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UNIVERSITY OF CALIFORNIA

CHEMICAL BIODYNAMICS DIVISION

ANNUAL REPORT
July 1982–June 1984

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CHEMICAL

BIODYNAMICS

DIVISION

George C. Pimentel
Division Head

ANNUAL REPORT

July 1982 - June 1984

LAWRENCE BERKELEY LABORATORY
University of California
Berkeley, CA 94720

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The Chemical Biodynamics Division of the Lawrence Berkeley Laboratory is also known as the Laboratory of Chemical Biodynamics, an Organized Research Unit of the Univerisity of California and the word "laboratory" as used in the text refers to both.

INTRODUCTION

Chemical Biodynamics was established in 1945 as a Division of Lawrence Berkeley Laboratory to conduct basic research on the dynamics of living cells and on the interaction of radiant energy with organic matter. The Division has made rich contributions to our understanding of the molecular mechanisms of photosynthesis and of the effects of environmental pollutants on plant and animal cells. Its interactions with various departments on the Berkeley campus of the University of California are enhanced by its physical location. Chemical Biodynamics draws its M.S. and Ph.D. candidates from over thirteen departments and groups of the University, with the single largest component being in chemistry. The Division attracts research scientists from all over the world; during the current year ten foreign postdoctoral and visiting faculty personnel from nine countries have conducted research in the Chemical Biodynamics laboratory.

Four broad aims guide the Division:

- to maintain the strong interdisciplinary character of the laboratory
- to build upon the expertise of the existing laboratory staff and to exploit the excellent array of facilities in Chemical Biodynamics
- to develop coherent and contemporary research themes that are fundamental in character, forward looking in their broad direction, and that optimize the likelihood of synergistic interaction across the disciplines represented in the laboratory.
- to strengthen collaborative ties to faculty research scientists in the biological sciences

Two themes are being developed that are consistent with these aims. Both are directed at fundamental areas of knowledge essential to the most effective utilizationof solar energy. The first theme, Photon Conversion, pertains to the fundamental aspects of the interaction of light with matter; the absorption, migration, relaxation, transformation, and storage of energy derived from photons. It will extend from photobiology, already a strong area within the laboratory, through photochemistry to photophysics.

The second theme, Genetics of Photosynthesis, is the application of recombinant DNA techniques to photosynthetic organisms. The staff's experience in molecular biology, plant cell culture, and the functional mechanisms of the chloroplast are being exploited as we investigate and attempt to control the genetic material from which the photosynthetic apparatus arises.

George C. Pimentel, Head
Chemical Biodynamics Division

OVERVIEW

OF

CHEMICAL BIODYNAMICS

ACTIVITIES

July 1, 1982 - June 30, 1984

I. PHOTON CONVERSION

A. Photosynthesis in Plants and Photosynthetic Bacteria

1. Structure and Organization of Photosynthetic Membranes

Professor Kenneth Sauer

Understanding of the mechanism of the conversion of solar energy into chemical energy in photosynthetic organisms requires a knowledge of the composition and organization of the membranes in which the light reactions occur.

Some of the significant accomplishments that we have made in this area during the past year include:

- development of an affinity chromatography method for isolating and rapidly purifying reaction centers in high yield
- partial sequence determination of each of the three reaction center proteins of Rhodopseudomonas capsulata, so far confirming the complete DNA sequences of the corresponding genes worked out by John Hearst and his group
- proposal of a sequence segment responsible for quinone binding, based on significant homologies to a chloroplast membrane protein
- initial exploration of crystallization of reaction center complexes
- development of a suicide quinone to label the peptide center complexes

The isolation of bacterial reaction centers in high yield and purity is now routinely used to accumulate sufficient material for biochemical and structural determinations. We have applied this approach successfully to reaction centers from Rhodopseudomonas sphaeroides, Rhodospirillum rubrum and Photosystem 1 of higher plant chloroplasts; however, we are using it primarily on the wild-type and on the blue-green (carotenoidless) mutants of Rps. capsulata. This is the same organism for which Prof. John Hearst and his group have recently isolated and sequenced the genes coding for the three reaction center polypeptides and those of one of the light-harvesting bacteriochlorophyll (BChl) proteins. Although the DNA sequence of the genes can be translated unambiguously into the amino acid sequence of the corresponding proteins, it is essential to confirm the predicted sequences, because a single missed or extra nucleotide would result in a shift in the reading frame and generate a nonsense segment. For this purpose we have used our affinity chromatograpy method followed by preparative gel electrophoresis to obtain pure preparations of each of the three reaction center polypeptides from Rps. capsulata. By direct amino acid sequencing we have confirmed the N-terminal sequences predicted by the DNA sequencing for each of these polypeptides. We are in the process of extending this to internal fragments generated by specific cleavage reactions and will also check the C-terminus sequences. We feel strongly that the predicted sequences are correct. This is the first photosynthetic reaction center for which complete sequence information is available. Other groups, expecially that of Feher at UC San

Diego, are working on reaction centers from different organisms and have published or announced partial sequence information.

Relevant Abstracts

Procedure for Rapid Isolation of Photosynthetic Reaction Centers Using Cytochrome c Affinity Chromatography. G. W. Brudvig, S.T. Worland, and K. Sauer. Proc. Natl. Acad. Sci. USA, 80, pp. 653-686, 1983.

Horse heart cytochrome c linked to Sepharose 4B is used to purify reaction centers from Rhodopseudomonas sphaeroides R-26. This procedure allows for an initial recovery of 80-90% of the bacterial reaction centers present in chromatophore membranes. High purity reaction centers ($A_{280}/A_{802} < 1.30$) can be obtained with a 30% recovery. Reaction centers from wild-type Rps. sphaeroides and Rps. capsulata also bind to a cytochrome c column. Cytochrome c affinity chromatography can also be used to isolate photosystem I complexes from spinach chloroplasts.

Protein Sequence Homologies between Portions of the L and M Subunits of Reaction Centers of Rhodopseudomonas capsulata and the Q_B-Protein of Chloroplast Thylakoid Membranes: a Proposed Relation to Quinone-Binding Sites. John E. Hearst and Kenneth Sauer. Z. Naturforsch 39 c, 421-424 (1984).

Sequence homology has been found between the L and M subunits of Rhodopseudomonas capsulata reaction centers and the Q_B-protein from all species thus far sequenced. The homology between the L subunit and the Q_B-protein is contained in the sequence PFHMLG---F----AMHG-LV-S and is believed to have survived three billion years of evolution. A model is presented which associates quinone bindng with this highly conserved sequence.

2. Photosynthetic Light Reactions - Kinetics and Mechanism
Professor Kenneth Sauer

Using spectroscopic methods, such as EPR and fluorescence decay, with the ability to monitor fast kinetic responses, we have explored the initial events in excitation transfer and primary charge separation in the photosynthetic light reactions.

Important accomplishments made during the past year include:

a) Fluorescence decay kinetics measurements using single-photon timing
- the state of chemical reduction profoundly influences the amplitude of the slow decay components of fluorescence from chloroplasts, but not the fast or middle components
- heterogeneity of Photosystem 2 reaction centers is evident
- changes in decay kinetics accompany the development of chloroplast membranes

- thylakoid membrane protein phosphorylation influences the decay pattern, reflecting changes in excitation migration

b) Electron paramagnetic resonance of unpaired electrons, radical-pair and triplet species in chloroplasts

- the orientation and temperature dependence of EPR transients characterize two primary acceptor species in Photosystem I
- kinetics of the rise of the rapid light-induced Signal II and the decay of $P680^+$ show that the two components are immediately adjacent to one another in Photosystem 2.

Charge separation in the reaction centers upon illumination produces EPR signals from the unpaired electrons present on the donor and acceptor species. In reaction centers of Photosystem I of plant chloroplasts this results in transient electron spin polarization originating in radical pair interactions involving the electron donors and acceptors. At low temperature we have resolved the decay kinetics of the polarization relaxation. Using samples containing oriented chloroplast membranes we have determined the orientation of the PS I spin polarization tensor in the membrane. Analysis of the orientation dependence of the signal relaxation as a function of magnetic field strength revealed the presence of a new electron acceptor in the PS I reaction center, a component that is more primary than the acceptor A_1 that we had discovered previously. The new electron acceptor, designated A_0, may be a chlorophyll molecule, whereas the EPR spectrum of A_1 is distinctly different from that of Chl^-.

We have completed our initial characterization of the fluorescence decay kinetics of chloroplast membranes using our single-photon timing spectrometer. We are now applying this approach to investigating pigment organization in the photosynthetic membranes, excitation transfer within pigment proteins and the complex nature of the excited state decay process. We have been able to show that the phosphorylation of the membrane protein that contains the Chl a/b light harvesting antenna quenches the slow fluorescence decay component in a way that reflects a decreased antenna size of Photosystem II. These changes reverse upon dephosphorylation of the membrane protein, and they are thought to reflect an important regulator of excitation partitioning between PS I and PS II. In another study we have found that the development from etioplast to chloroplast is accompanied by dramatic changes in Chl fluorescence decay, especially as the early forms of Chl become incorporated and connected progressively to functioning reaction centers.

The accompanying pigment C-phycocyanin, found in a variety of blue-green (cyanoacteria), is an attractive molecule for investigating the mechanism of excitation transfer among its chromophores (phycocyanobillins). We have studied steady-state and time resolved fluorescence of this hexameric molecule (18 chromophores) subunits. Fluorescence depolarization provides clear evidence of the decreasing extent of excitation transfer that accompanies dissociation of this pigment protein. Time-resolved studies exhibit some unexpected complexity, however. The subunit containing just a single chromophore nevertheless exhibits two exponential decay components. We find no evidence that this reflects partial denaturation of the sample, and we will pursue the origin of this unusual behavior in future studies. This work has

benefitted from a new association of Prof. Alex Glazer (Microbiology/Immunology Department) with the Chemical Biodynamics Laboratory.

In the light reaction associated with Photosystem 2 of higher plants, the primary donor P680 transfers an electron first to pheophytin and then to a quinone Q that serves as a fluorescence quencher in its oxidized form. The oxidized $P680^+$ becomes quickly re-reduced. The ultimate electron donor is water, but a complex and little understood reaction path intervenes. We have succeeded in determining that the immediate electron donor to $P680^+$ is the species designated Z that is the origin of EPR Signal II_{vf} (or II_f). This was accomplished in two stages. We first studied the kinetics of Signal II_f in tris-washed chloroplsts where O_2 evolution is inactivated. We were able to resolve the rise kinetics of Signal II_f (4-15 μsec) and show that they are identical to the decay kinetics of $P680^+$, which had previously been reported from optical measurements over a range of pH values. At the same time we discovered a new EPR signal attributable to $P680^+$ and that exhibits a decay rate that closely matches the Signal II_f rise. We then went on to study intact chloroplasts and O_2-evolving PS 2 particles. In this case the $P680^+$ decay kinetics is known from optical measurements to be much faster (30-600 ns). In a previous study we had seen rise kinetics for Signal II_{vf} that seemed to be significantly slower. We now find that those measurements were troubled by previous instrument limitations and apparent sample deterioration. Our new investigations indicate that the rise kinetics of signal II_{vf} are faster than our instrument time constant (2 μs). Thus, there is no longer any discrepancy with the conclusion, based on tris-washed chloroplasts, that Z is the immediate donor to $P680^+$. Using a new pulsed EPR spin echo spectrometer (under construction) we hope to have sufficient time resolution to verify this association for O_2-evolving preparations.

On the electron acceptor side of PS 2, we can monitor the participation of Q by virtue of its effect on chlorophyll fluorescence. In previous studies using our single-photon timing system we showed that the slowest (1-2 nsec) of the three resolved fluorescence decay components has an amplitude that increases dramatically as Q becomes reduced photochemically. The fast (50-100 psec) and middle (400-700 psec) decay components are not affected by the redox state of Q. We have gone on to investigate the behavior of Q as an electron acceptor and its relation to fluorescence quenching by carrying out chemical reductive titrations of this species and measuring the fluorescence decay kinetics at a set of known potentials. The titration curves show two very clear transitions with midpoint potentials at +120 mV and -350 mV (pH 8.0). The first of these is associated with an increase in amplitude of the slow fluorescence decay component that is about equal to that seen upon photochemical reduction. The second and low-potential transition seen electrochemically provides a near doubling of the slow component yield and a significant increase in its time constant. This stage does not correspond to any known physiological state of PS 2, and its origin is currently unknown. Over the full range of potentials investigated (+300 to -400 mV) there was no change in either the fast or the middle fluorescence decay component.

In another study we investigated the nature of the reported heterogeneity of PS 2 that is suggestive that there are two distinct classes of these reaction centers in chloroplasts. Because these were known to be affected by

the concentration of added divalent ions (Mg^{2+}), we studied the effect of ion concentration on the redox behavior of the fluorescence decay. Both midpoint potential transitions were seen under all conditions, indicating that we do not have a situation where each is associated with a particular type of PS 2 center. There was an effect of Mg^{2+} concentration on the value of the low midpoint potential, and we have interpreted this in terms of thylakoid structural rearrangements involved in cation-regulated grana stacking.

Relevant Abstracts

Potentiometric Titration of Photosystem II Fluorescence Decay Kinetics in Spinach Chloroplasts. Kerry K. Karukstis and Kenneth Sauer, Biochem. Biophys. Acta, 722, 364-371 (1983).

The fluorescence yield of chloroplasts reflects the redox state of the electron acceptor of the Photosystem II reaction center, with increasing yield as the acceptor is reduced. Chemical reductive titrations of fluorescence yield in chloroplasts at room temperature indicate two distinct midpoint potentials, suggesting the possibility of Photosystem II electron acceptor heterogeneity. We have carried out a potentiometric titration of the fluorescence decay kinetics in spinach chloroplasts using a continuous mode-locked dye laser with low-intensity excitation pulses and a picosecond-resolution single-photon timing system. At all potentials the fluorescence decay is best described by three exponential components. As the potential is lowered, the slow phase changes 30-fold in yield with two distinct midpoint potentials, accompanied by a modest (3-fold) increase in the lifetime. The titration curve for the slow component of the fluorescence decay of spinach chloroplasts is best characterized by two single-electron redox reactions with midpoint potentials at pH 8.0 of + 119 and -350 mV, with corresponding relative contributions to the fluorescence yield of 49 and 51%, respectively. There is little change in the fast and middle components of the fluorescence decay. We found that the oxidized form of the redox mediator 2-hydroxy-1,4-naphthoquinone preferentially quenches the fluorescence, causing an anomalous decrease in the apparent midpoint of the high-potential transition. This effect accounts for a significant difference between the midpoint potentials that we observe and some of those previously reported. The selective effect of reduction potentials on particular fluorescence decay components provides useful information about the organization and distribution of the Photosystem II electron acceptor.

3. Mechanism of Water Oxidation and Oxygen Evolution

Dr. Melvin Klein

The reaction by which absorbed visible light causes the splitting of water into molecular oxygen and reducing components at the level of molecular hydrogen is of great interest because of the possibility of applying knowledge

of its mechanism in photosynthesis to design artificial solar energy converters.

During the past year we have made the following accomplishments in this area:

- characterization of the kinetics and temperature dependence of a multiline EPR signal detetable at 10 K identified it as resulting from the S2 state of the water splitting complex
- the influence of added anions (Cl^-, Br^-, F^-, etc) on the multiline EPR show their direct involvement in the S2 center
- X-ray absorption edge energies of Mn correlate closely with the EPR signal amplitude, reflecting oxidation state changes that accompany S-state advancement
- EPR evidence for a new intermediate, probably containing iron in a rhombic (S=5/2) environment and apparently lying between the S2 species and the donors to Photosystem 2
- preliminary X-ray studies of Fe-containing species that participate in photosynthetic electron transport.

An important feature of the mechanism of photosynthetic water oxidation to molecular oxygen is the ability of chloroplast thylakoid membranes to store high-potential oxidizing intermediates in the four-electron transfer reaction. Several years ago a low temperaure EPR "multiline" signal was detected and attributed to the S2 state in the four step mechanism as formulated by Kok. The spectroscopic structure of this signal is suggestive of contribution by manganese to its origin. We have explored the temperature dependence of its formation upon flash or continuous illumination and its disappearance either by relaxation back to state S1 or its advancement to S3. In all respects the EPR signal behaves as one would expect from prior knowledge of the kinetic properties inferred for S2. As a further consequence of these studies involving low temperature illumination, we have recently trapped and identified a state that is a precursor to S2. Associated with this state is the appearance of a new light-induced EPR signal at g 4.3. Upon warming in the dark, the g 4.3 signal disappears concomitantly with the appearance of the S2 multiline signal. Thus, the g 4.3 species, which is thought to reflect an iron atom (S=5/2) in a rhombic environment, is a candidate for an electron carrier lying between the water-oxidation complex and the reaction center of Photosystem 2. This is the first evidence for the direct paticipation of Fe on the electron donor side of Photosystem 2 in a reaction leading to O_2 evolution.

The ability to generate reproducibly the multiline EPR signal which is associated with S2 provides a long sought marker for the presence and quantity of at least one of the S intermediates. Coupled with procedures for preparing samples enriched in Photosystem II components we were able to make comparisons between the magnitude of the EPR signal and the detailed features of the Mn X-ray K-edge spectra. Indeed, there are strong correlations between the intensity of the mn multiline signal and the inflection point energy and shape between samples prepared in states S1 and either S2 or S3, establishing, for the first time, a direct relationship between the local environment of Mn and S state composition. Samples in S2 and S3 exhibit a broadening of the

principal absorption peak and a shift to higher energy by as much as 2.5 eV relative to S1 samples. Changes of this nature are classic behavior for loss of ligand symmetry caused by metal oxidation and/or forced ligand geometry distortion. The effect of a shift of one of the components with respect to the other is to broaden the principal absorption maximum in a manner similar to that observed. The detailed sources and origins of these changes, be they purely oxidation state, ligand or protein conformation change, or a combination thereof, are topics of our planned future research. Based on these observations, and assuming that the multiline EPR spectrum arises from a pair of coupled Mn atoms, an interpretation based on our earlier EXAFS results, we have generated a model which is modified and refined from one previously postulated for the flow of electrons during the evolution of the oxygen as it progresses from S0 through S4. The essence of the model is that in states S0, S1 and S1*, the oxidation states of the Mn atoms are (+2,+3), while charge accumulates on donor molecules which are part of the complex. States S2 through S4 have manganese as (+3,+4) with additional photoinduced charges again accumulating on the donors. In effect, charge is transferred from the donors to hydroxyl oxygen converting them to the level of peroxide, as a binuclear peroxo-bridged species. Upon transfer of two more units of charge, the peroxy oxygens are converted to O_2 and the manganese pair returns to the (+2,+3) level.

We have also obtained low temperature EPR spectra for what appears to be two classes of manganese which are weakly bound to the photosynthetic membranes and thought to play no crucial role in oxygen evolution. Rather, they appear to be atoms adventitiously bound to the membranes of dislodged from their usual binding sites by various treatments. One of these occurs at fields lower than 400 G at 9 GHz while the second class occurs at about 700-800 G. Each is marked by Mn hyperfine structure. We have been able to simulate the very low field signal and ascertain its spin-Hamiltonian parameters. Additionally, we have measured the binding constants for the two classes of sites.

In order to ascertain the X-ray parameters for other situations in which Mn plays a crucial role, we have studied the edge and EXAFS of several Mn-containing superoxide dismutase enzymes. The resting reduced proteins are determined to be in the Mn(III) oxidation state; upon one electron reduction they become Mn(II) and exhibit K-edge shifts almost exactly as preducted from our earlier determination of binding energy vs coordination charge. The EXAFS of these proteins has been determined. Analysis has led to two equally valid sets of coordination environments. One contains oxygen and/or nitrogen ligation while the other environment contains a sulfur ligand. Interestingly, the protein isolated from yeast does not yield a minimum with sulfur ligation, nor are there any sulfur bearing amino acids in its sequence. Substitution of iron into the manganese enzyme yields data which analyze for the same coordination as the native metal, even though the cross substituted proteins are not enzymatically active. This work has raised some provocative questions relating to the evolutionary relationship between the Mn and Fe proteins, which have significant regions of homology. One can create a logical sequence of events based on a point mutation in the triplet codon for methionine leading to leucine or isoleucine. The former is suggested as the sulfur

ligand in the Mn form while the latter residues are at the homologous sites in the Fe forms of the protein.

Relevant Abstracts

The Effect of Temperature on the Formation and Decay of the Multiline EPR Signal Species Associated with Photosynthetic Oxygen Evolution. Gary W. Brudvig, John L. Casey and Kenneth Sauer. Biochem. Biophys. Acta 723, 366-371 (1983)

We have investigated the effects of temperature on the formation and decay of the light-induced multiline EPR signal species associated with photosynthetic oxygen evolution (Dismukes, G.C. and Siderer, Y. (1980) FEBS LETT. 121, 78-80). (1) The decay rate following illumination is temperature dependent: at 295 K the half-time of decay is about 40 s, at 253 K the half-time is approx. 40 min. (2) A single intense flash of light becomes progressively less effective in generating the multiline signal below about 240 K. (3) Continuous illumination is capable of generating the signal down to almost 160 K. (4) Continuous illumination after a preilluminating flash generates less signal above 200 K than at lower temperatures. Our results support the conclusion of Dismukes and Siderer that the S_2 state gives rise to this multiline signal; we find that the S_1 state can be fully advanced to the S_2 state at temperatures as low s 160 K. The S_2 state is capable of further advancement at temperatures above about 210 K, but not below that temperature.

The State of Manganese in the Photosynthetic Apparatus 3. Light-induced changes in X-ray Absorption (K-edge) Energies of Manganese in Photosynthetic Membranes. David B. Goodin, Vittal K. Yachandra, R. David Britt, Kenneth Sauer and Melvin P. Klein. Biochim. Biophys. Acta (BBA41615 in press) 1984.

Photosynthetic water oxidation by higher plants proceeds as though five intermediates, So-S4, operate in a cyclic fashion. In this study of the manganese involvement in the process, a low temperature EPR signal is used as an indicator of S-state composition for manganese X-ray absorption K-edge measurements of a spinach Photosystem II preparation. A dramatic change is observed in the edge properties between samples prepared in states S1 and either S2 or S3, establishing a direct relation between the local environment of Mn and the S-state composition. Samples in S2 or S3 exhibit a broadening of the principal absorption peak and a shift to higher energy by as much as 2.5 eV relative to S1 samples. The magnitude of these changes is directly related to the EPR signal intensity induced by illumination. Models are discussed in which these data may be interpreted in terms of a conformation-induced change in Mn ligation and/or oxidation during the S1 to S2 transition.

4. Regulation of Photosynthetic Carbon Metabolism

Dr. James A. Bassham

The life cycle of green plants requires precise physiological control of photosynthetic carbon metabolism in accordance with the plant's needs during growth and in response to environmental conditions. Long term control is achieved through regulation of expression of genes coding for enzymes which catalyze reactions of metabolism. Short term and fine control occurs via modulation of the activities of the enzymes for rate-limiting steps in accordance with conditions such as light, temperature, nutrition, location of the enzyme within plant organs and subcellular organelles, and the biosynthetic requirements of the plant.

We are determining sites and mechanisms of both types of control. Of particular interest are reactions catalyzed by enzymes such as pyruvate-phosphate dikinase (PPDK) which is synthesized in only one of two types of photosynthetic cells in certain plants called C_4 which employ a particularly efficient carbon dioxide collecting mechanism in photosynthesis. Once synthesized, this enzyme is subject to strong metabolic control by light and other factors. Moreover, this enzyme catalyzes a reaction that cannot be allowed to occur at the same time and place as another regulated reaction (mediated by pyruvate kinase, PK) since the simultaneous reactions would wastefully deplete the plant cell's ATP, causing cell death. These two enzymes and another related enzyme, phosphoenolpyruvate carboxylase (PEPC), are the focus of much of our work on both gene expression and metabolic regulation. Immunoprecipitation methods have been developed for visuallizing the polypeptide subunits of these enzymes following sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of extracted soluble proteins for screening the presence of these enzymes in tissues and organelles.

Last year we described a number of advances in understanding of the gene expression and regulation of these enzymes in photosynthetic cells and seeds of both C_3 (such as wheat) and C_4 (such as corn) tissues. These advances included: 1) progress in understanding the regulatory effects of ammonia in the cell cytoplasm, 2) isolation and regulatory properties of two cytoplasmic isozymes of PK, 3) presence of PPDK in wheat leaves and seeds and in several other C_3 plants, 4) proof that PPDK is a chloroplast enzyme and is nuclear encoded in maize, a C_4 plant, 5) PPDK has similar regulatory properties in some C_3 and C_4 tissues, 6) the level of PPDK protein in developing wheat seeds follows that of mRNA for PPDK during seed development, indicating control of gene expression at the level of DNA transcription. Many of these findings were extended and published during the past year.

In addition, during the past year we found that in wheat, just as had been reported for maize (a C_4 plant), when mRNA from leaves is extracted and translated *in vitro*, the resulting PPDK polypeptide (determined by PAGE and immunodetection) has a molecular weight of 110 kdaltons, compared to 94 kdaltons for the native polypeptide found in both leaves and seeds of both wheat and maize. In the case of maize, the extra 16 kdaltons of polypeptide length is considered to be a "leader" which is recognized by the chloroplast membrane and is removed during or after transit into the chloroplast. Our evidence suggests that a similar situation exists in leaf cells of wheat, a C_3 plant.

When we carried out similar studies with in vitro translation of mRNA extracted from seeds of wheat and kernels of maize, only 94 kdalton polypeptide was detected in both cases. It therefore appears that in both wheat and maize, there is a tissue-specific expression of PPDK polypeptide precursor. In leaf cells it is likely that the protein functions in the chloroplasts while in seeds it is in the cytoplasm. The regulation of this differential tissue-specific expression will be an important issue. Also it may be related to the inverse expression of PPDK and ribulose 1,5-bisphosphate carboxylase small subunit (RuBPCase-SS) in mesophyll and bundle-sheath leaf cells. The presence of PPDK in the chloroplasts of a C_3 plant has interesting implications for the possibility of some kind of intracellular C_4-type of metabolism in C_3 leaf cells, and possible improvement of photosynthetic efficiency in C_3 plants of agricultural importance through genetic engineering in the future.

The gene expression of PEPC has been followed in callus tissue culture of two species of Euphorbia (remii and degenerii), and compared with expression of parent plant tissues and in tissues of plants regenerated from tissue cutlure. There are two isozymes of PEPC, one of which is associated with general metabolism and is found in all cells, and one which is specifically associated with C_4 photosynthetic carbon metabolism. The latter is found in parent leaf cells and in cells of regenerated plants, but is not found in callus cells, even when they are quite green. It appears that in C_4 plant tissues, at least in these species, Kranz anatomy, associated with organized leaf structure, is required for the expression of this aspect of C_4 carbon metabolism.

Relevant Abstracts

Ammonia Regulation of Intermediary Metabolism in Photosynthesizing and Respiring Chorella pyrenoidosa: Comparative Effects of Methylamine. Tamotsu Kanazawa, Mark Distefano and James A. Bassham, Plant Cell Physiol. 24, 979-986 (1983).

Methylamine added to the medium of the unicellular alga Chlorella pyrenoidosa causes most of the shifts in carbon metabolism previously observed with ammonia addition. The most prominent and reproducible effect seen is a greatly increased flow of carbon from triose phosphates into alanine, aspartate, and glutamate, and acids of the tricarboxylic acid cycle including malate, fumarate, and citrate.

The large increase in synthesis of glutamine seen with ammonia addition does not occur with methylamine addition. It is concluded that accelerated glutamine synthesis per se is not the primary cause of the other regulatory shifts in carbon metabolism seen with ammonia (and now with methylamine) as has been proposed earlier.

Stimulation of phosphoenolpyruvate carboxylase by methylamine supports a previous proposal that the activity of this enzyme increases with a small rise in cytoplasmic pH

resulting from entry of ammonia into the cytoplasm. Increases in the activity of pyruvate kinase and the rate of oxidation of triose phosphate remain unexplained. If these changes are due to change in ATP/ADP ratio, the cause for the change in this ratio must be found elsewhere than in a stimulation of the rate of glutamine synthesis.

Pyruvate Orthophosphate Dikinase in Wheat Leaves. Kazuko Aoyagi and James A. Bassham. Plant Physiol. 73, 853-854 (1983).

Pyruvate orthophosphate dikinase (PPDK) was found in wheat (*Triticum aestivum* L. cv Cheyenne [CI 8885]) leaves both by activity assays and by the protein blot method. The specific activity of the wheat enzyme is comparable to that of PPDK from maize leaves. Of the total soluble protein in wheat leaves, about 0.05% was PPDK, comparable to the amount in the immature wheat seed and about 1/70th the amount found in mesophyll cells of maize. Immunoprecipitation of wheat PPDK with maize enzyme antiserum indicates partial identity, and the apparent subunit molecular weight is the same based on sodium dodecyl sulfate-polyacrylamide gell electrophoresis.

Spinach Pyruvate Kinase Isoforms, Partial Purification and Regulatory Properties. Chris Baysdorfer and James A. Bassham. Plant Physiol. 74, 374-379 (1984)

Pyruvate kinase from spinach (*Spinacea oleracea* L.) leaves consists of two isoforms, separable by blue agarose chromatography. Both isoforms share similar pH profiles and substrate and alternate nucleotide K_m values. In addition, both isoforms are inhibited by oxalate and ATP and activated by AMP. The isoforms differ in their response to three key metabolites; citrate, aspartate, and glutamate. The first isoform is similar to previously reported plant pyruvate kinases in its sensitivity to citrate inhibition. The K_i for this inhibition is 1.2 millimolar citrate. The second isoform is not affected by citrate but is regulated by aspartate and glutamate. Aspartate is an activator with a K_a of 0.05 millimolar, and glutamate is an inhibitor with a K_i of 0.68 millimolar. A pyruvate kinase with these properties has not been previously reported. Based on these considerations, we suggest that the activity of the first isoform is regulated by respiratory metabolism. The second isoform, in contrast, may be regulated by the demand for carbon skeletons for use in ammonia assimilation.

Pyruvate Orthophosphate Dikinase of C_3 Seeds and Leaves as Compared to the Enzyme from Maize. Kazuko Aoyagi and James A. Bassham, Plant Physiol. 75, 387-392 (1984).

Pyruvate orthophosphate dikinase (PPDK) was found in various immature seeds of C_3 plants (wheat, pea, green bean, plum, and castor bean), in some C_3 leaves (tobacco, spinach, sunflower, and wheat), and in C_4 (maize) kernels. The enzynme in the C_3 plants cross-reacts with rabbit antiserum against

maize PPDK. Based on protein blot analysis, the apparent subunit size of PPDK from wheat seeds and leaves and from sunflower leaves is about 94 kdatons, the same as that of the enzyme from maize, but is slightly less (about 90 kdaltons) for the enzyme from spinach and tobacco leaves. The amount of this enzyme per mg of soluble protein in C_3 seeds and leaves is much less than in C_4 leaves. PPDK is present in kernels of the C_4 plant, Zea mays in amounts comparable to those in C_4 leaves.

Regulatory properties of the enzyme from C_3 tissues (wheat) are similar to those of the enzyme from C_4 leaves with respect to in vitro cold lability (seeds and leaves).

Following incorporation of $^{14}CO_2$ by illuminated wheat pericarp and adjoining tissue for a few seconds, the labeled metabolites were predominantly products resulting from carboxylation of phosphoenolpyruvate, with lesser labeling of compounds formed by carboxylation of ribulose 1,5-bisphosphate and operation of the reductive pentose phosphate cycle of photosynthesis. PPDK may be involved in mechanisms of amino acid interconversions during seed development.

5. Hydrocarbons from Plants
Professor Melvin Calvin

This project involves the study of green plants as a renewable resource for high energy liquid fuel production. A great number of plant species have already been screened as candidates for energy use. The results that emerged from the research programs conducted over the past five years delineate the basic problems as well as the important areas for future research. In this field it is generally recognized that biomass research must be an integrated endeavor with agronomic, engineering, chemical, biochemical and environmental research components. However, one important limitation to the development of bioenergy crops for liquid fuel use is the apparent upper limit of approximately 15% of dry weight of reduced photosynthetic materials that plants surveyed to date seem to produce. An increase in this percentage, even without a corresponding increase in total biomass yield, is predicted to have an important effect on the final cost of a barrel, whether this barrel is used for chemicals or for fuel.

One major obstacle to rapid improvement of hydrocarbons yields is our limited knowledge of the fundamental biosynthetic mechanism, including regulation of secondary plant products. Such knowledge is imperative for any future work on the development of plants that overproduce hydrocarbons.

Research efforts have recently been focussed on the elucidation of terpene and terpene ester biosynthesis. Acetate and mevalonate have both been found to be efficient substrates for terpenoid production; both the triterpenols and the triterpenol esters are labeled when radioactive acetate or mevalonate is incubated in fresh latex. Since acetate can be utilized for fatty acid synthesis as well as isoprenoid synthesis, we have investigated the labeling pattern of triterpene esters. The triterpene esters were hydrolyzed

with methanolic potassium hydroxide and the resulting triterpenols and fatty acids were separated by thin layer chromatography. Only the triterpenols were labeled; therefore, acetate is not utilized in this system for the synthesis of the fatty acid moiety of the triterpene esters. Consequently, an endogenous pool of the fatty acids must be present in latex or must be transported from the surrounding cells to the laticifers. We have isolated another major new lipid class from latex, identified it as phospholipids and have shown that the phospholipids represent the endogenous fatty acid pool that is utilized for triterpene ester synthesis.

(a) Investigation of Triterpenoid Synthesis in Latex. A new triterpenol has been detected in Euphorbia lathyris latex by HPLC. The structure of this triterpenol was shown to be 24-methylene lanosterol by comparison of the latex compound with independently synthesized 24-methylene lanosterol.

(b) Investigation of Triterpenoid Production in Latex. In order to identify the site of triterpenoid synthesis in latex we have explored several ways of fractionating it. We have previously shown tha the site of triterpenol synthesis from the precursor mevalonate (MVA) is pelletable by centrifugation at 10,000-20,000 g. Even at 10,000 g, however, mitochondria start to pellet as well, so we have investigated the properties of latex that was centrifuged at only 5000 g for 15 min. MVA metbolism to triterpenols and triterpene esters (ST + STE) still occurs in the pellet, but according to our enzyme assays for mitochondria (fumarase) these are now exclusively in the supernatant. In some other plant systems mitochondria have been implicated as the site of terpenoid synthesis; this may be also true in latex for the early steps (Ac MVA), but the site of MVA metabolism is now separated from the mitochondria.

Even though we have established that the site of the MVA to triterpene process is the 5000 g pellet, we have observed some qualitative differences between triterpene synthesis in whole latex vs. this pellet. We have reported previously that in whole latex triterpene ester synthesis is significantly faster than that of the free triterpenols. The typical ratio in whole latex is 6:1; the corresponding ratio in the pellet is 3:1. This change in ratio is due to loss of triterpene ester synthesis only. On recombination of supernatant and pellet some increase in triterpene ester synthesis is observed, but the ratio is never restored to that seen in whole latex. The supernatant does not have an effect on the rate of free triterpenol production.

Examination of the synthesis of the six individual triterpenols in the pellet showed another difference between the properties of whole latex and pellet. The most striking difference is observed in the rates of synthesis of cycloartenol and 24-methylene cycloartenol. In the 5000 g pellet, the rate of synthesis of the 24-methylene compound is decreased, whereas that of cycloartenol is increased, compared to the rates observed in whole latex. The methyl donor in this step is S-adenosylmethionine (SAM), the substrate for the transmethylase in the conversion of cycloartenol to the 24-methylene cycloartenol. It appears that the pellet is deficient in SAM; the process of centrifugation and washing removed sufficient quantities of this substrate so that the rates of synthesis of the 24-methylene compounds are decreased. When

SAM is added to the pellet the rates are restored to that observed in whole latex.

Exogenously added SAM also affects the triterpene ester to triperpenol ratio. At the optimal concentration of 50 M SAM this ratio is restored to that observed in whole latex.

SAM has two functions in latex: methylation on nitrogen to form PC and methylation on carbon to form the 24-methylene triterpenes. The observed increase of total biosynthesis in the pellet upon addition of SAM provides a partial explanation for the different levels of terpenoid synthesis in whole and centrifuged latex. It is likely, however, that there is yet another factor that is needed at the site of terpenoid production so that a reconstituted system could be achieved.

Relevant Abstracts

New Sources for Fuel and Materials. Melvin Calvin, Science, 219, 24 (1983).

A selection of new plant sources with high potential for production of chemicals and liquid fuels is reviewed. Some productivity data are given, and suggestions are made for modification of both the product character and the productivity of the plants.

Terpenoid Biosynthesis in Euphorbia Latex. E.K. Nemethy, C. Skrukrud, G.J. Piazza and M. Calvin. Biochem. Biophys. Acta 760, 343-349 (1983)

The latex of *Euporbia lathyris* can utilize acetate, pyruvate and mevalonate for triterpene synthesis in vitro. Acetyl-CoA, hydroxymethylglutarate, hydroxymethylglutaryl-CoA and isopentenyl pyrophosphate were not effective as precursors for triterpene biosynthesis. Acetate is utilized only by the terpenoid pathway and by the tricarboxylic acid cycle; it is not used for fatty acid synthesis in this system. However, phospholipids were found to be efficient acyl donors for triterpene ester synthesis. The observed selectivity of precursor utilization as well as the observed rates for product formation indicate separate sites for triterpenol and triterpene ester synthesis and that one is not precursor for the other.

B. Artificial Photosynthesis

We wish to understand the essential elements of photosynthesis so that we can tailor and optimize them to human needs. Some of our studies mime natural photosynthesis closely. Others are more general and more fundamentally addressed to the chemistry of electronically excited molecules.

1. Photo-Induced Oxidation-Reduction Processes at Phase Boundaries
Professor Melvin Calvin, Dr. John Otvos

Using the basic knowledge we have acquired of the mechanism by which green plants convert quanta into stored chemical energy, we are devising totally synthetic systems to accomplish a corresponding result. In all cases, photoelectron transfer across a phase boundary (the system used by natural chloroplasts) is the conceptual basis of our effort. The process of energy storage is either the production of hydrogen from water or the reduction of carbon dioxide to functionalized organic compounds with concomitant production of an especially useful oxidizing agent or oxygen itself.

The phase boundaries under study are: (1) the lipid bilayer walls of phospholipid vesicles, which are also used to keep separate the initial photoproducts, (2) the surfaces of colloidal silica, various polyelectrolytes and surface charged micelles, and (3) the surfaces of functionalized polymeric colloids. With the vesicles we have found that energy exchange and migration take place laterally on the surface among adsorbed sensitizer molecules. Such exchange facilitates the reaction and produces an overall enhancement of the quamtum yield of electron transfer. The surface potential of polyelectrolytes and polymeric colloids can be used to retard back-reactions of the primary products of electron transfer. They may also hae advantages over colloidal silica by virtue of their adjustable charge density and useful pH range.

(a) Catalysts for oxygen production. In natural and artificial photosynthetic systems, water oxidation into molecular oxygen and protons plays an imporatnt role in the utilization of the photogenerated oxidizing power. We have been continuing work on metal complexes that can catalyze the photooxidation of water. In green plants, manganese is an essential component although the exact molecular nature of this catalyst remains unknown. Therefore, we have focussed our efforts on model manganese compounds, specifically manganese porphyrins. We have observed that when Mn(III)-tetramethylpyridylporphyrin chloride is oxidized chemically (hypochlorite or peroxydisulfate) in aqueous solutions spectral changes indicate that the oxidized form returns to the original Mn(III) porphyrin at rates dependent upon pH (slower at higher pH). An obvious possible product of this reaction is molecular oxygen.

$$\text{Oxidized Mn porphyrin} + 2\ H_2ZO \rightarrow \text{Mn(III) porphyrin} + O_2 + 4H^+$$

Our efforts are currently directed at identifying the products of this reaction and at investigating the mechanism with the aim of coupling this reaction with photogenerated oxidizing power.

(b) Electron transfer in rigid media. We have demonstrated photoinduced electron transfer across the phospholipid bilayer of a vesicle. The sensitizer was located in the vesicle wall, the electron donor inside the vesicle cavity and the acceptor in the continuous aqueous phase outside. We will eventually need stronger separating barriers between compartments than lipid bilayers and, therefore, we have begun to study the factors that determine the rates of electron transfer between donor-acceptor pairs in rigid media where the molecules are randomly distributed. In one such model system we have determined the distance dependence of electron transfer from triplet

zinc tetraphenylporphyrin (ZnTPP) to methylbenzoquinone (MBQ) in sucrose octaacetate glass. The zinc porphyrin molecules are far enough apart that they may be considered to act independently and to be influenced only by the nearest acceptor molecule. The quenching radius for electron transfer is 15 A measured by this model. This is the distance at which the electron transfer rate equals the rate of disappearance of triplet by other paths. So far we have only been able to study the electron transfer by observing this disappearance. We had hoped to monitor also the intermediate photoproducts, the porphyrin cation radical and the reduced quinone, to gain an understanding of the back-reaction and to measure its rate dependence with respect to distance. We will explore the use of other donor-acceptor pairs with more favorable absorption spectral properties as well as the possible application of EPR to the observation of intermediate photoproducts.

(c) Polyelectrolytes and Colloids as Interfaces for Retarding Back-Electron Transfer. We have continued our study on negatively charged polyelectrolytes (polystyrene sulfonate-PSS) as interfaces for stabillizing the initial products of photoinduced electron transfer reactions. Such materials are more versatile in the pH regime that can be applied than the sillica colloids we have used earlier. PSS, as well as silica, binds the sensitizer (zinc tetramethylpyridyl porphyrin) tightly and causes the photoreaction to occur in the vicinity of the polyelectrolyte from which the reduced acceptor, PVS^{π} (propylviologen sulfonate anion radical) is repelled. Steady-state experiments have now confirmed that PSS increases the overall quantum yield of the reaction but not nearly so much as the silica colloids do. Flash experiments show that while the back-electron transfer rate is decreased as expected in the presence of polyelectrolyte, so also is the forward reaction between excited porphyrin and PVS. This latter effect is not completely understood. It probably results from the combination of two factors. First, there is the dimensionality effect, which operates to reduce the bimoleculr rate constant when one of the reaction partners is immobilized by adsorption to a colloid surface. In addition, the envelopment of one of the reactants by the polyelectrolyte could also reduce the rate by interfering with the approach of the other. In an attempt to prepare a surface that is more analogous to that of the silica systems, we have sulfonated a cross-linked polystyrene colloid. The particles are about 2000 A in diameter with a high surface density of sulfonate groups. Thus they are structurally similar to silica particles but with a much lower pK. Their behavior in photochemical systems is now being studied. Smaller charged particles (40 A) are also being made from the silica particles by sulfonation of the phenylated colloid to permit photochemistry at lower pH, such as 6-7.

Relevant Abstracts

Artificial Photosynthesis: Quantum Capture and Energy Storage. Melvin Calvin, Review Article, Photochem. Photobiol. 37, 349-360 (1983)

Organized systems are described to achieve separation of the charges produced by quantum absorption in a sensitizer, and their stabilization in separated chemical form. These systems involve phase boundary charge separation by both membranes and charged particles, in both cases simulating the natural system.

Temperature Dependence of Photosensitized Electron Transport Across Lipid Vesicle Walls. Howard D. Mettee, William E. Ford, Tetsuya Sakai and Melvin Calvin, Photochem. Photobiol. 39, 679-683, 1984

An overall Arrhenius activation energy of 117 ± 19 kJ/mol(28.0 ± 4.5 kcal/mol) has been measured for photosensitized electron transport across phospholipid vesicle walls in the temperature range 18-38°C. A dynamic model for the overall process is proposed which accounts for the parabolic growth curves of the kinetically probed species, heptyl viologen radical. The temperature dependence of the initial quantum yield derived from these curves, and of fluorescence qenching behavior of the ruthenium tris-bipyridyl photosensitizer, is used to estimate an activatio energy of 67 ± 21 kJ/mol (16 ± 5 kcal/mol) for the electron transport step itself. The activation energy for the co-transport of charge compensating ions is estimated to contribute no more than 4.6 kJ/mol to this energy.

Quantum Capture and Redox Storage. Melvin Calvin, Can. J. Chem. 61, 873 (1983).

Over many years multiple investigations of the mechanism of the primary quantum conversion steps in natural photosynthesis have exposed the participation of some kind of manganese complex in the oxidation of water to molecular oxygen and the participation of a number of iron hemes in the electron transport chain between the two photosystems. It is almost certain that the participation of an iron-sulfur protein on the reduction side of the photosynthetic electron transfer scheme plays a role in the formation of reduced pyridine nucleotide through ferredoxin and in the evolution of hydrogen in plants deprived of CO_2 but having the hydrogenase present. The physical arrangement of the components is important as well, and the participation of at least one phase boundary (and probably two) in the same transport chain is likely. We have used this information to try to construct a synthetic chloroplast that would accomplish quantum conversion into stored energy in the form of intermediate oxidant and intermediate reductant. These could than be converted, respectively, into a useful oxidation product, or molecular oxygen, on the one hand, and molecular hydrogen, or a more useful reduction product, on the other.

2. The Chemistry of Electronically Excited Molecules

Professor George C. Pimentel and Dr. Heinz Frei

Using cryogenic matrix techniques, FTIR, Visible, and UV spectroscopy, and tuned laser selective excitation, we are studying chemical changes as they occur on electronic hypersurfaces with emphasis on endothermic reactions that

can be induced with near-infrared photons. These studies permit controlled examination of the factors that are involved in all photochemical storage of photon energy in the form of chemical energy. We are investigating chemistry initiated with electronically excited O_2, NO_2, and atomic Hg, Cd, and Zn. Also under study is the mapping of the electronic hypersurface of 2,3-dimethyl azirine.

The laboratory has now been equipped for laser excitation of cryogenic samples with Fourier Transform Infrared Spectroscopy (FTIR) analysis. An argon-ion CW laser is in use to pump a tunable dye laser for selective excitation anywhere from the near-infrared to near-ultraviolet spectral regions. A closed cycle refrigerator permits preparation, irradiation, and spectroscopic study of cryogenic samples over many hours and at sample temperatures from 10°K upward. Spectroscopic study of the cryogenic samples is conducted with an IBM FTIR spectrometer and with a Perkin and Elmer visible/UV spectrometer.

a) Singlet-Oxygen Reactions. The chemistry of electronically excited oxygen molecule, O_2, differs dramatically from that of the ground state. This chemistry has been explored considerably using sensitization and chemical generation of the $^1\Delta_g$, $^1\Sigma_g^+$, and ($^1\Delta_g$, $^1\Delta_g$) states. It has not, however, been coupled with direct photo-excitation, primarily because of the extremely low absorption coefficients. Using our tuned-laser and cryogenic techniques, endothermic reactions of electronically excited O_2 have been explored as prototype solar energy storage reactions. Interest is considerable since excitation occurs in the near infrared where most of the solar energy falls and at low photon energies at which chemical reactions are not readily induced.

Solid oxygen or oxygen in solid argon was selectively excited using tunable dye lasers to the $^1\Delta_g$, v'=0, $^1\Delta_g$, v'=1, $^1\Sigma_g^+$, v'=0, $^1\Sigma_g^+$, v'=1, and ($^1\Delta_g$, $^1\Delta g$, v'=0,0) states. When a small amount of furan, 2-methyl furan or 2,5 dimethyl furan is present in the solid, wavelength-dependent reaction occurs. The infrared spectrum identifies the products to be the corresponding 2,5-endoperoxides. In each case, the quantum yield was measured and found to be unity within experimental uncertainty. These endoperoxides are known to decompose on gentle warming, returning quantitatively the starting reagents with O_2 electronically excited in the $^1\Delta_g$ state. A new technique was developed, "reaction excitation" spectroscopy, in which reactivity as a function of exciting frequency revealed the absorption contour of a feature too weak to observe.

The same technique has now been applied to the endothermic reactions of O_2 with F_2 to produce F_2O_2 and FO_2. In this system, no reaction is observed when O_2 is excited to the $^1\Delta_g$, v'=0 state at 8000 cm^{-1}, to the $^1\Delta_g$, v'=1 state at 9500 cm^{-1} or the $^1\Sigma_g^+$,v'=0 state at 13,260 cm^{-1}. Tunable laser, selective excitation of the $^1\Sigma_g^+$,v'=1 state with 14,650 cm^{-1} causes reaction to occur. Detection limits indicate that $^1\Sigma_g^+$,v'=1 is at least 180 times more effective than $^1\Sigma_g^+$,v'=0 in promoting reaction with molecular fluorine and at least two or three orders of magnitude more efficient than selective excitation of $^1\Delta_g$,v'=0 or 1.

Reaction to form O_2F_2 also takes place near 15,860 cm^{-1} with a "reaction excitation" contour that identifies the absorption process, O_2 dimer ($^3\Sigma_g$,$^3\Sigma_g$,0,0) ($^1\Delta_g$, $^1\Delta_g$,0,0). Between this and that due to O_2 excitation to $^1\Sigma_g$.b'=1 at 14.650 cm^{-1}, the "reaction excitation" spectrum shows additional reaction not readily attributed to electronic excitation of oxygen. We believe this is due to O_2-induced absorption by F_2 to the repulsive part of the weakly bound $^3\Pi_{0^+u}$ state, more than 200 nm to longer wavelength than any electronic absorption of molecular fluorine thus far reported.

Relevant Abstracts

Selective Vibrational Excitation of the Ethylene-Fluorine Reaction in a Nitrogen Matrix. II. Heinz Frei, J. Chem. Phys. 79, 748-58 (1983)

The product branching between 1,2-difluoroethane and vinyl fluoride (plus HF) of the selective vibrationally stimulated reaction of molecular fluorine with C_2H_4 has been studied in a nitrogen matrix at 12 K and found to be the same for five different vibrational transitions of C_2H_4 between 1896 and 4209 cm^{-1}. The HF/DF branching ratio of the reaction of F_2 with CH_2CD_2, trans-CHDCHD, and cis-CHDCHD was determined to be 1.1, independent of precursor, $C_2H_2D_2$ isomer and particular mode which excited the reaction. These results, as well as the analysis of the mixtures of partially deuterated vinyl fluoride molecules produced by each $C_2H_2D_2$ isomer indicate that the product branching occurs by $_{12}$ elimination of HF[DF] from a vibrationally excited, electronic ground state 1,2-difluoroethane intermediate. Selective vibrational excitation of fluorine reactions in isotopically mixed matrices t-CHDCHD/C_2H_4/F_2/N_2, and in matrices C_2H_2/C_2H_4/F_2/N_2 revealed a high degree of isotopic and molecular selectivity. The extent to which intermolecular energy transfer occurred is qualitatively explained in terms of dipole coupled vibrational energy transfer. A study of the loss of absorbance of the $C_2H_4 \cdot F_2$ pairs in case of ν_9 as a function of both the laser irradiation frequency within the absorption profile, and the ethylene concentration showed that the $C_2H_4 \cdot F_2$ absorption is inhomogeneously broadened. Substantial depletion of reactive pairs which did not absorb laser light is interpreted in terms of Forster transfer.

Selective Vibronic Excitation of Singlet Oxygen-Furan Reactions in Cryogenic Matrices. Heinz Frei and George C. Pimentel. J. Chem. Phys. 79 3307 (1983).

The reactions of 2,5-dimethylfuran(DMF), 2-methylfuran (MF), and furan with molecular oxygen to form endoperoxides have been induced in Ar and O_2 matrices at 12 K with selective vibronic excitation of O_2 using near infrared light. Reaction was induced through excitation of the $^1\Delta_g$(v' =0, v' =1) and $^1\Sigma_g^+$(v' =0, v' =1) O_2 states near 8000 and 13,100 cm^{-1} respectively, as well as by the ($^1\Delta_g$, $^1\Delta_g$) simultaneous transitions of $(O_2)_2$ at 15,900 and 17,300 cm^{-1}. The $^1\Delta_g \leftarrow {}^3\Sigma_g^-$ vibronic progression in solid O_2 was recorded by FTIR spectroscopy, whereas members of the $^1\Sigma_g^+ \leftarrow {}^3\Sigma_g^-$ and ($^1\Delta_g$, $^1\Delta_g$) ← ($^3\Sigma_g^-$, $^3\Sigma_g^-$) progressions in Ar matrices were located by "reaction excitation" spectroscopy. The DMF+O_2 reaction is a single photon process, apparently with unit quantum yield, for all vibronic levels excited. For MF+O_2($^1\Delta_g$, v' =0) and furan + O_2($^1\Delta_g$, v' =0), quantum yields were high, ~0.6 and 0.4, respectively, but the reaction rate showed an $^{18}O_2$ isotope effect 0.78±0.15, which can be interpreted in terms of quantum mechanical tunneling on the lowest singlet hypersurface. The IR matrix spectra of DMF endoperoxide and its peroxidic ^{18}O counterpart are reported, and a partial vibrational assignment for this intermediate is given. The endoperoxide vibration that absorbs at 815.7 cm^{-1} is dominated by the bridge O-O stretching motion.

Excitation Spectrum of the Reaction F_2+O_2 in the Red Spectral Range. Heinz Frei, J. Chem. Phys., 80, 5616-22(1984).

A search has been made for new low-energy pathways of the reaction F_2+O_2 → O_2F+F or O_2F_2 through excitation of F_2 or O_2 in an oxygen matrix with red and near infrared photons. By monitoring the product yield as a function of laser irradiation frequency, a reaction excitation spectrum of O_2+F_2 was recorded which begins at 14500 cm^{-1} and extends through the entire red spectral range. It revealed two discrete absorptions O_2 $^1\Sigma_g^+$, v'=1 ← $^3\Sigma_g^-$, ν"=0 and $(O_2)_2$($^1\Delta_g$, $^1\Delta_g$)0,0 ← ($^3\Sigma_g^-$, $^3\Sigma_g^-$)0,0, and a continuous, oxygen-enhanced absorption of F_2 which is assigned to excitation to the repulsive part of the weakly bound $^3\Pi_{0^+u}$ state. The F_2 absorption detected in this experiment extends more than 200 nm to longer wavelengths than any electronic absorption of molecular fluorine reported so far.

b) Nitrogen Dioxide-Olefin Reactions. Nitrogen dioxide absorbs in its X^2A_1 → $^2B_2$ transition beginning in the near infrared at 12,000 cm^{-1}. This transition allows us to investigate the chemistry of this $^2B_2$ excited electronic state at frequencies far too low to permit nitrogen-oxygen bond rupture (which requires 25,000 cm^{-1} = 71 kcal/mole). Reactions with olefins (ethylene and tetramethylethylene) have been selected for first study.

It was first necessary to identify the product absorptions associated with photo-induced isomerization of N_2O_4 using broad-band photolysis light in the range 700-450 nm. Then, with NO_2 and ethylene at various concentrations in solid argon, the matrix mixture was irradiated using tuned laser light over the range 620-457 nm (16,100 - 21,900 cm^{-1}). Product growth is observed

for light at wavelengths shorter than 560 nm ($h\nu > 17,860\ cm^{-1} = 51$ kcal/mole). The product bands show that the ultimate products are nitric oxide, acetaldehyde and ethylene oxide but there is also evidence that an intermediate is trapped. This intermediate, tentatively identified as $C_2H_4NO_2$, is readily photolyzed at 514 nm (19,500 cm^{-1}).

With tetramethylethylene, reaction occurs at longer wavelengths, to 650 nm (15,400 cm^{-1} = 44 kcal/mole). No absorption due to nitric oxide is observed, so the product bands are assigned to intermediate species, presumably $C_2(CH_3)_4NO_2$. Again product features are removed by irradiation at shorter wavelength, 514 nm.

The structures of these intermediates, the extent of energy storage, and the chemistry of $^2B_2NO_2$ is under further study.

c) Hg*, Cd*, and Zn* Reactions with Haloethenes. The mercury atom-induced reactions have now been extended to its family counterparts, cadmium and zinc. The Cd and Zn matrix samples were prepared by evaporation from filaments of the appropriate wire.

Attention has recently focussed on 2-chloro-1,1 difluoroethene in solid krypton for which no photolysis occurs using a high pressure mercury lamp. When mercury, cadmium, or zinc atoms are added to the matrix, however, new features appear which we are tentatively attributing to an intermediate compound. Tentatively, we identify this compound as a pi complex in which the metal atom bridges across the double bond. Isotopic substitution will be used to characterize more definitively this structure.

d) Reaction of Imidogen with Dimethylacetylene. Photolysis of hydrazoic acid, HN_3, produces predominantly $NH(^1\Delta)$. The addition of electronically excited NH, so produced, to the triple bond of dimethylacetylene has been investigated in argon and xenon matrices. In each matrix, two or three products can be distinguished by their photolysis growth curves. Most important, the major product in argon is a minor product in xenon and the major product in xenon is not observed at all in argon. Infrared study, including ^{15}N and deuterium substitution show that in argon 3,3-dimethylketimine is produced in highest yield whereas the xenon major product is N-methyl-1-amino-1-propyne. In both matrices, the cyclic 2,3-dimethyl-1H-azirine is probably formed--if so, this is the first detection. We attribute the change of major product to spin-orbit coupling by the matrix to facilitate crossing from the reactive singlet hypersurface to a triplet hypersurface.

We assign all of the products to tautomeric forms of the primary addition product which, presumably is 2,3-dimethyl-1H-azirine. The system has the desired characteristics for our proposed hypersurface mapping.

Relevant Abstracts

The Imidogen-Dimethylacetylene Reaction in Ar and Xe Matrices: Possible Matrix-Inducing Intersystem Crossing. Susan T. Collins and George C. Pimentel, J. Phys. Chem. 88, 4258-4264 (1984).

The Photolysis of HN_3 in the presence of dimethylacetylene in 12 K matrices gives reaction products in Xe different from thse formed in Ar. In Xe the reaction results in N-methyl-1-propyne as the major product, whereas in Ar the primary product is 3,3-dimethylketenimine. In Xe, 3,3-dimethylketenimine is produced in low yield, and in both matrices another product, probably 2,3-dimethyl-H-azirine, is formed as a minor product. We postulate that, in Ar matrix, photolysis of HN_3 gives $NH(^1\Delta)$ which reacts ona singlet reaction surface to give, primarily, the ketenimine. In Xe matrix, however, the external heavy-atom perturbation promotes intersystem crossing so that reaction occurs on a triplet reation surface, leading to a different product distribution that favors the propyne.

3. Picosecond Photochemistry

Professor John Clark

Picosecond visible/UV absorption, emission, resonance Raman, and dynamic polarization spectroscopic techniques are being developed and used to probe the interplay between electronic and geometrical structure and the reaction dynamics of electronically excited molecules.

Excited-State Proton-Transfer Reactions. A major activity over the past two years has been the development of predictive models with which to explain the observed dependence of excited-state proton-transfer kinetics on electronic and geometrical structure. A model which explains the 500-fold difference in excited-state proton-transfer rates between 1-naphthol and 2-naphthol has been proposed. This model assumes that the controlling factor in determining excited-state proton-transfer rates is the charge density distribution on the excited molecule. This model not only explains all of the trends we have observed in excited-state proton-transfer rates, but also predicts that the quenching process we have observed in 1-naphthol is due to electrophilic attack of solvated protons at the 5 and 8 positions on the ring. This prediction has now been confirmed in independent experiments by Professor Laren Tolbert at the University of Kentucky. Professor Tolbert spent a sabbatical leave in our laboratory working with us to further develop and extend this model.

Relevant Abstracts

Ultrafast Excited-State Proton Transfer in 1-Naphthol. S.P. Webb, Sheila W. Yeh, Laura A. Philips. M. A. Tolbert, and J. H. Clark. J. Am. Chem. Soc., in press.

The first direct observation of the excited-state proton-transfer reaction of 1-naphthol in aqueous solution is reported. Using picosecond, time-resolved emission spectroscopy, the lifetime of 1-naphthol at pH = 7 is found to be 33 ± 5 psec, one to three orders of magnitude faster than previous determinations. Coupled with steady-state techniques,

direct, time resolved measurements provide a generally applicable method for the accurate determination of the numerous rate constants required for a complete description of the kinetics of electronically excited 1-naphthol in water. Comparison of the present results on 1-naphthol with previous work on 2-naphthol reveals striking differences in the excited-state reaction dynamics of these seemingly similar molecules.

Excited-State Dynamics of Ru-trisbipyridine. The generally accepted model of the photophysics of $Ru(bpy)_3^{2+}$ can be pictured as a simple Jablonski diagram. $Ru(bpy)_3^{2+}$ is excited from its ground singlet state (S_0) to its first excited singlet state (S_1). Intersystem crossing converts S_1 into the triplet state (T_1), which is the photoactive, luminescent state so often encountered in solar energy research. Previous work on the nanosecond timescale by Woodruff et al. had provided a resonance Raman spectrum of the photoactive state. In our initial picosecond Raman work, we obtained exactly the same Raman spectrum as Woodruff. Our interpretation of these results was (and is) that they are strong evidence for a single, mixed excited state in this system. However, since we had no unique Raman spectral signature for S_1, the separate state argument could not be disproved by the Raman spectrum alone. That is to say that in the absence of any idea of what the resonance Raman spectrum of S_1 was, we could not definitively prove that our Raman spectrum did not contain weak S_1 features buried in the strong T_1 spectrum.

However, by combining our earlier Raman work with new picosecond time-resolved emission studies, we can conclusively rule out a two-state model. The two-state model was originally invoked to explain the apparent discrepancy between the observed integrated absorption coefficient of the $Ru(bpy)_3^{2+}$ luminescence lifetime. The integrated absorption coefficient predicts a 90 nsec lifetime, while the observed lifetime is >6 μsec. If one models this system, one finds that even if the spectral signatures of the two states are identical, the two state system will have a unique temporal signature in its luminescence. This two state signature consists of a sharp spike at early times. The decay of the spike can be related to the intersystem crossing rate. With our present data and analysis, we can definitively rule out a two state model with an intersystem crossing time of longer than 1 psec. Since this time corresponds to an uncertainty principle spectral broadening of >15 cm^{-1}, the notion of separate states is rapidly becoming defunct. Further analysis of the data should allow us to determine whether the two-state model has any validity whatsoever.

Relevant Abstracts

Picosecond Time-Resolved Spectroscopy of Electronically Excited tris(2,2'-bipyridine)Ruthenium(II) Dichloride. L. A. Phillips, W.T. Brown, S.P. Webb, S. W. Yeh, and J. H. Clark, In: Ultrafast Phenomena, eds. D. H. Auston and K. B. Eisenthal, Springer, New York 1984 (in press).

The dynamics of electronicaly excited tris(2,2'-bipyridine) ruthenium(II) dichloride are probed with

picosecond time-resolved resonance Raman and emission measurements.

Energy Transfer in Phycobilisomes. Phycobilisomes are an important constituent of the light harvesting systems of cyanobacteria and red algae. They differ from the light havesting systems of green plants in that the different chromophores which make up the antenna system have quite distinct spectral properties. This allows the flow of energy within the phycobilisome structure to be followed in considerable detail.

In conjunction with Professor Alex Glazer, we are using picosecond time-resolved emission spectroscopy to follow the energy flow within phycobilismes. Because Professor Glazer has developed various mutants which lack known portions of the phycobilisome structure and becouse we can selectively excite various chromophores using our picosecond tunable source, we are able to assess the role of various substructures in the transport of energy through the antenna system.

Relevant Abstracts

Disc-to-Disc Transfer as the Rate-Limiting Step for energy flow in Phycobilisomes. A.N. Glazer, S.W. Yeh, S.P. Webb, and J. H. Clark, Science, Nov. 1984.

A broadly tunable picosecond laser source and an ultrafast streak camera were used to measure temporally and spectrally resolved emission from intact phycobilisomes and from individual phycobiliproteins as a function of excitation wavelength. Both wild-type and mutant phycobilisomes of the unicellular cyanobacterium Synechocystis 6701 were examined, as well as two biliproteins, R-phycoerythrin (240 kilodatons, 34 bilins) and allophycocyanin (100 kilodatons, 6 bilins). Measurements on intact phycobilisomes with known structural differences show that the addition of an average of 1.6 phycoerythrin discs in the phycobilisome rod increases the overall energy transfer time by 30 ± 5 ps. In the isolated phycobiliproteins, the onset of emission was found to be as prompt as that of a solution of rhodamine B laser dye and was independent of excitation wavelength. This imposes an upper limit of 8 ps (instrument limited) on the transfer time from ^{3}ensitizing to $_{6}$luorescing chromophores within these biliproteins. Together these results indicate that disc-to-disc transfer is the slowest energy transfer process within phycobilismes. In combination with previous structural analyses, these results show that with respect to energy transfer, the lattice of ~625 light-harvesting chromophores in the Synechocystis 6701 wild-type phycobilisome functions as a linear, five-point array.

Picosecond Tunable Source. We have developed a unique picosecond tunable source. This device exploits optical parametric processes to produce transform-limited optical pulses that are continuously tunable from 450-650 and 1550 to 780 nm. The technique employed involves spontaneous optical parametric fluorescence and high-gain optical parametric amplification (OPA)

in a pair of potassium dihydrogen phosphate (KDP) crystals pumped by single, ~20 ps, third harmonic (355 nm) pulses derived from a modelocked Nd:YAG laser. Broad spectral bandwidth spontaneous parametric fluorescence is produced by the annihilation of 355 nm photons at frequency $\omega 3$, and subsequent generation of photon pairs at frequencies $\omega 1$ and $\omega 2$, such that $\omega 1 + \omega 2 = \omega 3$. Parametric fluorescence collinear with the pump beam is amplified and spectrally narrowed via OPA in Type II phase-matched KDP. Energy conversion efficiencies in excess of 20 percent are easily achieved in a double crystal OPA device. The detailed interaction of the pump and generated beams leads to considerable pulse compression of the tunable beams. For $\omega 2$ pumping pulses 25 ps long, tunable pulses of less than 10 ps duration with transform-limited (~2 cm^{-1}) bandwidths are obtained.

II. MOLECULAR BIOLOGY

The Laboratory of Chemical Biodynamics has begun a program of research directed at the application of recombinant DNA techniques to photosynthetic organisms. The Laboratory has been a leader in clarifying the basic processes of photosynthesis and the subsequent metabolic chemistry so initiated. In addition, a number of LCB scientists have contributed to our present understanding of the control of information in biological cells. These studies range from the control of DNA synthesis in eucaryotic cells to the determination of the DNA interaction with proteins using X-ray crystallography. Techniques have been developed for characterizing how specific functions are regulated in cells. As research in these several areas continues to advance, it provides a rich setting in which to pursue our thrust in genetic engineering of the photosynthetic apparatus.

A. Recombinant DNA Techniques Applied to Photosynthetic Organisms

1. Understanding a Photosynthetic Gene Cluster

Professor John Hearst

The specific aims for the coming year are directed at learning as much as we can about the photosynthetic gene cluster of *Rhodopseudomonas capsulata*. In particular we are attempting to take best advantage of the nucleic acid sequences that this laboratory has determined, to take advantage of the map of 30 transposon insertion mutants which we have generated and characterized in this gene cluster, and to establish physical evidence for the location of the ubiquinone binding sites in the L and M subunits of the reaction center.

The first specific aim is the determination of an operon map for the photosynthetic gene cluster. In the portion of this gene cluster which we have sequenced, we are focusing our attention on the operon containing the H subunit gene. Using synthesized oligonucleotide primers it is our intention to determine the sequence of the 5' end of the messenger RNA for this gene. Dideoxy sequencing procedures using reverse transcriptase will be used. Regulation of the H subunit promoter will also be studied with emphasis on the time course of expression by this promoter during the transition from chemoheterotrophic growth to photosynthetic growth.

In the unsequenced portions of the gene cluster, operon structure will be studied by reverse transcription from a primer sequence which is complementary to the transposon so that only transposon interrupted mRNA will be primed. With one or a small number of transcripts from this primer per transposon mutant strain, a low resolution map of operons should be readily accessible. Sequence resolution information will be obtained by dideoxy sequencing using the same priming procedure when possible or secondary synthetic primers when necessary. The long term goal of this activity is the determination of the sequences of the promoters in this gene cluster and to the extent that is possible, differentiation between the oxygen regulated promoters and those which are constitutive.

The biosynthetic pathways for bacteriochlorophyll and for the carotenoids will be further characterized by the analysis of pigment products and pigment precursor products in the many transposon mutant strains that are available to our laboratory. The ultimate objective is the identification of a specific enzymatic function with each of the gene loci in our gene map.

I.Finally, with the use of suicide quinone reagents, it is our objective to establish by direct alkylation of the suicide quinones to their binding sites in the reaction center, exactly where in the proteins binding takes place. Since quinones serve as shuttles to the b-c_1 complex and at the water splitting side of PSII complexes, it is intended that our experience with these suicide quinones in reaction centers will ultimately be useful in defining other quinone binding sites in photosynthetic membranes and in mitochondria.

Relevant Abstracts

Tn5.7 Construction and Physical Mapping of pRPS404 Containing Photosynthetic Genes from Rhodopseudomonas capsulata. Krisztina M. Zsebo, Fonda Wu and John Hearst. Plasmid 11, 182-184 (1984).

A transposon, Tn5.7, has been constructed incorporating the transposition functions of Tn5 and the antibiotic-resistance factors from Tn7. It was used to mutagenize the plasmid of pRPS404 which contans the photosynthetic genes of Rhodopseudomonas capsulata and is $Kanamycin^R$. In conjunction with the mutagenesis, physical mapping of the restriction endonuclease recognition sites for XhoI, Bg/II, KpnI, and SstI has been accomplished.

Protein Sequence Homologies between Portions of the L and M Subunits of Reaction Centers of Rhodopseudomonas capsulata and the Q_B-Protein of Chloroplast Thylakoid Membranes: a Proposed Relation to Quinone-Binding Sites. John E. Hearst and Kenneth Sauer, Z. Naturforsch, 39, 421-424 (1984).

Sequence homology has been found between the L and M subunits of Rhodopseudomonas capsulata reaction centers and the Q_B-protein from all species thus far sequenced. The homology between the L subunit and the Q_B-protein is contained in the sequence PFHMLG---F----AMHG-LV-S and is believed to have survived three billion years of evolution. A model is presented which associates quinone binding with this highly conserved sequence.

Genetic-Physical Mapping of a Photosynthetic Gene Cluster from R. capsulata. Krisztina M. Zsebo and John E. Hearst. Cell, 37, 937-947 (1984).

Transposon mutagenesis and complementation analysis of the photosynthesis genes in Rhodopseudomonas capsulata is presented utilizing Tn5.7 mutagenized R-primes. The R-prime pRPS404 contains many of the genes necessary for the

differentiation of the photosynthetic apparatus. Utilizing homologous recombination, 30 independent copies of Tn5.7 were inserted into the R. capsulata chromosome with subsequent deletion of wild-type alleles. Mutants were characterized by absorption spectroscopy, SDS-PAGE, and determination of capability for photosynthetic growth. Many mutations in the bacteriochlorophyll and carotenoid biosynthetic pathways were isolated. A regulatory mutation was isolated affecting reaction-center synthesis as well as a 44 kd heme-containing polypeptide. Complementation analysis using various pRPR404::Tn5.7 plasmids has led to the postulation of transcriptional units.

Nucleotide and Deduced Polypeptide Sequences of the Photosynthetic Reaction-Center, B870 Antenna, and Flanking Polypeptides from R. capsulata. Douglas C. Youvan, Edward J. Bylina, Marie Alberti, Helmut Begusch, and John E. Hearst, Cell, 37, 949-957 (1984).

The complete nucleotide sequence (8867 bp) and the deduced polypeptide sequence are given for 11 proteins from the photosynthetic gene cluster of R. capsulata (46 kb), including the photosynthetic reaction-center L, M, and H subunits and the B870α and B870β polypeptides (light harvesting I). These polypeptides bind bacteriochlorophyll, bacteriopheophytin, carotenoids, and quinones that are involved in the primary light reactions of photosynthesis. Hydropathy plots indicate that the L and M subunits are transmembrane proteins that may cross the membrane five times, while the H subunit has only one hydrophobic section near the amino terminus, which may be transmembrane. The L and M subunits are homologous over their entire length and have a high degree of homology with the Q_B protein from photosystem II of higher plants. An additional six genes were identified that may have some unknown role in bioenergetics since only mutations that affect the differentiation of the photosynthetic apparatus are known to map to this gene cluster.

2. Light Regulation of Nuclear and Chloroplast Gene Expression

Dr. James C. Bartholomew

Even though the chloroplasts of photosynthetic organisms contain their own genome, many of the functions carried out in the organelle are encoded in the nucleus. Some of the proteins which function in chloroplast are encoded partially in the chloroplast and partially in the nucleus. The expression of both these genomes is regulated by light. Light stimulates mRNA synthesis as well as DNA synthesis in Euglena gracilis. Presumably, this light response is mediated by photopigments in the cells which receive the light and convert it into a signal which control nucleic acid activity in both the genome compartments. We have a series of bleached mutants of Euglena which lack various photopigments as well as have different levels of chloroplast DNA. We are using these mutants to study the involvement of the photopigments and

chloroplast DNA in the stimulation of nuclear DNA and RNA synthesis. First, we are characterizing the cell cycle response of these various mutants by flow cytometry. Wild type Euglena conditioned in the resting medium in the dark can be stimulated to reenter the cell cycle by light. We are comparing the kinetics of reentry into the cell cycle of the bleached mutants with that of the wild type to discern the role of the chloroplast supplied functions in regulating the DNA replication response in the nucleus. We are also comparing the light stimulated expression of nuclear encoded chloroplast functions in wild type vs bleached mutants to determine if the chloroplast plays a role in the regulation of the nuclear genes. We have begun to map the nuclear genes for the light harvesting chlorophyll a/b proteins in Euglena to determine if polymorphisms exist for this/these polypeptide(s). All of these studies are designed to help us understand the factors involved in the coordinate regulation of the genes in the two physically distinct compartments.

All of our studies are being done on cell populations which have been characterized with respect to cell cycle properties by flow cytometry. These characterizations become very important since other workers have shown that both illumination and cell cycle position affect the expression of photosynthetic genes. To begin with, we are studying the light-induced synthesis of messenger RNA (mRNA) for the Light-Harvesting Chlorophyll a/b Protein or LHCP in wild-type Euglena and a chloroplastless mutant, W3BUL. LHCP is a nuclear-encoded gene whose product in conjunction with chlorophyll functions as an antenna in the chloroplast membrane. LHCP mRNA has been found to be light inducible in both higher plants and the photosynthetic alga, Chlamydomonas reinhardii. The amount of LHCP mRNA synthesized by the mutant Euglena compared to the wild type will give an indication of the importance of a chloroplast in controlling nuclear transcription. As a control, the synthesis of a nuclear-encoded, non-photosynthetic gene for tubulin, a component of the Euglena flagella, is also being monitored.

In order to see if other nuclear genes are light-induced, we would like to clone any mRNA's that are made in light-exposed cells but not dark-adapted cells. This can be done by the method of cascade hybridization, in which cDNA copies of messages made by dark grown cells are mixed with messages made from light induced cells; homologous messages are removed by passage through a hydroxyapatite column that binds double stranded nucleic acids. The remaining single stranded messages are those that are made in light grown cells alone and will represent the light induced genes. These can then be copied into DNA, cloned, and used for further studies.

We would also like to determine the gene copy number for those photosynthesis genes that are located in the nucleus. Recently is has been shown that the major chlorophyll binding proteins in petunia are represented by as many as 16 different gene copies. Using a DNA probe we have for LHCP we would like to determine the number of copies for this class of proteins in Euglena. If, as in petunia, Euglena has multiple copies for this abundant protein, we would like to determine if all the copies are active, and whether different alleles are active in different stages of chloroplast development. These studies may help us understand how the nuclear genome is able to mount the transcriptional activity necessary to produced the large quantities of LHCP during chloroplast development. In addition, differences in the

structure of the LHCP encoded by different genes may suggest mechanisms for explaining the ability of the light harvesting complex to funnel the collected light energy to the reaction centers.

We are now transferring the LHCP gene cloned from bean into an M13 phage; this will allow more efficient incorporation of radioactive label ^{32}P into the gene than is possible in its current plasmid carrier. The M13 clone can be labelled to a ten-fold greater specific activity than the same clone in the plasmid pBR322. The bean LHCP clone will then be used to look for homology with the same gene in Euglena. Similarly, the two control genes will be recloned into M13. Expression of the genes in dark-adapted Euglena that are exposed to light for varying amounts of time will be monitored by isolating RNA from those cells and looking at the amount of hybridization in each set of RNA to the ^{32}P- labelled clones.

We have worked hard on purifying the nuclear and chloroplast DNA from Euglena. We have had great difficulty getting chloroplast DNA free of nuclear DNA sequences. Part of our problem is that the Euglena are difficult to break open without damaging the chloroplasts. Intact and clean chloroplasts are needed to progress through the purification scheme. We have obtained some DNA which has the expected restriction enzyme pattern and are progressing toward cloning the DNA both into pBR322 and M13. Once we have these libraries we can begin screening the W3BUL to confirm the absence of chloroplast DNA in these cells. We need these chloroplast DNA markers so that we can compare the kinetics of induction of the chloroplast genes with that of the nuclear genes in the wild type strain.

B. Mutagenesis and Carcinogenesis

1. Cellular Oncogene Expression During the Cell Cycle

Dr. James C. Bartholomew

Our research is designed to determine if cellular sequences homologous to viral oncogenes are normally involved with the regulation of transit of cells around the cell cycle.

Cellular oncogenes (c-onc genes) are DNA sequences in the genome of animal cells which share sequence homology with viral genes (v-onc genes) known to be responsible for the malignant transformation of cells infected with RNA tumor viruses. This sequence homology between genes from normal cells and transforming genes in retroviral genomes has suggested that oncogenic RNA viruses may have originally acquired transforming sequences from genes in normal cells which they infected. Since c-onc genes are evolutionarily conserved and transcribed into mRNA and translated into protein products in normal cells, oncogenes appear to have some important, but unknown functions in nontransformed cells.

The mechanism(s) by which changes in these normal genes bring about malignant transformation has not been elucidated. The hypotheses center around both a qualitative change in the genes as well as a quantiative change in the production of gene products. Elevated expression of several oncogenes has been shown to occur in different transformed cell lines. Probably the

most studied cellular oncogene is the c-myc oncogene which is homologous to the v-onc gene from the avian retrovirus MC29. C-Myc is amplified 16 to 32 fold in the genome of the cell line HL-60 derived from a human promyelocytic leukemia patient when compared to normal hematopoietic cells. RNA transcripts homologous to the c-myc DNA sequence in HL-60 cells are elevated in continuously growing cells but are dramatically reduced when these cells differentiate in response to stimuli. A similar elevation of both DNA and mRNA levels specific for c-myc have been observed in the COLO 320 cell line derived from a human colon tumor. The c-myc sequences in both Burkitt's lymphoma and mouse plasmacytoma cells are translocated from their normal location to a point adjacent to the gene coding for the immunoglobulin heavy chain. These translocations appear in some cases to alter the character of the gene product, and in other cases to increase the number of c-myc specific transcripts in the transformed cells. Thus, several malignant cell types are characterized by elevated levels of DNA copies or RNA transcripts for c-myc suggesting that quantitative differences in c-myc gene expression determine whether a cell continues cycling or stops in G_o.

The evidence for a role for the c-myc oncogene in the control of normal cell cycling has been supported by a recent report from Leder's lab. They have shown that the number of mRNA transcripts homologous to c-myc increased in quiescent Balb c/3T3 cells exposed to platelet derived growth factor (PDGF) and in mouse hematopoietic cells exposed to other mitogens. The increase in c-myc transcripts occurred within hours after stimulation of the cells to began cycling and fell to pre-stimulation levels before the cells began to synthesize DNA in preparation to divide. These results suggested that c-myc gene products may be part of the stimulation process by which cells leave quiescence (G_o), enter the G_1 phase, and progress to the DNA synthesis (S) phase hours after the peak of myc transcription.

Another oncogene that has been extensively studied for its association with malignant transformation is c-ras. C-Ras is different from c-myc in that specific point mutations in the gene have been shown to be associated with transformation. The protein product of c-ras appears to be membrane bound, and may be involved in transmitting growth signals from the membrane to the nucleus.

Although the circumstantial evidence that the c-onc gene products plays a role in the regulation of the cell cycle is growing, there is no direct evidence that can distinguish the cause and effect relationship between these gene products and cell cycling.

A number of approaches are being developed to study the involvement of c-onc genes in the regulation of the cell cycle. First, we are studying the expression of the c-onc genes, c-myc, n-myc, and c-ras as a function of the cell cycle. The N-myc gene is related to the c-myc gene, and has been shown to be amplified and overexpressed in neuronally derived tumors. It appears to carry out a similar function in these tumors as that of c-myc in lymphoid tumors. Second, we are determining the effect of G_1 transit modifying factors like TPA on the level of c-onc gene expression. And third, we are constructing plasmids that contain regulatible promoters linked to onc genes and transfecting them into cells to determine if we can control transit

through the cell cycle by controlling the expression of these transfected genes.

The role of the c-myc oncogene in the control of cell proliferation has been investigated by attempting to artificially perturb the level of c-myc expression in otherwise normal cells. Thus far, we have introduced plasmids containing c-myc and SV40 sequences into CV-1 cells using DNA transfection techniques. Numerous published reports have demonstrated that plasmids transfected (or microinjected) into cells are expressed by the recipient cell at reasonably high levels, at least transiently.

Our initial experiments suggested that CV-1 cells, transfected with plasmids containing SV40 (pJY1) or c-myc (pMC16), incorporated ^{3}H-thymidine into their DNA to a greater degree than did cells transfected with calcium phosphate with no DNA or with pBR322 DNA. This effect seemed to be most pronounced at 24 hrs; no effect was seen at 10 or 16 hrs.

For these reasons, the introduction of additional copies of the c-myc gene into cells which normally express c-myc at a low level provides the opportunity to investigate these questions directly. In our future experiments, we plan to use two promising techniques for introducing foreign genes into mammalian cells which will allow us to determine whether transfected DNA is expressed or not by using plasmids which express selectable genes. First, the transient early (24-48 hr) expression of transfected genes can be determined using a plasmid containing SV40 early promoter regions combined with the dihydrofolate reductase (dhfr) gene. The gene product of this plasmid can be detected by vital staining of the cells with a methotrexate analog which binds to dhfr and can be detected in the fluoresenct activated cell sorter (FACS). When the c-myc sequence is inserted into this plasmid, it will be possible to determine the proportion of transfected cells which express the dhfr gene and (probably) the c-myc gene as well. Staining of these cells with a second stain (propidium iodide, which intercalates into DNA) and analyzing the cells on the basis of two parameters in the FACS will allow us to determine both the point in the cell cycle for each cell and which cells express the plasmid for a whole population of cells. This technique will provide us with an excellent way of determining whether the cells which express the transfected plasmid are cycling in a way different from the cells in the same population which do not express the foreign DNA.

The second way in which selection procedures of this kind will be used to elucidate the effect of additional copies of the c-myc gene on the cell cycle involves a more long-term experimental approach. Another plasmid (pSV2 neo) is available which confers resistance to the antibiotic G418 to mammalian cells into which it is transfected. Thus, we will insert the c-myc sequence into the pSV2 neo plasmid, the resulting plasmid will be used to transfect cells with both genes. By growing the transfected population in the G418 antibiotic, only those cells which express this plasmid will survive. The surviving clones can then be grown in large numbers, and the effect of the added sequences can be assessed by measuring the cell cycle kinetics by flow cytometry as described above. These two approaches therefore provide the opportunity to assess the effects of transiently expressed foreign DNAs on the kinetics of recipient cells as well as to determine the effect of the introduced genes on cells stably expressing the c-myc gene over many generations.

We have also been studying the expression of N-myc during the growth of various retinoblastoma cells. Our initial studies indicate that N-myc is tightly regulated during the growth of these cells. When cells reach their saturation density, the level of transcription of N-myc is very low, even lower than that of c-myc and c-ras. Although we have not yet done a detailed cell cycle study on the expression of the N-myc, so far the regulation of this gene in the retinoblastoma cells appears similar to that of the c-myc in lymphoid cells.

If we are successful in developing an approach to studying the effect of specific DNA sequences on the cell kinetics, we would like to compare the response of different cell systems to the different sequences. We hope to address questions related to whether epithelial cells are controlled by different sequences than fibroblasts. Does development down a differentiation pathway take place by controlling which genes for growth regulation are dominant? There is clearly a lot that can be done if we can put specific genes back into cells and determine their effect on growth kinetics.

2. Carcinogen Induced Changes in the Fluidity of the Genome
Dr. James C. Bartholomew

Almost all phenotypic changes that occur in cells as a result of alterations in the genome require some form of DNA synthesis to implement. These changes include both alterations during normal development processes as well as changes resulting in an aberrant phenotype. Classical studies have focussed on the ability of chemical mutagens to alter the nucleotide sequence of DNA by changing the base pairing properties during replication. More recent work has indicated that phenotypic alterations can also occur as a result of a change in gene organization brought about during DNA synthesis. However, whether chemical carcinogens induce these processes is not known. Our studies are aimed at the role carcinogens may play in rearranging and amplifying sections of the genome during DNA replication. We make use of plasmids with defined genes and replication elements to sort out the effect of carcinogen DNA adducts on the organization of their genome. The purpose of our studies is to learn more about how the cell organizes the duplication of its genome during the S-phase of the cell cycle and how carcinogens affect this process.

In general, the replication of the DNA in a cell is a highly organized process leading to the faithful duplication of the genome into the two daughter cells. The control of this process takes place at several steps including the initiation of DNA synthesis, the timing of gene replication, and the gene copy number per cell. Alterations in the control of initiation of DNA synthesis leading to continuous growth of the cells is thought to be a prerequisite for the onset of malignancy. Recent studies indicate that c-onc sequences may be the genes involved with growth control and that malignancy may result from qualitative as well as quantitative changes in their gene products.

Changes in the genome that occur during the process of DNA synthesis are presumably the cause of the altered control of DNA synthesis initiation. Such

changes may include alterations in DNA sequence, gene organization, or gene copy number. Our focus is primarily on the effect of carcinogens on gene organization and copy number brought about by aberrant DNA synthesis.

Recent studies have indicated that the control of gene copy number may not be stringent. Numerous example have been observed in which the number of gene copies for a particular selectible marker has increased when cells are grown in the presence of a selecting agent. Whether gene amplification has a normal function in cells or how extensive it may be is not known. It has been shown that carcinogens or DNA damaging agents can induce either the stabilization of amplified genes, or the amplification process itself.

Virtually nothing is known about the control of gene rearrangements. At least some of the DNA gymnastics that takes place in cells have a function in normal developmental process and therefore may occur in a highly controlled fashion. A well known example is the rearrangements that take place during the development of the immune response to individual antigens. Another example is the control of expression of the surface antigens on Trypanosomes by gene rearrangements, and there are many others. All of these "normal" rearrangements appear to be very controlled and directed in the sense that they occur at a definite time in development or in response to a specific stimulus. Of particular interest as well is the gene rearrangements that correlate with certain forms of malignancies. Most notable among these is the amplification and/or rearrangement of cellular oncogenic sequences such as c-myc in various lymphomas. Although these gene rearrangements seem to occur at a certain constitutive level which depends on cell type, there is growing evidence that carcinogens can increase their frequency. These latter observations suggest that at least some cancers can result from carcinogen stimulation of gene rearrangement or as indicated above amplification possibly involving c-onc sequence.

We are attempting to quantify the effect of the carcinogen benzo[a]pyrene diol epoxide (BaP DE) on the amplification and rearrangement of gene sequences in mammalian cells growing in culture. We are using defined plasmids because they offer both the possibility of understanding the processes as well as controlling the genetic elements involved. We hope eventually to understand the biochemistry of these processes, and to determine the relationship between sequences that replicate during particular parts of the S-phase of the cell cycle and recombination. For example, does the c-myc sequence tend to recombine with the immunoglobin gene because the two sequence are normally replicated at the same time in S.

Specific Objectives

a. Measure the replication and integration efficiency of SV40 derived plasmids transfected into monkey cell lines. These studies will be carried out with BaP DE modified and unmodified plasmid DNAs as well as carcinogen treated and untreated recipient cells.

b. Compare the replication and integration of SV40 derived plasmids in DNA repair competent human cells as well as in repair defective *Xeroderma pigmentosum* human cells.

c. Design and construct plasmids sensitive to recombination activities. Measure the relationship between replication efficiency and recombinational activity in cells.

d. Begin mapping the timing in S-phase for the replication of specific DNA sequences. We will begin with the c-myc and the immunoglobin genes to test the hypothesis that recombination efficiency is controlled at the level of coordinate DNA synthesis.

Progress

Replication of BaP DE Treated Transfected DNA. Our experiments with transfecting plasmids containing SV40 sequences into monkey cells have been progressing well. We have shown that plasmids which contain the origin of replication of SV40 DNA can replicate efficiently in Cos 7 cells. We can routinely monitor the replication of as little as 10 ng imput plasmid DNA per 10^6 cells by Southern blotting the DNA extracted from the cells 48 hrs after transfection. Preliminary evidence has indicated that pretreatment of the Cos 7 cells with low levels of BaP DE (0.05 ug/ml of growth medium) increases the replication efficiency of transfected plasmids. Pretreatment of the DNA with BaP DE prior to transfection has led to an increased rate of disappearance of the plasmid from the Hirt supernatants. Whether the DNA is being digested or being incorporated into the cellular DNA present in the Hirt pellet is presently being examined. We have looked for induction of amplification of the integrated SV40 sequence present in Cos 7 cells caused by BaP DE treatment, but the compound does not appear to affect the copy number. This is presumably because the integrated copy does not contain and active orgin for DNA replication.

DNA Repair and the Replication of Exogenous DNA. Our studies with repair competent and deficient human cells have uncovered an interesting aspect of transformation of human cells by SV40 virus. In our analysis of both the GM 637 and XP12 RO cells we found that they both contain SV40 sequences which are Hirt extractible and run on a 1% agarose gel as Form I and Form II circular DNA. Neither of these cells were transfected with exogenous DNA prior to the analysis, and both cells lines were derived from independent transformations with wild type SV40 virus. When we analyzed the DNA extracted from these cells by restriction analysis, the DNA from both cell lines were shown to contain a deletion in the early region coding for the T-antigen. We have analyzed the DNA from the GM 637 cells and found that the deletion maps within the intron of the large T-antigen reading frame and just at the end of the small t-antigen region. We are presently sequencing the DNA to determine the precise location of the deletion. Preliminary studies indicate that transfection of this DNA into Cos 7 cells allows for efficient replication of the DNA, but when transfected into CV-1 cells no replication occurs. These studies indicate that although the GM 637 cells are transformed by wild type SV40 virus, they harbor a non-integrated SV40 DNA which contains a deletion and which appears to replicate efficiently enough to maintain its presence in the human cells but does not code for enough information to replicate in its

natural host, the CV-1 cells. We are presently looking for other copies of SV40 DNA in the GM 637 cells that may be integrated in the cellular DNA as a possible explanation of this phenomenon. If the cellular DNA contains authentic SV40 DNA with a ,ood T-antigen, then it becomes a mystery as to why human cells need these unintegrated copies. Also, why these two independent isolates of transformed cells would produce such a similar deletion in the SV40 DNA is unknown.

Our studies of the effect of DNA repair capacity and its effect on the replication of BaP DE treated DNA are just beginning. Our studies on the contruction of recombination sensitive probes are also just beginning and would be greatly helped by funds for a Postdoctoral Visitor.

Coordination of the Replication of DNA Sequences During S-Phase. This work has just begun with the arrival of a visiting scientist from Japan. We have begun by obtaining the COLO 320 DM cells which contain the amplified c-myc sequence on a double minute chromosome and a COLO 320 HSR cell line which presumably has the c-myc integrated into the chromosome at the site of the homogenously staining region. We have also obtained a c-myc containing plasmed from P. Duesberg of the UC Molecular Biology Department which he derived from chicken DNA. We have shown that the probe does hybridize to the human DNA from COLO 320 cells by dot blotting the DNA onto nitrocellulose and hybridizing with a ^{32}P-labelled probe. We are working on the isolation of double minute chromosomes from these cells and will begin restriction analysis of this DNA soon. We are also planning to do *in situ* hybridization to demonstrate that the sequences are where we think they are. If all works well we will then begin the cell cycle synthesis studies.

Relevant Abstracts

Flow Cytometric Measurement of the Metabolism of Benzo[a]pyrene by Mouse Liver Cells in Culture. Bartholomew, J.C., C.G. Wade, and K.K. Dougherty. In Biological Dosimetry, W.G. Eisert and M.L. Mendelsohn, Eds., Springer-Verlag Berlin Heidelberg 1984, pp. 311-323

The metbolism of benzo[a]pyrene in individual cells was monitored by flow cytometry. The measurements are based on the alterations that occur in the fluorescence emission spectrum of benzo[a]pyrene when it is converted to various metabolites. Using present instrumentation the technique could easily detect 1 X 10^6 molecules per cells of benzo[a]pyrene and 1 x 10^7 molecules per cell of the diol epoxide. The analysis of C3H 10T 1/2 mouse fibroblasts growing in culture indicated that there was heterogeneity in the conversion of the parent compound into diol epoxide derivatives suggesting that some variation in sensitivity to transformation by benzo[a]pyrene may be due to differences in cellular metabolism. The technique allows sensitive detection of metabolites in viable cells, and provides a new approach to the study of factors that influence both metabolism and transformation.

3. Molecular and Cellular Parameters Associated with the Onset of Cancer
Dr. Melvin P. Klein

The objectives of this research are to achieve a basic understanding of the molecular consequences and correlates of cellular transformation and to develop methods for determining the pertinent molecular and cellular parameters associated with both the onset and the progression of cancer. Measurements of cellular metabolism, bioenergetics, cellular membrane dynamics and interactions between membrane and extra cellular surfaces are performed *in vivo*, by a variety of biophysical spectroscopic methods. Recent accomplishments include the determination of the distribution of fluorescently labeled molecules in cultured cells by measurements of fluorescent lifetimes, the synthesis of a new fluorescently labeled lipid molecule which resides almost exclusively in the plasma membranes of an epithelial cell line, the use of this probe to monitor the results of cellular transformation by phorbol ester tumor promoters where a new class of immobile phospholipid accompanies the appearance of cell shape change. ^{31}P NMR spectra have been obtained from cultured cells and from llving rats using chronically implanted coils surrounding liver, kidney and heart. The metabolism and quantity of phosphorylated intermediates is measured *in situ* as are the rates of phosphorus exchange between these intermediates. It is hence possible to ascertain the response to stress or pathology in the living system. Superoxide dismutase may protect cells from chemical or radiation induced oxidants. The ligation about the metal atom of several iron and manganese forms of these enzymes has been ascertained by X-ray spectroscopy using synchrotron radiation. We expect to be able to detect many of the heretofore invisible phosphorylated intermediates using methods customarily employed for observing NMR in solids. Such measurements are anticipated to reveal the origins of the quantitative difference observed between normal and transformed cellular metabolism. These measurements will have a significant implication for both whole body NMR imaging and for combined imaging and spectroscopy. It is planned to determine how membrane mobility is modified by tumor promoter induced protein reorganization. The detailed metal environments of the Fe and Mn forms of superoxide dismutase will be studied by single crystal electron paramagnetic resonance and electron spin echo spectroscopy.

Relevant Abstracts

Intracellular Dye Heterogeneity Determined by Fluorescence Lifetimes. Beverly S. Packard, Kerry K. Karukstis and Melvin P. Klein. Biochim. Biophys. Acta. 769 201-208(1984).

The cellular localization of a fluorescent probe molecule depends on both the chemical structure of the dye and the cellular environment. To study the number and types of environments in an epithelial cell line, we have measured in Madin-Darby canine kidney (MDCK) cells the fluorescence lifetimes of three structurally distinct fluorescent dyes - rhodamine-B, 3,3'- dihexadecylindocarbocyanine-(C3) (diI), and Collarein - incorporated into these cells. The latter is a rhodamine-cardiolipin conjugate that we designed and

synthesized for the property of exclusive localization in the plasma membrane. The former two dyes required at lease two exponential components to fit their fluorescence decay curves, while the decay of Collarein was characterized by a single exponential. These data are consistent with fluorescence microscopic observations, in which diI and rhodamine-B exhibit heterogeneous spatial distributions, while Collarein appears to be located on the cell surface.

Plasma Membrane Reorganization Induced by Tumor Promoters in an Epithelial Cell Line. Beverly S. Packard, Michael J. Saxton, Mina J. Bissell and Melvin P. Klein. Proc. Nat'l. Acad. Sci. USA 81, 449-452 (1984).

The effects of phorbol ester tumor promoters on the lateral diffusion in plasma membrane lipid environments were examined by the technique of fluorescence recovery after photobleaching. To this end, the probe collarein, a fluorescent lipid analog that has the property of exclusive localization in the plasma membrane, was synthesized. Measured decreases in three parameters [percentage of fluorescene bleached (30%), percentage of recovery (52%), and half-time for recovery (52%)] connoted the appearance of an immobile fraction upon exposure to tumor promoters. These data are consistent with lipid reorganization in response to a reorganization of the intra- and perimembranous macromolecular scaffolding upon the interaction of cells with tumor promoters. The idea of induced reorganization is supported by experiments in which cell shape change, brought about by either exposure to cytochalasin B or growth on matrices of collagen, fibronectin, or laminin, resulted in values in the fluorescence recovery after photobleaching technique similar to those with active phorbol esters.

P-31 NMR Spectroscopy of Rat Organs, in situ, Using Chronically Implanted Radiofrequency Coils. A. P. Koretsky, S. Wang, J. Murphy-Boesch, M. P. Klein, T. L. James and M. W. Weiner. Proc. Nat'l. Acad. Sci. USA 80, 7491-7495 (1983).

A Technique for making P-31 NMR spectroscopic measurements in rat kidney, heart, and liver in vivo is presented. Two-turn solenoid coils were surgically implanted around the organ sufficiently in advance of NMR experiments to allow recovery of the animal. These chronically implanted coils allowed acquisition of high resolution spectra at 40.5 and 97.3 MHz. No resolution improvement occured at the higher field. Spectra were stable for up to 24 hr. during which time a variety of experiments could be performed. By accumulating spectra at 10-min intervals, the effects of intraperitoneal fructose injections were monitored; in kidney and liver, a rapid increase in sugar phosphates at the expense of Pi and ATP resulted. Fructose had no effect on heart metabolite levels. Spectra from the heart in vivo were obtained at systole and diastole by gating the spectrometer to the aortic pressure

wave; no differences in phosphate metabolites were detected. Finally, saturation transfer techniques were used to monitor the rate of ATP synthesis in the kidney. The unidirectional rate constant for the converstion of Pi to ATP was 0.12 ± 0.03/sec.

4. Fundamental Chemistry of Mutagenesis
Professor John E. Hearst and Professor Henry Rapoport

The objective of this program is to determine molecular mechanisms of mutagenesis. The approach is to achieve site specific modification of an infectious M13 viral DNA molecule by reaction with a psoralen. In the past year, techniques have been perfected for sequencing dideoxynucleotide DNA. Several site specifically modified M13 molecules have been formed, all of which have a psoralen added to a thymidine at one site in the genome. Furthermore, the stereochemistry of the adduct at each of those sites is known. The site of modification has been selected to be in the middle of a single hit restriction site in the intergenic region of M13 so no mutations which are lethal to the virus are expected. Transfection experiments are being performed at present.

Relevant Abstracts

Termination Sites of the in vitro Nick-Translation Reaction on DNA that had Photoreacted with Psoralen. Jacques G. Piette and John E. Hearst. Proc. Natl. Acad. Sci. USA 80, 5540-5544 (1983).

A double-stranded circular DNA having a single nick at a specific site has been photochemically induced to react with 4'-hydroxymethyl-4,5',8- trimethylpsoralen (HMT) and used as a substrate for nick-translation with *Escherichia coli* DNA polymerase I holoenzyme. By using the dideoxy chain-terminating sequencing procedure, it was possible to map the termination sites observed on the template that had photoreacted with HMT. These sites occur at nucleotides preceding potential psoralen crosslinking sites. Analysis of DNA products synthesized on templates that had photoreacted under conditions designed to maximize psoralen monoaddition revealed that DNA polymerase I is not stopped by this lesion. Psoralen monoadducts situated on the template strand act only as kinetic attenuators, whereas psoralen monoadducts localized on the nick-translated strand have no effect on the rate of synthesis. These data suggest that psoralen crosslinks are responsible for the lethal effects of psoralen photochemistry in *E. coli*. Mutagenesis may be associated, however, with the repair replication of psoralen monoadducts by *E. coli* DNA polymerase I.

Initiation of RNA Synthesis in vitro by Vesicular Stomatitis Virus: Single Internal Initiation in the Presence of Aurintricarboxylic Acid and Vanadyl Ribonucleoside Complexes. Sohel Talib and John E. Hearst. Nucleic Acids Research 11(20), 7031-7042 (1983).

In the presence of aurintricarboxylic acid (ATA) and vanadyl ribonucleoside complexes (VRC), we have isolated and characterized a small RNA, product of VSV *in vitro* transcription. This RNA is capped and lacks poly(A) at its 3'-end. Nucleotide sequence analysis revealed that this RNA corresponds to the 5'-terminal transcription product of the N-gene. The termination of the transcription occurs precisely at the 118th base from the 3'-end of the VSV genome. Analysis of the nucleotide sequence around this region reveals a potential secondary structure. Photoreaction of the VSV with 4'-substituted psoralen fails to inhibit the synthesis of the 68-mer RNA under conditions where full length mRNA synthesis is blocked, indicating that the psoralen binding site is located further into the N-gene. Since this RNA is the only *in vitro* transcription product synthesized under these conditions, the existence of two types of polymerase activities, one for the synthesis of leader RNA and one for mRNA is suggested.

Efficient Formation of a Crosslinkable HMT Monoadduct at the Kpn I Recognition Site. Howard Gamper, Jacques Piette and John E. Hearst. Photochemistry and Photobiology 40, 29-30 (1984).

The double-stranded Kpn I linker, CGGTACCG, is readily crossinked by 4'-hydroxymethyl-4.5',8-trimethylpsoralen (HMT). Under identical conditions, the corresponding Bam HI linker, CGGATCCG, is resistant to modification. The differential reactivity of HMT towards the two sequences is also observed with SV40 DNA where the Kpn I restriction site is ten-fold more susceptible to HMT modification than the Bam HI site. Selective reaction with the Kpn I site is attributed to preferential intercalation of HMT into the TpA sequence. The availability of crosslinked Kpn I linker has allowed us to characterize the kinetics of photoreversal. Upon irradiation at 254 nm. the diadduct reverses at a faster rate than the monoadduct, leading to an accumulation of the latter. The resultant monoadduct reforms crosslink rapidly upon irradiation at 365 nm. DNA oligomers like the Kpn I linker, which can be modified by the above protocol to contain a crosslinkable HMT monoadduct, could be exploited as photoreactive sequence-specific probes.

C. Structural Studies

1. Structural Biophysics
Professor Sung-Hou Kim

Structures of DNA Damaged by UV and Environmental Chemicals. Living organisms are constantly exposed to radiation and environmental chemicals that can cause damage to DNA. To understand the biological effects of these damages, we have been working on deriving structural models for the damaged

DNA. Our approach has been a combination of x-ray crystallographic results, molecular model building and energy minimization methods. Specifically, we have constructed a double stranded DNA molecule containing a psoralen photo-crosslink which shows a large kinking of the helical axis and unwinding at the crosslink site. Additionally, we have built a DNA model containing a thymine photodimer which shows similar, but smaller, distortions. Such sites of DNA structural impairment may have long-distance, as well as local, effects due to the changes they can induce in DNA supercoiling and overall architecture. They may also present good recognition sites for repair enzymes. S 1

Global Search of Conformational Surface for DNA and RNA. It is well known now that DNA and RNA assume many different conformations. To understand all possible conformations and their interconversion pathways, we have been working on the global search of conformational space for DNA and RNA.

We have been examining the conformational energies of the eight most common nucleosides--A, U, G, D, dA, dT, dG, dC--as monitored by a semi-empirical energy force field. These are the first calculations to fully explore the conformational spaces available to the nucleosides. We can now completely specify the conformation of any nucleoside, after some reasonable approximations, by three variables: torsion angle gamma (O5'-C5'-C4'-C3'), torsion angle xi (O4'-C1'-N9/N1-C4/C2), and pseudorotation angle of sugar P.

This line of studies is computation-intensive. In the beginning, we have to build over 46,000 molecular models representing entire conformation space of the sugar group in DNA and RNA. Potential energy of each model has to be calculated; several structures corresponding to local energy minima have to be identified; then energy minimization techniques applied to obtain each of all possible nucleotide structures.

Local Motional Analysis of DNA and RNA. Our view of DNA and RNA structure is undergoing rapid change. No longer is our concept of double hellical DNA limited to the uniform models derived from fiber diffraction data.

Even though our vision of nucleic acid structure has been improved by these recent developments, we still have only a static picture of DNA and RNA. We lack a view of nucleic acids as dynamic, flexible molecules, fluctuating between different conformations and perhaps even different helical forms. Only now are theoretical methods of molecular dynamics and vibrational mode analysis being applied to the static models of DNA.

What is needed at this time is a sound foundation of experimental data with which the theoretical results can be compared and evaluated. Some information about the local motion of DNA and RNA has been obtained from NMR measurements. However, for detailed information about the directions and magnitudes of motion of individual nucleotides, we now turn to X-ray crystallographic data.

In our approach, we represent the mobility of polynucleotides with an anisotropic segmented rigid body model, in which each segment, or subgroup, of the molecule undergoes translational and rotational motion as a rigid body.

We have been analyzing the mobility of nucleotides in various RNA and DNA fragments in several different crystal lattice environments based on X-ray crystallographic data. Our approach provided the first experimentally derived motional parameters of DNA and RNA.

Relevant Abstracts

Sharp Kink of DNA at Psoralen-cross-link Site Deduced from Crystal Structure of Psoralen-Thymine Monoadduct. S. H. Kim, S. Peckler, B. Graves, D. Kane, H. Rapoport, and J. E. Hearst, In: Cold Spring Harbor Symposia on Quantitative Biology, 1983.

Psoralens are a class of tricyclic aromatic heterocycles that have been used as chemical probes to study DNA and RNA structures. On exposure to UV light, psoralens form chemical cross-links to the DNA bases in three steps. The first step is intercalation of psoralen between two adjacent base pairs. The second step is formation of a monoadduct, i.e., one psoralen photoreacts to one strand of DNA. The third step is the cross-linking of the same psoralen to the other strand of DNA.

Light-induced cross-linking of double-stranded nucleic acids by psoralens has been exploited to locate, in vivo or in vitro, those double-helical regions of DNA or RNA that can accommodate any structural changes caused by the psoralen cross-links. To determine three-dimensional structural parameters of the cross-link, we have solved the crystal structure of the psoralen-thymine monoadduct formed in photoreaction between calf thymus DNA and 8-methoxypsoralen (8MOP).

There are eight possible configurations for psoralen-thymine monoadducts and 64 for diadducts. Spectroscopic and other studies have narrowed this down to a few possible configurations, and recent nuclear magnetic resonance (NMR) studies on psoralen monoadducts and crystallographic studies described here prove unambiguously that the biologically relevant structures have the "cis-syn" configuration. Such drastic reduction in the number of configurations comes from the fact that the double-helical DNA conformation imposes very stringent restrictions on modes of psoralen interaction. Thus, presumably, only a particular configuration can be formed.

Correlation Between Chemical Modification and Surface Accessibility in Yeast Phenylalanine Transfer RNA. Stephen R. Holbrook and Sung-Hou Kim. In: Biopolymers, 22, John Wiley Sons, Inc. 1145-1166 (1983).

Chemical reactivities of the functional groups of yeast phenylalanine transfer RNA are compared with surface accessibilities of the groups calculated with various probe radii representing effective radii of the chemical reagents used. We observe 97% agreement with the hypothesis that the chemically modified bases are those with the greatest surface

accessibility. This overall strong correlation supports the conclusion that base exposure is an important determinant of chemical modification in this polynucleotide.

The Local Mobility of Nucleic Acids as Determined from Crystallographic Data. I. RNA and B Form DNA. Stephen R. Holbrook and Sung-Hou Kim, J. Mol. Biol. (in press).

We have determined the directions and magnitudes of translational and rotational mobility of the phosphate, ribose, and base moities of several ribooligonucleotides and of the self-complementary DNA duplex, CGCGAATTCGCG, from single crystal X-ray diffraction data. To describe nucleic acid mobility, we have used a "segmented rigid body" model in which an oligonucleotide is divided into several subgroups, each of whose motion is constrained to translate and librate (partially rotate) as a unit. This approach sufficiently reduces the number of parameters to allow determination of the local mobility from available experimental data.
Our results show that double stranded DNA and double stranded RNA have similar local mobilities. Some of the types of mobility which we observe for double helices are:

1. Propeller twisting between paired bases and/or rolling of the base pairs as a unit.
   Buckling of the base pairs.
3. Sliding of the bases in the plane of the base pairs.
4. Coupled rotation of the sugar and base as a nucleoside unit.
5. Coupled translation of paired bases.
6. Fluctuation of the groove sizes or bending of the helices.
7. Unwinding/winding motion at the ends of helices.

We have also noted a dependence of mobility in double helices on base type as well as location of the residue relative to the helical ends.

These observed local mobilities can help us understand conformational changes during such processes as intercalation, melting, transition between different helical forms, and interaction of nucleic acids with proteins and other ligands.

Somewhat different mobilities are observed for RNA fragments which are not involved in Watson-Crick base pairing. For example, the major single stranded base libration axis corresponds to a simple rotation around the glycosyl bond.

Also, there does not appear to be a coupling of the librational mobility of the ribose and base like that observed for the double-stranded oligonucleotides. Differences in mobillity and flexibility between single and double stranded nucleic acids may be important for recognition and binding of nucleic acids by proteins.

"Interactive" Recognition in EcoRI Restriction Enzyme-DNA Complex. Rosalind Kim, Paul Modrich, and Sung-Hou Kim. Nucleic Acids Research, in press 1984.

Interaction between DNA and protein, which plays an important role in all living cells, can be divided into two categories. In specific recognition, a protein recognizes a particular DNA sequence several orders of magnitude tighter than in non-specific recognition, where the protein recognizes primarily the general features with little or no specificity for a particular base sequence. Interaction between the EcoRI restriction enzyme and DNA provides a good example of both recognitions.

In specific recognition the mechanism of how a protein finds a particular sequence of DNA is under intense study. The current view is that, initially, protein binds to DNA non-specifically, and then finds a cognate DNA sequence by sliding, short hopping and/or inter-strand jumping. The question we would like to address is what happens to DNA after a protein binds to its cognate DNA sequence in solution. Two consequences can arise. In one the protein may simply stop at the cognate DNA sequence without substantial conformational changes in DNA and/or protein (we'll call this "passive" recognition), and in the other, there may be an interactive process of recognition or selection between protein and DNA resulting in a conformation of DNA and/or protein different from the average conformation in free solution ("interactive recognition"). (The same question can be asked for non-specific interaction as well.) Depending on the function of the protein subsequent to the recognition step, additional conformational changes of DNA and/or protein may occur.

We report our observation that there is a definite distortion of DNA in the specific recognition complex between EcoRI endonuclease and DNA but no detectable distortion of DNA in the non-specific recognition complex. As a measure of conformational change on DNA, the angle of topological unwinding (or winding) of circular plasmid DNA was measured by electrophoresis on agarose gel according to the methods developed by Keller and Wandell and exploited so well by various investigators.

III. WORK FOR OTHERS

A. Structures of DNA and RNA
Professor Ignacio Tinoco

We have developed new methods to probe the structures of RNA, and we have found a significantly different, new structure for double-stranded RNA. After the finding in 1979 that double-stranded DNA can exist as a left-handed helix, there has been a search for a left-handed RNA helix. We recently found that in 6 M $NaClO_4$, 45°C the synthetic RNA molecule poly[r(G-C)] is a left-handed helix, with a similar structure to the corresponding DNA molecule. Circular dichroism and nuclear magnetic resonance spectra were used to make the identification. This left-handed RNA helix is the first significantly different structure found for a double-stranded RNA.

Determination of structure in naturally occurring RNA molecules which have both single- and double-stranded regions is very difficult. We have developed a sensitive method for specifically identifying guanine residues in single-stranded regions. Riboflavin is added to an RNA solution and a short pulse of light is applied. Using model compounds we found that the riboflavin is photo-excited and that it selectively polarizes protons of guanine residues in single strands. This nuclear polarization is detected by nuclear magnetic resonance (NMR); the NMR spectrum indicates the local environment of the guanine. We have applied this method to transfer RNA, but it is generally useful.

Relevant Abstracts

Chemically Induced Dynamic Nuclear Polarization Studies of Yeast $tRNA^{Phe}$. Elizabeth F. McCord, Kathleen M. Morden, Ignacio Tinoco, Jr., and Steven G. Boxer, Biochemistry 23,1935 (1984).

Chemically induced dynamic nuclear polarization (CIDNP) has been observed from yeast $tRNA^{Phe}$ following reaction with photoexcited riboflavin. At 20°C, several resonances of tRNA in the native form show polarization; previous work predicts that only guanosine and its derivatives in single-stranded regions are likely to become polarized [McCord, E.F., Morden, K. M., Pardi, A., Tinoco, I., Jr., & Boxer, S.G. (1984) Biochemistry]. The methyl protons of m^2_2G-26 show strong negative spin polarization, indicating that this residue is accessible. The solvent accessibility of this residue has not been previously demonstrated. In addition, two positively polarized aromatic resonances are observed, which are likely due to two or more G(H8) protons, including those of G-20, m^2_2G-26, and/or Gm-34. For temperatures below 50°C, a negatively polarized signal in the aromatic region is shown to arise from cross relaxation with the methyl group protons of m^2_2G-26. At higher temperatures, the CIDNP spectra show polarization of several additional G resonances, including those of m^2G-10.

These changes in the CIDNP spectra reflect melting of the tertiary and secondary structure of the tRNA. This work is the first use of CIDNP to study a large nucleic acid molecule and exemplifies the value of this technique in probing single-stranded and solvent-accessible regions of tRNA.

Circular Differential Scattering Can Be an Important Part of the Circular Dichroism of Macromolecules. Carlos Bustamante, Ignacio Tinoco, Jr., and Marcos F. Maestre. Proc. Natl. Acad. Sci. USA. 80, 3568-72 (1983)

Differential scattering of incident left and right circularly polarized light can be an important contribution to the circular dichroism of macromolecules. In principle both differential absorption and differential scattering of circularly polarized light contribute to circular dichroism, but differential scattering is increasingly important for particles whose dimensions are greater than 1/20th the wavelength of light. The scattering contribution is probably not important for unaggregated proteins and nucleic acids in solution. It can be very important for viruses, membranes, and other protein-nucleic acid complexes. Outside the absorption bands of the scattering, chiral particle, only differential scattering contributes to the circular dichroism. The sign and magnitude of the differential scattering is quantitatively related to the relative orientations and the distances between the scattering units of the particle. The interpretation of the circular differential scattering depends on a simple, classical method. Thus, in understanding a measured circular dichroism, it often will be easier to relate the differential scattering to the structure of a particle (such as a virus) than it is to relate the differential absorption to the structure.

Kinetics for Exchange of Imino Protons in Deoxyribonucleic Acid, Ribonucleic Acid, and Hybrid Oligonucleotide Helices. Arthur Pardi and Ignacio Tinoco, Jr., Biochemistry 21, 4686 (1982).

The lifetimes for opening of individual base pairs in a DNA(dCA_5G+dCT_5G), and RNA (rCA_5G+rCU_5G), and a hybrid DNA-RNA(rCA_5G+dCT_5G) helix have been measured by proton nuclear magnetic resonance. The lifetimes were obtained by saturation recovery experiments performed on the hydrogen-bonding imino protons of the Watson-Crick base pairs. In these oligonucleotide helices the observed relaxation rates were dominated by exchange with water, with the magnetic spin-lattice relaxation time of the imino protons possibly being important only at the lowest temperatures in the DNA helix. It was shown that three interior base pairs in the DNA heptamer dCA_5G+dCT_5G were in the open-limited region, which means that these imino protons exchange every time the base pair opens. The lifetimes of the terminal G-C base pairs in the DNA helix are much shorter than the interior A-T base

pairs. The pH dependence of the terminal base pairs indicated that the ends of the helix open and close many times before exchange of the imino protons with water takes place. The temperaure dependence of the lifetimes of the interior A-T imino protons in the DNA helix showed that these proteins exchange only when the double helix has dissociated into single strands. Thus, these lifetimes measure the rate for dissociation of the double helix. The activation energy for this process was found to be 47 kcal/mol. Comparison of the lifetimes of the interior protons in the DNA, RNA, and hybrid helices showed that the rates of dissociation of the RNA and hybrid helices are very similar at 5°C, whereas the rate for the DNA helix was approximately 1 order of magnitude smaller than that for the other two helices. The reasons for the differences in the kinetics of the three helices are discussed, as are the general dynamics of oligonucleotide helices in solution.

Secondary Structure of the Tetrahymena Ribosomal RNA Intervening Sequence: Structural Homology with Fungal Mitochondrial Intevening Sequences. Thomas R. Cech, N. Kyle Tanner, Ignacio Tinoco, Jr., Bruce R. Weir, Michael Zuker, and Philip S. Perlman. Proc. Natl. Acad. Sci. USA 80, 3903-3907 (1983).

Splicing of the ribosomal RNA precursor of *Tetrahymena* is an autocatalytic reaction, requiring no enzyme or other protein *in vitro*. The structure of the intervening sequence (IVS) appears to direct the cleavage/ligation reactions involved in pre-rRNA splicing and IVS cyclization. We have probed this structure by treating the linear excised IVS RNA under nondenaturing conditions with various single- and double-strand-specific nucleases and then mapping the cleavage sites by using sequencing gel electrophoresis. A computer program was then used to predict the lowest-free-energy secondary structure consistent with the nuclease cleavage data. The resulting structure is appealing in that the adjacent coding regions (exons) for ligation, and IVS cyclization can occur. The *Tetrahymena* IVS has several sequences in common with those of fungal mitochondrial mRNA and rRNA IVSs, sequences that by genetic analysis are known to be important cis-acting elements for splicing of the mitochondrial RNAs. In the predicted structure of the *Tetrahymena* IVS, these sequences interact in a pairwise manner similar to that postulated for the mitochondrial IVSs. These findings suggest a common origin of some nuclear and mitochondrial introns and common elements in the mechanism of their splicing.

B. National Tritium Labeling Facility
Dr. Richard M. Lemmon and Professor Henry Rapoport

This facility is available to scientists throughout the country who wish to prepare tritiated compounds at very high specific activities and radiopurities that are not available commercially. Research at the facility involves the study of novel methods of introducing tritium into organic compounds. Such methods include generation of active tritium radicals in a microwave plasma that is directed toward selected targets. Mechanisms such as addition, substitution, and dehalogenation by the excited tritons are under study. The facility also functions as a training laboratory for handling large quantities (i.e., hundreds of curies) of tritium safely, and for learning the latest techniques for purifying and storing radioactively labeled compounds.

Relevant Abstracts

Mechanism of Hydrogen-Atom Catalyzed Isotope Exchange in the Benzene Ring: Application to Tritium Labeling of Biologically-Important Aryl Componds. M. F. Powell, H. Morimoto, W. R. Erwin, B. E. Gordon, R. M. Lemmon, J. Phys. Chem. Accepted 1984.

Reaction of thermal tritium atoms, generated by microwave activation of T_2 gas, with benzene and biphenyl was studied at ~ -50°C and -196°C. The saturation reactions (i.e., benzene cyclohexane-t_6) predominated over isotope exc;hange (i.e. benzene benzene-t) at -196°C. However, significant exchange labeling occurred at ~ -50°C, with a concomitant reduction in the yields of saturated products. This reversal in labeled product yields at the different temperatures is due, in part, to the faster rate of H-expulsion from the intermediate cyclohexadienyl radical at -50°C, and to the increased mobility of the warmer matrix that retards multiple T• reactions with the same aryl molecule by covering up singly tritiated intermediates. The less volatile aryl compound, biphenyl, was labeled in a diffusionally-active matrix of either benzene or cyclohexane, whereas it could not be labeled otherwise.

A High-Level Tritium Facility at the Lawrence Berkeley Laboratory. R. G. Aune, B. E. Gordon, W. R. Erwin, C. T. Peng, and R. M. Lemmon, Synthesis and Applications of Isotopically Labelled Compounds. Proceedngs of an International Symposium, Kansas City, MO, U.S.A. June 6-11, 1982, W. P. Duncan and A.B. Susan (eds.) Elsevier Scientific Publishing Co., Amsterdam,, (1983) 437-438.

A high-level tritium labeling facility has been developed at the Lawrence Berkeley Laboratory. This facility can handle up to 20,000 curies of tritium gas (T_2) and 6,000 curies of tritiated water (HTO). Tritium-labeling reactions such as catalytic dehalogenation and catalytic reduction with T_2 gas, catalytic exchange with HTO, and solid-state exchange with tritium atoms generated in a microwave induced plasma have

been carried out using hundreds of curies, with radiological safety. The tritium facility is being used to study the mechanisms of tritium reactions, and is being upgraded to accommodate users nationwide and to add purification accessories and tritium nuclear magnetic resonance capability.

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1983

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Kahlow, M.A. M.S. Thesis. Photoinduced Electron Transfer in Rigid Solution: Time Related Quenching of Triplet Zinc Tetraphenylporphine. (LBL-17200) December 1983 (Melvin Calvin) Chemistry.

Pratum, Thomas Kingston. Ph. D. Some Nitrogen-14 Studies in Solids. (LBL-16998) November 1983 (Melvin Klein).

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Keller, David John. Ph.D. Thesis. Scattering Optical Activity of Chiral Molecules: Circular Differential Scattering and Circular Differential Imaging. LBL-17472 February 1984 (Ignacio Tinoco) Chemistry.

Oren-Shamir, Michal. M.S. Thesis. Characterizations of a Variant Form of SV40 DNA Isolated from Human Cells Transformed by Wild Type SV40 Virus. June 1984 (James A. Bartholomew) Comparative Biochemistry.

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1982

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1983

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1984

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STATISTICAL DATA

A. CHEMICAL BIODYNAMICS DIVISIONAL STAFF
July 1, 1982 - June 30, 1984

Faculty Associates
(Chemistry Department)

George C. Pimentel, Director
Melvin Calvin
John H. Clark
John E. Hearst
Sung-Hou Kim
Henry Rapoport
Kenneth Sauer
Ignacio Tinoco, Jr

Senior Scientific Staff

James C. Bartholomew
James A. Bassham
Edward L. Bennett
Melvin P. Klein
Richard M. Lemmon
John W. Otvos

Divisional Fellow

Heinz Frei

Administrative Staff

William Shanahan, Administrator
Evangeline Ceralde
Gloria Goldberg
Paul Hayes*
Beth Klingel
Bonita Ranken
Lois Soule
Marilyn Taylor

Scientific and
Technical Staff

Marie Alberti
Matt Ban
Phillip Eggers
Walace Erwin
Edward Fleischer
Benjamin Gordon
Everett Harvey
Stephen Holbrook
Rosalind Kim
Jean Lawson*
Ethel Lefall
Jorge Meaux
Hiromi Morimoto
Esther Nemethy
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Deborah Ang*
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Edwin Barnett
Mark Distefano*
Sherry Gee*
Mimi Kim*
Lisa Pascual
Alan Pepper*
David Pirkle
Michael Stelman
Fonda Wu*

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B. POSTDOCTORAL VISITORS: CHEMICAL BIODYNAMICS DIVISION
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Name	Source of Funding	Associate
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Barsky, Debonny	LBL	James Bartholomew
*Begusch, Helmut	UC	John Hearst
*Blackwell, Mary	UC	Kenneth Sauer
Blough, Neil	NIH	Kenneth Sauer
Collins, Susan	LBL	George Pimentel
Cimino, George	NIH/LBL	John Hearst
Cruz, Phillip	UC	Ignacio Tinoco, Jr.
DeVos, Abraham	UC	Sung-Hou Kim
Grant, Janice	LBL	Melvin Calvin
Graves, Bradford	NIH	Sung-Hou Kim
*Hatada, Marcos H.	UC	Sung-Hou Kim
Karukstis, Kerry K.	NIH	Kenneth Sauer
Maliyackel, Anthony	LBL	Melvin Calvin
Miyashita, Koichi		Melvin Calvin
Mowbray, Sherry	UC	Sung-Hou Kim
Nakanishi, Tomoko	Nippon Zeon Co. Ltd. Tokyo, Japan	James Bartholomew
O'Brien, Philip	LBL	John Hearst
Orton, Edward	LBL	George C. Pimentel
*Piazza, George	LBL	Melvin Calvin
*Rogers, Stephen	LBL	Melvin Calvin
*Schrems, Otto	Deutsche Forschugs-gemeinschaft	George C. Pimentel
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Shimer, George	UC	Ignacio Tinoco, Jr.
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Tang, Yui-Sing	LBL	Richard Lemmon
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Yachandra, Vittal	UC	Melvin Klein
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*Mary Blackwell	Chemistry	Kenneth Sauer
*John Blanding	Comparative Biochemistry	James Bassham
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Michael Boska	Chemistry	Kenneth Sauer
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Ward Brown	Chemistry	John Clark
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Lucinda Buhse	Chemistry	John Clark
*Edward Bylina	Chemistry	John Hearst
Harry Cartland	Chemistry	George Pimentel
John Casey	Chemistry	Kenneth Sauer
Thomas Casti	Chemistry	Melvin Calvin
Seunghuon Choe	Biophysics	Sung-Hou Kim
James Cole	Chemistry	Kenneth Sauer
James Corbett	Chemistry	Ignacio Tinoco
David Cook	Chemistry	John Hearst
Victoria DeRose	Chemistry	Kenneth Sauer
Susan Dexheimer	Physics	Melvin Klein
Marc Donsky	Chemistry	Henry Rapoport
*Richard Duclos	Chemistry	Henry Rapoport

Paul Feldman	Chemistry	Henry Rapoport
*Jeremy Gelbwaks	Chemistry	John Hearst
Miriam Gochin	Chemistry	Melvin Klein
*David Goodin	Chemistry	Kenneth Sauer
Ronald Guiles	Chemistry	Melvin Klein
Kathleen Hall	Biophysics	Ignacio Tinoco
Douglas Hawkins	Chemistry	Melvin Calvin
Marian Helfand	Chemistry	John Clark
Carolyn Hoener	Chemistry	George Pimentel
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Sara Hoover	Chemistry	Kenneth Sauer
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Sarah Tabbut	Chemistry	Kenneth Sauer
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*Bruce Watkins	Chemistry	Henry Rapoport
Steven Webb	Chemistry	John Clark
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Mu-Ching Yee	Chemistry	James Bartholomew
Shiela Yeh	Chemistry	John Clark
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*Kris Zsebo	Comparative Biochemistry	John Hearst

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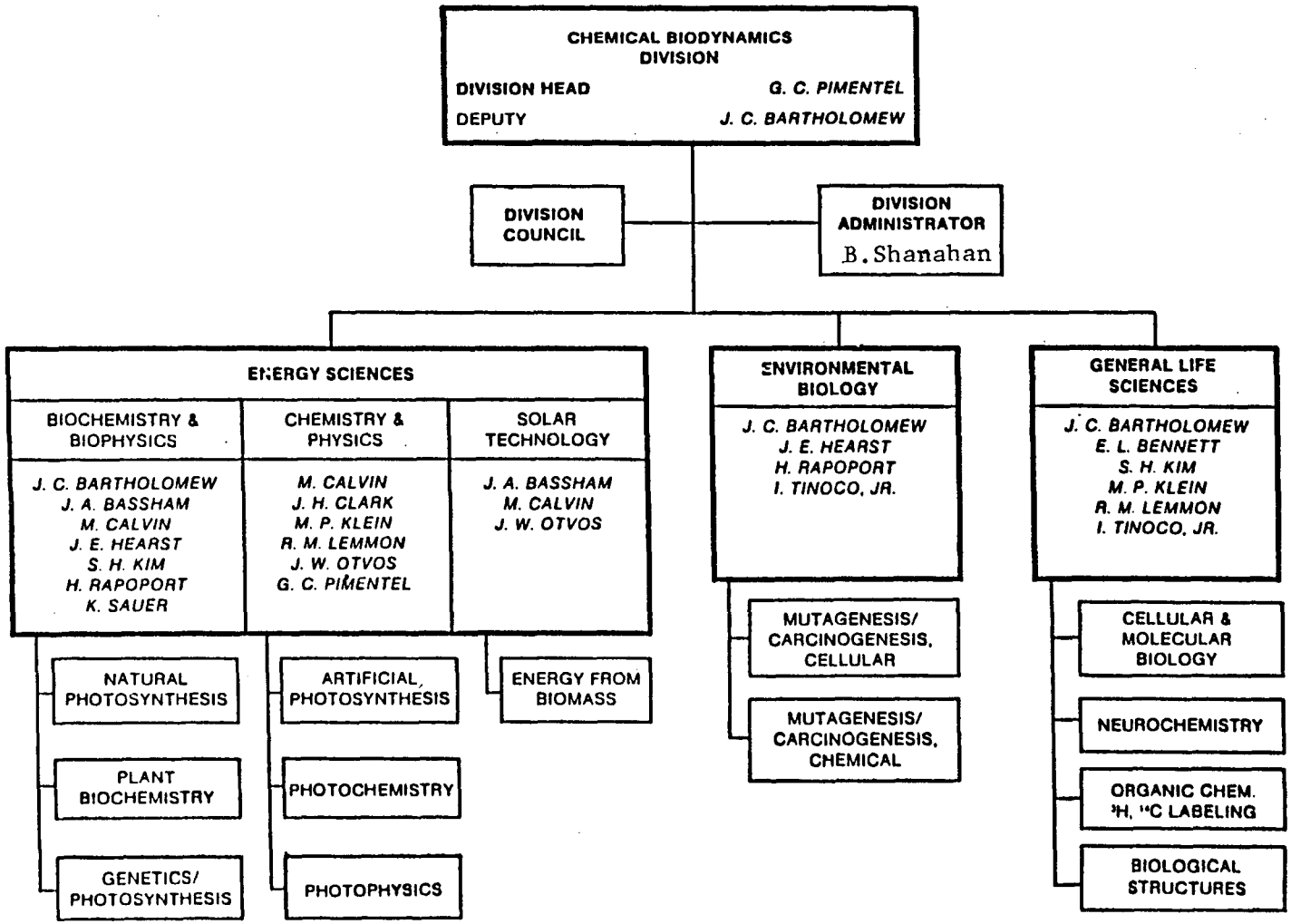

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