll a/b-protein complex (LHCP). LHCP-specific antibodies were raised in rabbits. Radioimmunological determinations of total LHCP and their polypeptide precursors have shown that the polypeptides were translated only during late growth or stationary phases of the cell cultures in the presence of the hormone. The appearance of the LHCP was strikingly parallel to the appearance of chlorophylls. We never detected any accumulation of LHCP precursors.

All together, the evidence suggests that cytokinin specifically stimulates the biosynthesis of some thylakoid polypeptides encoded in the nuclear genome. This stimulation might take place at some step regulating transcription of the DNA or maturation of the mRNA rather than the translation process itself. The splitting of the LHCP precursor polypeptides or their transfer into the chloroplast membranes are not affected by the hormone.

1351 LAMELLAR COMPONENTS DURING IRON NUTRITION MEDIATED CHLOROPLAST DEVELOPMENT, John N. Nishio, Scott E. Taylor, and Norman Terry, University of California, Berkeley, CA 94720

Development of chloroplast lamellae in sugarbeet (Beta vulgaris L.) was studied by resupplying iron to iron deficient plants. Changes in chlorophyll a and chlorophyll b; electron transport components, P700, Q and cytochrome f; in vivo photosynthesis; membrane lipids; and proteins were monitored. Using spectrophotometric techniques we observed that the synthesis of chlorophyll a, chlorophyllb, and Q exhibited a lag phase during the first 24 to 48 hours of iron resupply, while P700 and cytochrome f increased rapidly. Preliminary data suggests that thylakoid galactolipids and proteins were synthesized linearly. The monogalactolipid to digalactolipid ratio increased during iron resupply which correlated with grana stacking and a decrease in the chlorophyll a to b ratio. The relationship of the above findings to the synthesis of specific peptides was analyzed by lithium dodecyl sulfate polyacrylamide gel electrophoresis. Whole leaf photosynthesis was also examined.

1352 PHYSIOLOGICAL AND BIOHCEMICAL CHANGES DURING LEAF DEVELOPMENT OF PLASTOME-ENCODED PHOTOSYSTEM II MUTANTS, John H. Duesing, Cathy P. Chia and Charles J. Arntzen, MSU-DOE Plant Research Laboratory, East Lansing, Michigan 48824

In higher plant chloroplasts, several protein complexes are critical to the integrity and function of the photosynthetic membrane. As more becomes known about the components of the complexes, it should be possible to investigate the regulation required to accumulate the proteins and to assemble them into a functional complex. Our laboratory is specifically interested in the development of the Photosystem II chlorophyll protein complex and considers the use of mutants an important aid to our understanding.

Because several major polypeptide components of Photosystem II (PS II), are encoded in the chloroplast genome, a collection of maternally inherited chloroplast mutants of Nicotiana tabacum has been surveyed. A detailed analysis was made of mutant 77-13 at different stages of development. With increasing leaf age the mutant leaves undergo a loss of total chlorophyll, a decrease in the chlorophyll a/b ratio, the loss of variable fluorescence and a shift of the 685 nm 77°K fluorescence peak to 680 nm indicative of LHC disconnected from PS II reaction centers. Characterization of the thylakoid proteins revealed the gradual loss of all proteins generally assigned to the PS II complex except for the larger (50 Kd) of the two polypeptides considered to constitute the PS II reaction center. These changes occur without modification to the ultrastructure of the chloroplast.

We conclude from our findings that a PS II reaction center polypeptide can exist in the thylakoid membrane without full complex formation and is sufficient to sustain normal levels of LHC-mediated grana formation.

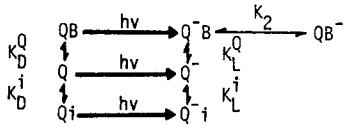
Biosynthesis of the Photosynthetic Apparatus: Molecular Biology, Development and Regulation

1353 LATERAL DISTRIBUTION OF THYLAKOID MEMBRANE PROTEINS, David R. Paterson and Colin A. Wraight, University of Illinois at Urbana Champaign, Urbana, IL 61801

It is generally believed that PSII and PSI tend to segregate into the grana and stroma-facing lamellae respectively. In spinach the segregation is apparently extreme, with PSII located exclusively in the grana and PSI present only in the stroma facing lamellae. However, in low-light grown peas we have analyzed granal and stromal lamellae preparations by SDS-PAGE and spectrophotometric assays and find that although PSII is almost exclusively located in the grana, PCI is evenly distributed between grana and stroma lamellae. It is very unlikely that this represents contamination of the grana preparation by stroma membranes, and we conclude that PCI is a normal component of appressed membranes. The relationship between PSI content of the grana and the light quality during growth will be discussed. We have also quantitated the distribution of CFo by SDS-PAGE of membrane fractions incubated with 14C-DCCD, and find it almost exclusively located in the stroma lamellae, along with a second labelled component (AMW = 17kD). One other DCCD-binding component (AMW = 22kD) was observed to partition almost exclusively into the grana.

1354 A BACTERIAL MODEL FOR PSII-HERBICIDE ACTIVITY, Randall R. Stein and Colin A. Wraight, University of Illinois at Urbana-Champaign, Urbana, IL 61801

The reaction centers of PSII and of purple photosynthetic bacteria have a remarkably similar electron acceptor complex consisting of two quinones. Electron transfer between these two quinones is blocked by many agents recognized as herbicides. The bacterial system, however, is less sensitive and more selective. On the basis of our studies on reaction centers from Rhodopseudomonas sphaeroides we have proposed a mechanism of action for the PSII-herbicides in which the secondary quinone is displaced from its binding site by herbicides (1):



This mechanism accounts for the "leak" from the inhibited state: the quinone and inhibitor binding equilibria allow eventual electron transfer from B which is very strongly bound. The leak rate is therefore a measure of K_L^Q and K_L^i, while the instantaneous inhibition is a measure of K_D^Q and K_D^i. A similar model has been proposed by Velthuys (2).

We have now demonstrated the essentially competitive nature of this interaction and have determined some of the binding and kinetic constants for three representative inhibitors: terbutryn, ioxynil and o-phenanthroline. We have also isolated and partially characterized several triazine-resistant mutants which show selective desensitization to triazines.

(1) Wraight, C.A. 1981. Israel J. Chem. 21:348 (2) Velthuys, B.R. 1981. FEBS Lett. 126:277

1355 PARTIAL CHARACTERIZATION OF A WATER OXIDIZING PHOTOSYSTEM II PREPARATION FROM SPINACH, Steven Berg and Paula Ogilvie, Biology Department, Univ. of Denver, Denver, CO 80208.

A water oxidizing photosystem II preparation is described which is prepared by Triton X-100 extraction of thylakoid membranes. The preparation has six major peptides with apparent molecular weights of 36, 31, 28, 27, 25, and 21 kD. Sucrose density gradient centrifugation indicates that the preparation is more homogeneous and more dense than control thylakoid membranes. The preparation photoreduces a number of known photosystem II oxidants including the Class I acceptor, ferricyanide; the Class II acceptor, 2,6-dichloroindophenol; and the Class III acceptor, 2,6-dichlorobenzoquinone. However, quinonediimines such as phenylenediimine are not reduced, suggesting that these substances are reduced at sites in the thylakoid membrane which are not found in the photosystem II preparation. All the oxygen producing reactions are sensitive to inhibition by 3-(3,4-dichlorophenyl)-1,1-dimethylurea. The photosystem II preparation is far more sensitive to inhibition by TRIS-hydroxymethylaminomethane and hydroxylamine than are thylakoids. However, the preparation is relatively insensitive to inhibition by trypsin and carboxypeptidase. SDS-PAGE analysis, following protease treatment, reveals that all six major peptides are sensitive to protease, although to variable extents. The inhibition studies suggest that no permeability barriers (membranes) remain in the photosystem II preparation and that most, or all of the peptides are exposed to proteases. Light microscopy reveals massive, flattened arrays of green materials. Thus the individual photosystem II complexes appear to be aggregated into membrane-like associations. However, as discussed above, this association is not characterized by providing a permeability barrier.

1356 DEVELOPMENT OF CHLOROPLASTS AT LOW TEMPERATURE IMPAIRS LIGHT-DRIVEN PROTON FLUX AND PHOTOPHOSPHORYLATION, William G. Hopkins and Norman P. Huner, University of Western Ontario, London, Canada N6A 5B7

Chloroplast suspensions were prepared from rye plants (Secale cereale) grown at either 5° (RH) or 25° (RNH). Photophorphorylation was measured by 32-P phosphate esterification. Initial rate of esterification in RNH chloroplasts is twice that of cold-grown chloroplasts. While RNH continues to phosphorylate for at least 2 min, RH chloroplasts no longer phosphorylate after 60 to 90 sec. ATP formation measured by light-induced proton uptake at pH 8 in the presence of ADP and K_2HPO_4 is not detectable in RH chloroplasts. The light-driven proton gradient in RH chloroplasts pH 7 is no more than one-third that of RNH chloroplasts. Mixing RH chloroplasts with RNH can cause as much as 75% inhibition of ATP formation. Mg^{2+} - ATPase activity is similar for both RNH and RH thylakoid membranes. The mechanism by which development at low temperature impairs proton pumping and photophosphorylation is not yet known.

1357 DEVELOPMENT OF CHLOROPLASTS AT LOW TEMPERATURE IMPAIRS ELECTRON TRANSPORT ACTIVITY AND ENERGY DISTRIBUTION, N. P. A. Huner, Univ. of Western Ontario, London, Canada.

Chloroplasts were prepared from rye plants (Secale cereale L. cv Puma) grown at either 5°C (RH) or 20°C (RH) for 8-10 weeks and 3-4 weeks respectively with a 16 hr photoperiod and a light intensity of 150 μE $m^{-2}s^{-1}$. Light-dependent electron transport activity in RH plastids was 20-40% less than in RNH plastids when measured as whole chain activity ($H_2O \rightarrow$ MV), PSI activity (ascorbate/DCPIP $\rightarrow$ MV), or PSII activity ($H_2O \rightarrow$ DCPIP; $H_2O \rightarrow$ KFeCN). Electron transport activity was higher when RH plastids were isolated in the presence of 10 mM $MnCl_2$ than in the presence of 10 mM NaCl. RNH plastids showed no such dependence. In addition, RH plastids were insensitive to uncoupling of electron transport by NH_4Cl whereas RNH plastids were sensitive. Isolation of these plastids in the presence of proteolytic inhibitors, BSA and/or PVP had no effect on these properties. Measurement of electron transport activity as a function of light intensity indicated that one major lesion in electron transport upon growth of rye at 5°C occurred between H_2O and the diphenyl carbazide donating site for PSII. Fluorescence induction curves indicated a decreased ability of RH plastids for Mg^{2+} regulation of energy distribution between PSII and PSI.

1358 ON THE MOLECULAR ORGANIZATION OF THE PHOTOSYSTEM I REACTION CENTER. Mark L. Richter & Peter H. Homann, Florida State Univ., Tallahassee, Fl. 32306

Detergent-solubilized thylakoid particles enriched in the photosystem I (PSI) reaction center were exposed for short times to glutaraldehyde at pH 7. This treatment caused the complete loss of P700 reduction by added plastocyanin (PC), even in the presence of divalent cations. However, addition of polylysine to glutaraldehyde-treated particles induced high rates of PC-dependent P700 reduction. These results indicate firstly that glutaraldehyde acts on the PC-binding site of the reaction center complex, and secondly that the stimulatory effects of divalent cations and polycations, which have been observed with untreated particles, occur via different mechanisms. We suggest that divalent cations stimulate P700 reduction by PC when mediated by the PC-binding polypeptide (subunit III), where polylysine opens up an alternative route to P700 reduction by PC, possibly by mimicking the function of the PC-binding polypeptide. Studies using PSI particles treated to remove this polypeptide have supported this contention.

Our results suggest that the polypeptide which mediates electron transfer between PC and P700 in situ possesses positively charged amino groups which are involved in recognition and binding of PC at the predominately negatively charged inner thylakoid membrane surface.

1359 ISOLATION OF PHOTOSYSTEM II REACTION CENTRE AND ITS RELATIONSHIP TO THE MINOR CHLOROPHYLL-PROTEIN COMPLEXES. Beverley R. Green and Edith L. Camm, Botany Dept. University of British Columbia, Vancouver, B.C., Canada, V6T 2B1

Photosystem II particles containing only the two minor chlorophyll a complexes CPa-1 and CPa-2 (CP47 and CP43) can be prepared from spinach and chlorophyll b-less barley by a simple procedure involving differential solubilization with octyl glucoside and centrifugation on a 10-30% sucrose gradient. The gradient fractions with highest PS II activity (as measured by the light-dependent reduction of 2,6-dichlorophenol-indophenol by diphenylcarbazide) contained both CPa-1 and CPa-2, but no other chlorophyll-protein complex. Distribution of PS II activity across the sucrose gradient was most closely matched by the distribution of CPa-1(CP 47), suggesting that this complex might carry the reaction centre chlorophylls.

Biosynthesis of the Photosynthetic Apparatus: Molecular Biology, Development and Regulation

1360 EFFECT OF ZINC IONS ON PHOSPHORYLATION OF THYLAKOID MEMBRANE PROTEINS, John P. Markwell and Merri P. Skrdla, University of Nebraska, Lincoln, NE 68583

It is well established that the ability of the photosynthetic membrane to regulate the distribution of light energy captured by the Light-Harvesting Chlorophyll a/b-Protein Complexes (LHCP's) to either Photosystem I or Photosystem II is correlated with the phosphorylation of thylakoid membrane proteins. Some groups have suggested that phosphorylation of the LHCP apoproteins brings about this quantal redistribution, termed the State 1-State 2 transition. Examination of the effect of zinc ions on thylakoid protein phosphorylation and the State 1-State 2 transition call this latter hypothesis into question. Zinc ions (2.5 mM) cause a marked stimulation of tobacco thylakoid protein phosphorylation at ATP concentrations less than 0.2 mM. The effect was independent of magnesium ion concentration, and at 0.025 mM ATP resulted in a 4 to 5-fold stimulation of total thylakoid protein and apo-LHCP phosphorylation. However, identical conditions resulted in an inhibition of the State 1-State 2 transition as monitored by the decrease in ATP-induced quenching of the Photosystem II fluorescence signal. Although total protein phosphorylation was increased, the phosphorylation of several proteins was inhibited (M_r = 58,000; 46,000; 42,000; and 9,000). Similar results were observed with spinach and pea thylakoids. The results appear to support a recent suggestion (Owens and Ohad. 1982. J. Cell Biol. 93:712-718) that phosphorylation of the LHCP may play a role in membrane organization rather than serve to control quantal distribution.

1361 THE TOPOGRAPHY OF THE PHOTOCHEMICAL MEMBRANE SYSTEM IN THE GREEN BACTERIUM CHLOROFLEXUS. R. C. Fuller and Reiner Feick, Univ. of Mass., Amherst, MA 01003

The photosynthetic apparatus of the green bacteria is organized on two cellular components. Antenna BChl c resides on the extramembranous structure, the chlorosome. The undifferentiated cytoplasmic membrane contains both antenna and reaction center BChl a as well as the secondary photochemical and respiratory electron transport system. Recently a model based on freeze-fracture electron microscopy has suggested the supramolecular organization of this system, namely that the chlorosome contains a linear array of BChl c protein and is oppressed against the cytoplasmic membrane. Isolation and purification of chlorosomes and both BChl a antenna and reaction center has been achieved. A model has been proposed that suggests energy capture and transfer via the following pathway: hV → BCHl c* → (chlorosome) → BChl a* → (90 chlorosome baseplate) → antenna BChl a* → reaction center BChl a* (P865-CM). Purified clorosomes contain only three polypeptides with apparent molecular weights of 3,700 KD;11,200 KD: 18,000 KD respectively. Proteolytic digestion experiments show that the latter two peptides are exposed on the surface and the 3,700 KD peptide is apparently sequestered. Protein cross linking followed by two-dimensional SDS PAGE analysis indicate the sequestered peptide is present as a dimer and contains undenatured BChl c. Purified reaction center shows reversible photochemical bleaching at P865 and one major band at 30,000 KD. The antenna BChl a protein appears to be present in the CM in the form of a tetramer. These biochemical experiments confirm the molecular arrangement of the photochemical components of these systems as originally suggested by both electron microscopic and physical chemical observations.

1362 CHARACTERIZATION OF A HIGHLY ACTIVE O_2-EVOLVING PSII PREPARATION FROM MAIZE, Terry M. Bricker, James G. Metz, Donald Miles and Louis A. Sherman, Division of Biological Sciences, University of Missouri, Columbia, MO 65211.

O_2-evolving PSII vessicles were isolated from maize by the Triton X-100 procedure of Kuwabara and Murata. A highly active preparation was obtained which evolved O_2 at 76% the rate of fresh chloroplasts ($H_2O \rightarrow DCBQ$) and which was very sensitive to DCMU. There was no detectable PSI activity ($DAD \rightarrow MV$) in these preparations. When analyzed by LiDS-PAGE, this preparation was shown to be devoid of CP 1 apoprotein, CF_1, and cytochromes f and b_6 (the absence of which was confirmed by difference spectroscopy). The preparation was enriched in the PSII reaction center polypeptides I and II, the "34 kD" species of Metz, Bishop and Wong, the Coomassie blue stainable "32kD" polypeptide, LHCP-associated polypeptides and cytochrome b_{559}. Polypeptides of unknown function at 40.5, 25, 24, 22, 16.6 and 14 kD were also enriched in the preparation. This O_2-evolving preparation was enriched in low mobility, UV-fluorescing, chlorophyll-proteins which are probably oligomers of light-harvesting chlorophyll-proteins. Additionally, at least 4 chlorophyll binding polypeptides are resolved in the LHCP region. When examined by the octylglucoside procedure of Camm and Green followed by LiDS-PAGE, the preparation was shown to contain CP 27, CP 29, CP II , D, and CPa-1 and CPa-2. Chlorophyll-proteins associated with PSI were not found. The 77 K fluorescence emission spectrum (Ex. = 435 nm) exhibits a strong F_{686} with no F_{695} shoulder and a broad, low intensity F_{735} emission. Electron microscopy with negative staining of this preparation reveals a homogeneous population of large, spherical vessicles which aggregate under stacking conditions. Supported by NIH Grant GM21827.

1363 VIRESCENT MUTANTS OF MAIZE WITH HIGH CHLOROPHYLL FLUORESCENCE, Mary L. Polacco, University of Missouri, Columbia, MO 65212

The mechanisms that regulate the stoichiometry of the photosynthetic membrane components are unknown. One possibility is that the rate of insertion of one (or more) functional components is closely regulated and its insertion directs the rate of insertion of other components. If this "obligate order" hypothesis is valid, there should be a class of mutants in chloroplast biogenesis where a photosynthetic activity is altered. While the existence of this class would not prove involvemnt of a functional component in assembly regulation, it would provide a basis for future investigation.

Chlorophyll fluorescence has been used to assess defects in photosynthesis. Virescent (v) mutants of maize where both mesophyll and bundle sheath chloroplasts green slowly were examined for high chlorophyll fluorescence during greening. Most v-mutants showed no increased increased fluorescence. Three independently isolated alleles (from the Neuffer collection) exhibited up to 5 times increased fluorescence than normal siblings. The amplitude of the fluorescence was inversely proportional to the extent of greening. Thylakoids isolated from fluorescing leaf segments showed no absence of any polypeptides when examined by gel electrophoresis (8-18% polyacrylamide, 30 cm long gel, lithium dodecyl sulfate, 4 C). Electron transport data will be presented.

1364 ASSEMBLY OF THE UBIQUINONE-CYTOCHROME b/c_1 OXIDOREDUCTASE COMPLEX AND DEVELOPMENT OF PHOTOSYNTHETICALLY COMPETENT *RHODOPSEUDOMONAS SPHAEROIDES* MEMBRANES, Robert A. Niederman, John R. Bowyer*, C. Neil Hunter and Tomoko Ohnishi*, Rutgers University, Piscataway, NJ 08854 and *University of Pennsylvania, Philadelphia, PA 19104

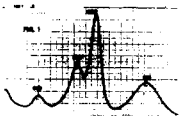
After rate-zone sedimentation of *R. sphaeroides* extracts, bacteriochlorophyll *a* (Bchl) is found with intracytoplasmic membrane (ICM)-derived chromatophores and an upper pigmented band (UPB) containing ICM growth initiation sites and unpigmented respiratory membrane. Essentially all components of light-driven cyclic electron flow have now been shown in UPB through redox potentiometry, EPR spectroscopy and flash-induced absorbance changes. EPR signals characteristic of light-induced reaction center (RC) Bchl triplet and $(Bchl)_2^+$ states were shown for UPB, but the Rieske iron-sulfur signal of the cytochrome bc_1 segment was present at a much reduced level than in chromatophores. Although flash-induced absorbance measurements demonstrated primary and secondary semiquinone anion signals, cytochrome b_{561} photoreduction and cytochrome c_1/c_2 reactions occurred at slow rates. The UPB was enriched in total *b*- and *c*-type cytochromes on a RC basis over chromatophores, but photoreducible *b* per RC in UPB was lower. Respiratory activity as measured from succinate-cytochrome *c* oxidoreductase was 6.6-fold higher in UPB per RC, but none of the RC-linked respiratory systems exhibited mature ICM kinetics. Thus, interaction of newly synthesized RC with respiratory membrane does not produce fully functional cyclic electron flow. This requires subsequent assembly of appropriate components of the chain, particularly those of the bc_1 segment. (Supported by PHS grant GM26248 (R.A.N.) and NSF grants PCM78-16779 (T.O.) and PCM79-03665 (R.A.N.).)

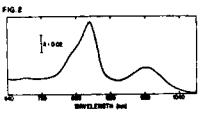
1365 NUCLEAR GENE MUTANTS REGULATING CHLOROPLAST PROTEIN COMPLEXES IN THYLAKOIDS OF MAIZE, Donald Miles and James G. Metz. Division of Biological Sciences, University of Missouri Columbia, MO 65211

Photosynthesis is being studies years using mutagen induced high chlorophyll fluorescence nuclear mutants of maize (Zea mays L.). A large number have been isolated and classified as mutants of photosynthetic electron transport, photophosphorylation, carbon dioxide fixation or of chloroplast structure. Recently it has become clear that a number of these nuclear mutants regulate the synthesis or assembly of macromolecular complexes of the thylakoid membrane. Four examples of such mutants are: hcf-3, which has a total loss of the PS-II complex including the 49kd and 45kd chlorophyll binding proteins, 32kd protein, 34kd (associated with Mn^{++}) 23kd, 16kd, 12kd and the 10kd cytochrome b-559 subunit. It is clear that at least four of these polypeptides are synthesized in the chloroplast on 70S ribosomes and two are mapped in the chloroplast DNA. hcf-19 shows a partial loss of the same PS-II complex but mapped to a different chromosome in the nucleus. Both of the mutants have normal PS-I. hcf-6 has normal PS-I and PS-II electron transport but has lost the cytochrome f, b6, Rieske Fe-S complex which functions between the photosystems. These proteins are also synthesized in the chloroplast but this nuclear mutation has eliminated them from the membrane. hcf-50 has normal PS-II but has lost the entire PS-I complex. This includes the 68kd apoprotein, 19kd, 16kd, 11kd as well as P-700 activity. We now see that there are specific nuclear genes which are altered by mutation and which regulate the synthesis or assembly of whole thylakoid membrane complexes of proteins synthesized in the chloroplast. Supported by NSF Grant PCM 8208910

Biosynthesis of the Photosynthetic Apparatus: Molecular Biology, Development and Regulation

1366 PHOTOCHEMICALLY ACTIVE PIGMENT-PROTEIN COMPLEXES FROM BACTERIOCHLOROPHYLL B-CONTAINING ORGANISMS, Richard E. B. Seftor, Univ. of California, Los Angeles, LA, Ca, 90024 The value of the BChl b-containing bacterium Rps. viridis to the studies of the primary processes of photosynthesis has caused the search for equivalent reaction center (RC) preparations from other BChl b-containing organisms to be continued. Of the three other known BChl b-organisms (Sulfoviridis, Ectothiorhodospira halochloris and Thiocapsa pfennigii), Th. pfennigii has been the best described and the most readily available. The isolation and characterization of its photosynthetic RC has been of primary importance and is reported in this poster. The photosynthetic complex is solubilized from broken membranes with the detergent Miranol S2M-SF and the RC is separated from the light harvesting component with the detergent LDAO. This is followed by ion-exchange chromatography on the DEAE anion-exchanger DE-51. The RC is stabilized with 10% glycerol plus 1mM dithiothreitol and is relatively stable at room temperature for hours at a time. Data will also be presented concerning the spectral characteristics of Th. pfennigii RC (Fig. 1) as compared with that of Rps. viridis (Fig. 2). In particular, the nature of the intermediary electron acceptor(s) will be discussed in relation to what is known from the work with Rps. viridis.





1367 PHOTOINHIBITION IN CHLAMYDOMONAS, David J. Kyle, Charles J. Arntzen and Itzhak Ohad, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI, 48824, U.S.A. and the Department of Biological Chemistry, Hebrew University of Jerusalem, Jerusalem, Israel. Incubation of Chlamydomonas reinhardii under conditions of very high light intensity (5,000 W/m^2/sec) leads to a loss of room temperature variable fluorescence and a quenching of the low temperature (77K) fluorescence emission arising from photosystem II (F687). The kinetics of the fluorescence decrease are complex, and include a component related to an alteration in energy transfer and one reflecting photoinhibition. The fluorescence changes associated with photoinhibition were accompanied by a loss of photosystem II activities (measured by the photoreduction of dichloroindophenol by water or diphenylcarbazide), but little damage to photosystem I activity occurred under these conditions.A decrease in photosystem II activity of 80-90% by the photoinhibition could be repaired within 3 hours by incubation of the cells in light at normal growth intensities (50W/m^2/sec). The degree of repair was reduced if the recovery occurred in the dark or in the light in the presence of chloramphenicol. We suggest that photoinhibition in Chlamydomonas is a complex multistep process involving the loss of chloroplast translated polypeptides participating in the formation of the photosystem II reaction center, connection of photosystem II antannae, and possibly the formation of the herbicide binding protein.

1368 ANTENNA ORGANIZATION AND PRIMARY PHOTOCHEMISTRY IN THE AEROBIC GREEN PHOTOSYNTHETIC BACTERIUM Chloroflexus aurantiacus Robert E. Blankenship†and R. C. Fuller‡ †Chemistry Dept. Amherst College, Amherst, MA 01002, ‡Biochemistry Dept., University of Massachusetts, Amherst, MA 01002.

Spectroscopic and photochemical studies show that the primary photochemistry in this organism is much more similar to that of the purple photosynthetic bacteria than to that of the anaerobic green bacteria. The pigment content (Bchl a and Bchl c) and membrane morphology (chlorosomes), however, are very similar to that found in the anaerobic green bacteria. The antenna organization, as revealed by absorption and fluorescence spectroscopy, suggests that the membrane is similar to that of the purple bacteria with the addition of the chlorosome antenna structures. The water-soluble Bchl a protein found in anaerobic green bacteria appears to be absent, and chlorosome-depleted membranes have an absorption spectrum strikingly similar to that of the purple bacterium Rps. sphaeroides.

This remarkable organism, containing a mixture of properties previously found only in very different organisms, may be useful in understanding the evolution of photosynthesis.

Supported by USDA Competitive Research Grant 59-2259--0-1-473-0 (REB) and NSF Grant PCM 7915326 (RCF).

1369 O_2-EVOLVING PSII PREPARATIONS--STRUCTURE AND FUNCTION, Michael Seibert*, Steven P. Berg*, Terry L. Goodman†, and L. Andrew Staehelin†, *SERI, Golden, CO 80401, *University of Denver, Denver, CO 80208, and †University of Colorado, Boulder, CO 80309.

O_2-evolving PSII preparations from spinach, extracted by high Triton (T) treatment (Berthold et al., 1981, FEBS Lett. 134, 231) or by sequential treatment with low concentrations of digitonin and triton (DT; Yamamoto et al., 1982 Biochim. Biophys. Acta 679, 347), were examined by spectroscopic, electrophoretic, and EM techniques. Room-temperature absorbance spectra show similar blue shifts of the 680 nm peak compared to control chloroplasts and cannot distinguish the two PSII preparations. Low-temperature fluorescence spectra show a substantial decrease in F-735 compared to chloroplasts. Slightly less F-735 is observed in the case of the T preparations. Solubilization of the PSII preparations in 30 mM octyl glucoside followed by SDS-PAGE demonstrates that both are depleted in PSI-related complexes, Chl a-P1 and Chl a-P1* (~6% vs 16% of the total Chl in whole thylakoids). In addition, Chl a/b-P2 and Chl a/b-P2** as well as Chl a-P2 and Chl a-P3 bands were present. Denaturing gels revealed the DT preparation to contain two major peptides (16 kD and 58 kD) not found in the T preparations. Freeze-fracture EM has shown that the PSII preparations consist of highly purified, stacked grana membranes and are devoid of non-appressed connecting regions. They exhibit normal looking EFs and PFs faces and normal ESs surfaces. The DT preparations show in addition some EFu regions, and the membranes are longer than those in the T preparations. Since both preparations exhibit high O_2 evolution and low Mehler rates, we conclude that they are double membranes from the grana region and that, in spite of some residual PSI, only PSII is functional in terms of secondary electron transport.

1370 ORIENTATION OF PIGMENT-PROTEIN COMPLEXES IN THE MEMBRANE OF *RHODOSPIRILLUM RUBRUM* G-9, R. Bachofen*, R. Brunisholz+, V. Wiemken* and H. Zuber+, University of Zürich* and Federal Institute of Technology+ Zürich, Switzerland

The localization of the reaction center in the membrane of the photosynthetic bacterium *R. rubrum* has been investigated using hydrophilic and hydrophobic membrane markers and proteolytic enzymes (R. Bachofen and V. Wiemken, Abstracts 4th Int. Symp. Photosynth. Procaryotes, Bordeaux 1982, C 3 and references cited there). In contrast to the proteins of the reaction center, for the two light harvesting polypeptides the complete amino acid sequences are now known (R. Theiler et al., Abstracts 4th Int. Symp. Photosynth. Procaryotes, Bordeaux 1982, C 40). The orientation of their C- and N-termini can therefore be determined. First results have been obtained using the hydrophilic marker DABS and proteases for the degradation of the accessible polypeptide chains (V. Wiemken et al., FEMS Lebb., in press, R. Brunisholz et al., Abstracts 4th Int. Symp. Photosynth. Procaryotes. Bordeaux 1982, C 38).

1371 HIGH POTENTIAL CYTOCHROME b559, AN INDICATOR OF PHOTOSYSTEM II HETEROGENEITY, Mary A. Selak, Barbara Koch-Whitmarsh, and John Whitmarsh, Botany Department, USDA, University of Illinois, Urbana, IL 61801

Oxidation kinetics of spinach cytochrome b559 (monitored at 561-572nm) following the addition of 100 μM ferricyanide reveal a marked biphasicity. About 50% of the cytochrome is oxidized rapidly with a halftime of about 10sec and approximately half is oxidized with a halftime of about 150sec. Spectra of the fast and slow oxidation phases indicate that the fast phase is largely attributable to cytochrome b559 while the slow phase is a mixture of both cytochrome b559 and cytochrome f oxidation. In the presence of 1mM ferricyanide, the oxidation kinetics are monophasic with a halftime of about 5sec. The stoichiometry of high potential b559: chlorophyll was found to be 1:295. No observable differences were noted in the ferricyanide oxidation kinetics of phosphorylated, dephosphorylated, and nonphosphorylated pea chloroplast samples. Thylakoid unstacking did not eliminate the biphasic cytochrome oxidation observed following 100 μM ferricyanide, but unstacking did decrease the velocity (three to four-fold) of both the fast and the slow oxidation phases. Heat treatment of chloroplasts abolished all responsiveness to ferricyanide. The slow phase of the ferricyanide oxidation response was eliminated in osmotically shocked chloroplasts and all cytochrome b559 oxidized in these samples was hydroquinone-reducible. The differential reactivity seen in the presence of ferricyanide appears to arise from different locations of homologous species of b559 within the plane of the thylakoid membrane. No information is available to determine whether the different species of b559 result from an inter or an intra-photosystem II heterogeneity. A close physical association between the oxygen evolving apparatus and high potential b559 is suggested by observations that inhibition of oxygen evolution is often accompanied by a decrease in the midpoint potential of cytochrome b559.