THE ROYAL SOCIETY

doi 10.1098/rstb.2000.0697

Balancing the two photosystems: photosynthetic electron transfer governs transcription of reaction centre genes in chloroplasts

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Chloroplasts are cytoplasmic organelles whose primary function is photosynthesis, but which also contain small, specialized and quasi-autonomous genetic systems. In photosynthesis, two energy converting photosystems are connected, electrochemically, in series. The connecting electron carriers are oxidized by photosystem I (PS I) and reduced by photosystem II (PS II). It has recently been shown that the oxidation–reduction state of one connecting electron carrier, plastoquinone, controls transcription of chloroplast genes for reaction centre proteins of the two photosystems. The control counteracts the imbalance in electron transport that causes it: oxidized plastoquinone induces PS II and represses PS I; reduced plastoquinone induces PS I and represses PS II. This complementarity is observed both *in vivo*, using light favouring one or other photosystem, and *in vitro*, when site-specific electron transport inhibitors are added to transcriptionally and photosynthetically active chloroplasts. There is thus a transcriptional level of control that has a regulatory function similar to that of purely post-translational 'state transitions' in which the redistribution of absorbed excitation energy between photosystems is mediated by thylakoid membrane protein phosphorylation. The changes in rates of transcription that are induced by spectral changes *in vivo* can be detected even before the corresponding state transitions are complete, suggesting the operation of a branched pathway of redox signal transduction. These findings suggest a mechanism for adjustment of photosystem stoichiometry in which initial events involve a sensor of the redox state of plastoquinone, and may thus be the same as the initial events of state transitions. Redox control of chloroplast transcription is also consistent with the proposal that a direct regulatory coupling between electron transport and gene expression determines the function and composition of the chloroplast's extra-nuclear genetic system.

Keywords: photosynthesis; photosystem stoichiometry; gene expression; redox signal; plastoquinone; state transitions

1. DISTRIBUTION OF ABSORBED LIGHT ENERGY BETWEEN PHOTOSYSTEMS: STATE TRANSITIONS

In photosynthesis in chloroplasts, two separate light-driven reactions each move an electron from a donor to an acceptor: the acceptor becomes chemically reduced and the donor becomes oxidized. Subsequent electron transfers between the acceptor of one reaction and the donor of the other is passive, thermodynamically 'downhill', and resembles respiratory electron transport in a number of ways. The two light-driven reactions, termed photosystem I (PS I) and photosystem II (PS II), are thus connected in series (Hill & Bendall 1960): the sequence of electron transfers, plotted on a scale of redox potential, gives a 'Z-scheme'.

Each photosystem contains a photochemical reaction centre and its own array of light-harvesting antenna pigments. Reaction centres and light-harvesting pigments are associated with specific protein complexes, and reaction centre complexes are always intrinsic membrane proteins. PS II, where the initial electron donor is water, absorbs and converts visible light at wavelengths up to 680 nm. PS I, which accepts electrons from PS II, uses light of greater wavelength, up to or beyond 700 nm: the action spectrum of PS I extends beyond the 680 nm 'red drop' in quantum yield of complete photosynthesis that is imposed by the wavelength maximum of PS II (Myers 1971). This arrangement presents an experimental opportunity for defining spectral bands that predominantly excite PS II ('light 2') or PS I ('light 1'). As predicted by the Z-scheme (Hill & Bendall 1960), light 2 causes reduction of electron carriers of intermediate redox potential, while light 1, when superimposed, reversibly induces their oxidation (Duysens & Amesz 1962).

The state of oxidation–reduction ('redox state') of one of the components of the connecting electron transport

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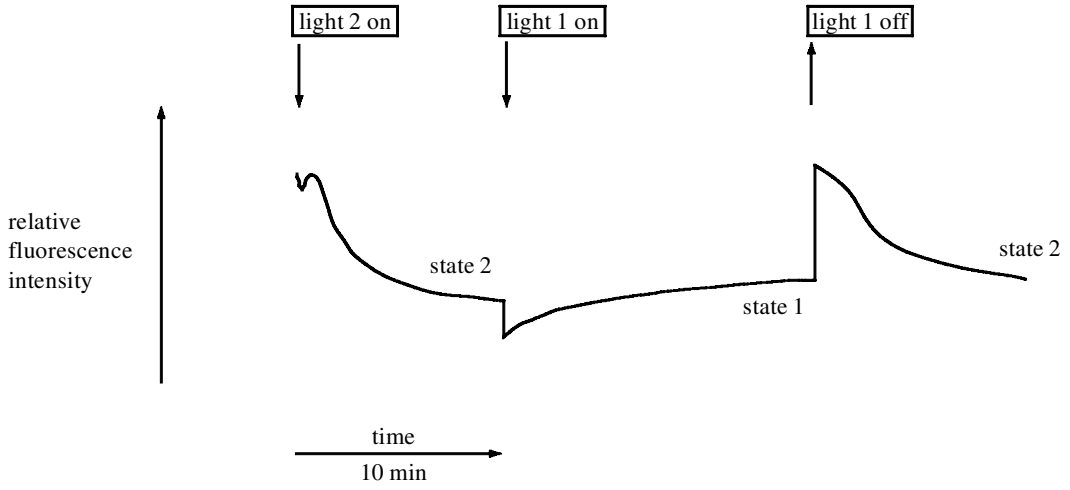

Figure 1. Model state 1–state 2 transitions, as monitored by chlorophyll fluorescence. Light 2 (e.g. $\lambda = 645$ nm) is modulated to combine the functions of fluorescence excitation and actinic illumination of PS II. Alternatively, modulated fluorescence excitation and continuous actinic illumination may be supplied from separate light sources. The fluorescence signal is obtained as the output from an amplifier locked in phase at the frequency of modulation of the excitation light. Effects of continuous light 1 (e.g. $\lambda = 710$ nm) are therefore indirect and indicate changes in yield of fluorescence from PS II. Rapid transients (half-time of seconds) indicate effects of light 1 on photochemical quenching of PS II fluorescence. The subsequent, slower phases, with a half-time of several minutes, indicate redistribution of excitation energy between PS I and PS II: the falling phase is the transition to state 2, which is redistribution of absorbed excitation energy to PS I at the expense of PS II; the rising phase is the transition to state 1, where absorbed excitation energy is redistributed to PS II at the expense of PS I. Qualitatively similar results have be obtained with many oxygen evolving, 2-light reaction species, from cyanobacteria to leaves of higher plants. The phenomenon can be demonstrated in isolated chloroplasts, and also in isolated thylakoids provided ATP is present as a substrate for the LHC II kinase. The wavelengths described above are suitable for LHC II-containing organisms. In phycobilin-containing organisms (cyanobacteria and red algae) light 2 should be specific for phycobilin absorption (e.g. within the range 500–610 nm) and any chlorophyll-absorbed light (e.g. blue, centred on 440 or 480 nm, or red, above 640 nm) will function as light 1. Redrawn from Allen (1992).

chain, plastoquinone, controls the activity of a protein kinase that catalyses phosphorylation of a mobile light-harvesting pigment–protein complex (Allen *et al.* 1981). The light-harvesting of the PS II complex (LHC II) provides absorbed excitation energy to PS II when dephosphorylated and to PS I when phosphorylated. This redox control of LHC II kinase activity, by a rapid and purely post-translational mechanism, tends to make distribution of absorbed light energy between PS I and PS II self-regulating.

Redistribution of absorbed excitation energy between PS I and PS II was demonstrated in the green alga *Chlorella pyrenoidosa* by Bonaventura & Myers (1969) and in the red alga *Porphyridium cruentum* by Murata (1969). Figure 1 presents a summary of this phenomenon as monitored by changes in variable chlorophyll fluorescence emission from PS II. Dark-adapted cells are illuminated with modulated light at 645 nm, absorbed predominantly by chlorophyll *b* in the LHC II complex. Chlorophyll fluorescence falls slowly from an initial maximum, and oxygen yield (not shown) increases with approximately the same kinetics as those with which energy is redistributed to PS I at the expense of PS II. PS I, which is initially rate limiting, becomes more effective in capturing light energy and transporting electrons as the redistribution proceeds. The chlorophyll fluorescence from PS II decreases as a result of the combined decrease in excitation energy transfer to PS II and increased photochemical quenching of PS II fluorescence. The state of minimum fluorescence (and maximum oxygen yield) under PS II light, light 2, is termed 'state 2'. The transition to state 2 is thus a process of redistribution of excitation energy in favour of PS I. Upon addition of continuous light 1 at 710 nm, further quenching of PS II fluorescence occurs and there follows a fluorescence rise that indicates redistribution of excitation energy back to PS II. The new state under PS I light, light 1, is termed 'state 1'. The transition to state 1 is thus a process of redistribution of excitation energy in favour of PS II. In whole cells both the state 1 and state 2 transitions result in increased yield of oxygen (Bonaventura & Myers 1969).

The transition to state 2 can be explained by redox-controlled phosphorylation of LHC II, according to the scheme in figure 2, as follows. Where light 2 drives PS II momentarily faster than PS I, plastoquinone becomes reduced, the LHC II kinase is activated, and LHC II becomes phosphorylated. Phospho-LHC II then moves away from PS II and supplies excitation energy to PS I instead. Conversely, the transition to state 1 occurs because light 1 drives PS I momentarily faster than PS II, plastoquinone becomes oxidized, and the LHC II kinase is inactivated. The LHC II phosphatase, which is continually active (Silverstein *et al.* 1993), then catalyses dephosphorylation of LHC II, thereby returning excitation energy to PS II.

2. ACCLIMATION BY ADJUSTMENT OF PHOTOSYSTEM STOICHIOMETRY

The rationale for photosystem stoichiometry adjustment is similar to that for state transitions: for maximum efficiency, equal rates of electron flow must result from

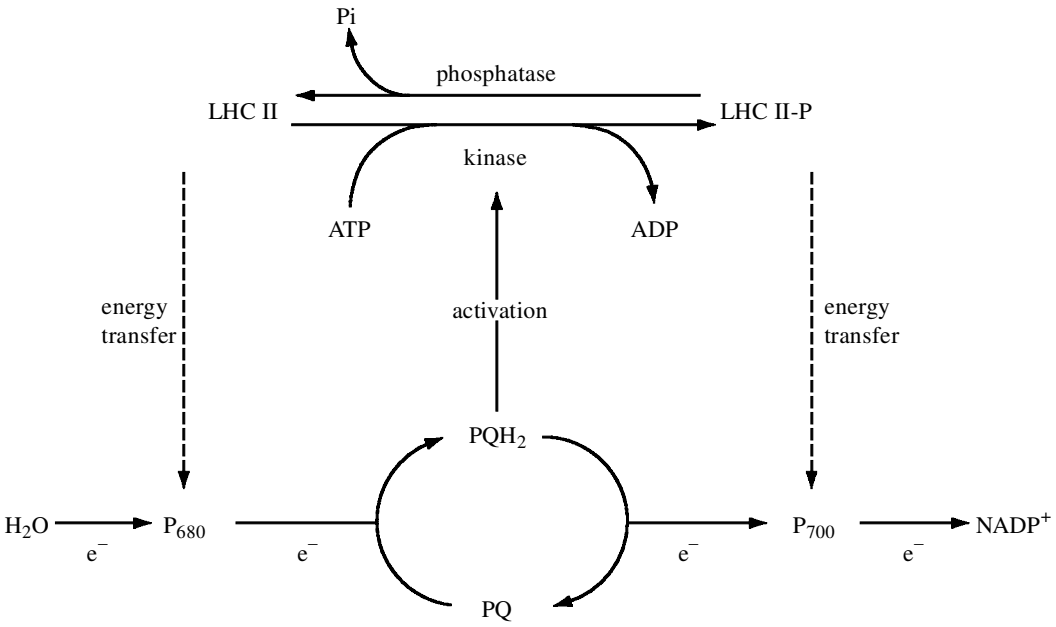

Figure 2. A scheme for control of the LHC II protein kinase by the redox state of plastoquinone. LHC II transfers absorbed excitation energy primarily to PS II, driving electron transport at the reaction centre chlorophyll, P_{680}. Reduction of plastoquinone (PQ) to plastoquinol (PQH_2) by imbalance in excitation energy distribution leads to activation of the LHC II protein kinase. Phospho-LHC II (LHC II-P) transfers absorbed excitation energy primarily to PS I, where it drives electron transport at the reaction centre chlorophyll, P_{700}. Increased excitation energy transfer to PS I will tend to oxidize plastoquinone, inactivate the kinase, and allow the LHC II-phosphatase reaction to predominate. Excitation energy distribution between PS I and PS II will therefore tend to be self-regulating. Redrawn from Allen (1992).

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.

In chlorophyll *b*-containing plants and algae, chlorophyll *a* is found in both photosystems, while chlorophyll *b* is mostly confined to PS II (Anderson *et al.* 1973). An increase in the stoichiometry of PS II to PS I therefore produces a decrease in the ratio of chlorophyll *a*/*b*. This effect is known to be produced by exchanging light 2 for light 1 (Anderson 1986) or by decreasing the intensity of white light (Melis & Harvey 1981). The stoichiometry of PS II to PS I may also be measured as the stoichiometry of phaeophytin or Q_A (from fluorescence induction) to P_{700} (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).

It has been proposed by Fujita *et al.* (1987) that adjustment of photosystem stoichiometry is a response to changes in the redox state of interphotosystem electron carriers. Fujita *et al.* (1987) measured photosystem stoichiometry in the cyanobacterium *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. From the 2-*n*-heptyl-4-hydroxyquinoline-N-oxide (HQNO)-sensitivity of increased PS I formation, Murakami & 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 or in binding of chlorophyll *a* to one or more PS I apoproteins (Murakami & 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. These results 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).

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 fluorescence (Allen *et al.* 1989). These results suggest that state transitions and photosystem stoichiometry adjustment may operate simultaneously, and may both be triggered by changes of redox state of interphotosystem electron carriers (Allen 1995).

3. PLASTOQUINONE REDOX CONTROL OF CHLOROPLAST GENE EXPRESSION

The same redox signal of imbalance of excitation energy, the redox state of the plastoquinone pool, now appears to control both state transitions and photosystem stoichiometry.

In experiments with mustard (*Sinapis alba*) seedlings, Pfannschmidt *et al.* (1999*a*) devised an experimental system for growth of seedlings in a cabinet supplied with low irradiance (35 $\mu E\,m^{-2}\,s^{-1}$) light 1 or light 2. Seedlings were grown for five days in each photosystem-selective light regime; the light 1 was then exchanged for light 2 and *vice versa*. The changes in illumination produced changes in chlorophyll *a*/*b* ratio that occurred with a half-time of about 12 h, and which took place in directions consistent with the induction of changes in photosystem stoichiometry: transfer from light 1 to light 2 caused an increase in chlorophyll *a*/*b* ratio as expected for a decrease in PS II–PS I ratio, while transfer from light 2 to light 1 caused a decrease in chlorophyll *a*/*b* ratio, consistent with an increase in PS II–PS I (Pfannschmidt *et al.* 1999*a*,*b*). Measurement of the effects of each 'light switch' (light 1 to light 2; light 2 to light 1) on chlorophyll fluorescence emission *in situ* (Pfannschmidt *et al.* 1999*b*) confirmed that each light was functionally specific for one photosystem, and that the switch itself also induced a transition to state 2 (light 1 to light 2) or to state 1 (light 2 to light 1), following the pattern depicted in figure 1.

Using probes specific for the reaction centre genes *psbA* (encoding the D_1 polypeptide of PS II) and *psaAB* (encoding the A and B polypeptides of PS I), Pfannschmidt *et al.* (1999*a*) showed that the same light switches induced changes in mRNA abundance. These changes occurred in directions consistent with the observed changes in chlorophyll *a*/*b* ratio and photosystem stoichiometry. Thus the change from light 1 to light 2 caused a decrease in *psbA* mRNA and an increase in *psaAB* mRNA, while the change from light 2 to light 1 caused an increase in *psbA* mRNA and a decrease in *psaAB* mRNA. Furthermore, the rate of transcription of each gene showed the same pattern in transcriptional run-on assays with isolated chloroplasts.

Figure 3 summarizes the complementarity of the responses of the gene expression systems for PS I and PS II in the experiments of Pfannschmidt *et al.* (1999*a*). The data presented shows the effect observed *in vivo* of moving from rate limitation by PS II (left-hand column PS II-I) to rate limitation by PS I (right-hand column PS I-II). The columns are as follows (from left to right for each panel): plants grown in light 2 for 5 days and then in light 1 for 2 days (PS II-I); plants grown for 7 days in light 1 (PS I); plants grown in white light (the control, W); plants grown in light 2 for 7 days (PS II); plants grown in light 1 for 5 days and then in light 2 for 2 days (PS I-II). Three quantities associated with each photosystem are plotted: rate of run-on transcription ('transcription'); quantity of RNA ('RNA'); and quantity of reaction centre measured as Q_A for PS II and P_{700} for PS I ('protein'). As the site of rate limitation changes incrementally from PS II (PS II-I) to PS I (PS I-II), all PS I-related values increase (left-hand panel) while all PS II-related values decrease (right-hand panel). For PS II, the exception to this general trend is that PS I-II plants do not show a further decrease compared to PS II plants. The reasons for this departure from complete symmetry in the two data sets are not known. Figure 3 also shows that the greatest changes for *psbA* occur at the level of RNA, which suggests the additional involvement of post-transcriptional events.

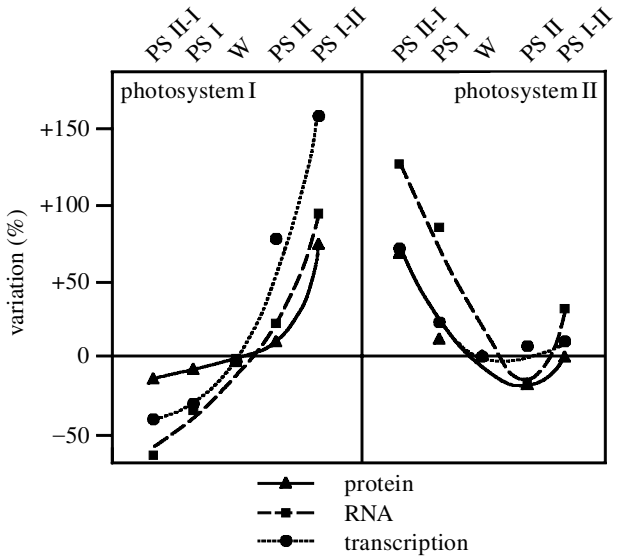

Figure 3. Comparison of relative changes in rates of transcription (filled circles), transcript pool sizes (filled squares) and photosynthetic reaction centre numbers (filled triangles) under various light-quality environments in mustard seedlings. Changes in values for each of the three quantities were induced by a distinct light-quality environment (top of figure) and are given as percentages of the corresponding value under white light (W). Growth light conditions were seven days in light 1 (PS I) or light 2 (PS II); five days in light 1 followed by two days in light 2 (PS I-II); and five days in light 2 followed by two days in light 1 (PS II-I). Left-hand panel ('photosystem I'), changes in PS I-related quantities; P_{700}, *psaAB* transcript, *psaAB* transcriptional rate. Right-hand panel ('photosystem II'), changes in PS II-related quantities; Q_A, *psbA* transcript, *psbA* transcriptional rate. 'Protein' means measured quantities of P_{700} for PS I and Q_A for PS II. Results are calculated from the published data of Pfannschmidt *et al.* (1999*a*).

Figure 4 outlines a scheme for the relationship between state transitions and photosystem stoichiometry adjustment. The model is consistent with the LHC II-phosphorylation model of state transitions (figure 2) and with recent data, described here, indicating control of photosystem stoichiometry by means of control of transcription of reaction centre genes by the redox state of the plastoquinone pool.

4. COMPARISON OF THE KINETICS OF STATE TRANSITIONS AND OF TRANSCRIPTIONAL REDOX CONTROL

If (as depicted in figure 4) LHC II phosphorylation and state transitions comprise the post-translational arm of an integrated redox control system that extends also to transcription of reaction centre genes, it becomes useful to ask if the two levels of response operate simultaneously or on different time-scales. One simple expectation might be that the post-translational response occurs first and that a transcriptional response follows if, and only if, the post-translational response is insufficient in amplitude to restore redox poise and thus to remove the signal of imbalance in excitation energy distribution. If this

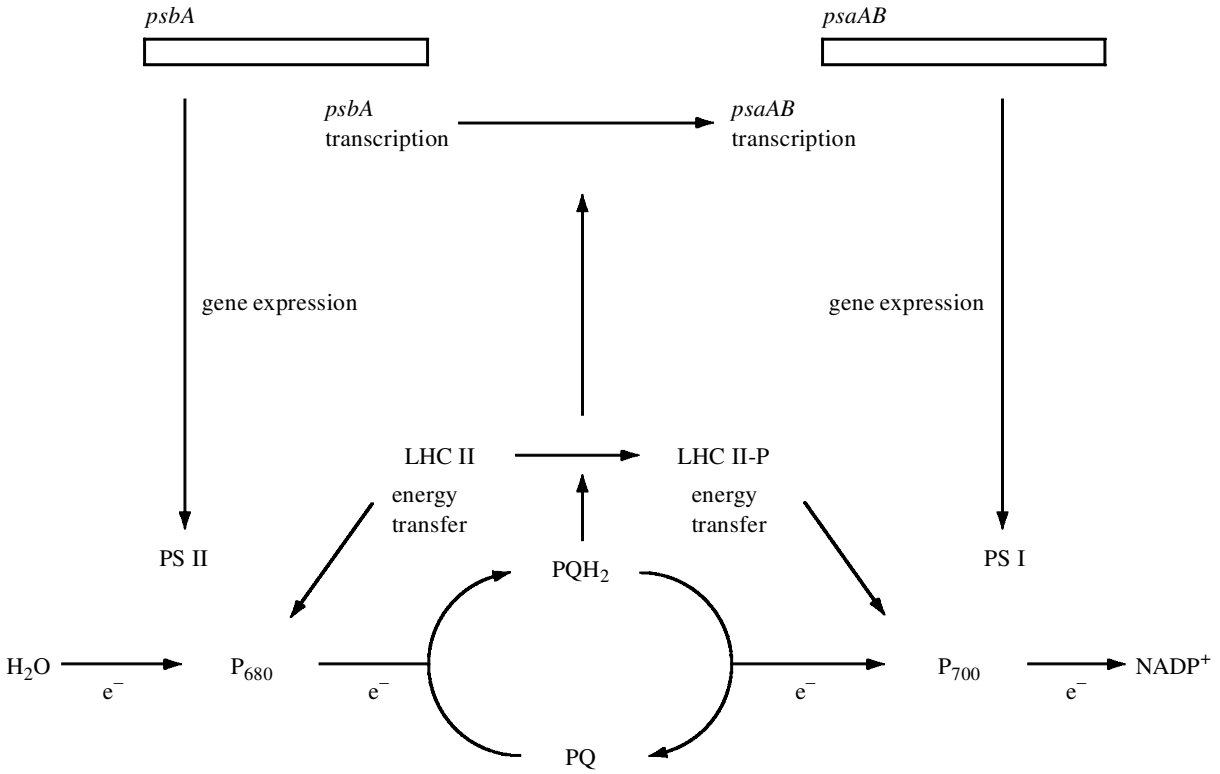

Figure 4. Redox control of photosystem stoichiometry by effects on transcription of genes for reaction centre components. *psbA* encodes the D_1 protein of PS II; *psaAB* encodes the A and B subunits of the reaction centre of PS I. Reduction of plastoquinone (PQ) to plastoquinol (PQH_2) by imbalance in excitation energy distribution leads phosphorylation of LHC II (see figure 2) and also to activation of transcription of *psaAB* and repression of *psbA*. Increased excitation energy transfer to PS I will tend to oxidize plastoquinone, which leads to dephosphorylation of LHC II (see figure 2) and also to activation of transcription of *psbA* and repression of *psaAB*. The stoichiometry between PS I and PS II will therefore tend to be self-regulating, and will adjust itself as if to compensate for a changed light regime with a spectral distribution favouring either PS I or PS II.

expectation were correct, we might expect a time-delay between state transitions and the transcriptional responses that are brought into play: it would seem not to make energetic sense for the costly machinery of gene expression and protein synthesis and assembly to be switched on and off when the possibility still exists that post-translational mechanisms may provide a sufficient response that restores maximum photosynthetic efficiency.

Figures 5 and 6 show changes in chlorophyll fluorescence and in the rate of transcription of reaction centre genes induced by changes in the light regime that select for PS I and PS II. The two dependent variables, fluorescence and transcriptional rate, are plotted in the same time-axis. Figure 5 shows the responses that occur after light 1 is replaced by light 2 ('PS I-II'), that is, when the state 2 transition occurs, and when the rate of transcription of *psaAB* increases and that of *psbA* decreases. Although the changes in rate of transcription appear to lag behind the fluorescence changes, transcription is certainly affected before completion of the fall in fluorescence that reports on the transition to state 2. Figure 6 shows the corresponding changes induced by the opposite light switch, that is, after light 2 is replaced by light 1 ('PS II-I'). Increased rate of *psbA* transcription and decreased rate of *psaAB* transcription certainly occur whilst the slow rise in fluorescence that accompanies the state 1 transition is still underway. These results (figures 5 and 6) indicate that the simple expectation that gene transcription follows only after state transitions are complete is certainly incorrect.

The unexpected rapidity of the transcriptional responses (figures 5 and 6) may reflect a functional state of 'readiness' for subsequent alterations in the pattern of gene expression, since the time-scale of changes in chlorophyll *a*/*b* ratio (Pfannschmidt *et al.* 1999*b*) suggest that changes in photosystem stoichiometry are much slower than state transitions. Nevertheless, the rapid changes in rate of transcription occur in the correct directions to account for subsequent changes in mRNA abundance and, eventually, in photosystem stoichiometry. Post-translational and transcriptional responses to perturbation of the redox state of the plastoquinone pool occur contemporaneously, with half-times measured in minutes (figures 5 and 6).

5. REDOX CONTROL AND THE FUNCTION OF CHLOROPLAST GENOMES

The results described (figures 3, 5 and 6) and reviewed here (Pfannschmidt *et al.* 1999*a*,*b*) indicate a functionally intelligible link between plastoquinone redox state and control of chloroplast gene expression at the transcriptional level. Previous work on effects of light and redox conditions on photosynthesis gene expression in eukaryotic systems has tended to focus on whole algal cells. Danon & Mayfield (1994) obtained evidence for

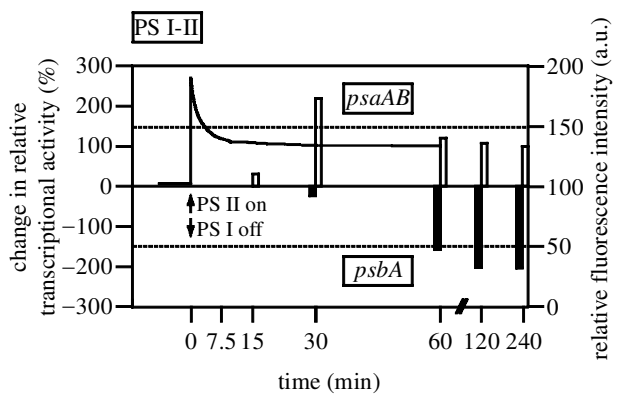

Figure 5. Kinetics of changes in fluorescence (state transition) and transcriptional rates in response to reduction of the plastoquinone pool after a growth light switch from light 1 to light 2. Chlorophyll fluorescence emission at 700 nm was obtained by weak modulated excitation emission at 580–600 nm of seven-day old mustard seedlings. State 1–state 2 transitions were induced by switching between continuous actinic lights 1 and 2 (PS I off, PS II on). Parallel changes in transcriptional rates of chloroplast genes *psbA* (closed bars) and *psaAB* (open bars) were determined at several time intervals after the light switch and are given as a percentage of the transcriptional rate just before the light switch. Results are calculated from the published data of Pfannschmidt *et al.* (1999*b*).

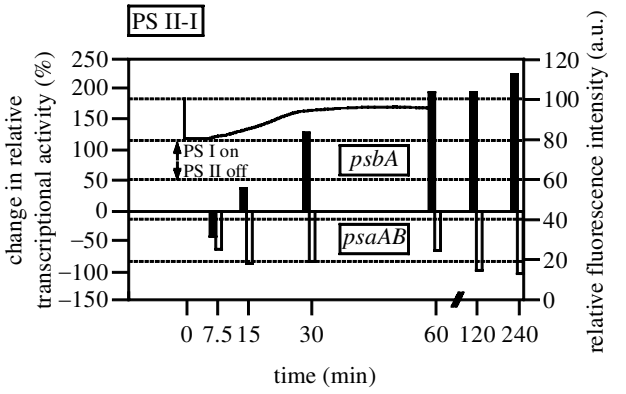

Figure 6. Kinetics of changes in fluorescence (state transition) and transcriptional rates in response to oxidation of the plastoquinone pool after a growth light switch from light 2 to light 1. Chlorophyll fluorescence emission at 700 nm was obtained by weak modulated excitation emission at 580–600 nm of seven-day old mustard seedlings. State 1–state 2 transitions were induced by switching between continuous actinic lights 1 and 2 (PS I on, PS II off). Parallel changes in transcriptional rates of chloroplast genes *psbA* (closed bars) and *psaAB* (open bars) were determined at several time intervals after the light switch and are given as a percentage of the transcriptional rate just before the light switch. Results are calculated from the published data of Pfannschmidt *et al.* (1999*b*).

translational control of *psbA* gene expression in *Chlamydomonas*, implicating the acceptor side of PS I, via ferredoxin and thioredoxin. Previous evidence for control of photosynthesis gene expression control at the level of plastoquinone has been obtained for the nuclear-encoded LHC II of green algae (Escoubas *et al.* 1995; Maxwell *et al.* 1995). Chloroplast redox control of nuclear gene expression is not addressed by the experiments described here, but it is possible that the redox signalling pathway that we have shown to control chloroplast transcription extends to the nuclear–cytosolic system of photosynthetic eukaryotes. Previous *in vitro* experiments on redox control of organelle gene expression have been confined to studies of *de novo* protein synthesis in isolated chloroplasts and mitochondria (Allen *et al.* 1995; Escobar Galvis *et al.* 1998). In prokaryotes, a role for plastoquinone redox state in controlling photosystem stoichiometry through effects on biosynthesis of chlorophyll *a* has been proposed for cyanobacteria (Fujita *et al.* 1987). In view of the prokaryotic ancestry of chloroplasts, cyanobacterial redox control of photosystem gene transcription might now usefully be considered as a possible basis for control of cyanobacterial photosystem stoichiometry.

There is an evolutionary implication of our finding that chloroplast reaction centre gene expression is rapidly and directly regulated by the redox state of a component of the photosynthetic electron transport chain. The ancestor of eukaryotic cells acquired many genes upon its merger (Whatley *et al.* 1979; Cavalier-Smith 1987; Martin & Müller 1998) with the prokaryotic, eubacterial ancestors of chloroplasts (Ellis 1984) and mitochondria (Attardi & Schatz 1988). Of the genes subsequently retained, most have now been removed to the cell nucleus, but a small and relatively constant subset of genes has remained *in situ*, within the organelle. So why do chloroplasts and mitochondria retain any genes at all? It has been suggested that the function of chloroplast (Allen 1993*a*,*b*) and mitochondrial (Allen 1993*a*; Allen & Raven 1996) genetic systems is rapid and direct regulation of expression of genes whose products must assume a particular stoichiometry in order for electron transport to function safely and efficiently. This stoichiometry may be different under different environmental conditions, and a plausible signal for adaptation is departure of electron carriers from optimal redox poise. Photosynthetic control of chloroplast reaction centre gene transcription is completely consistent with this hypothesis. Here we present evidence that plastoquinone is the site of a photosynthetic redox control of chloroplast gene expression that leads to adjustment of photosystem stoichiometry. The exact location of the redox signal is likely to be the same as that involved in state transitions (Allen & Nilsson 1997). Evidence from site-directed mutants of *Chlamydomonas* suggests that the Q_o plastoquinone binding site of cytochrome b_6 is the primary point of control (Zito *et al.* 1999).

The rapidity of the transcriptional responses shown here (figures 5 and 6) is reminiscent of those of prokaryotic systems. Such close and direct control may depend upon a chloroplast location for the genes affected. In contrast, the genes for the components of the signal transduction pathway need not, themselves, be carried by the chloroplast genome. Indeed, there is every reason to suppose that these regulatory genes fall into the major class of genes for chloroplast components, that is, that they originated from the photosynthetic (cyano)bacteria that evolved into chloroplasts but are now located in the cell nucleus (Allen 1993*a*,*b*; Allen & Raven 1996). Photosynthetic control of gene expression may thus, as a general principle, help to explain the maintenance, in evolution, of the chloroplast genetic system.

Supported by grants to J.F.A. from The Swedish Natural Sciences Research Council (NFR), The Swedish Council for Planning and Co-ordination of Research (FRN) and the Crafoord Foundation, and to T.P. from Deutsches Forschungsgemeinschaft. We thank A. Nilsson for spectroscopic determination of P_{700} and Q_A (figure 3) and G. Link for discussions and for providing seeds and mustard gene probes.

REFERENCES

Allen, C. A., Håkansson, G. & Allen, J. F. 1995 Redox conditions specify the proteins synthesised by isolated chloroplasts and mitochondria. *Redox Rep.* **1**, 119–123.

Allen, J. F. 1992 Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta* **1098**, 275–335.

Allen, J. F. 1993*a* Control of gene expression by redox potential and the requirement for chloroplast and mitochondrial genomes. *J. Theor. Biol.* **165**, 609–631.

Allen, J. F. 1993*b* Redox control of gene expression and the function of chloroplast genomes—an hypothesis. *Photosynth. Res.* **36**, 95–102.

Allen, J. F. 1995 Thylakoid protein phosphorylation, state 1–state 2 transitions, and photosystem stoichiometry adjustment: redox control at multiple levels of gene expression. *Physiol. Plant.* **93**, 196–205.

Allen, J. F. & Nilsson, A. 1997 Redox signalling and the structural basis of regulation of photosynthesis by protein phosphorylation. *Physiol. Plant.* **100**, 863–868.

Allen, J. F. & Raven, J. A. 1996 Free-radical-induced mutation vs redox regulation: costs and benefits of genes in organelles. *J. Mol. Evol.* **42**, 482–492.

Allen, J. F., Bennett, J., Steinback, K. E. & Arntzen, C. J. 1981 Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. *Nature* **291**, 25–29.

Allen, J. F., Mullineaux, C. W., Sanders, C. E. & Melis, A. 1989 State transitions, photosystem stoichiometry adjustment and non-photochemical quenching in cyanobacterial cells acclimated to light absorbed by photosystem I or photosystem II. *Photosynth. Res.* **22**, 157–166.

Anderson, J. M. 1986 Photoregulation of the composition, function, and structure of thylakoid membranes. *A. Rev. Plant Physiol.* **37**, 93–136.

Anderson, J. M., Goodchild, D. J. and Boardman, N. K. 1973 Composition of the photosystems and chloroplast structure in extreme shade plants. *Biochim. Biophys. Acta* **325**, 573–585.

Attardi, G. & Schatz, G. 1988 Biogenesis of mitochondria. *A. Rev. Cell Biol.* **4**, 289–333.

Bonaventura, C. & Myers, J. 1969. Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*. *Biochim. Biophys. Acta* **189**, 366–389.

Cavalier-Smith, T. 1987 The simultaneous symbiotic origin of mitochondria, chloroplasts, and microbodies. *Annls NY Acad. Sci.* **503**, 55–71.

Chow, W. S., Melis, A. & Anderson, J. M. 1990. Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. *Proc. Natl Acad. Sci. USA* **87**, 7502–7506.

Danon, A. & Mayfield, S. P. 1994 Light-regulated translation of chloroplast messenger RNAs through redox potential. *Science* **266**, 1717–1719.

Duysens, L. N. M. & Amesz, J. 1962 Function and identification of two photochemical systems in photosynthesis. *Biochim. Biophys. Acta* **64**, 243–260.

Ellis, R. J. 1984 The nuclear domination of chloroplast development. *Sci. Prog. Oxf.* **69**, 129–142.

Escobar Galvis, M. L., Allen. J. F. & Håkansson, G. 1998 Protein synthesis by isolated pea mitochondria is dependent on the activity of respiratory complex II. *Curr. Genet.* **33**, 320–329.

Escoubas, J.-M., Loumas, M., LaRoche, J. & Falkowski, P. G. 1995 Light intensity regulation of cab gene transcription is signaled by the redox state of the plastoquinone pool. *Proc. Natl Acad. Sci. USA* **92**, 10 237–10 241.

Fujita, Y., Murakami, A. & Ohki, K. 1987 Regulation of photosystem composition in the cyanobacterial photosynthetic system: the regulation occurs in response to the redox state of the electron pool located between the two photosystems. *Plant Cell Physiol.* **28**, 283–292.

Hill, R. & Bendall, F. 1960 Function of the two cytochrome components in chloroplasts, a working hypothesis. *Nature* **186**, 136–137.

Kim, J. H., Glick, R. E. & Melis, A. 1993. Dynamics of photosystem stoichiometry adjustment by light quality in chloroplasts. *Plant Physiol.* **102**, 181–190.

Martin, W. & Müller, M. 1998 The hydrogen hypothesis for the first eukaryote. *Nature* **392**, 37–41.

Maxwell, D. P., Laudenbach, D. E. & Huner, N. P. A. 1995 Redox regulation of light-harvesting complex II and cab mRNA abundance in *Dunaliella salina*. *Plant Physiol.* **109**, 787–795.

Melis, A. & Harvey, G. W. 1981 Regulation of photosystem stoichiometry, chlorophyll *a* and chlorophyll *b* content in relation to chloroplast ultrastructure. *Biochim. Biophys. Acta* **637**, 138–145.

Murakami, A. & Fujita, Y. 1993 Regulation of stoichiometry between PS I and PS II in response to light regime for photosynthesis observed with Synechocystis PCC 6714: relationship between redox state of cyt b_6f complex and regulation of PS I formation. *Plant Cell Physiol.* **34**, 1175–1180.

Murata, N. 1969 Control of excitation transfer in photosynthesis. I. Light-induced change of chlorophyll *a* fluorescence in *Porphyridium cruentum*. *Biochim. Biophys. Acta* **172**, 242–251.

Myers, J. 1971 Enhancement studies in photosynthesis. *A. Rev. Plant Physiol.* **22**, 289–312.

Pfannschmidt, T., Nilsson, A. & Allen, J. F. 1999*a* Photosynthetic control of chloroplast gene expression. *Nature* **397**, 625–628.

Pfannschmidt, T., Nilsson, A., Tullberg, A., Link, G. & Allen, J. F. 1999*b* Direct transcriptional control of the chloroplast genes *psbA* and *psaAB* adjusts photosynthesis to light energy distribution in plants. *Int. Union Biochem. Mol. Biol. Life* **48**, 271–276.

Silverstein, T., Cheng, L. & Allen, J. F. 1993 Chloroplast protein phosphatase reactions are redox-independent and kinetically heterogeneous. *FEBS Lett.* **334**, 101–105.

Whatley, J. M., John, P. & Whatley, F. R. 1979 From extracellular to intracellular: the establishment of mitochondria and chloroplasts. *Proc. R. Soc. Lond.* B **204**, 165–187.

Zito, F., Finazzi, G., Delosme, R., Nitschke, W., Picot, D. & Wollman, F.-A. 1999 The Q_o site of cytochrome b_6f complexes controls the activation of the LHC II kinase. *EMBO J.* **18**, 2961–2969.

Discussion

A. Laisk (*Department of Plant Physiology, University of Tartu, Estonia*). It is not the number (density) of PS I and PS II that determines the balance of the Z-scheme but the relative antenna cross-sections of PS I and PS II. Since your data show that the density of PS II–PS I changes, may it be interpreted as showing that illumination with PS I or PS II light does not influence antenna sizes but it induces genes that control the number of centres? In plant canopies there is constantly a type of adaptation that leads to changes in antenna sizes.

J. F. Allen. No, our data should not be interpreted in this way. There is no incompatibility between changes in density of the two photosystems and changes in their antenna cross-sections. The equivalence and coexistence

of these two processes is an important point, and this is implicit in the investigations I have described. Certainly state transitions occur, and can be best described as complementary changes in the antenna size of PS II and PS I, both in chloroplasts and cyanobacteria (for a review, see Allen 1992). In searching for phosphoproteins that might help to explain the 'mobile phycobilisome' model for changes in cyanobacterial antenna size (Allen *et al*. 1985), members of my Leeds laboratory discovered the first evidence for a two-component regulatory system in cyanobacteria, the Ntr system (Harrison *et al*. 1990; Tsinoremas *et al*. 1991). We also learned an important lesson in microbial physiology, and one which, I think, has continued to apply to chloroplasts—determining, in fact, their genetic composition. The lesson is this: if there is a post-translational mechanism for a response to an environmental challenge, then there almost certainly a transcriptional response, too—a solution to the same problem, one which is more expensive in the short term, but longer lasting and therefore cheaper in the long run. One could thus regard photosystem stoichiometry adjustment as the transcriptional arm of an integrated set of redox responses, where change in antenna cross-section is its post-translational equivalent (Allen 1995; Allen & Nilsson 1997). In other words, state transitions are a cheap and interim solution to a problem that can also be solved by a more profound reconfiguration of the photosynthetic apparatus. To my mind, this raises the apparently teleological questions 'Does the system choose between the two levels of response? If so, how?' One might anticipate that the fast, cheap solution is tried first, and the slow, expensive one comes into play if, and only if, the problem persists. The data in figures 5 and 6 indicate that this is not the case: redox control of transcription begins to be exerted even when changes in absorption cross-section are still taking place. If a decision is made about which redox control pathway to deploy, we must therefore look for one or more additional inputs. One might be time: arbitration between competing levels of response by the output from a biological clock would represent invaluable 'foresight', that is, the anticipation of the likely duration on the environmental change (Allen 1998). Returning to leaf canopies, changes in relative antenna size must surely take place, but so do changes in photosystem stoichiometry; in chloroplast morphology and the number of chloroplasts per cell—also in leaf morphology and, in deciduous trees, for example, whether you actually have any leaves at all. I think temporal and other inputs have to play some part in the choice of an appropriate level of response. Trees do not shed their leaves every day, at dusk.

B. Osmond (*Research School of Biological Sciences, Australian National University, Australia*). What is the role of state transitions in regulation of excess photon loads? It is often stated that state transitions deflect excitation from PS II (which is more sensitive to damage) to PS I (which has a higher activity of photon use).

J. F. Allen. I view the function of the adaptations that I have described as maximizing quantum yield of photosynthesis, which is of clear selective value when light is a limiting factor. This would apply both to state transitions and to photosystem stoichiometry adjustment. If this is correct, I am, in a sense, disqualified from a meeting on 'photoprotection of the photosynthetic apparatus' but may have something to say on 'alternative photon and electron sinks'. In fact we routinely take care, in our experiments, in order to ensure that light intensity is limiting for growth and rate limiting for photosynthesis. The light 1 and light 2 used here were adjusted to give a mere $35\,\mu E\,m^{-2}\,s^{-1}$. Yes, it is often argued that the state 2 transition (a decrease in absorption cross-section of PS II and increase in that of PS I) helps to prevent PS II from photodamage. However, I think the magnitude of the change is far too small, at maybe a 20% decrease in PS II antenna size, to make much difference. I would imagine that a sun fleck, for example, can increase the photon flux density at surface of a shaded leaf easily by two orders of magnitude (20 to $2000\,\mu E\,m^{-2}\,s^{-1}$, for example). State transitions and photosystem stoichiometry adjustments are delicate and subtle fine-tuning devices, which help the plant make best use of limiting light. When huge increases in light intensity occur, threatening to tear the fabric of the system by photo-oxidative damage, I am sure high energy state (q_E) quenching and the xanthophyll cycle are qualitatively and quantitatively far more important, and the urgent requirement is to decrease the quantum yield of photochemistry, that is, to downregulate PS II. Responding to a sun fleck with a state transition would be rearranging the deckchairs on the *Titanic*, to borrow a metaphor from elsewhere. Equally, when the shade returns, photosynthetic energy conversion must be as efficient as possible. I think the two processes (energy conservation and energy dissipation) occur at different times and for different reasons. I do not think one can help to explain the other.

C. H. Foyer (*Department of Biochemistry and Physiology, IACR-Rothamsted, UK*). Your model for signal transduction favours the bacterial analogy yet the plastoquinone redox state is a well-characterized modulator of protein kinase activity. Do you have any evidence to suggest that protein kinase activation is not part of the signal transduction process?

J. F. Allen. No. However, the redox-activated LHC II kinase acts on its substrate, LHC II. I would think that this event lies 'downstream' from the branch point of the bifurcated signal transduction pathway. It is the signal itself, the redox sensor, which lies upstream, and which the two levels of response have in common. This is what we learn from, for example, the bacterial Ntr system (Tsinoremas *et al*. 1991), and it seems to make physiological sense. In principle, of course, the LHC II kinase itself could be part of the mechanism of transcriptional control—perhaps even LHC II. I think these possibilities are unlikely. The 'bacterial analogy' is more than an analogy. I have a conviction that chloroplasts are still photosynthetic prokaryotes at heart. This is obviously true when you consider the fundamental features of energy conversion, particularly water oxidation and oxygen evolution. Everything I have argued here is based on the assumption that the same is true for the chloroplast genetic system. In fact, this is why we embarked on these experiments—to test the evolutionary hypothesis (Allen 1993*a*) that the function of the chloroplast genome is to

maintain a sequestered set of genes for key proteins—genes whose expression must always have been kept under strict redox control. The loss of that control would be the penalty to be paid for removal of those genes to the nucleus. The plant cell has, in a sense, no choice but to tolerate the continued quasi-independence of its prokaryotic guest.

C. H. Foyer. Can you speculate on the general role of quinones as signal transducing components in plant cell membranes?

J. F. Allen. Yes. I think that quinone redox state is of huge significance, in both mitochondria and chloroplasts. Plastoquinone or ubiquinone redox state can be perturbed transiently, but must be restored to its optimal value by any means available. Apart from considerations of quantum yield and so on, one very important reason for maintaining a balance between quinone oxidation and quinol reduction is that conversion of the energy of electron transfer into the proton motive force by Mitchell's Q-cycle (Mitchell 1976) requires the semiquinone anion radical ($Q^{\cdot -}$) as an obligatory intermediate. $Q^{\cdot -}$ must be very short lived: it reacts rapidly with oxygen to form the superoxide anion radical, as you know. In animals, this may be the chief source of oxidative stress in ageing and degenerative disease, and I am sure it does similar damage in plants. This underlines my conviction that redox homeostasis is an important perspective for cell evolution: the danger of free-radical induced damage is an unavoidable consequence of the need to convert energy. I would argue that risk assessment (redox sensing) and damage limitation (adaptation) have driven some of the most fundamental changes in evolution, and shape many aspects of life as we know it (Allen 1996, 1998).

E. Garcia-Mendoza (*Department of Microbiology, University of Amsterdam, The Netherlands*). I suggest that state transitions do play an important role in photoprotection by controlling part of the xanthophyll cycle reactions. This suggestion is based on data obtained in a green microalga (Garcia-Mendoza, unpublished data) that the dissociation of LHC II-b enhances the violaxanthin to zeaxanthin reaction.

J. F. Allen. This refers back to the question of Professor Osmond. Yes, LHC II is clearly involved in both energy conservation and energy dissipation. I do think that phosphorylation of LHC II may cause dissociation of LHC II trimers, and that it is monomeric phospho-LHC II that leaves PS II and attaches to PS I, functioning there as an addition to its light-harvesting antenna (Nilsson *et al.* 1997). If the dissociation of LHC II also enhances quenching of excitation energy by promoting de-epoxidation of violaxanthin, then there is an important mechanistic link between the two processes. Many people have objected to the lateral movement of phospho-LHC II (usually in defence of a 'spillover' model in contrast to one of altered absorption cross-section) on the grounds that a species of LHC II unconnected to a reaction centre should have very long fluorescence lifetime, and thus become visible in steady-state fluorescence emission spectra. Since this is not observed, the argument is that LHC II never detaches from PS II. What you suggest may be a solution to this problem: the excitation energy absorbed by free monomeric LHC may be subject to non-radiative decay. We should also note that some workers are still sceptical about the increased absorption cross-section of PS I (see Allen 1992). It is possible to imagine a sort of state transition mechanism in which the redox state of photochemical quenching is restored purely by decreased energy transfer to the reaction centre of PS II. However, it is difficult to see how this could lead directly to the increase in quantum yield of oxygen evolution that characterizes the state 2 transition in whole cells. If an extra increment of excitation energy is thrown away, the quantum yield should not increase. If, instead, it is taken from PS II and given to PS I, then an overall energetic gain is easily explained. As in my reply to Professor Osmond, I do think the functions of the two processes (state transitions and energy dissipation) are quite separate. It could be, of course, that some other factor determines whether phospho-LHC II quenches excitation energy by non-photochemical means or by means of excitation energy transfer to PS I. As I pointed out, the latter makes sense only when light intensity is limiting. Perhaps there is an effect of light intensity or thylakoid lumen pH, and this determines the fate of excitation energy absorbed by monomeric phospho-LHC II. We also have recently shown that LHC II is phosphorylated on tyrosine as well as threonine (Tullberg *et al.* 1998). It is possible that the tyrosine phosphorylation occurs at high light and plays some part on triggering energy dissipation.

Additional References

Allen, J. F. 1996 Separate sexes and the mitochondrial theory of ageing. *J. Theor. Biol.* **180**, 135–140.

Allen, J. F. 1998 Light, time and micro-organisms. In *Microbial responses to light and time* (ed. M. X. Caddick, S. Baumberg, D. A. Hodgson & M. K. Phillips-Jones), pp. 1–31. Cambridge University Press.

Allen, J. F. & Nilsson, A. 1997 Redox signalling and the structural basis of regulation of photosynthesis by protein phosphorylation. *Physiol. Plant.* **100**, 863–868.

Allen, J. F., Sanders, C. E. & Holmes, N. G. 1985 Correlation of membrane protein phosphorylation with excitation energy distribution in the cyanobacterium *Synechococcus* 6301. *FEBS Lett.* **193**, 271–175.

Harrison, M. A., Keen, J. N., Findlay, J. B. C. & Allen, J. F. 1990 Modification of a *glnB*-like gene product by photosynthetic electron transport in the cyanobacterium *Synechococcus* 6301. *FEBS Lett.* **264**, 25–28.

Mitchell, P. 1976 Possible molecular mechanisms of the protonmotive function of cytochrome systems. *J. Theor. Biol.* **62**, 327–367.

Nilsson, A., Stys, D., Drakenberg, T., Spangfort, M. D., Forsén, S. & Allen, J. F. 1997 Phosphorylation controls the three-dimensional structure of plant light-harvesting complex II. *J. Biol. Chem.* **272**, 18350–18357.

Tsinoremas, N. F., Castets, A. M., Harrison, M. A., Allen, J. F. & Tandeau de Marsac, N. 1991 Photosynthetic electron transport controls nitrogen assimilation in cyanobacteria by means of post-translational modification of the *glnB* gene product. *Proc. Natl Acad. Sci. USA* **88**, 4565–4569.

Tullberg, A., Håkansson, G. & Race, H. L. 1998 A protein tyrosine kinase of chloroplast thylakoid membranes phosphorylates light harvesting complex II proteins. *Biochem. Biophys. Res. Commun.* **250**, 607–622.