Chloroplast redox signals: how photosynthesis controls its own genes

Thomas Pfannschmidt

Department of Plant Physiology, Friedrich-Schiller University of Jena, Dornburger Str. 159, 07743 Jena, Germany

The photosynthetic apparatus of higher plants and algae is composed of plastid- and nuclear-encoded components, therefore the expression of photosynthesis genes needs to be highly coordinated. Expression is regulated by various factors, one of the most important of which is light. Photosynthesis functions as a sensor for such light signals, and the redox state of photosynthetic electron transport components and redox-active soluble molecules act as regulating parameters. This provides a feedback response loop in which the expression of photosynthesis genes is coupled to the function of the photosynthetic process, and highlights the dual role of photosynthesis in energy fixation and the reception of environmental information.

In higher plants and algae, photosynthesis takes place in specialized organelles, the chloroplasts, which evolved from a prokaryotic, cyanobacterium-like ancestor that was taken up by the eukaryotic host cell [1]. Chloroplasts contain a highly organized thylakoid membrane system that harbours all components of the light-capturing photosynthetic apparatus and provides all structural properties for optimal light harvesting [2]. In addition, as a remnant of their prokaryotic origin, chloroplasts have their own small genomes, the so-called plastome, which (in higher plants) contains a relatively stable set of 100–120 genes [3]. They also contain the complete machinery to express this genetic information [4], although the vast majority of chloroplast proteins is encoded in the nucleus [5] and has to be imported post-translationally into the organelle via an envelope-located import machinery [6].

The photosynthetic apparatus, like all multimeric plastid protein complexes, is a patchwork of plastome- and nucleus-encoded proteins. As a general species-overriding pattern, the sequencing of several plastomes has revealed that proteins that are located at, or close to, photosystem reaction centres are encoded on the plastome, whereas peripheral proteins are encoded in the nucleus [3]. This dual location of photosynthesis genes complicates their concerted expression in many ways and causes immense costs for the cell to coordinate it. The question therefore arises of why a particular set of photosynthesis genes was retained on the plastome and not transferred into the nucleus during evolution, as most other genes were.

In 1993, John Allen theorized that the plastome (and the chondriome of mitochondria) was maintained throughout evolution because of their regulatory dependence on redox-active components within the organelle [7], analogous to the situation in many photosynthetic and non-photosynthetic bacteria [8]. In the same year, Colin Pearson *et al.* [9] showed for the first time that chloroplast transcription is connected to photosynthetic redox reactions. This was the beginning of a new research field that has developed greatly in recent years. An increasing number of reports shows that the redox state of plastoquinone, the cytochrome b_6f complex, other unidentified components of the electron transport chain and electron-flow-dependent soluble compounds [e.g. thioredoxin, glutathione and reactive oxygen species (ROS)] affect chloroplast gene expression. In addition, several of these parameters also affect nuclear gene expression and therefore represent a new type of so-called plastid signal(s). This article focuses on the influences of photosynthetic redox reactions on the various expression levels of photosynthetic genes in both the chloroplast and the nucleus. It shows the importance of this photosynthetic control and describes how photosynthesis acts as a light-dependent regulator in addition to known photoreceptors such as phytochromes and cryptochromes.

Redox parameters controlling photosynthesis

Photosynthesis functions optimally when the prevailing environmental conditions meet its species-specific requirements. However, environmental conditions can fluctuate on a timescale ranging from seconds to days or even years, and many of these changes decrease photosynthesis. Because of the central role of photosynthesis as an energy source, photosynthetic organisms have developed many strategies to acclimate to a broad range of environmental conditions. Their effect is to maintain photosynthetic efficiency at as high a level as possible, especially under adverse conditions. The light-driven photosynthetic process in chloroplasts of plants and algae involves a complex chain of redox reactions (Fig. 1). Many environmental changes result, directly or indirectly, in a change of the redox potential (Box 1) of components of this electron transport chain or of pools of photosynthesis-coupled redox-active compounds (thioredoxin or glutathione) by affecting the electron transport efficiency.

Low temperature or CO_2 availability, for instance, reduce the activity of the Calvin cycle, which in turn results in a decrease in the production of the final electron acceptor $NADP^+$. This leads to an over-reduction of the photosynthetic electron transport chain, and electrons from the temperature- and CO_2-independent light reaction

Corresponding author: Thomas Pfannschmidt (thomas.pfannschmidt@uni-jena.de).

 1360-1385/02/$ - see front matter PII: S1360-1385(02)00005-5

Glossary

Chloroplast and nuclear genes:

- *Apx1*, *Apx2*: nuclear genes for cytosolic ascorbate peroxidases. Like their chloroplast counterparts, these enzymes are scavengers of reactive oxygen species that are generated in the chloroplast.
- *Fed1*: nuclear gene for ferredoxin. Ferredoxin accepts the electrons from photosystem I (PSI). The transfer is mediated by the PsaE subunit (encoded by the nuclear gene *PsaE*). Reduced ferredoxin is used as substrate to reduce $NADP^+$ and oxidized thioredoxin.
- *Lhcb*: nuclear gene(s) for the chlorophyll *a*/*b* binding proteins of light-harvesting complex II of photosystem II.
- *PetE*: nuclear gene for plastocyanin, which transfers electrons from the cytochrome b_6f complex to photosystem I.
- *Por*: nuclear gene for NADPH–protochlorophyllide oxidoreductase, which reduces protochlorophyllide to chlorophyllide.
- *psaAB*: plastid genes for P700 apoproteins A and B of photosystem I. These two proteins form a heterodimer that binds the reaction centre P700 (a chlorophyll dimer) and the electron acceptors A_0 (a chlorophyll a molecule), A_1 (a phylloquinone molecule) and F_x (a 4Fe–4S iron–sulfur cluster). Together with the PsaC subunit (encoded by the plastid gene *psaC*), PsaA and PsaB represent the core of photosystem I.
- *psbA*, *psbD*: Genes for the D1 and D2 proteins of photosystem II. The photosystem-II reaction centre is formed by a D1–D2 heterodimer that binds all essential cofactors for the electron transfer from the water-splitting complex to the plastoquinone pool.
- *PsaD*: nuclear gene for subunit II of photosystem I. The PsaD protein is located at the stromal side of photosystem I and provides the essential site for ferredoxin docking.
- *PsaF*: nuclear gene for subunit III of photosystem I. The PsaF protein is located at the lumenal side of photosystem I and provides the docking site for plastocyanin.
- *rbcL*: plastid gene for the large subunit of Rubisco. The RbcL subunit carries the catalytic site for CO_2 fixation.

Fenton reaction: In this reaction, highly reactive hydroxyl radicals are generated from H_2O_2 with Fe^{2+} as the major cellular catalyst (Box 1).

Mehler reaction: In this reaction, molecular oxygen is reduced and therefore consumed by photosynthesis (Box 1).

Mehler valve: The Mehler reaction is thought to provide an additional valve for dissipating excess electrons from photosynthetic electron transport, and thus helps to avoid photoinhibition.

are then transferred to oxygen at photosystem I (PSI) or via the MEHLER REACTION (see Glossary) which generates ROS [10,11] (Box 1). ROS induce oxidative stress and damage because of their high reactive potential and so are detoxified by scavenging mechanisms such as superoxide dismutase and the glutathione–ascorbate cycle [12]. ROS are also produced under drought conditions, which increase the cellular concentration of Fe^{2+} ions, resulting in an increase of electron transfer to oxygen via the FENTON REACTION (Box 1). Similar effects can be observed under starvation of nutrients, such as Mg^{2+} or Fe^{2+}, which are essential co-factors for several redox-active proteins in the photosynthetic electron transport chain [13]. However, the most immediate environmental changes are changes in illumination, such as in light quantity or light quality, which result from shading by clouds, neighbouring plants, tree canopies or different depths of aquatic environments. Such changes directly affect the photosynthetic light reaction and are those that have been investigated the most to date.

High-light conditions (like low-temperature conditions) lead to an overexcitation of the photosynthetic apparatus, which can result in a photoinhibition of the photosynthetic electron transport by, for example, the destruction of the D1 protein in photosystem II (PSII) [14]. To avoid this stress phenomenon, several quenching processes dissipate excess electrons via heat or the MEHLER VALVE [15]. By contrast, under low-light conditions, a light-quality-dependent imbalance is often observed in the excitation of the two photosystems that is counteracted by state transitions [2,16] or by adjusting the stoichiometry of the photosystems [17]. State transitions are a short-term response (occurring in minutes) in which the imbalance in excitation energy absorption between the two photosystems is counterbalanced by the lateral migration of the mobile part of light-harvesting complex II antenna complexes. By contrast, photosystem stoichiometry adjustment is a long-term response (occurring in hours or days) in which the relative number of photosystems is changed in favour of one that is rate limiting. In this way, the efficiency of photosynthesis is enhanced [18]. Both processes are regulated by the redox state of the

Box 1. Redox potential of photosynthetic components and 'dangerous' byproducts of photosynthesis redox chemistry

The redox potential *E* of an electron- or hydrogen-transferring component is described in general by the Nernst equation (Fig. Ia) and depends on the component-specific mid-point potential (E_m), the number of transferred electrons (n) and the concentration ratio of oxidized [ox] to reduced [red] forms of the component. Environmental factors that influence or participate in the electrochemistry of the component will result in a change of its respective redox potential. Because most redox-active components exhibit proper function only in a relatively small range of its redox potential, organisms have several mechanisms to keep the redox potential stable. However, reactive oxygen species are unavoidable side products of oxygenic photosynthesis [a].

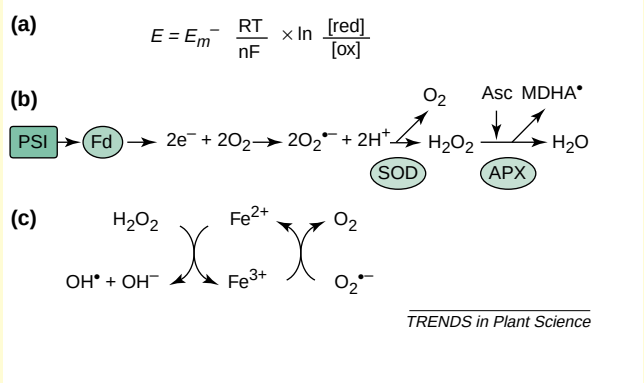

Fig. I.

In the Mehler reaction (Fig. Ib), superoxide is mainly formed at photosystem I (PSI), either directly or via ferredoxin, and is rapidly detoxified by superoxide dismutases (SODs), which produce hydrogen peroxide. Ascorbate peroxidases (APXs) then reduce hydrogen peroxide to water via the oxidation of ascorbate (Asc) to monodehydroascorbate radicals (MDHA$^\bullet$), which are reduced back to ascorbate via glutathione.

Under various stress conditions, the cellular concentration of ROS increases and can overcome the antioxidant-defence mechanisms. In the Fenton reaction (Fig. Ic), hydrogen peroxide can then be transformed into highly reactive hydroxyl radicals using divalent iron ions as catalysts. These radicals cause oxidative damage by oxidizing fatty acids or amino acids, which can impair membrane structure or proper protein function.

Reference

a Baier, M. and Dietz, K.J. (1999) The costs and benefits of oxygen for photosynthesizing plant cells. *Prog. Bot.* 60, 282–314

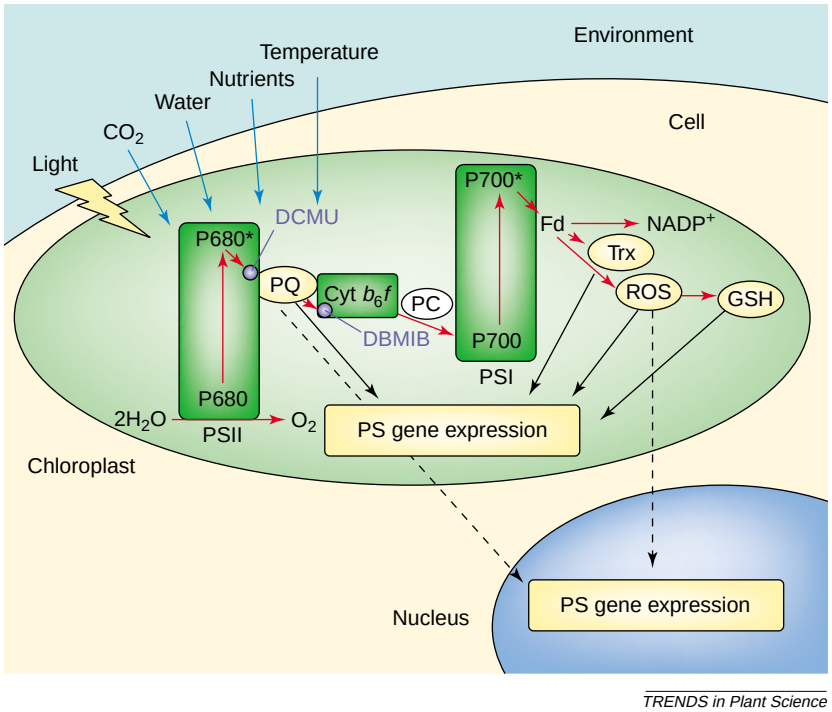

Fig. 1. Factors in photosynthetic redox chemistry that influence photosynthesis gene expression. The electron transport chain of a chloroplast is drawn schematically according to the Hill–Bendall Z scheme and the electron flow is represented by red arrows. During photosynthesis, water is split into protons, electrons and oxygen by light-induced charge separation in the reaction centre (P680 → P680$^+$) of photosystem II (PSII). Electrons are then transferred to the cytochrome b_6f (Cyt b_6f) complex by the mobile electron carrier plastoquinone (PQ). Its redox state depends on linear electron flow and can be manipulated chemically by site-specific electron transport inhibitors 3-(3′,4′-dichlorophenyl)-1,1′-dimethyl urea (DCMU) and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) (purple). DCMU inhibits the reduction of PQ by binding to the Q_B site of the D1 protein, whereas DBMIB inhibits its oxidation by binding to the PQ oxidation site at the cytochrome b_6f complex [73]. Electrons from the cytochrome b_6f complex are transferred to photosystem I (PSI) by plastocyanin (PC). After activation through a second light reaction (P700 → P700$^+$), they are accepted by the electron end acceptor $NADP^+$ via ferredoxin (Fd). Fd also reduces thioredoxin (Trx), which controls, for example, the activity of several enzymes of the Calvin cycle. Under extreme conditions (e.g. high light intensity or low temperatures), electrons of PSI can also be transferred to oxygen, which results in the generation of reactive oxygen species (ROS). To avoid the accumulation of ROS, they are detoxified by redox buffer compounds such as glutathione, which can result in changes of the ratio of its reduced to its oxidized state. Unbroken black arrows indicate influences on chloroplast gene expression, broken black arrows indicate influences on nuclear gene expression. Various environmental influences on photosynthetic electron flow are indicated by blue arrows. Abbreviations: GSH, reduced glutathione; PS gene, photosynthesis gene.

plastoquinone (PQ) pool, which reflects the balance of light use between both photosystems.

All these examples of acclimatory mechanisms are based on environmentally induced changes in the redox state of photosynthetic components or the generation of ROS. Several of these regulatory redox-reactive key molecules (Figs 1,2, yellow) are signals that activate or inactivate the expression of CHLOROPLAST AND NUCLEAR GENES. All the genes reported to date that show redox-regulated expression characteristics (Fig. 2) are directly involved in or connected with photosynthesis. This adds an important new aspect to the regulatory network that controls photosynthesis: the functional state of the photosynthetic process has a direct impact on the expression of genes encoding its functional constituents. Thus, the chloroplast not only provides energy but also represents a sensor for environmental information, and chloroplast redox signals help to acclimatize the organism to changes in environmental factors [19–22].

Redox-controlled genes and their affected expression levels

Photosynthesis is now recognized to be a regulator of the expression of photosynthesis (and other) genes, but which levels of gene expression are affected? For a long time, it was believed that nuclear genes were regulated exclusively at the transcriptional level and chloroplast genes at the post-transcriptional level. However, studies from many laboratories show that this view is simplified. Expression of genetic information in living organisms is a complex, integrated network that is influenced by various endogenous and exogenous stimuli. Gene expression involves several steps (Fig. 2), starting with the transcription of a gene or operon into a pre-mRNA that then is processed into a mature mRNA molecule by mechanisms including splicing and editing. The pool size of the mRNA molecule further depends on its stabilization or degradation. Finally, to obtain a functional polypeptide, the mRNA must be loaded onto polyribosomes and translation must be initiated and, after successful elongation, correctly terminated. Most of these steps in gene expression have been found to be highly regulated, both in the nucleus and in the chloroplast. Recent studies now show that, in photosynthetic organisms, chloroplast redox signals initiate important regulatory events that act on most of these gene expression levels by a range of mechanisms (Fig. 2). Further studies should show whether the unconfirmed steps (question marks in Fig. 2) are also affected.

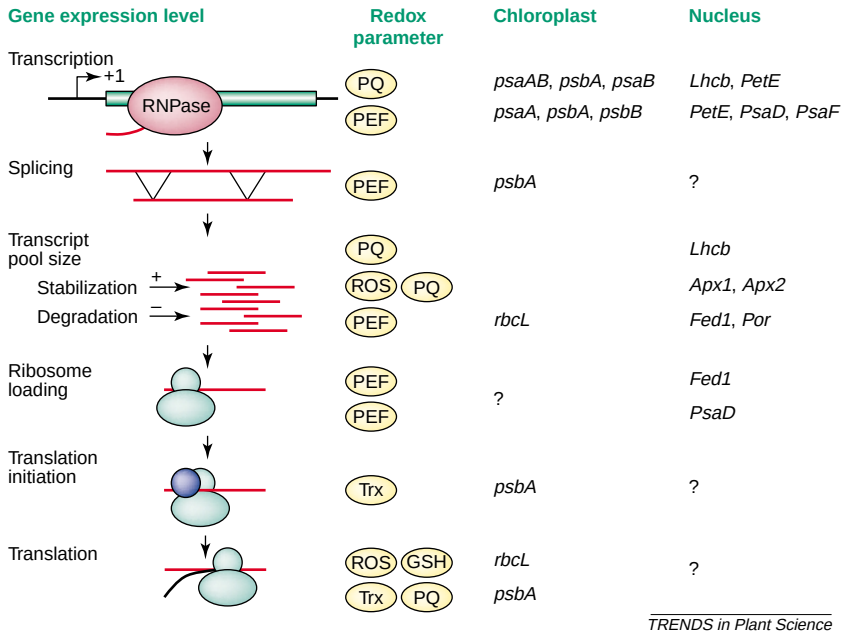

Fig. 2. Summary of redox-regulated expression levels of nuclear and chloroplast photosynthesis genes in higher plants and algae. The left-hand column represents the various steps of general gene expression, beginning with transcription and ending with translation. The green bar represents a gene that is transcribed by the RNA polymerase, with +1 as the transcription start site. The resulting transcript is represented by a red line including introns (black). The small and large subunits of a ribosome are shown as light-blue circles and ovals, respectively. Additional factors for translational initiation are represented by a dark-blue circle. The generated polypeptide chain is shown as a black line. The second column summarizes the known redox parameters that affect the gene expression level (compare with Fig. 1). Columns 3 and 4 list the affected photosynthesis genes that are known to date, listed by chloroplast [23–26,36,74,76,85,86] and nuclear location [64,71,75–84], respectively. Affected genes and regulatory redox factors are drawn at the same height. Abbreviations: GSH, glutathione; PEF, photosynthetic electron flow; PQ, plastoquinone; RNPase, RNA polymerase; ROS, reactive oxygen species; Trx, thioredoxin.

The present data clearly show that redox regulation of photosynthesis genes occurs at multiple expression levels and suggest the existence of a complex signalling network. However, in spite of many reports, our actual state of knowledge represents a collection of single observations that have not yet provided a complete picture of cellular redox signalling. The integration of these data into a coherent model will be one of the great challenges in the future. At present, this is complicated by several facts. Some results are species specific (e.g. the *psbA* gene splicing in *Chlamydomonas* [23], which does not occur in higher plants) or are still not conclusive enough to identify the signalling redox parameter exactly (marked with PEF in Fig. 2). In several cases, experiments by different research groups have produced contradictory or inconclusive results, especially in the identification of the origin of redox signals from the PQ pool and/or the cytochrome b_6f complex [22] (Fig. 3). In most cases, only a few genes or even a single gene were investigated, leaving these observations at the stage of pilot studies. In addition, a specific regulation at a certain gene expression level is not necessarily reflected on the next one. Therefore, it is important to analyse each single step (e.g. transcription, transcript pool size and translation of redox-regulated genes) in several organisms to obtain a complete picture of this and, in principle, all other types of regulation. Our increasing genomic competence combined with transcriptomic and proteomic approaches should provide such global information to understand the underlying molecular network of chloroplast redox signals.

Inner-chloroplastic transduction of photosynthetic redox signals

How are redox signals from the thylakoid membrane or from pools of redox-active compounds transduced to their target gene expression level? A few studies have already determined that proteins can act as signal-mediating molecules and give a first idea of the transduction of redox signals. The translation initiation of *psbA* in *Chlamydomonas* (Fig. 4) was analysed *in vitro*, showing that a multiprotein complex binds to the 5′-untranslated region of the *psbA* message and enhances its translation. *In vitro* and *in organello* studies showed that the binding activity of this complex and the resulting *psbA* translation are increased under reducing conditions, such as in the light (Fig. 4), when D1 protein is needed most [24,25]. Recent results demonstrate that this light activation is mediated by two signals: a ‘priming’ signal generated by the PQ pool and a dithiol signal from thioredoxin. The priming signal regulates an oxidation activity that has yet to be identified, which oxidizes the protein complex, making it susceptible to reduction by thioredoxin [26]. Inactivation of complex-binding activity in the dark is mediated by phosphorylation through an ADP-dependent protein kinase [27] and it is hypothesized that, by this mechanism, high ADP levels in chloroplasts in the dark inhibit *psbA* translation and that a high content of reducing equivalents in the light promote its translation. Recent studies in *Arabidopsis* show that a comparable protein complex binds to the *psbA* message in this organism in a redox-dependent manner, suggesting that this type of regulation also exists in higher plants [28].

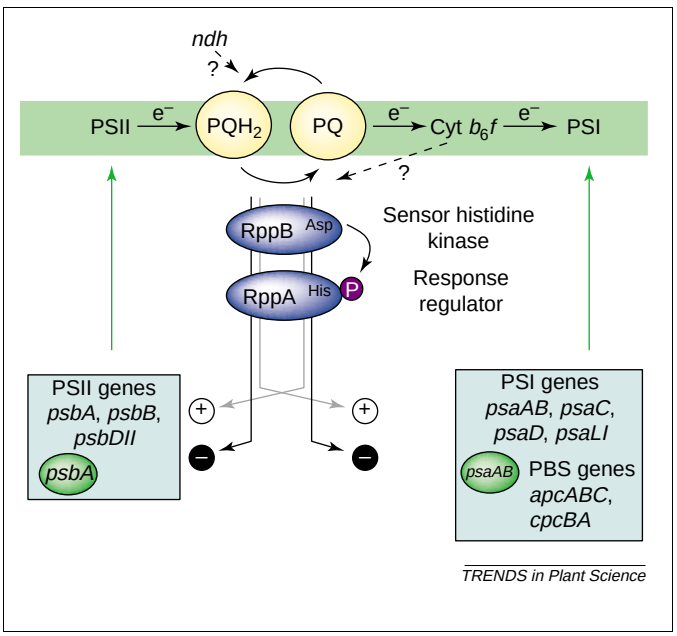

Fig. 3. Plastoquinone (PQ) redox control of photosystem reaction-centre gene transcription in cyanobacteria and chloroplasts. This model is a compilation of recent results from chloroplasts and cyanobacteria. It confirms and expands the original theoretical models that John Allen deduced from bacterial systems [7,87,88]. The photosynthetic electron transport chain is drawn as in Fig. 4, the predominant redox states of the plastoquinone (PQ) pool [reduced (PQH_2) and oxidized (PQ)] are highlighted in yellow. When the PQ pool is reduced by light, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) or glucose [in cyanobacteria via a NADH dehydrogenase (*ndh*)], the transcription of photosystem I genes is activated (grey arrow, +) and that of photosystem II genes is repressed (black arrow, −). When the PQ pool is oxidized by PSI–light or 3-(3′,4′-dichlorophenyl)-1,1′-dimethyl urea (DCMU), the opposite is the case. This has been shown for the cyanobacterial genes *psbA*, *psbD*, *psaA* and *psaB*, which encode the reaction-centre proteins D1, D2, PsaA and PsaB (light-blue boxes) [41] and the chloroplast genes *psbA* and *psaAB* (green oval insets) [36,74]. Data from another study in *Synechocystis* also show that the redox state of a photosynthetic electron carrier activates *psbA* in its oxidized state and *psaE* in its reduced state, supporting this model of opposite redox regulation of PSI and PSII genes [89]. However, the origin of the signal was located between the PQ pool and PSI. From the present data, this regulation mechanism seems to work only under low- or mid-light conditions. In the dark or under high light, it is likely that other or additional mechanisms overtake the control [22]. In addition, there are the interesting questions of how this model of transcriptional regulation fits with that of translational regulation (Fig. 4), and of whether and how these regulating mechanisms cooperate. To date, it is not clear how the PQ redox signal leaves the thylakoid membrane, nor whether or how the cytochrome b_6f (Cyt b_6f) complex is involved (indicated by question mark). In *Synechocystis* PCC6803, a two-component system (dark-blue ovals) consisting of a sensor histidine kinase (RppB) and a response regulator (RppA) is involved in the transduction of the PQ redox signal. Activation in bacterial two-component systems typically occurs by a transfer of a phosphoryl group (P) from an aspartate residue (Asp) of the sensor kinase to a histidine residue (His) of the corresponding response regulator [90]. From studies of cyanobacterial deletion *rppA* and *rppB* mutants, the RppA response regulator protein is tentatively thought to control photosynthesis stoichiometry by depressing PSII transcription and activating PSI transcription. In addition, it activates phycobilicyanin (PBC) transcription and inactivates phycobilisome (PBS) degradation. However, its detailed functions still needs to be resolved [41]. Abbreviations: Asp, aspartate; e^-, electrons; Fd, ferredoxin; GSH, reduced glutathione; GSSG, oxidized glutathione; His, histidine; PC, plastocyanin; PSI, photosystem I; PSII, photosystem II; ROS, reactive oxygen species; Trx, thioredoxin.

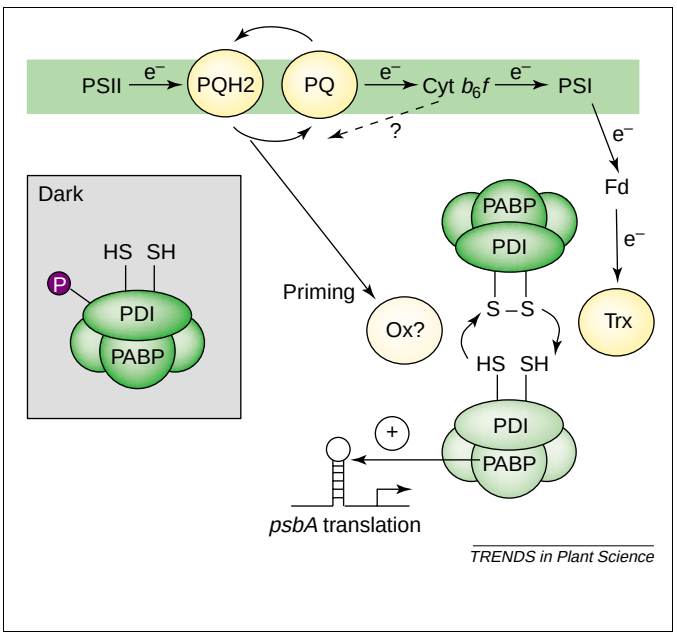

Fig. 4. Light-mediated redox control of chloroplast *psbA* translation in *Chlamydomonas*. In this model, a multiprotein complex consisting of a 60 kDa protein with homology to protein disulfide isomerases (PDI) [91], a 47 kDa protein with homology to poly(A)-binding proteins (PABP) [92] and two unknown proteins of 38 kDa and 55 kDa, binds to the 5′-untranslated region of the mature *psbA* transcript in *Chlamydomonas*. PABP mediates the binding of the protein complex and regulation of binding activity is mediated by PDI. In the light, electrons from the photosynthetic electron transport chain are transferred via ferredoxin (Fd) and thioredoxin (Trx) to a vicinal dithiol group of PDI. This requires prior dithiol activation oxidation of PDI by an unknown component. This component is activated upon illumination by a 'priming' signal starting from a reduced plastoquinone (PQ) pool (PQH_2) [26]. In its reduced active form, PDI then transmits its SH-group signal to PABP, resulting in an increased binding activity that finally leads to an increased translation of the message. In the dark (grey box), PDI is inactivated via phosphorylation by an ADP-dependent protein kinase, which is activated by an increasing ADP:ATP ratio in the dark and inactivated by the decrease in the ADP:ATP ratio after illumination [27]. Inactive protein complexes are shown in dark green, active complexes in light green. Abbreviations: Cyt b_6f, cytochrome b_6f complex; e^-, electrons; Ox, unknown oxidizing activity; P, phosphoryl group; PSI, photosystem I; PSII, photosystem II; S–S, disulfide bond.

Proteins have also been found to mediate redox control during RNA maturation or transcription. In the higher plant mustard, a sequence-specific endoribonuclease (p54) is involved in the 3′ formation of *trnK* and *rps16* precursor transcripts *in vitro* [29]. Further studies have shown that the p54 activity can be enhanced by phosphorylation and by the oxidized form of glutathione, whereas dephosphorylation and treatment with reduced glutathione (GSH) decrease it [30]. Furthermore, biochemical studies demonstrated that a protein kinase termed plastid transcription kinase is associated with the plastid-encoded RNA polymerase in mustard chloroplasts [31]. This kinase can phosphorylate σ-like factors *in vitro*, and this activity is regulated *in vitro* by phosphorylation and the redox state via GSH [32]. These σ-like factors are thought to play a prominent role in the regulation of chloroplast gene transcription [33,34], providing an attractive link between photosynthetic control and gene expression. Further investigations have shown that high light levels induce variations in the chloroplast GSH content, resulting in chloroplast transcription changes, suggesting that this GSH-mediated redox control also accounts for *in vivo* conditions [35].

Redox signalling pathways in cyanobacteria – a model for chloroplasts?

In spite of these first models, signal transduction in most redox-controlled chloroplast gene-expression events is not understood, as in the case of the redox-regulated transcription of chloroplast reaction-centre genes [36]. Manipulation of photosynthetic electron transport by light sources that predominately excite either PSI or

PSII, or treatment with the electron-transport inhibitors 3-(3′,4′-dichlorophenyl)-1,1′-dimethyl urea (DCMU) and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) led to the conclusion that the redox state of the PQ pool generates a signal that activates *psaAB* transcription in its reduced state and *psbA* transcription in its oxidized state (Fig. 3). In higher plants and algae, these genes are uniformly encoded on the plastome. Because these genes encode the core of both photosystems, the regulation of its expression is of exceptional importance for the correct assembly and stoichiometry of the photosystems, and the determination of its redox-regulated expression perfectly meets the predictions of Allen's model [7]. However, the mechanism of the transduction of this PQ redox signal is unknown.

Cyanobacteria are the ancestors of chloroplasts and therefore might be a useful model to elucidate the development and molecular structure of chloroplast redox-signalling pathways. Several similarities support this assumption. As in chloroplasts, the transcription of many cyanobacterial photosynthesis genes is influenced by light and photosynthetic electron transport [37–39]. Under photoautotrophic conditions, DCMU and DBMIB can be used to manipulate the redox state of the PQ pool in the same way as in chloroplasts [40,41]. Furthermore, the sequencing of the *Arabidopsis* genome revealed that many genes of cyanobacterial origin have been maintained throughout evolution, suggesting that, at least in part, its gene products could still have their former function [42]. Experimental support for this came from a study in *Synechocystis* PCC 6803 that revealed a redox control pathway for reaction-centre genes with striking similarities to the chloroplast pathway described above. A reduced PQ pool, like in chloroplasts, activates the transcription of PSI reaction-centre genes whereas an oxidized PQ pool activates the transcription of PSII reaction centre genes [41] (Fig. 3). Obviously, this regulation represents a highly conserved mechanism.

Additional mutant analyses uncovered components responsible for the transduction of the signal: a two-component system consisting of a sensor kinase (RppB) and a response regulator (RppA) [41] (Fig. 3). Is there such a system in plants or algae? Indeed, there are many (nuclear-encoded) two-component systems in the genomes of higher plants [43–45], but direct homologues of RppB and RppA could not be identified by BLAST searches (T. Pfannschmidt and R. Oelmüller, unpublished). Nevertheless, sequence analyses show that some of these genes recruited potential organellar target sequences, suggesting that they exist in chloroplasts and mitochondria [46,47]. At present, nothing is known about how RppB actually senses the PQ redox state. We will have to wait for further genetic and biochemical developments in this field to get a better understanding of how this actually works. Therefore, other well-investigated prokaryotic redox responding regulator systems such as the ArcB–ArcA system of *E. coli* or the various two-component systems of purple bacteria are useful as additional models for this type of regulation in cyanobacteria and in chloroplasts [7,8,22].

In spite of the strong similarities mentioned above, the model character of cyanobacteria is limited because, in contrast to chloroplasts, many components of their electron transport chain are shared by both photosynthesis and respiration. Therefore, the redox status of electron transport components (i.e. the PQ pool, the cytochrome b_6f complex and plastocyanin) depend on photosynthetic light harvesting and on the metabolism of respiratory substrates [48,49]. Thus, the redox state of the PQ pool more or less reflects the energy state of the cell. The reduction of the chloroplast PQ pool in the dark by stromal substrates via *ndh* (Figs 3,4) in so-called chlororespiration is still a matter of debate [50] and requires further investigation. In recent years, it has emerged that electron transport (especially the redox state of the PQ pool) in cyanobacteria plays a combined role as a sensor of the cellular status (which depends on the environment) and as a regulator of the photosynthetic capacity. This agrees with the view of chloroplast electron transport as an environmental sensor, underlining the dual function of photosynthesis in energy fixation and gene regulation. However, redox regulation of cyanobacterial gene expression is not restricted to the PQ pool alone. Additional redox signals come from PSII excitation pressure and the thiol state of the cell [51,52]. Furthermore, as well as the reaction-centre genes, several other genes are also regulated by signals from electron transport, generated either photosynthetically or from glucose metabolism. These include photosynthesis genes such as *trxA* (encoding thioredoxin) [53] but also non-photosynthesis genes such as glutamine synthetase [54], *dnaA*-like [55], fatty acid desaturase [56] and RNA-helicase genes [57]. Whether this is the case for plants and algae still has to be proved.

Chloroplast redox control of nuclear photosynthesis genes

Photosynthesis genes in the nucleus are also affected by chloroplast redox signals (Fig. 2), so such signals represent one new class of plastid signals [58–60]. This task has been reviewed recently in detail [61–63], so only a few important features are discussed here. All nuclear genes that have been shown to be under chloroplast redox control encode components that are either directly involved in photosynthesis (Fig. 2) or coupled to chloroplast functions such as light perception during development or stress defence. Because chloroplasts participate in many cellular metabolic pathways, many more genes will probably be found that show redox-dependent expression. At present, the transduction of chloroplast redox signals is an unexplored area.

The redox-regulated *Lhcb* transcription in *Dunaliella salina* might involve an unidentified phosphorylatable protein, but the molecular connections are not understood [64]. Photosynthesis-derived H_2O_2, as a membrane-permeable component, can pass freely through the envelope, activating a cytosolic mitogen-activated-protein kinase cascade [65] in a stress-defence reaction. However, this does not fully describe how H_2O_2 influences the expression of at least 175 open reading frames as found in an *Arabidopsis* cell culture [66]. We are just beginning to understand the redox signalling routes between chloroplasts and the nucleus. Nevertheless, we can imagine the role that these signals play in the intracellular communication. They report the functional state of all chloroplasts

in the cell to the nucleus. Here, the signals (which can vary between the individual chloroplasts) are integrated and translated into a common signal that induces a general response of the nucleus (i.e. through the targeted expression of distinct genes). Gene products located in the cytosol fulfil the overall demands of the cell, whereas chloroplast-located products are imported and used in a per-plastid fashion, in which the single chloroplast decides (e.g. by import or assembly regulation) how much of this product is appropriate for its individual metabolic state.

Perspectives

This review has focused on the expression of photosynthesis genes that are known to be regulated by chloroplast redox signals. This clearly represents only a small piece of the puzzle of plant gene redox control. Many more photosynthesis and non-photosynthesis genes might also be regulated by these signals. The exact origin of the respective regulation is still unknown in many cases. Furthermore, chloroplasts represent only one source of redox signals in the cell. Mitochondria and peroxisomes are other potent sources for such signals [67–69]. Because these three cellular compartments are functionally (and perhaps spatially) connected (e.g. during photorespiration), it would be not surprising if they all contribute to an intracellular redox-signalling network that senses and communicates the relative activities of the three organelles to each other under various metabolic situations. The connection between photosynthesis and respiration is well known [22,70], but the mutual influences between these processes are still only partly understood. Furthermore, these signals can be expected to connect to other important signalling pathways of the cell that communicate the present state of carbohydrate supply, stress-related ROS (e.g. generated by pathogen attack or wounding) [71,72] or photoreceptor-mediated illumination. From our current state of knowledge, redox signals are likely to play one of, if not the, most important roles in the integration of intracellular light and energy signals in plant cells. Future work will show whether, and how far, this holds true.

Acknowledgements

I apologize that it was not possible to cite all relevant primary literature in this review. I thank Utta Berchner-Pfannschmidt and Ralf Oelmüller for critical reading and helpful discussions. The work of my research group is supported by a grant from the Deutsche Forschungsgemeinschaft.

References

1 Martin, W. *et al.* (2002) Evolutionary analysis of *Arabidopsis*, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. *Proc. Natl Acad. Sci. U.S.A.* 99, 12246–12251
2 Allen, J.F. and Forsberg, J. (2001) Molecular recognition in thylakoid structure and function. *Trends Plant Sci.* 6, 317–326
3 Race, H.L. *et al.* (1999) Why have organelles retained genomes? *Trends Genet.* 15, 364–370
4 Stern, D.B. (1997) Transcription and translation in chloroplasts. *Trends Plant Sci.* 2, 308–315
5 Abdallah, F. *et al.* (2000) A prediction of the size and evolutionary origin of the proteome of chloroplasts of *Arabidopsis*. *Trends Plant Sci.* 5, 141–142
6 Jarvis, P. and Soll, J. (2001) Toc, Tic, and chloroplast protein import. *Biochim. Biophys. Acta* 1541, 64–79
7 Allen, J.F. (1993) Control of gene expression by redox potential and the requirements for chloroplast and mitochondrial genomes. *J. Theor. Biol.* 165, 609–631
8 Bauer, C.E. *et al.* (1999) Mechanisms for redox control of gene expression. *Annu. Rev. Microbiol.* 53, 495–523
9 Pearson, C.K. *et al.* (1993) NAD turnover and utilisation of metabolites for RNA synthesis in a reaction sensing the redox state of the cytochrome b_6f complex in isolated chloroplasts. *Eur. J. Biochem.* 218, 397–404
10 Smirnoff, N. (2000) Ascorbate biosynthesis and function in photoprotection. *Philos. Trans. R. Soc. London B Biol. Sci.* 1402, 1455–1464
11 Badger, M.R. *et al.* (2000) Electron flow to oxygen in higher plants and algae: rates and control of direct photoreduction (Mehler reaction) and Rubisco oxygenase. *Philos. Trans. R. Soc. London B Biol. Sci.* 1402, 1433–1446
12 Noctor, G. and Foyer, C.H. (1998) Ascorbate and glutathione: keeping active oxygen under control. *Annu. Rev. Plant Physiol.* 49, 249–279
13 Baier, M. and Dietz, K.-J. (1999) The costs and benefits of oxygen for photosynthesizing plant cells. *Prog. Bot.* 60, 282–314
14 Aro, E.-M. *et al.* (1993) Photoinhibition of photosystem II. Inactivation, protein damage and turnover. *Biochim. Biophys. Acta* 1143, 113–134
15 Niyogi, K.K. (2000) Safety valves for photosynthesis. *Curr. Opin. Plant Biol.* 3, 455–460
16 Haldrup, A. *et al.* (2001) Balance of power: a view of the mechanism of photosynthetic state transitions. *Trends Plant Sci.* 6, 301–305
17 Allen, J.F. and Pfannschmidt, T. (2000) Balancing the two photosystems: photosynthetic electron transfer governs transcription of reaction centre genes in chloroplasts. *Philos. Trans. R. Soc. London B Biol. Sci.* 1402, 1351–1359
18 Chow, W.S. *et al.* (1990) Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. *Proc. Natl Acad. Sci. U.S.A.* 87, 7502–7506
19 Arnon, D.I. (1982) Sunlight, earth, life: the grand design of photosynthesis. *The Sciences* 22, 22–27
20 Anderson, J.M. *et al.* (1995) The grand design of photosynthesis: acclimation of the photosynthetic apparatus to environmental cues. *Photosynth. Res.* 46, 129–139
21 Huner, N.P.A. *et al.* (1998) Energy balance and acclimation to light and cold. *Trends Plant Sci.* 3, 224–230
22 Pfannschmidt, T. *et al.* (2001) Principles of redox control in photosynthesis gene expression. *Physiol. Plant* 112, 1–9
23 Deshpande, N.N. *et al.* (1997) Evidence for light/redox-regulated splicing of *psbA* pre-RNAs in *Chlamydomonas* chloroplasts. *RNA* 3, 37–48
24 Danon, A. and Mayfield, S.P. (1994) Light-regulated translation of chloroplast messenger RNAs through redox potential. *Science* 266, 1717–1719
25 Trebitsh, T. *et al.* (2000) Translation of chloroplast *psbA* mRNA is modulated in the light by counteracting oxidizing and reducing activities. *Mol. Cell. Biol.* 20, 1116–1123
26 Trebitsh, T. and Danon, A. (2001) Translation of chloroplast *psbA* mRNA is regulated by signals initiated by both photosystems II and I. *Proc. Natl Acad. Sci. U.S.A.* 98, 12289–12294
27 Danon, A. and Mayfield, S.P. (1994) ADP-dependent phosphorylation regulates RNA-binding: implications in light-modulated translation. *EMBO J.* 13, 2227–2235
28 Shen, Y. *et al.* (2001) RNA binding-proteins interact specifically with the *Arabidopsis* chloroplast *psbA* mRNA 5′-untranslated region in a redox-dependent manner. *Plant Cell Physiol.* 42, 1071–1078
29 Nickelsen, J. and Link, G. (1993) The 54 kDa RNA-binding protein from mustard chloroplasts mediates endonucleolytic transcript 3′-end formation *in vitro*. *Plant J.* 3, 537–544
30 Liere, K. and Link, G. (1997) Chloroplast endoribonuclease p54 involved in RNA 3′-end processing is regulated by phosphorylation and redox state. *Nucleic Acids Res.* 25, 2403–2408
31 Baginsky, S. *et al.* (1997) Transcription factor phosphorylation by a protein kinase associated with chloroplast RNA polymerase from mustard (*Sinapis alba*). *Plant Mol. Biol.* 34, 181–189
32 Baginsky, S. *et al.* (1999) PTK, the chloroplast RNA polymerase associated protein kinase from mustard (*Sinapis alba*), mediates

redox control of plastid *in vitro* transcription. *Plant Mol. Biol.* 39, 1013–1023
33 Link, G. (1996) Green life: control of chloroplast gene transcription. *BioEssays* 18, 465–471
34 Allison, L.A. (2000) The role of sigma factors in plastid transcription. *Biochimie* 82, 537–548
35 Baena-Gonzalez, E. *et al.* (2002) Chloroplast transcription at different light intensities. Glutathione-mediated phosphorylation at the major RNA polymerase involved in redox-regulated organellar gene expression. *Plant Physiol.* 127, 1044–1052
36 Pfannschmidt, T. *et al.* (1999) Photosynthetic control of chloroplast gene expression. *Nature* 397, 625–628
37 Golden, S.S. (1995) Light-responsive gene expression in cyanobacteria. *J. Bacteriol.* 177, 1651–1654
38 Fujita, Y. (1997) A study on the dynamic features of photosystem stoichiometry: accomplishments and problems for future studies. *Photosynth. Res.* 53, 83–93
39 Mullineaux, C.W. (2001) How do cyanobacteria sense and respond to light? *Mol. Microbiol.* 41, 965–971
40 Alfonso, M. *et al.* (2000) Redox control of *psbA* gene expression in the cyanobacterium *Synechocystis* PCC 6803. Involvement of the cytochrome b_6/f complex. *Plant Physiol.* 122, 505–515
41 Li, H. and Sherman, L.A. (2000) A redox-responsive regulator of photosynthesis gene expression in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *J. Bacteriol.* 182, 4268–4277
42 Rujan, T. and Martin, W. (2001) How many genes in *Arabidopsis* come from cyanobacteria? An estimate from 386 protein phylogenies. *Trends Genet.* 17, 113–120
43 Chang, C. and Stewart, R.C. (1998) The two-component system. Regulation of diverse signaling pathways in prokaryotes and eukaryotes. *Plant Physiol.* 117, 723–731
44 D'Agostino, I.B. and Kieber, J. (1999) Phosphorelay signal transduction: the emerging family of plant response regulators. *Trends Biochem. Sci.* 24, 452–456
45 Lohrmann, J. and Harter, K. (2002) Plant two-component signaling systems and the role of response regulators. *Plant Physiol.* 128, 363–369
46 Oelmüller, R. *et al.* (2001) Novel aspects in photosynthesis gene regulation. In *Signal Transduction in Plants* (Sopory, S.K. *et al.*, eds), pp. 259–277, Kluwer Academic Publishers
47 Forsberg, J. *et al.* (2002) Redox signalling in chloroplasts and mitochondria: genomic and biochemical evidence for two-component regulatory systems in bioenergetic organelles. *Biochem. Soc. Trans.* 29, 403–407
48 Scherer, S. (1990) Do photosynthesis and respiratory electron transport chains share redox proteins? *Trends Biochem. Sci.* 15, 458–462
49 Schmetterer, G. (1994) Cyanobacterial respiration. In *The Molecular Biology of Cyanobacteria* (Bryant, D.A., ed.), pp. 409–435, Kluwer Academic Publishers
50 Nixon, P.J. (2000) Chlororespiration. *Phil. Trans. R. Soc. London B Biol. Sci.* 1402, 1541–1547
51 Campbell, D. *et al.* (1995) Electron transport regulation exchange of two forms of photosystem II D1 protein in the cyanobacterium *Synechococcus*. *EMBO J.* 14, 5457–5466
52 Sippola, K. and Aro, E.-M. (1999) Thiol redox state regulates expression of *psbA* genes in *Synechococcus* sp. PCC 7942. *Plant Mol. Biol.* 41, 425–433
53 Navarro, F. *et al.* (2000) Electron transport controls transcription of the thioredoxin gene (*trxA*) in the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Mol. Biol.* 43, 23–32
54 Reyes, J.C. and Florencio, F.J. (1995) Electron transport controls transcription of the glutamine synthetase gene (*glnA*) from the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Mol. Biol.* 27, 789–799
55 Richter, S. *et al.* (1998) Transcriptional analysis and mutation of a *dnaA*-like gene in *Synechocystis* sp. strain PCC 6803. *J. Bacteriol.* 180, 4946–4949
56 Kis, M. *et al.* (1998) Light-induced expression of fatty acid desaturase genes. *Proc. Natl Acad. Sci. U.S.A.* 95, 4209–4214
57 Kujat, S.L. and Owttrim, G.W. (2000) Redox-regulated RNA helicase expression. *Plant Physiol.* 124, 703–713
58 Oelmüller, R. (1989) Photooxidative destruction of chloroplasts and its effect on nuclear gene expression and extraplastidic enzyme levels. *Photochem. Photobiol.* 49, 229–239
59 Taylor, W.C. (1989) Regulatory interactions between nuclear and plastid genomes. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 406, 211–233
60 Goldschmidt-Clermont, M. (1998) Coordination of nuclear and chloroplast gene expression in plant cells. *Int. Rev. Cytol.* 177, 115–180
61 Durnford, D.G. and Falkowski, P.G. (1997) Chloroplast redox regulation of nuclear gene transcription during photoacclimation. *Photosynth. Res.* 53, 229–241
62 Rodermel, S. (2001) Pathways of plastid-to-nucleus signaling. *Trends Plant Sci.* 6, 471–478
63 Pfannschmidt, T. *et al.* Chloroplast redox control of nuclear gene expression – a new class of plastid signals in interorganellar communication. *Antiox. Redox Signal.* (in press)
64 Escoubas, J.-M. *et al.* (1995) Light intensity regulation of cab gene transcription is signaled by the redox state of the plastoquinone pool. *Proc. Natl Acad. Sci. U.S.A.* 92, 10237–10241
65 Kovtun, Y. *et al.* (2000) Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. *Proc. Natl Acad. Sci. U.S.A.* 97, 2940–2945
66 Desikan, R. *et al.* (2001) Regulation of the *Arabidopsis* transcriptome by oxidative stress. *Plant Physiol.* 127, 159–172
67 Delledonne, M. *et al.* (1998) Nitric oxide functions as a signal in plant disease resistance. *Nature* 394, 585–588
68 Corpas, F.J. *et al.* (2001) Peroxisomes as a source of reactive oxygen species and nitric oxide signal molecules in plant cells. *Trends Plant Sci.* 6, 145–150
69 Maxwell, D.P. *et al.* (2002) Evidence of mitochondrial involvement in the transduction of signals required for the induction of genes associated with pathogen attack and senescence. *Plant J.* 29, 269–279
70 Padmasree, K. *et al.* (2002) Essentiality of mitochondrial oxidative metabolism for photosynthesis: optimization of carbon assimilation and protection against photoinhibition. *Crit. Rev. Biochem. Mol. Biol.* 37, 71–119
71 Oswald, O. *et al.* (2001) Plastid redox state and sugars: Interactive regulators of nuclear-encoded photosynthetic gene expression. *Proc. Natl Acad. Sci. U.S.A.* 98, 2047–2052
72 Mullineaux, P. *et al.* (2000) Are diverse signalling pathways integrated in the regulation of *Arabidopsis* antioxidant defence gene expression in response to excess excitation energy? *Philos. Trans. R. Soc. London B Biol. Sci.* 355, 1531–1540
73 Trebst, A. (1980) Inhibitors in electron flow: tools for the functional and structural localization of carriers and energy conservation sites. *Methods Enzymol.* 69, 675–715
74 Tullberg, A. *et al.* (2000) Photosynthetic electron flow regulates transcription of the *psaB* gene in pea (*Pisum sativum* L.) chloroplasts through redox state of the plastoquinone pool. *Plant Cell Physiol.* 41, 1045–1054
75 Pfannschmidt, T. *et al.* (2001) A novel mechanism of nuclear photosynthesis gene regulation by redox signals from the chloroplast during photosystem stoichiometry adjustment. *J. Biol. Chem.* 276, 36125–36180
76 Kovacs, L. *et al.* (2000) Short- and long-term redox regulation of photosynthetic light energy distribution and photosystem stoichiometry by acetate metabolism in the green alga, *Chlamydobotrys stellata*. *Photosynth. Res.* 65, 231–247
77 Yang, D-H. *et al.* (2001) The redox state of the plastoquinone pool controls the level of the light-harvesting chlorophyll *a/b* binding protein complex II (LHCII) during photoacclimation – cytochrome b_6f deficient *Lemna perpusilla* plants are locked in a state of high-light acclimation. *Photosynth. Res.* 68, 163–174
78 Maxwell, D.P. *et al.* (1995) Redox regulation of light-harvesting complex II and *cab* mRNA abandance in *Dunaliella salina*. *Plant Physiol.* 109, 787–795
79 Karpinski, S. *et al.* (1997) Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in *Arabidopsis* during excess light stress. *Plant Cell* 9, 627–640
80 Karpinski, S. *et al.* (1999) Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. *Science* 284, 654–657

81 Petracek, M.E. *et al.* (1998) *Ferredoxin-1* mRNA is destabilized by changes in photosynthetic electron transport. *Proc. Natl Acad. Sci. U.S.A.* 95, 9009–9013
82 Eguchi, S. *et al.* (2002) Photosynthetic electron transport regulates the stability of the transcript for the protochlorophyllide oxidoreductase gene in the liverwort, *Marchantia paleacea* var. *diptera*. *Plant Cell Physiol.* 43, 573–577
83 Petracek, M.E. *et al.* (1997) Light-regulated changes in abundance and polyribosome association of ferredoxin mRNA are dependent on photosynthesis. *Plant Cell* 9, 2291–2300
84 Sherameti, I. *et al.* Polyribosome loading of *PsaD* mRNA is controlled by the photosynthetic electron transport. A crucial *cis* element in the spinach *PsaD* gene is located in the 5′-untranslated region. *Plant J.*, (in press)
85 Salvador, M.L. and Klein, U. (1999) The redox state regulates RNA degradation in the chloroplast of *Chlamydomonas reinhardtii*. *Plant Physiol.* 121, 1367–1374
86 Irihimovitch, V. and Shapira, M. (2000) Glutathione redox potential modulated by reactive oxygen species regulates translation of Rubisco large subunit in the chloroplast. *J. Biol. Chem.* 275, 16289–16295
87 Allen, J.F. (1992) Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta* 1098, 275–335
88 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
89 El Bissati, K. and Kirilovsky, D. (2001) Regulation of *psbA* and *psaE* expression by light quality in *Synechocystis* species PCC 6803. A redox control mechanism. *Plant Physiol.* 125, 1988–2000
90 Stock, A.M. *et al.* (2000) Two-component signal transduction. *Annu. Rev. Biochem.* 69, 183–215
91 Kim, J. and Mayfield, S.P. (1997) Protein disulfide isomerase as a regulator of chloroplast translational activation. *Science* 278, 1954–1957
92 Yohn, C.B. *et al.* (1998) A poly(A) binding protein functions in the chloroplast as a message-specific translation factor. *Proc. Natl Acad. Sci. U.S.A.* 95, 2238–2243