Chloroplast redox signals: how photosynthesis controls its own genes

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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_{6}f 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...
Detoxification 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 photo-inhibition of the photosynthetic electron transport by, for example, the destruction of the D1 protein in photosystem II (PSII) [14]. To avoid this stress phenomen, 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 counterbalanced 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 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].

In the Mehler reaction (Fig. 1b), superoxide is mainly formed at photosystem I (PSI), either directly or via ferrodoxin, and is rapidly detoxified by superoxide diamutases (SODs), which produce hydrogen peroxide. Ascorbate peroxidases (APXs) then reduce hydrogen peroxide to water via the oxidation of ascorbate (Asc) to monodehydroascorbate radicals (MDHA), which are reduced back to ascorbate via glutathione.

Under various stress conditions, the cellular concentration of ROS increases and can overcome the antioxidan-defence mechanisms. In the Fenton reaction (Fig. 1c), 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
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.
Chloroplast Nucleus

chloroplast redox signals. Information to understand the underlying molecular network of proteomic approaches should provide such global information combined with transcriptomic and, in principle, all other types of regulation. Our increasing ability to analyse each single step (e.g. transcription, transcript splicing in Chlamydomonas (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 b6f 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].

![Diagram of gene expression level and redox parameter](http://plants.trends.com)

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 occurring at multiple expression levels and suggest the existence of a complex signalling network. 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) 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.
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 PSII reaction-centre genes whereas an oxidized PQ pool activates the transcription of PSI 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 organelar 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 b6f 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 H2O2, 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 H2O2 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 fulfill 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 not be 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.

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