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Minireview

Thylakoid protein phosphorylation, state 1–state 2 transitions, and photosystem stoichiometry adjustment: redox control at multiple levels of gene expression

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In photosynthesis in chloroplasts, control of thylakoid protein phosphorylation by redox state of inter-photosystem electron carriers makes distribution of absorbed excitation energy between the two photosystems self-regulating. During operation of this regulatory mechanism, reduction of plastoquinone activates a thylakoid protein kinase which phosphorylates the light-harvesting complex LHC II, causing a change in protein recognition that results in redistribution of energy to photosystem I at the expense of photosystem II, thus tending to oxidise the reduced plastoquinone pool. These events correspond to the transition from light-state 1 to light-state 2. The reverse transition (to light-state 1) is initiated by transient oxidation of plastoquinone, inactivation of the LHC II kinase, and return of dephosphorylated LHC II from photosystem I to photosystem II, supplying excitation energy to photosystem II and thereby reducing plastoquinone. State 1–state 2 transitions therefore operate by means of redox control of reversible, post-translational modification of pre-existing proteins. A balance in the rates of light utilization by photosystem I and photosystem II can also be achieved, on longer time-scales and between wider limits, by adjustment of the relative quantities, or stoichiometry, of photosystem I and photosystem II. Recent evidence suggests that adjustment of photosystem stoichiometry is also a response to perturbation of the redox state of inter-photosystem electron carriers, and involves specific redox control of de novo protein synthesis, assembly, and breakdown. It is therefore suggested that the same redox sensor initiates these different adaptations by control of gene expression at different levels, according to the time-scale and amplitude of the response. This integrated feedback control may serve to maintain redox homeostasis, and, as a result, quantum yield. Evidence for the components required by such systems is discussed.

Key words – Chloroplast, electron transport, gene expression, light-harvesting complex, photosynthesis, photosystem I, photosystem II, protein degradation, protein kinase/phosphatase, redox sensor/response regulator.

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Introduction

In oxygenic photosynthesis, two separate photoelectrochemical reaction centres are linked in series, driving electron transport from water to $NADP^+$, with coupled proton translocation across the thylakoid membrane generating a proton motive force for synthesis of ATP. Each reaction centre is served by a distinct set of light-harvesting antenna pigments, giving each photosystem a distinct absorption and action spectrum. Photosystem I (PS I) utilizes light energy absorbed predominantly by chlorophyll *a* to drive electron transport from the cytochrome

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b$_6$*f* complex to ferredoxin and then NADP$^+$, while photosystem II (PS II) utilizes light energy absorbed by chlorophyll *a* and chlorophyll *b* to drive electron transport from water to the plastoquinone pool. In certain algal phyla, chlorophyll *b* is replaced by chlorophylls *c* or *d*, while in red algae and cyanobacteria, it is usually replaced by phycobilins. In all systems, PS II becomes limiting at incident wavelengths greater than about 680 nm (the 'red drop' in quantum yield), where PS I is thus selected. In red algae or cyanobacteria any purely chlorophyll-absorbed light is also selective for PS I. The composition of the light-harvesting systems of different photosystems II gives an action spectrum for the enhancement of photosynthetic yield by PS I-absorbed light that is characteristic of the plant, bacterial, or algal group concerned (reviewed in Myers 1971).

It has been known for many years that a physiological adaptation occurs when plants, algae or cyanobacteria are moved between illumination conditions favouring either PS I or PS II (Bonaventura and Myers 1969, Murata 1969, Myers 1971). This adaptation occurs typically on a timescale of a few min, during which time the quantum yield of oxygen evolution increases while the yield of chlorophyll fluorescence emission either decreases (during the adaptation to PS II light) or increases (during the adaptation to PS I light). The increase in quantum yield of oxygen indicates that the mechanism of the adaptation involves a redistribution of absorbed excitation energy between the two photosystems, such that the light-limited photosystem receives more energy while the light-saturated photosystem receives less. Thus PS I becomes favoured under PS II light, while PS II becomes favoured under PS I light. Since the variability in chlorophyll fluorescence arises from PS II at room temperature, the fluorescence emission changes are also consistent with redistribution of excitation energy that serves to compensate for light-limitation at one or other photosystem. The state of adaptation to PS I light is referred to as the light 1-state, or state 1, and the state of adaptation to PS II light is referred to as the light 2-state, or state 2. The transitions between the two adaptation states are therefore known as light-state transitions, or state 1–state 2 transitions.

More recently, it has also become clear that a functionally similar reconfiguration of the relative capacity of each photosystem for light-energy conversion occurs on much longer time-scales, typically many hours, and involves a change in the relative quantities of photosystems I and II, whether measured spectroscopically or by protein composition. Both reaction centre and light-harvesting components of each photosystem are affected, though the internal photosynthetic unit size may also be subject to long-term control. The long-term mechanism compensating for unequal utilization of light by the two photosystems involves an increase in the relative quantity of PS II in PS I light, and an increase in the relative quantity of PS I in PS II light (Melis and Harvey 1980, Chow et al. 1990). The mechanism of adjustment of photosystem stoichiometry is unknown, but the response occurs on time-scales measured in hours, consistent with regulation at an early stage of gene expression. Furthermore, a role for the redox state of the plastoquinone pool or cytochrome *b*$_6$*f* complex is suggested by the observation that mutants impaired in the PS II light-harvesting complex are locked into the state in which PS II is abundant compared with PS I, and show no further changes in photosystem stoichiometry (Kim et al. 1993).

Thus, viewed from the control point of the redox state of plastoquinone, photosystem stoichiometry adjustment serves the same purpose as light-state transitions, but operates over longer time periods. Balanced excitation energy distribution may therefore be achieved by a combination of the fine, short-term control of state transitions with the coarse, long-term control of photosystem stoichiometry. In the general terminology of biological adaptations: state transitions are physiology; photosystem stoichiometry adjustment is development.

This minireview is not a comprehensive account of recent work on the topics of protein phosphorylation (cf. Allen 1992a), state transitions, and photosystem stoichiometry. In each area a number of important questions are left unaddressed. For example, there is still the question of whether state transitions necessarily lead to increased quantum yield in vivo, and, if so, under what conditions (Andrews et al. 1993, Rouag and Dominy 1994). A further omission is the role of protein structural changes in molecular recognition (for a general review see Johnson and Barford 1993; applied to LHC II, see Allen 1992a,b). The present paper examines different roles of redox control of thylakoid membrane protein phosphorylation and their possible interactions. In view of the functional similarities between state transitions and photosystem stoichiometry adjustment, the possibility is considered here that these processes share a common redox sensory component and other initial steps of a subsequently branching signal transduction pathway. The evidence for redox control of specific gene expression and the role of protein phosphorylation in such control is considered. The location of the genes for the reaction centre core proteins of photosystems I and II in chloroplasts, rather than in the nucleus, is convenient for regulation of their expression by redox state of components of the photosynthetic electron transport chain. This may be no coincidence: the requirement for redox regulation of gene expression, as exemplified by photosystem stoichiometry adjustment, may be the primary reason for the retention, in evolution, of chloroplast genetic systems.

Abbreviations – CP43, PS II core antenna chlorophyll-protein of approximately 43 kDa (psbC); D1, (for diffuse band 1) PS II reaction centre protein psbA; D2, (for diffuse band 2) PS II reaction centre protein psbD; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, 2,6-dichlorophenolindophenol; HQNO, 2-*n*-heptyl-4-hydroxyquinoline N-oxide; LHC II, light-harvesting complex of PS II; Q_A, first quinone accepting electrons in reaction centre of PS II; Q_B, second quinone accepting electrons in reaction centre of PS II; Q_i, inside (stromal side) quinone of the cytochrome *b*$_6$*f* complex.

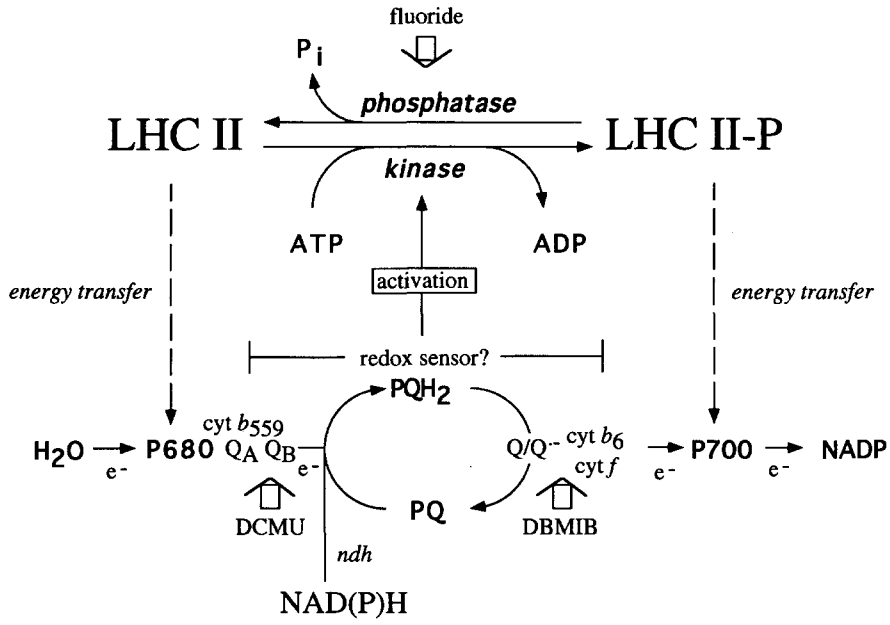

Fig. 1. Control of distribution of excitation energy, absorbed by LHC II, between the reaction centres of PS I (P700) and PS II (P680). A redox sensor that is close to, but not identical with, the plastoquinone pool, activates the LHC II kinase when plastoquinone (PQ) is in its reduced form (PQH_2 – plastoquinol), resulting in phosphorylation of LHC II. The phosphorylated form of LHC II transfers excitation energy to PS I at the expense of PS II, serving to oxidise the plastoquinone pool. The LHC II phosphatase is redox-independent. When plastoquinone is oxidised, the kinase is inactive, and dephosphorylation of LHC II predominates, thus returning excitation energy to PS II and increasing the rate of reduction of plastoquinone. The plastoquinone pool is oxidised by PS I and reduced by PS II and by NAD(P)H dehydrogenase (*ndh*). Electron transport from PS II to plastoquinone is inhibited by DCMU; electron transport from plastoquinone to PS I is inhibited by DBMIB. The LHC II phosphatase is inhibited by fluoride. For further explanation of symbols, see Abbreviations and text. Adapted from Allen et al. (1981) and Allen (1992a).

State transitions and redox control of thylakoid protein kinase

The light-stimulation of LHC II phosphorylation (Bennett 1977) was suspected to be a redox-stimulation from the ability of ferredoxin with NADPH to activate the reaction in darkness (Bennett 1979). The redox control was demonstrated more directly using dithionite to activate phosphorylation in darkness and ferricyanide to inhibit phosphorylation in the light, and the site of redox control was suggested to be plastoquinone on the basis of low DBMIB sensitivity and of activation by duroquinol but not DCPIP/ascorbate (Allen et al. 1981). The location of the site of redox control between PS I and PS II is of significance for the function of LHC II phosphorylation, since the phosphorylation reaction itself causes a redistribution of excitation energy towards PS I at the expense of PS II (Bennett et al. 1980). The conclusion of control by plastoquinone redox state was also supported by the results of potentiometric redox titration of LHC II phosphorylation (Horton et al. 1981). Control of the LHC II kinase by redox state of plastoquinone thus provides a concrete mechanism for coupling excitation energy transfer to the two reaction centres with redox changes (Fig. 1), as suggested in general terms by Murata (1969) and Duysens (1972).

Following the identification of a number of other thylakoid phosphoproteins (reviewed in Bennett 1991 and Allen 1992), Silverstein et al. (1993a) carried out further redox titrations in order to see which of the thylakoid protein phosphorylation reactions were under redox control, and how many points of redox control might be involved. In place of autoradiography and scintillation counting of excised bands, Silverstein et al. (1993a) also used a phosphorimager, a more sensitive technique allowing independent measurement of the quantity of ^{32}P-labelling in any number of protein bands on an SDS-PAGE slab gel. Surprisingly, all 13 pea thylakoid phosphoproteins observed by this technique showed evidence of redox control. Both separately and together, the titration curves gave a single midpoint-potential at pH 7.6 (E_m) of approximately +40 mV (+38 ± 4 mV), and a value for the number of electrons transferred (n) very close to unity (0.95 ± 0.06). Five of these proteins were identified on the basis of electrophoretic mobility and by comparison with previous results: LHC II, D1, D2, CP43, and the 9 kDa phosphoprotein. Both the E_m and n values are incompatible with redox control being exerted by the bulk quinone pool itself, but instead are compatible with three known redox couples of the photosynthetic chain: the $PQ/PQ^{\cdot -}$ couple of the Q_i site of the cytochrome b_6/f complex, the $PQ/PQ^{\cdot -}$ couple of the Q_B site of the reaction centre of PS II, and the low-potential form of cytochrome b_{559} of PS II. All are close to, but not identical

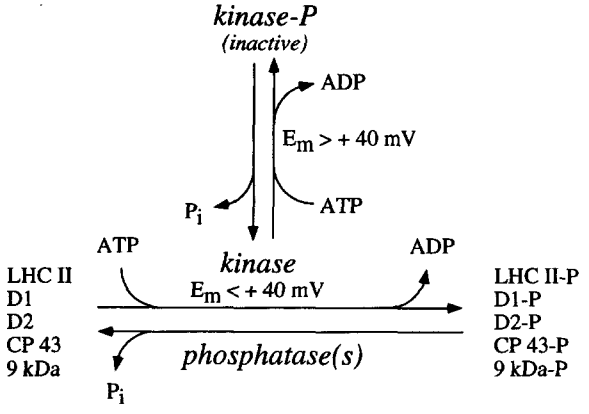

Fig. 2. Redox activation of thylakoid protein phosphorylation reactions by dephosphorylation of a protein kinase with broad substrate specificity. Activation of the kinase results from its own dephosphorylation under reducing conditions; inactivation of the kinase results from its (auto)phosphorylation under oxidising conditions. The thylakoid phosphoprotein phosphatase is redox-independent (Silverstein et al. 1993b). This scheme is suggested by the results of Silverstein et al. (1993a,b) on the assumption that the kinase is one of the proteins seen to be phosphorylated under oxidising conditions (Silverstein et al. 1993a). The redox sensor in Fig. 1 either is, or regulates activation of, the kinase.

with, the bulk quinone pool: redox control 'at the level of plastoquinone' may be retained as an approximation for the time being.

Of the three primary sites of redox control most consistent with the data of Silverstein et al. (1993a), none can be excluded immediately on the basis of previous work, and each has both advantages and disadvantages when considered as a candidate for explaining state transitions and related phenomena. The Q_i (alias Q_n) site of the b_6/f complex would be most easily reconciled with the known requirement for an intact b_6/f complex for full reconstitution of redox-activated LHC II kinase activity in vitro (Frid et al. 1992), and with the finding that any mutant deficient in any part of the cytochrome b_6/f complex seems to be unable to phosphorylate LHC II (Gal et al. 1987). However, it is possible to envisage an indirect requirement for the b_6/f complex in redox control exerted primarily at the Q_B site or at cytochrome b_{559} in PS II. The Q_B site is well-characterised in terms of the interaction of the bound plastoquinone with herbicides and by analogy with the Q_B site of the purple bacterial reaction centre, and occupancy of the Q_B probably plays an important regulatory role in degradation of D1 (Jansen et al. 1993). Cytochrome b_{559} has no known function (Cramer et al. 1993), but *b*-type cytochromes are thought to act as sensors in other systems, for example, as an oxygen sensor in mammalian neutrophils via generation of $O_2^{\cdot-}$ and H_2O_2 in the respiratory burst (Acker 1994). The α-chain (psbE) of cytochrome b_{559} of PS II has 82 amino acids and a relative molecular mass of 9 300. Although the '9 kDa phosphoprotein' is now generally taken to refer to the *psbH* gene product (reviewed in Allen 1992), it has not been shown that cytochrome b_{559}-α cannot become phosphorylated, and both the α- and β-chains (the latter is psbF) contain the protein kinase C-type phosphorylation motif T-X-R as a conserved sequence near the stromal side of the membrane helix, where the T (the putatively phosphorylated threonine) lies 7 amino acids before the histidine haem axial ligand (Cramer et al. 1993).

The redox titration of thylakoid protein phosphorylation by Silverstein et al. (1993a) also revealed two thylakoid proteins showing reverse redox dependency: their E_m and n values were similar to those of the other phosphoproteins, but the sign on the potential scale was reversed, showing phosphorylation at high potentials (oxidising conditions) and dephosphorylation at low potentials (reducing conditions). Although redox titrations have not yet been performed, protein phosphorylation under oxidising but not reducing conditions has been seen in isolated purple bacterial chromatophores (Holmes and Allen 1988, Cortez et al. 1992, Ghosh et al. 1994) and mitochondrial inner membranes (A. Struglics and K. Fredlund, unpublished results). The apparent molecular masses of the two pea thylakoid 'reverse titrators' in the experiment of Silverstein et al. (1993a) were 63 kDa and 46 kDa. An LHC II kinase activity has been demonstrated in detergent-solubilized preparation from spinach thylakoids containing the cytochrome b_6/f complex together with plastoquinol and an autophosphorylated protein of 64 kDa (Gal et al. 1992). If the 63 kDa 'reverse titrator' is indeed the LHC II kinase described by Gal et al., it would follow that the kinase acts on a wide range of other thylakoid phosphoproteins, and is inactive in its phosphorylated form. The redox-activation of the whole spectrum of thylakoid phosphoproteins could then be attributed to the activation by dephosphorylation at low potentials of a key protein kinase, and dephosphorylation of the major thylakoid phosphoproteins would result from inactivation of the kinase by autophosphorylation, as depicted in Fig. 2.

The conclusion that all thylakoid protein phosphorylations are redox-controlled is also important for understanding the various of roles of the different phosphorylation reactions. Giardi (1993) found evidence for heterogeneity in phosphorylation of the PS II core in spinach thylakoids, suggesting that phosphorylation of the reaction centre proteins D1 and D2 results in loss of the 9 kDa phosphoprotein from the core, a state associated with protection from photoinhibition. A role for phosphorylation of core polypeptides in partial protection of PS II from photoinhibition is also suggested by the work of Aro et al. (1993), and for D1 this is directly contrary to the suggestion of Elich et al. (1992) that phospho-D1 is preferentially degraded during photoinhibition. The role of phosphoproteins other than LHC II has been considered elsewhere (Allen 1992a). Since the known thylakoid phosphoproteins all function in the entire PS II complex, phosphorylation may be a means of dissociation or regrouping of components during regulation. It may there-

fore be that the tacit assumption 'one phosphoprotein, one regulatory function' is incorrect, with different phosphorylation reactions playing a concerted role in determining precise alterations in function through effects on protein-protein recognition.

Redox- and light-independence of the thylakoid protein phosphatase

Implicit in most descriptions of state transitions in terms of protein phosphorylation (Allen et al. 1981, Allen 1992a) is the redox- and therefore light-independence of the phosphoprotein phosphatase that removes the phosphate group from phospho-LHC II during the transition to state 1 (Fig. 1). The evidence for this rests largely on the fact that fluoride, which inhibits the protein phosphatase (Bennett 1980) inhibits the transition to state 1, while the the rate of the fluorescence decrease that reflects the transition to state 2 is greater in the presence of fluoride, suggesting that the phosphatase is continually active at a low rate (Telfer et al. 1983). The assumption of a redox-independent phosphatase was tested directly by Silverstein et al. (1993b), using redox titration of ^{32}P-labelling measured by phosphorimaging. The E_m and n values of LHC II phosphorylation were found to be essentially the same whether or not fluoride (10 m*M*) was present in the redox cuvette, that is, whether the phosphatase was active or inactive. Furthermore, after a pre-illumination in the presence of (γ-^{32}P)ATP, the rates of dark dephosphorylation of all thylakoid phosphoproteins were found to be strictly redox-independent, though different phosphoproteins became dephosphorylated at different rates, with LHC II dephosphorylation being the fastest. In a second experiment, where phosphorylation was induced not by light, but by a pre-incubation of the thylakoids at a poised potential of −140 mV, similar differences between various phosphoproteins in rate of dephosphorylation were observed after the potential of the medium was adjusted to +200 mV. These experiments provide a definite confirmation that redox control of thylakoid protein phosphorylation is exerted through the kinase, and not the phosphatase.

In contrast, Elich et al. (1993) concluded on the basis of studies of D1 phosphorylation in *Spirodela* that the D1 phosphatase is light-activated in vivo. In their experimental protocol, Elich et al. (1993) pre-incubated whole *Spirodela* plants with ^{35}S-methionine (to measure newly-synthesised D1) or ^{32}P-orthophosphate (to measure protein phosphorylation), added DCMU, then illuminated or dark-incubated the plants, after which thylakoids were isolated and D1 and other thylakoid protein phosphorylations were measured. In the presence of DCMU, which inhibits electron transfer from Q_A to Q_B on the acceptor side of PS II, light will serve to oxidise inter-photosystem electron carriers by driving PS I. The results of Elich et al. (1993) can therefore be attributed to light-driven inactivation of the thylakoid protein kinase by oxidation of the plastoquinone pool, and do not demonstrate a light-activated phosphatase. Indeed, the reported inhibition of the light-induced dephosphorylation by DBMIB (inhibiting oxidation of plastoquinol) is consistent with this interpretation, as are the wavelength studies indicating that PS I light preferentially leads to protein dephosphorylation (Elich et al. 1993). The LHC II and other thylakoid protein kinase(s) should be expected to remain in the active form in darkness because of reduction of the plastoquinone pool by chloroplast NAD(P)H dehydrogenases, in 'chlororespiratory' electron transfer. In the presence of DCMU, light would drive net oxidation of the plastoquinone pool, and hence cause kinase inactivation, leading to a predominance of the light- and redox-independent phosphatase. The importance of distinguishing between light-reduction of the plastoquinone pool in vitro and light-oxidation of plastoquinone in vivo has been appreciated for some years in studies of green algae (Wollman and Delepelaire 1984, Williams and Allen 1987) and cyanobacteria (Mullineaux and Allen 1986). In the case of *Spirodela* plants illuminated after preincubation with DCMU, none of the data reported by Elich et al. (1993) conflict with the conclusion that the thylakoid kinase is the site of control while the phosphatase is light-independent. This is not to say that the thylakoid phosphatase is not regulated in some way. As discussed elsewhere (Allen 1992a), there are many precedents for control of protein phosphorylation by phosphatase regulation, but there is as yet no evidence for this for thylakoid protein phosphorylation, where the available data suggests firmly that redox control of the kinase is sufficient to explain light-activation of both phosphorylation and dephosphorylation, as depicted in Fig. 1.

Photosystem stoichiometry adjustment

Although a naïve interpretation of the Z-scheme might suggest that the reaction centres of photosystems I and II should be present in a 1:1 stoichiometry, it is now clear from many different lines of evidence that the photosynthetic electron transport chain involves a number of gates and branch points, that different chains can have pools of certain components in common, and that diffusion of small molecules, even via restricted paths, connects macromolecular redox complexes which may themselves move and reassociate in various ways. All the Z-scheme requires is a version of Kirchoff's laws for current flow: the sum of the rates in equals the sum of the rates out, since electrons are neither created nor destroyed. Thus only the rates of PS II and PS I electron transport need to be equal for purely linear electron flow and in the steady-state. The rationale for photosystem stoichiometry adjustment is then similar to that for state transitions: for maximum efficiency, equal rates of electron flow must result from equal rates of energy transfer to the two reaction centres, and, since any randomly-chosen spectral band will favour one or other antenna system, a mechanism is required to equalize energy transfer. A further need for flexibility arises from the fact that the electrical

circuit is more complex, and adjustable: real-life electron transfer switches between various combinations of linear and cyclic electron flow according to metabolic demands, and PS II is down-regulated by various mechanisms associated with photoinhibition. An additional complication is the likely operation of chloroplast NAD(P)H dehydrogenases under some conditions, as yet undefined (Matsubayashi et al. 1987). Clearly there is no 'single' electron transport chain, but various options, selected according to environmental and metabolic circumstances. It should thus be no surprise if photosynthetic electron transport in chloroplasts and cyanobacteria turns out to respond to environmental changes in the same manner as the branched respiratory and photosynthetic chains of bacteria (Gunsalus 1992, Iuchi and Lin 1993).

The stoichiometry of PS II to PS I may be measured as the stoichiometry of phaeophytin or Q_A (from fluorescence induction) to P700 (from absorption spectroscopy). Such measurements give values that often depart significantly from unity. The PS II/PS I stoichiometry has been reported to vary from 1.1, in thylakoids isolated from pea plants grown in PS II light, to 2.5, in the corresponding thylakoids from plants grown in PS I light (Chow et al. 1990). Similar values are described for wild-type barley (Kim et al. 1993). In the cyanobacterium *Agmenellum quadruplicatum*, thylakoids isolated from wild-type cells had a PS II/PS I stoichiometry that varied from 0.32, from PS II-light grown cells, to 0.57, from PS I-light grown cells. For mutants deficient in the light-harvesting antenna of PS II, the PS II/PS I stoichiometry was high, and much less dependent on spectral composition during growth: between 2.7 (PS II-light grown) and 3.1 (PS I-light grown) for the chlorophyll *b*-less *chlorina f2* mutant of barley, and 1.1 (PS II-light grown) for a phycobilisome-deficient mutant of *A. quadruplicatum* that could not be grown under PS I light (Kim et al. 1993). It would be useful to examine state transtions in these mutants, since the proposed role of excitation energy distribution predicts that the inter-photosystem pool will be predominantly oxidised, light-state 1 will prevail, but light utilization by the impaired PS II will still be insufficient to cause the compensatory re-reduction of plastoquinone. Increased PS II/PS I stoichiometry will then tend to maximize electron transport into the plastoquinone pool.

In cyanobacteria, PS I-light grown cells with a high PS II/PS I stoichiometry and PS II-light grown cells with a low PS II/PS I stoichiometry both exhibit state transitions, but the amplitude of the fluorescence changes is greater in PS I-light grown cells with their larger PS II antenna and greater contribution of PS II variable fluorescence to total room-temperature fluoresence (Allen et al. 1989). These results indicate that state transitions and photosystem stoichiomery adjustment may operate simultaneously, and may both be triggered by changes of redox state of inter-photosystem electron carriers.

Several authors have proposed that adjustment of photosystem stoichiometry is a response to changes in the redox state of inter-photosystem electron carriers. Fujita et al. (1987) based this proposal on measurement of photosystem stoichiometry in the cyanonbacterium *Synechocystis* PCC 6714 grown under different nutritional conditions, from purely autotrophic growth, giving a PS II/PS I stoichiometry of 0.31, to photoheterotrophic growth with succinate, giving a PS II/PS I stoichiometry of 1.1. The redox state of the plastoquinone pool in each cell type was estimated from fluorescence induction, and a decreased PS II/PS I stoichiometry was correlated with growth conditions that gave a more reduced pool. Fujita et al. (1987) subsequently carried out alternative measurements of redox state, and, from the HQNO-sensitivity of increased PS I formation, Murakami and Fujita (1993) suggest that a signal for PS I assembly is the reduced form of cytochrome b_6. Control of the assembly process itself may involve a rate-limiting step either in chlorophyll *a* synthesis (Fujita et al. 1990) or in binding of chlorophyll *a* to one or more PS I apoproteins (Murakami and Fujita 1993). Kim et al. (1993) suggest that the effect of the PS II light-harvesting mutations in causing a high and constant PS II/PS I stoichiometry may be a consequence of the control of photosystem stoichiometry being dependent upon the initial distribution of excitation energy distribution between the two photosystems. All groups seem to favour the redox state of the interphotosystem electron transport chain as the factor initiating the signal transduction pathway (Fujita et al. 1987, Chow et al. 1990, Kim et al. 1993). Further, a number of proteins are phosphorylated under redox control, but too slowly to be implicated directly in state transitions both in cyanobacteria (Sanders et al. 1989) and chloroplasts. Among these, phosphorylation of the 9 kDa phosphoprotein in particular seems to play a role in long-term complex formation and breakdown (Giardi 1993). The suggestion of Murakami and Fujita (1993) that cytochrome b_6 redox state controls photosystem stoichiometry in *Synechocystis* PCC 6714 may be of wide importance, since Q-cycle function may be a prime site for redox homeostasis in view of the potentially indiscriminate reactivity of the semiquinone species involved. Cytochrome b_6 should be considered seriously as a primary point of redox control. There is also the observation that cytochrome b_6 becomes slowly phosphorylated in *Lemna* thylakoids (Gal et al. 1992).

Redox control of synthesis and degradation of photosystems I and II

If, during photosystem stoichiometry adjustment, the net rate of synthesis and assembly of PS I relative to that of PS II increases as a consequence of reduction of the plastoquinone pool, the resulting decrease in PS II/PS I stoichiometry and hence PS II/PS I relative electron transport rates would tend to re-oxidise plastoquinone, in precisely the same way as in the state 2 transition, but on a longer time-scale, and with less rapid reversibility. Conversely, an inhibition of PS I synthesis and/or increase in PS II synthesis by oxidation of plastoquinone

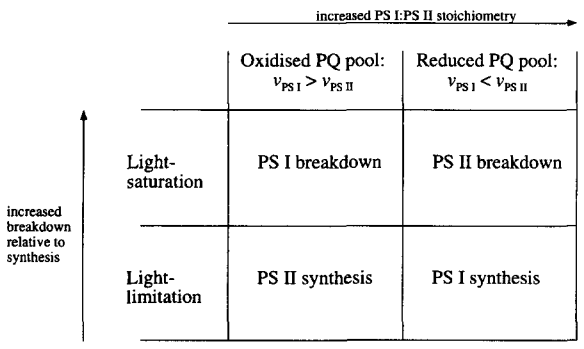

Fig. 3. Proposed balance between synthesis and breakdown of PS I and PS II during photosystem stoichiometry adjustment. At high light, photosystem stoichiometry adjustment is achieved by changes in the relative rates of breakdown of the two photosystems. At low light, changes in relative rates of synthesis predominate.

would tend to increase plastoquinone reduction. Net synthesis and assembly involves many steps: in principle a plastoquinol-accelerated PS II breakdown with plastoquinol-inhibited PS I breakdown could provide the necessary feedback control of net photosystem stoichiometry. However, since the end-product of control is increased efficiency, continual synthesis of the two photosystems accompanied by controlled rates of breakdown makes little energetic sense, though it is possible that such control of rates of breakdown may be useful to the cell in avoiding cytotoxic effects of unbalanced electron flow under conditions of energy surplus. For steady-state photosynthesis at moderate light intensity, control of biosynthesis makes more physiological sense, with control of relative rates of synthesis of PS I and PS II being superimposed on constant and low rates of degradation. In practise, both synthesis and degradation of each photosystem must presumably be selectively controlled, since an alternating requirement for increased and decreased PS II/PS I stoichiometry would be unlikely to be satisfied optimally by the mere addition of more of each photosystem in turn: there must exist also an optimal stoichiometry of the two photosytems to other membrane-bound and soluble complexes. In view of the propensity of PS II to breakdown, we might therefore predict that reduction of the plastoquinone pool promotes a combination of PS II breakdown with PS I synthesis, while oxidation of the plastoquinone pool retards PS II breakdown and promotes PS II synthesis, perhaps also promoting breakdown of PS I, though the capacity of PS I for energy-dissipating cyclic electron flow may make it more accommodating than PS II to prolonged over-excitation. In addition, a selection of synthesis versus breakdown as the predominant means of photosystem stoichiometry adjustment could be made by a control system sensing the unused capacity for increased photochemistry in each photosystem, such that breakdown would predominate where energy dissipation is most important, at high light intensities. Figure 3 presents a contingency table showing the possible intersection of the effects of light intensity and plastoquinone redox state on synthesis and degradation during photosystem stoichiometry adjustment. If phosphorylation of PS II reaction centre proteins promotes their dissociation and breakdown, as proposed by Giardi (1993), then the activation of D1 and D2 kinase by reduced plastoquinone (Silverstein et al. 1993a) would be consistent with this scheme, and the kinetic differences between the various thylakoid protein phosphorylation reactions might be sufficient to determine their roles in either state transitions or the degradative aspects of photosystem stoichiometry adjustment.

For the role of de novo protein synthesis in photosystem stoichiometry adjustment, a number of possible mechanisms can be described. An activation of *psbA* translation via mRNA-binding under reducing conditions in vitro has been described for *Chlamydomonas reinhardtii* by Danon and Mayfield (1994). This would tend to increase D1 synthesis under conditions of reduction of the inter-photosystem electron transport chain, the opposite of that required for increased PS II/PS I stoichiometry adjustment. Obviously the photosystems must be synthesised and assembled in a concerted fashion, so the result of Danon and Mayfield (1994) should also be seen for translation of other *psb* messenger RNAs, particularly that of *psbD*, which encodes D2, the partner of D1 in the PS II reaction centre heterodimer. Thioredoxin-mediated control of translation may also be a non-specific property of the protein synthetic system, though thioredoxin-like effects on transcription have also been described in mammalian systems (Ng et al. 1993). Expression of different *psbA* genes in response to photoinhibitory conditions has been demonstrated in the cyanobacterium *Synechococcus* PCC 7942 (Clarke et al. 1993), and could be a result of redox signals to different *psbA* promoters.

A transcriptional level of control of the relative expression of *psa* and *psb* genes has been proposed as a basis for redox control of photosystem stoichiometry (Allen 1992a). This level of control could plausibly share initial components of the redox signal transduction pathway with state transitions, since the combined operation of post-translational and transcriptional levels of response to the same environmental signal is a common occurence in prokaryotic systems (reviewed in Allen 1993a). Clear evidence for phosphorylation of components of the chloroplast transcriptional apparatus has been provided by Tiller and Link (1993), who also demonstrated phosphorylation-associated changes in protein binding to the promoter region of *psbA* by altered DNase footprinting. Pearson et al. (1993) describe evidence for redox-dependent changes in total chloroplast RNA synthesis, with the site of redox control being narrowed down to the cytochrome b_6/f complex by use of electron transport inhibitors. Maximum labelling of a putative RNA fraction under conditions of oxidation of the cytochrome b_6/f complex (Pearson et al. 1993) would be consistent with the major mRNA component being that for PS II proteins, if one accepts the arguments outlined here for the

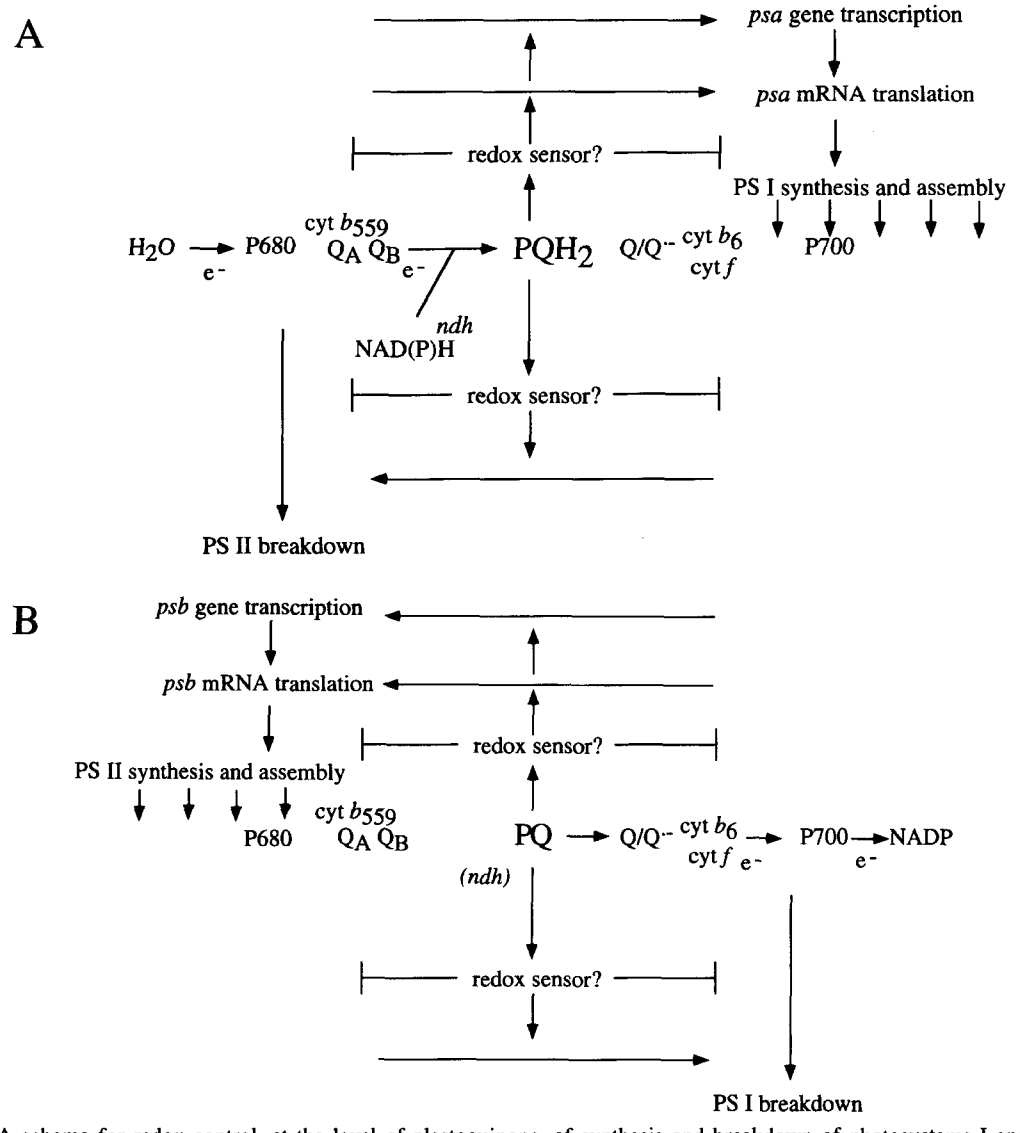

Fig. 4. A scheme for redox control, at the level of plastoquinone, of synthesis and breakdown of photosystems I and II during adjustment of photosystem stoichiometry. A: Plastoquinone is in its reduced form, favouring synthesis of PS I and breakdown of PS II. B: Plastoquinone is in its oxidised form, favouring synthesis of PS II and breakdown of PS I. Possible elements of the pathways of control of synthesis and breakdown are discussed in the text: protein phosphorylation may be involved in any of these pathways. The control of LHC II phosphorylation in state transitions (Fig. 1) can be viewed as a short-term adjustment with a similar function to the long-term effects of photosystem stoichiometry adjustment. The redox sensor and other initial steps in the signal transduction pathway may be common to both systems.

function of the redox control of transcription in photosystem stoichiometry adjustment (Fig. 4).

One may wish to conclude from these isolated pieces of evidence that redox control of phosphorylation of thylakoid proteins usefully extends to both protein degradation and gene expression, at either the level of translation, transcription, or both, and that some or all of these levels of control may participate in the developmental adaptation, or acclimation, of photosystem stoichiometry adjustment. This possibility is outlined in Fig. 4.

Redox regulation and the function of chloroplast genomes

The hypothesis that chloroplast gene expression is subject to redox control was formulated to provide for self-regulating distribution of excitation energy during photosystem stoichiometry adjustment, which may act therefore as a long-term counterpart to state 1–state 2 transitions (Allen 1992a). This hypothesis turns out to have a number of additional implications which have recently

been described elsewhere (Allen 1993b,c). An absolute requirement of the scheme in Fig. 4, for example, is that a direct regulatory link is possible between the redox sensor of the chloroplast thylakoid and the transcription of genes for proteins whose concerted function determines the redox state of the redox sensor itself: only by the operation of such a feedback loop could redox homeostasis be achieved by these means.

The functions of a genetic level of control for redox homeostasis could be the same as those for the post-translational level of control: to maximize quantum yield under light-limiting conditions by achieving optimal rates of turnover of the two reaction centres; and to minimize destructive effects of inappropriate electrochemistry under light-saturing conditions. This homeostatic mechanism would therefore confer a considerable selective advantage on any photosynthetic organism that could maintain and optimize redox control of synthesis, assembly, functional interactions and degradation of those of its proteins which carry out potentially rate-limiting and damaging steps in energy convertion, and whose respective stoichiometries must be capable of responding therefore to changes in the intensity and quality of the light regime of different natural environments. It has been proposed (Allen 1993b,c) that chloroplast genomes encode precisely this sub-set of chloroplast proteins, together with certain components of the chloroplast genetic system itself. Photosystem stoichiometry adjustment may therefore be a prime example of the requirement for the maintenance, in evolution, of extra-nuclear genetic systems: the encoding in situ of proteins whose synthesis is thereby able to respond rapidly to changes in redox potential. Such changes occur in response to changes in light quality and quantity, and in oxygen and carbon dioxide concentrations. Organelle genes encoding components of their own genetic systems may serve a secondary function in permitting the maintenance and replication of the extra-nuclear genetic systems whose primary function is maintenance of correct redox balance in eukaryotic cells.

The experimental predictions of the proposed mechanistic link between state transitions and photosystem stoichiometry adjusment (Fig. 4) include redox control of selective protein turnover, both by synthesis at a transciptional and/or translational level of gene expression, and by protein degradation. These problems are of intrinsic interest for regulation of photosynthesis, and of wider biological relevance, since achievement of redox homeostasis by gene control may also apply to mitochondria and bacteria. The discovery of 'bacterial' two-component systems (Alex and Simon 1994) in yeast (Ota and Varchavsky 1993) and *Arabidopsis* (Chang et al. 1993) and the two-component sensor module of phytochrome (Schneider-Poetsch 1992) support the proposal that a two component redox regulatory system (Allen 1993a) such as Arc (Iuchi and Lin 1993) may also be present in eukaryotes. The coordination of the organelle redox genetic response with nuclear redox-regulated transcription (Ng et al. 1993) must be important for the general integration of bioenergetic systems in the maintenance and defence of eukaryotic cellular function.

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