Homeostasis of adenylate status during photosynthesis in a fluctuating environment

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Abstract

This review describes and assesses pathways likely to influence and stabilize the ATP/reductant balance during whole cell photosynthesis. The sole reductive step of the Calvin cycle occurs during the conversion of 3-phosphoglycerate to triose phosphate. Photophosphorylation linked to this reaction can undoubtedly supply most of the ATP required by the Calvin cycle and other chloroplastic reactions. Small but crucial contributions must come from several other pathways, some of which involve co-operation between the chloroplast and the rest of the cell. Extrachloroplastic compartments can contribute to chloroplastic ATP requirements by supplying ATP directly or, probably more significantly, by accepting reducing equivalents and so supporting ATP synthesis within the chloroplast.

Key words: Photosynthesis, whole cells, ATP/reductant balance, Calvin cycle.

Introduction

Reductants (ferredoxin, NADPH, NADH) and ATP are the principal energetic links between membrane-associated redox reactions and metabolism in the soluble phase of the cell. These two types of molecule, which may be considered as the cell’s energetic currencies, are generated simultaneously in the chloroplast during light-dependent electron transport and photophosphorylation. They are utilized in the reductive assimilation of inorganic elements (carbon, nitrogen, sulphur) into cellular matter, from which ATP and reductant can be regenerated by oxidative processes such as respiration. Respiration includes oxidative phosphorylation in the mitochondria, which enables the reducing power of NAD(P)H to be converted into ATP.

Much attention has been paid to the reaction pathways responsible for producing ATP and NADPH at the stoichiometry required by carbon assimilation. In leaves from C₃ plants, however, considerable energy is also used to power the non-assimilatory photorespiratory pathway, which is initiated by RuBP oxygenation. Moreover, foliar assimilation of nitrogen, which is generally believed to occur in photosynthetic cells alongside CO₂ fixation, can account for a significant portion of photosynthetic energy. Both photorespiration and nitrogen assimilation require ATP and reductant at ratios that are different from that needed for carbon assimilation.

Theory predicts that small changes in flux will cause big changes in ratios

Chloroplastic adenylate and reductant pools turn over quickly during photosynthesis. This means that a small change in the ratio at which ATP and NADPH are produced, relative to their ratio of consumption, could quickly impact upon adenylate and redox status. Figure 1 shows the predicted effect on the ATP/ADP ratio if the amount of ATP generated relative to NADPH increased slightly (by 1%) while the ratio of their utilization remained the same. The starting point of ATP/ADP = 2.4 is typical of measured stromal ratios in photosynthesizing cells (for references, see legend to Fig. 1). Despite the buffering effect of adenylate kinase activity, the ATP/ADP ratio would increase 10-fold within 30–40 s. Even assuming that the increased phosphorylation status of the adenylate pool were not accompanied by a decrease in inorganic Pi concentration, the 10-fold increase in the [ATP]/[ADP][Pi] ratio should favour a significantly enhanced transthylakoid
proton gradient (ΔψH⁺). This example shows that even a very slight imbalance in ATP:NADPH production and consumption could rapidly exert marked effects on electron transport and associated reactions.

The example shown in Fig. 1 is hypothetical. In the photosynthetic cell, significant rapid fluctuations in adenylate or redox status are only observed during fairly severe perturbation of the photosynthetic system; for example, an abrupt change in light intensity (Takahama et al., 1981; Stitt, 1986; Furbank and Horton, 1987). One reason for this stability is that photosynthetic electron transport can occur through several pathways which produce ATP and NAD(P)H at different ratios. A second reason is that stromal reductant and adenylate pools are not isolated from the rest of the cell. Previous analyses have generally placed far greater emphasis on the first feature of photosynthetic metabolism. This review will consider both aspects, in assessing the numerous factors that confer flexibility upon ATP production and consumption in the illuminated photosynthetic cell.

**How many ATP are formed during non-cyclic electron transfer to NADP?**

The amount of ATP generated during the transfer of two electrons from H₂O through both photosystems is still not known with certainty. The uncertainty stems from two principal gaps in our knowledge: (1) The number of protons translocated across the thylakoid membrane during electron transfer through the cytochrome bc₊ complex; (2) The H⁺/ATP ratio of the chloroplast coupling factor.

Mitochondrial electron transfer is considered to involve an obligatory Q cycle at the cytochrome bc₁ complex (Mitchell, 1976), resulting in the translocation of two protons from the matrix to the intermembrane space for each electron that passes from ubiquinone to cytochrome c. There is abundant evidence that an analogous pathway can occur during chloroplastic electron transfer from the plastoquinone pool to plastocyanin through the cytochrome bcₐ complex, notably the considerable structural similarity between the mitochondrial and chloroplastic complexes and the phenomena of oxidant-induced reduction of cytochrome b haems and the slow phase of the electrochromic shift (reviewed by Cramer et al., 1996). If electron transfer involved the Rieske FeS centre and cytochrome f only, there would be no apparent function for the cytochrome b haems in non-cyclic electron transport.

Despite general agreement that a Q cycle (or similar mechanism) is possible, H⁺/e⁻ ratios have been obtained that are inconsistent with its obligatory operation, although the interpretation of these data has been questioned (Rich, 1988). Perhaps primarily because of methodological difficulties, it remains to be demonstrated whether a Q cycle occurs under steady-state conditions, particularly at higher light intensities. Apart from some intrinsic differences between the bc₁ and bcₐ complexes, the environments in which they operate may be significant. For example, most of the mitochondrial ΔψH⁺ is due to an electrical potential difference, whereas in the chloroplast the gradient mainly reflects the low pH maintained on the thylakoid interior. It has been suggested that electron paths through the cytochrome bcₐ complex may, at least partly, depend on whether the complex exists in a monomeric or dimeric form (Cramer et al., 1996; Heimann et al., 1998).

Historically, values reported for the number of protons translocated per ATP synthesized by Fₐ-type ATPases range from about two to approximately four (see Table 1 in Haraux and de Kouchkovsky, 1998, and the accompanying discussion of methods and their associated problems). Current opinion, at least for the chloroplast coupling factor, favours four H⁺/ATP, though this figure probably cannot as yet be considered definitive (Haraux and de Kouchkovsky, 1998). The synthesis of sugar phosphate from CO₂ in the Calvin cycle requires ATP and NADPH at a ratio of 1.5. If four protons are required per ATP synthesized, then at least some Q cycle activity probably must occur to raise the yield of ATP/NADPH above 1.0. In fact, an obligatory Q cycle would raise the ATP/NADPH ratio to exactly 1.5, which seems a seduct-
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In addition to possible flexibility conferred by a facultative Q cycle (variable $H^+/e^-$ ratio), the ATP yield associated with NADP reduction may be affected by variable $H^+/ATP$ values. This could result from changeable membrane permeability to protons. Alternatively, variability could be due to $H^+$ translocation through the coupling factor without concomitant ATP synthesis (so-called ‘proton slip’). This phenomenon may explain relatively high ‘basal’ rates of electron transport in the absence of uncouplers or ATP synthesis (Evron and Avron, 1990). Proton slip is perhaps a self-regulating mechanism allowing $\Delta$$\mu$$H^+$ dissipation to continue under conditions of high ATP/ADP ratios or low phosphate availability (Strotmann et al., 1986; Heineke et al., 1989). The physiological relevance of proton slip has, however, been questioned (Groth and Junge, 1993).

**Facultative cyclic and pseudocyclic photophosphorylations?**

Two further sources of flexibility in the production of ATP and NADPH by the thylakoid are cyclic photophosphorylation and ATP synthesis associated with reduction of acceptors other than 1,3-bisphosphoglycerate (Fig. 2).

Cyclic electron transport and its relationship to the Q cycle have been recently reviewed in depth (Bendall and Manasse, 1995). Although higher rates are undoubtedly possible in isolated thylakoids or chloroplasts, it was concluded that under most conditions this pathway probably occurs at only a few per cent of the rate of non-cyclic electron flow (Bendall and Manasse, 1995). This notion is consistent with measurements of the quantum yields of both photosystems in leaves in air (Harbinson and Foyer, 1991). Such low rates would suggest that the contribution of cyclic photophosphorylation to ATP synthesis is minor and it has therefore been proposed that the pathway may be more important in sustaining a large enough ApH to control photosystem II activity (Heber and Walker, 1992). Nevertheless, even low rates of cyclic photophosphorylation could be significant in chloroplast metabolism (Furbank and Horton, 1987), given the effect of slight variations in ATP yields on adenylate status (Evron and Avron, 1990). Proton slip is perhaps a self-regulating mechanism allowing $\Delta$$\mu$$H^+$ dissipation to continue under conditions of high ATP/ADP ratios or low phosphate availability (Strotmann et al., 1986; Heineke et al., 1989). The physiological relevance of proton slip has, however, been questioned (Groth and Junge, 1993).

As well as NADP-linked 3-phosphoglycerate reduction, other photosystem I electron acceptors include oxaloacetate, nitrite, oxoglutarate, and $O_2$. Reduction of the first three is considered in subsequent sections. Oxygen reduc-

**Fig. 2.** Electron transport in the thylakoid membrane and electron sinks in the stroma. The proton gradient necessary for ATP synthesis can be sustained by a variety of oxidants (shown in boxes) that accept electrons derived from the splitting of water, and by cyclic electron transport (dashed line). P680, P700, reaction centres of photosystems II and I, respectively; OEC, oxygen-evolving complex; CYT b$_{2f}$, cytochrome $b_{2f}$ complex; Fd, ferredoxin; 1,3bPGA, 1,3-bisphosphoglycerate.
tion (pseudocyclic electron transport) is favoured by conditions where the ATP/NADPH demand is relatively high (Egneus et al., 1975) and is inhibited in the presence of NADP, nitrate, nitrite or oxaloacetate (Behrens et al., 1982; Furbank and Badger, 1983; Backhausen et al., 1994). The initial product of oxygen reduction is superoxide, from which hydrogen peroxide is formed by dismutation or reduction (Allen and Hall, 1973; Asada et al., 1974). Ascorbate peroxidase uses ascorbate to reduce hydrogen peroxide to water, and reduced ascorbate is regenerated through several possible pathways, all of which depend on the electron transport chain (Anderson et al., 1983). For each O$_2$ molecule ultimately reduced to water at photosystem I, four electrons are transferred from water cleaved at photosystem II. The overall reaction supports Δ$\mu$H$^+$ formation and ATP synthesis without net O$_2$ exchange or NADP reduction (Furbank and Badger, 1983; Hormann et al., 1994; Forti and Elli, 1995). Protonation of superoxide on the thylakoid interior may act to offset the Δ$\mu$H$^+$ formed during O$_2$ reduction (Hormann et al., 1994, and references therein). This effect will probably be relatively minor, given the likely H$^+$/e$^-$ stoichiometries of non-cyclic electron transport (discussed further in Foyer and Noctor, 1999a), and ATP/2e$^-$ ratios associated with O$_2$ reduction are fairly similar to those observed during electron flow to NADP (Furbank and Badger, 1983).

Both cyclic electron transport and O$_2$ reduction may be particularly important when high amounts of ATP are needed to support CO$_2$-concentrating mechanisms (in algae or in C$_4$ plants). In C$_4$ photosynthesis, CO$_2$ fixation requires ATP/NADPH ratios of approximately 2.5. In C$_3$ plants, oxygen reduction may be important as an ‘energy valve’ when light input exceeds metabolic capacity, although it remains to be satisfactorily explained how electron flow to oxygen can continue if ATP sinks are strongly limiting. With respect to the ATP/reductant balance, the preferential reduction of NADP, oxidants of NADPH (oxaloacetate) or oxidants of ferredoxin (nitrite), suggests that like cyclic electron transport, oxygen reduction may be most important as a facultative mechanism that can be engaged in response to an inadequate production of ATP relative to NADPH. A metabolic shortage of ATP would theoretically favour over-reduction of the NADP pool, which may be prevented by diversion of electrons to oxygen or the cyclic pathway.

**Metabolic branchpoints and bypasses modify ATP yields**

In the photosynthetic cell, ATP and NAD(P)H participate in innumerable reactions. The aim here is to consider those pathways whose flux will be most significant during photosynthesis. In species that form large amounts of starch, the stromal ADP-glucose pyrophosphorylase reaction will increase the chloroplast’s ATP/NADPH requirements somewhat. Under many conditions, most of the carbon fixed in the chloroplast is exported to the cytosol, mainly as triose phosphate via the Pi translocator (Flügge and Heldt, 1991; Stitt, 1997). This exported carbon can flow in two directions. Either it can be converted glucoseoxygenetically to sucrose, requiring ATP but no reductant, or it can flow oxidatively in the glycolytic direction to produce substrates for processes such as nitrogen assimilation. Oxidative carbon flow will produce both ATP and NAD(P)H, but yields can vary considerably, depending notably on the extent of 3-phosphoglycerate formation through NADP-linked glyceraldehyde-3-phosphate dehydrogenase activity (which does not produce ATP), the path of carbon flow into pyruvate, the completeness of carbon oxidation in the citric acid cycle, and ATP yields during mitochondrial electron transport (see below).

At least two shuttle systems enable transfer of reductant out of the chloroplast (Fig. 3). These are triose phosphate exchange for 3-phosphoglycerate through the Pi translocator (Flügge and Heldt, 1991), and the ‘malate valve’ composed of malate dehydrogenases and malate-oxaloacetate exchange transporters (Scheibe, 1987). Glutamate is also exported at high rates in net exchange for oxoglutarate, a crucial process in photorespiratory nitrogen recycling. Because extrachloroplastic regeneration of oxoglutarate occurs predominantly through aminotransferases rather than dehydrogenases, this shuttle is much less important in exporting reductant than triose phosphate/phosphoglycerate and malate/oxaloacetate exchange (Fig. 3). All three shuttles could, however, complement the ATP requirements of the Calvin cycle, since the ratios at which they require chloroplastic ATP and NADPH are lower than 1.5. Here, stromal malate formation, which does not require ATP, will be particularly powerful. For example, to supplement a hypothetical ratio of 1.33 ATP formed per NADP reduced and reach the ratio of 1.5 required by the Calvin cycle in steady state, the triose phosphate/3-phosphoglycerate shuttle would need to operate at four times the rate of the malate/oxaloacetate shuttle (this would necessitate 50% of the triose phosphate produced from 3-phosphoglycerate exiting the chloroplast and re-entering as 3-phosphoglycerate, to be reduced once more). Glutamate/oxoglutarate exchange will produce a similar effect to the triose phosphate/3-phosphoglycerate shuttle, since the reductive conversion of oxoglutarate to glutamate also requires 1 ATP and the equivalent of 1 NADPH.

Photorespiration is generally held to increase the ATP/reductant requirements of photosynthesis. Engagement of the photorespiratory pathway introduces an ATP sink at glyceraldehyde kinase and also increases the ATP/NADPH ratio required by the Calvin cycle. These increases in the required ATP/reductant ratio are partly offset by electron transport and ATP synthesis linked to photorespiratory
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Fig. 3. Shuttle systems connect chloroplastic reductant and adenylate status to the rest of the cell. ATP synthesis is coupled to electron transport to ferredoxin (Fd). Oxidation of Fd can be linked to reductant export, allowing electron acceptors outside the chloroplast to support chloroplastic ATP synthesis. The most active shuttles are glutamate-oxoglutarate exchange (functioning in photorespiration and nitrogen assimilation: shown in blue), malate-OAA exchange (shown in purple) and triose phosphate-PGA exchange (shown in red). GS, glutamine synthetase; GOGAT, glutamate synthase; OAA, oxaloacetate; PGA, 3-phosphoglycerate; Pi, inorganic phosphate.

nitrogen recycling, as discussed above. Conditions favouring photorespiration are associated with an increased activation state of stromal NADP-malate dehydrogenase (Backhausen and Scheibe, 1999). Enhanced electron flow to oxaloacetate and ‘overflow’ export of malate may contribute to the ATP/reductant balance in these conditions. Likely electron acceptors coupled to malate oxidation are hydroxypyruvate (in the peroxisome), O₂ (in the mitochondrion) and nitrate (in the cytosol).

At least part of the glycine metabolized during photorespiration may be oxidized via the mitochondrial electron transport chain, accounting for increases in the cytosolic ATP/ADP ratio under photorespiratory conditions (Gardeström and Wigge, 1988). Not only could this process produce abundant amounts of ATP in the mitochondrion, it would also necessitate another source of electrons for hydroxypyruvate reduction in the peroxisome. It is not clear how significant this alternative source must be, since in the absence of a corresponding ATP sink, measurable increases in ATP/ADP ratios may result from relatively low rates of ATP synthesis (Fig. 1). According to this view, only a small proportion of the total rate of glycine oxidation would be linked to the mitochondrial electron transport chain. More recently, however, it has been suggested that the oxidation of photorespiratory glycine may be predominantly linked to electron transport pathways of very low ATP yields (see below) that effectively uncouple mitochondrial electron flow from oxidative phosphorylation (Igamberdiev et al., 1997). In this case, hydroxypyruvate reduction coupled to redox shuttles would be able to support high rates of chloroplastic ATP formation without net formation of NADPH (discussed further in Noctor and Foyer, 1998).

The photorespiratory pathway may further influence ATP/reductant ratios by cycling of glyoxylate and glycollate via glycollate oxidase and glyoxylate reductase, which amounts to reduction of oxygen by cytosolic or peroxisomal NAD(P)H. This cycle may be prevented in many conditions by efficient channelling of metabolites in the peroxisome (Heupel and Heldt, 1994).

Branchpoints in the mitochondrial electron transport chain permit flexible ATP yields

Electron transfer to O₂ in the mitochondrion must occur to some extent in the light (Krömer et al., 1988; Krömer, 1995; Noctor and Foyer, 1998), whether the reductant originates from glycolytic and citric acid cycle activities or the photorespiratory pathway. The associated ATP yield can vary considerably. One reason is that distinct dehydrogenases exist which transfer electrons from NAD(P)H to different components of the chain. In addition to the rotenone-sensitive NADH dehydrogenase (complex I), there are rotenone-insensitive dehydrogenases that oxidize matrix NAD(P)H and others that are able to accept electrons from cytosolic NADH or NADPH (Douce and Neuburger, 1989; Lambers, 1997). The maximum yield of ATP per 2e⁻ (or ADP/O ratio) is probably slightly less than three (rotenone-sensitive
NADH oxidation) or just below two (rotenone-insensitive oxidation of NAD(P)H).

These yields assume electron transfer to O$_2$ via cytochrome oxidase. It now seems, however, that a portion of the electrons passing through the ubiquinone pool flows to the alternative oxidase (Day et al., 1994). This enzyme is subject to complex regulation that allows electron transfer and ATP yields to be adjusted to cellular carbon status and redox state (Millar et al., 1993; Vanlerberghe et al., 1995; Rhoads et al., 1998). Oxygen reduction via the alternative oxidase probably yields at most about 1 ATP/2e$^-$, depending on the point of NAD(P)H oxidation. Entry and exit branches in the mitochondrial chain may be important in allowing necessary carbon fluxes to occur, either through the photosynthetic pathway or through respiration. The long-debated question of whether respiration continues in the light is probably better reformulated thus: which respiratory pathways are most important in the light and which are more significant in the dark?

In isolated leaf mitochondria, ADP/O ratios are dependent on the nature of the substrate supplied (Krömer and Heldt, 1991). The highest ADP/O ratios were observed when mitochondria were oxidizing glycine; the lowest were associated with oxidation of external NADH (Krömer and Heldt, 1991). While high ADP/O ratios for glycine oxidation appear at first sight to argue against an important role for the alternative oxidase in photosynthesis, the variability in these ratios emphasizes the metabolic flexibility that leaf mitochondria confer on the photosynthetic cell. The potential for mitochondrial ATP formation in the leaf cell is far from negligible: the maximum capacity of oxidative phosphorylation has been estimated at about 25% of the leaf’s capacity for non-cyclic photophosphorylation at high light, while a value of 10% was derived from experiments with mitochondria respiring at likely cytosolic substrate concentrations (Krömer and Heldt, 1991).

Nitrogen and carbon: complementary electron acceptors, respiratory carbon flows

As well as the processes discussed above, several other reaction pathways could impact upon adenylate status and redox state in photosynthetic cells. These include the oxidative pentose phosphate pathway and sulphate reduction. The former process is probably less important in illuminated photosynthetic cells than in the dark or in heterotrophic cells. Similarly, while the reduction of one sulphate to the level of a thiol requires eight electrons and probably the equivalent of two ATP, rates are 100–1000 times lower than those of carbon fixation. More significant will be photorespiration and nitrogen assimilation which, together with primary carbon metabolism, must account for most of the light energy used by photosynthetic cells. The ATP/reductant balance during these photosynthetic processes has recently been analysed in this laboratory, as a first attempt to extend traditional considerations of this aspect of chloroplast metabolism to the whole of the cell (Noctor and Foyer, 1998). Based on an ATP/2e$^-$ ratio of 1.33, which is close to values experimentally determined in isolated chloroplasts (Davenport and McCarty, 1984), the calculations show that primary nitrogen assimilation and its associated respiratory activity could potentially complement the ATP/reductant requirements of photosynthetic carbon metabolism. The predicted influence of nitrogen assimilation depends on several factors, including its rate relative to CO$_2$ fixation, how reduced the nitrogen source is, and the nature of the amino acids formed.

The effect of nitrate assimilation on the cellular ATP balance is due in part to the fact that the generally accepted pathway of nitrate reduction to the level of an α-amino group requires ten electrons but only one ATP. The amount of ATP required per electron is therefore 7.5 times greater for reduction of CO$_2$ than nitrate. Nitrogen assimilation may also influence cellular ATP status due to linked respiratory carbon flow that is necessary to produce oxo-acids for amino acid synthesis (Fig. 4). The ATP yield of this respiratory flow will depend on the route of carbon flow and also on electron transfer path-
ways in the mitochondrion, as discussed above. One particularly influential factor is the source of NADH for nitrate reductase activity. If this is generated during the oxidative flow of carbon required to generate amino acceptors, then the potential ATP yield is much lower than if NADH is supplied by the malate valve (Noctor and Foyer, 1998). Whichever pathways operate, some ATP must be produced. When excised tobacco leaves were fed sucrose, nitrate assimilation was reported to account for about 25% of electrons during photosynthesis at low light (Morcuende et al., 1998). Although this condition is undoubtedly exceptional rather than typical (Foyer et al., 1999), it does emphasize the potential significance of energy partitioning to the photosynthetic assimilation of nitrate. Respiratory activity necessary for such high rates of nitrogen assimilation may produce considerable amounts of ATP. Indeed, slight increases in ATP levels associated with sucrose feeding to tobacco leaves have been explained in terms of stimulation of flux to organic acids (Morcuende et al., 1998). While some of the ATP produced during respiratory activity in the light may be utilized in sucrose synthesis in the cytosol (Kröm er, 1995), under many conditions this ATP sink may not be sufficiently powerful to account for turnover of all the ATP generated during respiratory activity.

**Could ATP produced in the cytosol or mitochondrion make a significant contribution to chloroplastic fixation of CO$_2$?**

Unlike NAD(P)H, ATP can cross the chloroplast envelope directly, in exchange for ADP (Heldt, 1969). The presence of the ATP/ADP translocator in the chloroplast envelope has still to be adequately explained. Although its primary function in the leaf may be to import ATP into the darkened chloroplast, it is not yet clear how similar flux in the light can be excluded, particularly since cytosolic ATP/ADP ratios are higher than stromal ratios in the illuminated cell (Hampp et al., 1982; Stitt et al., 1982; Heinke et al., 1991).

Maximum *in vitro* rates of the ATP translocator vary with plant age and between species (Huber and Edwards, 1976; Robinson and Wiskich, 1977; Woldegiorgis et al., 1985; Flügge and Heldt, 1991). Direct measurement of rates of ATP import into C$_4$ mesophyll chloroplasts gave values of 33 μmol mg$^{-1}$ chlorophyll h$^{-1}$, though inferred rates were 80–140 (Huber and Edwards, 1976). The latter rates would allow extrachloroplastic ATP sources to make a substantial contribution to carbon assimilation in C$_4$ photosynthesis, which has a high ATP/NADPH requirement (it may nevertheless be noted that the ratio necessary for reactions thought to take place in the mesophyll chloroplast of many C$_4$ species is probably close to 1.5). In C$_3$ species, the chloroplast ATP translocator exhibits lower rates, of the order of 10 and 20 μmol mg$^{-1}$ chlorophyll h$^{-1}$ in spinach and pea, respectively (Huber and Edwards, 1976; Woldegiorgis et al., 1985). These activities would be sufficient to support only a few percent of the light-saturated rate of photosynthesis, though at lower rates of photosynthesis (e.g. at lower light intensities) the proportional contribution would be greater. For instance, ATP import rates of 20 μmol mg$^{-1}$ chlorophyll h$^{-1}$ would support 10% of the ATP demand of the Calvin cycle fixing CO$_2$ at 67 μmol mg$^{-1}$ chlorophyll h$^{-1}$. At this photosynthetic rate, therefore, ATP import could theoretically allow the ATP/NADPH demands of the Calvin cycle to be satisfied by the production at the thylakoid of only 1.35 ATP per NADPH. Interestingly, the activity of the pea chloroplast ATP translocator has been reported to be as high as 4°C as at 22°C (Woldegiorgis et al., 1985), suggesting that ATP import could become increasingly significant as photosynthetic rates slow at lower temperatures. In intact spinach chloroplasts, inhibition of cyclic electron transport decreased CO$_2$ fixation by more than 50% (Backhausen et al., 1994). This effect was largely prevented by the presence of alternative electron acceptors (nitrite or oxaloacetate), but could also be partially reversed by exogenous ATP (Backhausen et al., 1994).

Inferred or measured changes in the rate of chloroplastic adenylate exchange during the development of wheat and pea leaves suggest that the source of ATP for chloroplast metabolism may shift gradually. In young, expanding leaves, elements of heterotrophic metabolism may be retained, in which extrachloroplastic processes may make a substantial contribution to stromal ATP requirements. As development into mature leaf tissue progresses, and biosynthetic processes become increasingly dominated by carbohydrate production, the ATP requirements of the chloroplast *in vivo* probably become satisfied exclusively by photophosphorylation.

**Balancing the books: exchange of phosphate**

Net import of ATP in exchange for ADP would entail net influx of Pi into the chloroplast, in conflict with the abundant evidence suggesting that the total amount of Pi (inorganic + organic) remains more or less constant in this organelle. As has been previously noted (Stitt, 1997), this problem also applies to heterotrophic plastids where the ATP/ADP translocator imports ATP for processes such as starch synthesis (Emes and Neuhaus, 1997; Tjaden et al., 1998). It may also be relevant to ATP/ADP exchange in C$_4$ chloroplasts. The mitochondrial inner membrane has an active carrier that translocates Pi without exchange for other phosphates, but there is, at present, scant evidence for such an activity in plastids. The predominantly chloroplastic location of sulphate assimilation requires sulphate import, which probably occurs mainly in exchange for Pi via the Pi translocator.
(Hampp and Ziegler, 1977; Dumas et al., 1989). Given the comparatively low rates of sulphate assimilation, however, this exchange is likely to make only a limited contribution to the chloroplast–cytosol Pi balance. Isolated spinach chloroplasts become depleted of phosphate on storage, perhaps due to very slow efflux of Pi through the Pi translocator (Mourioux and Douce, 1981). Interestingly, spinach chloroplasts may also transport pyrophosphate without exchange for other phosphates (Lunn and Douce, 1993). The capacity of this activity is more than 10-fold lower than Pi exchange through the Pi translocator, although it is greater than the relatively slow transport of adenylates by spinach chloroplasts (Lunn and Douce, 1993). In view of the gradient between cytosolic and chloroplastic pyrophosphate concentrations in the leaf (Weiner et al., 1987), pyrophosphate transport is likely to result in net influx into the chloroplast and it has been suggested that this activity may be necessary to balance any slow efflux of Pi, as well as Pi exported in exchange for sulphate (Lunn and Douce, 1993). It is therefore unclear which routes act to balance net Pi influx that would result from import of ATP in exchange for ADP. In pea chloroplasts, the adenylate translocator has been reported to translocate pyrophosphate and phosphoenolpyruvate (Woldegiorgis et al., 1985). Although counterexchange of these compounds would result in net movement of phosphate, this would presumably compete with adenylate transport and, as discussed above, the direction of pyrophosphate transfer is likely to be into, rather than out of the chloroplast. Efflux of Pi may therefore be as important as the capacity of the translocator itself in determining the rate of ATP import into plastids. As in many other areas of photosynthesis (Walker, 1976; Stitt, 1986, 1997), Pi could play a central regulatory role, perhaps setting an upper limit on the contribution the rest of the cell can make to the ATP demands of plastidic metabolism. In the case of chloroplasts, limitations on the contribution made by external ATP might simply lead to greater engagement of internal pathways able to increase the amount of ATP produced per NADPH. This would entail the chloroplast and mitochondrion becoming more autonomous in their ATP relations and continued respiratory carbon flow would need to occur through pathways of lower ATP yield. Knowledge of relations between chloroplastic and mitochondrial ATP production is likely to be advanced by work with various types of transgenic plants that have recently been produced (Vanlerbergh et al., 1995; Tjaden et al., 1998; Backhausen and Scheibe, 1999).

### Adenylate status and redox state in signal transduction

Cellular ATP/NADPH balance is not only crucial in metabolism but may also be important in signal transduction. It has been known for some time that LHClI kinase activity is controlled by the redox states of components close to plastoquinone and the cytochrome b6f complex (Horton et al., 1981). Growing evidence suggests that redox components in this region, as well as those close to the ubiquinone/cytochrome bc1 region of the mitochondrial chain, may exert influence over transcription and/or translation (Vanlerbergh and McIntosh, 1994; Danon and Mayfield, 1994; Escoubas et al., 1995; Karpinski et al., 1997, 1999; Pfannschmidt et al., 1999; Foyer and Noctor, 1999b). It has been noted that these electron transport components are ideally suited to sensing redox state, because their midpoint redox potentials are intermediate on the cellular redox scale (Allen, 1995). While this is true, these components are also those whose redox state should be most responsive to adenylate status due to changes transmitted via transmembrane electrochemical potentials.

### Conclusion

Simple theoretical calculations for an isolated adenylate pool dictate that even slight imbalances between ATP generation and utilization will lead to marked increases in ATP/ADP ratios (Fig. 1). This is not observed in practice because the multitude of ATP-linked pathways in the photosynthetic cell confers a high degree of flexibility on cellular adenylate status. Energetic flexibility means that relatively large changes in flux through a given pathway involving ATP and ADP will probably be required to override the stabilizing influence of complementary changes in other pathways and to cause even minor changes in ATP/ADP ratios. Disruption of metabolism due to excessive fluctuations in redox state and adenylate status in an unstable environment (changes in light intensity, temperature, nutrient availability, water potential) is therefore minimized. Prolonged exposure to stressful conditions that perturb this homeostasis will lead to redox-mediated modifications in gene expression.

The relative significance of the pathways that act to balance ATP/redundant status can vary with conditions, with plant development, or between species, making assignment of specific roles to specific pathways difficult, perhaps even pointless. In the cellular biochemical economy, as in fiscal economies, currency can be used and recycled by numerous processes whose individual contributions are often very difficult to gauge. This review has attempted to emphasize that even if the two central currencies of the cellular economy have relatively invariant energetic values, their local exchange rates may be considerably more plastic.

### References


