Scientific Background

Introduction

Higher plants conduct photosynthesis according to the empirical equation

$$2nH_2O + nCO_2 + \text{light} \rightarrow (CH_2O)_n{}^{1} + nH_2O + nO_2$$

But, the process of photosynthesis actually occurs in two distinct phases, viz., the harvesting of light and the reduction of CO_2. We will review both of these processes as well as touching on relevant aspects of carbon oxidation.

You will need to know and understand the fundamentals of photosynthesis (and oxidation processes[2]) in order to design your research project well. Because of the immediate importance of these processes to this course, we will spend about two or three hours reviewing them. The aim is to bring you to about the competence level of an "A" in PCB 2010. It is my experience that one learns more thoroughly when the material is approached from more than one perspective, so you are urged to review your PCB 2010 text and notes. Some of you have had other university exposures to photosynthesis (e.g., in Cell Structure and Function), and you will want to use these resources also. Finally, you are referred to the suggested-reading list in these notes, which is optional.

I will now introduce the phases of photosynthesis.

(a) light harvesting

$2\ H_2O \rightarrow O_2\uparrow + 4\ e^- + 4\ H^+$ (extraction of electrons from water, "splitting water")

$2\ NADP^+ + 4\ e^- + 4\ H^+ \rightarrow 2\ NADPH + 2\ H^+$ (reduction of pyridine nucleotide)

(continued, next page)

$3\ ADP + 3\ Pi \rightarrow 3\ ATP$[3]

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[1]For puposes of discussion, carbohydrate ("CH_2O" = empirical formula) is usually considered the end-product of photosynthesis. *In planta*, myriad products (acids, amino acids, lipids) at various levels of oxidation are formed as transport molecules, storage molecules, and feedstocks for many processes.

[2]You must review! The lectures are predicated on a knowledge of the carbon oxidation pathways that are covered in a prerequisite, PCB 2010. If necessary, review your notes and reread your 2010 text.

[3]There is not a rigidly fixed stoichiometry of NADPH:ATP. I have chosen "3" as a convenience that will be revealed later.

In brief, the energy in absorbed photons of light is used to drive the energetically uphill redox reaction, $H_2O + NADP^+ \rightarrow ½O_2 + NADPH$. As a matter of perspective, this redox couple, in the direction shown, has a $\Delta G° \approx +53$ kcal mol^{-1}. This free-energy change is equivalent to the reduction of NAD^+ to NADH, the oxidation of which in the mitochondrion is coupled, recall, to the phosphorylation of 3 ADPs.

During these so-called light reactions, ATP is also synthesized according to the Mitchell hypothesis. As we will briefly describe later, the $\Delta\mu H^+$ that drives the phosphorylation of ADP in the chloroplast develops as a consequence of the transfer of electrons along the electron-transport "chain" that connects H_2O and $NADP^+$.

(b) carbon fixation and reduction[2]

$CO_2 + 4e^- \rightarrow (CH_2O)$

$2\ NADPH \rightarrow 2\ NADP^+ + 4e^-$

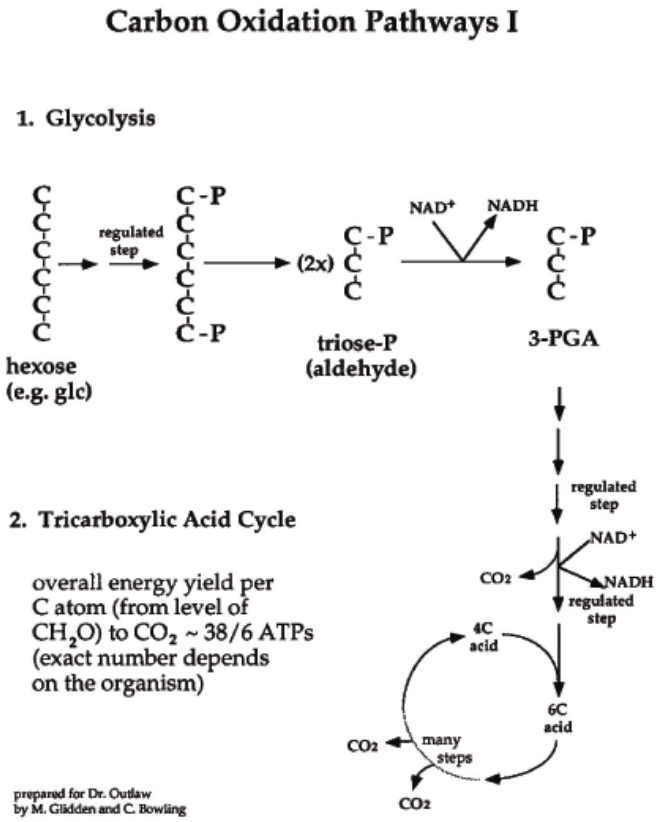
$3\ ATP \rightarrow 3\ ADP + Pi$

During the reactions that accomplish the foregoing steps ("Reductive Pentose Phosphate Pathway" or "Calvin Cycle"), the energetically uphill reduction of the carbon atom of CO_2 is driven by the free-energy loss associated with the oxidation of NADPH. The absolute values (under standard conditions[4]) of these two reactions (oxidation of 2x NADPH and reduction of 1 CO_2) are approximately equal. Energetically, the additional use of ATP "drives" the reaction.

The reduction of carbon cannot be considered in isolation. It is necessary to consider also the oxidation of carbon, which we now review (and will not return to later).

[4]The energetic arguments are intended to be very general. A more complete and accurate description (which we neither are prepared for nor have the time for) would lead to the same conclusion.

Overhead: Carbon oxidation pathways. I Glycolysis and the TCA



This overhead shows an outline of the carbon oxidation pathways that you learned in PCB 2010. These two pathways, glycolysis and the TCA, account for about 80% of carbon oxidation. In addition, they form substrates for other cellular processes; e.g., pyruvate is the immediate precursor of alanine, an amino acid required for protein synthesis.

In brief, consider that the starting substance is glucose (indeed, glycolysis literally means sugar splitting). Two preparatory steps (isomerization and 2x phosphorylation) require an input of energy. **Importantly**, metabolism is regulated. If a cell were a simple sack of unconstrained catalysts and substrates, life would quickly run down. Formation of the bisphosphate (fru 1,6-P_2) constitutes a "commitment" to oxidize carbon. Various biochemical signals are integrated in the "decision" whether to form fru 1,6-P_2. The enzyme that catalyzes fru 1,6-P_2 formation is controlled by "downstream" metabolites, particularly citrate. If citrate is abundant, the overall pathway is "full" and the upstream process should be slowed. Such feed-back inhibition is common. This facet of fru 6-P phosphorylation was well worked out by Oliver Lowry and his colleagues in the late 1960's. As the overall pathway of carbon oxidation had been worked out for a long time, it was a big surprise to learn, courtesy of Heyrs and van Shaftingen, in the early 1980's, that fru 6-P phosphorylation was strongly regulated by the hitherto unknown signal

substance fru 2,6-P_2, which is active at the nmolar level (i.e., as potent as a hormone). We will return to this step later; for the moment, the key point is the notion that metabolism must be regulated, and that one way to regulate metabolism is through the influence of metabolites and signal substances on the rate of enzymic catalysis. Said more generally, the chemical (*p*H, etc.) and physical environment (temperature, compartmentation) regulates flux through pathways.

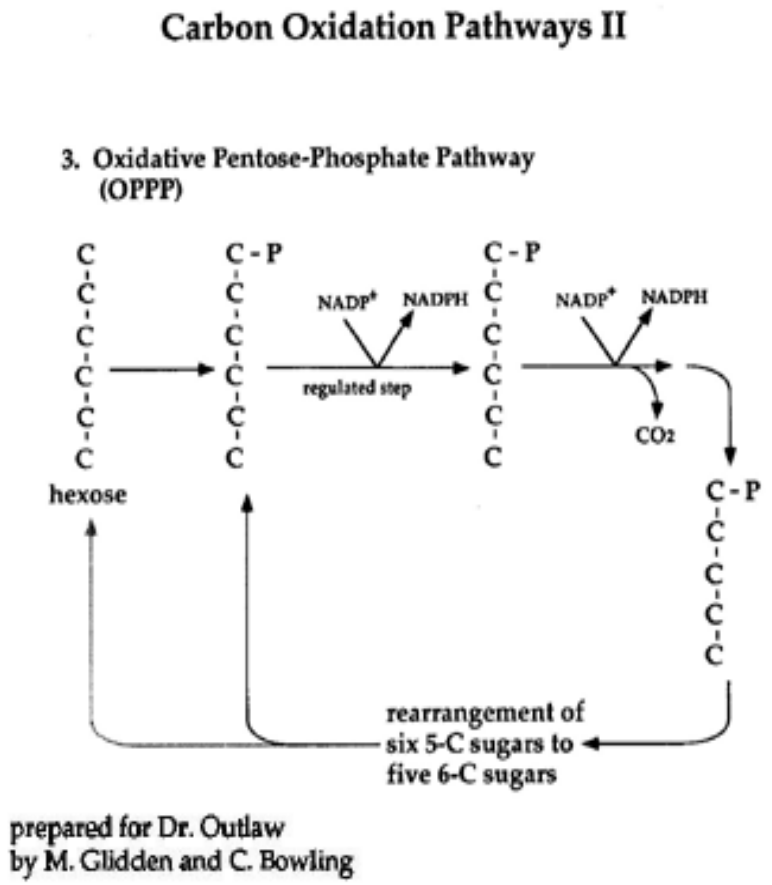
Continuing, fru 1,6-P_2 is split into two 3-C compounds, both still at the oxidation level of carbohydrate. These two 3-C compounds are interconvertible. One of these triose-Ps, **p**hospho**g**lycer**al**dehyde (PGal), is oxidized to **p**hospho**g**lyceric **a**cid (PGA) in two steps. This is **the** oxidative step of glycolysis; the oxidation of PGal[5] is coupled to the reduction of NAD^+. Eventually, PGA is oxidized to CO_2 through the agency of the pyruvate dehydrogenase complex and the TCA.

The energy yield of oxidizing one molecule of glucose to 6 CO_2 is in the range of 34-38 ATPs, depending on the organism. This value is equivalent to about the energy *yield* of respiring in the mitochondrion 12 NADH or 2 NADH/C atom.[6] The free-energy loss associated with the complete combustion of a mole of glucose is 673 kcal mol^{-1}, whereas the energy yield is nominally 250 kcal mol^{-1} glucose (36 mol ATP mol^{-1} glucose x 7 kcal mol^{-1} ATP). An **important** point is that an energy loss is associated with the oxidation of glucose--from a cell's perspective, it "makes no sense" to burn glucose unless there is a compelling demand for energy.

[5]The #3 carbon in PGal is (a) double-bonded to an O atom, which "draws" electrons from the C atom, as O is more electronegative, and (b) single-bonded to a H atom, which is less electronegative than C. The #3 carbon of PGA is bonded to two O atoms. Thus, the #3 C of PGA has a smaller share of electrons than the #3 C atom of PGal; i.e., PGal is more reduced.

[6]In this context, the stoichiometry is fortuitous. However, the reduction of CO_2 to carbohydrate is a four-electron reduction and, thus, would require the oxidation of two two-electron carriers.

Overhead: Carbon oxidation pathways. II the **O**xidative **P**entose **P**hosphate **P**athway (OPPP)



You may not have encountered the OPPP, which accounts for about 20% of carbon oxidation and, as with the other pathways, provides intermediates for other metabolism, notably the phosphopentose that is used in the synthesis of nucleic acid.

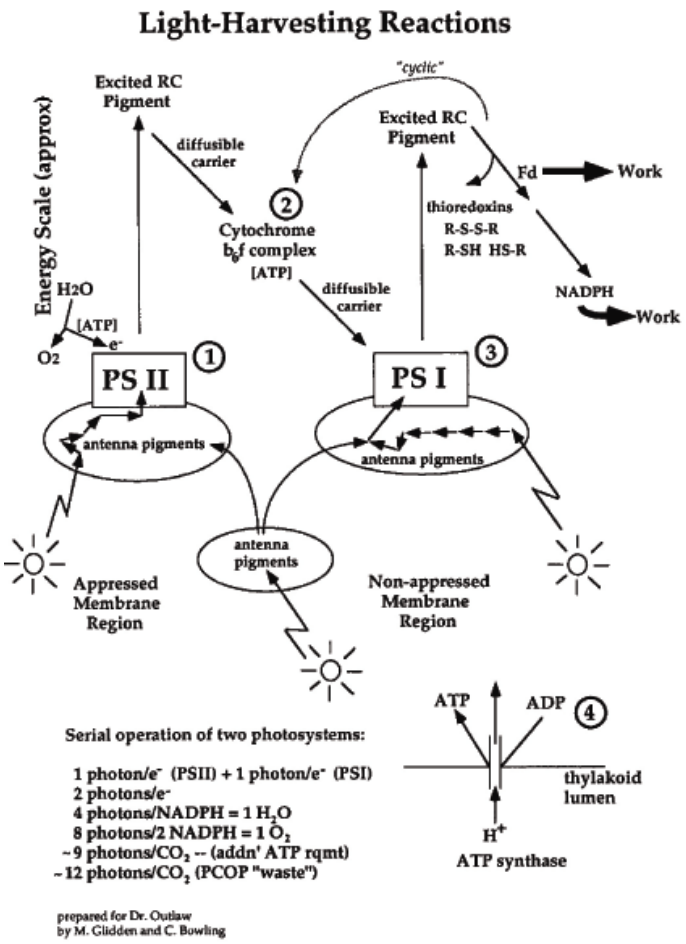
In brief, starting with glucose, a preparatory step (phosphorylation) is followed by two oxidation steps, both coupled to the reduction of $NADP^+$, the same two-electron carrier used in the RPPP. The product of these two oxidation steps is a 5-C sugar phosphate and a CO_2. The remainder of the pathway simply rearranges the 5-C sugar phosphate to the hexose-P (glc 6-P) that "feeds" into the pathway. The rearrangement steps neither consume nor yield energy. Thus, the energy yield of the OPPP is the same as that of glycolysis + TCA, i.e., 2 NADPH/C atom. Although NAD and NADP are to some extent interconvertible, the usual fate of NADPH is utilization in biosynthetic processes and not ATP synthesis.

As in glycolysis + TCA, the "committed" step in the OPPP is regulated. Glucose 6-P dehydrogenase (G6PDH) is reversibly post-translationally modified. The enzyme is relatively inactive if a certain disulfide is reduced to the respective SH functional groups. The S's, located on different parts of the protein, lock the protein into a competent form when a bond bridges the two S's. In the reduced enzyme (R_1-SH + R_2-SH), the protein assumes a noncatalytic conformation. Later, I will show you how--under what

conditions--G6PDH is activated. In summary, in addition to control of pathway flux by chemical and physical environment, post-translational modification of regulated enzymes[7] is an important mechanism.

Light Harvesting (review)

Overhead: Light-Harvesting Reactions



Photosynthesis in higher plants involves four major protein complexes: (a) PSII, including the Oxygen Evolving Complex, (b) PSI, (c) the cytochrome complex, and (d) the ATP synthase. The first three of these act serially in the harvesting of light and utilization of this energy (a) to extract electrons from water and use them to reduce $NADP^+$, and (b) to develop a transthylakoid $\Delta\mu H^+$, mostly ΔpH. The electrochemical potential difference of H^+ across the thylakoid membrane is relaxed through the fourth component, viz., the ATP synthase. This electroenzyme complex converts the energy of

[7]A third mechanism, intuitive, is coarse control by the level of enzyme. This control can be manifested in two ways: (a) through development (e.g., roots have only the small levels of photosynthetic enzymes that would be attributed to a "leaky" promoter) and (b) through expression of temporal patterns (e.g., the key enzyme in the reduction of NO_3^- varies over the course of a day in concert with the level of the mRNA for this protein).

the proton gradient into the bond energy of the terminal phosphoanhydride bond of ATP, at the expense of ADP and Pi.

Incident radiation is absorbed by antenna pigments (in higher plants, chlorophylls or carotenoids) that are complexed with different types of proteins in the membrane. As a matter of perspective, there are on the order of 300 chlorophyll molecules complexed specifically with different proteins that are associated with PSII; roughly the same overall situation exists with PSI, although the arrangement and the complexed proteins are different. Let us say that a photon of light[8] is absorbed by an antenna chlorophyll molecule that is associated with PSII.[9] The absorbed photon causes a change in electronic configuration of the molecules. This higher-energy "excited" molecule transfers its "excitement" to another molecule; this transfer causes the original molecule to return to ground state. The "exciton" moves by random walk through the pigment bed. In the end, a special chlorophyll molecule is excited. This special molecule, the so-called reaction-center pigment (RC pigment), by virtue of its chemical environment and location, may become oxidized by a primary electron acceptor **if** it is excited. In summary:

$chl_1 + light \rightarrow chl_1^*$

$chl_1^* + chl_2 \rightarrow chl_1 + chl_2^*$ *and so forth until chl_n is in the excited state*

$chl_n^* + \text{RC Chl} \rightarrow chl_n + \text{RC Chl}^*$

$\text{RC Chl}^* + \text{primary acceptor}_{oxidized} \rightarrow \text{RC Chl}^+ + \text{primary acceptor}_{reduced}$

$\text{RC Chl}^+ + e^- \text{ (from } H_2O) \rightarrow \text{RC Chl}$ *and the process repeats itself*

The $\text{primary acceptor}_{reduced}$ is oxidized in the process of reducing a mobile electron carrier, which transfers electrons to the cytochrome complex. Through a different diffusible carrier (in higher plants, the Cu-protein plastocyanin) the cytochrome complex is the source of electrons for PSI, just as H_2O is the source of electrons for PSII.

The operation of PSI is conceptually the same as the operation of PSII. Impinging radiation is absorbed by antenna pigments, and the reaction-center pigment becomes excited. In the excited state, the RC Chl can be oxidized by a primary electron acceptor. Electrons are "shuttled" downhill to ferredoxin (Fd), which, in turn, can be used to reduce $NADP^+$. Alternatively, ferredoxin can itself be a source of reducing power. **Importantly**, in the context of this course, when the pool of Fd is substantially reduced, $Fd_{reduced}$ reduces stromal proteins, the thioredoxins, which come in two basic varieties.

The above description is of linear electron transport, aka noncyclic transport, whereby electrons are extracted from water and are used to reduce $NADP^+$, which serves as the

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[8]As you know, chlorophyll absorbs light in the blue (440 nm) and the red (650-690 nm) regions of the spectrum. The shorter wavelengths are more energetic, but photosynthesis proceeds as a quantum process--absorption of a red photon is just as effective in driving photosynthesis as absorption of a blue photon. Because of time demands and lack of direct relevancy to this exercise, we can not discuss the dissipation of the excess energy.

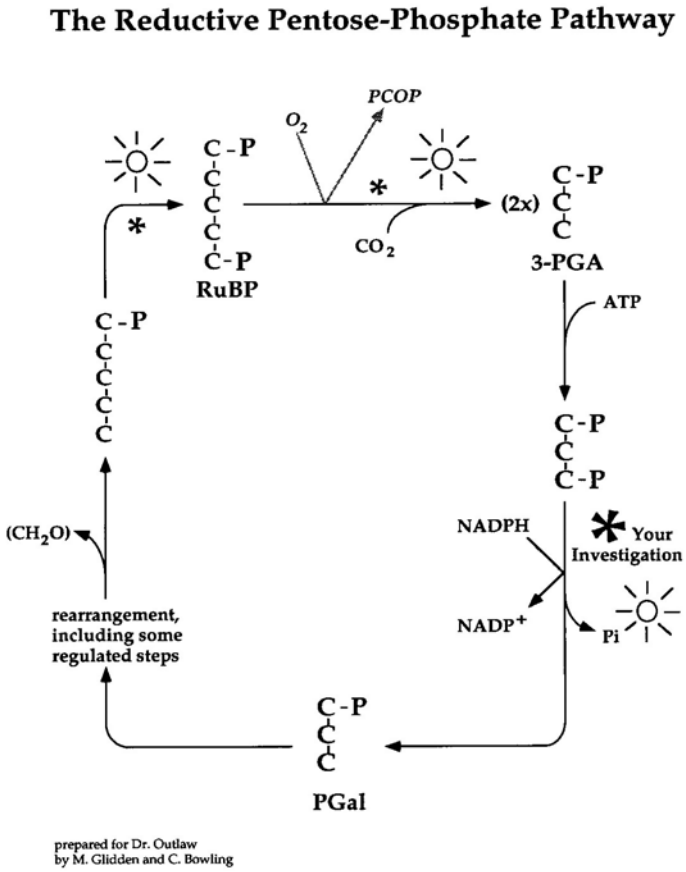
[9]There are various degrees of association. The absorbing pigment may be in a core antenna, a peripheral antenna, or the mobile antenna $LHCII_\beta$.

reductant for the RPPP. As you note on the overhead, in two places, I have placed "[ATP]" to indicate the "positions" along the "chain" that causes lumenal H^+ accumulation. As mentioned, the H^+ electrochemical potential gradient is used to drive ATP synthesis through the agency of the fourth protein complex, the ATP synthase. Linear electron transport would provide for the provision of ATP and NADPH at fixed stoichiometry. Carbon reduction is not the only use to which photosynthetic electron transport is put, and, indeed, the requirements of the RPPP[10] do not exactly match the output of linear electron transport. Shown by the dotted line (and marked "cyclic") on the overhead is a second path of electrons in photosynthesis. Instead of being used to reduce Fd, electrons can be used in a cyclic fashion, feeding into the electron transport chain just "upstream" of the ATP site in the interchain carrier system. Thus, light "pumps" RC Chl to an excited state, from which it is oxidized, but the electron does not go to a terminal acceptor, like $NADP^+$. Like never-ending Monopoly™, each time the electron passes the energy-conservation site, protons are collected in the lumen, and these may be cashed in for ATP via the ATP synthase. Succinctly, the light energy is converted to an ion gradient, which is converted to ATP--nothing more basic in biology.

[10]This statement is correct, if misleading to the sophisticated reader. Inextricably associated with the RPPP is a consequential cycle, the Photosynthetic Carbon Oxidation Pathway (PCOP). The extent to which the latter operates with regard to the former is not fixed. We will not discuss the PCOP because time is of the essence in the coverage of this scientific background. Regardless of the level at which you read these notes, the essential truth--that there is not a fixed ATP:NADPH ratio for the various fates of photosynthetic electron transport--is intact.

The reduction of carbon (review)

Overhead: The reductive pentose-phosphate pathway (RPPP)

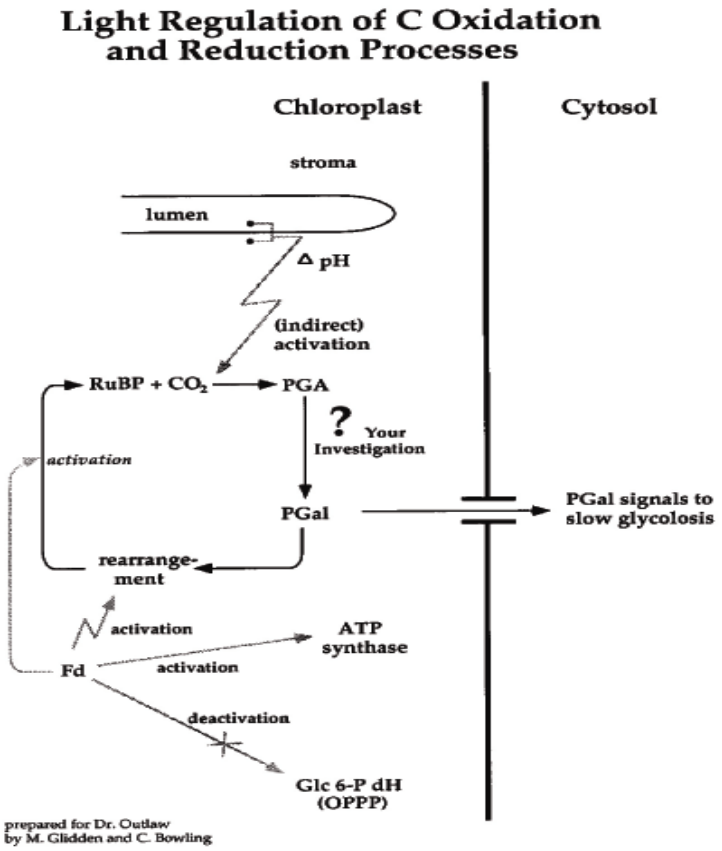


An outline of the RPPP is given on this overhead. The acceptor molecule for CO_2 is the five-carbon carbohydrate RuBP, which is unique to photosynthesis. The fixation of CO_2 is catalyzed by an enzyme dubbed "rubisco," and the products are two identical molecules of 3-**p**hospho**g**lyceric **a**cid (PGA). This step is light activated in complex ways that may involve phosphorylation, that do involve changes in the stromal environment, and that do involve a reversible post-translational modification, carbamylation, that may involve association and dissociation with other proteins.... Light activation is indicated by the sun icon, and the star indicates that rubisco is unique to the RPPP.

Fixation of CO_2 does not constitute overall reduction. RuBP is at the oxidation level of a carbohydrate, CO_2 is the most oxidized form of carbon, and the product PGA is intermediate between CH_2O and CO_2. Reduction of carbon occurs at one place, and at one place only: the step catalyzed by PGal DH, the subject of this course and indicated by the humongous star. This step, as you well know now, is also light activated, a phenomenon that you will investigate. It is, except for pyridine nucleotide specificity, the "reverse" of the oxidative step of glycolysis. PGal is a carbohydrate. Incorporating 1 CO_2 into organic form and reducing back to the level of carbohydrate requires 4 electrons-- two PGAs are formed for each CO_2 incorporated. In addition, there are

requirements for ATP: to regenerate RuBP, to "prep" PGA for reduction. Altogether, the RPPP requirements to fix one CO_2 by the RPPP are 2 NADPH and 3 ATP. Actually, the costs are higher overall because of the associated pathway, the PCOP, alluded to. As an approximation, the costs are about 30% higher. A sweeping summary statement is that the four-electron reduction of CO_2 has a direct energy cost equivalent to approximately 12 ATPs. In sharp contrast, recall, the energy yield of oxidizing one CH_2O to CO_2 is equivalent to only 6 or so ATPs. In other words, a hypothetical plant would burn up 2x the CH_2O in oxidative processes that would be required to build one CH_2O. Obviously, there are control mechanisms.

Overhead: Light regulation of C oxidation and reduction processes



This final overhead provides some integration of the oxidative and reductive processes. From the foregoing discussion, it follows that, in the presence of stable chemical energy formed by the light-harvesting processes, carbon reduction (very expensive) should occur, but carbon oxidation (yielding comparatively little) should not occur. Should the two processes occur, they would constitute a grand futile cycle: CO_2 + large energy$\rightarrow$ CH_2O, at the same time, $CH_2O \rightarrow CO_2$ + small energy.

In brief summary, oxidation of C via the OPPP is avoided in light because the committed-step enzyme is light-deactivated. This deactivation is brought about by $\text{thioredoxin}_{\text{reduced}}$, a condition that prevails only when the pool of Fd is mostly reduced. Fd is mostly reduced only during active photosynthetic electron transport. Thus,

thioredoxin is a signaling link between the oxidation of carbon via the OPPP and a "measurement" of the availability of reducing power from the photosynthetic electron transport chain. *In vitro*, G6PDH can be deactivated by the strong reductant DTT.

Oxidation of carbon via glycolysis[11]is slowed by the export of PGal from the chloroplast. PGal causes a fall in the concentration of fru 2,6-P_2, the signal substance described that speeds glycolysis[12] (which see).

Via the thioredoxin system, light activates the enzyme that catalyzes the synthesis of RuBP and two of the rearrangement steps, which we did not discuss. The thioredoxin system is also involved in the regulation of ATP synthase, which seems to be governed also by the ΔpH across the thylakoid membranes.

In the third overhead (light-harvesting reactions), a mobile light-harvesting antenna was shown. Whether this antenna is docked with PSII or PSI--a means equalizing energy distribution between the serial systems--is a function of the redox state of the hydrophobic mobile carrier that links PSII with the cytochrome complex.

Your task is to describe, or better yet, to discover, the mechanism of light activation of PGal DH. It is light activated, albeit to a limited extent, so your measurements must be careful. The choice of this enzyme lies in its importance (it **is** the reductive step), its historical significance (it was the **first** enzyme to be discovered to be light activated), its simplicity of assay (which permits real-time spectrophotometry). Good luck, and happy hunting.

[11]In fact, there is some question concerning the extent to which glycolysis and the TCA function in light. I am relating the "story" in a qualitative fashion, whereas the truth is probably a description of the extent of light deactivation. Recall that intermediates in all the oxidative pathways are used in other biosynthetic processes, which may impose a requirement during the light.

[12]Apologies, again, for skipping interesting and important details. Fru 2,6-P_2 does not affect the SAME enzyme in plants that it does in animals, but the functional result is the same. As it turns out, plants are more complicated in the processing of carbon than are animals. Plants have alternative steps for phosphorylating fru 6-P, for oxidizing malate (a step in the TCA), and for mitochondrial respiration at the level of cytochrome oxidase.