The Importance of Energy Balance in Improving Photosynthetic Productivity[1][W]

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Current proposals to improve photosynthesis to meet our energy and food needs include the following: (1) improving the performance of Rubisco; (2) decreasing photorespiration by turning C_3 plants into C_4 plants, installing algal or cyanobacterial carbon-concentrating mechanisms into higher plant chloroplasts, or redesigning photorespiratory metabolism; and (3) adding new biosynthetic pathways to increase the flow of carbon into useful products, like starch or oils, etc. While introducing or modifying pathways for these processes will be an important step forward, it is important to note that these approaches may also substantially alter the energetic demands placed on photosynthesis. To successfully translate these modifications into enhanced photosynthesis requires that chloroplasts can meet these altered demands. Chloroplasts have already evolved significant energy flexibility mechanisms, as discussed below, but these are activated under specific environmental and metabolic challenges. We need knowledge of the mechanisms regulating these processes in order to modulate them for increased energy efficiency. Ultimately, we could adjust chloroplast performance to meet altered needs by altering gene regulation or by introducing new balancing systems. It is thus useful to review what is known about energy balance in the chloroplast and project how these might be adjusted.

The light reactions involve highly reactive species, and if not controlled properly, they can produce deleterious reactive oxygen species. In addition, the synthesis of ATP and NADPH in linear electron flow is tightly coupled (i.e. one cannot occur without the other). If, for example, the substrates for the ATP synthase (ADP, inorganic phosphate) become limiting, then the proton motive force (*pmf*) builds up, inhibiting electron transfer to $NADP^+$. Likewise, if $NADP^+$ is limiting, photosynthetic electron carriers become reduced, slowing electron transfer and associated proton translocation, thus limiting ATP synthesis. Linear electron flow produces a fixed ATP/NADPH ratio, and each metabolic pathway directly powered by photosynthesis consumes different fixed ATP/NADPH ratios. However, since fluxes through these pathways vary between species and under different physiological conditions, substantial mismatches in the production and demands for ATP/NADPH could arise. Chloroplasts have very limited pools of ATP and NADPH. Consequently, such mismatches will rapidly (within seconds) inhibit photosynthesis (Avenson et al., 2005b; Cruz et al., 2005; Amthor, 2010). The chloroplast must balance the production and consumption of both ATP and NADPH by augmenting production of the limiting intermediate (e.g. by cyclic electron flow) or dissipating the intermediate in excess.

Here, we consider recent progress in understanding the mechanisms used by plants and algae to match ATP/NADPH supply with demands, with the aim to guide future efforts at optimizing these processes. Engineered plants may exacerbate the situation by creating demands for ATP/NADPH that differ from those to which the chloroplast has adapted. For example, adding a C_4 pathway to a C_3 plant would increase the ATP/NADPH demand, whereas increasing the storage of energy in saturated fats or oils would decrease it. Thus, we need to consider two distinct situations: (1) when the ATP/NADPH production ratio is lower than that needed to support downstream metabolism; and (2) when the ATP/NADPH production ratio exceeds demand. Both situations lead to metabolic congestion requiring different flexibility mechanisms. To make the situation more complex, the transthylakoid *pmf*, which drives ATP synthesis, is also the main "signal" regulating the light reactions (Kramer et al., 2004). Acidification of the lumen associated with a buildup of *pmf* activates the photoprotective energy-dependent excitation quenching (q_E) response (Müller et al., 2001). Down-regulating the conductance of ATP synthase alters electron flux relative to the *pmf* by reducing quantum efficiency and slowing electron transfer but does not alter the ATP/NADPH output ratio (Kramer et al., 2004).

THE ATP/NADPH RATIO

There have been long-standing arguments in the literature over whether the textbook linear electron

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flow pathway produces sufficient ATP/NADPH to support CO_2 fixation and other metabolic processes, and thus whether ancillary processes are needed. Attempts to infer ATP/NADPH production and consumption at low irradiance require assumptions about how light is distributed between the two photosystems, how many protons are translocated to the lumen per electron, and how many protons are required to form ATP. Estimates of these requirements have changed over time, and it is timely to revisit them again in light of recent results.

The absorption of four quanta by PSII and four by PSI extracts four electrons from two molecules of water, releasing four protons and oxygen into the thylakoid lumen. Linear transport of these four electrons from PSII to PSI can reduce two $NADP^+$ and pump eight protons into the thylakoid lumen through the action of the Q cycle (Sacksteder et al., 2000). On the basis of the rotational catalysis model with the chloroplast integral membrane portion (CF_O) of the ATP synthase having 14 c-subunits (which form a ring structure in the F_O subcomplex), 14 protons are required to synthesize three ATP (4.67 H^+/ATP; Seelert et al., 2000; Vollmar et al., 2009). Consequently, eight quanta absorbed for linear electron flow can generate two NADPH and 2.57 ATP (12 protons × 3 ATP/14 protons). This was observed experimentally with spinach (*Spinacia oleracea*) thylakoids supplied with NADP (Furbank and Badger, 1983). However, other estimates of only four H^+/ATP (Steigmiller et al., 2008) have been observed, which would yield three ATP/two NADPH. The discrepancy between theoretical and measured H^+/ATP is important both for the mechanism of the ATP synthase and the energetics of photosynthesis and obviously needs to be addressed in future work.

The production of three ATP/two NADPH exactly matches consumption by the Calvin-Benson cycle, whereas that of 2.57 ATP/two NADPH requires a mechanism to supply additional ATP (Allen, 2002; Kramer et al., 2004; Amthor, 2010). However, one also needs to consider photorespiration, nitrogen and sulfur reduction, and the synthesis of amino acids, proteins, and other molecules. Considering just CO_2 assimilation and photorespiration, the demands for ATP and NADPH are given by $3+7\Gamma_*/C$ and $2+4\Gamma_*/C$, respectively (Farquhar et al., 1980). Γ_* is the CO_2 photocompensation point of Rubisco, which characterizes the relative rates of carboxylation and oxygenation by Rubisco, and C is the partial pressure of CO_2 at Rubisco. Γ_* increases with temperature. To illustrate the impact of photorespiration, the ATP/NADPH ratio required is shown as a function of C for two values of Γ_* (Fig. 1A). As C increases, the ratio decreases from 1.67 to 1.51 as the proportion of photorespiration decreases. Regardless of whether four or 4.67 H^+ are required to synthesize each ATP, additional ATP is needed to meet the demands of CO_2 assimilation and photorespiration.

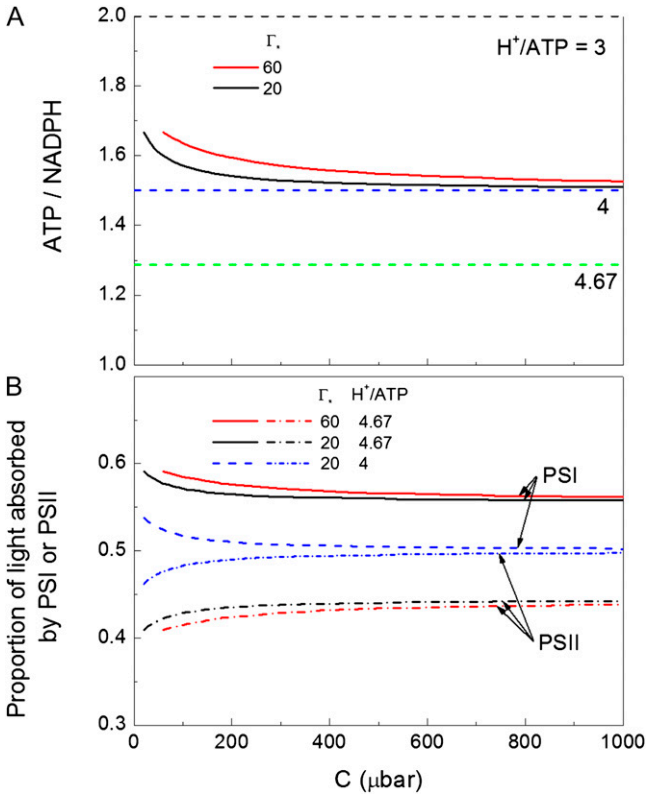

Figure 1. A, The ATP/NADPH ratio required to satisfy CO_2 assimilation and photorespiration as a function of CO_2 partial pressure at Rubisco. The ratio depends on the value assumed for Γ_* (μbar), the CO_2 photocompensation point, which increases with temperature. The ratios produced by linear electron flow are shown assuming three, four, and 4.67 H^+/ATP. B, The proportion of light absorbed by PSI and PSII necessary to satisfy the linear electron flow and cyclic electron flow required to provide the ATP/NADPH for different values of Γ_* and likely H^+/ATP ratios. A constant value is frequently used to calculate linear electron flow from chlorophyll fluorescence.

MECHANISMS TO SUPPLY EXTRA ATP

Five mechanisms have been proposed to augment ATP production. The first four are cyclic electron flow around PSI, the water-water cycle (WWC), the malate valve, and plastoquinol oxidase (Fig. 2). These processes might all operate under different conditions or in parallel. A fifth mechanism would be to alter the H^+/ATP ratio required by the ATP synthase, but this would require the modification of CF_O subunits.

Cyclic Electron Flow

Cyclic electron flow around PSI produces ATP without net reduction of NADPH. Plastoquinone (PQ) is reduced by ferredoxin (Fd) or NADPH via one or more enzymes collectively called PQ reductase, rather than by PSII, as in linear electron flow. From PQH_2, electrons return to PSI via the cytochrome b_6f complex. As this appears to be the major ATP/NADPH-balancing

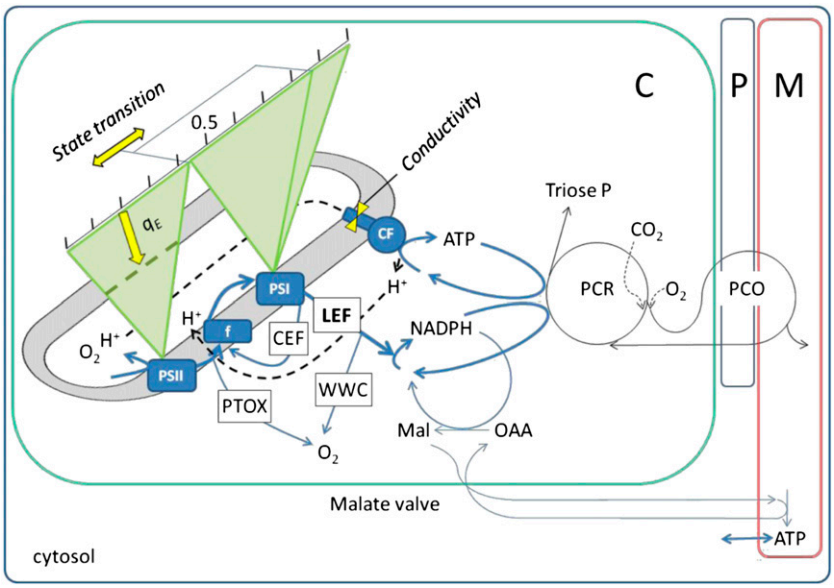

Figure 2. The coupling between light capture, electron flow, and photophosphorylation, which produce NADPH and ATP with their consumption by CO_2 assimilation (photosynthetic carbon reduction [PCR]) and photorespiration (photosynthetic carbon oxidation [PCO]) in a cell. Three of the cell organelles are shown: C, chloroplast; P, peroxisome; M, mitochondrion. The photosynthetic electron transport chain is represented in a thylakoid membrane, with electron flow from water through PSII, the cytochrome *b*/*f* complex (f), and PSI to NADPH, and proton flow into the lumen and out through the ATPase (CF) to generate ATP. The dominant path for electron flow is linear electron flow (LEF), while two alternative paths are shown: (1) cyclic electron flow around PSI (CEF); and (2) electrons can leave from PQH_2 via the plastoquinol oxidase (PTOX), which oxidizes plastoquinol and reduces oxygen to water. Some electrons from LEF can return to oxygen via the WWC or can be exported from the chloroplast via the malate valve to generate ATP in mitochondria (Mal, malate; OAA, oxaloacetate). Equal photon capture by PSII and PSI is required for LEF, and additional photon absorption by PSI is required for cyclic electron flow. The proportion of photons delivered to PSII reflects the relative amounts of chlorophyll associated with the two photosystems. State transitions can dissociate chlorophyll protein complexes from PSII, which may contribute to PSI, thereby enabling greater cyclic electron flow. The q_E quenching in the light-harvesting chlorophyll protein complexes associated with the xanthophyll cycle, which is activated by low pH in the lumen, reduces the effective efficiency of the antenna. The conductivity of the ATPase can be varied to alter the luminal pH relative to the rate of ATP synthesis, thus providing feedback via the q_E mechanism.

pathway, we will return to it after briefly describing the other mechanisms.

WWC

In the WWC, also called the Mehler peroxidase reaction or pseudocyclic electron transfer, electrons from PSI are transferred from Fd and NADPH to reduce oxygen. The superoxide formed in this reaction is detoxified by superoxide dismutase and ascorbate peroxidase, consuming NADPH (Asada, 1999). The consumption of NADPH by the WWC allows linear electron flow to continue, thereby producing a net gain in ATP. WWC is known to operate in isolated thylakoids (Asada, 1999), but its rate in vivo is less clear. Chloroplasts isolated from C_3 plants generally show higher capacity for the WWC than protoplasts or intact leaves (Backhausen et al., 2000; Badger et al., 2000). In C_3 leaves, the WWC contributed less than 5% of linear electron flow even when CO_2 fixation was inhibited (Ruuska et al., 2000; Clarke and Johnson, 2001). Both cyclic electron flow and WWC appear to operate in rice leaves during photosynthetic induction (Makino et al., 2002). For some C_4 plants under environmental stress, a substantial fraction of linear electron flow can be shunted away from CO_2 fixation and into alternative acceptors, most likely into WWC (Farage et al., 2006).

Malate Valve

In the malate valve (Scheibe, 2004), NADPH is consumed by the reduction of oxaloacetate to malate, which is exported from the chloroplast. Some of this malate is oxidized in the mitochondrion to synthesize ATP. It can also be oxidized back to oxaloacetate in the cytosol, generating NADH. The malate valve appears to have limited capacity to balance the ATP/NADPH budget (Scheibe et al., 2005) and cannot compensate for loss of NADPH dehydrogenase complex (NDH) in high cyclic electron flow mutants (Livingston et al., 2010a). However, malate valve components may also be up-regulated in response to environmental stresses (Scheibe, 2004). Although relatively little work has been done on this pathway in recent years, it appears to be a viable option for increasing ATP production in modified plants.

Plastoquinol Oxidase

Chloroplasts contain an enzyme that can oxidize PQH_2 and reduce oxygen to water (Cournac et al., 2000; Joet et al., 2002). High levels of plastoquinol oxidase have been found in the alpine plant species *Ranunculus glacialis*, where it is proposed to act as an effective alternate acceptor for photosynthetic electron transfer (Streb et al., 2005). However, in "typical" C_3 plants (e.g. Arabidopsis [*Arabidopsis thaliana*] or tomato [*Solanum lycopersicum*]), plastoquinol oxidase is expressed at very low levels and appears to play roles in biosynthetic pathways (Josse et al., 2000). In principle, plastoquinol oxidase could contribute to ATP production, albeit with lower yield than other processes, because only one proton should be deposited in the lumen for each electron transferred through PSII.

ATP Synthase Composition

The number of protons required to synthesize an ATP by the ATP synthase is thought to be determined by the number of c-subunits in the ATP synthase complex (Stock et al., 2000). Since the c-subunit stoichiometry varies between species, changing the ATP synthase "gear ratio" (which determined the H^+/ATP ratio) by substituting a foreign ATP synthase could possibly be used to alter ATP/NADPH output. In an extreme case, substituting the mammalian ATP synthase, with eight c-subunits per CF_O (Watt et al., 2010), could increase ATP production by 14/8 = 1.75. However, to maintain a given free-energy storage in ATP would require approximately 75% greater *pmf*, either from a much lower steady-state lumen pH or increased electric field. This would necessitate other regulatory changes in the chloroplast, since lumen pH is central to the control of energy dissipation (Kramer et al., 1999).

CYCLIC ELECTRON FLOW AROUND PSI

The major role proposed for cyclic electron flow is to increase ATP supply (Kramer et al., 2004). Cyclic electron flow appears to be minimally engaged (less than 14% of linear electron flow) under nonstressed conditions in C_3 plants (Cruz et al., 2005; Fan et al., 2007; Laisk et al., 2007; Livingston et al., 2010a, 2010b), either because linear electron flow nearly meets the ATP required for chloroplast metabolism or because other processes (WWC, malate valve) are sufficient to balance the ATP/NADPH budget. However, cyclic electron flow appears to be important for C_4 photosynthesis, carbon-concentrating mechanisms in green algae, and coping with environmental stress (Rumeau et al., 2007; Jia et al., 2008; Kohzuma et al., 2009), where additional ATP may be required.

Cyclic Electron Flow May Trigger, But Is Not Essential for, Photoprotection

Another proposed role of cyclic electron flow is to acidify the thylakoid lumen to initiate the photoprotective q_E response and regulate electron transfer at the cytochrome b_6f complex (Golding et al., 2004; Kramer et al., 2004). These two roles for cyclic electron flow are not independent, since a balanced ATP/NADPH budget is critical for maintaining proper levels of *pmf* and thus regulating q_E. However, q_E is clearly activated in the absence of high cyclic electron flow and in plants lacking complexes thought to catalyze cyclic electron flow (Avenson et al., 2005a; Ishikawa et al., 2008). We thus conclude that cyclic electron flow is not "essential" for photoprotection, but its absence may hinder the formation of *pmf* (and q_E) under certain conditions. Other mechanisms that can control *pmf* and regulate q_E include altering the conductivity of the ATP synthase to protons or the proportion that $\Delta\psi$ (the electric field across the thylakoid membrane) and ΔpH contribute to *pmf*.

Progress Identifying Pathways for Cyclic Electron Flow

The literature supports at least four distinct pathways for cyclic electron flow, diverging at the key PQ reductase step, where electrons from PSI are transferred into the PQ pool (Fig. 3). This lack of consensus, or perhaps diversity of biochemical solutions, has been a major source of confusion. Much recent progress has been made identifying and characterizing these pathways. Unfortunately, the results lead to a more complex rather than a simpler picture.

Pathway 1: Thylakoid Type 1 NDH

Thylakoids from cyanobacteria and higher plant chloroplasts contain a type 1 NADPH dehydrogenase, termed the NDH complex, which is partially homologous to bacterial and mitochondrial complex I and is thought to participate in cyclic electron flow. Complex I is found in mitochondria and bacteria and serves to oxidize NADH for complex I and reduce ubiquinone. The redox reactions are coupled to proton translocation in two ways. First, protons are taken up on the negatively charged side of the membrane during quinone reduction and released on the positive side of the membrane during quinol oxidation (i.e. by the first step in the Q cycle catalyzed by the cytochrome bc_1 or b_6f complex). Second, four additional protons are directly pumped by mechanical action for each pair of electrons transferred to the quinone. If the same proton-pumping action occurs in NDH, four protons should be translocated for each electron transferred through the cycle, two via the reduction and oxidation of PQ and the Q cycle and two more via the NDH proton pump. This proton/electron stoichiometry is twice that expected for a PQ reductase without a proton pump and halves the rate of cyclic electron flow needed to balance the ATP/NADPH budget (i.e. a large amount of balancing can be achieved with very little cyclic electron flow). Comparison of NDH genes with the recent crystal structure of bacterial complex I and the accompanying mechanistic model (Efremov

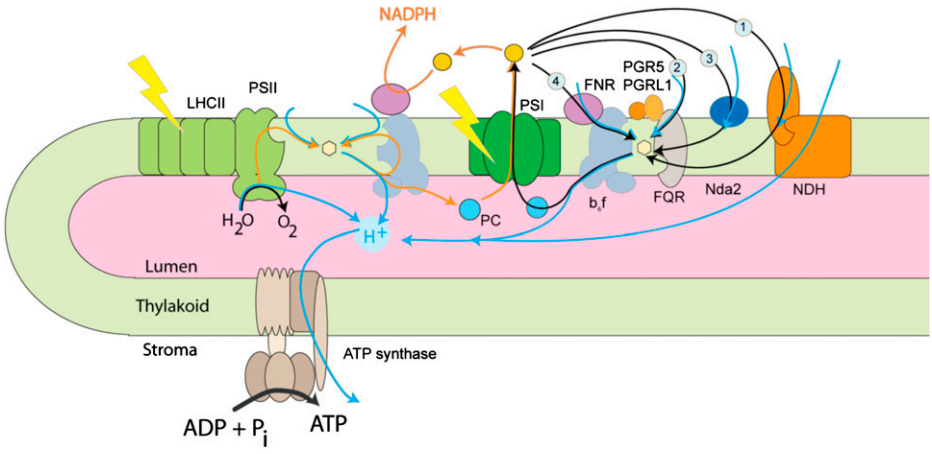

Figure 3. Details of the linear (orange arrows) and cyclic (black arrows) electron flow pathways that produce NADPH and ATP. Proton movement is denoted by blue arrows. Four different cyclic pathways have been proposed and may operate in parallel: (1) NDH, PQ is reduced by NAD(P)H:PQ oxidoreductase; (2) FQR/PGR5, electrons are transferred from Fd to PQ via the FQR; (3) Nda2, a type 2 NAD(P)H:PQ oxidoreductase; (4) the Q_i (PQ reductase) site of the cytochrome b_6f complex is used to reduce PQ and may involve the Fd:$NADP^+$ oxidoreductase (FNR). Other thylakoid components in the diagram include the ATP synthase, PSI, PSII, PQ (yellow hexagon), plastocyanin (PC), and light-harvesting complexes associated with PSII (LHCII).

et al., 2010) suggests that the proton-pumping reactions are conserved in the thylakoid.

Key advances in the biochemistry of NDH include the discovery of new subunits or proteins involved in the synthesis and stability of the complex (Nixon and Rich, 2007; Suorsa et al., 2009). Expression of NDH is low under nonstressed conditions, and mutants deficient in the NDH complex only show strong effects on photosynthesis when under environmental stress. High NDH expression is also seen in the *hcef1* mutant, which shows continuous high cyclic electron flow (Livingston et al., 2010a).

Pathway 2: The Fd-PQ Oxidoreductase

The Fd-PQ oxidoreductase (FQR) pathway has been proposed to catalyze the antimycin A-sensitive reduction of the PQ pool by Fd, although no actual FQR protein complex has yet been isolated. Munekage et al. (2002) isolated a mutant, *pgr5*, lacking a strong q_E response, suggesting a deficiency in the establishment of the proton gradient, consistent with a deficiency in cyclic electron flow. This suggestion was supported by assays in isolated thylakoids of chlorophyll fluorescence changes upon the addition of NADPH and Fd, which should reflect the reduction of the PQ pool. Based on these results, Munekage et al. (2002) concluded that PGR5 catalyzes the "major route" for cyclic electron flow. PGR5 does not appear to house redox cofactors or substrate-binding sites and thus has been proposed to regulate cyclic electron flow, perhaps by mediating the formation of supercomplexes containing PSI and other proteins (Shikanai, 2007). DalCorso et al. (2008) showed that PGR5 forms a complex with the related PGRL1 protein and suggested that they work together to catalyze cyclic electron flow. It has also been proposed that PGR5 is involved in redox "poising" of the electron transfer chain (Breyton et al., 2006) or in the formation of supercomplexes (Laisk, 1993; Joliot and Joliot, 2002) that promote cyclic electron flow.

However, the story is more complex. While PGR5 clearly plays a role in coping with fluctuating light (Tikkanen et al., 2010), there is conflicting data on what role PGR5 plays in cyclic electron flow. In the assays of Munekage et al. (2002), only a small (approximately 5%–10%) fraction of the PQ pool is reduced by NADPH and Fd (T. Shikanai, personal communication; D. Strand and D.M. Kramer, unpublished data), suggesting that the majority of PQ reductase is deactivated in isolated thylakoids. Avenson et al. (2005a) showed that the loss of q_E in *pgr5* is not related to changes in cyclic electron flow but to a change in the rate of proton efflux from the lumen through the thylakoid ATP synthase. In addition, cyclic electron flow has been shown to operate quite rapidly in the complete absence of PGR5, both during induction from a dark-adapted state (Nandha et al., 2007) and in steady state in mutants with elevated cyclic electron flow (Livingston et al., 2010a). This indicates that PGR5 is not needed for cyclic electron flow. While it is certainly possible that multiple cyclic electron flow pathways operate under different conditions, one cannot say that the PGR5 pathway constitutes the major pathway for cyclic electron flow.

Pathway 3: Nda2

Some green algae, including *Chlamydomonas reinhardtii* (Maul et al., 2002), and conifers, such as *Pinus thunbergii* (Wakasugi et al., 1994), lack the chloroplast NDH complex. In *Chlamydomonas*, PQ reduction in cyclic electron flow has been proposed to occur via a type 2 NADH:PQ oxidoreductase (Nda2; Desplats

et al., 2009). It is related to those found in bacteria and mitochondria and does not pump protons. It would catalyze a cyclic electron flow pathway with 2 H^+/e^- and would have to run twice as fast as a cycle with a proton-pumping NDH (see above). However, type 2 complexes are structurally much simpler than complex I (one subunit with a single flavin cofactor compared with at least 11 protein subunits, nine FeS clusters, and a flavin). Nda2, therefore, may be a more tractable (albeit less efficient) system to introduce into plants for energy balancing.

Pathway 4: The Cytochrome b_6f Complex and Fd NADP Reductase

A pathway that uses the Q_i (PQ reductase) site of the cytochrome b_6f complex to reduce PQ has been proposed (Zhang et al., 2004; Iwai et al., 2010). Electron transfer to Q_i probably involves the newly discovered heme c_i, which is located in a seemingly ideal position for this reaction (Kurisu et al., 2003; Stroebel et al., 2003). It also seems well placed to allow electrons to flow from Fd or Fd NADP reductase to the bound PQ (Zhang et al., 2004). This pathway probably involves the formation of a special cyclic electron flow supercomplex (Iwai et al., 2010), as discussed below.

STATE TRANSITIONS

Reduction of the PQ pool activates a kinase that phosphorylates thylakoid proteins, resulting in the dissociation of antenna complexes from PSII. In *Chlamydomonas*, state transitions also affect the rate of cyclic electron flow (Finazzi et al., 2002) and the formation of the cyclic electron flow supercomplex described by Iwai et al. (2010). This regulation makes physiological sense because, under ATP deficit conditions, NADPH should build up, slowing PSI electron transfer and allowing PQ to be reduced. Activating state 2 transition decreases PSII excitation, while increased cyclic electron flow should alleviate the ATP deficit and increase *pmf*, thus down-regulating PSII antenna via the q_E mechanism (Fig. 2).

There are a growing number of mutants in Arabidopsis associated with state transitions. Orthologs to thylakoid-associated kinases in *Chlamydomonas* have been identified in Arabidopsis, such as STN7 (Bellafiore et al., 2005). STN7 is related to a kinase that phosphorylates light-harvesting complexes in the light or when PSII is preferentially excited, causing it to dissociate from PSII and migrate (Tikkanen et al., 2010). An increase in the mobile fraction of pigment protein complexes is seen following photoinhibitory treatments (Goral et al., 2010). The fact that *stn7* mutants were less fit in a fluctuating light environment (Wagner et al., 2008) suggests that STN7 might help in coping with changing ATP/NADPH demands or light balancing.

CYCLIC ELECTRON FLOW IN C_4 PLANTS

There is also confusion over which PQ reductase catalyzes the cyclic electron flow required to power C_4 photosynthesis in bundle sheath chloroplasts. Maize (*Zea mays*) shows strong bundle sheath expression of NDH genes (Majeran et al., 2008). Takabayashi et al. (2005) reported that expression of NDH, but not PGR5 (see above), correlated with the expected requirement for cyclic electron flow in different C_4 species, suggesting that NDH was the major PQ reductase for cyclic electron flow in C_4 plants. In contrast, Munekage et al. (2007) found that both NDH-H and PGR5 were expressed at higher levels in NAD-malic enzyme C_4 compared with C_3 and C_3-C_4 intermediate species of *Flaveria*. This suggests a role for both NDH and FQR in C_4 photosynthesis.

DEALING WITH EXCESS ATP

The case of insufficient ATP per NADPH has received the most attention in the literature, but the opposite case (too much ATP per NADPH) can also become a major problem. Any process that consumes less than the produced ratio of ATP/NADPH (e.g. nitrite reduction) can lead to this situation. This has already become an issue with the introduction of H_2 production in algae (Ghirardi and Mohanty, 2010). Here, the depletion of ADP can lead to slowing of the ATP synthase, resulting in high *pmf*, lumen acidification, and subsequent slowing of electron transfer at the b_6f complex. Excessive acidification of the lumen can also lead to catastrophic "acid photodamage" of PSII (Krieger and Rutherford, 1997). This area has not been well studied, and it is unclear how chloroplasts deal with this situation. It is possible that excess ATP can be remedied by introducing dissipative mechanisms. A controlled proton leak, perhaps induced by the expression of mitochondrial uncoupler proteins (Hourton-Cabassa et al., 2009) in the chloroplast or "slip" of the ATP synthase itself (Evron et al., 2000), could be activated. Futile metabolic cycles, such as that recently proposed by Livingston et al. (2010a) to cycle around chloroplast glyceraldehyde 1,3-bisphosphate, could hydrolyze excess ATP. A variety of other enzymes that hydrolyze ATP could be introduced to the chloroplast. Although these approaches appear to be energetically wasteful, one must keep in mind that chloroplasts perform analogous processes, such as dissipating "excess" light energy to avoid deleterious effects of overexcitation.

IMPLICATIONS FOR PHOTON REQUIREMENTS AND DISTRIBUTION BETWEEN PHOTOSYSTEMS

The photon requirements for the various electron transport paths producing NADPH and ATP are listed in Table I. In the absence of photorespiration, 1.5 ATP are required per NADPH, but linear electron flow only

Table I. *NADPH and ATP yields depending on where photons are absorbed and which pathway is used*

Due to the uncertainty in the H^+/ATP required in chloroplasts, two alternative options are given. Both photosystems are assumed to operate with 100% efficiency.

Path	PSII Photons	PSI Photons	NADPH	H^+	cATP[a] 4 H^+/ATP	cATP[a] 4.67 H^+/ATP	mATP[b]
Linear electron flow	2	2	1	6	1.5	1.28	
Cyclic electron flow, NDH[c]		2		8	2	1.71	
Cyclic electron flow pathways 2 to 4[d]		2		4	1	0.86	
WWC	2	2		6	1.5	1.28	
Malate valve	2	2		6	1.5	1.28	2.5
Plastoquinol oxidase	2			2	1	0.86	

[a]Chloroplast production of ATP. Structural evidence favors 4.67 H^+/ATP. [b]Mitochondrial production of ATP or one cytosolic NADH per NADPH equivalent exported from the chloroplast via malate and returned as oxaloacetate. [c]Assuming a proton-pumping NDH. [d]FQR, type 2 NADH:PQ oxidoreductase, cytochrome b_6f, and Fd NADP reductase (see paths 2, 3, and 4 in Fig. 3).

supplies 1.285 ATP, assuming 4.67 H^+ are required to synthesize each ATP. This deficit in ATP could be met in several ways. The photon requirement for CO_2 assimilation in the absence of photorespiration would be 8.2 to 8.7 photons. This is slightly less than experimental observations (10.4 in Skillman, 2008; but see Amthor, 2010). Under normal CO_2 conditions, additional ATP is required to meet the demand from photorespiration.

The pathway used to supply the additional ATP has implications on the photon requirement for CO_2 assimilation and the proportion of light required by each photosystem at low irradiance. The distribution of light depends on the composition of the antenna complexes associated with each photosystem, which depends on growth irradiance and state transitions. At higher irradiance, the effectiveness of those excitations will be influenced by photoprotective exciton-quenching processes (such as the q_E response, which decreases the quantum efficiency of the antenna) and the relative fractions of each photosystem in photochemically active or "open" states (which is influenced by downstream processes that consume their photoexcited products). If cyclic electron flow with a proton-pumping NDH (see above) is used, then the fraction of light that PSII should process is about 0.44, which is consistent with the fraction of chlorophyll associated with PSII (Albertsson, 2001). As photorespiration increases, either due to lower CO_2 or higher temperature raising Γ_*, the fraction of light that PSII should process need only decline by a few percentage points (Fig. 1B). However, if linear electron flow-WWC is used, or if only four H^+ are required to synthesize each ATP, then light needs to be distributed almost equally between both photosystems to achieve the observed photon yields. This distribution factor is used when converting photochemical efficiency measured by chlorophyll fluorescence into a rate of electron transport. Laisk and Loreto (1996) provide one method of estimating this, and their estimates ranged from 0.42 to 0.6. It has also been used to calculate PSI electron transport rate from the reduction state of P700 multiplied by the fraction of light absorbed by PSI and irradiance. Values are assumed for these fractions, and they are assumed to be constant. These assumptions are difficult to verify and may result in incorrect estimates of electron transport rates.

C_4 photosynthesis adds further levels of complexity. First, two ATP are required by the C_4 cycle pump for each CO_2 fixed by phospho*enol*pyruvate carboxylase, and a variable proportion of the CO_2 pumped into the bundle sheath leaks back out (Cousins et al., 2006; Tazoe et al., 2008; Pengelly et al., 2010). Second, electron transport is distributed between the mesophyll and bundle sheath chloroplasts, and the distribution of chlorophyll and thylakoid protein complexes differs between these two cell types depending on the decarboxylation type (Ghannoum et al., 2005). Reducing equivalents can also be supplied to the bundle sheath via the phosphoglycerate/glyceraldehyde shuttle and NADP-malic enzyme. If cyclic electron flow were the main pathway producing the extra ATP in C_4 leaves, the proportion of light captured by PSII would only need to be 0.27 rather than 0.44. As no methods currently exist that allow us to measure the proportion of light captured by PSII in C_4 leaves, we can only speculate on the exact paths for electron flow.

CONCLUSION

Unlike a power station, which supplies only one form of energy, electricity, the chloroplast must supply both ATP and NADPH in precisely the right proportion to match consumption. There are clear indications that "improving" the photosynthetic properties of organisms may affect energy budgets, requiring us to extend the flexibility of the energy-producing systems. The apparent complexity of pathways utilized by photosynthesis to balance energy supply to meet demand continues to grow. This provides us with new opportunities to meet the demands that may be placed by metabolic engineering of photosynthesis and may enable novel regulation of the system.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Table S1. Provides a list of abbreviations used in the text.

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