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The balancing act: redox poise and signalling

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Introduction

Biological electron transfer requires continual monitoring and adjustment of redox state of electron carriers. Maintenance of optimal efficiency is exemplified in photosynthesis by light-state transitions and by adjustment of photosystem stoichiometry. Adjustment of relative and absolute light-harvesting antenna size and density of photosynthetic units takes place in response to changing light quality or quantity, and in response to altered metabolic demand for ATP relative to reducing power. Plastoquinone redox state governs both antenna size and the relative rates of synthesis and breakdown of the two photosystems. Plastoquinone and its binding sites in the cytochrome b_6-f complex and in photosystem II are located between the two photosystems, and the redox signals emanating from this point in the photosynthetic electron transport chain form part of homeostatic negative feedback loops that operate at a number of levels of gene expression. These redox signalling pathways serve to maintain an optimal redox state of plastoquinone. They also serve to balance the light-harvesting capacity of the two photosystems and the stoichiometry of their reaction centres. These different levels of control appear to ensure maximal quantum yield of photosynthesis, which is selectively advantageous under conditions of limiting light intensity.

Post-translational redox control of relative antenna sizes of the two photosystems: state transitions

Complementary changes in light-harvesting antenna size are produced by post-translational modification of light-harvesting proteins. Phosphorylation of chloroplast light-harvesting complex II (LHC II) (Bennett 1977) is activated when plastoquinone becomes reduced (Allen et al 1981). Potentiometric redox titration of 32P-labelling of LHC II and a number of other thylakoid proteins supports this location of redox control (Silverstein et al 1993a). Further evidence (Vener et al 1997, Zito et al 1999) strongly implicates the Q_o plastoquinol binding site of the cytochrome b_6-f complex as the primary site of control, suggesting that initial events of the redox signalling pathway occur within the cytochrome b6-f complex itself (Finazzi et al 2001, Wollman 2001). Equally, the original assumption (Allen et al 1981) of the light-independence and redox-independence of the phosphoprotein phosphatase is supported by results from redox titration of [32]P-

labelling remaining in darkness after light-activated membrane protein phosphorylation (Silverstein et al 1993b).

In 1992 it was stated that "…it is likely that sequence information on the LHC II kinase will soon become available, and there is therefore little point here in speculation concerning the location of the phosphorylation site, membrane disposition, location of the kinase gene and the possible identity of the protein with known thylakoid components" (Allen 1992). In fact, the kinases catalyzing phosphorylation of LHC II and other thylakoid membrane proteins have proved surprisingly elusive. A number of thylakoid-associated kinases (TAKs) has been identified by Kohorn and co-workers (Snyders and Kohorn 1999). These enzymes contain a glycine-serine motif characteristic of signalling by the eukaryotic TGF-β system (Snyders and Kohorn 1999). Antisense-RNA repression of the gene for TAK-1 in *Arabidopsis thaliana* indicates that this enzyme is specifically required for phosphorylation of LHC II and for the associated changes in chlorophyll fluorescence reporting on the transition to light-state 2 (Snyders and Kohorn 2001).

Another approach to identification of components of the redox signalling pathway involved in state transitions is to image chlorophyll fluorescence as a means of identification of a phenotype unable to carry out these adaptations. This has been applied successfully in the cyanobacterium *Synechocystis* 6803 (Emlyn-Jones et al 1999) and in the green alga *Chlamydomonas reinhardtii* (Kruse et al 1999).
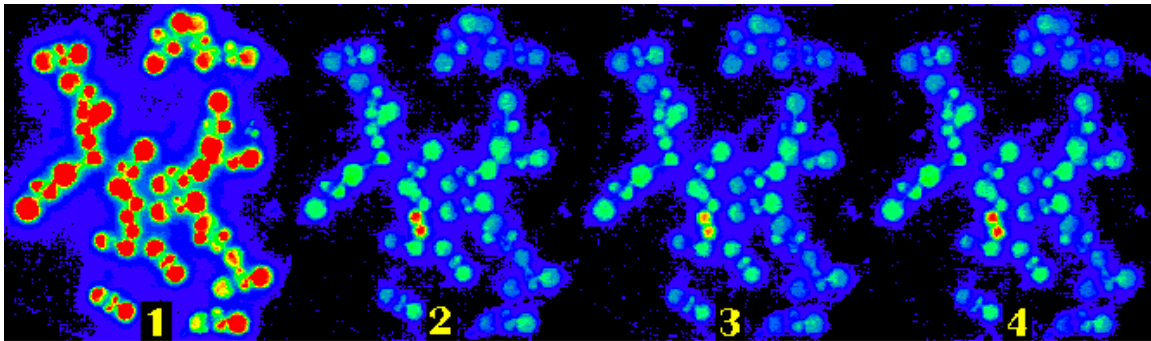

Fig. 1. Using chlorophyll fluorescence imaging to screen for state transition mutants in *Arabidopsis thaliana*. The technique used allows visualisation of chlorophyll fluorescence during the full course of state transitions that are induced by addition of light 1 (710 nm) to a continuous, combined light 2 and excitation beam from a quartz-halogen lamp and defined by a blue Corning 4-96 filter. Chlorophyll fluorescence is imaged by a Photonic Science ISIS red-extended CCD camera blocked by 660 nm narrow-band interference filter. The imaged sequence was acquired processed by the program NIH-Image using an Apple Macintosh computer. Fluorescence is initially high (seen as the red colour in image 1), and falls after six minutes in the light combined excitation bean and light 2 (image 2), as LHC II becomes phosphorylated (giving state 2). Light 1, undetected by the camera, is switched on, and fluorescence falls as photosystem 2 traps open (image 3), to rise slightly after a further two minutes (image 4), as LHC II becomes dephosphorylated (giving state 1). One plant, whose two leaves are seen near the centre, behaves differently, and its fluorescence is consistently higher and less variable than that of others. This T-DNA mutant is tagged in an *Arabidopsis* homologue of *slr1645* of *Synechocystis* 6803. The false colour represents fluorescence intensity (red is maximum signal; blue is minimum signal) at 660 nm. See also http://plantcell.lu.se/research/imaging.html

If the mutant phenotype is produced by a tagged mutation, then isolation of the inserted T-DNA or transposon tag should reveal a component necessary for the state transition that the mutant is unable to perform. This approach has been applied to Arabidopsis thaliana using a fluorescence imaging technique that is capable of following a full time-course of state transitions induced by addition of a background light 1 to a combined light 2 and excitation beam (Allen et al 1995). One high-fluorescence and state-transition-depleted phenotype (Fig. 1) was produced by T-DNA insertion into *slr1645*, a homologue of cyanobacterial *psbZ* (Nakamura et al 1998), an 11 kDa protein associated with photosystem II (Davison P, personal communication). Other studies directed at isolation of tagged, state-transition-minus mutants are in progress in a number of laboratories, and it will be important to see if these independently reveal components with similarities to the TAK system (Snyders and Kohorn 2001), to other putative thylakoid kinases, or to any corresponding system of cyanobacteria (Emlyn-Jones et al 1999).

Molecular recognition

Upon phosphorylation, chloroplast light harvesting complex II undergoes a change in 3-D structure (Nilsson et al 1997). This structural change is likely to be the basis of the increase in the affinity of LHC II for photosystem I and the decrease in its affinity for photosystem II (Allen and Forsberg 2001). Antisense repression of the *PsaH* gene in *Arabidopsis* inhibits the state 2 transition not by affecting kinase activation, but by preventing productive interaction of phospho-LHC II with photosystem I (Lunde et al 2000). The PsaH protein is a subunit of chloroplast photosystem I and is therefore likely to act as an anchor, or binding site, for phospho-LHC II (Haldrup et al 2001). These results support the idea that short-range redistribution of LHC II between the photosystems occurs by means of lateral diffusion and thylakoid protein-protein molecular recognition (Allen 1992, Allen and Forsberg 2001). In addition to redox-activation of the LHC II kinase, a light-induced conformational change in LHC II facilitates its phosphorylation (Zer et al 1999). It has also become clear that LHC II is likely to be phosphorylated at multiple sites (Dilly Hartwig et al 1998), including both threonine and tyrosine residues (Tullberg et al 1998). Phosphorylation of LHC II on tyrosine seems to be an additional factor in the state 2 transition (Forsberg and Allen 2001). LHC II is clearly a very versatile and flexible protein. Not only does LHC II play a key role in thylakoid stacking and in maintaining quantum yield at low light intensities, LHC II and homologous proteins also participate in protective, energy-dissipating reactions that offset destructive photochemistry when light intensity is too high for photosystem II too convert it all in productive charge separation (Niyogi 1999, Horton 2000).

Transcriptional control of reaction centre gene expression: photosystem stoichiometry adjustment
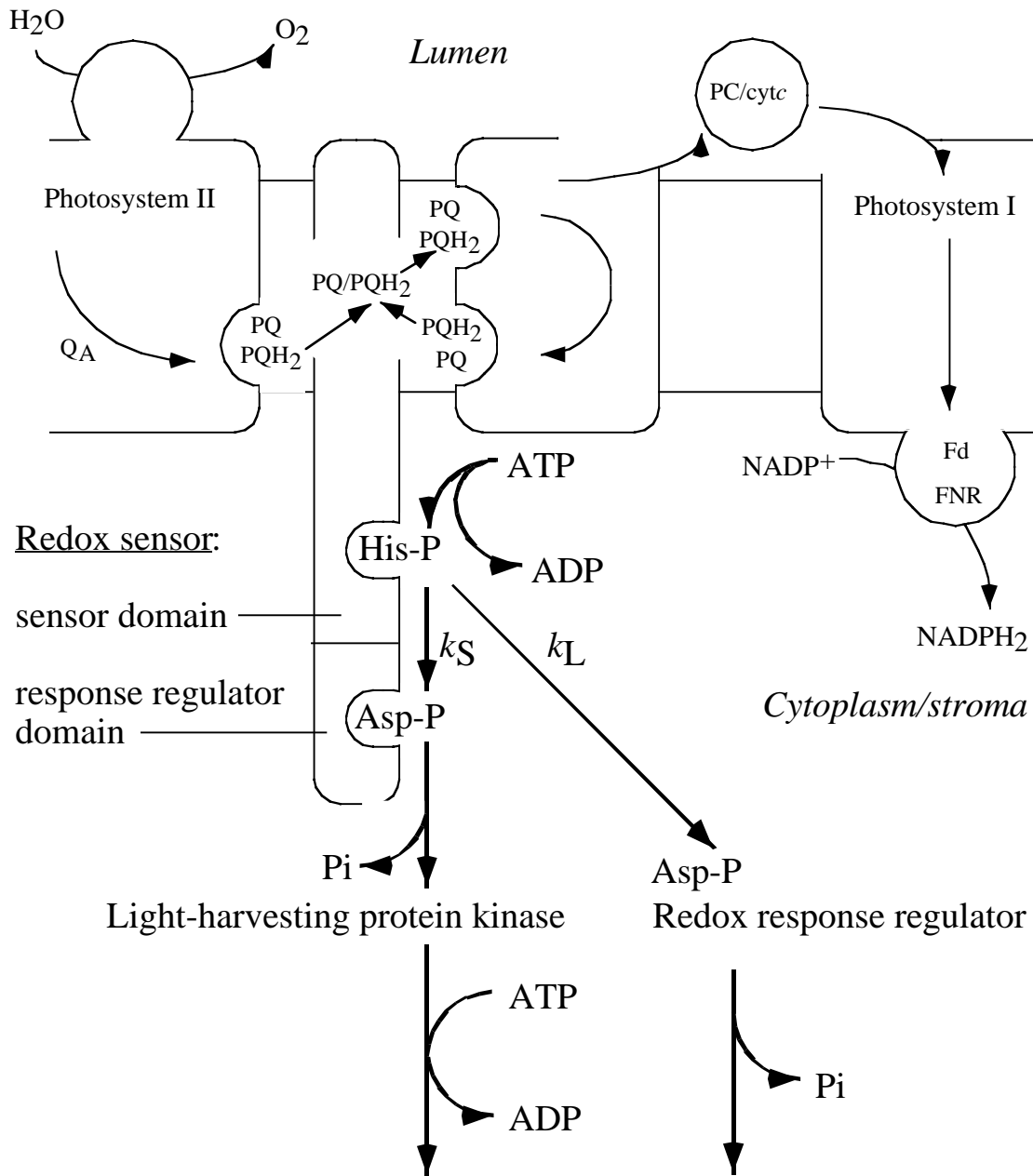
Rapid and complementary control of reaction centre gene transcription appears to be retained entirely within chloroplasts (Pfannschmidt et al 1999, Tullberg et al 2000). The direction of redox control is intelligible functionally in precisely the same way as the direction of excitation energy redistribution in state transitions. Thus reduction of plastoquinone is a signal that photosystem II electron transfer is faster than that in

photosystem I, and its consequence is to repress photosystem II reaction centre gene transcription and to induce photosystem I reaction centre gene transcription (Pfannschmidt et al 1999). Conversely, oxidation of plastoquinone induces photosystem II and represses photosystem I. Furthermore, the changes in reaction centre gene transcription are remarkably rapid, with rates of run-on transcription responding to shifts between lights 1 and 2 on the same time-scale, measured in a few minutes, as changes in fluorescence emission that report on state transitions and LHC II phosphorylation (Allen and Pfannschmidt 2000). It is also clear that plastoquinone redox effects on transcription extend to nuclear genes, both to Lhc genes in algae (Escoubas et al 1995, Maxwell et al 1995) and to photosystem I genes in higher plants (Pfannschmidt et al 2001)

Two-component regulatory systems

Two-component redox regulatory systems exist whose genes are nuclearly encoded, but whose sensors are likely to be thylakoid proteins, and whose response regulators may be targeted to thylakoids and to the chloroplast stroma (Forsberg et al 2001). Such systems are prime candidates for the redox sensor-response regulator pathways predicted to underlie state transitions and photosystem stoichiometry adjustment (Allen 1993, Allen 1995). A redox-responsive two-component system has been identified in the cyanobacterium Synechocystis 6803, and seems to be required for photosystem stoichiometry adjustment in response to alteration in the redox state of the plastoquinone pool (Li and Sherman 2000).

   In view of the reliance of purple photosynthetic bacteria on two-component redox regulatory systems to control expression of genes for components of photosynthetic units (Bauer et al 1999, Eraso et al 2000), it seems reasonable to consider that evolutionary conservation of these control pathways has maintained a chloroplast quinone redox sensor as the initial input into mechanisms that adjust light-harvesting capacity and photosystem stoichiometry. Each level of response may involve a specific response regulator (Allen and Nilsson 1997). Fig 2 shows a possible mechanism for bifurcated redox signal transduction in oxygenic photosynthesis and involving a hybrid histidine kinase-response regulator, and a second response regulator that might function as an alternative phosphate group acceptor. One of the best-characterised redox two-component system is the Arc system of *Escherichia coli*. The sensor ArcB is a hybrid histidine kinase such as that depicted in Fig 2. ArcB is now known to respond specifically to the redox state of the *E. coli* respiratory ubiquinone pool (Georgellis et al 2001).

H$_2$O

O$_2$

Lumen

PC/cyt*c*

Photosystem II

PQ
PQH$_2$

PQ/PQH$_2$

PQ
PQH$_2$

PQH$_2$
PQ

Q$_A$

Photosystem I

ATP

NADP$^+$

Fd
FNR

Redox sensor:

His-P

ADP

NADPH$_2$

sensor domain

k_S

k_L

response regulator
domain

Asp-P

Cytoplasm/stroma

Pi

Asp-P

Light-harvesting protein kinase

Redox response regulator

ATP

ADP

Pi

Decreased PS II antenna size;
increased PS I antenna size;
transition to light state 2

Decreased *psb* transcription;
increased *psa* transcription.
Increased PS I: PS II
stoichiometry

*Short-term adaptation
- state transitions*

*Long-term adaptation
("acclimation")
- photosystem stoichiometry
adjustment*

Fig. 2. A possible mechanism for the branch-point of the redox signalling pathways controlling state transitions and photosystem stoichiometry, and for the selection of the level of response deployed. If the putative redox sensor of two-light reaction organisms contains both sensor and response regulator domains, and if the kinetically favoured pathway for phosphoryl transfer (k_S) is intramolecular, then phosphorylation of the redox sensor's own aspartate would initiate the state 2 transition, for example by activation of the chloroplast LHC II kinase. If full activation of the light-harvesting kinase then still fails to restore redox poise, then phosphoryl transfer (k_L) may occur to the aspartate of the response regulator that regulates photosystem stoichiometry. An assumption of the proposed mechanism is that intramolecular phosphoryl group transfer is kinetically favoured over intermolecular transfer: $k_S > k_L$. Modified from Allen and Nilsson (1997). A more general model for arbitration between short-term (physiological) and long-term (developmental) adaptations is given in Allen (1998).

Genetic systems in bioenergetic organelles may depend upon redox signalling pathways that have continued to operate, and therefore been conserved, through the major evolutionary transition from prokaryotic to eukaryotic cells (Allen 1993).

Acknowledgements

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