Regulation by redox signalling

Photosynthesis is light-driven redox chemistry. Molecular redox signalling, the coupling of gene expression to electron transfer, is now implicated in the adaptation of photosynthesis to variation in light quality and quantity.

Photosynthesis is the conversion of light energy into chemical energy. The primary event is light-driven electron transfer — a redox reaction — and it sets in motion a chain of electron transfers upon which all life ultimately depends. The last decade has seen huge strides forward in understanding the hardware of photosynthesis. The structure of a reaction centre, where the primary redox reaction occurs, has been solved [1]. There are now also structures available for light-harvesting proteins of plant chloroplasts [2] and purple bacteria [3], which capture photons and feed the light energy to the photosynthetic reaction centres. A structural description of a complete photosynthetic unit is, it seems, on the horizon.

Crystallography yields still photographs, whereas life is, almost by definition, dynamically responsive to environmental change. For example, the number of chlorophyll molecules that harvest light for each photosynthetic reaction centre is variable. This variability provides a gain control that is turned up to increase photosynthetic efficiency in dim light, and down to postpone destructive redox chemistry in bright light. An amplifier's gain control is not an optional extra, and regulation is not an afterthought to photosynthesis: it was built into the hardware from early in its evolution. The primary redox chemistry of photosynthesis would be useless or positively destructive without it.

A photosynthetic gain control may operate at the level of expression of the genes that encode the protein machinery of photosynthesis. In studies of the light-regulation of photosynthesis genes, there has been a tendency to focus on specialized photoreceptors, such as phytochrome, which coincidentally sense light. But increasing evidence from a range of systems suggests that photosynthesis is also self-regulating, through molecular redox signalling (Fig. 1).

Two-component redox signalling

Purple bacteria have the delightful ability to switch between chemotrophy and phototrophy — from an animal to a plant lifestyle — which was always fun in the bad old days of the cold war between botanists and zoologists. When masquerading as plants, purple bacteria express genes for photosynthetic reaction centre [1] and light-harvesting complex [3] proteins; the genes reside in just two operons. It has long been known that the photosynthetic apparatus is synthesized from scratch in the light and the absence of oxygen, and long suspected that this is a response to decreased redox potential somewhere in the cell — in other words, a response to something going from an oxidized to a reduced state.

Recent work with *Rhodobacter capsulatus* [4,5] confirms this suspicion, and shows that redox control over photosynthesis genes is exerted by two new members of the growing family of two-component regulatory systems. RegA is a 'response regulator', phosphorylated on aspartate by transfer of a phosphate from its partner, RegB. RegB is a 'sensor kinase', and is autophosphorylated on a histidine residue under anaerobic conditions [4,5]. Mutations in either *regA* or *regB* prevent anaerobic induction of photosynthesis genes [4]. Reg thus works like Arc, the two-component redox regulatory system that mediates aerobic respiratory control in *Escherichia coli* [4,6].

Inoue *et al.* [5] have recently reported that purified, truncated RegB undergoes autophosphorylation *in vitro*, and that the incorporated phosphate is transferred directly from RegB to purified RegA. Truncated RegB requires just [γ-^{32}P]ATP and Mg^{2+} to become phosphorylated under reducing conditions. A reducing agent, such as

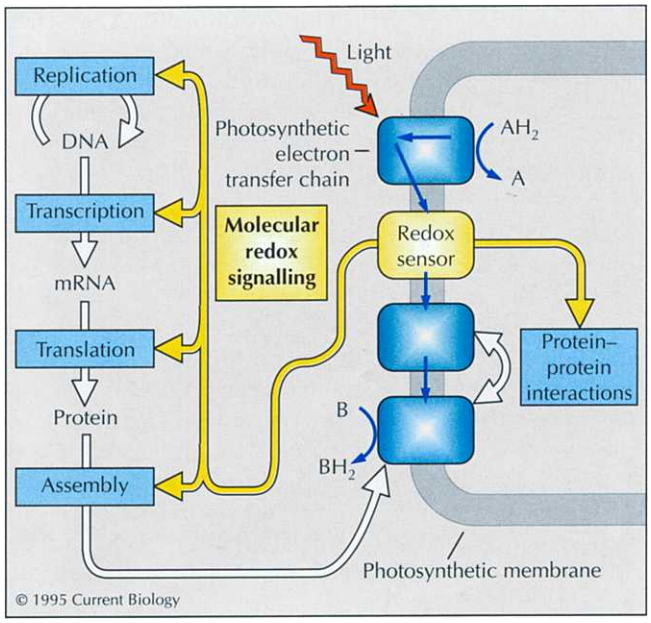

Fig. 1. Molecular redox signalling is proposed to couple the expression of photosynthesis genes to electron transfer. This coupling allows regulatory control to be exerted in both directions: gene expression controls electron transfer, and electron transfer controls gene expression. Gene expression can be controlled at the level of DNA replication, transcription, translation, complex assembly or protein–protein interactions. AH_2 represents the initial electron donor (A is its oxidized state), and B the final electron acceptor (BH_2 is its reduced state), of the photosynthetic electron transfer chain. Electon transfer reactions are represented by blue arrows.

dithiothreitol, substitutes *in vitro* for anaerobic conditions *in vivo*, even in the presence of oxygen, and an oxidizing agent such as ferricyanide suppresses phosphorylation, even in the absence of oxygen (C.E. Bauer, personal communication). It follows that RegB is not an oxygen sensor like the FixL sensor of *Rhizobium meliloti* [4], but a strict redox sensor [6], and RegA is its cognate redox response regulator [6]. There is still a missing link between RegA and the control of transcription — unlike most other response regulators, RegA is not itself a DNA-binding protein [4]. Perhaps RegA directly controls the activity of a protein that binds to the promoter of the primary photosynthesis operon, the *puf* operon [4].

Redox signals in the chloroplast

Chloroplast thylakoid membranes contain a well-established system for redox control of the post-translational modification of a number of proteins [7], including those of the major light-harvesting complex, LHC II [2]. When the pool of plastoquinone between the two photosynthetic reaction centres — I and II — becomes reduced to plastoquinol, a protein kinase is activated and phosphorylates LHC II. Phosphorylation causes LHC II to change its allegiance: it moves from photosystem II to photosystem I. This movement of phospho-LHC II supplies photosystem I with more light, and photosystem II with less. As the plastoquinone pool is reduced by photosystem II and oxidized by photosystem I, this movement counteracts the reduction of plastoquinone, the signal that initiated the response. This purely post-translational feedback mechanism explains the ability of plants and algae rapidly to redistribute light energy equitably between their two photosystems [7]. Two recent papers [8,9] suggest that redox signalling extends to the translation and transcription of chloroplast genes, which should be no surprise if chloroplasts are, as is thought, the descendants of bacteria.

Danon and Mayfield [8] have used the eccentric, but cooperative, unicellular green alga *Chlamydomonas reinhardtii* to study the binding of translational activator proteins to the 5′ untranslated region of *psbA* mRNA, the message for the D_1 reaction-centre protein of photosystem II. D_1 is a chloroplast-encoded homologue of the L subunit of the purple bacterial reaction centre [1]. The binding of nuclear-encoded chloroplast activator proteins to *psbA* mRNA was monitored by gel-shift assays, and *psbA* mRNA translation was measured by pulse-labelling of newly-synthesized D_1 with [^{14}C]acetate. Danon and Mayfield [8] found that binding was promoted by the reducing agent dithiothreitol, but not by β-mercaptoethanol, and inhibited by the oxidizing agent dithionitrobenzoate. They also found that dithiothreitol can reverse oxidant-induced inhibition of binding, and that the redox-regultory protein thioredoxin accelerates the reversal. As thioredoxin accepts electrons from photosystem I, the conclusion that chloroplast thioredoxin may regulate translation is consistent also with the failure of light to activate *psbA* translation in a photosystem I-deficient *Chlamydomonas* mutant [8]. So the light activation of translation of specific mRNAs, including that of *psbA*, is, in fact, redox activation. Thioredoxin is no stranger here, having a well established role in the redox activation, and therefore in the light activation, of enzymes of the Calvin cycle, among others.

Although many groups (see [8], for example) start from the assumption that chloroplast mRNAs are generally long lived, and hence that control cannot be exerted at the level of transcription, the results of Pearson *et al.* [9], among others, suggest otherwise. Pearson *et al.* gave lettuce chloroplasts radiolabelled NAD^+, and found that the label appeared in adenosine and then in acid-insoluble material susceptible to RNase digestion, suggesting that they were looking at RNA synthesis. Greater rates were observed in darkness than in the light. Rates were also increased by the presence of any of a number of electron transport inhibitors that prevent electron flow into the cytochrome b_6/f complex. Ferricyanide increased apparent RNA synthesis in the light, whereas dithiothreitol decreased it. High dithiothreitol concentrations decreased apparent RNA synthesis in both light and dark. Pearson *et al.* [9] conclude that chloroplast transcription is redox-controlled, being activated when the cytochrome b_6/f complex becomes oxidized.

Whereas Pearson *et al.* [9] conclude that total RNA synthesis in lettuce chloroplasts is activated by oxidation of the cytochrome b_6/f complex, Danon and Mayfield [8] conclude that mRNA translation in *Chlamydomonas* chloroplasts is activated by reduction of thioredoxin. These conclusions are not necessarily incompatible with each other — even if we resist the temptation to plead species differences, citing the evolutionary distance between *Chlamydomonas* and lettuce. It is unclear, for example, whether Danon and Mayfield's results can be extrapolated from *psbA* to all chloroplast genes. It is also possible that chloroplast transcription is rapid under oxidizing conditions — such as at low light levels or when light absorption favours photosystem I — but that the mRNA products are translated when light levels are higher and thioredoxin becomes reduced.

There are several reasons to expect that the cytochrome b_6/f complex will turn out to be a key player in the redox control of gene expression. It is located at a strategic point in the electron transfer chain, midway between photosystems I and II, and, like plastoquinone, it is oxidized by photosystem I and reduced by photosystem II during linear electron transport. Under certain conditions, photosynthetic electron transport is not linear, but cyclic. During cyclic electron transport, which drives ATP synthesis independently of reduction of the terminal electon acceptor $NADP^+$, the cytochrome b_6/f complex is both oxidized and reduced by photosystem I. A redox sensor located in or near the cytochrome b_6/f complex could help regulate the proportion of electrons recycled into the cytochrome b_6/f complex from the acceptor side of photosystem I, and hence the production of ATP relative to NADPH. Similar cytochrome *b/c* complexes are located at branch points in respiratory electron transport chains.

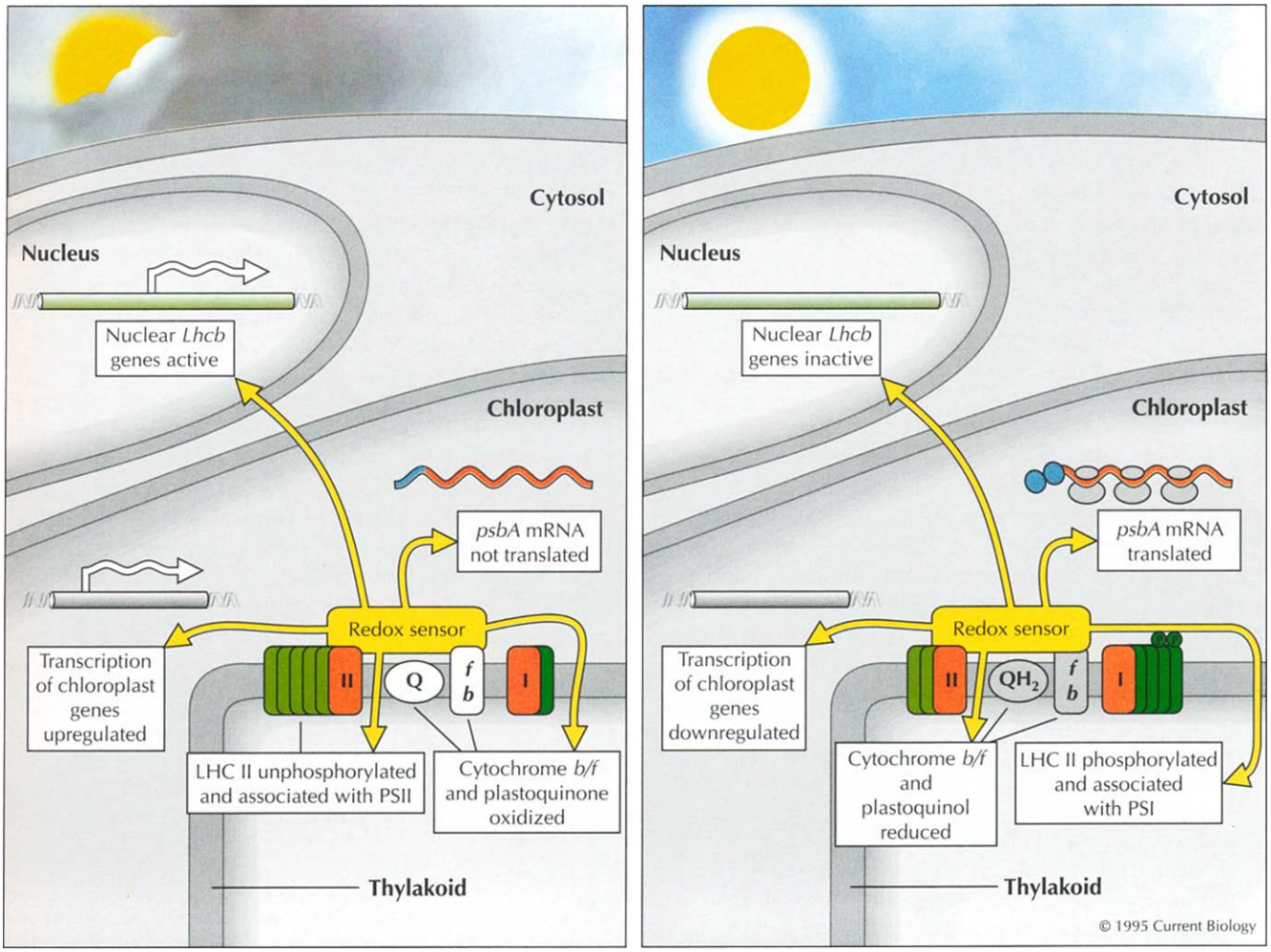

Fig. 2. Photosynthesis adapts to changes in the level and quality of the illuminating light by redox signalling. For example, an increase in light intensity (right) increases the rate of electron transfer from the photosynthetic reaction centes (PSI and PSII, red). The 'dark' reactions that oxidize NADPH with electrons from PSI become rate-limiting, and quinone (Q) is reduced to to quinol (QH_2). This activates a kinase that phosphorylates, and thus modulates, the light-harvesting complexes (green). This redox signalling also regulates the transcription of genes encoding the reaction centre proteins (which are chloroplast-encoded in eukaryotes). In eukaryotes, a decrease in light intensity activates transcription of the nuclear genes that encode the light-harvesting-complex proteins. This activation appears to involve one or more cytosolic protein phosphatases. The redox sensor may be an electron carrier located between PSI and PSII. For translation of *psbA* mRNA, thioredoxin may also be involved, sensing the redox state of ferredoxin in PSI.

Another reason for looking more closely for possible redox sensors associated with cytochrome *b/c* complexes is that these contain quinone-binding sites, and will respond directly to changes in the redox states of quinone pools. There is also evidence that the chloroplast LHC II kinase requires an intact cytochrome b_6/f complex to be activated by reduction of the plastoquinone pool [7].

Chloroplast RNA polymerase has sigma factor subunits, like those that regulate the promoter specificity of bacterial RNA polymerase. Sigma factors of mustard chloroplasts undergo reversible phosphorylation during light-regulated chloroplast development [10], providing further evidence that chloroplast gene expression is controlled at the transcriptional level. Perhaps the constitutive transcription from chloroplast genes of long-lived mRNAs, which Danon and Mayfield [8] assumed to occur in their system, is just another *Chlamydomonas* eccentricity. If the jury is still out on the regulation of chloroplast transcription, in the next courtroom redox signals are showing clearly that they habitually do something to chloroplast genes.

Redox signals to the nucleus

The chloroplast light-harvesting complex LHC II [2], like most chloroplast proteins, is imported. LHC II apoprotein precursors are synthesized, in the cytosol, from mRNA transcripts of nuclear *Lhcb* genes. Using *Dunaliella tertiolecta*, a green alga that proves God likes biochemists, Escoubas *et al.* [11] have obtained evidence that LHC II apoprotein synthesis is regulated at the level of nuclear transcription by redox reactions far away in the chloroplast. At high light intensities, *Lhcb* transcription is repressed — the gain control is turned down. Lower the light intensity, and something turns the gain up — *Lhcb* transcription is induced. But put in the thylakoid electron transport inhibitor DBMIB, which blocks oxidation of plastoquinol to plastoquinone by photosystem I, and the gain stays low even when the lights go down. Conversely,

keep the light intensity high, but throw in a little DCMU, which blocks the reduction of plastoquinone to plastoquinol by photosystem II, and *Lhcb* begins to be transcribed as if the cells think they have been put in the shade.

Thus, the redox state of plastoquinone determines not only whether LHC II is phosphorylated [7], but also whether the nuclear genes encoding its protein components are transcribed [11]. Another way to override the control marked '*Lhcb* up' is to apply the protein phosphatase inhibitors okadaic acid, microcystin L-R or tautomycin [11]. The implications are that a redox signal is transmitted from the chloroplast to the nucleus, and that the pathway involves protein kinases and phosphatases.

Maxwell *et al.* [12] have demonstrated chloroplast redox control of nuclear *Lhcb* transcription by quite independent means, in the equally compliant alga *Dunaliella salina*. Their approach was ingenious, did not depend on chemical inhibitors and employed the historically important strategy of controlling both light intensity and temperature. Temperature influences the rate of the 'dark' reactions of photosynthesis, CO_2 assimilation, whereas the 'light' reaction, electron transport, is for all practical purposes temperature-independent. Thus, what is a low light intensity for cells growing at high temperature can be a high light intensity for cells growing at low temperature.

Like Escoubas *et al.* [11], Maxwell *et al.* [12] found that both *Lhcb* mRNA and LHC II apoprotein levels increase when the light intensity is decreased. They then chose a constant, moderate light intensity, and increased the temperature from 13 °C to 30 °C, switching on *Lhcb* mRNA and apoprotein synthesis. Using an established chlorophyll fluorescence technique to measure the redox state of electron acceptors of photosystem II, Maxwell *et al.* chose their light intensities so that the acceptor Q_A is 10 % reduced at moderate light intensity and 30 °C as well as at low light intensity and 13 °C. This final state can be arrived at by decreasing light intensity from high to moderate at 30 °C or from moderate to low at 13 °C, or by raising temperature from 13 °C to 30 °C at moderate light intensity. In all cases, as the percentage of reduced Q_A drops to 10 %, LHC II apoprotein synthesis increases six fold, and *Lhcb* mRNA increases eight fold: the gain is turned up. Maxwell *et al.* conclude that light intensity itself has no direct effect of *Lhcb* expression, but that a redox sensing and signalling mechanism governs *Lhcb* transcription.

The nucleus must delegate

Despite their undoubted ability to signal to the nucleus, it may be no accident of evolution that chloroplasts retain genes for key proteins of photosynthetic electron transport. One view is that a requirement for direct redox control of gene expression has determined the composition of both chloroplast and mitochondrial genomes [13] — the removal of a gene to the nucleus would produce no obstacle to eventual re-import of its product to the organelle, but would slow down and weaken its redox control. We have a preliminary set of observations that seem to support this idea: redox conditions select which proteins are synthesized in isolated chloroplasts and mitochondria incubated with [^{35}S]methionine [14].

Now that we have clear evidence for redox signals from photosynthesis (Fig. 2), what are the mechanisms by which they operate? Purple bacteria are distant relatives of chloroplasts and mitochondria. Have eukaryotic organelles inherited two-component redox signalling from the precursors of regA and regB? If so, their redox sensors and response regulators are probably now nuclear-encoded [13]. What of the elusive chloroplast redox kinase [7]? Is it a redox sensor, or does it merely require a separate sensor for activation? Does either of these putative sensors fulfil one or more of the multiple requirements for control at the transcriptional, translational and post-translational levels? And where are the signal-processing steps that decide which responses to use? Does nuclear transcription respond independently to redox changes, or is the signal processed through the chloroplast genetic system? These problems are worth solving for their own importance, and the detailed answers that we get may also provide insights into the roles of molecular redox signalling more generally in cell regulation.

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