BOT 4503
4 Hours
Photosynthetic electron transport

Objectives

1. Name the four major protein complexes that are involved in photosynthetic electron transport and briefly describe the function of each.

2. Draw the generalized structure of a chloroplast and identify the three aqueous spaces. Give two interpretations of granum formation. Why was the more recent one proposed? Name the various types of plastids and their functions.

3. Describe the technique of freeze-fracture. What did we learn from this technique about the heterogeneity of the distribution of proteins in the different membranes of chloroplasts?

4. Draw a generalized glycerol-derived membrane lipid. What broadly based generalizations might you make from Table 1?

5. Describe the Engelmann experiment and results.

6. Discuss van Niel's insight in the similarity of oxygenic photosynthesis and non-oxygenic photosynthetic organisms.

7. What is the Hill reaction? How did Hill's work corroborate the insight of van Niel?

8. Discuss the several reasons that there might be discrepancies between an action spectrum and the absorption spectrum of a relevant pigment.

9. What is the Emerson Enhancement Effect? . . . far-red drop? How do we interpret these results today?

10. Describe the periodicity of O_2 evolution with light flashes. Draw Kok's S-state model of photosynthetic oxygen evolution.

11. Give a general description of the chlorophyll molecule. Considering its chemical structure, why do you expect that it would absorb visible light over a wide spectrum? Identify other biomolecules that resemble chlorophyll. How do chl a and chl b differ (structure, energy required to make the ground-state to "lower" excited-state transition)? What are carotenoids? Draw an absorption spectrum of chl and of carotenoids. To what extent are carotenoids involved in the primary act of light harvesting? Are pigments in addition to those above involved in photosynthesis in various organisms?

12. Describe the overall pigment distribution of chlorophyll**s** in PSII (reaction center, proximal antennae, and peripheral antennae). . . . PSI.

13. Write a brief essay on the association of pigments and proteins in the thylakoid membranes. Describe light absorption and excitation of the RC pigment.

14. Discuss the general problem of optimizing the energy allocation between two serial photosystems that have different spectral properties. How is this problem "solved" by plants?

15. Draw a simple energy diagram that shows the ground state of chlorophyll, the "higher" excited state of chlorophyll, and the "lower" excited state of chlorophyll. Describe the mechanisms that cause interconversion of these energy states.

16. Briefly describe in a generic way the two kinds of photosystems. Do plants have both types? Are there analogous photosystems in other organisms?

17. What is PAR? Identify two assumptions that are implicit in its usage?

18. Draw the Z-scheme of photosynthesis and explain the axes. Why is "chain," as in the photosynthetic electron transport chain, a misnomer?

19. Describe the process of charge separation in PSII with as much molecular and biophysical detail as you can.

20. Describe the two energy-conservation sites in the photosynthetic electron transport chain.

21. Make an argument by analogy with non-oxygenic bacteria that the OEC is not D1/D2 as most researchers think? (By asking this question, I do not mean to imply that the OEC is not D1/D2.)

22. What are the probable redox components of the OEC?

23. How is energy distributed efficiently between the two photosystems? . . . what is the sensing mechanism?

24. Describe briefly the signal transducing system by which the OPPP is deactivated in light and the RPPP is activated in light.

Lecture

At no risk of hyperbole, one can assert that *the* central—most important, whatever superlative that you wish to supply—life process is the conversion of light energy to stable chemical energy. It is,

therefore, no surprise that this area of experimental plant biology has enjoyed, along with phloem transport and water relations, a very long history of inquiry. Indeed, the dawn of all "modern" experimental science is tied intimately with photosynthesis. Lavoisier, regarded as the founder of modern chemistry, was concerned with the isolation of gases in air, and as you know, virtually all atmospheric O_2 has accumulated as a result of oxygenic photosynthesis[1,2]. Priestly, in 1771, showed that air "exhausted" by the burning of a candle could be "renewed" by plants. Less than a decade later, Ingenhousz, a Dutchman, showed that light was important to the process. Thus, our present knowledge of photosynthesis comes from more than two centuries of serious study.[3]

We will approach the learning of photosynthetic electron transport in the following way. First, chloroplast structure itself will be reviewed. (As you recall, perhaps, the anatomy review only touched lightly on chloroplast structure, as I wanted to give the details immediately before this topic.) Second, a

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[1]Cyanobacteria and all photosynthetic eukaryotes conduct "oxygenic" photosynthesis. I.e., they extract electrons from H_2O and the byproduct is O_2. Although I have referred to this as photosynthesis without qualification, you should at least be aware that some prokaryotes utilize other than H_2O as an electron donor. As an example, one taxon extracts electrons from H_2S; the product, elemental S, is found in large deposits owing to these organisms, just as O_2 in the atmosphere accumulated from oxygenic organisms. We will not discuss non-oxygenic photosynthesis, except in a historical context, in this course in *plant* physiology. However, as we have stressed, biochemistry is very similar among all organisms. Indeed, important insights into plant photosynthesis have come from studies with bacteria. The first integral membrane protein to be crystallized is a part of the photosynthetic machinery of a non-oxygenic photosynthetic bacterium. The crystallization permitted detailed structural analysis. As an unabashed plant chauvinist, I would take the position that this work was most important because inferences about the structure of part of the photosynthetic machinery ("Photosystem II") of plants could be drawn from this bacterium's photosynthetic apparatus. This work was recognized by a 1988 Nobel Prize (to Michel and Deisenhofer, of München). (A not-so-subliminal message is that advances in understanding often rely on technical advances--in this case, protein crystallization. You will recall the importance of the patch-clamp technique to investigations of membrane transport. This work, too, was recognized by a Nobel Prize (to Neher, of Göttingen).)

[2]The irony is that the metric system stems from the French revolutionists who took Lavoisier's head in 1794.

[3]Over the years, FSU has produced valuable contributions in a number of areas of plant biology. By way of example, Wiese was a pioneering researcher on *Chlamydomonas*, Menzel was a renown cotton geneticist; if I can presume to be able to judge, Godfrey was the most knowledgeable field botanist I have walked with, with some reservations held out for Wilber Duncan (of the University of Georgia). Friedmann was a leading expert on photosynthetic microorganisms that are adapted to cold and dry environments. It is, therefore, with no intent to diminish the individual contributions of many former and present colleagues that I obviously have a great deal of respect for that I offer the opinion that perhaps FSU's most lasting legacy to plant biology is in the area of photosynthetic electron transport. There are two bases on which I offer this opinion. First, one may gauge roughly the importance of a scientific work by the number of times that other scientists refer to it in their publications. The Institute of Scientific Information keeps a running tab; a paper from Homann's lab has been designated a "citation classic." (Briefly, a number of other factors must be considered in addition to the raw number of citations. E.g., guard-cell biochemistry is the focus of no more than, say, 25 labs world wide; regardless of its quality or impact, no paper in this area will be cited very frequently. On the other hand, an average paper on the modification of a widely used technique or an average paper in a "popular" area of research, such as HIV, may be widely cited. Second, a university can be judged by the success of its students.

few "old" experiments that provide the foundation for modern inquiry will be described. Third, the pigments responsible for the initial events--absorption of light--will be covered. Fourth, four major protein complexes of photosynthesis ((1) the **o**xygen **e**volving **c**omplex [OEC] + **p**hotosystem **II** [PSII][4], (2) **p**hotosystem **I** [PSI], (3) the cytochrome **b_6-f complex** [b-f complex], and (4) the ATP synthase [ATPase] will be described.

Chloroplasts are but one type of the generalized organelle, the plastid. Proplastids (in meristematic cells and give rise to other types of plastids), amyloplasts (starch-storing, often in storage tissue), leucoplasts (colorless, synthesize essential oils), etioplasts ("abnormal," represent an arrested stage when the proplast does not develop into a chloroplast) and chromoplasts (colored, in fruits and flowers) are other types of plastids. Plastids in plants have two surrounding membranes, ribosomes, a small genome, are self-replicating, and semi-autonomous. One type of plastid may convert to another type of plastid. Plastids in various protista display a range of characteristics that often distinguish them from plant chloroplasts.

As mentioned above, we will focus only on the photosynthetic properties of plastids, but be aware that they are involved in other essential functions also, such as being the sole site of synthesis of fatty acids (required for membranes) and for the reduction of nitrite (formed from nitrate in the cytosol).

Overhead: General chloroplast structure

The cartoon at bottom depicts a "cut-away" chloroplast view. The outer envelope, as you know, is permeable to metabolites and even small proteins up to 10kD that pass through nonselective pore proteins. Although it is beyond the scope of this course, nuclear-encoded cytosolically translated proteins are imported into the plastid. Special proteins facilitate this transport; in doing so, they form the only contact points of the inner and outer envelope. It is also beyond the scope of this course, but consider that the imported proteins must be targeted to particular locations, such as the stroma, thylakoid membrane and so forth. We focused on the transporters of the inner envelope of the chloroplast, which is continuous with the extensive membranous system of the interior, a point that we will return to later. As we discussed before, the aqueous interior of the chloroplast is the stroma, the site of the RPPP. Photosynthetic electron

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Without elaboration, FSU alumni Melis and Theg are professors at the University of California (Berkeley and Davis, respectively).

[4]Please do not infer that the OEC and PSII are distinct entities. As will be discussed later, the OEC has no bacterial counterpart, whereas the "remainder" of PSII does.

transport occurs in (or across) the thylakoid membranes of the interior. These membranes exist as sheets (like the ER), and as the cartoon shows, they enclose a lumen ("cavity"). By way of review, then, there are three aqueous spaces: (1) the space between the envelope membranes, (2) the stroma, and (3) the thylakoid lumen. As you will see, the thylakoid membranes not only harvest light, but they separate two regions in which the electrochemical potentials of H^+ differ considerably as a consequence of electron transport. At this point, please note that there is considerable organization in the interior membranes: Some sheets traverse the stroma, ("stromal lamella") and are not associated with others. Some of the membranes form stacks; stacks of thylakoids are called grana (sing. granum—from the grainy appearance in the light microscope).

A preliminary interpretation of the ultrastructure of the inner membranes is given in the center panel. There are two points that I would like for you to take from this diagram: (1) separate grana are continuous by way of the stromal lamellae, and (2) membranous regions that are "appressed" (i.e., in contact with other membranes) can be distinguished from non-appressed regions.

Now, the center and the top panels need to be considered as a unit. Consider the center diagram a portion of an entire system in an intact chloroplast that is in an intact cell. Imagine that the living tissue sample is quickly frozen (to quench metabolism, to avoid structural changes, to avoid diffusion).[5] As a later step, application of a force against the tissue will fracture it; thus, the straightforward name for this procedure is freeze-fracture. The tissue will fracture unevenly along planes of least resistance, which is on membrane:aqueous-space interface (surfaces of membrane) or in the bilayer itself. The darkened region in the center diagram depicts fracture planes. After suitable methods to enhance visualization in the scanning electron microscope, the fractured surfaces can be examined. The top panel is an electron micrograph. The topography can be summarized: some membrane surfaces have small bumps (protein complexes), whereas other membranes are enriched with large protein complexes. Quantitative microscopy has also shown that some membranes (e.g., of the outer envelope) have few proteins, and other membranes (e.g., of the inner envelope) have many more. Corroboratively, the results obtained from the physical isolation of membranes (see below) show that some membranes are diverse in their protein complement, whereas others (particularly, the outer envelope) have a few major proteins. Thus, just as we can distinguish gross

[5]Depending on the purpose, different freezing methods are used. Often, simply submersion in liquid N_2 (-196° C) is sufficient. Detailed structural studies require more demanding methods that result in faster freezing (in the range of ms). One approach is to "blast" a thin tissue sample with a stream of very cold liquid propane.

morphological differences (granal vs. stromal lamellae), we see that there is a difference in protein complement as well; any model of photosynthesis must accommodate this spatial heterogeneity.

The preliminary interpretation of the membrane organization in the previous overhead does not accord with the observation that grana can unstack.

Overhead: Membrane organization

A different model, such as the one on this overhead, must be presented. This model provides for regions of membrane appression and non-appression and is consistent with the fact that grana need not be permanent inalterable structures.

As mentioned in the introduction, we will discuss four major components of the photosynthetic electron transport system. These components are shown as the different symbols keyed in the upper right hand corner of the diagram. Two techniques (freeze-fracture and two-phase polymer systems that enable investigators to isolate physically very similar biological structures [such as the different chloroplast membranes or the cis vs. trans faces of Golgi] have shown that the four components are not equally distributed through out the membranes. To facilitate our later discussions[6], I would ask you to commit to memory now: (1) The ATPase and PSI are localized exclusively in the non-appressed regions. (2) PSII is localized mostly in the appressed regions. (3) The b-f complex is abundant in both the appressed and non-appressed regions.[7]

Not only the protein complement, but also the lipid constituents of the various cellular membranes are different. Eukaryotic membrane lipids are mostly glycerol derivatives, in which carbons may be represented in the following fashion:

C—[OH or ester bond-RX]

where RX is a carbon chain. Thus, there may be mono-, di-, or triglycerides. Other substitutions are common. Of particular importance is the phospholipid: the OH of the #1 carbon is esterified to Pi. The result is the familiar polar-headed molecule with the two hydrophobic tails. The phosphate ester residue

[6]Although I try to avoid apparent mindless memorization, any pedagogical scheme (structure or biochemistry first?) can be faulted on these grounds.

[7] This interpretation was questioned in 2001 by a leader in the field. As far as I can determine, there is no evidence to support his objections and his new interpretation has not been accepted by scientists working in the field.

itself may be simply protonated, or it may be bonded to other substances such as ethanolamine or choline. In addition to the phospholipid, there is another broad class of glycerol-based membrane lipids—those in which a carbohydrate (e.g., the hexose galactose) is bonded to the #1 carbon. Again, the result is a polar head (the polyhydroxylic nature of carbohydrate and the ester linkages themselves) and two hydrophobic tails. Finally, some membranes contain "neutral" glycerolipids--simply esters of fatty acids. With this background, I will make the point that membrane composition as well as protein complement shows considerable variation among the membranes of plants, with the focus on the uniqueness of the chloroplast membranes.

Table 1. Distribution of glycerolipids in membranes of a plant cell.

MEMBRANE	PHOSPHOLIPID	GALACTOLIPID	NEURAL LIPID
plasma	46	18	36
mitochondrial	98	2	-
chlpt envelope (outer)	56	44	-
chlpt envelope (inner)	20	80	-
chlpt lamella	17	83	-
nuclear	58	13	29
microsomal	67	13	20

This table provides the basis for the following conclusions: (1) the endomembrane lipids (plasma, nuclear, microsomal≡ER) show general similarities, and (2) the chloroplast inner envelope and lamella membrane lipids are broadly similar, but these are different from the outer envelope. Thus, the functionally and evolutionarily related membranes are most similar in lipid content.

The second topic of this lecture provides for a historical overview of some of the important observations that provide a foundation for our current understanding.

Overhead: The Engelmann experiment

As discussed in the stomatal physiology lecture, a first basic question to ask in the elucidation of the mechanism of a light-driven process is to determine the quality (color, or wavelength[8]) and quantity (overall energy) of the incident radiation required to drive the process. Brilliant in its simplicity, the Engelmann experiment, conducted in the 1880's, provided an answer for the first question. The fresh-

[8]You may want to pause here and review the concept of light as an electromagnetic wave packaged in discrete units (photons), the energy of which can be described by wavelength, or its reciprocal, frequency. Your textbook for this course, as well as supplemental material from previous courses, would be an easily accessible resource.

water filamentous green alga *Spirogyra* has a single spiral chloroplast. (As we have emphasized so many times before, the proper choice of experimental organism is all-important. For the experimental results to be unambiguously interpretable, the capacity to carry out photosynthesis must have been uniform—clusters of chloroplasts or spaces between chloroplasts would be been fatal to the simple demonstration he provided.) Certain bacteria are aerotactic; that is, they move toward a region of high oxygen concentration. With the knowledge that light drives O_2 evolution, he separated white light by use of a prism into a continuum of colored light, with which he illuminated the algal filament. His observation under the microscope revealed that bacteria congregated especially on the filament where it was illuminated by blue (ca. 450 nm) and red (ca. 670 nm) light. As far as I am aware, this was the first action spectrum taken of photosynthesis. The implication was that the pigment responsible for absorbing the light that drives O_2 evolution must have an absorption spectrum similar to the action spectrum that he obtained.[9]

In the 1920's, C.B. van Niel provided an important early insight into the nature of the chemical mechanism of photosynthesis. By the end of the 19th century, the overall process of photosynthesis had been described:

$$nCO_2 + nH_2O + \text{light} \rightarrow (CH_2O)n + nO_2$$

where (CH_2O) represents a carbon reduced to the level of carbohydrate. van Niel recognized that the preceding equation was stoichiometrically identical to this equation:

$$nCO_2 + 2\,nH_2O + \text{light} \rightarrow (CH_2O)n + nO_2 + nH_2O$$

and he saw the similarity between this equation for higher plant photosynthesis and photosynthesis carried out by some bacteria, thus:

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[9]Despite the development of sophisticated instrumentation, simple approaches like that used by Engelmann still have application. By way of example, Rüdiger Hampp of the Karls-Eberhart University has used aerotactic bacteria to examine the viability of single plant cells. In brief, after certain manipulations, the intactness of cells may be called into question. A low rate of photosynthetic oxygen evolution may stem from some cells that are conducting photosynthesis at a normal rate being mixed with other cells incapable of evolving oxygen. Else, the suspension may comprise cells that are uniformly depressed in the ability to evolve oxygen. By mixing aerotactic bacteria with an illuminated cell suspension, one can easily determine with a microscope which of these two possibilities obtain.

$$nCO_2 + 2nH_2S + light \rightarrow (CH_2O)n + 2nS + nH_2O$$

He inferred correctly that CO_2 was not the source of the evolved O_2, or alternatively said, the initial oxidation-reduction reactions did not involve CO_2. In other words, the conversion of light energy to biosynthesis using CO_2 is not obligatory.

Shortly afterwards, Robin Hill showed that a variety of substances stimulated photosynthetic oxygen evolution (by acting as electron acceptors). Thus, any substance that accepts H_2O-derived electrons from the photosynthetic electron transport chain is termed a Hill reagent, and oxygen evolution is called the Hill reaction. Clearly, one set of reactions (which we now call photosynthetic electron transport) harvests light and produces an intermediate reductant (which we now know is NADPH) and another set of reactions (the RPPP) uses the reductant to reduce CO_2. Of course, CO_2 is the common natural Hill reagent. (Nitrite, mentioned earlier, is also a Hill reagent because the reduction nitrite to the level of ammonia requires a reductant, with the electrons ultimately coming from water.)

With some qualifications[10], one would expect concordance between an action spectrum for photosynthesis and an absorption spectrum of the molecule that drives the process, chlorophyll.

Overhead: Emerson enhancement effect

In the 1950's, Emerson studied the relationship between the *in vivo* absorption of chlorophyll and the action spectrum of photosynthesis. As the overhead shows, chlorophyll absorption is still relatively

[10]First, in whole-leaf photosynthesis, a number of pigments—not just chlorophyll—harvest light. Thus, the action spectrum is a summation of all the light harvesting carried out by the different pigments. Second, to a certain extent, light that enters the leaf is trapped there, because the internal leaf surfaces reflect light. Because chlorophyll absorbs light only weakly in the green portion of the spectrum, the internal light is somewhat enriched with green (because blue and red light is strongly absorbed). To simplify (maybe too much), blue and red get fewer passes through chlorophyll before they are absorbed, but green--getting more "opportunities"--will still be absorbed to a limited extent. Third, the exact absorption spectrum by a molecular species will depend on the chemical environment. E.g., there are modest differences in the absorption by different types of chlorophyll (a topic we will cover later), and even a single type of chlorophyll will absorb slightly differently depending on whether it is solubilized in ether, pyridine, acetone, ethanol . . . complexes with protein in a lipid bilayer.

high at λ>680 nm, but that absorbed far-red light is not efficiently[11] used for O_2 evolution.[12] This phenomenon is known as the far-red drop. He and his group discovered that concomitant illumination with a shorter wavelength light increases the efficiency of the far-red illumination. This synergism—far-red plus shorter wavelength light gave a rate of O_2 evolution greater than the sum of far-red alone plus shorter wavelength alone—has come to be known as the Emerson Enhancement Effect. Although it was not interpreted as such right away, it provides the first evidence for two photosystems acting cooperatively in the transfer of electrons from H_2O to a stable reductant, as illustrated sketchily on the bottom portion of the overhead. (PSII and PSI, recall, are two of the four protein complexes that we will cover in the final portion of this lecture.) The idea of two photosystems was proposed in 1960, after it was discovered that intermediates (b-f complex, a third of the four complexes) that we now know shuttle electrons between the photosystems would either be oxidized or reduced, depending on the wavelength of light applied.

A final historical question regards the stoichiometry of electron transport. The oxidation of 2 H_2Os to 1 O_2 is a four-electron process. Usual biochemical oxidation/reduction reactions involve one or two electrons. This situation implies that there must be a mechanism to "store" electrons. The following overhead is the widely accepted Kok model (1970) that provides as explanation of the Joliot (1960's) data depicted above it.

Overhead: The S-state model of photosynthetic oxygen evolution

The oxidation/reduction of chlorophyll (see later) is a one-electron process. As will be explained, absorption of light "excites" chlorophyll, in which state it is easily oxidized. An electron donor must replace the electron lost in the oxidation. (When this donor gives up the electron, it, in turn, must be reduced by a donor . . . which, ultimately, is water.)

The data show that one brief flash of light (just long enough for one photochemical event in each of the two photosystems) results in no O_2 evolution. A second flash of light also causes no O_2 evolution. On the third flash, however, there is oxygen production. Again, on the fourth flash, there is no oxygen

[11]As we will discuss later, absorbed light energy drives "useful" processes, like the photochemistry that leads to the ultimate production of a stable reductant (NADPH). However, absorbed light energy may be dissipated in other "wasteful" ways.

[12]The discrepancy in the 450-550 nm region is caused by the absorption of light by carotenoids, as will be discussed in the next section of the lecture. For the moment, ignore this "noise."

production. In such an experiment, there was O_2 evolution only every fourth flash. (Eventually, of course, the pattern dampened because occasionally some individual photosystems would absorb two photons or no light at all.) These data provide the interpretation that the OEC ("on" PSII) exists in five states. The S_1-state is most stable. In darkness, S_2 and S_3 revert to the S_1-state, and, in all conditions, S_4 spontaneously "decays" to S_0, another stable state. In darkness, however, OECs are in the S_1-state, being three flashes away from O_2 evolution. In brief summary, evolution of 1 O_2, as we have said so often, requires the absorption of 8 photons—each photosystem absorbs one photon for each electron "transported" from water to $NADP^+$.

The third topic of this lecture is a brief description of the light-absorbing pigments, with the primary focus on chlorophyll.

Overhead: photosynthetic pigments

The primary pigment involved in photosynthetic light absorption is chlorophyll. In higher plants, there are two versions of chlorophyll, chl a and chl b. The ratio a/b is in the range of 2.5-4.5, with the higher values being typical of C_4 plants[13]. Chl a and Chl b are almost identical—at the "top" (as shown) of the ring structure, Chl a has a methyl functional group that on Chl b is an aldehyde[14]. Although we draw many molecules with an alternating double bond system (conjugated system of bonds), we recognize that the bonding is not simply "single-double-single-double . . . ," instead, all the bonds in the system have a partial-double-bond nature, with electrons being "shared through out." Such a conjugated system of bonds is typical of pigments that absorb visible light (e.g., hemoglobin and the cytochromes). Chlorophyll (and the parenthetical referenced compounds) has a similar four-ring structure.[15] In chlorophyll, the N atoms are coordinated with a Mg atom. The ring-structure of chl is planar, whereas the phytol tail—note easily its derivation from isoprenoid units—should not be restricted in movement.

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[13]Chl b is preferentially associated with PS II, and C_4 plants are somewhat enriched with PS I.

[14]All oxygenic organisms contain the pigment chl a. These are all photosynthetic eukaryotes and the cyanobacteria. All plants, green algae, and cyanobacteria in the genus *Prochloron* contain Chl b. Some protists contain different chlorophylls.

[15]The "inward-facing" N atoms of the cytochromes are coordinated with an atom of Fe^{2+} (reduced state) or Fe^{3+} (oxidized) state. They are electron carriers and function variously in biological systems. Our focus, on photosynthetic electron transport, will be on their use as electron carriers that shuttle electrons from PSII to PSI (b-f complex). At this point, it useful for you to take note of the fact that some substances, like cytochrome, are oxidized and reduced simply

This overhead also shows the pigment β-carotene, which is one type of carotenoid. (The other type, xanthophylls, is very similar in structure[16], just with the end rings being oxidized.) Earth without carotenoids would be dull; these pigments impart color to fruits, flowers, fungi, birds' feathers, fish scales. The carotenoids that we see are in the minority. Most carotenoids occur in green leaves, where the abundant chlorophyll there masks them[17].

We will turn now to two aspects of these pigments, first, their physical organization, and second, their light-harvesting and energy transducing attributes.

Overhead: Overall pigment distribution in PSII (highly schematic)

N.B.: Do not infer structural relationships from this figure. It (and the following one) is intended to make your understanding of the distribution of these pigments easy to grasp. For both PSII and PSI, structural diagrams will be provided later.

A photosystem, as Thornber simplifies, may be thought of as a (a) a reaction-center pigment, which is, in essence, chl that becomes excited by light (indirectly), and which—in the "excited state"—can be easily oxidized (i.e., it can reduce another chemical) + (b) a core complex that houses the reaction-center pigment plus other components of the redox "chain" and some light-harvesting chlorophyll molecules (core-complex antenna) + (c) light-harvesting pigment complexes (e.g., **L**ight-**H**arvesting **C**omplex (PS**I**) —LHCI). The bulk of all the pigments is in these peripheral antenna complexes or LHCs.

An important principle is that the pigments (both the chlorophylls and carotenoids) do not simply float around in the membrane. They are bound specifically in protein complexes of which about 15 have been identified. By way of example, an important chlorophyll-binding protein in PSII (see overhead) is CP47 (**c**hlorophyll-**p**rotein with a Mr of **47** KD). Pairs of His, separated by 12-13 residues (4 turns of an α-helix), bind pairs of chlorophyll molecules—one chlorophyll toward the cytoplasmic side and the other

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by electrons. Others that we will discuss later involve an H-atom (i.e., a H^+ in addition to the electron). Yet others, like $NADP^+ \rightarrow$NADPH, involve an unequal number of electrons and protons.

[16]You need not be concerned (for testing), but it *appears* that carotenes are found solely or predominantly in certain regions (core complexes) and the xanthophylls are found solely or predominantly in other regions (the LHCs).

[17] Our focus will be on the role of carotenoids in harvesting light. However, they have other roles. Plants sometimes absorb too much light, and a cycle based on the conversion of carotenoids dissipates this excess energy (as discussed later). In addition, these compounds are the building blocks for other important substances, such as ABA. Finally, one lab has proposed that they are the blue-light receptor in some tissues.

toward the lumenal side of the membrane. This arrangement holds for CP43 and for LHCII. In some cases, Asn may be a functional homolog of His.

In both photosystems, chl a is found in both the complex antennae and in the peripheral antenna (LHCs). However, chl b is found only in the LHCs. In PSII, there are two reaction-center proteins; depending on the wash stringency, these two proteins (usually called D1 and D2) have 4-6 molecules of a chl a (P_{680}) that are in a special environment, and as the name implies, absorb maximally at 680 nm. Although it may not mean too much to you now, please remember that P_{680} is the "reaction-center pigment," a concept briefly alluded to above. The two core-complex chlorophyll-binding proteins in PSII are, as mentioned, CP47 and CP43. These each bind to about 40 chl a molecules, which is about 5% of the total chlorophyll[18] There are three kinds of LHCs that are associated with PSII. LHCIIα represents about 7 % of total chlorophyll, and has an a/b ratio of 2.5. LHCIIγ, of uncertain abundance, has a very low a/b, 1. The bulk of chlorophyll—40-50% of the total chlorophyll in a leaf—is associated with LHCIIβ[19]. This complex is very special: It can "collect" light energy and channel that energy to PSII, as shown and as the name implies, or it may migrate to PSI, where it can dock and transfer the energy to one of the LHCIs. In this way, the two photosystems—acting serially—can be balanced[20].

Carotenoids are present in all protein-pigment complexes of PSII (with the possible exception of LHCIIγ).

Overhead: Overall pigment distribution in PSI (highly schematic)

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[18]Both the number of bound chlorophylls and the %-representation given in this and the following overhead I derived for several sources. You will note on close examination that some minor discrepancies, owing to the different sources, have crept in. Thus, my intent is to give you an overall, and not a precise, image. E.g., I have "assigned" 40 chl a each to CP 43 and CP47, but another source indicates that together they contain 40-50 chl a. Thus, use these numbers in a general way.

[19]Formerly, LHC a/b. This nomenclature is continuing to evolve.

[20]As learned from the Emerson Enhancement Effect, the two photosystems preferentially absorb light at slightly different wavelengths. PSI (with P_{700}) is able to utilize light of less energy (longer wavelength); it has a relatively low abundance of chl b (excitation maximum in the red of 650 nm) and a relatively high abundance of chl a (excitation maximum in the red of 670 nm). When PSI is not harvesting enough energy, it can not oxidize (gain electrons from) PSII, which renders PSII nonfunctional. On the other hand, when PSII is not harvesting enough light, electrons are not being extracted from H_2O and are thus unavailable for the rereduction of PSI. This (and other causes) demonstrates the need to "redistribute" the light-harvesting apparatus. This redistribution is brought about by the phosphorylation of LHCIIβ; charged thus, this protein moves from the appressed regions of the membranes (where resides PSII) to the stromal lamellae (where resides PSI).

The pigment distribution in PSI is quite unlike that in PSII[21]: There are two core complexes, but only one has antenna chl associated with it. Core Complex I (CCI) binds to approximately 100 chl a molecules (but no carotenoids); CC1-RC has not only reaction-center chlorophyll, but also 12-15 carotenoids. There are four types of LHCIs, and current thinking is that there is one of each type associated with each of the two core-complex proteins. Altogether, the LHCIs bind about the same number of chl molecules as CCI, i.e., 100, which is on the order of 15-20% of the total chlorophyll. LHCI also has bound carotenoids.

The biochemical distributions that I presented above imply that PSII and PSI each act as a (separate) unit in absorbing light. These data were gained through biochemical fractionation techniques (the tricky isolation of membrane complexes with detergents without (presumably) the loss of the pigment). Much earlier, however, physical methods had already given rise to the notion of a photosynthetic unit. In brief, use of controlled illumination and knowledge of the chlorophyll cross-sectional area permitted the conclusion that about 2500 chlorophyll molecules were involved in the evolution of an O_2 molecule. This fact reconciles—in broad terms, please recall the disclaimer—that there are two photosystems, each with nominally 300 molecules of chlorophyll, and each of which must absorb four photons of light (300 x 2 x 4 = 2400). We will now look at how light energy is absorbed, and how any one of many "affiliated" molecules may absorb a photon and pass that energy on to the reaction-center pigment, P_{680} in PSII or P_{700} in PSI.

Overhead: Energy states of chlorophyll

Because of the large number of electronic configurations (which reflects the extensive double-bonding throughout the large molecule), chlorophyll absorbs light over a wide visible wavelength

[21]There are two kinds of known photosystems--PSII is representative of one type, and PSI is representative of the second. Both types are very similar in the early stages of charge separation and stabilization . [As you will see, excited reaction-center chlorophyll becomes oxidized as it reduces a proximal primary electron acceptor. In these early stages (measured in the 10^{-12} second range), the reverse reaction can occur. The primary acceptor passes the gained electron to a more distant (in distance, but also lower in energy) secondary electron acceptor, &c, until the charge separation is stabilized.] One type of photosystem is represented by the water-splitting PSII of plants and by the reaction center of purple bacteria. In this type, two electrons are transported consecutively to reduce a quinone to a hydroquinone (a 2-electron step), which is the "final" bound electron acceptor. In the second type, represented by PSI of plants, and by the reaction center of green sulfur bacteria, electron transfer occurs as a series of one-electron steps; characteristic intermediate redox components are so-called Fe-S centers. The final bound redox component is a one-electron acceptor. In brief summary, both PSII and PSI have bacterial counterparts.

spectrum[22]. Absorption of an energetic blue photon takes chlorophyll from the "ground" state to a highly "excited" state. This highly excited state decays to a lower excited state by heat loss. The lower excited state corresponds to the same state caused by the absorption of a red photon by a ground-state chlorophyll. This lower excited state is the one of interest. The energy represented by the lower excited state may be lost as fluorescence (which, overall, is a process of low magnitude, especially at low light, say, 5% of lower excited state chlorophyll molecules return to ground state by fluorescence). As the preceding discussion caused you to infer, most of the chlorophyll molecules act as antenna to harvest light—they, themselves, are not involved in the light-driven oxidation-reduction reactions that characterize photosynthesis. The bottom panel in this overhead shows that an incoming photon excites an antenna chlorophyll molecule. The excitation energy is transferred by a "random walk"—the drunken sailor paradigm—to another chlorophyll molecule, and so forth, until the excitation energy is transferred to the special reaction-center pigment, which, because of its location (and the energy represented by the excited state) can reduce an electron acceptor; in the process, of course, the chlorophyll becomes oxidized.

Carotenoids have two roles in photosynthesis. They, themselves, harvest light in the roughly 430-530 nm range. Their excitation energy can be passed along to chl a (to push it to the lower excited state). Because they absorb further into the green than do chlorophylls, the contributions to photosynthesis can be significant. E.g., although the carotenoids constitute only 23 (mol) % of the photosynthetic pigments in LHC of lettuce, 43% of photosynthesis in the 390-530 nm range results from carotenoid absorption. A second role of carotenoids is in their protective role against damage caused by light in the presence of oxygen (precisely those conditions that obtain in plants)[23]. In addition to the deactivation processes of chlorophyll that we described (fluorescence plus excitation transfer, photochemistry), a third electronic-state rearrangement (a term I use here for simplicity, but not a formal term; "third" simply refers to its numerical order in our discussion, nothing else) of chlorophyll is possible. This third state, derived from the "lower excited state" that we discussed, can be deactivated by energy transfer to carotenoids, which can lose the energy nondestructively. In the absence of carotenoids, the third state of chlorophyll reacts with O_2, to form a highly reactive species ("excited singlet oxygen") that wreaks havoc on unsaturated fatty

[22]Plant physiologists use the term, **P**hotosynthetically **A**ctive **R**adiation (PAR), which is a count of photons per time unit and area unit in the 400-700 nm range. Implicit in this usage is the fact that plants do use for photosynthesis a wide range of light , and secondly, that absorption of a red photon (low energy) is just as effective for photosynthesis as absorption of a blue photon (high energy). Thus, on an energy basis, blue light is less efficiently used than red light is.

[23]Some commercial herbicides ("weed-killers") work by blocking the biosynthesis of carotenoids. Treated plants bleach (lose chlorophyll) and die.

acids (e.g., of membranes) and a host of other biomolecules. Any formed of the highly reactive species of O_2 is "detoxified" (by conversion to ordinary O_2) by carotenoids. Of the two protective roles of carotenoids, the quantitatively most important during photosynthesis is the deactivation of the third state of chlorophyll, and the lesser role is destruction of singlet oxygen[24].

We turn now, for summary and orientation to the overall process of photosynthetic electron transport, to the fourth and final topic, i.e., a functional description of how three of the four major protein complexes function to transport electrons from H_2O to $NADP^+$.

Overhead: The Z-scheme of photosynthesis

The Z-scheme shown on this overhead is the convenient way that plant photosynthetic electron transport is depicted, but some orientation is required for you to appreciate the information presented. You may think of the X-axis as representing "distance" or position along an electron's path in photosynthesis. Thus, water, the source of electrons, is shown on the far left and NADPH, the final reduced product, is shown on the far right. The Y-axis is an energy axis. The Z-scheme is arranged so that an energetically "downhill" reaction (redox couple) will "fall" in the scheme. The precise term that is used for the y-axis is the so-called "mid-point" potential, which we will not discuss in a rigorous fashion. Chemicals with more negative values (at the top of the scheme) have a tendency to give up electrons (i.e., reduce other substances), whereas those at a lower vertical position have a tendency to take electrons (i.e., oxidize other substances)[25]. As a relevant example, (the unexcited) P_{680} does not have a tendency to become oxidized whilst reducing another substance. However, P_{680}* (the excited state of P_{680} that results from the absorption of a red photon--ca. 45 Kcal mol^{-1}) is at the top of the scale (most negative), which means that it will "readily" give up an electron to any oxidized substance lower [or not much higher] on the scale than it is. E.g., pheophytin (a Mg^{2+}-less chlorophyll) —like all the other components of the "chain" may exist in an oxidized or reduced state. Because of its position on the scale, you know that pheophytin has a tendency to give up an electron, and does not have a tendency to take one. However, the tendency for P_{680}*

[24]Not all researchers would agree with this statement, which is the latest word that I have been able to get.
[25]Thinking of the y-axis as an energy scale, compare the differences in midpoint potential between the redox couples $H_2O/½O_2$ & $NADPH/NADP^+$. Four photoacts are required to reduce $NADP^+$, which is 2x the energy of $P_{680} \rightarrow P_{680}^*$ plus 2x the energy of $P_{700} \rightarrow P_{700}^*$. From this analysis, you can see that about 30% of the red-photon energy absorbed may be used in the reduction of pyridine nucleotide.

to be oxidized is stronger than the tendency of $pheo^+$ (the oxidized form of pheo) to resist reduction. The take-home message is that a loss of G occurs when $P_{680}^* + pheo^+ \rightarrow P_{680}^+ + pheo$.[26]

Now, we are ready to discuss the essence of plant photosynthesis. Absorption of a photon of light by the antenna chl of PSII results in the excitation of the RC pigment. In this excited state, the RCII pigment has a propensity to oxidize, which is coupled to the reduction of pheophytin, which, in turn, reduces Q_A, which, in turn, reduces Q_B[27]. Meanwhile, the oxidized RCII pigment is reduced by the OEC. The excited state of P_{700}—caused by a PSI photon-absorption event—is sufficiently energetic to reduce Ao^+, the reduced form of which reduces . . . . Electron transport from water to $NADP^+$ is called linear (or non-cyclic) electron transport.

The dotted line shows an alternative flow of electrons—not from Fd to NADP, but from the Fd region to the b-f complex region (which, note, is energetically downhill). Light-driven electron flow around PSI is called cyclic electron transport. There are two "energy-conservation sites" in the electron-transport chain. An energy-conservation site is so denoted when the redox activities of the electron-transport chain result in the uptake of H^+ from the stroma and its deposition in the thylakoid lumen. The first site was in the oxidation of water (recall $4\ H^+\ O_2^{-1}$). The second site is upstream of the b-f complex. Thus, the light absorption that drives cyclic flow of electrons contributes to a proton gradient across the membrane, which is used for ADP phosphorylation. The balance of linear:cyclic electron flow will determine the NADPH:ATP output of the photosynthetic electron transport chain. Cyclic electron flow may play only a minor role in the overall process of photosynthesis.

Overhead: Photosystem II[28]

As mentioned previously, both PSII and PSI represent two distinct types of photosystems and each has a bacterial counterpart. That statement holds for the "acceptor" side of PSII (to the right on the Z-

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[26]Recall that $\Delta G << 0$ implies that a reaction "will go to completion," i.e., the concentration of reactants at equilibrium is small. $\Delta G >> 0$ implies that the reaction "will not occur," the concentration of the reactants at equilibrium is large. ΔG of about 0 means that a substantial amount of reactant and product both remain at the equilibrium.

[27] The herbicide diuron (DCMU) works by blocking electron flow between Q_A and Q_B. This is also the site blocked by another herbicide, atrazine (the most widely used herbicide in the U.S.).

[28]Whereas the pigment distributions in PSII and PSI were highly schematic, as was, to a lesser extent, the Z-scheme, this model of PSII and the following one of PSI are intended to more-or-less portray spatial relationships as we currently understand them. N.B.: Some components await discovery perhaps, and others await assignment of function, so these models should be interpreted as provisional.

scheme), but does not hold for the "donor" side of PSII, since only chl a-containing organisms extract electrons from water. We will briefly examine PSII in two parts; first, we will cover the OEC and then the remainder of PSII.

The direct electron donor to P_{680}^+ is Z, a tyrosyl residue on D1[29]. In turn, the one-electron donor Z is reduced by the water-splitting system. As we have mentioned earlier, the OEC stores oxidizing equivalents, the different redox levels being denoted as S-states. Most researchers agree that there are 4 Mn^{2+} ions per PSII complex and that Mn^{2+} undergoes redox changes. Perhaps the four redox states of the OEC are accounted for by Mn^{2+} redox, or one of the state changes may involve the redox of an amino acid residue, His. Although this figure does not portray it exactly thus, most researchers favor the concept that D1 and D2 are the coordinating ligands for the Mn^{2+}s. This implies that D1 and D2 constitute the water-oxidizing enzyme, but an alternative model is that the water-oxidizing enzyme is distinct from D1 and D2—perhaps CP47 has this additional role or some other of the polypeptides of PSII carry out this function[30].

The overall function of a photosystem is to separate charge. As you imagine, P_{680} is located near pheo. As mentioned, P_{680}* reduces (within about 3 ps) $pheo^+$, the oxidized form of pheo. Since this reaction is a relatively small energy change (see the Z-scheme), the back reaction can occur. However, if pheo reduces Q_A, (within about 300 ps), and Q_A, Q_B (within another 100 ps) . . . , there is a large free energy difference between that and P_{680}+, so that overall back reaction will not occur. Thus, the light energy has been utilized to drive a stable redox reaction. At the expense of redundancy, the transfer of the electron to Q_B constitutes the irreversible charge separation.

Q_A is a tightly bound quinone that undergoes a single-electron redox reaction. (Quinone + 1 electron $\rightarrow$ semiquinone). Q_B is a loosely bound quinone that undergoes a two-electron redox reaction. (Quinone + 1 electron $\rightarrow$ semiquinone; semiquinone + 1 electron $\rightarrow$ hydroquinone). Whereas Q_A is permanently bound to D2, where it is a single-electron gate, fully reduced Q_B dissociates from D1 and migrates in the hydrocarbon region of the thylakoid membrane. There is an excess of Q_B in the membrane[31], so, as the

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[29]There is a homologous tyr on D2 that shows very slow (hour vs. ms) redox kinetics. D1, but not D2, of this heterodimer complex is regularly being degraded and replaced. We do not know whether this is a mechanism of repairing damage that may result because of photosynthesis or whether it is a mechanism for regulating PSII.

[30]D1 and D2 are analogs of the bacterial proteins, L and M. In the cognate bacteria, the H_2S-oxidizing enzyme **is** distinct from L and M. This would suggest to some that the H_2O-oxidizing enzyme would be expected to be distinct from D1 and D2.

[31]During photosynthesis, a pool of Q that is oxidized would indicate that PSI is "outworking" PSII. On the other hand, if the pool of Q is rather reduced, the indication is that PSI is not keeping up with PSII. Thus, the relative oxidation

reduced molecule migrates away, the binding site on D1 is occupied by another (oxidized) Q_B.[32] In brief summary, two photoacts of PSII yield the PSII "product," fully reduced Q_B.

The reduction of Q_B to the hydroquinone requires not only electrons, but protons, also. These protons are taken up from the stromal side of the membrane. When Q_B is oxidized by a complicated cycle, a total of four (?) H^+s are released into the lumen. This contribution to the ΔpH is the second energy conservation site. Q_B is the first of two mobile carriers that we will discuss.

The b-f complex acts as an inter-photosystem electron carrier. We are not entirely certain of the mechanism of electron and proton flow through this complex, although analogous processes in the similar complexes of bacteria and mitochondria are well described. Because of time pressures, we will not focus on this complex; the "product" is plastocyanin[33], the second diffusible intermediate. Plastocyanin is a small protein (~ 10 KD) that forms the redox connection between b-f complex and PSI.

Overhead: Photosystem I

Having the essence of a photosystem covered in the previous discussion, our attention to PSI will be cursory. Plastocyanin is the direct electron donor to P_{700}^+, formed from the oxidation of P_{700}*. Just as PSII can be considered a light-driven H_2O:Q_B oxidoreductase, PSI can be considered a light-driven plastocyanin:ferredoxin oxidoreductase. The physical separation of reduced ferredoxin on the stromal side of the membrane and oxidized plastocyanin on the lumenal side of the membrane is the final crucial step in charge separation. (As a reminder, PSI is a one-photoact/one electron photosystem, whereas PSII is a two-photoact/two electron system.) Through a series of intermediates, ferredoxin[34] (a one-electron redox protein) is used to reduce $NADP^+$.

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state of Q provides an indication of how well balanced the photosystems are. It is this mechanism of sensing the energy distribution between the photosystems that drives the LHCIIβ to associate with one or the other photosystem, through, as we discussed earlier, phosphorylation (which causes movement out of the region of membrane appression [and PSII]) and dephosphorylation (which allows the complex to move into the region of membrane appression).

[32]At any given time, about 60-70% of the Q_B-binding sites are occupied. In about 30% of the cases, Q_A undergoes the back-reaction because its half-life is fairly short.

[33]Some photosynthetic eukaryotes use a "substitute" instead of plastocyanin.

[34]The oxidation state of ferredoxin provides a gauge of the rate of photosynthesis. When the pool of ferredoxin is mostly reduced (as it is soon after a dark→light transition), some of the electrons are not used for the reduction of $NADP^+$; instead, they reduce proteins called thioredoxins. These proteins, in turn, reduce disulfides on target proteins to sulfhydryls. Notably, Glc 6-P dehydrogenase (the "entry point" to the OPPP) is thus inactivated. The RPPP is thus

In overall (final) summary, 8 photoacts (two per electron, four per photosystem) drives the reduction of 2x $NADP^+$ and the deposition of ~ 8 H^+ in the lumen. The H^+s, 4/ATP, "flow" down an electrochemical potential gradient[35] through the ATPase in the phosphorylation of ADP chemiosmotically. This provides a unit stoichiometry for NADPH:ATP. As you will see in the following section, the stoichiometry requirement for reducing CO_2 is variable (depending the pathway [C_3, etc] and photorespiration). The theoretical requirement is 1 CO_2: 2 NADPH: 3 ATP if the Calvin cycle alone is considered.

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activated (e.g., P-ribulokinase, PGal dehydrogenase, and the well studied P'ases). As we discussed during membrane transport, primary ion pumps can function as either ion pumps or as ATP synthases, depending on the energetics. As there is only a small ΔpH across the thylakoid membranes in darkness, the energetics would favor "wasteful" ATP hydrolysis. It is therefore necessary that this electroenzyme be regulated. One mechanism that is involved in the light-activation of this pump is thioredoxin-mediated.

[35]The large difference in the electrochemical potential of H^+ across the thylakoid membrane owes mostly to the concentration term (>2.5 *p*H units are required to drive ATP synthesis). Although proton movement considered alone is electrogenic, recall that there is a counter-flux of Mg^{2+}.