ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 5 & 6, 2005

Forum Review

Redox Regulation and Modification of Proteins Controlling Chloroplast Gene Expression

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ABSTRACT

Chloroplasts are typical organelles of plant cells and represent the site of photosynthesis. As one very remarkable feature, they possess their own genome and a complete machinery to express the genetic information in it. The plastid gene expression machinery is a unique assembly of prokaryotic-, eukaryotic-, and phage-like components because chloroplasts acquired a great number of regulatory proteins during evolution. Such proteins can be found at all levels of gene expression. They significantly expand the functional and especially the regulatory properties of the "old" gene expression system that chloroplasts inherited from their prokaryotic ancestors. Recent results show that photosynthesis has a strong regulatory effect on plastid gene expression. The redox states of electron transport components, redox-active molecules coupled to photosynthesis, and pools of reactive oxygen species act as redox signals. They provide a functional feedback control, which couples the expression of chloroplast genes to the actual function of photosynthesis and, by this means, helps to acclimate the photosynthetic process to environmental cues. The redox signals are mediated by various specific signaling pathways that involve many of the "new" regulatory proteins. Chloroplasts therefore are an ideal model to study redox-regulated mechanisms in gene expression control. Because of the multiple origins of the expression machinery, these observations are of great relevance for many other biological systems. *Antioxid. Redox Signal.* 7, 607–618.

INTRODUCTION

Plant cells possess a unique compartment that is not present in animal or fungal cells—the plastid(s). The most prominent representative of this morphological and functional heterogeneous group of cell organelles is the chloroplast, which can be found in green tissues or cells (40). The chloroplast is the site of photosynthesis and provides the structural and functional properties for this complex process that converts light energy of the sun into chemical energy and, by this means, fixates the energy for almost all organisms on earth. In addition, chloroplasts are involved in many other biosynthetic or metabolic pathways, such as amino acid and pyrimidine biosynthesis or sulfate and nitrate reduction. Therefore, they represent an indispensable compartment of a plant cell. As further specific characteristics, chloroplasts possess a double envelope membrane and their own small genome, the so-called plastome, both of which are remnants of the prokaryotic ancestry of plastids (61). They also possess a complete machinery to express the genetic information encoded in the plastome (41, 55, 88). In higher plants, the plastome is organized as a circular plasmid that exists in up to 100 copies per plastid. It typically contains ~100–120 genes, which encode mainly components of the photosynthetic apparatus and of the gene expression machinery (89). The vast majority of chloroplast proteins, however, are encoded in the nucleus and have to be imported from the cytosol via a specialized import machinery (44). Plastids therefore are considered to be genetically semiautonomous.

During photosynthesis, a light-driven chain of reduction/oxidation (redox) reactions takes place that split water into oxygen, electrons, and protons. Electrons and protons are

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used to generate reduction ($NADPH_2$) and energy equivalents (ATP). The photosynthetic apparatus that performs these reactions is located in a complex intraorganellar membrane system (the thylakoid system) and consists of four major multisubunit protein complexes: photosystem II (PSII), the cytochrome b_6f complex (cytb_6f), photosystem I (PSI), and the ATP synthase (ATPase). The first three are connected by mobile electron carriers: plastoquinone (PQ) transfers the electrons from PSII to cytb_6f, and plastocyanin then transfers the electrons from the cytb_6f complex to PSI, which, finally, reduces $NADP^+$ to $NADPH_2$. During this electron transport, a proton gradient over the membrane is generated that is used by the ATPase to generate ATP (16). All these protein complexes consist of plastid- and nucleus-encoded subunits. As a common picture, it emerged from the plastome sequences of several organisms that the inner proteins of the photosystems are encoded in the plastome, whereas all peripheral subunits are encoded in the nucleus (77). This split location of photosynthesis genes exacerbates a controlled expression of the involved genes in many aspects and requires a high coordination between the two genetic compartments. The coordination is achieved by an exchange of information between nucleus and chloroplasts, in both an anterograde (nucleus-to-chloroplast) and retrograde (chloroplast-to-nucleus) manner (35, 36, 90).

Recent studies show that the photosynthetic process has a direct impact on the expression of genes for photosynthetic components, in both the chloroplast and the nucleus (13, 19, 30, 58, 74, 81, 91). As initial signals, changes in the redox state of the components of the electron transport chain itself, other photosynthesis-coupled redox-active, soluble components such as thioredoxin or glutathione, and reactive oxygen species (ROS) that are unavoidable by-products of photosynthesis have been identified. This regulation provides a feedback loop that couples the actual function of photosynthesis to the expression of its own constituents. Redox signals therefore allow the organism to acclimate the photosynthetic process to varying environmental cues that negatively affect the photosynthetic process (69).

This review focuses on redox signaling pathways and their components that control the expression of chloroplast genes. We summarize the present knowledge about the mechanistic understanding of redox signal transduction in chloroplasts and provide working models for future research in the field of plastid gene expression.

MODES OF REDOX CONTROL

To date, at least three different general modes of redox control in chloroplast gene expression are known, and are summarized in Fig. 1. These modes are connected by several interrelationships and form a complex redox signaling network that is aimed to acclimate photosynthetic efficiency and presumably other important chloroplast functions to adverse environmental conditions.

Photosynthetic redox control

Beside its function as an energy fixation device, photosynthesis operates also as an environmental sensor. Variations in environmental cues often induce changes in the efficiency of photosynthetic electron flow (PEF) that affect the redox state of involved PEF components. Such changes in redox state initiate signaling cascades that, in turn, affect the expression of genes. In this case, the input of the signaling cascade is redox-dependent and directly generated within the electron transport chain. The transduction of the redox signal

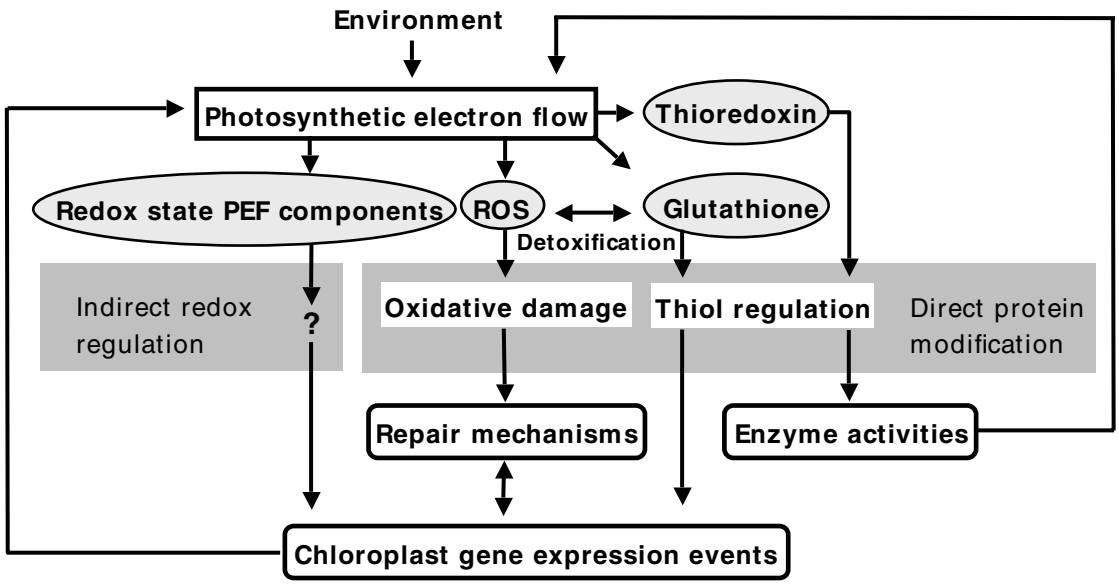

FIG. 1. Summary of typical redox control modes in chloroplasts. The scheme depicts important general pathways of redox regulation that control enzyme activities, repair mechanisms, and gene expression in response to environmentally induced changes in photosynthesis. Connections between pathways and the feedback effects of molecular responses on the photosynthetic process are indicated.

is still unknown (question mark in Fig. 1), and it is very likely that it is translated into a different signal, *e.g.,* the redox signal could be sensed by a sensor kinase activity that translates the redox signal into a phosphorylation signal, as is known for state transitions (3, 8, 38). As the final effect on the regulatory protein(s) can be different from the input signal, we assume in this case an indirect redox regulation of chloroplast gene expression.

Thiol group modification

Redox-active molecules such as thioredoxins, a group of small proteins with a redox-active disulfide bridge in their active site, or glutathione, a multifunctional tripeptide acting as redox buffer and sulfur sink, are reduced when photosynthesis is taking place. In this form, they are active and affect the function or activity of their target protein(s) by thiol group modification(s) via electron donation, *i.e.,* activation of the target by the formation of dithiol residues upon reduction (15, 85). Here we have a direct redox modification of the protein in question. Thus, the output of the signaling cascade is redox-dependent. A well-known example of this is the thioredoxin-mediated activation of the Calvin–Benson-cycle enzymes upon illumination (20, 83, 84). Thiol group modification of regulatory proteins therefore provides a different tool for redox control in chloroplast gene expression.

Oxidative damage

The generation of ROS during photosynthesis is unavoidable; therefore, photosynthetic organisms developed a number of scavenging mechanisms. Under various stress conditions, however, these protection mechanisms are overridden and ROS can accumulate that often result in oxidative damage of proteins. Such an oxidation reaction is characterized by its deleterious and irreversible effect on the protein(s), in contrast to oxidation of dithiol groups into disulfide bridges, which can be reversed. Oxidative damage therefore induces repair mechanisms that often involve changes in gene expression. The most prominent example in photosynthesis is the D1 protein turnover. This reaction center protein of PSII is subjected to continuous oxidative damage through singlet oxygen and has to be replaced in a complex repair cycle in order to avoid photoinhibition of PSII (7). As a side effect, accumulating ROS also affect the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) because GSH is involved in scavenging and detoxification of ROS. This can have an indirect effect on the thiol group regulation of regulatory proteins in gene expression and provides a link between stress and acclimation responses.

Many other oxidative molecules, such as oxylipins, hydrogen peroxide, or nitric oxide, have been shown to affect gene expression in plant cells (29, 75, 79). A specific role for these redox-reactive molecules in chloroplast gene expression, however, has not been identified yet. Further new key players in this context are also the peroxiredoxins, a family of low-efficiency peroxidases, which are located in several compartments of the plant cell, including the chloroplasts. They detoxify several peroxides by reduction; therefore, they have an important role in antioxidative responses of plant cells and substantially contribute to the intracellular redox signaling network. Their impact on gene expression events, however, is mainly unknown (30, 31). Further research will uncover if and how these molecules affect chloroplast gene expression. In the following, we describe in detail those redox control mechanisms in plastid gene expression that are identified and characterized to date.

TRANSCRIPTIONAL REDOX REGULATION

Transcription in plastids is driven by two different RNA polymerases: a bacteria-type multisubunit enzyme and a phage-type single subunit enzyme. The core complex of the bacteria-type enzyme is comprised of four subunits (α, β, β′, β″) encoded by the plastid genes *rpoA, rpoB*, $rpoC_1$, and $rpoC_2$ and, therefore, is termed plastid-encoded polymerase (PEP) (Table 1). The phage-type polymerase belongs to the class of T7-like RNA polymerases and is encoded in the nucleus. In *Arabidopsis,* three copies exist from which one is directed to chloroplasts, one to mitochondria, and one to both organelles. Consequently, this polymerase is termed nucleus-encoded polymerase (NEP) (21, 41, 55). Despite this classification, promoter recognition of the PEP enzyme still relies on the interaction of its core complex with nuclear-encoded sigma factors, which have to be imported into the organelle (6, 57). Furthermore, it could be shown in mustard that the PEP enzyme exhibits the bacterial subunit structure only in plastids of dark-grown plants, the so-called etioplasts. Upon

Table 1. Gene Symbols, Encoded Proteins, and Protein Function

Gene symbol	*Encoded component*	*Function/process*
clpP	Chloroplast protease subunit	ATP-dependent protein degradation
psaAB	p700 apoproteins of PSI	Binding of cofactors, reaction center of PSI
psbA	D1 protein of PSII	Binding of cofactors, reaction center of PSII
rbcL	Large subunit of RubisCO	Catalytic subunit for CO_2 fixation
rpoA	α subunit of PEP	Structural protein
rpoB	β subunit of PEP	Catalytic subunit for RNA synthesis
$rpoC_1$	β′ subunit of PEP	Unknown
$rpoC_2$	β″ subunit of PEP	Putative DNA-binding subunit
rps16	Protein 16 of small ribosome subunit	Unknown
trnK	tRNA lysine	Translational insertion of lysine

illumination and subsequent chloroplast maturation, the enzyme recruits additional subunits resulting in an enzyme complex with up to 15 subunits, which was termed PEP-A (70). Similar complex RNA polymerases have been purified from several other higher plants, too (43). The additional proteins most probably have regulatory functions in transcription and adapt the bacterial RNA polymerase to the specific redox conditions in chloroplasts. Among these proteins, an iron superoxide dismutase, an annexin-like protein, an RNA-binding protein, and a CK2-type kinase (59, 67, 73) have been identified in mustard (see below).

Promoters of most chloroplast genes and operons, *e.g.,* those for photosynthesis, have bacteria-like −10 and −35 *cis*-elements, which are recognized by the PEP enzyme. The NEP enzyme recognizes a different promoter element called YRTA-motif, which can be found upstream of several genes with PEP promoters (54), indicating that those genes are transcribed by both polymerases, however, as expression analyses revealed, to different degrees. Transcription of only a few genes is driven exclusively from a NEP promoter including the *rpo* operon, which encodes the PEP subunits, and the *clpP* gene, which encodes an important plastid protease. Although many aspects of plastid transcription are still not understood, present data point to the PEP enzyme as the major active polymerase in mature chloroplasts and prominent target for regulation signals, including redox control (21, 41, 55). The NEP enzyme, in contrast, seems to play a dominant role in the early stages of plastid development when the expression machinery including PEP has to be built up. Its role in mature chloroplasts is still enigmatic because of its low expression level. Nevertheless, the existence of exclusively NEP-transcribed coding regions suggests that the NEP enzyme is necessary for proper gene expression and regulation also in mature chloroplasts (21, 41, 55).

Photosynthetic redox control of transcription via *the redox state of the PQ pool*

Redox control of gene expression in chloroplasts and mitochondria was hypothesized early in analogy to bacterial systems and interpreted as the major selection process by which organelles have retained genomes throughout evolution (2). A first confirmation for such a redox control in plastid gene expression was obtained in lettuce. It was shown that illumination promoted the incorporation of radioactively labeled NADH into the RNA fraction of chloroplasts, whereas this was not the case in nonilluminated control samples (68). Gene-specific effects then were demonstrated by treatment of mustard seedlings with a more sophisticated growth-light regimen in which an imbalance in excitation energy distribution between the photosystems was generated (71, 72). This approach used the different light absorption properties of the reaction center chlorophylls of PSII and PSI (680 and 700 nm, respectively). As both photosystems work electrochemically in series, a preferential excitation of PSII results in a reduction of the electron transport chain, whereas a preferential excitation of PSI results in its oxidation. Both situations decrease photosynthetic efficiency and are counteracted in the short term (on the order of minutes) by so-called state transitions. In this acclimation process, a part of the light-harvesting antenna of PSII (LHCII) can be moved between the photosystems, resulting in a redistribution of light energy. This is controlled by a redox-dependent kinase activity that phosphorylates the mobile part of LHCII under reducing conditions and, hence, induces its lateral migration to PSI. Despite long-ongoing research, the "real" LHCII kinase is still not identified (5). Recent results rather suggest that a complex kinase network is responsible for this control (27, 87). In the long term (on the order of hours and days), the stoichiometry of photosystems is readjusted, resulting in the same but longer-lasting effect as state transitions, *e.g.,* when PSII is predominantly excited, the PSII:PSI ratio decreases and vice versa. The study on mustard seedlings showed that the change in photosystem stoichiometry correlated with respective changes in the transcriptional rates and transcript amounts of the plastid genes for the reaction center proteins of PSII and PSI, *psbA* and *psaAB*. Further, *in organello* run-on transcription experiments with isolated chloroplasts demonstrated that the transcriptional regulation was independent of cytosolic factors, such as photoreceptors. The use of specific electron transport inhibitors 3-(3′,4′-dichlorophenyl)-1,1′-dimethyl urea (DCMU; inhibiting the reduction of the PQ pool) and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB; inhibiting the oxidation of the PQ pool) revealed that the redox state of this mobile carrier is the major determinant for the changes in gene expression. When the PQ pool is mainly reduced, transcription of the *psaAB* operon is promoted, whereas in the opposite case *psbA* transcription is increased (Fig. 2). An equivalent opposite regulation of these genes

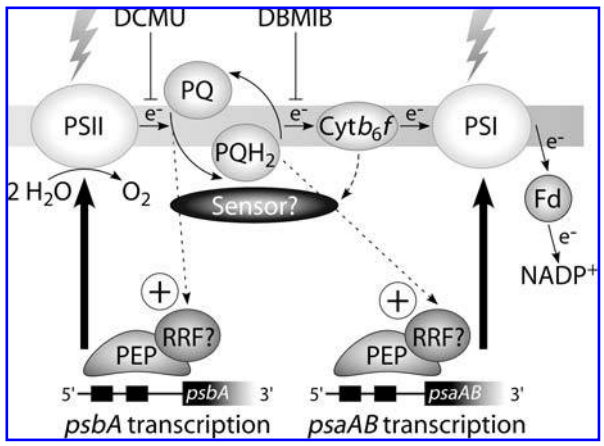

FIG. 2. Photosynthetic redox control of chloroplast transcription. The model provides a present view of how imbalances in energy distribution between the photosystems are counteracted in a long-term response via changes in the transcription of reaction center protein genes *psbA* and *psaAB*. Upon reduction of the PQ pool *psaAB* transcription is enhanced, whereas under opposite conditions *psbA* transcription is promoted. Enhanced transcription leads to increased amounts of the respective photosystem. Perception and transduction of the redox signal from the PQ pool may be mediated by yet unknown sensor protein(s) (Sensor) and redox-responsive factors (RRFs). Besides differential excitation of photosystems, electron transport inhibitors DCMU and DBMIB can be used to manipulate the redox state of the PQ pool (see text). e^-, electrons; Fd, ferredoxin; PQH_2:PQ, reduced to oxidized PQ ratio.

under comparable or similar conditions has been recently found also in pea (95), *Chlamydobotrys stellata* (50), and *Synechocystis* PCC 6803 (32, 52), suggesting that this mechanism represents an evolutionary old regulation.

These data provide a first model of how plants acclimate to light quality gradients that often occur in natural environments under low light intensities. The signal transduction from the PQ pool toward the level of transcription is not clear yet; however, it is conceivable that the long-term response represents an extended branch of the short-term response (the state transition), which is also regulated by the redox state of the PQ pool (4, 76). It was shown that the PQ oxidation site at the cyt b_6f complex functions as the sensor for the PQ redox state during a state transition (97, 102). A small 9-kDa protein of PSII, TSP9, has been discussed to be a putative candidate as signal transducer toward transcription. TSP9 was shown to be partially released from PSII upon PQ reduction in spinach and, in addition, to possess a putative DNA binding domain (22, 101). In spinach, a protein of 31 kDA was identified that is capable of sequence-specific binding of the *psaAB* promoter region (23), suggesting the existence of yet unidentified transcription factors that may transduce the redox signal. Extensive further studies, however, are necessary to resolve this important question.

Thiol group regulation via *the plastid transcription kinase (PTK)*

Phosphorylation of sigma factors and the PEP enzyme itself has been shown to be an important regulatory event in chloroplast transcription (11, 92). A CK2-type kinase has been identified to be a component of the PEP-A complex of chloroplasts of mustard (67). This kinase, PTK, is able to phosphorylate purified sigma-like factors, as well as subunits of the PEP-A complex. In *in vitro* assays, the activity of this enzyme could be modified by pretreatments with heterologous kinase and phosphatase activities or GSH (12). The modifications of phosphorylation and SH-group redox state were shown to work antagonistically (Fig. 3). A nonphosphorylated enzyme appeared to be active, whereas it was inhibited after additional treatment with GSH. In contrast, a phosphorylated nonactive enzyme could be reactivated by adding GSH. These data could be correlated with *in vivo* observations (9). PTK isolated from plants grown under moderate light conditions effectively phosphorylated the associated PEP-A, whereas this was not observed with PTK from plants subjected to 3 h of high light. Results from *in organello* run-on transcription experiments with chloroplasts from both plant sources revealed higher transcriptional activity in high light-treated mustard seedlings. In parallel, the light intensity affected the GSH:GSSG ratio, *i.e.,* the GSH:GSSG ratio increased by the high light treatment, whereas the total glutathione content decreased. Together with the *in vitro* data, a further model of trancriptional regulation in chloroplasts has been proposed (Fig. 3) (9, 59), which complements the low-light PQ redox control described above (Fig. 2). Short exposure to high light inactivates the PTK through effects on the glutathione redox state of mustard chloroplasts. This subsequently leads to a low phosphorylation state of PEP-A and an enhanced transcription of chloroplast genes. The enhancement is aimed to efficiently replace photosynthesis proteins

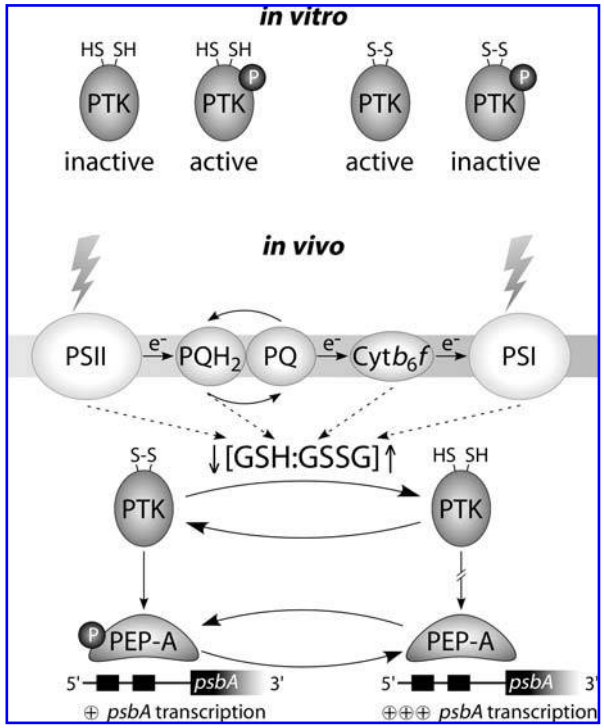

FIG. 3. Thiol regulation of chloroplast transcription. The central regulator in this model is the PTK. Its activity can be modified *in vitro* by phosphorylation and redox state. *In vivo* excessive supply of photons by illumination with high light affects chloroplast transcription also by the combined action of phosphorylation and glutathione-mediated thiol regulation. PTK serves as a sensor for changes in the redox state of glutathione induced by not yet defined influences from the photosynthetic electron flow. Its phosphorylation activity is decreased upon GSH-mediated reduction of a yet unidentified thiol site, resulting in a decreased phosphorylation state of the PEP-A complex. Less phosphorylated PEP-A [responsive phosphorylation site(s) are unknown to date] exhibits higher transcriptional activity and provides more chloroplast transcripts that may serve as matrix for enhanced protein production. e^-, electrons; GSH:GSSG, reduced to oxidized glutathione ratio; P, phosphoryl group.

destroyed during the excessive illumination by new gene products and helps to compensate the high light stress. To date, it is still unresolved how the high light effect is mediated. In *Arabidopsis,* it was shown that treatment with excess light results in the generation of ROS, which are scavenged by glutathione, thus decreasing the total GSH:GSSG ratio (46). However, in the mustard study, the organellar GSH:GSSG ratio was observed to react in the opposite way (9); therefore, other ways of control that might include yet unknown factors must be assumed.

POSTTRANSCRIPTIONAL REDOX-REGULATION

To a great extent, regulation of plastid gene expression has been assigned to posttranscriptional processes, such as RNA stabilization and RNA editing, and to translational control

mediated by nucleus-encoded plastid RNA-binding proteins (cpRBPs). Over the past 10 years, a number of cpRBPs have been identified and characterized both by biochemical approaches and by analyzing photosynthetic mutants. Most cpRBPs seem to be organized in supramolecular complexes, which consist of heterogeneous nuclear ribonucleoprotein (hnRNP)-like small cpRBPs with more general function in stabilizing and folding RNA messages, as well as cpRBPs with distinct functions in splicing, editing, or RNA maturation, such as exo- or endoribonucleases (39, 63, 80). The diverse group of differentially regulated plastid RNA-binding proteins reflects the complex regulatory network of the intracellular communication system between the nucleus and the plastids. To date, several distinct mechanisms involved in the regulation of plastid posttranscriptional processes are known. Apart from phosphorylation (26, 45, 53, 60) and cofactor requirements (17, 42), light, *i.e.,* redox processes, seems to play an important role in the regulation of cpRBP activities (10, 28, 33, 53, 62, 82, 86, 93, 94). Despite this common aspect, however, it becomes increasingly clear that the underlying details can be quite variable. It is likely that in plastids a complex signaling network with multiple and/or split signal transduction pathways exists. In this chapter, we want to give an overview on such pathways by focusing on well-studied cpRBPs regulated by redox poise.

Posttranscriptional control by p54, a 3′ RNA-binding protein

One of the proteins known to respond to phosphorylation and redox reagents *in vitro* is a 3′ endoribonuclease from mustard (*Sinapis alba*), which was first found to bind specifically to a conserved U-rich sequence element (UUUAUCU) of chloroplast *trnK* and *rps16* precursor transcripts (T_1R) (64–66). This protein, which purifies as a monomeric polypeptide of an apparent molecular size of 54 kDa, was subsequently termed p54 (53, 56). Purified p54 was shown to be activated by phosphorylation or oxidation by GSSG and inhibited by either dephosphorylation or reduction by GSH (53, 56). Interestingly, kinase pretreatment of p54 prior to oxidation with GSSG resulted in the highest levels of activation, suggesting that phosphorylation and redox state act together to control p54 activity (Table 2). Puzzling at first, neither dithiothreitol nor thioredoxin was able to induce similar effects on p54 RNA binding and processing abilities. The observed effect of the glutathione redox state, *i.e.,* the ratio between GSH and GSSG, on p54 activity resembles very much the GSH regulation of PTK (see above) (57, 59), indicating that this regulation may be active on several levels of gene expression.

How does the regulation of p54 fit into the overall picture? It is conceivable (Fig. 4) that both the preactivation of p54 by phosphorylation and the subsequent further enhancement of processing activity by oxidation by GSSG occur in response to environmental changes, such as light intensity. They may, however, not necessarily be mediated by the same sensors. In addition to a fine-tuned redox sensor possibly associated with the PQ pool and/or the cytb_6f complex controlling a sensor kinase (69), a more global redox regulation by the GSH:GSSG ratio as a sensor for biological stress could be involved. Dual control may be one mechanism by which plastid function is regulated in response not only to stress conditions, but

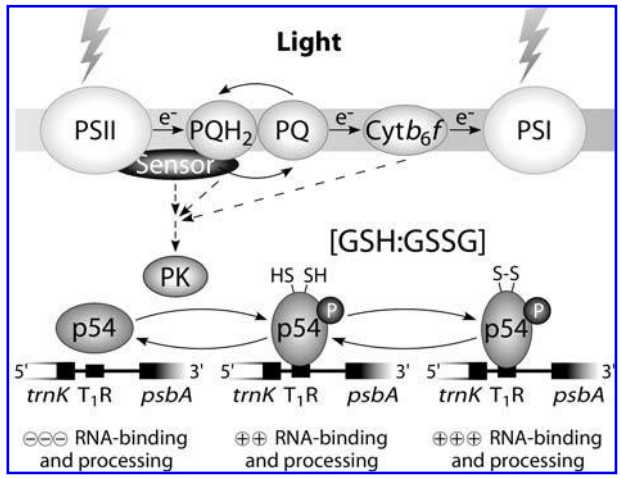

FIG. 4. Model for regulation of *trnK* 3′-RNA processing by phosphorylation and redox state of p54. This model aims at giving a view of possible connections between the photosynthetic apparatus and RNA processing via phosphorylation and redox state. The inactive (reduced and unphosphorylated) form of p54 (left) is phosphorylated by a plastid kinase (PK), perhaps exposing sequestered sulfhydryl groups (SH). Although p54 is now preactivated (middle), the SH groups now accessible must be converted into disulfide groups (S-S) by GSSG in order to reach full p54 activity (right). In both its pre- and fully activated forms, p54 is capable of sequence-specific cutting of the long *trnK* precursor (*i.e.,* a *trnK-psbA* cotranscript) at a site termed T_1R. PK might be involved in signaling cascades, which may be connected to the photosynthetic apparatus via the PQ pool (PQH_2/PQ), the cytb_6f complex (Cytb_6f), or a hypothetical redox sensor (Sensor) on the stromal side of the photosynthetic apparatus. e^-, electrons; GSH:GSSG, reduced to oxidized glutathione ratio; P, phosphoryl group.

TABLE 2. POSTTRANSLATIONAL MODIFICATIONS MODULATE THE ACTIVITY OF THE P54 ENDORIBONUCLEASE

Modification	*Treatment*	*Effect*
Phosphorylation	PKA	+ +
Dephosphorylation	CIAP	− − −
Reduction	GSH	− − −
Oxidation	GSSG	+
Phosphorylation/reduction	PKA/GSH	− − −
Phosphorylation/oxidation	PKA/GSSG	+ + +
Reduction/phosphorylation	GSH/PKA	− −
Oxidation/phosphorylation	GSSG/PKA	+ +
Dephosphorylation/reduction	CIAP/GSH	− − −
Dephosphorylation/oxidation	CIAP + GSSG	− −
Reduction/dephosphorylation	GSH/CIAP	− − −
Oxidation/dephosphorylation	GSSG/CIAP	− −

The positive (+) or negative (−) response of p54 to the various treatments as detailed in Fig. 4 is indicated by the number of symbols. CIAP, calf intestinal alkaline phosphatase; PKA, protein kinase A.

also to normal developmental or environmental changes, thus increasing the flexibility of gene expression responses to environmental signals.

As a riboendonuclease, p54 is involved in the formation of the *trnK* and *rps16* precursor transcript 3′-ends (66). However, it is not yet clear which specific consequences regulation of p54 has on the expression of its target genes. Hitherto, as the encoding gene has yet to be identified, functional analysis of p54 *in vivo* by knockout or antisense mutants is pending. Nevertheless, it is notable that p54 is capable of cleaving a bicistronic RNA precursor containing the genes *trnK* and *psbA* (65). As a result, two *psbA* RNA species exist, which differ in the length of their 5′-untranslated region (UTR). The shorter, originating from the *psbA* promoter, is much more abundant than the longer one, generated by this cleavage. Having the potential for alternative folding, these different 5′-regions may be starting points for translational and posttranscriptional control (63). Besides, it was shown that a functional interaction between 5′ and 3′ RNA sequences could provide a means for translational control (37, 47). Hence, apart from its role in RNA maturation, p54 may play a role in the regulation of *psbA* expression.

Translational control by 5′ RNA-binding complexes

To date, the most comprehensively studied photoregulation of plastid gene expression is that of *psbA* in *Chlamydomonas reinhardtii*. Danon and Mayfield purified a multicomponent protein complex, which specifically binds to the 5′-noncoding region of *psbA* and consists of four subunits, RB60, RB55, RB47, and RB38 (24–26). Among these, RB47 was found to be the major RNA-binding protein. RB47 is highly homologous to eukaryotic poly(A)-binding proteins (cPABP), whereas RB60 belongs to a class of protein disulfide isomerases (cPDI) (48, 98). The binding activity of the complex was decreased in nuclear D1-synthesis mutants, supporting its function in *psbA* translation initiation *in vivo* (25, 99).

As with p54, this RNA-binding protein complex was shown to be regulated by phosphorylation and redox state *in vitro*, however, in the opposite way. Phosphorylation and/or oxidation inhibited its RNA binding activity. Interestingly, the serine/threonine kinase responsible for phosphorylation of RB60 within the complex was shown to utilize ADP, instead of ATP, as the phosphate donor. Furthermore, the abolished RNA binding activity of the oxidized complex could be most effectively restored by reduced thioredoxin (33, 94). The proposed model of regulation of D1 synthesis links the photosynthetic activity to the translation initiation via the ATP:ADP ratio and a ferredoxin–thioredoxin system (Fig. 5). Whereas high ADP levels in the dark inhibit *psbA* translation, high content of reducing equivalents generated in the light promote its translation. Results obtained by inhibition experiments of the photosynthetic electron transport demonstrate that indeed two pathways mediate light activation of D1 synthesis. The first, termed priming, probably initiates on reduction of the PQ pool and is required to allow the second, the thiol-mediated pathway, which is generated by PSI and transduced by thiol-containing proteins. Therefore, both the linear photosynthetic electron transport, through the ferredoxin–thioredoxin system, and the relative activities of PSI and PSII, via the redox state of the PQ pool, control *psbA* translation (93). Moreover, recent data show that RB60 (cPDI) possesses thioredoxin-like domains, which are capable of catalyzing the redox-regulated RNA binding activity of RB47 (cPABP). These results suggest that RB60 is the end point of the thiol-mediated pathway, directly modulating the binding of RB47 to the *psbA* 5′-UTR in response to the reducing potential generated by PSI (49).

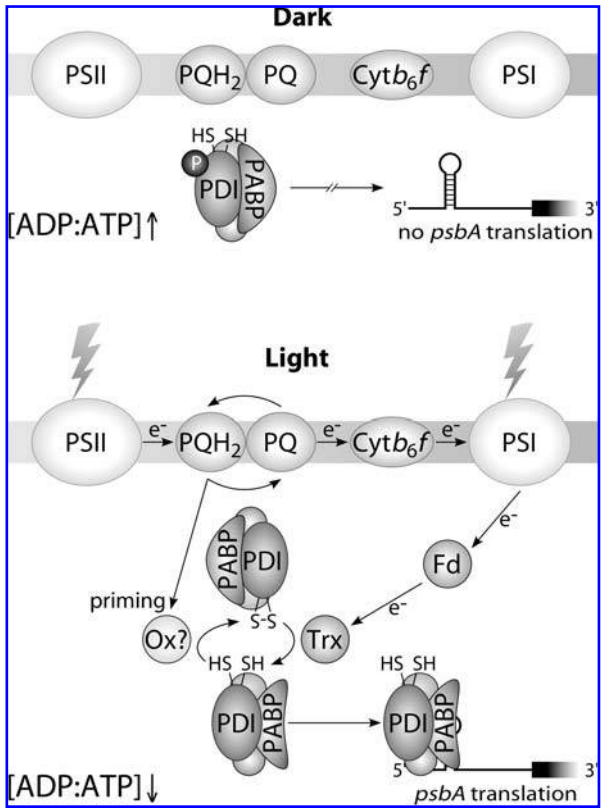

FIG. 5. Light-mediated redox control of chloroplast *psbA* translation in *Chlamydomonas*. In the dark (**upper panel**), PDI (RB60) is phosphorylated (P) by an ADP-dependent kinase, activated by an increased ADP:ATP ratio (173), and its oxidation inactivated, which results in an inactive RNA-binding complex (PDI, 60-kDa disulfide isomerase homologue; PABP, 47-kDa poly(A)-binding protein homologue; and two unknown proteins of 38 and 55 kDa). Light regulates *psbA* translation via two pathways: a "priming" signal starting from a reduced PQ pool (PQH_2), and a second redox-dependent pathway. Priming activates the RNA-binding complex by dephosphorylation and specific oxidation of PDI by an unknown component (Ox?). In the redox-dependent pathway, electrons (e^-) from the photosynthetic electron transport chain are transferred via ferredoxin (Fd) and thioredoxin (Trx) to reduce a vicinal dithiol (S-S) group of PDI. The thiol group (SH) signal is transmitted to PABP, resulting in an increased RNA-binding activity to the 5′-UTR of the *psbA* mRNA. Activation of RNA binding of the complex consequently leads to activation of *psbA* mRNA translation. Therefore, in the light, reducing and oxidizing pathways modulate the redox state of the PDI pool and thus the translation of *psbA* mRNA, in response to fluctuating light intensities.

Studies in *Arabidopsis thaliana* revealed an analogous redox regulation of an RNA-binding complex on the *psbA* 5′-UTR consisting of at least two proteins of 43 and 30 kDa (86). However, to date not much more is known about the mechanisms of redox regulation involved in control of *psbA* expression in *Arabidopsis*. One has to be careful to extrapolate the data derived from *Chlamydomonas* to higher plants. Unique mechanisms such as light-dependent chlorophyll biosynthesis, as well as tissue-specific gene expression, are likely to add to the complexity of regulation in multicellular higher plants. Therefore, additional factors and pathways that may be involved in all levels of plastid gene regulation need to be considered.

Interesting data were obtained recently in *Chlamydomonas* in an effort to examine the regulation of *rbcL*, the gene encoding the ribulose biphosphate carboxylase/oxygenase (RubisCO) large subunit (LSU) (100). A group of cpRBPs (81, 62, 51, and 47 kDa) specifically interact with the *rbcL* 5′-UTR *in vitro*. However, binding of these proteins was abolished under oxidizing conditions using the redox reagent GSSG, which resulted in a new 55-kDa protein interacting with the *rbcL* RNA. Western blot analysis of two-dimensional protein gels suggested that this protein might be the RubisCO LSU itself. Indeed, it was shown that the RubisCO LSU has an N-terminal RNA-binding domain, which enables the purified protein to bind RNA under oxidizing conditions. Structural analysis of RubisCO LSU suggested that the N-terminus is obscured within the holoenzyme and exposed under oxidizing conditions, thus mediating the RNA-binding capability. There are two possible physiological roles for the RNA-binding activity of RubisCO. Firstly, it could have a specific function in an autoregulatory pathway causing a translational arrest. Secondly, RNA binding by RubisCO could serve a broader function as an RNA chaperone protecting RNA from damages that occur during oxidative stress.

It is not immediately obvious why two such different pathways represented by glutathione and thioredoxin have evolved in the redox regulation of plastid gene expression. However, this may be reflected by their different redox potentials and their role in plant metabolism. Thioredoxin goes through subtle, but noticeable, light-dependent changes in its redox state, maybe serving as a fine-tuned modulator of plastid gene expression,whereas glutathione as an antioxidant protects the organism during periods of photoinhibition induced by oxidative stress under high light conditions. Therefore, it may play a more general role in regulating plastid (and nuclear) gene expression under extreme environmental conditions (18, 46, 61).

Redox regulation of plastid splicing?

A key sensor of the photosynthetic redox chemistry regulating plastid gene expression seems to be located within the electron transport chain in chloroplasts. Deshpande *et al*. (28) obtained interesting data on light regulation in another post-transcriptional RNA processing step. Splicing of the intron-containing *psbA* pre-mRNA in *Chlamydomonas* is accelerated in light as compared with the dark. Inhibitors of the electron transport (DCMU, DBMIB) abolished this effect. Moreover, photosynthesis-deficient *Chlamydomonas* mutants showed similar splicing rates under light and dark conditions. Restoration of photoautotrophic growth in one of the mutants resulted in regained light-accelerated splicing, providing evidence that indeed *psbA* splicing is under redox control defined by an intact photosynthetic pathway (28). Mutants of one of the *psbA* intron sequences showed the importance of efficient splicing for photosynthetic growth of *Chlamydomonas* (51). Yet it is not clear how the photosynthetic electron transport is stimulating the splicing rates. However, as the response to light is rapid (within minutes) (28), one may speculate that thioredoxin is involved in the regulation of this process. The PSI-linked thioredoxin system could modulate *psbA* splicing by altering the redox state of yet to be found splicing factors specific for this pre-mRNA.

PERSPECTIVES

Chloroplasts represent only one compartment of the plant cell. They are integrated into a complex redox signaling network to which redox signals from mitochondria, peroxisomes, and cytosolic processes also contribute. Multiple interactions between these compartments exist, and we are just beginning to understand the relationships in more detail (30, 34, 78, 96). Chloroplast redox signals are important retrograde signals toward the nucleus and couple the expression of nuclear genes for chloroplast proteins to the photosynthetic function (69). Our understanding of interaction with other signaling networks (*e.g.,* with sugar or photoreceptor signals) is beginning to emerge and will be improved in the next few years through the availability of a large collection of *Arabidopsis* signaling mutants (69). Whether chloroplast gene expression is affected, however, by anterograde redox signals is unknown to date. We also still need to know all regulatory components involved in chloroplast gene expression. The completed genome sequences of *Arabidopsis*, rice, and *Chlamydomonas* provide powerful tools for future research in this field. An assessment of the number of nuclear genes encoding products with putative N-terminal chloroplast transit peptides in the *Arabidopsis* genome resulted in an estimate of ~3,500 genes encoding products with chloroplast location (1). Biochemical and genetic approaches indicate that a considerable number of them code for regulatory proteins, such as eukaryotic transcription factors, RNA-binding proteins, and assembly factors (14). Most of them have been poorly investigated so far. Nevertheless, this supports the view that the chloroplast gene expression machinery is an assembly of phage, prokaryotic, and eukaryotic components that have been combined during evolution. The enormous progress in mass spectrometric techniques will help to identify such unknown factors in chloroplast protein preparations, although it will be a difficult task because such regulatory proteins may be expressed in substoichiometric amounts or only under specific conditions. Does this knowledge help us to understand redox regulation of gene expression? All mechanistic principles summarized in this review can be found also in other organisms. Therefore, we can use models obtained in other biological systems to prove and understand their function in chloroplasts. On the other hand, the unique regulatory network in chloroplasts with its phage, prokaryotic, and eukary-

otic origins promises to lead to the discovery of regulatory coherences that will be relevant for all other biological systems. Together with the genome-covering knockout line resources and the established plastid transformation techniques, chloroplasts represent a fascinating model system to understand redox-regulated gene expression networks in nature.

ACKNOWLEDGMENTS

Research in the authors' laboratories is supported by grants from the Deutsche Forschungsgemeinschaft (T.P., FOR 387; K.L., SFB 429).

ABBREVIATIONS

ATPase, ATP synthase; cpRBP, chloroplast RNA-binding protein; cytb_6f, cytochrome b_6f complex; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3′, 4′-dichlorophenyl)-1,1′-dimethyl urea; GSH, reduced form of glutathione; GSSG, oxidized form of glutathione; LHCII, light harvesting complex of photosystem II; LSU, large subunit; NEP, nucleus-encoded RNA polymerase; PABP, poly-(A)-binding protein; PDI, protein disulfide isomerase; PEF, photosynthetic electron flow; PEP, plastid-encoded polymerase; PQ, oxidized plastoquinone; PQH_2, reduced plastoquinone; PSI, photosystem I; PSII, photosystem II; PTK, plastid transcription kinase; ROS, reactive oxygen species; RubisCO, ribulose bisphosphate carboxylase/oxygenase; UTR, untranslated region.

REFERENCES

1. Abdallah F, Salamini F, and Leister D. A prediction of the size and evolutionary origin of the proteome of chloroplasts of *Arabidopsis*. *Trends Plant Sci* 5: 141–142, 2000.
2. Allen JF. Control of gene expression by redox potential and the requirement for chloroplast and mitochondrial genomes. *J Theor Biol* 165: 609–631, 1993.
3. Allen JF and Forsberg J. Molecular recognition in thylakoid structure and function. *Trends Plant Sci* 6: 317–326, 2001.
4. Allen JF and Pfannschmidt T. Balancing the two photosystems: photosynthetic electron transfer governs transcription of reaction centre genes in chloroplasts. *Philos Trans R Soc Lond B Biol Sci* 355: 1351–1359, 2000.
5. Allen JF and Race HL. Will the real LHC II kinase please step forward? *Sci STKE* 2002: PE43, 2002.
6. Allison LA. The role of sigma factors in plastid transcription. *Biochimie* 82: 537–548, 2000.
7. Andersson B and Aro EM. Photodamage and D1 protein turnover in photosystem II. In: *Regulation of Photosynthesis*, edited by Aro E-M and Andersson B. Dordrecht, Netherlands: Kluwer Academic Publisher, 2001, pp. 377–393.
8. Aro EM and Ohad I. Redox regulation of thylakoid protein phosphorylation. *Antioxid Redox Signal* 5: 55–67, 2003.
9. Baena-Gonzalez E, Baginsky S, Mulo P, Summer H, Aro EM, and Link G. Chloroplast transcription at different light intensities. Glutathione-mediated phosphorylation of the major RNA polymerase involved in redox-regulated organellar gene expression. *Plant Physiol* 127: 1044–1052, 2001.
10. Baginsky S and Gruissem W. Endonucleolytic activation directs dark-induced chloroplast mRNA degradation. *Nucleic Acids Res* 30: 4527–4533, 2002.
11. Baginsky S, Tiller K, and Link G. Transcription factor phosphorylation by a protein kinase associated with chloroplast RNA polymerase from mustard (*Sinapis alba*). *Plant Mol Biol* 34: 181–189, 1997.
12. Baginsky S, Tiller K, Pfannschmidt T, and Link G. 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, 1999.
13. Baier M and Dietz KJ. The costs and benefits of oxygen for photosynthesizing plant cells. *Prog Bot* 60: 283–314, 1998.
14. Barkan A and Goldschmidt-Clermont M. Participation of nuclear genes in chloroplast gene expression. *Biochimie* 82: 559–572, 2000.
15. Bergmann L and Rennenberg H. Glutathione metabolism in plants. In: *Sulfur Nutrition and Assimilation in Higher Plants*, edited by DeKok LJ, Stulen I, Rennenberg H, Brunold C, and Rauser WE. The Hague: SPB Academic Publisher, 1993, pp. 109–124.
16. Blankenship RE. *Molecular Mechanisms of Photosynthesis.* Oxford: Blackwell Science, 2002.
17. Bollenbach TJ and Stern DB. Divalent metal-dependent catalysis and cleavage specificity of CSP41, a chloroplast endoribonuclease belonging to the short chain dehydrogenase/reductase superfamily. *Nucleic Acids Res* 31: 4317–4325, 2003.
18. Bowler C and Chua NH. Emerging themes of plant signal transduction. *Plant Cell* 6: 1529–1541, 1994.
19. Bruick RK and Mayfield SP. Light-activated translation of chloroplast mRNAs. *Trends Plant Sci* 4: 190–195, 1999.
20. Buchanan BB, Schürmann P, and Jacquot JP. Thioredoxin and metabolic regulation. *Semin Cell Biol* 5: 285–293, 1994.
21. Cahoon AB and Stern DB. Plastid transcription: a ménage à trois? *Trends Plant Sci* 6: 45–46, 2001.
22. Carlberg I, Hansson M, Kieselbach T, Schroder WP, Andersson B, and Vener AV. A novel plant protein undergoing light-induced phosphorylation and release from the photosynthetic thylakoid membranes. *Proc Natl Acad Sci U S A* 100: 757–762, 2003.
23. Cheng MC, Wu SP, Chen LF, and Chen SC. Identification and purification of a spinach chloroplast DNA-binding protein that interacts specifically with the plastid psaA-psaB-rps14 promoter region. *Planta* 203: 373–380, 1997.
24. Danon A and Mayfield SP. Light regulated translational activators: identification of chloroplast gene specific mRNA binding proteins. *EMBO J* 10: 3993–4001, 1991.
25. Danon A and Mayfield SP. Light-regulated translation of chloroplast messenger-RNAs through redox potential. *Science* 266: 1717–1719, 1994.

26. Danon A and Mayfield SP. ADP-dependent phosphorylation regulates RNA-binding in vitro—implications in light-modulated translation. *EMBO J* 13: 2227–2235, 1994.
27. Depege N, Bellafiore S, and Rochaix JD. Role of chloroplast protein kinase Stt7 in LHCII phosphorylation and state transition in *Chlamydomonas. Science* 299: 1572–1575, 2003.
28. Deshpande NN, Bao YJ, and Herrin DL. Evidence for light/redox-regulated splicing of psbA pre-RNAs in *Chlamydomonas* chloroplasts. *RNA* 3: 37–48, 1997.
29. Desikan R, Mackerness SAH, Hancock JT, and Neill SJ. Regulation of the *Arabidopsis* transcriptome by oxidative stress. *Plant Physiol* 127: 159–172, 2001.
30. Dietz KJ. Redox control, redox signalling, and redox homeostasis in plant cells. *Int Rev Cytol* 228: 141–193, 2003.
31. Dietz KJ. Plant peroxiredoxins. *Annu Rev Plant Biol* 54: 93–107, 2003.
32. El Bissati K and Kirilovsky D. Regulation of psbA and psaE expression by light quality in *Synechocystis* species PCC 6803. A redox control mechanism. *Plant Physiol* 125: 1988–2000, 2001.
33. Fong CL, Lentz A, and Mayfield SP. Disulfide bond formation between RNA binding domains is used to regulate mRNA binding activity of the chloroplast poly(A)-binding protein. *J Biol Chem* 275: 8275–8278, 2000.
34. Foyer CH and Noctor G. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol Plant* 119: 355–364, 2003.
35. Goldschmidt-Clermont M. Coordination of nuclear and chloroplast gene expression in plant cells. *Int Rev Cytol* 177: 115–180, 1998.
36. Gray JC, Sullivan JA, Wang JH, Jerome CA, and MacLean D. Coordination of plastid and nuclear gene expression. *Philos Trans R Soc Lond B Biol Sci* 358: 135–144, 2003.
37. Gunkel N, Yano T, Markussen F-H, Olsen LC, and Ephrussi A. Localization-dependent translation requires a functional interaction between the 5′ and 3′ ends of *oskar* mRNA. *Genes Dev* 12: 1652–1664, 1998.
38. Haldrup A, Jensen PE, Lunde C, and Scheller HV. Balance of power: a view of the mechanism of photosynthetic state transitions. *Trends Plant Sci* 6: 301–305, 2001.
39. Hayes R, Kudla J and Gruissem W. Degrading chloroplast mRNA: the role of polyadenylation. *Trends Biochem Sci* 24: 199–202, 1999.
40. Herrmann RG, Westhoff P, and Link G. Biogenesis of plastids in higher plants. In: *Cell Organelles*, edited by Herrmann RG. Wien, Austria: Springer Verlag, 1992, pp. 275–349.
41. Hess WR and Borner T. Organellar RNA polymerases of higher plants. *Int Rev Cytol* 190: 1–59, 1999.
42. Horlitz M and Klaff P. Gene-specific trans-regulatory functions of magnesium for chloroplast mRNA stability in higher plants. *J Biol Chem* 275: 35638–35645, 2000.
43. Igloi GL and Kössel H. The transcriptional apparatus of chloroplasts. *Crit Rev Plant Sci* 10: 525–558, 1992.
44. Jarvis P and Soll J. Toc, Tic, and chloroplast protein import. *Biochim Biophys Acta* 1590: 177–189, 2002.
45. Kanekatsu M, Ezumi A, Nakamura T, and Ohtsuki K. Chloroplast ribonucleoproteins (RNPs) as phosphate acceptors for casein kinase II: purification by ssDNA-cellulose column chromatography. *Plant Cell Physiol* 36: 1649–1656, 1995.
46. Karpinski S, Escobar C, Karpinska B, Creissen G, and Mullineaux PM. Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in *Arabidopsis* during excess light stress. *Plant Cell* 9: 627–640, 1997.
47. Katz YS and Danon A. The 3′-untranslated region of chloroplast psbA mRNA stabilizes binding of regulatory proteins to the leader of the message. *J Biol Chem* 277: 18665–18669, 2002.
48. Kim JM and Mayfield SP. Protein disulfide isomerase as a regulator of chloroplast translational activation. *Science* 278: 1954–1957, 1997.
49. Kim J and Mayfield SP. The active site of the thioredoxin-like domain of chloroplast protein disulfide isomerase, RB60, catalyzes the redox-regulated binding of chloroplast poly(A)-binding protein, RB47, to the 5′ untranslated region of psbA mRNA. *Plant Cell Physiol* 43: 1238–1243, 2002.
50. Kovacs L, Wiessner W, Kis M, Nagy F, Mende D, and Demeter S. 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, 2000.
51. Lee J and Herrin D. Mutagenesis of a light-regulated psbA intron reveals the importance of efficient splicing for photosynthetic growth. *Nucleic Acids Res* 31: 4361–4372, 2003.
52. Li H and Sherman LA. A redox-responsive regulator of photosynthesis gene expression in the cyanobacterium *Synechocystis* sp strain PCC 6803. *J Bacteriol* 182: 4268–4277, 2000.
53. Liere K and Link G. Chloroplast endoribonuclease p54 involved in RNA 3′-end processing is regulated by phosphorylation and redox state. *Nucleic Acids Res* 25: 2403–2408, 1997.
54. Liere K and Maliga P. In vitro characterization of the tobacco rpoB promoter reveals a core sequence motif conserved between phage-type plastid and plant mitochondrial promoters. *EMBO J* 18: 249–257, 1999.
55. Liere K and Maliga P. Plastid RNA polymerases in higher plants. In: *Regulation of Photosynthesis*, edited by Aro EM and Andersson B. Dordrecht, Netherlands: Kluwer Academic Publishers, 2001, pp. 29–49.
56. Liere K, Nickelsen J, and Link G. Chloroplast p54 endoribonuclease. In: *Ribonucleases, Part B*, edited by Nicholson AW. New York, NY: Academic Press, 2001, pp. 420–428.
57. Link G. Green life: control of chloroplast gene transcription. *Bioessays* 18: 465–471, 1996.
58. Link G. Redox regulation of photosynthetic genes. In: *Regulation of Photosynthesis*, edited by Aro EM and An-

dersson B. Dordrecht, Netherlands: Kluwer Academic Publishers, 2001, pp. 85–107.
59. Link G. Redox regulation of chloroplast transcription. *Antioxid Redox Signal* 5: 79–87, 2003.
60. Lisitsky I and Schuster G. Phosphorylation of a chloroplast RNA-binding protein changes its affinity to RNA. *Nucleic Acids Res* 23: 2506–2511, 1995.
61. Martin W, Rujan T, Richly E, Hansen A, Cornelsen S, Lins T, Leister D, Stoebe B, Hasegawa M, and Penny D. 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, 2002.
62. Monde RA, Zito F, Olive J, Wollman FA, and Stern DB. Post-transcriptional defects in tobacco chloroplast mutants lacking the cytochrome b6/f complex. *Plant J* 21: 61–72, 2000.
63. Nickelsen J. Chloroplast RNA-binding proteins. *Curr Genet* 43: 392–399, 2003.
64. Nickelsen J and Link G. Interaction of a 3′ RNA region of the mustard trnK gene with chloroplast proteins. *Nucleic Acids Res* 17: 9637–9648, 1989.
65. Nickelsen J and Link G. RNA–protein interactions at transcript 3′ ends and evidence for trnK-psbA cotranscription in mustard chloroplasts. *Mol Gen Genet* 228: 89–96, 1991.
66. Nickelsen J and Link G. The 54 kDa RNA-binding protein from mustard chloroplasts mediates endonucleolytic transcript 3′ end formation in vitro. *Plant J* 3: 537–544, 1993.
67. Ogrzewalla K, Piotrowski M, Reinbothe S, and Link G. The plastid transcription kinase from mustard (*Sinapis alba* L.)—a nuclear-encoded CK2-type chloroplast enzyme with redox-sensitive function. *Eur J Biochem* 269: 3329–3337, 2002.
68. Pearson CK, Wilson SB, Schaffer R, and Ross AW. 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, 1993.
69. Pfannschmidt T. Chloroplast redox signals: how photosynthesis controls its own genes. *Trends Plant Sci* 8: 33–41, 2003.
70. Pfannschmidt T and Link G. Separation of two classes of plastid DNA-dependent RNA polymerases that are differentially expressed in mustard (*Sinapis alba* L.) seedlings. *Plant Mol Biol* 25: 69–81, 1994.
71. Pfannschmidt T, Nilsson A, and Allen JF. Photosynthetic control of chloroplast gene expression. *Nature* 397: 625–628, 1999.
72. Pfannschmidt T, Nilsson A, Tullberg A, Link G, and Allen JF. Direct transcriptional control of the chloroplast genes psbA and psaAB adjusts photosynthesis to light energy distribution in plants. *IUBMB Life* 48: 271–276, 1999.
73. Pfannschmidt T, Ogrzewalla K, Baginsky S, Sickmann A, Meyer HE, and Link G. The multisubunit chloroplast RNA polymerase A from mustard (*Sinapis alba* L.)—integration of a prokaryotic core into a larger complex with organelle-specific functions. *Eur J Biochem* 267: 253–261, 2000.
74. Pfannschmidt T, Allen JF, and Oelmuller R. Principles of redox control in photosynthesis gene expression. *Physiol Plant* 112: 1–9, 2001.
75. Polverari A, Molesini B, Pezzotti M, Buonaurio R, Marte M, and Delledonne M. Nitric oxide-mediated transcriptional changes in *Arabidopsis thaliana. Mol Plant Microbe Interact* 16: 1094–1105, 2003.
76. Pursiheimo S, Mulo P, Rintamaki E, and Aro EM. Coregulation of light-harvesting complex II phosphorylation and *lhcb* mRNA accumulation in winter rye. *Plant J* 26: 317–327, 2001.
77. Race HL, Herrmann RG, and Martin W. Why have organelles retained genomes? *Trends Genet* 15: 364–370, 1999.
78. Raghavendra AS, Padmasree K, and Saradadevi K. Interdependence of photosynthesis and respiration in plant cells: interaction between chloroplasts and mitochondria. *Plant Sci* 97: 1–14, 1994.
79. Reymond P, Weber H, Damond M, and Farmer EE. Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis. Plant Cell* 12: 707–720, 2000.
80. Rochaix JD. Post-transcriptional regulation of chloroplast gene expression in *Chlamydomonas reinhardtii. Plant Mol Biol* 32: 327–341, 1996.
81. Rodermel S. Pathways of plastid-to-nucleus signaling. *Trends Plant Sci* 6: 471–478, 2001.
82. Salvador ML and Klein U. The redox state regulates RNA degradation in the chloroplast of *Chlamydomonas reinhardtii. Plant Physiol* 121: 1367–1374, 1999.
83. Scheibe R. Redox-modulation of chloroplast enzymes. A common principle for individual control. *Plant Physiol* 96: 1–3, 1991.
84. Schurmann P. Redox signaling in the chloroplast: the ferredoxin/thioredoxin system. *Antioxid Redox Signal* 5: 69–78, 2003.
85. Schurmann P and Jacquot JP. Plant thioredoxin systems revisited. *Annu Rev Plant Physiol Plant Mol Biol* 51: 371–400, 2000.
86. Shen YX, Danon A, and Christopher DA. 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, 2001.
87. Snyders S and Kohorn BD. Disruption of thylakoid-associated kinase 1 leads to alteration of light harvesting in *Arabidopsis*. *J Biol Chem* 276: 32169–32176, 2001.
88. Stern D, Higgs D, and Yang J. Transcription and translation in chloroplasts. *Trends Plant Sci* 2: 308–315, 1997.
89. Sugiura M. The chloroplast genome. *Plant Mol Biol* 19: 149–168, 1992.
90. Surpin M and Chory J. The co-ordination of nuclear and organellar genome expression in eukaryotic cells. *Essays Biochem* 32: 113–125, 1997.
91. Surpin M, Larkin RM, and Chory J. Signal transduction between the chloroplast and the nucleus. *Plant Cell* 14: S327–S338, 2002.
92. Tiller K and Link G. Phosphorylation and dephosphorylation affect functional characteristics of chloroplast and

etioplast transcription systems from mustard (*Sinapis alba* L.). *EMBO J* 12: 1745–1753, 1993.
93. Trebitsh T and Danon A. 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, 2001.
94. Trebitsh T, Levitan A, Sofer A, and Danon A. Translation of chloroplast psbA mRNA is modulated in the light by counteracting oxidizing and reducing activities. *Mol Cell Biol* 20: 1116–1123, 2000.
95. Tullberg A, Alexciev K, Pfannschmidt T, and Allen JF. Photosynthetic electron flow regulates transcription of the psaB gene in pea (*Pisum sativum* L.) chloroplasts through the redox state of the plastoquinone pool. *Plant Cell Physiol* 41: 1045–1054, 2000.
96. van Lis R and Atteia A. Control of mitochondrial function via photosynthetic redox signals. *Photosynth Res* 79: 133–148, 2004.
97. Vener AV, van Kan PJ, Rich PR, Ohad II, and Andersson B. Plastoquinol at the quinol oxidation site of reduced cytochrome *bf* mediates signal transduction between light and protein phosphorylation: thylakoid protein kinase deactivation by a single-turnover flash. *Proc Natl Acad Sci U S A* 94: 1585–1590, 1997.
98. Yohn CB, Cohen A, Danon A, and Mayfield SP. 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, 1998.
99. Yohn CB, Cohen A, Rosch C, Kuchka MR, and Mayfield SP. Translation of the chloroplast psbA mRNA requires the nuclear-encoded poly(A)-binding protein, RB47. *J Cell Biol* 142: 435–442, 1998.
100. Yosef I, Irihimovitch V, Knopf JA, Cohen I, Orr-Dahan I, Nahum E, Keasar C, and Shapira M. RNA binding activity of the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit from *Chlamydomonas reinhardtii*. *J Biol Chem* 279: 10148–10156, 2004.
101. Zer H and Ohad I. Light, redox state, thylakoid-protein phosphorylation and signaling gene expression. *Trends Biochem Sci* 28: 467–470, 2003.
102. Zito F, Finazzi G, Delosme R, Nitschke W, Picot D, and Wollman F-A. The Qo site of cytochrome b_6f complexes controls the activation of the LHCII kinase. *EMBO J* 18: 2961–2969, 1999.

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Received for publication November 23, 2004; accepted November 30, 2004.

This article has been cited by:

1. Juan de Dios Barajas-López, Nicolás E. Blanco, Åsa Strand. 2012. Plastid-to-nucleus communication, signals controlling the running of the plant cell. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* . [CrossRef]
2. Éva Kiss, Péter B. Kós, Min Chen, Imre Vass. 2012. A unique regulation of the expression of the psbA, psbD, and psbE genes, encoding the D1, D2 and cytochrome b559 subunits of the Photosystem II complex in the chlorophyll d containing cyanobacterium Acaryochloris marina. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* . [CrossRef]
3. C. H. Foyer, J. Neukermans, G. Queval, G. Noctor, J. Harbinson. 2012. Photosynthetic control of electron transport and the regulation of gene expression. *Journal of Experimental Botany* **63**:4, 1637-1661. [CrossRef]
4. Peter Kindgren, Dmitry Kremnev, Nicolás E. Blanco, Juan de Dios Barajas López, Aurora Piñas Fernández, Christian Tellgren-Roth, Ian Small, Åsa Strand. 2011. The plastid redox insensitive 2 mutant of Arabidopsis is impaired in PEP activity and high light-dependent plastid redox signalling to the nucleus. *The Plant Journal* no-no. [CrossRef]
5. Karsten Liere, Andreas Weihe, Thomas Börner. 2011. The transcription machineries of plant mitochondria and chloroplasts: Composition, function, and regulation. *Journal of Plant Physiology* **168**:12, 1345-1360. [CrossRef]
6. J. Qiao, C. Ma, M. Wimmelbacher, F. Bornke, M. Luo. 2011. Two Novel Proteins, MRL7 and Its Paralog MRL7-L, Have Essential but Functionally Distinct Roles in Chloroplast Development and Are Involved in Plastid Gene Expression Regulation in Arabidopsis. *Plant and Cell Physiology* **52**:6, 1017-1030. [CrossRef]
7. Arthur R. Grossman, Claudia Catalanotti, Wenqiang Yang, Alexandra Dubini, Leonardo Magneschi, Venkataramanan Subramanian, Matthew C. Posewitz, Michael Seibert. 2011. Multiple facets of anoxic metabolism and hydrogen production in the unicellular green alga Chlamydomonas reinhardtii. *New Phytologist* **190**:2, 279-288. [CrossRef]
8. Yvonne Schröter, Sebastian Steiner, Kevin Matthäi, Thomas Pfannschmidt. 2010. Analysis of oligomeric protein complexes in the chloroplast sub-proteome of nucleic acid-binding proteins from mustard reveals potential redox regulators of plastid gene expression. *PROTEOMICS* **10**:11, 2191-2204. [CrossRef]
9. Éva Kiss, Péter B. Kós, Imre Vass. 2009. Transcriptional regulation of the bidirectional hydrogenase in the cyanobacterium Synechocystis 6803. *Journal of Biotechnology* **142**:1, 31-37. [CrossRef]
10. Ning Shao, Christoph F. Beck, Stéphane D. Lemaire, Anja Krieger-Liszkay. 2008. Photosynthetic electron flow affects H2O2 signaling by inactivation of catalase in Chlamydomonas reinhardtii. *Planta* **228**:6, 1055-1066. [CrossRef]
11. H SHAO, L CHU, M SHAO, C JALEEL, M HONGMEI. 2008. Higher plant antioxidants and redox signaling under environmental stresses. *Comptes Rendus Biologies* **331**:6, 433-441. [CrossRef]
12. T. Pfannschmidt, K. Brautigam, R. Wagner, L. Dietzel, Y. Schroter, S. Steiner, A. Nykytenko. 2008. Potential regulation of gene expression in photosynthetic cells by redox and energy state: approaches towards better understanding. *Annals of Botany* **103**:4, 599-607. [CrossRef]
13. D.-P. Lu, D. A. Christopher. 2008. The effect of irradiance and redox-modifying reagents on the 52 kDa protein disulfide isomerase of Arabidopsis chloroplasts. *Biologia Plantarum* **52**:1, 42-48. [CrossRef]
14. F MUH, T RENGER, A ZOUNI. 2008. Crystal structure of cyanobacterial photosystem II at 3.0 Å resolution: A closer look at the antenna system and the small membrane-intrinsic subunits. *Plant Physiology and Biochemistry* **46**:3, 238-264. [CrossRef]
15. A. Bruce Cahoon, Elizabeth M. Takacs, Richard M. Sharpe, David B. Stern. 2008. Nuclear, chloroplast, and mitochondrial transcript abundance along a maize leaf developmental gradient. *Plant Molecular Biology* **66**:1-2, 33-46. [CrossRef]
16. N. P. Yurina, M. S. Odintsova. 2007. Plant signaling systems. Plastid-generated signals and their role in nuclear gene expression. *Russian Journal of Plant Physiology* **54**:4, 427-438. [CrossRef]
17. Monika Swiatecka-Hagenbruch, Karsten Liere, Thomas Börner. 2007. High diversity of plastidial promoters in Arabidopsis thaliana. *Molecular Genetics and Genomics* **277**:6, 725-734. [CrossRef]
18. Barry A. Logan, Dmytro Kornyeyev, Justin Hardison, A. Scott Holaday. 2006. The role of antioxidant enzymes in photoprotection. *Photosynthesis Research* **88**:2, 119-132. [CrossRef]
19. Mario Giordano, Yi-Bu Chen, Michal Koblizek, Paul G. Falkowski. 2005. Regulation of nitrate reductase in Chlamydomonas reinhardtii by the redox state of the plastoquinone pool. *European Journal of Phycology* **40**:4, 345-352. [CrossRef]
20. Kenneth Hensley , Robert A. Floyd . 2005. Oxidative Modification of Proteins in Cell Signaling. *Antioxidants & Redox Signaling* **7**:5-6, 523-525. [Citation] [Full Text PDF] [Full Text PDF with Links]