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Protein phosphorylation and optimal production of ATP in photosynthesis

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For green-plant photosynthesis, phosphorylation of LHCP is now widely accepted as a physiologically important mechanism for the control of distribution of excitation energy between the two photochemical reactions that drive non-cyclic electron transport (Allen, 1983*b*; Barber, 1983; Bennett, 1983; Haworth *et al.*, 1982; Horton, 1983). The proposal that this phosphorylation reaction is the basis of state 1–state 2 adaptations (Allen *et al.*, 1981; Horton & Black, 1980) depended originally upon an observed regulation of the thylakoid protein kinase by the redox state of plastoquinone, an observation confirmed by redox titration (Horton *et al.*, 1981; Millner *et al.*, 1982) and by use of site-specific electron donors and inhibitors (Allen & Horton, 1981).

Control of the protein kinase by plastoquinone redox state completes a negative feedback loop, which makes distribution of excitation energy self-correcting within certain limits. Thus the transition to light-state 2 (during which excitation energy is redirected to photosystem I) is brought about by reduction of plastoquinone and by phosphorylation of LHCP, while the transition to light-state 1 (during which excitation energy is redirected to photosystem II) is brought about by oxidation of plastoquinone and by dephosphorylation of LHCP. The predicted phosphorylation and dephosphorylation reactions do indeed accompany their respective light-state transitions (Telfer *et al.*, 1983).

Protein phosphorylation and photophosphorylation of ADP

The success of this hypothesis in explaining state 1–state 2 transitions has perhaps diverted attention from the obvious suggestion that LHCP phosphorylation may also regulate cyclic electron transport (Allen, 1983*a*,*b*; Bennett, 1983; Horton, 1983). Since cyclic photophosphorylation (that is, ATP synthesis) depends directly on electron transport only through photosystem I, and since LHCP phosphorylation diverts excitation energy to photosystem I at the expense of photosystem II, protein phosphorylation may play a role in photophosphorylation by determining, in part, the ratio of cyclic to non-cyclic electron transport. There are strong *a priori* grounds for making this suggestion: cyclic photophosphorylation is known to be regulated by redox poise of electron carriers in the cyclic chain (reviewed in Allen, 1983*a*), while the effect of LHCP phosphorylation during state 1–state 2 transitions must be to maintain redox poise of the plastoquinone pool if the current view of these adaptations is correct.

Abbreviation used: LHCP, light-harvesting chlorophyll *a*/*b* binding protein; PS I, photosystem I.

Two possible roles for LHCP phosphorylation in control of cyclic photophosphorylation may be distinguished: (i) control of the relative efficiency of cyclic photophosphorylation; (ii) control of electron flow through the cyclic chain and hence of the relative extent of cyclic photophosphorylation.

Efficiency of cyclic photophosphorylation. Although it has been held that cyclic photophosphorylation is unnecessary (Reeves & Hall, 1978), there is now good evidence (Allen, 1983*a*) that it occurs under physiological conditions where it may serve to increase the overall stoichiometry ATP/NADPH from about 1.3 (for non-cyclic photophosphorylation alone) to about 1.5 (for non-cyclic with 15% cyclic photophosphorylation).

The stoichiometry ATP/NADPH of 1.5 is the requirement of the reductive pentose phosphate pathway. In principle, the 'extra' ATP could be produced either by cyclic or by pseudocyclic photophosphorylation. (Pseudocyclic photophosphorylation is coupled to non-cyclic electron transfer where oxygen is the terminal electron acceptor.) Pseudocyclic photophosphorylation has the same P/2e ratio as non-cyclic photophosphorylation, and so a 15% contribution of pseudocyclic would require a 15% share of total absorbed quanta. Cyclic photophosphorylation would produce the same amount of ATP with the same share of total absorbed quanta only if the extra excitation energy could be directed entirely to photosystem I. If absorbed excitation energy were directed equally to photosystems I and II, cyclic photophosphorylation would effectively require a 30% share, with half the quanta being directed wastefully to photosystem II. The quantum yield of cyclic photophosphorylation would then be only half that of non-cyclic or pseudocyclic photophosphorylation. The effect of redistributing 15% of excitation energy from photosystem II to photosystem I would therefore be to raise the quantum yield of ATP from 0.29 to 0.33 for mixed cyclic and non-cyclic photophosphorylation (Table 1).

Regardless of the precise value assigned, any change in the contribution of cyclic to total photophosphorylation will require corresponding change in distribution of excitation energy if maximal efficiency is to be maintained.

Extent of cyclic photophosphorylation. It is possible that LHCP phosphorylation also controls the relative extent of cyclic photophosphorylation. At the simplest level, an increase in rate of turnover of photosystem I relative to that of photosystem II should increase cyclic as a proportion of total electron flow. The control of the protein kinase by redox state of plastoquinone may then complete a second negative feedback loop by which the ATP/NADPH ratio may be maintained at the correct value for CO_2 fixation and other assimilatory processes.

Consider any change in total demand for ATP during photosynthesis. If ATP production came to exceed its consumption, for example, then the limiting factor for CO_2

Table 1. *Possible quantum yields for photophosphorylation: the need for redistribution of excitation energy if cyclic photophosphorylation is to make a contribution to total ATP synthesis*

	Non-cyclic	Non-cyclic plus 15% pseudocyclic	Non-cyclic plus 15% cyclic without redistribution of excitation energy to PS I	Non-cyclic plus 15% cyclic with redistribution of excitation energy to PS I
Stoichiometry ATP/NADPH	1.3	1.5	1.5	1.5
Maximum quantum yield of ATP	0.33	0.33	0.29	0.33
Minimum quantum requirement of O_2 or CO_2	8.0	9.2	10.4	9.2

fixation would become reduction of $NADP^+$. Plastoquinone could then tend to be oxidized since the high $NADP^+$/NADPH ratio would increase turnover of photosystem I. Oxidation of plastoquinone would then switch off the protein kinase, and LHCP dephosphorylation would increase turnover of photosystem II, thereby supplying electrons to make good the limiting rate of $NADP^+$ reduction. Conversely, too low an ATP/ADP ratio would be expected to inhibit NADPH oxidation by the triose phosphate dehydrogenase reaction. Decreased turnover of photosystem I would then lead to reduction of plastoquinone, and hence to activation of the protein kinase and to phosphorylation of LHCP. Redistribution of excitation energy to photosystem I would then favour cyclic photophosphorylation, thereby tending to correct the initial ATP deficiency.

Is the protein kinase regulated by plastoquinone redox state or by ATP/ADP?

A more direct control of LHCP phosphorylation by the ATP/ADP ratio has been proposed (Baker *et al.*, 1982; Markwell *et al.*, 1982) as an alternative to regulation by plastoquinone redox state. It is further suggested that control by plastoquinone redox state serves merely to inactive wasteful ATP hydrolysis by the protein kinase in darkness (Markwell *et al.*, 1982). How the ATP/ADP ratio might be involved in state 1–state 2 transitions remains unclear (Baker *et al.*, 1982), and the observation of state 1–state 2 changes in uncoupled thylakoids (Telfer *et al.*, 1983) is in any case inconsistent with this idea.

With regard to the problem of regulation of the ATP/NADPH stoichiometry, a role for protein phosphorylation controlled by ATP/ADP suffers a serious theoretical disadvantage: by positive feedback, any imbalance in the ATP/NADPH ratio would be self-augmenting rather than self-correcting if protein kinase activity were dependent on the ATP concentration maintained by photophosphorylation. If too much ATP were being produced, then the high ATP/ADP ratio would promote phosphorylation of LHCP and hence favour distribution of excitation energy to photosystem I. Increased cyclic electron flow would then tend to increase ATP synthesis relative to reduction of $NADP^+$, leading to a greater imbalance and to further inhibition of photosynthesis. Conversely, if ATP synthesis became limiting then a decreased protein kinase activity would exacerbate the problem by causing a diversion of excitation energy away from the cyclic chain.

Control of protein kinase activity by the ATP/ADP ratio can be demonstrated under appropriate conditions *in vitro* (Baker *et al.*, 1982; Markwell *et al.*, 1982), and can also be inferred from the inhibitory effect of ribose 5-phosphate (an ATP-sink) on LHCP phosphorylation in a reconstituted chloroplast system (Horton & Foyer, 1983). However, neither system distinguishes between plastoquinone redox state and ATP/ADP ratio with respect to their effects *in vivo*. In the reconstituted chloroplast system, control of LHCP phosphorylation by plastoquinone redox state can be inferred from inhibition by the electron acceptor 3-phosphoglycerate (Horton & Foyer, 1983).

In intact chloroplasts, titration of LHCP phosphorylation and of CO_2-dependent oxygen evolution with uncouplers shows that LHCP phosphorylation can proceed at uncoupler concentrations (e.g. NH_4Cl at 40mM) sufficient to abolish photosynthesis (Allen, 1984). This in turn implies that the LHCP kinase has a greater affinity for ATP than has phosphoglycerate kinase and phosphoribulokinase. It is difficult therefore to envisage the ATP/ADP ratio exerting any important effect on LHCP phosphorylation and on excitation energy distribution *in vivo*.

Phosphorylation of LHCP is clearly an important regulatory reaction in photosynthesis, and its role in state 1–state 2 transitions may be explained by redox control of the thylakoid protein kinase. A role for LHCP phosphorylation in regulation of ATP synthesis has yet to be demonstrated experimentally, though control of the relative contribution of cyclic photophosphorylation and of its efficiency would seem likely. In all cases plastoquinone redox state is the most plausible link between the electron transport chain and LHCP phosphorylation.

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