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Photosynthesis of ATP—Electrons, Proton Pumps, Rotors, and Poise

Minireview

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Light-driven electron transport is coupled to ATP synthesis in chloroplasts. While the nature of the coupling and the structures of key components are now known, there has long been disagreement over pathways of electron transport. Recent results now put an old idea back on the agenda—cyclic electron transport around photosystem I.

Apart from a few ancient chemolithotrophs, which subsist on hydrogen and carbon dioxide, the profusion of life on Earth is supported entirely by radiant energy from the sun. Broadly, the greater the flux of solar energy, the greater the profusion—with tropical forests and coral reefs at one end of the scale, and polar ice caps and tundra at the other. Photosynthesis is the process that converts the energy of sunlight, storing it for use by all living things. Photosynthesis is also responsible for the global redox imbalance now seen as abundant, free oxygen—our planet's signature of life, unique in the solar system. Photosynthesis is light-driven electron transfer, or redox chemistry, and the free oxygen comes from its transfer of electrons from water. An outstanding introduction to photosynthesis has recently been published (Blankenship, 2002).

In plants and algae, which are eukaryotes, photosynthesis takes place in cytoplasmic organelles called chloroplasts. Chloroplasts themselves resemble photosynthetic bacteria and are descended from them. The light-capturing photosystems of chloroplasts and bacteria are proteins that are plugged through internal, energy-transducing membranes called thylakoids. Each photosystem is a complete structural and functional unit that contains two essential components. The primary component is the reaction center, which allows an energized chlorophyll molecule to lose an electron to an acceptor molecule, thus initiating and powering electron transport. The other component of a photosystem is its array of around 300 light-harvesting, or antenna, chlorophylls. These pigment molecules absorb light, and keep the reaction center supplied with excitation energy.

In all oxygenic photosynthetic organisms, two distinct photosystems are connected, in series, within the whole electron transport chain (Hill and Bendall, 1960). This connection is easily visualized if the sequence of electron transfers is plotted on a horizontal scale of redox potential, and the components are joined by lines or arrows. The resulting pathway resembles a letter "Z". The name "Z scheme" is retained even where a clockwise rotation of the diagram produces, instead, the letter "N." The upper line of the "Z" is photosystem I, and the lower line is photosystem II. At the lower right of the "Z," electrons originate from water, and oxygen is produced. The electrons are moved leftward (or upward, which is more intuitive, in the "N") by the reaction centers of photosystems I and II. The photosystems themselves are connected by the oblique line of the intermediary chain. The electrons arrive finally at the upper left of the "Z," where they produce NADPH. Thus, photosystem I receives electrons from photosystem II, and the two photosystems work together to give oxidation of water to oxygen and reduction of a terminal electron acceptor (such as $NADP^+$ to NADPH).

Cycles, Noncycles, and Pseudocycles

Synthesis of ATP in photosynthesis is called photosynthetic phosphorylation (Arnon et al., 1954)—photophosphorylation for short. Cyclic photophosphorylation does not require water oxidation and oxygen evolution, and works with light (symbolized by the jagged, red arrow in Figure 1) of wavelength beyond that required for complete photosynthesis. In contrast, noncyclic photophosphorylation (Arnon et al., 1957) is linked to oxygen evolution, water oxidation, and reduction of an acceptor. With the subsequent discovery of the Z scheme, it became clear that noncyclic photophosphorylation requires both photosystem I and photosystem II, and ATP synthesis is coupled to electron transport between them (Hill and Bendall, 1960). In contrast, cyclic photophosphorylation is driven by photosystem I, which returns electrons to itself through the site of proton translocation and ATP synthesis (Figure 1). It is interesting to ask whether it is the physically the same site of proton translocation, and the same photosystem I, that participates in the cyclic and noncyclic pathways (Albertsson, 2001).

There is a variant of noncyclic photophosphorylation. Molecular oxygen will readily accept electrons from ferredoxin, and then oxygen acts as the terminal electron acceptor, in place of $NADP^+$. When oxygen is thus reduced completely back to water (and not just to an intermediate, such as hydrogen peroxide), then oxygen is consumed by photosystem I as fast as it is produced by photosystem II. This is pseudocyclic electron transport, and pseudocyclic photophosphorylation. In pseudocyclic photophosphorylation, like cyclic photophosphorylation, nothing is finally oxidized or reduced; oxygen is simultaneously evolved and taken up. However, pseudocyclic photophosphorylation is really a kind of noncyclic photophosphorylation, since there is a linear electron path from water to oxygen, and this path incorporates both photosystem I and photosystem II. Therefore pseudocyclic photophosphorylation, like noncyclic photophosphorylation, is sensitive to inhibitors that act on photosystem II, and stops working when the wavelength of light exceeds about 670 nm, the longwave limit of light-harvesting by photosystem II. Photosystem II light ($\lambda < 670$ nm) is indicated by the orange, jagged arrow in Figure 1.

True noncyclic photophosphorylation is clearly the

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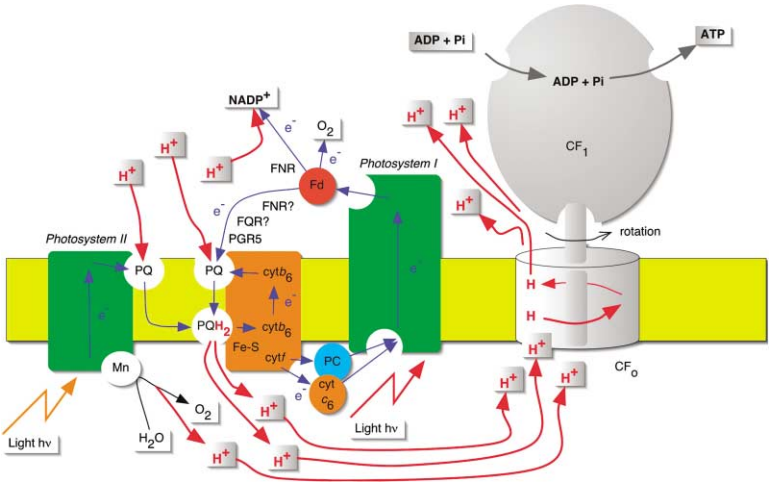

Figure 1. Photosynthesis–From Light to ATP

Electron transport (e^-) (blue) is arranged vectorially in the chloroplast thylakoid membrane (yellow). Proton (H^+) (red) translocation from the chloroplasts stroma (above the membrane) into the lumen (below the membrane) establishes a proton motive force that couples electron transport to ATP synthesis. The implied stoichiometry $3H^+/e^-$ is for noncyclic electron transport alone (cf. Figure 2). FQR is a hypothetical ferredoxin-quinone-oxidoreductase. Other abbreviations as in text. Junge's animations *Rotary ATP Synthase* and *From Light to ATP* are recommended viewing: http://www.biologie.uni-osnabrueck.de/Biophysik/Junge/overheads.html

core of oxygen-evolving photosynthesis, the path from water to NADPH and ATP. Are the cyclic and pseudocyclic alternatives real, or experimental artifacts? This is an old question. Many of the pioneers had definite views, and all possibilities are still defended today. One view is that noncyclic electron transport is coupled to ATP synthesis with a stoichiometry of ATP/NADPH of at least 3/2. This stoichiometry (ATP/NADPH = 3/2) exactly satisfies the predicted demands for ATP and NADPH by carbon dioxide assimilation by the well-established Benson-Calvin cycle. So perhaps cyclic photophosphorylation and pseudocyclic photophosphorylation are unnecessary, and are experimental artifacts. Arnon's view, in contrast, was always that noncyclic photophosphorylation has an ATP/NADPH ratio of unity, and that his lab's original discovery, cyclic photophosphorylation, makes the extra ATP required for carbon dioxide assimilation. Others agree that extra ATP is required, but think it comes from pseudocyclic photophosphorylation —from electron flow to oxygen, where no NADPH is produced. Everyone wants their favorite reaction to take place in the real world. What is the evidence for the existence of cyclic and pseudocyclic photophosphorylation in vivo? One recent clue comes from structural studies on the components of ATP synthesis.

Rotary Motors and the Velocity Ratio of Chemiosmotic Coupling

In photosynthesis, as in respiration, there is a universal intermediate that couples electron transport with ATP synthesis. This intermediate is a transmembrane electrochemical potential gradient of hydrogen ion concentration; that is, the proton motive force of Peter Mitchell's chemiosmotic hypothesis. The proton motive force is generated by vectorial electron and proton transport. In the case of photosynthesis, the reaction center cores of the two photosystems indeed move electrons across the thylakoid membrane (Figure 1). This conclusion is decisively confirmed by their structures, now described at near-atomic resolution, as reviewed by Blankenship (2002) and Heathcote et al. (2002). Connecting the two photosystems, an intramembrane recycling of electrons (the "Q cycle") through the cytochrome b_6-f is also vectorial, and gives a total of four protons translocated for each pair of electrons transferred from photosystem II to photosystem I. Figure 1 incorporates the two photosystems and the Q cycle, and summarizes the mechanistic relationship between photosynthetic electron transport and ATP synthesis as it is currently understood.

How then does the proton motive force make ATP? The key to understanding ATP synthesis is the coupling ATPase, also called ATP synthase. This protein complex has a large, water-soluble, membrane-extrinsic domain with pure ATPase activity. This "coupling factor" is termed F_1 for mitochondria and CF_1 for chloroplasts. Its membrane-intrinsic partner is correspondingly called F_o or CF_o. The subscript "o" denotes binding of an inhibitor, oligomycin.

The X-ray structure (Abrahams et al., 1994) of beef heart mitochondria F_1-ATPase has a three-fold rotational symmetry, with each 120° sector containing a different ligand binding site associated with the catalytic subunit, β. The shape of each site is determined by the asymmetry of the single γ subunit. Rotation of γ within the central axis of F_1 induces sequential changes in the conformation of each of its three $\alpha\beta$ heterodimers. Each heterodimer binds ADP and phosphate loosely; then ADP and phosphate, then ATP, tightly; and, finally, ATP is released from an open site to which ADP and phosphate may bind again. The γ subunit acts as a camshaft, inducing the cycle of conformational changes in each of the $\alpha\beta$ dimers in turn.

In coupled ATP synthesis, what drives rotation of the central γ subunit, thus inducing the changes in conformation and ligand binding? The answer is the membrane-intrinsic (C)F_o (Junge, 1999). In animal mitochondria, F_o is thought to have a twelve-fold rotational symmetry. The inner ring, with twelve proton or hydrogen atom binding sites, is coupled mechanically to F_1-γ. One complete rotation of the F_o ring, and of F_1-γ, must give three ATP molecules, and is driven by translocation of twelve protons across the membrane. The H^+/ATP ratio is therefore 12/3, which is 4. The sense of rotation of the inner ring of F_o and of F_1-γ is counterclockwise, as viewed from the top of Figure 1, during ATP synthesis in both mitochondria and chloroplasts (McCarty et al., 2000).

However, there seems to be an interesting difference

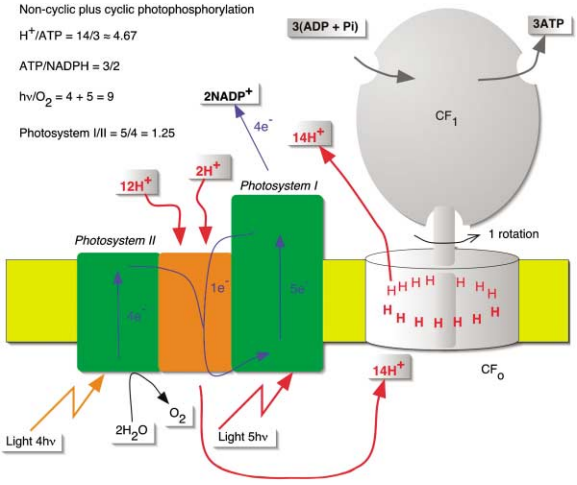

Figure 2. Coupling Quanta, Electrons, Protons, and ATP

Combined cyclic and noncyclic photophosphorylation, assuming $H^+/3ATP = 14$. Stoichiometries are depicted for four electrons transferred from H_2O to $NADP^+$ (cf. Figure 1). This gives one O_2 molecule and two NADPH molecules. Three ATP molecules will be made, provided photosystem I recycles one electron in order to contribute two protons to the proton motive force.

between F_o and CF_o. Seelert and coworkers have shown by atomic force microscopy that spinach chloroplast CF_o has fourteen-fold rotational symmetry (Seelert et al., 2000), not twelve-fold, as generally assumed for animal mitochondrial F_o (though for yeast mitochondria the value is 10). In noncyclic electron transport in chloroplasts, four electrons make one oxygen molecule and two NADPH molecules, and the same four electrons pump a total of 12 protons into the thylakoid lumen from the stroma (Figure 2). Now, if these 12 protons gave one rotation of CF_o and CF_1-γ, as would be expected from mitochondrial F_o and F_1-γ, then synthesis of three molecules of ATP will accompany production of two NADPH molecules, and noncyclic photophosphorylation alone would satisfy the demands of the Benson-Calvin cycle. However, if the twelve protons corresponding to two NADPH drive CF_o through only 0.851 of a rotation, or 308°, with 12/14 steps, then production of two NADPH molecules must accompany synthesis of only $3 \times (12/14)$ ATP molecules, not three. It follows that the true ATP/NADPH ratio of noncyclic phosphorylation is not 3/2, but $3 \times (6/14)$, that is, 9/7, or approximately 1.29. So there is a shortfall of ATP from noncyclic photophosphorylation alone, and something else must account for about 14% of the total ATP required (3 ATP per 2 NADPH) for carbon dioxide fixation in the Benson-Calvin pathway. If this ATP deficit is compensated for by cyclic photophosphorylation, and if cyclic pumps two protons for one electron (through the Q cycle operating alone), then photosystem I must recycle one electron in five. This analysis predicts that there is 20% more photosystem I than photosystem II. This conclusion is in remarkable agreement with recent estimates, based on biochemical, biophysical, and ultrastructural studies (Albertsson, 2001). According to these studies, there are between 14% and 20% more chlorophylls in photosystem I than in photosystem II, and the ratio of photosystem I to photosystem II reaction centers is between 1.1 and 1.2.

Oxygen, State Transitions, and Redox Poise

One distinctive feature of cyclic photophosphorylation is that each electron carrier is both a donor and an acceptor. It follows that cyclic electron transport is zero when its components are completely reduced, since there is nowhere for the electrons to go. Likewise, cyclic electron transport is zero when its components are completely oxidized, since there are no electrons to cycle.

Overoxidation of the cyclic chain can be cured by introduction of electrons as a poising pulse of reductant, or by restricting electron flow out of the chain. Overreduction can be cured by removal of electrons with a poising pulse of oxidant, or by restricting electron flow into the chain (Whatley, 1995). Overreduction is expected when the Benson-Calvin cycle is unable to utilize NADPH, and one reason for this is insufficient ATP. Poising by oxygen, which readily bleeds off surplus electrons, is a plausible function for pseudocyclic electron transport (Heber and Walker, 1992).

In all "Z scheme" photosynthetic organisms (plants, algae, and cyanobacteria), there is a phenomenon called state transitions (Allen and Forsberg, 2001; Finazzi et al., 2002; Wollman, 2001), by which posttranslational modification of light-harvesting, chlorophyll-protein complexes maintains redox poise of the plastoquinone pool between photosystem I and photosystem II. There is also a transcriptional mechanism acting on photosystem reaction center genes in chloroplast DNA that maintains a poised plastoquinone pool (Pfannschmidt et al., 1999). State transitions achieve maximal yield even of purely noncyclic electron transport and photophosphorylation (Allen and Forsberg, 2001). The requirement for a poised photosystem I cyclic chain adds another function for state transitions (Finazzi et al., 2002).

New Ways of Re-Cycling Electrons around Photosystem I—PGR5

Chlorophyll fluorescence emission at room temperature represents light energy that photosystem II has no capacity to convert, so it reemits it, slightly red-shifted. It is possible to image fluorescence with a video camera, and the search is on for interesting tagged mutants that are high in fluorescence. Such mutants must be impaired, in some way, in the conversion of absorbed light energy—they re-emit too great a fraction of this energy as fluorescence. A successful hunt of this type has located a gene for a protein, psbS, which seems to be required for disposing of excess energy at high light intensity (Li et al., 2000). This downregulation of photosystem II produced by psbS involves an interconversion of carotenoids to an excitation-dissipating form—the so-called "xanthophyll cycle" (Li et al., 2000)—and is also linked to a decreased pH in the thylakoid lumen. Cyclic electron flow around photosystem I establishes transthylakoid ΔpH, and may help to dissipate excess energy in photosystem II (Munekage et al., 2002).

Using fluorescence screening with *Arabidopsis thaliana*, a key gene for cyclic electron flow has now been identified by Yuri Munekage and coworkers (Munekage et al., 2002). The predicted protein is termed PGR5, for proton gradient regulation. The *pgr5* mutant phenotype is caused by a single nucleotide substitution, replacing glycine with serine, which results in loss of the novel

PGR5 protein. Several independent lines of evidence show that this loss results in decreased photosystem I cyclic electron transport. For example, cyclic flow of electrons back from the acceptor side of photosystem I to the intermediary carrier, plastoquinone, can also be demonstrated by measurement of chlorophyll fluorescence. When NADPH with ferredoxin is added to chloroplast thylakoids, fluorescence increases, because photosystem II then has fewer electron acceptors available to support the successful conversion of absorbed light energy. This increase in fluorescence upon chemical reduction of plastoquinone is seen in chloroplast thylakoids isolated from wild-type plants, but not to the same extent in thylakoids from the *Arabidopsis pgr5* mutant. Furthermore, this reduction of plastoquinone by the cyclic pathway is usually sensitive to the inhibitor antimycin A. The residual reduction of plastoquinone in the *pgr5* mutant is no longer antimycin A-sensitive. If cyclic photophosphorylation is indeed required for normal photosynthetic growth, then the *pgr5* mutant must have found an alternative to cyclic photophosphorylation as the source of extra ATP. Perhaps the general function of a combined cyclic and noncyclic photophosphorylation is to permit flexibility in the ATP/NADPH ratio, and the *pgr5* mutant is poor at adapting to changed metabolic demand for ATP.

The PGR5 protein is described as a membrane protein, but appears not to be membrane-intrinsic, nor does it have any obvious motif suggesting a redox-active prosthetic group. PGR5 can accumulate independently of the major complexes intrinsic to thylakoid membranes—it does not seem to be an integral part of photosystem I, the cytochrome b_6-*f* complex, or anything else. Munekage et al. suggest a role for the protein in electron transport from ferredoxin-$NADP^+$-oxidoreductase (FNR) (Zhang et al., 2001) to the cytochrome b_6-*f* complex (Munekage et al., 2002). In addition, a cytochrome c_6 is present in *Arabidopsis*, and it is now known to function as an alternative to plastocyanin (PC), the well-established, copper-containing electron donor to photosystem I (Gupta et al., 2002). Chloroplast-encoded NAD(P)H dehydrogenase subunits revealed by plastid genome sequencing may also play some part in cyclic photophosphorylation. Mutants deficient in these subunits have some of the properties of the *pgr5* mutant (Munekage et al., 2002)—electrons do not readily return from photosystem I to plastoquinone. However, Munekage et al. (2002) conclude that "the PGR5-dependent pathway is the main route of cyclic electron flow around photosystem I, while the NDH-mediated pathway may have compensatory functions."

PGR5 puts cyclic electron transport and photophosphorylation firmly on the map, both for ATP synthesis and for protective energy dissipation at high light intensity. Further characterization of this novel protein and its pathway of electron transport must be eagerly awaited. In particular, it is important to know how PGR5 fits and interacts with the beautiful and intricate structures of photosystem I and the proton-motive cytochrome b_6-*f* complex. Clearly, these are interesting times for understanding the inherent flexibility of photophosphorylation. New inputs from structural biology and molecular genetics must be expected to fuel a renaissance in understanding photosynthesis of ATP. The spectacular, recent advances in structure and function of protein complexes must be included in any integrated picture of how photosynthesis works—and adapts—inside the living plant cell.

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