LECTURES

I. GEOLOGICAL HISTORY OF PHOTOSYNTHESIS

A. Macrofossil Evidence
   1. Stromatolites – structural and functional stratification of oxygenic and anoxygenic photosynthesizers, and aerobic and anaerobic heterotrophs

B. ^{12}C / ^{13}C Discrimination
   1. Indirect evidence for carbon dioxide fixation due to ^{12}C preference

C. Oxygen production and geochemistry
   1. Marine Fe(II) oxidation to Fe(III) as a major sink for early oxygen production

The range of evidence creates a cohesive picture of atmospheric oxygen, created from oxygenic photosynthesis, appearing in concert with life forms more complex than the prokaryotic groups.

II. PHOTOSYNTHETIC DIVERSITY

A. Introduction to Anoxygenic Prokaryotic Groups
   1. Bacteriochlorophylls
   2. H_2S and H_2 as electron donors
   3. Unique carbon fixation mechanisms including reverse Krebs cycle and the Hydroxypropionate pathway

The emphasis on prokaryotic diversity highlighted the major primordial contributors to photosynthesis (anoxygenic). In the context of bioengineering, these groups may be useful, for biomimetics and possibly as engineered microbes.

III. LIGHT

A. The Physics of Light
   1. Dipole origins
   2. Wave and particle: The photoelectric effect and the energy of a photon

The physical properties of light were introduced in detail, including an introduction to Maxwell's field equations and a detailed presentation of the photoelectric effect and its use to determine the energy of absorption events.

B. Light and Photosynthesis
   1. Absorption by chlorophyll and exciton fates
      a. fluorescence, exciton transfer, radiationless loss, triplet formation (phosphorescence) and photochemistry
      b. photooxidation

The properties of light are closely intertwined with multiple events during the light reactions of photosynthesis, including photochemistry, the desired outcome of an absorption event. Absorption events and outcomes were presented in the context of useful and useless outcomes, including the photooxidation damage caused by long-lived triplet states.

III. PIGMENTS OF PHOTOSYNTHESIS

A. Carotenoids
   1. Synthesis and role in protection from photooxidation

B. Bilins
   1. Introduction to the pyrrole motif of linear and cyclic tetrapyrroles
   2. Phylogenetic uniqueness of bilin distribution

C. Chlorophylls
   1. Introduction to the diversity of cyclic tetrapyrroles
      a. hemes (Fe insertion)
      b. vitamins and vanadyl porphyrins
      c. chlorophylls (Mg insertion)
   2. Synthesis
      a. delta-aminolevulinic acid, the first committed precursor
         i. succinyl CoA and glycine pathway
         ii. glutamyl-tRNA mediated pathway
      b. deamination, decarboxylation and unsaturation to form the porphyrin foundation of the chlorophyll molecule

The central role of tetrapyrroles in multiple biochemical processes in organisms was emphasized, with reference to the evolution of diverse synthesis pathways that occur in tandem in photosynthetic eukaryotic cells.

IV. LIGHT HARVESTING
A. Emerson and Arnold's Classic Experiments on Light and O_2 production
   1. Dark interval required to complete O_2 production
   2. Chlorophyll antenna
      a. light-saturating conditions
      b. chlorophyll molecules per oxygen molecule produced
B. Light-Harvesting Complexes
   1. SDS-PAGE characterization
   2. X-ray crystallographic structures
   3. Regulation of the architecture of photosynthesis
      a. PQH_2/PQ ratio and granal stacking to effect modulation of electron fate through cyclic (ATP synthesis) and non-cyclic (NADPH production) photosynthesis

The classic basis for light harvesting complexes was presented in detail, including reading of the original papers by Emerson and Arnold. This was followed by an in depth description of the composition of light-harvesting complexes from major phylogenetic lineages and finally their role in regulation of the light reactions of photosynthesis in higher plants.

V. REACTION CENTERS
A. Evidence for Two Reaction Centers in Oxygenic Photosynthesis
   1. Red drop and red light enhancement
   2. Cytochrome oxidation
B. The Structure of the *Rhodopseudomonas* (*Rhodobacter*) Reaction Center
   1. X-ray crystallography (Michel and Deisenhofer)
C. The Structure of the PS II Reaction Center
   1. X-ray crystallography
   2. Water-splitting: Structure, kinetics and mechanism

The historical evolution of our understanding of the reaction center was presented, with emphasis on how structural elucidations have informed the present 'state-of-art' regarding the physical mechanisms of photochemistry and the charge separation after the photochemical event.

VI. NAPDH AND ATP PRODUCTION
- A. e^- and H^+ Transfer Mechanisms
  - 1. Mediators of redox reactions
    - a. NADPH: structure and function
    - b. flavins: structure and function
    - c. ubiquinone (plastoquinone): structure and function
    - d. iron-sulfur centers: structure and function
    - e. hemes
  - 2. H^+ shuttling
    - a. cytochrome b_6f complex
    - b. plastoquinone

Emphasis was placed on the mediators of redox reactions, rather than the linear sequences of redox transfers that occur in the electron transport chain, since these vary depending upon the organism (principally between anoxygenic and oxygenic organisms).

- B. Chemiosmotic Theory and the Coupling Factor
  - 1. ATP bioenergetics
  - 2. ATP synthetase
    - a. kinetic mechanism
    - b. mechanical mechanism

The relation between 'osmotic' gradients and chemical synthesis were presented, as well as the kinetic steps in ATP synthesis and the concept of a mechanical (rotatory) linkage between the proton motive force and ATP production.

VII. DARK REACTIONS
- A. Carbon Dioxide Fixation by RuBisCO
  - 1. Carboxylase reaction
  - 2. Structure of the enzyme, and its regulation

Details of the mechanism of carboxylation within the active site, including the mechanism of enzyme activation by carbamylation were presented in detail.

- B. Reductive Pentose Phosphate Pathway (the Calvin Cycle)
  - 1. Enzymatic reactions and the central role of 3-phosphoglyceraldehyde
  - 2. Evidence in support of the Calvin Cycle
- C. Photorespiration
  - 1. The oxygenase reaction of RuBisCO
  - 2. Carboxylase/Oxygenase specificity
  - 3. Regeneration of 3-phosphoglycerate
- D. C4 Pathways of Carbon Dioxide Fixation
  - 1. PEPCase reaction
    - a. comparisons of C3 and C4 lifestyles
  - 2. Mechanisms of carbon dioxide recycling to RuBisCO
  - 3. C4 ecophysiology
- E. Carbon Dioxide Concentrating Mechanisms
  - 1. CO_2 / HCO_3^- transport
    - a. *Eremosphaera viridis* – case study

There is a vast scope of carbon dioxide fixing strategies. All were presented at the level of transformation of chemical structures. With the exception of some of the anoxygenic prokaryotes, the Calvin Cycle is central. Any variations amount to biochemical and/or transport mechanisms designed to concentrate CO_2 at the site of RuBisCO.

VIII. METABOLIC FLUX

A. Utilization of Reducing Equivalents of Photosynthesis

1. Nitrogen assimilation

a. nitrate reduction and amino addition to glutamate

The crucial role of reducing equivalents produced in the light reactions to create utilizable forms of nitrogen was described in detail.

B. Fates of Photosynthates

1. Starch / sucrose synthesis

C. C3 Pathway Bioengineering

1. Flux control coefficients

2. Modifying activity of C3 pathway enzymes

a. sedoheptulose 1,7 bisphosphatase

The integration of the multiple biochemical requirements for life, utilizing the ATP and reducing equivalents provided by photosynthesis, were presented. The results of experiments bioengineering plants by transformation to modulate expression of key enzymes of the Calvin Cycle and the impact on biomass production were discussed.

LABORATORY EXERCISES (3 hours per week)

Chloroplasts in Photosynthetic Tissues
- Imaging chloroplasts in situ and in vitro (**Week One**)

Fluorescence and Reaction Centers
- Determining Excitation and Emission Spectra (**Week Two**)
- Delayed Fluorescence (**Week Three**)

Oxygen Electrode: Pathways of Photosynthetic Electron Transport
- P/O ratios (**Week Four**)
- Carbon dioxide coupling in chloroplasts and algae (**Week Five**)
- Pathways in photosynthetic electron transport (**Addenda Week**)

Photophosphorylation
- Light-induced ATP production in isolated chloroplasts (**Week Six**)
- Acid-base induced ATP production in isolated chloroplasts (**Week Seven**)

Chloroplast Molecular Biology
- Isolation of chloroplast DNA(**Week Eight**)
- Chloroplast genome mapping (**Week Nine**)

The laboratory exercises provided a detailed introduction to the scope of experimental techniques used to explore the primary processes in photosynthesis: From the light reactions, synthesis of chemical energy, and integration with carbon dioxide fixation, to, finally, the genomic identity of the chloroplast. The techniques included fluorometry, oxygen electrode, ATP assays with luciferin-luciferase, and genome mapping using restriction endonucleases.

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