Plastid-to-Nucleus Retrograde Signaling

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Annu. Rev. Plant Biol.
2006. 57:739–59

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

doi: 10.1146/annurev.arplant.57.032905.105310

First published online as a Review in Advance on February 7, 2006

1543-5008/06/0602-0739$20.00

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Key Words

Mg-ProtoporphyrinIX, redox signaling, *gun* mutants

Abstract

Plant cells store genetic information in the genomes of three organelles: the nucleus, plastid, and mitochondrion. The nucleus controls most aspects of organelle gene expression, development, and function. In return, organelles send signals to the nucleus to control nuclear gene expression, a process called retrograde signaling. This review summarizes our current understanding of plastid-to-nucleus retrograde signaling, which involves multiple, partially redundant signaling pathways. The best studied is a pathway that is triggered by buildup of Mg-ProtoporphyrinIX, the first intermediate in the chlorophyll branch of the tetrapyrrole biosynthetic pathway. In addition, there is evidence for a plastid gene expression-dependent pathway, as well as a third pathway that is dependent on the redox state of photosynthetic electron transport components. Although genetic studies have identified several players involved in signal generation, very little is known of the signaling components or transcription factors that regulate the expression of hundreds of nuclear genes.

Contents

INTRODUCTION

It is generally believed that plastids evolved from the endosymbiosis of a unicellular free-living photosynthetic bacterium by an ancient eukaryotic cell. The ancestral prokaryotic genome presumably contained all the information necessary to support an independent photoautotrophic lifestyle. However, over evolutionary time, subsequent to the endosymbiotic relationship that gave rise to green algae, and later to higher plants, the plastid genome has undergone progressive and drastic reduction in coding capacity. Several genes not necessary for the endosymbiotic existence may have been lost and a vast majority was transferred to the nuclear genome of the host. The present-day plastid genome of higher plants encodes fewer than 100 open reading frames, whereas the rest of the >3000 polypeptides found in the chloroplast, the differentiated photosynthetically active plastid, are transcribed from nuclear genes and imported post-translationally (1, 46). This division of labor presents a challenge to plant cells because essential photosynthetic and metabolic complexes, which are located in plastids, are composed of subunits encoded by both genomes. Such an arrangement necessitates tightly coordinated communication between plastids and the nucleus to ensure coregulated expression of genes whose products function together.

Plastid development and gene expression are largely under nuclear control (22, 47). The nucleus encodes most of the genes required for chloroplast gene expression, e.g., components of the transcription and translation machinery, as well as components of the protein import apparatus. In addition to such "anterograde" control, "retrograde" control mechanisms have evolved by which chloroplast functional and developmental states can regulate expression of nuclear genes encoding plastid-localized proteins.

Control of nuclear gene expression in response to the developmental and functional state of plastids is critical for establishing the photoautotrophic lifestyle, and for efficiently allocating available resources under conditions of reduced organelle metabolic function. Later in development, retrograde signaling may be involved in fine-tuning nuclear gene expression in response to changes in chlorophyll biosynthetic flux, or for modulating nuclear gene expression in response to changes in photosynthetic flux or when chloroplasts are damaged in high light or by pathogens. Thus, intracellular communication between the organelles establishes the proper balance of gene expression in a changing environment.

Over 20 years ago, it was proposed that a plastid-generated factor could trigger such a retrograde signaling pathway. It is now clear, from both genetic and biochemical studies in a number of organisms, that there are several pathways of communication between chloroplasts and the nucleus. In this review we present a comprehensive discussion of our current understanding of chloroplast-to-nucleus signaling and provide a perspective for future work aimed at a complete understanding of the pathways.

HISTORICAL OVERVIEW

The first evidence for the existence of plastid signals controlling nuclear gene expression came from studies on the *albostrians* and *Saskatoon* mutants of barley (*Hordeum vulgare* L. cv. Haisa) (8). The recessive nuclear mutations in *albostrians* and *Saskatoon* prevent the accumulation of carotenoids, resulting in seedlings with either completely white leaves or white stripes (16). White areas in *albostrians* leaves contain undifferentiated small plastids that lack ribosomes, have only trace amounts of chlorophyll and as a result are photosynthetically inactive. Although the *albostrians* phenotype is caused by a recessive nuclear allele, the undifferentiated plastids are maternally inherited so mutant lines can contain green or white plastids. Activity of two plastid-localized nuclear-encoded enzymes is significantly reduced in white sectors of the *albostrians* leaves (8), and expression of several nuclear genes encoding chloroplast-localized proteins, including the *Lhc* gene family (Light-harvesting chlorophyll *a/b*-binding protein), the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RbcS), and Calvin cycle enzymes, is downregulated (26). Reduced levels of nuclear-encoded photosynthetic transcripts are not a result of phytochrome impairment, suggesting that a signal from developed plastids is required for the light induction of these genes (27).

Subsequent studies using mutant plants impaired in carotenoid biosynthesis, which causes photobleaching in plastids, have demonstrated that the expression of several nuclear-encoded photosynthetic genes is dramatically reduced in the absence of functional chloroplasts (51, 62, 63, 86). Similar observations have been made by inducing carotenoid deficiency by growing seedlings in the presence of norflurazon (NF), an inhibitor of the enzyme phytoene desaturase in the carotenoid biosynthesis pathway (62, 76).

In addition to photobleaching, arresting chloroplast development by inhibiting plastid gene expression (PGE) also leads to inhibition of nuclear photosynthetic gene expression. When mustard (*Sinapis alba* L.) seedlings are treated with chloramphenicol (CF), chloroplast development is inhibited, and so is the activity of nuclear-encoded chloroplast-localized enzymes (62). Activity of phytochrome-induced cytoplasmic enzymes like chalcone synthase is not affected and in some cases is even increased by the CF treatment (62). In pea (*Pisum sativum* L.), CF could inhibit both red- and blue-light-induced expression of a gene encoding for an Early Light-Induced Protein (ELIP) (3). Other inhibitors of chloroplast gene expression also inhibit expression of nuclear genes. These include tagetitoxin (75), lincomycin (an inhibitor of chloroplast protein translation), and nalidixic acid (an inhibitor of DNA

NF: norflurazon

PGE: plastid gene expression

CF: chloramphenicol

PET: photosynthetic electron transport

Mg-ProtoIX: Mg-ProtoporphyrinIX

Mg-ProtoIXme: Mg-ProtoporphyrinIX methyl ester

DP: dipyridyl

replication and transcription in the chloroplast) (23). A specific role for chloroplast gene expression in the generation of a plastid signal was demonstrated in pea using erythromycin applications, which specifically inhibit plastid protein translation but not mitochondrial protein translation (84).

Regulation of nuclear gene expression by the redox state of photosynthetic electron transport (PET) components in chloroplasts is also a proposed retrograde signaling pathway (17). Since the original observations in the unicellular green alga *Dunaliella tertiolecta* more than 10 years ago, several approaches have been taken to manipulate the chloroplast redox state in different species, including higher plants, to determine the effects of chloroplast redox states on nuclear gene expression. From these efforts, it has been shown that several nuclear-encoded photosynthesis-related and stress-responsive genes are regulated by chloroplast redox states (35, 49, 72).

Given the number of recent reviews written in the area of plastid-to-nucleus signaling, there is considerable interest in these signaling pathways, yet very little is actually known. Recent genetic and biochemical studies, in the unicellular green alga *Chlamydomonas reinhardtii* and the reference plant *Arabidopsis thaliana*, have identified one signal generated by photobleached plastids. Yet, signal transduction mechanisms are still unknown, and no mutants in signaling components have been reported. Identification of cytosolic and/or nuclear factors involved in these pathways is crucial to understand the signaling mechanisms and to determine possible interactions between the various proposed pathways.

CHLOROPHYLL BIOSYNTHETIC PRECURSORS IN RETROGRADE SIGNALING

One presumably important function of retrograde signaling is to coordinate the biosynthesis of chlorophyll with expression of nuclear-encoded chlorophyll-binding proteins (e.g., LHCA and LHCB proteins). It is not surprising then that considerable evidence over the years has implicated the chlorophyll biosynthetic precursors, Mg-ProtoporphyrinIX (Mg-ProtoIX) and Mg-ProtoporphyrinIX-methylesters (Mg-ProtoIXme), as regulators of nuclear gene expression (42, 83).

Chlorophyll Biosynthetic Precursors Regulate Nuclear Gene Expression in *C. reinhardtii*

The first evidence for the involvement of chlorophyll biosynthetic precursors in retrograde signaling came from work in *C. reinhardtii* (32, 33). In light-dark synchronized cultures of *C. reinhardtii*, *Lhcb* mRNA begins to accumulate about two hours after the transition to light, primarily due to transcriptional activation (31). Adding inhibitors of several early steps in chlorophyll biosynthesis to *C. reinhardtii* cultures does not inhibit *Lhcb* expression (**Figure 1**) (31–33). In contrast, treating algal cultures with dipyridyl (DP), which inhibits the conversion of ProtoIX to heme and also the conversion of Mg-ProtoIX to Protochlorophyllide (**Figure 1**), resulting in accumulation of Mg-ProtoIX and Mg-ProtoIXme (13), prevents *Lhcb* mRNA accumulation (33). *C. reinhardtii* cultures grown under anaerobic conditions also accumulate Mg-ProtoIXme (13) and prevent light induction of *Lhcb* and *RbcS* expression (32). These results strongly suggest that accumulation of porphyrin intermediates between ProtoIX and Mg-ProtoIXme is necessary for *Lhcb* repression. The *C. reinhardtii* mutant *brs-1*, defective in the H-subunit of Mg-chelatase (11), and consequently in the conversion of ProtoIX to Mg-ProtoIX, is impaired in light-induced *Lhcb* expression (33), suggesting that ProtoIX accumulation can also repress *Lhcb* transcription.

In *C. reinhardtii*, there is also evidence that the induction of HSP70 genes requires Mg-ProtoIX and Mg-ProtoIXme. The heat-shock

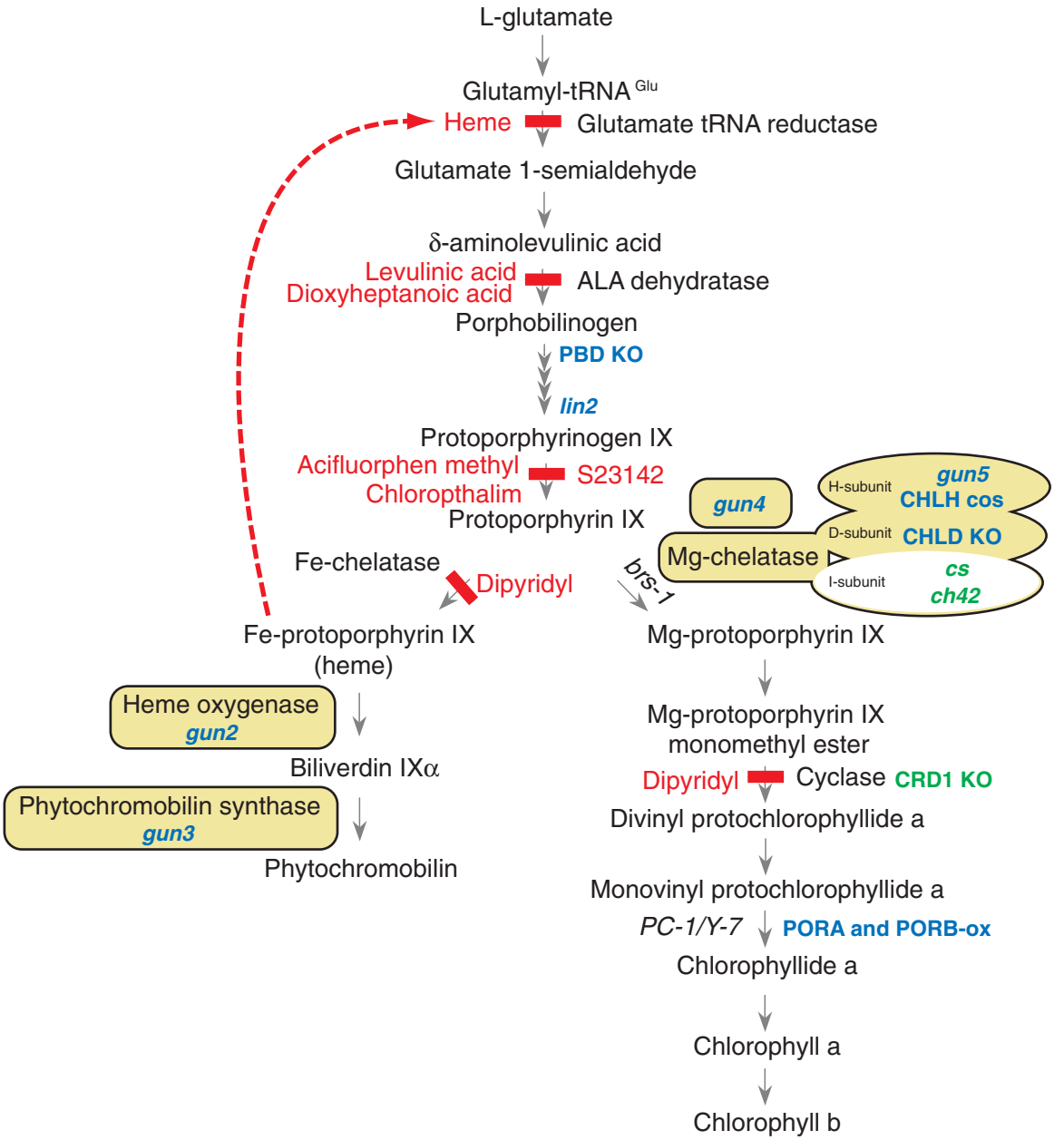

Figure 1

Tetrapyrrole biosynthetic pathway. Steps inhibited by specific inhibitors are indicated in red. Mutants with a *gun* phenotype are shown in blue, and mutants that do not show a *gun* phenotype are indicated in green. *brs-1* and *PC-1/Y-7* are *C. reinhardtii* mutants. PBD KO, T-DNA knockout of porphobilinogen deaminase; *lin2*, lesion in coproporphyrinogen oxidase; CHLD KO, T-DNA knockout of D-subunit of Mg-Chelatase; CHLH cos, cosuppression lines for H-subunit of Mg-Chelatase; CRD KO, T-DNA knockout of one subunit of the cyclase complex. *cs* and *ch42* are alleles of the I-subunit of Mg-Chelatase. PORA-ox and PORB-ox indicate overexpression of Protochlorophyllide oxidoreductase A and B, respectively.

genes *HSP70A* and *HSP70B*, encoding cytosolic and plastid-localized proteins respectively, can be induced by light via a pathway that is independent of heat-shock induction. Light induction of *HSP70* is impaired in the *brs-1* mutant (41, 42); however, *HSP70* light induction is normal in another chlorophyll biosynthesis mutant *PC-1/Y-7*, deficient in converting protochlorophyllide to chlorophyllide. These observations suggest that light induction of *HSP70* requires accumulation of Mg-ProtoIX (41). Indeed, addition of Mg-ProtoIX in the dark is sufficient to induce expression of *HSP70* genes (41). The effect is specific to Mg-ProtoIX because addition of ProtoIX, protochlorophyllide, or chlorophyllide is unable to substitute for light in *HSP70* induction (41). Interestingly, exogenously added ProtoIX is converted to Mg-ProtoIX and Mg-ProtoIXme, presumably within the chloroplast, but is still unable to elicit induction of *HSP70* in the dark (41). One possible explanation is that the Mg-ProtoIX made from exogenously added ProtoIX is sequestered within the chloroplasts, and is dependent on light for its release, whereas exogenously added Mg-ProtoIX has direct access to cytosolic and presumably the nuclear compartments to elicit the effect. This suggests that Mg-ProtoIX can function outside the chloroplast in this pathway and that light plays an additional role, perhaps in allowing plastidic Mg-ProtoIX to access the cytosol or the nucleus.

Other Studies Supporting a Role for Mg-ProtoIX as a Plastid-Generated Signal

Evidence for chlorophyll precursors in retrograde signaling also comes from studies on higher plants. Treating etiolated cress seedlings with thujaplicin inhibits protochlorophyllide synthesis and results in accumulation of Mg-ProtoIX and Mg-ProtoIXme and reduces the light-induced accumulation of *Lhcb* mRNA (66). Etiolated cress seedlings, when treated with δ-aminolevulinic acid (ALA), accumulate 50% less *Lhcb* mRNA after light induction, compared to water-treated seedlings (38). Etiolated barley seedlings treated with amitrole, a carotenoid biosynthesis inhibitor, accumulate ALA, Mg-ProtoIX, and Mg-ProtoIXme and are impaired in light-induced *RbcS* and *Lhcb* expression (44). *Arabidopsis* lines overexpressing *PORA* or *PORB*, encoding isoforms of protochlorophyllide reductase, also fail to repress *Lhcb* in the presence of the herbicide NF, perhaps because these plants can metabolize Mg-ProtoIX more efficiently (**Figure 1**) (53). Lastly, lesions in specific enzymes in the tetrapyrrole biosynthetic pathway lead to loss of plastid control over nuclear *Lhcb* gene expression, as discussed below.

Arabidopsis gun (*genomes uncoupled*) Mutants

Using a genetic approach, Susek et al. (85) identified several nuclear-encoded genes required for plastid-to-nucleus signaling in *Arabidopsis*. An *Lhcb* promoter fused to both a selectable and screenable marker was integrated into the nuclear genome and seeds mutagenized with ethyl methanesulfonate (EMS). These lines were then used to isolate mutants in which *Lhcb* expression is uncoupled from the functional state of the chloroplast. Wild-type plants grown on NF, under continuous light, have low expression of reporters driven by the *Lhcb* promoter, owing to photobleaching of the chloroplast. In contrast, mutants have high levels of *Lhcb* expression on NF-containing medium, even though the chloroplasts are photobleached. From this initial screen, five nonallelic loci impaired in retrograde signaling were identified and named *genomes uncoupled* or *gun* mutants (85).

Four *gun* mutants have been characterized at the molecular level so far and all four involve lesions in plastid-localized enzymes that function in porphyrin biosynthesis. *gun2* and *gun3* are allelic to *hy1* and *hy2* and have mutations in the heme oxygenase and

phytochromobilin synthase, respectively (**Figure 1**). These enzymes are involved in the synthesis of phytochromobilin; in their absence, plastids accumulate heme (56). Heme accumulation leads to negative feedback regulation of chlorophyll biosynthesis (**Figure 1**) (73, 87, 90). *gun5-1* has a point mutation in the *ChlH* gene encoding the porphyrin-binding subunit of the Mg-chelatase, the enzyme that introduces Mg^{2+} into ProtoporphyrinIX as the first committed step of chlorophyll biosynthesis (56). Lastly, *GUN4* encodes a Mg-ProtoIX-binding protein that can significantly stimulate the Mg-chelatase activity in vitro (45, 92). Thus, mutations in all four of these genes would lead to a decreased accumulation of Mg-ProtoIX. A second role for GUN4 in signaling or transport of Mg-ProtoIX has also been proposed because a significant portion of GUN4 is not associated with the Mg-chelatase (45).

The activities of the GUN2/3/4/5 (**Figure 1**) proteins indicate that accumulation of Mg-ProtoIX is important for repressing nuclear photosynthetic genes. Consistent with this idea, Strand and colleagues (83) observed that wild-type *Arabidopsis* seedlings grown on NF have about 15-fold more Mg-ProtoIX as compared to untreated controls, whereas *gun2* and *gun5* seedlings accumulate much less Mg-ProtoIX on NF. Lesions in other enzymes leading up to the synthesis of Mg-ProtoIX also impair plastid control of *Lhcb* expression. Mutations in porphobilinogen deaminase (PBD), in the D-subunit of the Mg-chelatase (CHLD), and in coproporphyrinogen oxidase (*lin2*) all impair retrograde signaling (**Figure 1**) (83). Additionally, a T-DNA knockout mutant in the CRD subunit of the cyclase complex that catalyzes the conversion of Mg-ProtoIXme to divinyl protochlorophyllide does not exhibit a gun phenotype when grown on NF (**Figure 1**) (Å. Strand, personal communication).

Contrary to expectation, retrograde signaling is not impaired in two *Arabidopsis* mutants, *cs* and *ch42*, that have lesions in the *ChlI* gene, which encodes the third subunit of Mg-Chelatase (56). *Lhcb* mRNA levels are similar to wild-type levels in *cs* mutants grown on NF, despite a demonstrably less active Mg-chelatase in *cs* than in *gun5-1* (56). The stronger allele, *ch42*, is albino but has a fully functional nuclear response (56). This suggests that GUN5 has a specific role in either sensing elevated Mg-ProtoIX levels or transmitting the signal, besides being required for its synthesis. Alternatively, sequence redundancy may provide an explanation for why *ChlI* mutants do not have a *gun* phenotype. A second *ChlI* gene (*ChlI2*) that is 82% similar to *ChlI1* exists in *Arabidopsis* (78). Although expression of *Chl12* is not sufficient to support viable levels of chlorophyll synthesis in a *ChlI1* mutant background, it may be sufficient under conditions in which retrograde signaling has been measured (78).

Inhibitor feeding experiments in *Arabidopsis* seedlings also point to a key role for Mg-ProtoIX in retrograde signaling. Wild-type, *gun2*, and *gun5* seedlings grown on DP (see above) in the presence of NF are all able to repress *Lhcb* gene expression (83), likely because inhibition of later steps in chlorophyll biosynthesis allows *gun* mutant seedlings to accumulate enough Mg-ProtoIX (14). Indeed, combined addition of DP, NF, and a potent inhibitor of protoporphyrinogen oxidase, S23142, prevents accumulation of Mg-ProtoIX and restores the *gun* mutant phenotype (83). Finally, direct addition of Mg-ProtoIX to leaf protoplasts is sufficient to repress *Lhcb* gene expression, whereas addition of porphobilinogen, heme, or ProtoIX does not repress expression of a luciferase gene driven by the *Lhcb* promoter (83).

PLASTID GENE EXPRESSION-DEPENDENT SIGNALING

A second retrograde pathway that requires chloroplast gene expression has also been reported. Inhibition of chloroplast protein

translation by CF or lincomycin results in repression of nuclear-encoded photosynthetic gene expression. The response to plastid gene expression occurs only early in seedling development, and it is light independent. This latter observation was first made using two constitutively photomorphogenetic mutants, the pea *lip1* and the *Arabidopsis cop1-4*. These mutants accumulate significant levels of the light-induced *Lhcb1.2* transcript when grown in the dark, but its levels are reduced when seedlings are exposed to lincomycin (84). Unlike the screens done on NF, no *gun* mutants have been reported from CF- or lincomycin-based genetic screens. However, *gun1* does have a phenotype on CF and lincomycin (23, 24, 85), suggesting that GUN1 has a role in the plastid gene expression-dependent pathway as well as the Mg-ProtoIX pathway. Genetic studies, using *gun1 gun4* and *gun1 gun5* double mutants, have implicated the existence of two separate but partially redundant pathways (56). In support of this interpretation, an early microarray study in *Arabidopsis* suggested that there was little overlap in the set of genes misregulated in *gun1* and *gun5* mutants (83). Recently, ABI4, an AP2-like transcription factor was implicated in the Mg-ProtoIX dependent pathway (2). ABI4, like GUN1, also appears to play a role in the PGE pathway (S. Koussevitzky, T. Mockler, F. Hong, Y. Huang & J. Chory, unpublished data). That GUN1 and ABI4 are required for both the gene expression-dependent and the Mg-ProtoIX pathways, suggests that these two signaling pathways converge. Consistent with this idea, analysis of many mutants and conditions using a gene-sequence-tag array of more than 2600 nuclear sequences encoding for chloroplast proteins suggests that a "master switch" controls the expression of many of these genes in response to the chloroplast signal (77). Based on gene expression patterns in these experiments, *gun1* and *gun5* cluster together, whereas a mutant in which *Lhcb* is underexpressed shows a complementary expression pattern (77).

RETROGRADE-RESPONSIVE *CIS*-ELEMENTS

Several studies have demonstrated that the *cis*-elements required for retrograde regulation are either identical to, or largely overlapping with, light-responsive elements, and in every case that has been examined the light- and plastid-responsive elements have been inseparable (7, 43, 81, 89). Minimal promoter regions from *Arabidopsis Lhcb* and *HEMA1* genes are sufficient for both light- and plastid-responsive expression (52). A 52-bp promoter element containing an I- and a G-box that was identified in the promoter of *rbcS 8B* in *Nicotiana plumbaginifolia* was able to confer phytochrome-, cryptochrome-, and retrograde-controlled reporter expression in *Arabidopsis* (48). In *C. reinhardtii*, the same promoter region is required for light- and Mg-ProtoIX-dependent expression of *HSP70* genes and it is distinct from the region required for heat-shock response (41). In *C. reinhardtii*, there are two *RbcS* genes, the light-inducible *RbcS1*, and light-independent *RbcS2*. Only the accumulation of *RbcS1* is affected in DP-treated cultures whereas *RbcS2* mRNA levels are unaffected (32). Even in the case of post-transcriptional regulation of the pea *PetE* in transgenic *Arabidopsis*, the same sequence in the 5′ untranslated region of the transcript is responsive to light and plastid signals (9).

Examination of individual promoter elements shows that tetramers of G-box or the GATA element, fused to a minimal nopaline synthase promoter (NOS), conferred light- and plastid-responsive expression, whereas another element that directed high-level expression in the dark did not respond to plastid signals (74). There are at least two well-characterized light-responsive elements in the *Lhcb* promoter, the CUF1 (cab upstream factor 1, CACGTA) element, which is similar to a G-box, and the GATA element. The CUF1 element is important for high-level expression in the light while being dispensable for phytochrome- and circadian-regulated *Lhcb*

expression (5). When *gun5* seedlings were grown on NF, accumulation of luciferase driven by an *Lhcb1* promoter with a mutated CUF1 element was reduced to wild-type levels, clearly emphasizing a role for the G-box in retrograde signaling (83). The 5′ end of the CUF1 element contains a CCAC motif that resembles the recently identified S-box (2), an element important for sugar and ABA responsiveness in *rbcS 8B* promoter (2).

Conflicting results from other studies suggest that mutation or deletion of the G-box element alone does not completely abrogate light and plastid responsiveness. Transgenic tobacco plants expressing β-glucuronidase (GUS) driven by spinach *RbcS1* promoter fragment (−296/+80) fused to a 90-bp cauliflower mosaic virus minimal promoter (CaMV) showed light-inducible and plastid-responsive expression. Mutation of the conserved G-box element in this region, or truncation of the promoter fragment to exclude the G-box, dramatically reduced the expression of GUS but did not qualitatively alter the dark repression or repression on NF (43).

MODEL FOR Mg-ProtoIX AND PLASTID GENE EXPRESSION-DEPENDENT RETROGRADE SIGNALING

The current model posits that reduced chloroplast function leads to an increase in accumulation of Mg-ProtoIX. Mg-ProtoIX then either diffuses or is actively transported to the cytoplasm, where it is bound by other, as yet unidentified, proteins that elicit regulation of nuclear gene expression (**Figure 2**) (82, 83). This is consistent with the proposed role for Mg-ProtoIX outside the chloroplast in *C. reinhardtii* (41). The fact that GUN4 is found in the stroma, thylakoid, and envelope fractions of the chloroplast suggests that GUN4 might be involved in intraplastidic transport of Mg-ProtoIX (45).

Indirect evidence indicates that at least some porphyrins are able to exit the chloroplast and export of some porphyrin

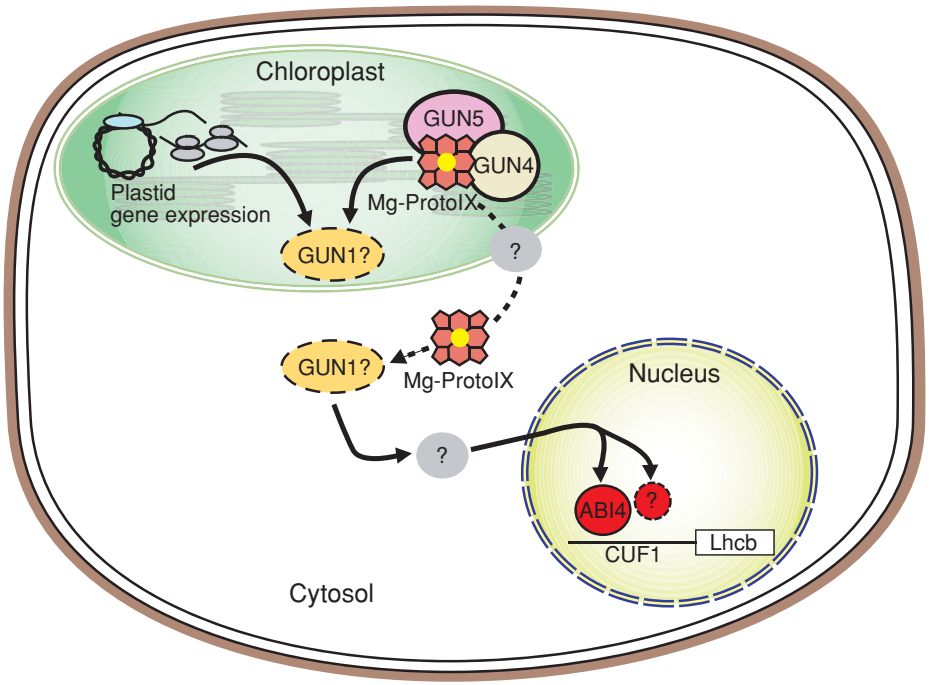

Figure 2

gun mutants in retrograde signaling. Because the identity of GUN1 is not yet known, its localization is depicted to be either in the plastid or the cytosol. GUN4 is found in stroma, thylakoid, and envelope fractions of chloroplasts. Other unidentified cytosolic components may also be involved in the signaling pathway.

ROS: reactive oxygen species

compounds from isolated chloroplasts has been demonstrated (30). The initial steps in the biosynthesis of heme up to ProtoIX occur exclusively in plastids whereas the rest of the pathway can occur in both plastids and mitochondria. Presence of heme in the mitochondria implies that at least some intermediates are transported to the mitochondria from the chloroplasts. Also, phytochromobilin, the chromophore for phytochromes, is synthesized in chloroplasts and transported to the cytosol, where it binds phytochromes.

Evidence from other systems also supports a role for porphyrins outside the organelles in interorganelle signaling. Studies on mitochondrial retrograde signaling show that heme can modulate activator or repressor activity of bound proteins (20, 95). Heme can bind to the yeast transcription factor HapI and increase its affinity for DNA and its ability to activate transcription (28). Heme also binds to the bZIP transcription factor Bach1 and reduces its ability to activate transcription (64).

We do not yet know the signal in the gene expression-dependent pathway. Perhaps it is a plastid transcript, as suggested by Bradbeer and colleagues (8). It appears likely that the Mg-ProtoIX signal and the signal generated by the plastid gene expression-dependent pathway are integrated at some point prior to GUN1 (**Figure 2**). Presumably some as yet unidentified cytosolic components are involved in signal transduction. In the nucleus the retrograde signal activates a transcriptional repressor and/or inactivates a transcriptional activator, leading to repression of nucleus-encoded photosynthetic genes. One of the transcriptional repressors may be ABI4, but its weak *gun* phenotype suggests that there are other transcription factors that might act redundantly within the nucleus (**Figure 2**). Retrograde signaling culminates on promoter elements very close to, or on the, G-box itself, and perhaps binding of ABI4 to elements close to the G-box prevents light-dependent activation of nuclear genes.

CHLOROPLAST REDOX SIGNALS IN RETROGRADE SIGNALING

The reduction/oxidation (redox) states of PET components regulate gene expression within chloroplasts (71). Redox states of PET components have also been proposed as chloroplast signals influencing nuclear gene expression (17, 18, 70). Furthermore, reactive oxygen species (ROS) generated in chloroplasts also play a role in chloroplast redox signaling (35, 91). In the following sections, we discuss identified and possible chloroplast redox signals regulating nuclear gene expression, as well as the genes responding to these signals.

Linear photosynthetic electron transport begins in photosystem II (PSII) and electrons are delivered to the cytochrome b_6f complex (cyt b_6f) through the plastoquinone (PQ) pool (**Figure 3**). Plastocyanin (PC) mediates electron transport from cyt b_6f to photosystem I (PSI). The electrons are then transferred to ferredoxin (FD) and finally delivered to $NADP^+$ to generate NADPH (**Figure 3**). During this PET, protons (H^+) are pumped to the thylakoid lumen to generate a proton gradient that is then utilized for ATP synthesis. Oxygen molecules (O_2), instead of $NADP^+$, can accept electrons from PSI to form superoxide ($O_2^{\bullet -}$). In addition, excited chlorophyll can transfer energy to O_2 to create singlet oxygen ($^1O_2$) (6, 60). These ROS must be removed to prevent oxidative stress. $O_2^{\bullet -}$ is detoxified by superoxide dismutase (SOD) to hydrogen peroxide (H_2O_2) and then reduced to H_2O by various types of peroxidases in chloroplasts. $^1O_2$ returns to its ground state after energy transfer to antioxidants such as carotenoids (60).

Photosynthetic Electron Transport Components as Redox Signals

The involvement of redox states of PET components in nuclear gene expression has been shown in several different ways.

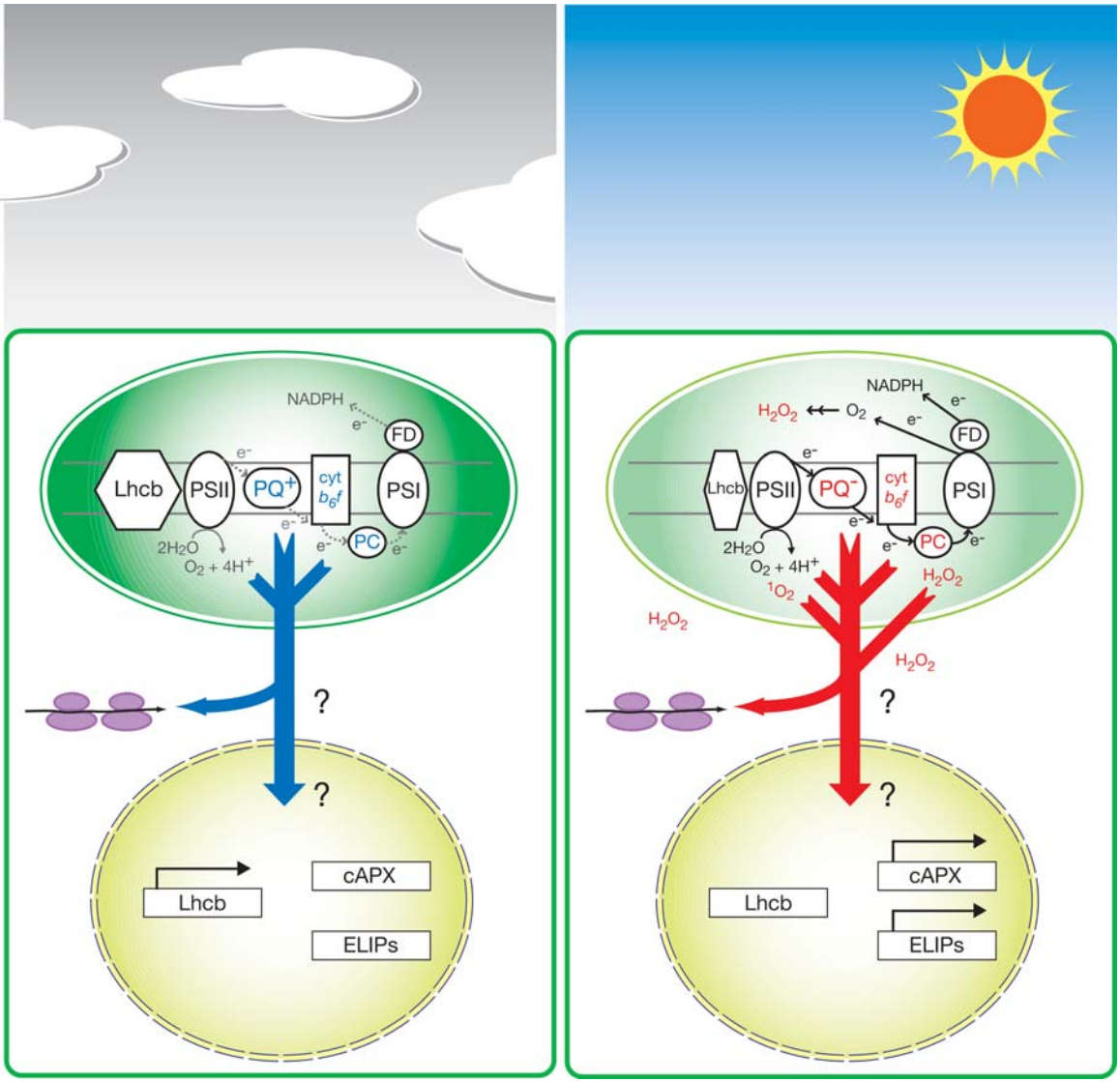

Figure 3

Under low light (*lefthand figure*), the rate of photosynthetic electron transport (PET) is low and most PET components are in oxidized states, e.g., the plastoquinone (PQ) pool is in an oxidized state (PQ^+). In contrast, in high-light conditions (*righthand figure*), due to higher excitation pressure, PET components are generally in reduced states, e.g., the PQ pool is in a reduced state (PQ^-). In addition to PET components, changes in cellular redox states are caused by different levels of reactive oxygen species (ROS) such as $O_2^{\bullet -}$ and H_2O_2 (34). Under low light (*lefthand figure*), ROS are seldom generated and, even if they are generated, most of them are detoxified by antioxidant systems (6). Under high light, however, much more ROS are generated than the antioxidant systems can deal with. These redox states may report the functional states of chloroplasts to the nucleus.

Low-light-grown plants were transiently exposed to high light or vice versa to change redox states of PET components (17, 34, 35, 49). PSII- or PSI-specific light and a shift between these PS-specific light conditions have also been utilized to adjust the redox states of PET components (72). In addition, other environmental conditions such as low temperature, sugar starvation, and/or reduced levels of electron acceptors such as O_2 and CO_2 have been used to determine the effects of the chloroplast redox states on nuclear gene expression (50, 58, 67). Furthermore, herbicides 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (DCMU), and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) have been applied directly to plants or in combination with other treatments mentioned above to manipulate the redox states (17, 49, 72). These herbicides specifically block electron transport before (DCMU) or after (DBMIB) the PQ pool (88), mimicking the effect of low- or high-light intensities, respectively. The PQ pool is mostly oxidized in DCMU-treated plants and mostly reduced in DBMIB-treated plants.

The redox state of the PQ pool is one of the major determinants of PET-derived retrograde signaling (17, 35, 36, 72). Genes that are induced by high light are also induced by DBMIB treatment in the absence of high light; in contrast, DCMU treatment inhibits high-light-induced gene expression (17, 35, 36, 49). In addition, modifying the redox state of the PQ pool by shifting from PSI- to PSII-specific light causes an opposite effect on nuclear gene expression compared to a shift from PSII- to PSI-specific light (72).

The importance of the redox state of PET as a source of chloroplast signal(s) to the nuclear compartment was recently corroborated by gene expression analyses of 2661 genes encoding chloroplast proteins and 631 genes encoding nonchloroplast proteins in *Arabidopsis* (19). Using very strict environmental conditions, including PS-specific light and DCMU, genes responsive to PET redox state were identified. Among the tested genes, 286 genes were identified as directly regulated by the redox state of PET. It would be interesting to see how many among these 286 genes are specifically regulated by the redox state of the PQ pool. The total complement of nuclear genes controlled by the PET redox state is yet to be determined.

Among the PET components, the redox state of the PQ pool seems to be the most obvious source of retrograde signal(s) (17, 49, 67, 72). However, in at least one case, reduction or oxidation of the PQ pool by DCMU, DBMIB, or PS-specific light quality shows similar effects, i.e., DCMU and DBMIB both reduce nuclear gene expression (72). This suggests that the overall redox states of PET components other than the PQ pool can also generate retrograde signals.

Reactive Oxygen Species as Retrograde Signals

Besides the redox states of PET components, ROS can also function as chloroplast redox signals. Photochemical reactions in chloroplasts generate ROS and this helps relieve excitation pressure of PET components under high-light stress (6, 61). Among ROS, H_2O_2 and singlet oxygen ($^1O_2$) generated in chloroplasts by high light could act as chloroplast redox signals (**Figure 3**). H_2O_2 can be reduced to H_2O by chloroplast ascorbate peroxidases (APXs) and excess H_2O_2 diffuses through the chloroplast envelopes to the cytoplasm. Owing to its highly reactive nature, most $^1O_2$ appears to be exclusively inside of chloroplasts (39). External H_2O_2 treatment can induce expression of nuclear genes related to stress responses such as the *cytosolic APX* (*cAPX*) whose expression is also upregulated by high light (35, 37). H_2O_2 generated in chloroplasts by high light seems to play a role in nuclear gene expression. This was shown by infiltration into high-light-exposed leaves in which catalase abolished the induction of *APX2*, an *Arabidopsis cAPX*, by high light, whereas SOD did not (35).

In addition, a correlation between *Arabidopsis APX2* expression and H_2O_2 generation was shown by comparing the expression pattern of a luciferase gene driven by the *APX2* promoter and H_2O_2-specific staining pattern under high light (21). Thus, H_2O_2 could be a retrograde signaling molecule under high-light conditions.

H_2O_2 is also generated in other organelles and by other stimuli such as pathogen attack (54). However, a specific role for chloroplast-generated H_2O_2 has been shown by using transgenic tobacco plants expressing catalase or thylakoid-type APX in chloroplasts and by using methyl viologen (MV), which accelerates the generation of $O_2^{\bullet -}$ and H_2O_2 in chloroplasts (93). In the transgenic plants, cellular H_2O_2 levels under high light were significantly lower than in wild-type, and the *cAPX* gene induction was saturated much earlier than in wild type. In addition, *cAPX* expression pattern correlated with cellular H_2O_2 generated by MV. Thus, it is clear that chloroplast-generated H_2O_2 can be a redox signal that induces *cAPX* expression in the nucleus.

$^1O_2$ is most likely retained exclusively within chloroplasts considering its short lifetime and distances traveled (39, 55); however, $^1O_2$ generated in plastids can also influence nuclear gene expression. In the *Arabidopsis flu* mutant, the chlorophyll biosynthetic precursor protochlorophyllide accumulates as a free pigment and it is possible to increase the amount of $^1O_2$ without affecting the amount of other ROS such as H_2O_2 (65). Accumulation of $^1O_2$ significantly changes the expression of a number of nuclear genes. This implies that $^1O_2$ initiates a retrograde signal from the plastids. The details of the signal transduction mechanisms initiated by H_2O_2 and $^1O_2$ still need to be determined. Recently, the *Arabidopsis* EXECUTER 1 (EX1) protein was identified as a component in the $^1O_2$-mediated signaling pathway (91). Characterization of EX1 may help determine the $^1O_2$ mediated chloroplast-to-nucleus signal transduction pathway.

Nuclear Genes Regulated by Chloroplast Redox Signals

A number of nuclear genes encoding chloroplast-localized proteins are regulated by the chloroplast redox state. Under certain environmental conditions, photosynthesis-related genes are regulated to ensure efficient photosynthesis (4, 60). Under extreme conditions such as high light, the *Lhc* genes are downregulated to reduce the size of the light-harvesting complex as a protection mechanism. In addition, plants respond to the same conditions by accumulating more antioxidant molecules and by expressing stress-response proteins, both of which protect plants from harmful effects of reactive molecules (60). Thus, chloroplast redox signals are crucial not only for efficient metabolism, but also for photoprotection.

Photosynthesis-Related Genes

In *Dunaliella*, transcript levels of the *Lhcb* and the *chlorophyll a oxygenase* (*CAO*) are regulated by chloroplast redox states (12, 17, 49, 50). The effect of PET inhibitors, DCMU and DBMIB, indicate that the redox state of the PQ pool is the initial signal for regulating *Lhcb* and *CAO* expression (12, 17, 49), with possible involvement of other signals such as *trans*-thylakoid membrane potential (12).

Unlike green algae, the redox-dependent response in higher plants is more complicated. The reduced and oxidized PQ pool differentially regulates spinach *PetE* promoter activity. In contrast, the promoter activities of spinach *PsaD* and *PsaF* decreased in both DCMU and DBMIB treatments. These results indicate that spinach *PetE* expression is regulated by the redox state of the PQ pool, while the *PsaD* and *PasF* genes are regulated by the redox state of PET components other than the PQ pool (72).

The *Lhcb* gene is also under the control of the chloroplast redox state in higher plants (58, 67, 94). Surprisingly, different treatments that generate the same chloroplast

redox state result in different expression levels, suggesting a strong effect of experimental conditions (58, 67). For example, in barley, high-light-induced reduced state decreases *Lhcb* mRNA levels, whereas a reduced state generated by low amount of electron acceptors does not affect transcript levels (58). In addition, sugar starvation induces the *Arabidopsis Lhcb* and *PetE* genes in mature leaves, whereas sugar does not affect *PetE* accumulation in PS-specific light shift experiments (67, 72). Furthermore, the spinach *PetH* gene encoding ferredoxin-$NADP^+$-oxidoreductase (FNR) does not respond to the redox signal (72), whereas the *Arabidopsis PetH2* gene is responsive to the chloroplast redox state (19). In conclusion, although the redox state of PET generates a signal that controls the expression of several nuclear photosynthetic genes, the specific genes affected vary between species and experimental conditions.

It seems that not all photosynthesis-related genes are regulated by chloroplast redox signals at the transcriptional level (49). The redox states of chloroplasts can influence post-transcriptional steps including stability and polyribosome loading of mRNAs of several photosynthesis-related genes (15, 68, 69, 79). The stability and polyribosome association of pea *ferredoxin* (*Fed-1*) mRNA are PET dependent (68, 69). It remains to be seen if other photosynthesis-related genes are also regulated post-transcriptionally by the chloroplast redox state.

Stress-Response Genes

Among stress-response genes induced by high light, the *Arabidopsis cAPX* and *ELIP* are best characterized. Chloroplast-type *APXs* are constitutively expressed, whereas expression of *cAPXs* is highly responsive to environmental conditions (80). DCMU and DBMIB treatments show that the expression of an *Arabidopsis cAPX* gene, *APX2*, is under the control of the redox state of the PQ pool (34, 35). The *ELIP* genes encode chloroplast proteins that are similar to members of the LHC protein family (57). Under high light, expression of *ELIP* is induced, whereas most LHC genes are repressed (25). This is consistent with a proposed photoprotective function for ELIPs (29). Similar to *APX2*, the expression of *Arabidopsis ELIP2* is also regulated by the redox state of the PQ pool (36). Interestingly, both *APX2* and *ELIP2* are induced by H_2O_2 in addition to the reduced PQ pool (35, 37). A recent report shows two phases in the induction of tobacco *cAPX*. Early response is induced by the reduced PQ pool and the later response is mediated by the cellular level of H_2O_2 (93). This suggests that the two signals can be temporally separated. It would be interesting to determine whether the induction of *Arabidopsis APX2* and *ELIP2* can be divided into similar phases.

POSSIBLE CHLOROPLAST REDOX SIGNAL TRANSDUCTION PATHWAYS

Very little is known about how chloroplast redox signals are delivered to the nucleus. One clue comes from pharmacological approaches, which indicate that a phospho-relay signaling pathway may be involved. In *Dunaliella*, it was shown that protein phosphatase inhibitors block a low-light acclimation process controlled by the redox state of the PQ pool (17). In addition, a protein kinase inhibitor also prevents *Dunaliella Lhcb* and *CAO* induction following high to low-light shift (49). In tobacco, it has been demonstrated that GUS reporter gene expression driven by the spinach *PsaF* promoter, which is regulated by the PET redox state (72), is affected by both kinase and phosphatase inhibitors (10).

ROS-mediated chloroplast-to-nucleus signal transduction also appears to involve protein phosphorylation. H_2O_2 activates mitogen-activated protein kinases (MAPKs) in *Arabidopsis* protoplasts (40) although it still needs to be determined whether $^1O_2$ can also activate a protein phosphorylation cascade. The fact that NPR1 (nonexpressor of PR

genes1) activates pathogen-related (PR) gene expression and that its activation is mediated by reduction of NPR1 to a monomeric form by cellular redox components suggests a mechanism by which the chloroplast redox signal might regulate nuclear gene expression (59). Taken together, these results strongly implicate a protein phosphorylation cascade in chloroplast-to-nucleus signal transduction initiated by the redox states of PET components. Identifying specific protein targets for the proposed phosphorylation relay will lead to a better understanding of redox-dependent retrograde signal transduction.

CONCLUSIONS AND PERSPECTIVES

Our understanding of plastid-to-nucleus retrograde signaling pathways is still rudimentary. We know a handful of proteins and one signaling intermediate involved in the pathway, but do not know any cytoplasmic signaling components. Importantly, we do not know how signals get out of the plastids. Several questions need to be addressed. Does Mg-ProtoIX exit the plastids or does accumulation of Mg-ProtoIX result in a secondary signal? If Mg-ProtoIX is transported, what are the specific transport proteins required? Is GUN4 involved in such transport? Where and how are the Mg-ProtoIX and PGE signals integrated? What are the other signal transduction components between GUN1 and ABI4?

Identifying GUN1 will further our understanding of the plastid gene expression-dependent pathway and its relation to the Mg-ProtoIX-dependent pathway. Identifying proteins that interact, genetically or physically, with the known GUN proteins may reveal important new components. Biochemical identification of transcription factors that can bind to specific DNA elements required for retrograde signaling should uncover new proteins that are perhaps redundantly encoded and which have eluded identification by genetic means. The redox pathway has thus far not been amenable to genetic screens. The identification of robust redox-responsive genes should allow large-scale screening for mutants using selectable reporters. Several different approaches, including genetic screens for suppressors, interacting protein screens, and identification of putative porphyrin-binding proteins, will no doubt lead to identification of novel components and a better understanding of the plastid-to-nucleus retrograde signaling pathways. Together, these approaches should elucidate the mechanisms by which plants respond to changing, and often stressful, environments.

SUMMARY POINTS

1. Plastids emit signals that can control the expression of hundreds of nuclear genes.
2. Accumulation of Mg-ProtoporphyrinIX is one signal that elicits a nuclear response.
3. Functional plastid gene expression during the transition from heterotrophic to photoautotrophic growth modes is required for expression of nuclear-encoded photosynthetic genes.
4. Photosynthetic electron transport components and several reactive oxygen species have been proposed as putative redox-dependent retrograde signaling molecules.
5. Retrograde signals impinge on the same promoter elements that confer light-responsive gene expression.
6. Retrograde signaling pathways are crucial for allowing plants to respond to changing, often stressful, environments.

ACKNOWLEDGMENTS

Our work on retrograde signaling is supported by grants from the Department of Energy (J.C.) and the Howard Hughes Medical Institute (J.C.), by Damon Runyon Cancer Research Foundation (A.N.), and by a long-term fellowship from the European Molecular Biology Organization (S.K.). We would like to thank Jennifer Nemhauser and Jesse Woodson for critical comments and Sheila Moles for help in preparing the manuscript.

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Annual Review
of Plant Biology

Volume 57, 2006

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