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Mini review

The mechanisms contributing to photosynthetic control of electron transport by carbon assimilation in leaves

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Abstract

'Photosynthetic control' describes the processes that serve to modify chloroplast membrane reactions in order to co-ordinate the synthesis of ATP and NADPH with the rate at which these metabolites can be used in carbon metabolism. At low irradiance, optimisation of the use of excitation energy is required, while at high irradiance photosynthetic control serves to dissipate excess excitation energy when the potential rate of ATP and NADPH synthesis exceed demand. The balance between ΔpH, ATP synthesis and redox state adjusts supply to demand such that the [ATP]/[ADP] and [NADPH]/[$NADP^+$] ratios are remarkably constant in steady-state conditions and modulation of electron transport occurs without extreme fluctuations in these pools.

Abbreviations: FBPase – Fructose-1,6-bisphosphatase; PS I – Photosystem I; PS II – Photosystem II; Pi – inorganic phosphate; PGA – glycerate 3-phosphate; PQ – plastoquinone; Q_A – the bound quinone electron acceptor of PS II; q_P – Photochemical quenching of chlorophyll fluorescence associated with the oxidation of Q_A; q_N – non-photochemical quenching of chlorophyll fluorescence; q_E – non-photochemical quenching associated with the high energy state of the membrane; RuBP – ribulose-1, 5-bisphosphate; TP – triose phosphate; Φ_p – intrinsic quantum yield of PS II; Φ_s – quantum yield of electron transport; Φ_{CO_2} – quantum yield of CO_2 assimilation

Introduction

An essential feature of the process which ensures coupling of electron transport to ATP synthesis in energy transducing membranes is that the proton motive force can bring about feedback inhibition of electron flux. In respiratory systems, the operation of the regulatory circuit between electron transport and the proton motive force was deduced from the restriction of electron transport rate observed under conditions of ADP limitation and its relief upon addition of ADP or uncoupling agents which dissipate the proton motive force. This phenomenon was called 'respiratory control' and it conferred physiological benefit since respiratory substrates would only be oxidised at a rate determined by the cellular demand for ATP and thus energy was conserved. An equivalent process of 'photosynthetic control' can be demonstrated in isolated thylakoids. This thermodynamic constraint on thylakoid electron transport by the develop-

ment of the proton motive force is an established and accepted phenomenon. The proton motive force across the thylakoid membrane consists almost entirely of a ΔpH and it is, therefore, the chemical potential of protons accumulated in the thylakoid lumen that is involved in the regulation of electron transfer. Although the mechanism of this regulation remains uncertain it has been recognised that the decrease in rate of oxidation of plastoquinol at the cytochrome *b–f* complex with decreasing lumen pH is a fundamental feature of the restriction of electron transport (Bendall 1982, Rich 1982). However, unlike respiratory control, photosynthetic control would not seem to give a 'physiological' advantage since 'light' cannot be stored. Moreover, restricted electron flow at the site of plastoquinol oxidation is likely to be detrimental since it would lead to a build-up of closed PS II reaction centres and photoinhibition (Kyle et al. 1985). However, recent work has indicated that PS II centres remain open even under high light (Weis et al. 1987), indicating that other restrictions may exist which are offsetting the effects of any limitation at the site of plastoquinol oxidation. Furthermore, the light-saturated rate of photosynthetic electron transport in vivo approaches the rate of uncoupled electron transport in vitro, suggesting that the lumen pH may be exerting only minimal restriction on the rate of electron transport.

It is therefore relevant to consider whether the classical concept of photosynthetic control is an important factor during photosynthesis in vivo. It is clear that in vivo the electron transport system is integrated with the metabolic processes of the plant cell (Horton 1985a,b) and that 'passive' control of this kind may be insufficient or even detrimental to the efficient operation of photosynthesis. Two additional types of control have been described. Firstly, adjustments of the efficiency of light harvesting and primary photochemistry have been identified which seem to enable efficient light utilisation in limiting light and effective dissipation in saturating light. Secondly, the enzymes of the Benson–Calvin cycle have been shown to undergo light-dependent regulation in response to stromal redox and energy states. More generally, it has been recognised that optimisation of the levels of intermediates (ΔpH, ATP/ADP, Pi, NADPH/NADP) is necessary if high rates of their production and utilisation are to co-exist; thus whilst a high ΔpH and high assimilatory power would undoubtedly favour high rates of carbon assimilation, this would be impossible since there would be a restriction on electron transport. An additional complication is the requirement of providing ATP and reducing power in the correct stoichiometry; changes in metabolic demand in this case are accommodated by changes in the pattern of electron transport, either via a PS I cycle, the Mehler reaction or within the *b–f* complex itself. The potential for imbalance exists here too; for example in isolated chloroplasts it has been shown that an excess ΔpH built up by the activity of PS I cyclic electron transport can reduce the rate of photosynthesis (Slovacek and Hind 1980). These considerations lead us to suggest that 'photosynthetic control' should be re-defined to describe those processes which serve to modify thylakoid membrane reactions in order to co-ordinate the synthesis of ATP and NADPH with their rate of use in carbon assimilation. This broader definition allows an integrated view to be taken of a variety of adaptive, protective and regulatory processes that have been described in photosynthetic systems. In this review, these mechanisms are outlined and it will be suggested how they allow effective photosynthetic activity under the changing conditions of the natural environment.

Metabolic aspects of the state transition

The state transition defines the process by which the distribution of excitation between PS II and PS I can be adjusted. It is a process that corrects for imbalance between rates of excitation of the photosystems and therefore ensures maximum quantum efficiency under light-limiting conditions. The molecular basis of the state transition is the reversible phosphorylation of LHC-II, the protein kinase being controlled by the redox state of an electron carrier operating between the two photosystems (Allen et al. 1981, Horton and Black 1980, Telfer et al. 1983). There is abundant evidence that phosphorylation causes a 'mobile' pool of LHC-II to dissociate from the PS II core and migrate laterally into the un-

appressed membranes. There is still some uncertainty as to whether phospho-LHC-II transfers excitation energy to PS I. Nevertheless, the state transition does change the relative absorption cross sections of PS II and PS I (Horton 1983). The existence of the state transition in vivo is well-documented under special conditions of low intensity narrow spectral bandwidth irradiance chosen to specifically excite PS II or PS I. More recently, it has been possible to demonstrate a state 1–2 transition upon transfer from darkness to illumination with broadband white or red light (Horton and Hague 1988). The size of the decreases in fluorescence observed indicated that there may be complete phosphorylation of the mobile LHC-II. The state transition appears to saturate at very low light intensity (approx. 5–10% saturating for CO_2-dependent O_2 evolution), suggesting that it is necessary for high efficiencies of light utilisation at low irradiance. However, there is still much more work needed to assess the role of the state transitions in vivo . It is also clear that the control over the LHC-II kinase by the redox state of the plastoquinone pool (Allen et al. 1981, Horton and Black 1980) does not appear to fully account for many subsequent observations, especially the inhibitory effect of ΔpH (Fernyhough et al. 1984). In fact, the activation of the kinase by antimpycin A (Oxborough et al. 1987) and the lack of activation in mutants deficient in the cytochrome b_6f complex (Bennett et al. 1988) suggest redox control is via a component in or associated with this complex possibly the protein responsible for ferredoxin-quinone reductase activity. There appears to be a link between the control of LHC-II phosphorylation and of the energy-dependent quenching of chlorophyll fluorescence (q_E), that may be central to photosynthetic control (Horton 1989, Horton et al. 1989). Thus it is necessary to consider what is meant by the notion that protein phosphorylation corrects for imbalance between PS II and PS I. For linear electron transport to NADPH this means ensuring *equal* rates of excitation but this might not necessarily apply in vivo . There is evidence obtained from intact maize mesophyll chloroplasts that the phosphorylation of LHC-II is promoted by an increase in the demand for ATP in excess of that for NADPH (Fernyhough et al. 1984). This was rationalised in terms of increasing the provision of ATP by increasing the extent of PS I-cyclic electron transfer. It was further shown that the increase in ΔpH which results when the ATP demand was low was the factor reducing the level of protein phosphorylation (Fernyhough 1984, Oxborough et al. 1987). These observations have led to the hypothesis that the distribution of excitation between PS II and PS I, determined by the phosphorylation of thylakoid polypeptides responds to the metabolic requirement for ATP and NADPH (Horton 1985a,b, 1989, Horton et al. 1988, 1989a,b); furthermore, it is suggested that the transition to state 2 occurs not only to correct a redox imbalance but to raise and stabilise the ΔpH in low light, under conditions of high ATP usage (e.g., assimilation of NH_3 into protein) or perhaps under stress conditions when there may be partial uncoupling.

PS II and energy dissipation

At light levels approaching saturation and beyond another physiological reason arises for regulation of thylakoids. Excess light is generally acknowledged to be deleterious. Not only can antenna pigments become photooxidised but the reaction centre of PS II is sensitive to light-induced inactivation (photoinhibition), most likely a result of damage to the D1 polypeptide (Kyle et al. 1985, Powles 1984). Moreover, the requirement for control of redox poise and the avoidance of over-reduction is as important in high light as in low light. Processes that prevent over-reduction are hence beneficial and these are of two kinds:

a) alternative electron transfer pathways that keep Q_A oxidised and prevent redox feedback to PS II due to limitation by electron transport beyond PS II and by carbon assimilation;
b) dissipation of excitation energy by non-radiative decay in the antennae pigments or in the reaction centre.

We propose that such mechanisms should be viewed not only in terms of having a long-term role in photo-protection but also as providing the basis for short-term 'photosynthetic control' of

electron transport either independently of, or in addition to, control of plastoquinol oxidation by intrathylakoid acidification. This feedback control of PS II would be responsive to potential imbalance between the level of irradiance and the capacity of carbon assimilation.

The quantitative analysis of chlorophyll fluorescence quenching provides a means of assessing these processes (Horton and Hague 1988, Krause et al. 1988, Oxborough and Horton 1987, Weis et al. 1987, Weis and Berry 1987). The quantum yield of non-cyclic electron transport is a function of the concentration of open PS II reaction centres and the efficiency by which absorbed excitation is transfered to, and utilised (productively) by, the open PS II reaction centres. Changes in the quantum yield of non-cyclic electron flow can be followed by the measurement of simple chlorophyll fluorescence parameters, quantitatively related to measurements of O_2 evolution or CO_2 uptake. For example, as the level of irradiance of a leaf is increased, a compensatory relationship between photochemical quenching (q_P) and non-photochemical quenching (q_N) is observed, implying that non-radiative dissipation is increased as photochemistry becomes light-saturated. Of importance is that q_P does not decrease in proportion to the measured quantum yield of electron transport as would be predicted theoretically and as is observed when measurements are made on uncoupled thylakoids or isolated PS II particles (Horton et la. 1989a,b). In other words, the decline in photosynthetic efficiency in leaves is not only due to closure of PS II reaction centres, but to a decrease in quantum yield of the open centres (Weiss et al. 1987). Thus, in high light, there is an estimated decline in intrinsic yield of PS II (Φ_p), defined as the quantum yield of electron transport (Φ_s) measured from the rate of O_2 evolution or CO_2 uptake normalised upon q_P.

The decrease in Φ_p has now been observed in the leaves of a variety of species (Genty et al. 1989, Horton et al. 1988, Peterson et al. 1988, Sharkey et al. 1989, Weis et al. 1987, Weis and Berry 1987), in algal cells (Horton et al. 1989b), in isolated protoplasts (Horton and Hague 1988), intact cholorplasts (Krause and Laasch 1987, Krause et al. 1988) and coupled thylakoids (Horton et al. 1989a, Oxborough and Horton 1988, Rees and Horton 1990, Noctor and Horton 1990). Restricting the turnover of the products of the thylakoid reactions at constant light intensity elicits a similar decrease in Φ_p. For example, decreasing the supply of CO_2 to leaves causes a decrease in Φ_p (Peterson et al. 1988, Sharkey et al. 1989). In intact chloroplasts illuminated in the absence of bicarbonate but in the presence of glycerate 3-phosphate, the rate of O_2 evolution rapidly declined from a peak value while q_P remains relatively constant (Foyer et al. 1988). When intact chloroplasts are illuminated in the absence of Pi, ΔpH (measured by 9-aminoacridine fluorescence quenching) increased as Pi became limiting, correlating with a decline in the rate of electron transport (Furbank et al. 1987). However Q_A remained relatively *oxidised*, despite the decreasing rate of electron transport. Thus, decreases in Φ_p occured when thylakoid ΔpH was high, under conditions where classical 'photosynthetic control' of electron transport would lead to a restriction on plastoquinol oxidation, tending to cause an increased *reduction* of Q_A. Table 1 shows a series of treatments of intact spinach chloroplasts where the rate of electron transport and non-photochemical quenching were varied by the addition of glycerate 3-phosphate, CO_2 and antimycin A, an inhibitor of cyclic photophosphorylation and energy dependent fluorescence quenching (Oxborough and Horton 1987). It is seen that in the presence of bicarbonate compared to glycerate-3-P, the rate of O_2 evolution is doubled yet q_P increases by only 25%; i.e., there is a large increase in Φ_p. Even more dramatic is the effect of antimycin A which stimulates O_2 evolution and yet causes reduction of Q_A. Similar observations have been made upon uncoupler-stimulated O_2 evolution in both intact chloroplasts (Krause and Laasch 1987) and thylakoids (Horton 1989a).

The mechanistic basis for the apparent decline in the efficiency of PS II has been the subject of much debate. It is difficult, especially in vivo to distinguish between changes in the delivery of excitation to the reaction centre and the inefficient use of the excitation by the centre in giving rise to measured photosynthesis. Similarly, it is difficult to identify the event or process which is signalling the balance between incident irradiance and metabolic capacity and so trigger-

Table 1. Variations in the relationship between q_P and the rate of O_2 evolution intact spinach chloroplasts. In each case additions were made prior to illumination except in treatment 1 where $NaHCO_3$ was added after 7 min in the light. Quenching coefficients (calculated according to Schreiber et al. (1986) were determined at the point in the time-course where the rate of oxygen evolution was maximum. O_2/q_P is the instantaneous rate of O_2 evolution divided by q_P

Treatment	Additions	q_N	q_P	O_2*	O_2*/q_P
1	glycerate 3-phosphate (0.3 mM)	0.8	0.4	41	103
	+HCO_3(10 mM)	0.6	0.5	90	181
2	glycerate 3-phosphate (0.3 mM) +antimycin A (0.5 mM)	0.05	0.18	76	420
3	+HCO_3(10 mM)	0.4	0.35	75	214
4	+HCO_3(10 mM) +Pi(0.3 mM)	0.4	0.43	88	206

* in μmol O_2 h^{-1} mg^{-1} chlorophyll.

ing the decline in efficiency. All work, so far, has dealt with the correlations made between the changes in the various parameters of chlorophyll fluorescence yield using simple mathematical models to interpret them. A striking correlation was first reported between the decline in Φ_p and the development of q_N, leading to the suggestion that it is an increase in thermal dissipation that decreases photochemical efficiency. The linearity of the relationship between q_N and Φ_p suggested that in high light the PS II reaction centre changes into a photochemically inactive, quenched state (Krause et al. 1988, Weis et al. 1987, Weis and Berry 1987). Broadly similar (though quantitatively different) data showed that Φ_p was directly proportional to the steady-state F_v/F_m ratio (Genty et al. 1989), a result that could be interpreted to mean that changes in dissipation in the antenna chlorophylls of PS II were involved. In fact, it can be concluded from this latter data that in leaves non-photochemical dissipation can completely account for the observed changes in PS II efficiency.

Observations of isolated thylakoids, however, indicate that a decrease in Φ_p in high light does not necessarily require formation of q_N but may involve instead an alternative electron transfer pathway such as PS II cycle which would increase photochemistry but not photosynthesis, therefore lowering Φ_p (Horton et al. 1988, 1989a,b, Oxborough and Horton 1988). Evidence for cycling in high light (Falkowski et al. 1988), in the presence of ATP (Schreiber and Rienits 1986) and upon donor side limitation (Heber et al. 1979) has also previously been presented, but it remains to be shown whether a cycle of sufficient capacity exists in vivo. There are other possibilities for photochemical dissipation, for example, reduction of O_2 by PS I and the reduction of H_2O_2 by ascorbate peroxidase has a high capacity (Anderson et al. 1983) and would increase q_P and not Φ_s (see also Fig. 1). In these cases, to refer to a decline in Φ_p is misleading since there is no change in either light-harvesting or electron transfer in PS II. The decline in Φ_p probably is the composite result of effects imposed by a number of processes. Dissipation of excitation energy by both non-photochemical and photochemical mechanisms simultaneously according to the metabolic and thermodynamic restrictions placed on the system (Horton 1989, Horton et al. 1989b). What is clear is that in all types of control there is an obligatory requirement for the thylakoid ΔpH. Energy-dependent quenching (q_E), that depends on the ΔpH, is the major component of q_N under most conditions (Quick and Horton 1984) and in isolated thylakoids the decline in Φ_p is eliminated by uncoupling agents (Horton et al. 1989a, Oxborough and Horton 1988). ΔpH has been shown to build up with increasing irradiance in vitro and to increase when the rate of ATP consumption is suppressed. Thus, it is ΔpH, or more probably, the lumen pH that is the regulatory signal involved in control of PS II, although it should be pointed out that the link between ΔpH and q_E is not understood. From the experiments described below it is also likely that there is little change in ΔpH upon the change in irradiance in vivo, which elicit significant changes in q_E. It is pos-

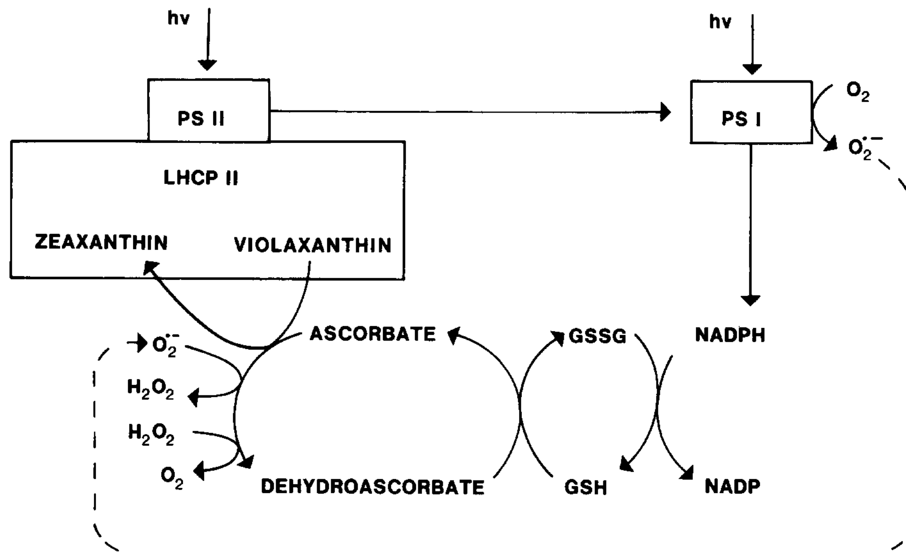

Fig. 1. Diagrammatic representation of the central position of ascorbic acid in the dissipative processes in PS II and PS I. The primary product of ascorbate peroxidase activity is monodehydroascorbate and the enzyme monodehydroascorbate reductase takes part in the regeneration of ascorbate using NAD(P)H as electron donor (Hossain et al. 1984).

sible to explain this by the existence of a localised domain of protons specifically controlling Φ_p (e.g. Dilley et al. 1987). Alternatively, an irradiance-dependent switch (e.g., changes in redox state) could be involved that could increase q_E with little change in ΔpH once q_E formation has been initiated by the high energy state of the membrane. The relationship between q_E and ΔpH has been shown to be variable; dibuccaine decreases ΔpH but not q_E (Krause et al. 1988) and antimycin A removes q_E but not ΔpH (Oxborough and Horton 1987). Oxidising conditions seem to increase the sensitivity of q_E to ΔpH (Oxborough and Horton 1988). It has been suggested that the light-induced formation of zeaxanthin from violaxanthin is in some way involved in q_E (Demmig-Adams et al. 1990). It has been shown that the presence of zeaxanthin increases the sensitivity of q_E to ΔpH (Rees et al. 1989) so that an increase in q_E may result from an increase in zeaxanthin rather than in ΔpH.

There is also evidence that light-induced formation of zeaxanthin, a process also thought to be dependent on the lumen pH, is also associated with the development of a more slowly reversible dissipation of excitation energy in the thylakoid. Yamamoto (1979) first suggested that the fact that the cycle is sensitive to events both before and after PS I could facilitate a regulatory role, possibly through effects on membrane properties. Quenching of fluorescence caused by the increase in non-radiative energy dissipation have been correlated with the formation of the carotenoid, zeaxanthin under photoinhibitory conditions (Demmig et al. 1987). Zeaxanthin formation, which in this way prevents photoinhibitory damage to the PS II reaction centres requires ascorbic acid as substrate (Yamamoto 1979). The regeneration of ascorbic acid occurs via the ascorbate-glutathione cycle which is, in turn, dependent on the concentration of NADPH (Foyer and Halliwell 1976). The ascorbate-glutathione cycle can itself provide a mechanism for the dissipation of excess reducing power on the chloroplast via detoxification of H_2O_2 (Anderson et al. 1983). Hence, ascorbate could play a role not only in the regulation of the redox state of the stroma but also in photosynthetic control if it were to be limiting for violaxanthin de-epoxidation. Therefore, in situations of excess irradiance ascorbate could firstly serve to detoxify O_2^- and H_2O_2 generated as a result of increased electron drainage to O_2 in the Mehler reaction and secondly could participate in the decrease in PS II activity through a stimulation of the xanthophyll cycle (Fig. 1). The integration of the control of excitation energy dissipation and the enzymic processes involved in

protection against oxidative damage is to be expected; the deleterious effects of the generation of extreme redox potentials in the chloroplast will be amplified by the presence of high exciton densities in the pigment beds.

In summary, although the molecular mechanisms involved are poorly understood, it is clear that the changes in quantum efficiency of electron transport are associated not only with a closure of PS II reaction centres but also with active regulation of the capture and productive use of excitation energy by PS II. This regulation of thylakoid function is dependent on the ΔpH. Thus, although the structure of the photosynthetic apparatus allows the achievement of a remarkably high quantum yield of photosynthesis, reversible decreases in the efficiency of light harvesting and photochemistry by PS II, brought about by at least three different regulatory mechanisms, should be seen as normal metabolic events in the life of plants.

Regulation of PS I electron flow

Important insights into the regulation of thylakoid electron transport have come from measurements of the redox state of the primary donor of the PS I reaction centre, P_{700}. Following the transfer of excitation energy from the PS I pigment bed, and given the availability of PS I electron acceptors P_{700} becomes oxidized. This oxidation can be monitored in vivo using light-induced absorbance changes around 820 nm (Harbinson and Woodward 1987).

The steady state level of P_{700} will be principally determined by three processes:

i) the rate of photochemical oxidation of P_{700};
ii) the rate of charge recombination;
iii) the rate of reduction of photochemically oxidized P_{700} by electrons derived from plastocyanin.

The first process is function of irradiance (provided that there is a pool of oxidised PS I acceptors) and occurs with a quantum efficiency of 1 at wavelengths (~700 nm) where PS II does not absorb and compete. In a nitrogen atmosphere, or when the Benson–Calvin cycle is inactive, P_{700} oxidation does appear to be restricted due to a limitation of acceptor pool activity, but in air under steady-state conditions, there is little evidence for restriction of electron transport of the reducing side of PS I (Harbinson and Hedley 1989). Although a series of back reactions from the PS I acceptor pool have been described when electron transport from P_{700} has been blocked in vitro, charge recombination is probably not significant when there are stable PS I electron acceptors available. The third process is determined by the rate of the reaction between plastoquinol and the cytochrome b_6/f complex (Rich 1982) and is controlled by the concentration of plastoquinol and the intrathylakoid pH (Bendall 1982). This reaction is considered to be the rate-limiting step for thylakoid electron transport (Stiehl and Witt 1969). Following the removal of excitation, a fast phase ($t_{1/2}$ of 20–800 μs) of reduction of oxidized P_{700} has been reported and is due to any electrons resident on plastocyanin or cytochrome f (Haehnel 1982, Haehnel et al. 1980). Once this pool is exhausted it must be replenished by reductant derived from the plastoquinol pool via the Reiske–FeS centre. The half-time for the reduction of oxidized P_{700} in this phase is variable but is greater than 4 ms (Harbinson and Hedley 1989). Measurements of the decay of P_{700}^+ following a light/dark transition with millisecond time resolution, therefore, affords a means of determining the kinetics of the rate-limiting step of thylakoid electron transport in vivo. In addition, because the rate of P_{700} oxidation is linearly dependent on light intensity, the degree of increase in the steady state level of P_{700}^+ upon increasing irradiance will also provide an estimate of $t_{1/2}$ for its re-reduction. If carbon metabolism modulates the rate of thylakoid electron transport then it would be expected to alter the rate of P_{700}^+ reduction at this step, so changing the resistance to the flux of reducing equivalents between the two photosystems. Such a change in resistance could be brought about by a decrease in intrathylakoid pH.

In leaves in air, direct measurements of P_{700}^+ reduction following a light–dark transition show that $t_{1/2}$ of the phase dominated by the kinetics of the reaction between plastoquinol and the cytochrome b–f complex is independent of irradiance (Harbison and Hedley 1989). This is so even though the $t_{1/2}$ for P_{700}^+ reduction decreases with the duration of irradiance of dark-adapted

leaves (Harbinson and Hedley 1989). Even after 90 s of irradiance of dark-adapted leaves when almost all the P_{700} pool is oxidized at high irradiances the $t_{1/2}$, though larger than under steady state conditions, is still independent of irradiance. This suggests that over the range of irradiances employed there is no change in the resistance for electron flow between the photosystems in the thylakoid membranes. The fact that there is little change in the degree of control exercised by the Benson–Calvin cycle over electron transport even though light saturation has occurred shows that carbon assimilation is able to keep pace with electron transport over a large range of irradiance. We conclude that it is the (constant) resistance of electron transport between platoquinol and the cytochrome *b–f* complex that limits CO_2 fixation and determines the profile of the irradiance curve for photosynthesis under conditions that inhibit photorespiration. Consistent with this rationale is the observation that a close linear relationship exists between the increasing oxidation state of P_{700} and the declining quantum efficiency of CO_2 fixation at increasing irradiance (Weis et al. 1987).

The constancy of the $t_{1/2}$ of P_{700}^+ reduction, and by implication, the kinetic restriction of intersystem electron flow suggests that there may be no change in the back-pressure on electron flow as the light intensity is increased. This implies that the intrathylakoid pH is also independent of irradiance, or at least does not change sufficiently to bring about a detectable change in rate. The measured $t_{1/2}$ of 4–5 ms is low and close to the maximum value obtained in vitro in uncoupled thylakoids and is consistent with the fact that the light saturated rate of electron transport in vivo is close to the uncoupled rate of electron flow in vitro. It is possible, however, that an inhibitory effect of the ΔpH is masked by the antagonistic effect of an increase in the reduction state of plastoquinone. The redox state of the plastoquinone pool is not well-characterised in vivo but it saturates at only 15 $\mu mol\ m^{-2}\ s^{-1}$ irradiance in the rhodophyte *Porphyridium aeruginosum* and in spinach thylakoids (Amesz et al. 1972). If this is also the situation in higher plants in vivo then all the changes resistance to electron flux between PS II and PS I will be due to changes in intrathylakoid pH and constant resistance indicates an unchanged lumen pH. A further argument to support this contention comes from the observation that the value of 5 ms for the $t_{1/2}$ of P_{700}^+ in vitro was determined when the plastoquinone pool was fully reduced by duroquinol.

Measurements of the irradiance response of P_{700} oxidation over a range of CO_2 partial pressures in 2% O_2 show that the P_{700}^+ pool increases more rapidly with irradiance as the CO_2 partial pressure is decreased (Fig. 2A). Decreasing CO_2 partial pressures act to increase the resistance to electron flow between PQH_2 and P_{700}^+. A decreased flux through the Benson–Calvin cycle is paralleled by a larger $t_{1/2}$ for P_{700}^+ reduction. So as with photosynthetic induction, there is an interaction between the Benson–Calvin cycle and electron transport even though it is not changed by irradiance. The linear relationship between the percentage reduction of P_{700} and the quantum efficiency of CO_2 fixation is consistent even at low CO_2 partial pressures (Figs. 2A and B). Restriction of electron flow on the electron acceptor side of PS I is not observed under steady-state conditions in air even at low temperatures (0°C) when photosynthesis is severely limited; the half-time for P_{700}^+ reduction was again independent of irradiance even though the value is greater under these conditions.

The observations showing the 'resistance' for electron flow between PQH_2 and P_{700}^+ is firstly, independent of irradiance but, secondly, is increased by decreased CO_2 partial pressures and also decreases during photosynthetic induction appear to be contradictory. However, decreased CO_2 partial pressure results in an increase of the assimilatory power within leaves, which could result in an increased ATP/ADP ratio and an increase in the transthylakoid ΔpH, thereby decreasing the rate of reaction between PQH_2 and the cytochrome *b–f* complex. Light scattering and q_E, (both of which are determined by ΔpH) increase as the CO_2 partial pressure decreases (Dietz et al. 1985, Sivak et al. 1985). Also during induction when CO_2 assimilation is minimal the ΔpH has been shown to be maximal both in vitro and in vivo. At low CO_2 partial pressures the decreased rates of electron flux imposed by a lower intrathylakoid pH may be necessary to maintain a large phosphorylation potential to

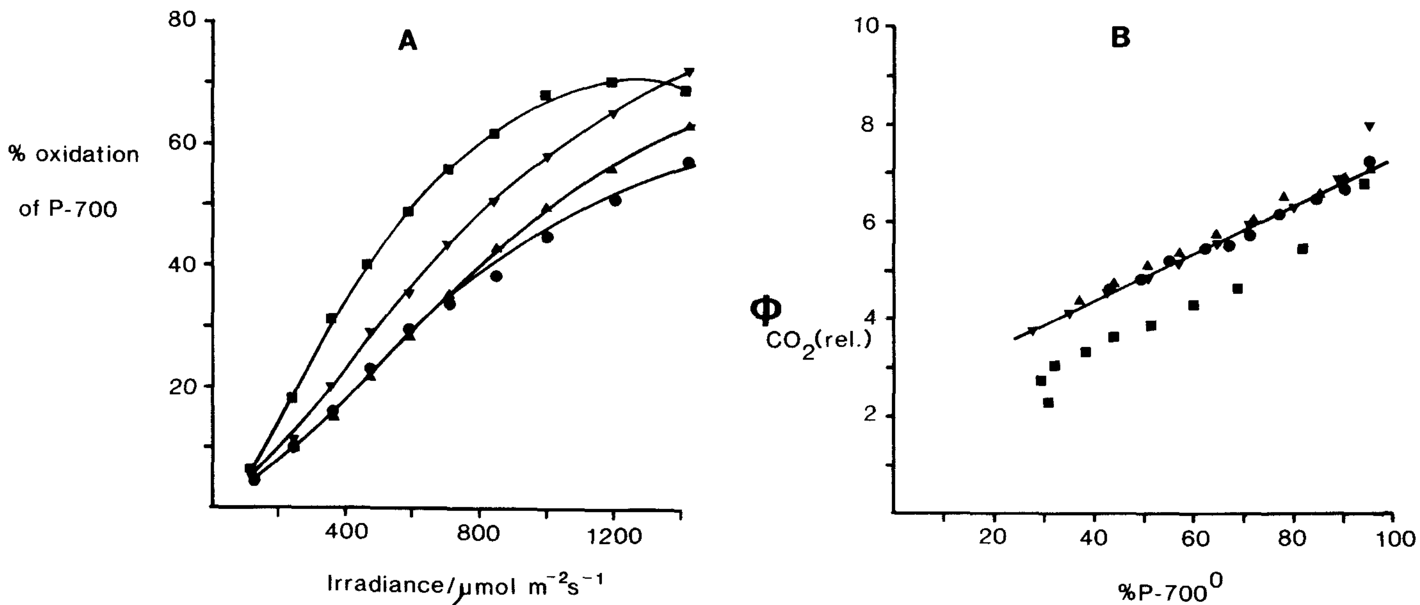

Fig. 2. (A) The irradiance dependence of the percentage oxidation of P_{700} under steady-state conditions at four different CO_2 partial pressures in 2% O_2 and N_2. (●) 400 ppm CO_2, (▲) 300 ppm CO_2, (▼) 180 ppm CO_2, (■) 80 ppm CO_2. (B) The dependence of the quantum efficiency of CO_2 fixation on the percentage non-oxidation of P_{700} (P_{700^o}). The leaf was exposed to gas containing 400, 300, 180 and 80 ppm CO_2 (symbols as in A) in 2% O_2 and N_2. Each data point corresponds to the quantum efficiency of CO_2 fixation and the percentage non-oxidation of P_{700} under steady state conditions at a range of irradiances; (the failure to intersect at the origin is due to an edge-effect of the irradiances on the leaf).

drive the Benson–Calvin cycle when the CO_2 concentration becomes progressively limiting.

In summary, the stability of the resistance of electron flow between PS II and PS I with increasing irradiance and the linear relationship between Φ_s and P_{700} suggest that the progressive saturation of the CO_2 fixation rate is due to a limitation within the electron transport system. If there were to be limitation within the Benson–Calvin cycle then at light saturation either the ΔpH would increase or PS I acceptors would accumulate in the reduced state. The quantum efficiency of the photosystems declines because of the relatively slow rate of P_{700}^+ reduction and is associated with closure of reaction centres and also, in the case of PS II, with a decrease in the intrinsic photochemical efficiency. However, the resistance of electron flow can be increased if the activity of the Benson–Calvin cycle is suppressed by CO_2-limitation or when the catalytic capacity of the component enzymes is low, for example, during induction. In these cases photosynthetic control may be expressed at the level of plastoquinone oxidation.

Regulation via the cycling of adenine and pyridine nucleotides

The ATP and NADPH produced by the light reactions of photosynthesis are used primarily for the reduction of CO_2 in the Benson–Calvin cycle. CO_2 assimilation can be limited either by the supply of CO_2 or by the availability of ATP and NADPH. It was shown above that the electron transport system is poised to accommodate changes in the balance between energy input and energy utilisation by metabolism. Similarly, there is abundant evidence that the activity of the Benson–Calvin cycle is regulated to respond to fluctuations in energy supply via the modulation of enzymes. The redox-mediated activation of fructose-1,6-bisphosphatase and sedoheptulose-1,6-bisphosphatase through the thioredoxin system provides an attractive mechanism by which changes in the flux through the electron transport system may be communicated to the Benson–Calvin cycle. Similarly, regulation of the activation state of RuBP carboxylase may respond to the availability of ATP in the stroma (Robinson and Portis 1988). Measurements of enzyme activities made simultaneously with those of the quantum efficiencies of PS II and PS I have shown that with increasing irradiance the activation states of the thiol-modulated enzymes NADP-malate dehydrogenase and fructose-1,6-bisphosphatase and also ribulose-1,5-bisphosphate carboxylase increase linearly in relation to the estimated electron flux through the photosystems (Fig. 3). The modulation of enzyme activities may be essential in order to stabilise the pool sizes of metabolites and to

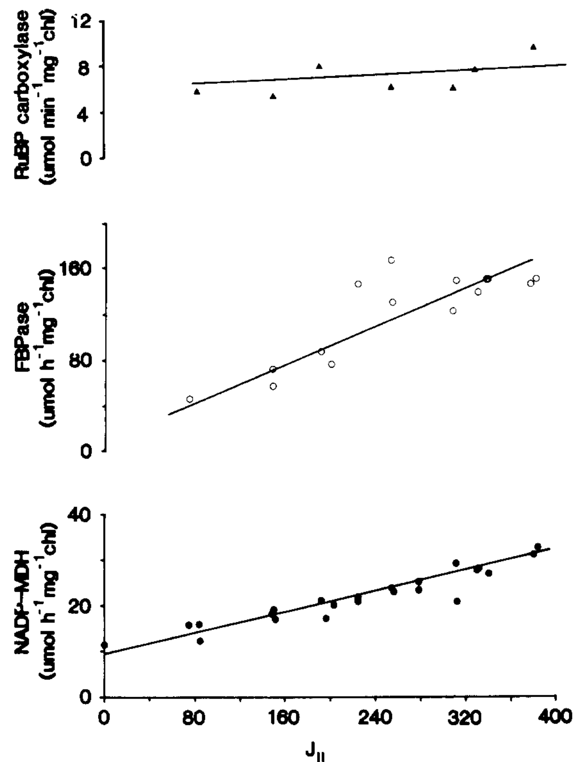

Fig. 3. The relationship between the flux of electrons through PS II (J_{II}) and the activities of the stromal enzymes NADP-malate dehydrogenase (NADP–MDH ●), fructose-1,6-bisphosphatase (FBPase ○) and ribulose-1,5-bisphosphate carboxylase (RuBP carboxylase ▲) in pea leaves. J_{II} was obtained from the product of the irradiance and the quantum efficiency of PS II, calculated from chlorophyll fluorescence (Genty et al. 1989). Simultaneous measurements were made on leaf samples at steady state photosynthesis in air and varying irradiance from darkness to 1400 μmol $m^{-2} s^{-1}$.

prevent the build-up of one or more metabolites at the expense of all the others (Woodrow and Berry 1985).

It should be emphasised that the localisation of the electron transport system within the same compartment as that of CO_2 assimilation requires that a compromise be reached between the levels of assimilatory force ([ATP/[ADP][Pi] × [NADPH]/[NADP]) that can be achieved whilst still allowing electron transport to proceed. Therefore, co-ordination of the rates of ATP and NADPH production and the demand for these metabolites must be the result of a concerted response to the phosphorylation and redox potentials of the stroma. It has been demonstrated that intact chloroplasts do not adjust phosphorylation potentials in the stroma to high levels (Giersch et al. 1980). This is in strong contrast to the very high ATP/ADP ratios generated by isolated thylakoids (Giersch et al. 1980) and may result from metabolic constraints on ATP synthesis. During CO_2-dependent O_2 evolution in protoplasts and leaves the [ATP]/[ADP] ratios are typically found to be in the range of 1–3. The activation state of the stromal enzyme NADP-malate dehydrogenase may be used as an indicator of the redox state of the chloroplast stroma since the enzyme activity responds only to the forces of reductive activation via the thioredoxin system and modulation by NADP (and hence the NADP–NADPH ratio) (Rebeille and Hatch 1986a,b, Scheibe and Stitt 1988). Although the activation state of the NADP-malate dehydrogenase increases with increasing electron flux, even at high irradiance the activation state does not exceed 30% of maximal activity indicating that under steady-state conditions in leaves, the stroma remain oxidised (Fig. 3). This is in agreement with the observation that the restriction of the photochemical oxidation of P_{700} by a shortage of electron acceptors only occurs in air during photosynthetic induction. Thus, neither the [NADPH]/[NADP] ratio nor the phosphorylation potential reach extreme values in steady-state conditions and remain relatively constant over a wide range of irradiances (Brooks et al. 1988, Dietz and Heber 1984, 1986, Foyer et al. 1989, Furbank et al. 1987, Heber et al. 1986). It would seem that 'photosynthetic control' serves to prevent the extreme buildup of assimilatory power and restricts the stromal [ATP]/[ADP] and [NADPH]/[NADP] ratios within a fairly narrow range.

Particularly interesting responses are seen when intact chloroplasts are subjected to limitation by Pi or CO_2. When CO_2-dependent O_2 evolution is limited by the availability of Pi, O_2 evolution is completely inhibited while [ATP]/[ADP] ratios are comparable with those found in the dark (0.5–0.8) (Furbank et al. 1987). Similarly, with gylcerate 3-phosphate-dependent O_2 evolution in the absence of CO_2, O_2 evolution becomes progressively limited as the glycerate 3-phosphate concentration is increased yet there is little change in the accompanying [ATP]/[ADP] ratio or in the activation state of NADP-malate dehydrogenase (Foyer et al. 1989). We must conclude from such observations that the thylakoid membrane is extremely sensitive to the balance between energy input and energy utilisation and that small changes in either the [ATP]/[ADP] ratio, the availability of Pi or the [NADPH]/[NADP] ratio will inhibit non-cyclic electron flow and O_2 evolution. The liberation of Pi from phosphorylated metabolites in the pathways of end-product (sucrose and starch) synthesis can be limiting when light and CO_2 levels are high (Dietz and Foyer 1986, Foyer 1988, Stitt 1988, Sivak and Walker 1986). The sensitivity of non-cyclic electron flow to inhibition by ΔpH is exacerbated by the decreasing affinity of the ATP synthase for Pi with increasing electron transport rate and increasing ΔpH (Selman and Selman-Reimer 1981). This can be compounded further in intact systems by the decreasing availability of Pi as substrate for photophosphorylation under conditions of optimal irradiance and CO_2 where the capacity of CO_2 fixation exceeds the regulated rate of sucrose synthesis (Foyer 1988). However, when Pi becomes limiting it is clear that control is exerted over both the activity of the Benson–Calvin cycle and electron transport simultaneously such that they are regulated-down in unison. In this situation the ability of the Benson–Calvin cycle to act as a sink for ATP and NADPH is decreased in response to the limitation and modulation of the activities of enzymes such as ribulose 1,5-bisphosphate car-

boxylase and fructose 1,6-bisphosphatase serves to limit the capacity of the cycle (Furbank et al. 1987, Giersch and Robinson 1987). Ribulose 1,5-bisphosphate carboxylase activity would be modulated both directly by the level of Pi (Heldt et la. 1978) and also by the level of ATP via the activase protein (Brooks et al. 1988, Parry et al. 1988, Robinson and Portis 1988).

We suggest that it is the integrated interaction between redox components in the stroma and the membranes and the transthylakoid ΔpH that results in the regulation of electron flow. ATP deficits automatically result in the decreased turnover of NADPH and a suppression of non-cyclic electron transport. The increased [NADPH]/[NADP] ratio may initiate an increase in transthylakoid ΔpH by cyclic electron flow. In this way small increases in the [NADPH]/[NADP] ratio could act to increase cyclic flow around PS I, increase ΔpH, increase phosphorylation potential and stabilise the [NADPH]/[NADP] ratio; the inverse is also possible. It is clear that inhibition of non-cyclic electron flow can occur if there is a small imbalance between the stoichiometry of ATP/NADPH production and consumption. Because of this the stoichiometry of ATP synthesis and NADPH production is confined to within very narrow limits. This could in part provide an explanation for the progressive failure of intact chloroplasts to metabolise glycerate 3-phosphate in the absence of CO_2 (Foyer et la. 1989). In such circumstances O_2 evolution ceases while glycerate 3-phosphate and Pi are abundant within the chloroplast stroma and the [ATP]/[ADP] ratio and redox state of the NADP system are changed very little from a situation of maximal O_2 evolution (Foyer et al. 1989). Of particular importance is the suggestion that intact chloroplasts are 'overenergised' with regard to the proton-motive force such that the transthylakoid ΔpH is not in equilibrium with the stromal phosphorylation potential even in the absence of a sink for ATP (Giersch et al. 1980). Indeed, low concentrations of uncouplers will stimulate CO_2 fixation in isolated chloroplasts at concentrations that lower the ΔpH (Krause and Laasch 1987, Tillberg et al. 1977). It is likely that the explanation for such observations again lies in the tendency for imbalance arising between the stoichiometries of NADP/ATP production and consumption with the possibility of increased restriction of electron transport by ΔpH. It is therefore significant that inhibition of PS I cyclic electron flow with antimycin A can stimulate photosynthesis in intact chloroplasts (Slovacek and Hind 1980) (Table 1).

The regulation of the activities of the ATP synthase and ferredoxin-NADP reductase may also make a crucial contribution to the maintainance of stability in the [ATP]/[ADP] and [NADPH]/[NADP] ratios. Thiol-reduction of the thylakoid ATP synthase has been shown to lower the magnitude of the ΔpH (by 0.3 pH units) required to activate the enzyme (Mills and Mitchell 1984). In turn, the ΔpH appears to stabilise thiol reduction in the light such that it resists oxidation in circumstances where the thiol-modulated Benson–Calvin cycle enzymes become oxidised (Noctor and Mills, 1987, 1988). This mechanism ensures maximum capacity for ATP synthesis over a range of irradiances and results in a high level of ATP synthase activity that is subject only to thermodynamic control. The activation state of the ferredoxin-NADP reductase, changes in its affinities for its substrates and its roles in both cyclic and non-cyclic paths of electron flow are also of importance in terms of regulation. PS I, like PS II, requires protection against photodamage but PS I has several alternative pathways through which to dispose of electrons. Without doubt there must be sensitive discrimination and poising of electron transfer routes between NADP reduction, cyclic electron flow around PS I, electron drainage to O_2 and reductive activation of enzymes. Because of the low redox potentials of thioredoxin and the thioredoxin-modulated enzymes, keeping these enzymes in the reduced state in the stromal environment may be viewed as a constant problem (Noctor and Mills 1987, 1988, Rebeille and Hatch 1986a,b). High [NADPH]/[NADP] ratios will favour reactions involving electron donation to O_2 and hence formation of the potentially deleterious products O_2^- and H_2O_2. Thus, it is advantageous that the steady-state [NADPH]/[NADP] ratios maintained in the stroma are relatively low.

Dynamic aspects of control

There is clearly abundant evidence for the operation of regulatory mechanisms that co-regulate electron transport and carbon assimilation when different steady states are examined. Of importance in the natural environment, when conditions are rarely constant are the dynamic aspects of regulation (Horton 1985a, Horton et al. 1988). Remarkably little work has been done on this aspect of regulation and has mostly concentrated on observation of the effects of single large transitions in light intensity or CO_2 level.

High [ATP]/[ADP] and [NADPH]/[NADP] ratios occur following the transition from dark to light (Furbank et al. 1987, Quick and Horton 1986) and following the induction of oscillatory behaviour when steady-state photosynthesis is perturbed by a change in light or CO_2 (Furbank and Foyer 1986). However, such high ratios are transient and are accompanied by inhibition of O_2 evolution until the adenylate and pyridine nucleotide pools attain a reduced energy-status (Furbank and Foyer 1987, Quick and Horton 1986). With leaves in air, restriction of electron transport on the acceptor side of PS I is only observed as a transitory effect following a dark to light transition or a sudden increase in irradiance. Following abrupt increases in irradiance deviations in the half-time for P_{700}^+ reduction do occur and Q_A is seen to go reduced. Following such perturbations any restriction in electron transport on the acceptor side of PS I or any change in half-time for P_{700}^+ re-reduction is followed by relaxation to the pre-perturbance value. This recovery can be relatively slow, in the order of several minutes and reflects the time taken for the adjustment in enzyme activities, metabolite pools and thylakoid organisation. The fact that no change in the half-time of P_{700}^+ reduction occurs over a wide range of conditions implies that either the ATP synthase complex or the enzymes of the Benson–Calvin cycle have very high gain in control terms, i.e., small perturbations produce large effects. One of the consequences of having this high gain is an increased tendency to become unstable and oscillate. However, this can be avoided by increased damping which increases the response time of the system. It is probably significant that photosynthetic systems can be induced to oscillate quite easily under relatively atypical physiologically conditions where normal control processes may be unable to operate effectively to balance the system.

While the kinetic behaviour of photosynthesis upon increases in irradiance reflects the time constants for the turning on of regulatory processes, a decrease in light intensity is followed by a period when the system is inappropriately regulated. The nature of this transient limitation on photosynthesis on lowering the light intensity is not fully understood; certainly the suppression of light capture and photochemical efficiency of PS II in high light would result in a transitory decrease in photosynthetic rate. However, there are also the potential metabolic imbalances to consider, that may result in periods of excessive consumption of ATP (Doncaster et al. 1989).

It is to be emphasised that each of the regulatory processes discussed in this review will have its own characteristic time responses, and that these may vary between species or under different conditions. Responses to changes in environmental conditions on different timescales can then be accomodated. Consequently, steady-state data such as the irradiance response curve may tell us little about how a plant photosynthesises in its natural environment when there will be sizable deviations in photosynthetic rate about the steady state values (Horton et al. 1988). Some species may be particularly adapted to making full use of light that comes in the form of sunflecks (Pearcy 1988).

Discussion

In this review we have tried to rationalise observations of the mechanisms of photosynthetic control in order to provide an integrated model of the interactions between light, electron transport and carbon assimilation. It is clear that at all levels regulation is serving to maximise efficiency while striving to avoid damage to the photosystems. We may state several tenets on which our model for photosynthetic control of electron transport is based:

1. The protection of PS II from photodamage is a major priority in all circumstances. Photosynthetic control is geared to energy dissipation in conditions that approach or exceed light-saturation, allowing a relatively oxidised state of Q_A to persist against increasing restriction to electron flow in the remainder of the electron transport chain. Decreasing the quantum efficiency of PS II serves to counterbalance the increasing failure to dissipate energy through photosynthetic O_2 evolution. It follows that the plastoquinone pool could be largely reduced in situations where Q_A is still relatively oxidised. High transthylakoid ΔpH therefore does not cause reduction of PS II as expected from control of the oxidation of plastoquinol but rather acts as a trigger to initiate dissipative processes.
2. The quantum efficiencies of PS II and PS I decrease in unison such that when q_E is high and F_v/F_m decreases, P_{700} becomes oxidised. The regulation of the redox poise of the intersystem carriers is essential for the maintenance of the correct provision of ATP and NADPH and is achieved by control over the delivery of excitation to the reaction centres and by their photochemical efficiency.
3. The [ATP]/[ADP] ratio and assimilatory power are fairly constant over a wide range of irradiances and metabolic states, indicating that these components are buffered against change by modulation of PS II at one extreme and Benson–Calvin cycle activity at the other. The maintenance of constant ratios of [NADPH]/[NADP] and [ATP]/[ADP] in situations of excess and sub-optimal irradiance clearly requires active feedback control of thylakoid processes co-ordinated with feed-forward modulation of carbon assimilation (Fig. 4). The enzymic activities of the cycle are able to adjust to match the rate at which ATP and NADPH can be provided; excitation energy is captured by PS II at a rate that also matches this provision.
4. It is suggested that the ΔpH is the factor that controls electron transport, via its affect on light-harvesting and photochemistry by PS II. Direct ΔpH control over the rate of oxidation of plastoquinone does not appear to be a factor of major importance under many conditions. We suggest this because not only is the restriction on P_{700} reduction constant over a range of light intensities but because the electron flux rate is indicative of minimal control by the intrathylakoid pH. Only when the Benson–Calvin cycle itself is restricted is there evidence that the ΔpH can bring about control at the plastoquinone oxidation site.
5. The notion of an intrathylakoid pH which saturates at low irradiance is at variance with the observed increase in q_E which occurs only to any significant extent once photosynthesis is approaching saturation. It is possible that the driving forces for q_E formation and for control of electron transport plastoquinol oxidation are different or that there is control over the sensitivity of q_E to the ΔpH. This control would appear to be necessary if the thylakoid membrane is able to simultaneously carry out high rates of electron transport and high rates of energy dissipation. Conversely, in low light a ΔpH high enough to drive ATP

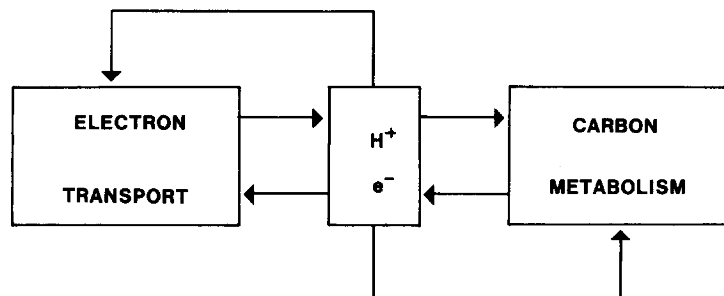

Fig. 4. Interactions between electron transport and carbon assimilation. The proton motive force and redox state in the chloroplast, determined by the rate of light-driven electron transport and the rate of carbon assimilation, exerts feed-back control over light-harvesting and photochemical efficiency and feed-forward control over the activities of Benson–Calvin cycle enzymes.

synthesis can be maintained without wasteful dissipation of excitation energy. In this situation the phosphorylation of thylakoid polypeptides may serve to maximise the efficiency of ΔpH formation. Therefore, the dissipation of excitation energy that we suggest to be a major element of photosynthetic control whilst requiring the presence of a ΔpH may actually be controlled in vivo by another factor and it is this factor which changes with irradiance.

In summary, we have presented a model, based on currently available data of how individual regulatory and dissipative processes act in synchrony to exert control over photosynthetic electron flow reconciling large variations in energy supply and demand for the products of electron flow, ATP and NADPH (in terms of the activity of the Benson–Calvin cycle and sucrose synthesis). 'Photosynthetic control' is specifically imposed on chloroplasts carrying out CO_2 fixation in order to match the production of ATP and NADPH to its utilisation in the Benson–Calvin cycle and prevent imbalance in the system. But why is such tight control necessary? The answer undoubtedly resides in four features of photosynthesis; firstly, the light-harvesting and reaction centres in the thylakoid have to be protected from the lethal combination of high excitation energy levels, extreme redox potentials and O_2. Secondly, ΔpH cannot be allowed to rise in high light to a value that restricts the maximum rate of electron transport at the site of plastoquinol oxidation (instead ΔpH mainly controls the *quantum yield* of electron transport), yet it has to be high enough to drive ATP synthesis at rates in concert with the rate of photosynthesis. Thirdly, because the synthesis and consumption of ATP and NADPH can be neither spatially nor temporally separated, the assimilatory force has to be low enough to allow high rates of electron transport, yet high enough to sustain the Benson–Calvin cycle. Finally, the Benson–Calvin cycle, driven by the products of electron transport has to provide triose phosphate to the cytoplasm at a rate that ultimately balances the rate of CO_2 fixation and O_2 evolution, a process that requires maintainance of appropriate levels of metabolites as well as the recycling of Pi. In essence, it is the necessity to achieve stoichiometric balance between irradiance, electron and proton fluxes, CO_2 fixation and carbohydrate synthesis in an environment that is both hazardous and constantly changing that rationalises the existence of photosynthetic control. Further work is now required to address the quantitative aspects of this control network, to describe the limits to its robustness and the effects of environmental stress and to assess the relationships between regulation and the adaptation and acclimation of plants to the environment.

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