Cyclic, pseudocyclic and noncyclic photophosphorylation: new links in the chain

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Photosynthetic electron transport is coupled to ATP synthesis. This process – photosynthetic phosphorylation – proceeds by several alternative electron-transport pathways in isolated chloroplasts. The question: 'Which of these works in real life?' has long occupied students of photosynthesis. Recent results from structural biology and genomics suggest that the answer is 'All of them'. The interplay between the pathways might explain the flexibility of photosynthesis in meeting different metabolic demands for ATP.

In 1953, the year of the double helix and the conquest of Everest, photosynthesis as electron transfer was gaining ground over the idea of photolysis of carbon dioxide. Light-driven electron transfer – photoelectrochemistry – had been demonstrated independently by Robert Hill, working with chloroplasts isolated from plants, and Cornelius van Niel, working with bacteria. Fritz Lipmann had produced evidence that ATP is a universal energy currency in living cells. Therefore, synthesis of ATP was probably also required for photosynthetic assimilation of carbon dioxide into carbohydrates and other cellular material, including macromolecules. The most plausible scenario, widely accepted until 1953, was that photosynthetic electron transfer generates reduced electron acceptors, and that these are supplied as substrates for respiratory electron transport in mitochondria. Therefore, complete photosynthesis was thought to depend on mitochondrial oxidative phosphorylation to supply the ATP. However, the problem was that leaves are full of chloroplasts but have few mitochondria.

Then Daniel Arnon, Mary Belle Allen and Bob Whatley, in Berkeley, California, showed that isolated chloroplasts are capable of direct, light-dependent synthesis of ATP, a reaction they called photosynthetic phosphorylation [1]. Albert Frenkel then found the same direct, light-driven ATP synthesis in bacterial membranes [2]. The concept of direct coupling of ATP synthesis to absorption of light by chlorophyll (Fig. 1a) suggested a cyclic reaction. The Berkeley group then showed that photophosphorylation in chloroplasts can also be coupled to linear, noncyclic electron transport [3] (Fig. 1b). So, ATP synthesis accompanies light-dependent oxygen evolution, and reduction of an electron acceptor. However, it worried people that there was no obvious, energetically 'downhill' electron flow to do the work. This problem, among others, was solved by the revolutionary 'Z scheme' of Robert Hill and Faye Bendall [4].

The Z scheme (Fig. 2) explains both cyclic and noncyclic reactions [5,6]. Cyclic photophosphorylation involves only chloroplast photosystem I. By contrast, noncyclic photophosphorylation requires both photosystem I and photosystem II, which are linked in series. This linkage produces net oxidation of an electron donor (of water to oxygen) and reduction of $NADP^+$ to NADPH. Both types of electron transfer pass through the 'coupling site' of ATP synthesis (Fig. 2). To complete this classical picture of photosynthesis of ATP, there is just one, apparently trivial,

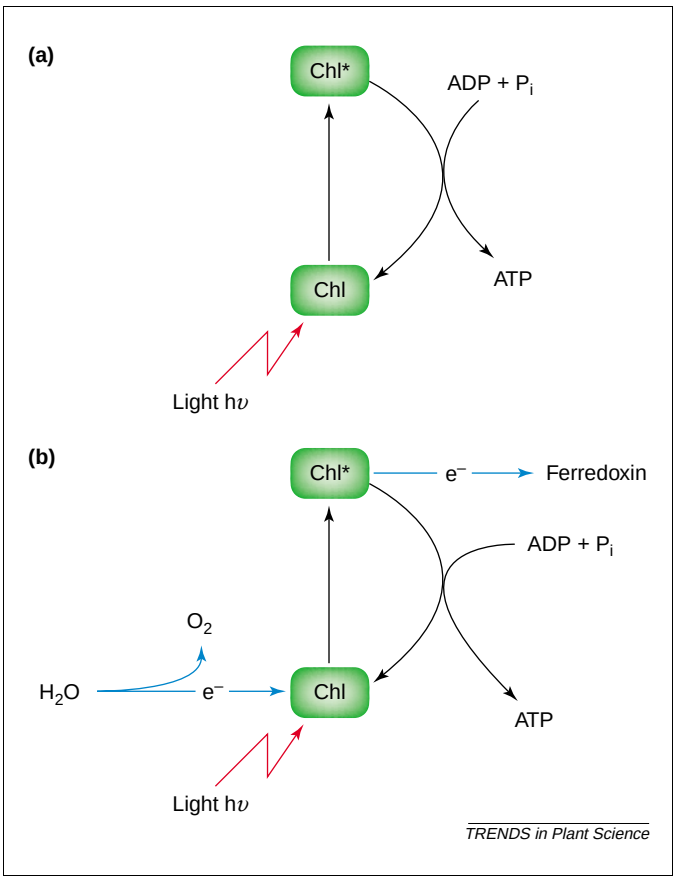

Fig. 1. (a) Photosynthetic phosphorylation of ADP (photophosphorylation) – cyclic synthesis of ATP by light. (b) ATP synthesis is also coupled to oxygen evolution and ferredoxin reduction in noncyclic photophosphorylation. Abbreviations: Chl, chlorophyll; Chl*, the excited state of Chl; e^-, electron; P_i, inorganic phosphate.

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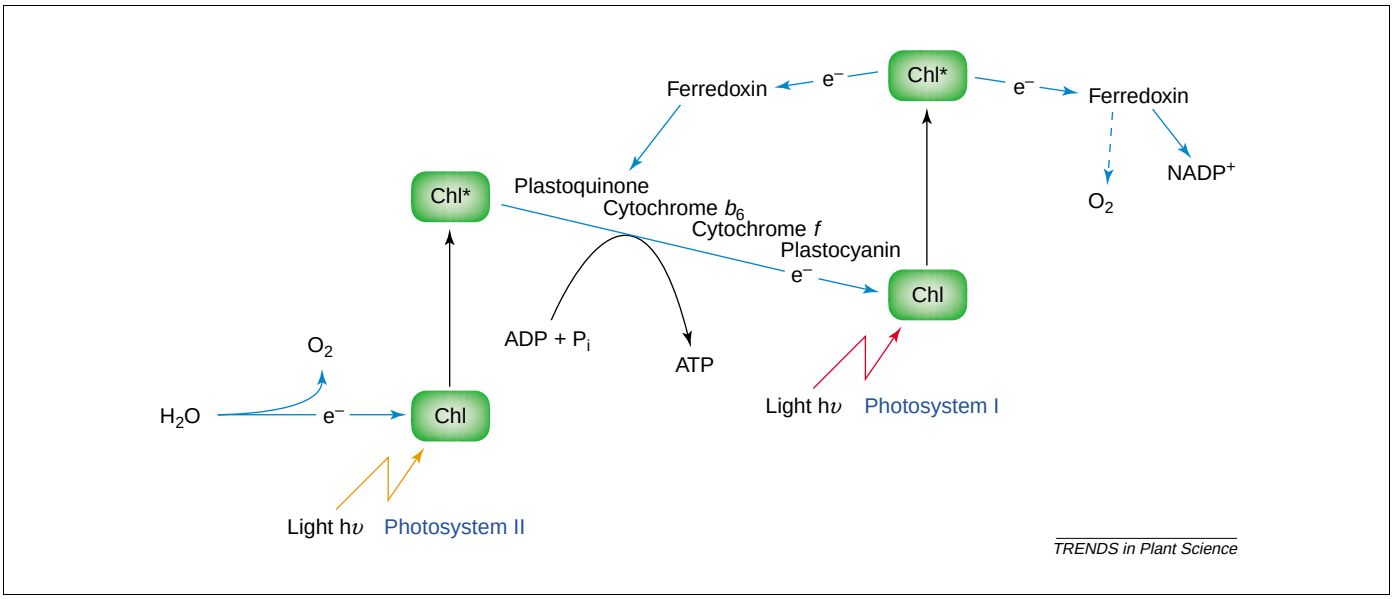

Fig. 2. The Z scheme for photosynthetic electron transfer encompasses ATP synthesis by cyclic (Fig. 1a) and noncyclic (Fig. 1b) photophosphorylation. Noncyclic photophosphorylation is revealed to use two photosystems, not one (Fig. 1b). In pseudocyclic photophosphorylation, the terminal electron acceptor is O_2 instead of $NADP^+$. Abbreviations: Chl, chlorophyll; Chl*, the excited state of Chl; e^-, electron; P_i, inorganic phosphate.

addition. One of the many electron acceptors that will work in place of $NADP^+$ is molecular oxygen. This is pseudocyclic electron transport, and pseudocyclic photophosphorylation. All three modes of electron transport (cyclic, noncyclic and pseudocyclic) are coupled to ATP synthesis (Table 1) and can readily be demonstrated *in vitro*, in isolated chloroplasts.

Noncyclic photophosphorylation produces oxygen, NADPH and ATP. Are the cyclic and pseudocyclic alternatives experimental artefacts? One view is that noncyclic electron transport is coupled to ATP synthesis with an ATP:NADPH stoichiometry of at least 3:2 and perhaps even 4:2 [7]. Another view is that noncyclic photophosphorylation has an ATP:NADPH ratio of 1:1, and cyclic photophosphorylation makes extra ATP [8]. A third possibility is that extra ATP is required but that it comes from pseudocyclic photophosphorylation [9]. Let us now fast-forward, to a time of structural biology and genomics, and see how these ideas have fared.

Stoichiometries in chemiosmotic coupling

John Walker and Paul Boyer shared part of the 1997 Nobel Prize for Chemistry for a structural and mechanistic breakthrough in understanding how ATP synthesis is coupled to electron transport. The universal intermediate in this coupling is a transmembrane electrochemical potential gradient of hydrogen-ion concentration, the proton-motive force of Peter Mitchell's chemiosmotic hypothesis (which itself won him the Nobel Prize for Chemistry in 1978). The Walker group's X-ray structure [10] of beef-heart mitochondrial F_1-ATPase shows 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, which forms a 'bearing' inserted through the central core of the cylindrical $\alpha_3\beta_3$ domain. Rotation of γ within the central axis of F_1 induces sequential conformational changes in each of the three αβ heterodimers; three ATP molecules are made for each 360° rotation of γ. Several independent lines of evidence [11] support this model, for both chloroplasts and mitochondria [12]. Oxidative and photosynthetic phosphorylation are not so different, after all.

The two photosystems of the Z scheme for photosynthetic electron transport (Fig. 2) have also now been described at near-atomic resolution [13–16]. Connecting the two photosystems, the chloroplast cytochrome b_6f complex is also essentially the same as its mitochondrial counterpart (the cytochrome bc_1 complex) in its fundamental features [17]. In particular, vectorial electron transport that includes the two quinone-binding sites and the two cytochromes b_6 (the 'Q cycle') gives two protons translocated for each electron transferred from photosystem II to photosystem I. Photophosphorylation works

Table 1. Properties of cyclic, noncyclic and pseudocyclic photophosphorylation

	Cyclic	Noncyclic	Pseudocyclic
Requires photosystem I	Yes	Yes	Yes
Requires photosystem II, inhibited by DCMU	No (except to obtain redox poise)	Yes	Yes
Oxygen produced	No	Yes	Yes
Oxygen consumed	No (except to obtain redox poise)	No	Yes
Electron acceptor	None, but cofactors include ferredoxin, flavins, quinones, Marmite™ [5]	$NADP^+$, ferredoxin, ferricyanide, quinones	Oxygen, via cofactors including ferredoxin, bypyridyls (viologens), flavins

Abbreviation: DCMU, 3-(3′,4′-dichlorophenyl)-1,1′-dimethyl urea.

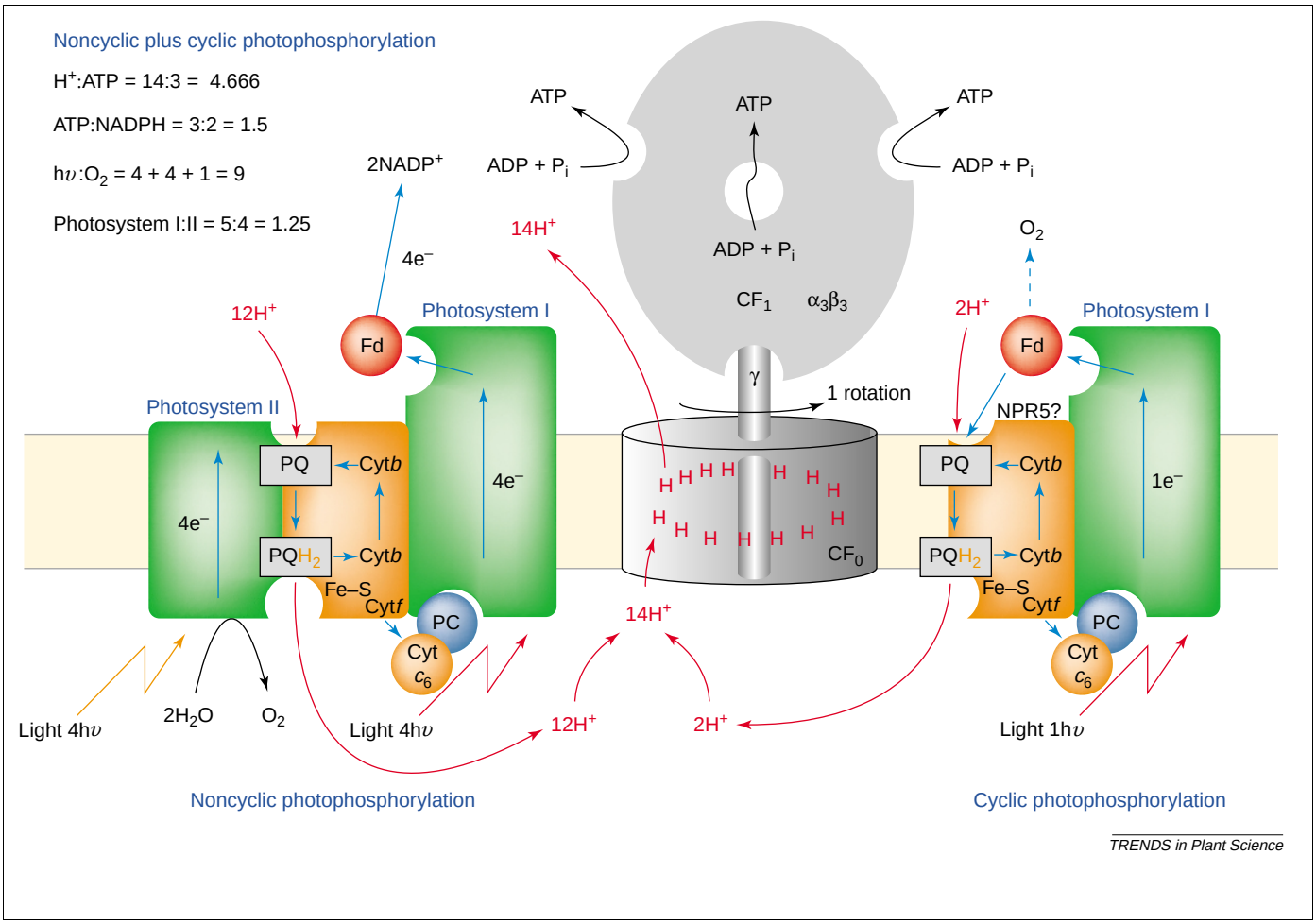

Fig. 3. A simplified scheme for noncyclic photophosphorylation giving 12 protons translocated for every four electrons, with combined photosystem-I cyclic phosphorylation translocating two protons per electron. Stoichiometries are depicted for four electrons transferred through the noncyclic chain from H_2O to $NADP^+$and one electron cycled through photosystem I alone. Therefore, H^+:ATP = 14 and ATP:NADPH = 3:2 when noncyclic and cyclic photophosphorylation are combined. Non-cyclic photophosphorylation alone would give ATP:NADPH = 9:7 (see text).; Abbreviations: cyt, cytochrome; e^-, electron; Fd, ferredoxin; P_i, inorganic phosphate; PQ, plastoquinone.

because electrons move from the inner to the outer side of the thylakoid membrane and protons are pumped in the opposite direction, into the thylakoid lumen (Fig. 3). Apart from leakage, or partial uncoupling, the protons move out again only through the CF_o component of the ATPase.

How does this understanding (Fig. 3) help us with the old problem of deciding whether noncyclic photophosphorylation, by itself, makes enough ATP? If we take the mitochondrial model for the ATPase and apply it, without modification, to chloroplasts, we arrive at an ATP:NADPH ratio for noncyclic phosphorylation of exactly 3:2. This is because two electrons are required for each NADPH molecule. Four electrons passing through the complete noncyclic electron transport chain should pump 12 vectorial protons – four released into the lumen by oxidation of water in photosystem II, four bound at the acceptor side of photosystem I, and eight pumped completely across the thylakoid membrane in the Q cycle of the cytochrome b_6f complex. Twelve protons equate to one 360° rotation of a 12-fold CF_o. One 360° rotation of CF_1-γ gives three ATP molecules. Therefore, synthesis of two NADPH molecules accompanies a single turn of CF_1-γ, and ATP:NADPH = 3:2. This is the worst-case scenario for devotees of cyclic and pseudocyclic photophosphorylation – their reactions are redundant. For conventional, complete photosynthesis in C_3 plants, cycles and pseudocycles are out of a job.

One foundation of this view has now been removed. Spinach chloroplast CF_o has 14-fold, not 12-fold, rotational symmetry [18]. Even mitochondrial F_o components are far from universally 12-fold: in yeast, they have tenfold symmetry [19]. It would be interesting to know whether the ring size is under physiological, developmental or genetic control, as it might be in *E. coli* [20]. However, let us now recalculate (Fig. 3). The 12 protons transported by four electrons in the non-cyclic chain must drive a 14-fold CF_o through 12 of its 14 steps. This will give six-sevenths of a rotation, or 308°. The true ATP:NADPH ratio of noncyclic phosphorylation is then $(3/2) \times (6/7)$, or 9:7. In decimal form, and rounded up to seven places, this is 1.2857143. This value would have seemed absurd in the days when it was thought that there were chemical intermediates in photosynthetic and oxidative phosphorylation. An integer was taken for granted, and there were high stakes on whether the answer was one or two. Even 3:2 was regarded as a facile compromise, with no obvious mechanistic basis.

So it seems that something has to supply the additional ATP for CO_2 fixation by the Benson–Calvin pathway, which requires ATP:NADPH = 3:2. If the answer is

Box 1. Redox poise

The velocity, *v*, of any simple, first-order electron transfer will be proportional to the product of the activities of the donor and acceptor.

$v \alpha [\text{donor}] \times [\text{acceptor}]$

Consider a component *Q* of a cyclic chain. The velocity will be given by:

$v \alpha [Q_{red}] \times [Q_{ox}]$

However, *Q* is either reduced, Q_{red}, or it is oxidized, Q_{ox}.

$Q_{red} + Q_{ox} = 1$

$\therefore Q_{ox} = 1 - Q_{red}$

Substituting, and omitting square brackets to approximate activity as concentration,

$v \alpha Q_{red} \times (1 - Q_{red})$

$\therefore v/C = Q_{red} - Q_{red}^2$

where C is a constant of proportionality. This is the equation of a parabola (Fig. I).

Thus, *v* is at a maximum when $Q_{red} = 0.5$ (i.e. when *Q* is 50% oxidized and 50% reduced). When *Q* is completely reduced ($Q_{red} = 1$) or completely oxidized ($Q_{red} = 0$), $v = 0$. This analysis was originally suggested by J. Bennett (pers. commun.).

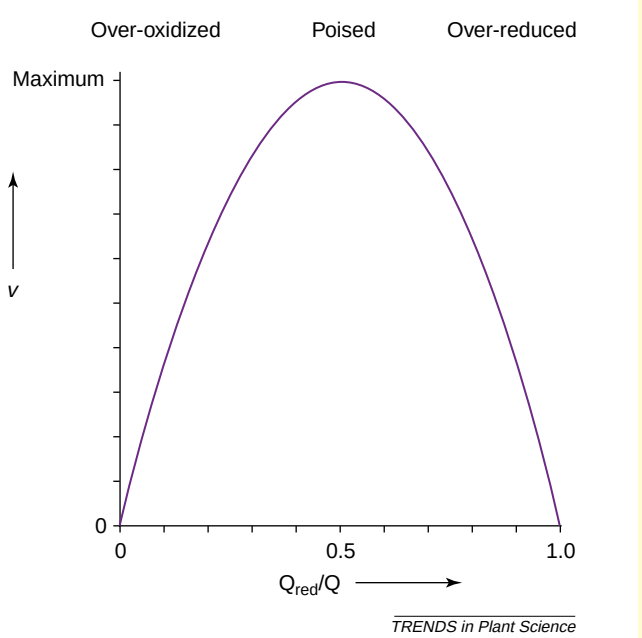

Fig. I.

pseudocyclic electron transport, 14% of electrons must end up on oxygen and not on $NADP^+$. If the answer is cyclic electron transport and cyclic electron transport pumps two protons for each electron (through the Q cycle operating alone), photosystem I must recycle one electron in five (Fig. 3). For maximum efficiency, this would mean that there is 20% more photosystem I than photosystem II. This conclusion is in agreement with recent estimates [21]. Perhaps the CF_o ring size of 14 is not as anomalous as it first appears.

Poised for action

Cyclic photophosphorylation requires a balance in its input and output of electrons (Box 1). Cyclic electron transport is zero when its components are completely reduced because there is nowhere for the electrons to go. Similarly, cyclic electron transport is zero when its components are completely oxidized because there are no electrons to cycle [22]. Photosynthetic systems seem to be obsessed with avoiding these two extremes. Pure kinetics (Box 1), post-translational modification [23–25] and even redox control of reaction-centre gene expression [26] are all deployed to maintain a poised plastoquinone pool. In spite of these control mechanisms, over-reduction is expected when the Benson–Calvin cycle is unable to use NADPH, and one reason for this is insufficient ATP. Therefore, if cyclic phosphorylation is required for the Benson–Calvin cycle to regenerate an electron acceptor ($NADP^+$) for noncyclic electron transport, then failure of cyclic electron transport will cause the whole system to stall – the surplus electrons from NADPH will have nowhere to go. A poising pulse of oxygen to bleed off surplus electrons and compensate for an over-reduced chain is sufficient to kick-start whole photosynthesis by restoring redox poise, allowing cyclic electron transport to generate the extra ATP required for the Benson–Calvin cycle to oxidize NADPH. Poising is thus a plausible function for pseudocyclic electron transport [27,28].

New genes for new components

Chlorophyll fluorescence emission at room temperature represents light energy that is re-emitted because photosystem II is unable to convert it photochemically. One factor that influences the yield of fluorescence is a protein, psbS, that seems to be required to dispose of excess energy at high light intensities by thermal or non-radiative decay [29]. The downregulation of photosystem II produced by psbS correlates with decreased pH in the thylakoid lumen. Cyclic electron flow around photosystem I establishes a pH gradient (ΔpH) and might help to dissipate excess energy in photosystem II [27]. It is possible that a ΔpH above a certain threshold is a switch to dissipate an unusable proportion of the light absorbed by chlorophyll molecules of photosystem II. However, restricting electron flow into the cyclic chain increases the rate of cyclic phosphorylation if it is otherwise limited by over-reduction.

Another gene required for efficient photosynthesis is described by Munekage and co-workers [30]. The predicted protein is termed PGR5, for proton gradient regulation. The *pgr5* mutant phenotype is one of decreased photosystem-I cyclic electron transport, as shown by its inability to increase fluorescence yield when NADPH and ferredoxin are added

to thylakoids. In normal thylakoids, the plastoquinone pool becomes reduced and fluorescence rises because photosystem II has nowhere to send electrons, indicating an electron-transport pathway from ferredoxin to plastoquinone, just as required by the cyclic chain. If cyclic photophosphorylation is required for normal photosynthetic growth, the *pgr5* mutant is expected to have found an alternative source of extra ATP. Possibilities include constitutive pseudocyclic phosphorylation and a decreased CF_o ring size. Perhaps the general function of a combined cyclic and noncyclic photophosphorylation is to permit flexibility in the ATP:NADPH ratio according to metabolic demands. The *pgr5* mutant would then be expected to be a poor adaptor to changes such as altered nitrogen supply. Ammonium assimilation requires mostly ATP for glutamine-synthetase activity, whereas nitrate or nitrate assimilation requires electrons from ferredoxin, and noncyclic electron transport.

The PGR5 protein is membrane bound but it does not have an extensive hydrophobic sequence, which suggests that it is not intrinsic to the thylakoid. Nor does PGR5 have any obvious motif suggesting a redox-active prosthetic group. PGR seems to be a candidate for the long-sought ferredoxin–quinone oxidoreductase [31] that must exist in some form if cyclic photophosphorylation is a reality. However, PGR5 is thought to have a role in electron transport from ferredoxin–$NADP^+$oxidoreductase to the cytochrome b_6f complex [30]. A functional association between these two complexes has been indicated [32]. In contrast to PGR5, cytochrome c_6 is a well-characterized electron carrier. For many years, cytochrome c_6 was thought to be exclusive to cyanobacteria, but its gene is present in *Arabidopsis* [33] and it is now known to function as an alternative to plastocyanin in chloroplasts [34]. Another factor to be considered is the chloroplast-encoded NAD(P)H dehydrogenase subunits revealed by plastid genome sequencing [30]. These might represent a 'long cycle' around photosystem I but their role in classical cyclic photophosphorylation is unclear. One function for a chloroplast NAD(P)H dehydrogenase might be to add electrons to the cyclic chain to produce poise and ATP synthesis when photosystem II is switched off by non-photochemical quenching or photoinhibition.

Therefore, all three modes of photosynthetic phosphorylation – cyclic, non-cyclic and pseudocyclic – are likely to be at work *in vivo*. Although the original distinction between oxidative and photosynthetic phosphorylation still holds, there is a fundamental unity in the mechanism of energy coupling and in the structures of the generators and consumers of the proton-motive force. What makes photosynthesis special is the way in which the electrons are set in motion in the first place, and its need to adapt to wide-ranging quantities and quality of light.

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