Review in Advance first posted online on February 4, 2013. (Changes may still occur before final publication online and in print.)

# Intracellular Signaling from Plastid to Nucleus

Wei Chi, Xuwu Sun, and Lixin Zhang

Photosynthesis Research Center, Key Laboratory of Photobiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China; email: zhanglixin@ibcas.ac.cn



Annu. Rev. Plant Biol. 2013. 64:10.1–10.24

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

This article's doi: 10.1146/annurev-arplant-050312-120147



## Keywords

gene expression, membrane-bound transcription factor, retrograde signaling, signal transduction

## Abstract

Intracellular signaling from plastids to the nucleus, called retrograde signaling, coordinates the expression of nuclear and plastid genes and is essential for plastid biogenesis and for maintaining plastid function at optimal levels. Recent identification of several components involved in plastid retrograde generation, transmission, and control of nuclear gene expression has provided significant insight into the regulatory network of plastid retrograde signaling. Here, we review the current knowledge of multiple plastid retrograde signaling pathways, which are derived from distinct sources, and of possible plastid signaling molecules. We describe the retrograde signaling–dependent regulation of nuclear gene expression, which involves multilayered transcriptional control, as well as the transcription factors involved. We also summarize recent advances in the identification of key components mediating signal transduction from plastids to the nucleus.

## Contents



## INTRODUCTION

Chloroplasts are responsible not only for photosynthesis but also for the synthesis of many essential compounds, such as fatty acids, vitamins, tetrapyrroles, and amino acids (79, 94). Thus, formation of normal chloroplasts is crucial for the growth and development of vascular plants. Plant chloroplasts evolved from a free-living cyanobacterial ancestor following a single endosymbiotic event (56, 115). After this event, most genes in the endosymbiont genome were either lost or transferred into the host nuclear genome (56, 115). As a result, plastid genomes of vascular plants encode fewer than 100 open reading frames, whereas nuclear genomes encode the vast majority of plastid proteins, which are translated in the cytoplasm and imported into plastids (1). This compartmentalization of genes and gene products requires the coordination of plastid and nuclear gene expression to maintain plastid development and function. It is postulated that plastid development and gene expression are largely under nuclear control, referred to as anterograde control (79, 109, 148). However, the coordination is also mediated by retrograde control, which functions in the opposite direction. In retrograde control, signals generated in plastids are transduced to the nucleus and modulate nuclear gene expression.

The concept of plastid retrograde signaling appears to be simple but has been greatly expanded since its original conception. Plastid retrograde signaling was first inferred from the study of two barley mutants (the *albostrians* and *Saskatoon* mutants of *Hordeum vulgare* L. cv. Haisa) that contain undifferentiated plastids lacking ribosomes (15). These mutants exhibit reduced accumulation of a set of proteins encoded by photosynthesis-associated nuclear genes (PhANGs), including light-harvesting chlorophyll *a/b*-binding protein (encoded by *Lhc*), the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (encoded by *RbcS*), and Calvin-Benson-cycle enzymes, suggesting that a signal originating from plastids is involved in the regulation of nuclear gene expression (41, 42). Further studies showed that in addition to occurring in plastids that are impaired owing to genetic defects, such a retrograde signal initiates in plastids that are impaired because a particular process has been inhibited by chemical treatment. For instance, retrograde signals are induced by norflurazon (NF) (96, 97), an inhibitor of the phytoene desaturase enzyme involved in carotenoid biosynthesis, and by tagetoxin and lincomycin (LIN) (114, 132), which affect plastid gene transcription and translation, respectively.

Perturbation of plastid functions via biotic stresses can also influence the expression of

**Norflurazon (NF):** an inhibitor of phytoene desaturase, a carotenoid biosynthesis enzyme

**Lincomycin (LIN):** an inhibitor of organelle translation

several nuclear genes (28, 54, 55, 107). In this case, the nuclear genes subjected to this control are not limited to PhANGs, and stress-related genes are included as well. The signals derived from plastids under stress conditions have been considered to be retrograde signals: They act as stress-induced signals capable of eliciting stress responses, including changes in nuclear gene expression. This represents an expansion of the idea and significance of plastid retrograde signaling in that it not only coordinates the expression of nuclear and plastid genes, which is essential for plastid biogenesis, but also maintains plastid function at optimal levels according to fluxes in metabolites and changes in environmental conditions (18, 81, 110).

During the past decade, genetic and biochemical studies in the unicellular green alga *Chlamydomonas reinhardtii* and the model plant *Arabidopsis thaliana* have significantly advanced our understanding of plastid signaling (9, 11, 18, 30, 38, 47, 60, 74, 77, 95, 103, 110, 130, 134, 148). It appears that distinct plastid signals are produced from different sources and that multiple, partially redundant plastid-to-nucleus signaling pathways exist (95). In this review, we summarize recent progress on identifying the molecular mechanisms of the origination of retrograde signals in plastids, nuclear responses to plastid signals, and retrograde signal transduction from plastids to the nucleus.

## GENERATION OF RETROGRADE SIGNALS IN PLASTIDS

### Multiple Plastid Signaling Pathways

Four distinct putative plastid retrograde signaling pathways have been traditionally recognized based on the sources of the signals: tetrapyrrole intermediate biosynthesis, plastid gene expression (PGE), plastid redox state, and reactive oxygen species (ROS) (60, 95, 103, 110, 148). However, in addition to these classical pathways, several novel plastid retrograde signaling pathways have been proposed. The SAL1–3′-phosphoadenosine 5′-phosphate (PAP) and methylerythritol cyclodiphosphate (MEcPP) pathways are two examples (29, 150).

**The tetrapyrrole intermediate signaling pathway.** Four classes of tetrapyrroles—chlorophyll, heme, siroheme, and phytochromobilin—are derived from a common biosynthetic pathway that resides in plastids (89, 137). In all living organisms, the tetrapyrrole synthesis begins with the first committed precursor, 5-aminolevulinic acid (ALA) (89, 137) (**Figure 1**). Chlorophyll, heme, and phytochromobilin synthesis share a common pathway up to protoporphyrin IX (proto IX) (89, 137). At the point of metal iron insertion, the pathway diverges: One route is directed to the synthesis of chlorophyll, and the other goes on to synthesize heme and phytochromobilin. There are two sets of evidence for the involvement of tetrapyrrole intermediates in retrograde signaling. First, inhibitors of several steps of the tetrapyrrole biosynthesis pathway can impair the normal expression profiles of nuclear genes, and this impairment can be eliminated via feeding-specific tetrapyrrole intermediates. Second, retrograde signaling is disrupted in some mutants that are defective in tetrapyrrole biosynthesis.

In 1984, Johanningmeier & Howell (51) found that light induction of *Lhcb* mRNA accumulation is prevented in *Chlamydomonas* upon treatment with the chlorophyll biosynthesis inhibitor α,α-dipyridyl, which blocks late steps in the chlorophyll biosynthesis pathway and leads to accumulation of the porphyrin intermediate Mg–proto IX methyl ester. This prevention was not detected when chlorophyll synthesis was blocked in early steps prior to the formation of Mg–proto IX methyl ester, suggesting that Mg–proto IX methyl ester and its immediate precursors in chlorophyll synthesis may act as negative factors required for the repression of *Lhcb* transcription (48, 50, 51). The involvement of Mg–proto IX in *Chlamydomonas* plastid signaling was further supported by studies on the expression of two heat shock proteins, HSP70A and HSP70B, which can be induced by light via a heat-independent pathway (67, 68). Light induction of *HSP70* is impaired in the *brs-1* mutant, which is defective in Mg–proto IX synthesis owing to a mutation in the

**Reactive oxygen species (ROS):** molecules that are highly reactive owing to the presence of unpaired valence shell electrons

**3′-Phosphoadenosine 5′-phosphate (PAP):** a derivative of AMP that is phosphorylated at the 3′ position and has a sulfate group attached to the 5′ phosphate

**Methylerythritol cyclodiphosphate (MEcPP):** a precursor of isoprenoids produced by the plastidial methylerythritol phosphate pathway

***Lhcb*:** a group of nuclear genes encoding the chlorophyll *a/b*–binding proteins of photosystem II

**Mg–protoporphyrin IX (Mg–proto IX):** a chlorophyll intermediate

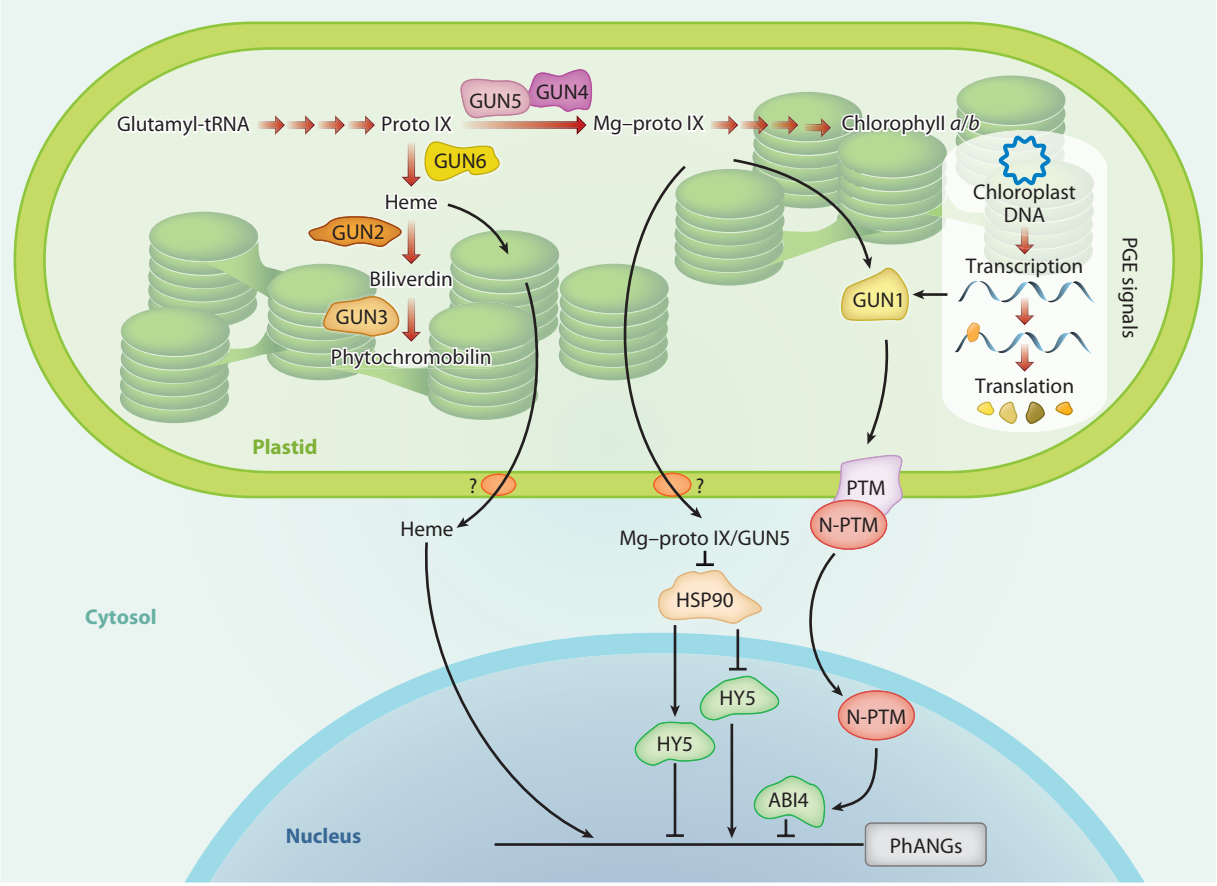


**Figure 1**

Plastid retrograde signaling derived from tetrapyrrole biosynthesis and plastid gene expression (PGE). The tetrapyrrole intermediates are involved in retrograde signaling. GUN6 (ferrochelatase), GUN2 (heme oxygenase), and GUN3 (phytochromobilin synthase) are involved in the heme branch of tetrapyrrole biosynthesis. Heme acts as a positive regulator of photosynthesis-associated nuclear genes (PhANGs), but the nuclear components involved in heme signaling and the exact mechanism by which heme reaches the nucleus are unknown. GUN5/CHLH and GUN4 (which binds the substrate and product of the reaction catalyzed by the Mg-chelatase and activates the Mg-chelatase) are involved in the biosynthesis of Mg–proto IX from the chlorophyll branch of tetrapyrrole biosynthesis. Mg–proto IX is suggested to be exported from plastids via an unknown mechanism and then to bind to HSP90 and trigger its changes, resulting in a HY5-dependent activated repression and/or inhibited activation of PhANG expression. The signals arising from PGE are largely unknown. Those signals and tetrapyrrole signals may converge on GUN1, which either generates or transmits a second signal. This signal activates a proteolytic process in which the N-terminal part of the PTM protein (N-PTM) is released. The processed PTM is able to travel to the nucleus and modulate nuclear gene expression by inducing the ABI4 transcription factor.

gene encoding the H subunit of Mg-chelatase (CHLH) (67). In addition, the feeding of Mg–proto IX or its dimethyl ester is sufficient to induce *HSP70* expression in both wild-type and *brs-1* cells. Nevertheless, subsequent study showed that heme feeding in the dark also induces the accumulation of *HSP70A* mRNA in wild-type cells, although the accumulation upon heme feeding is delayed compared with that in Mg–proto IX feeding (141). Thus, both heme and Mg–proto IX may be involved in retrograde signaling in *Chlamydomonas*.

Similar to *Chlamydomonas*, etiolated cress (*Lepidium sativum*) seedlings treated with

thujaplicin, which causes the accumulation of Mg–proto IX and Mg–proto IX methyl ester, are inhibited (by at least 50%) in light-induced accumulation of several *Lhcb* transcripts (99). In addition, the light-induced expression of *RbcS* and *Lhcb* is impaired in etiolated barley seedlings treated with amitrole, which inhibits carotenoid biosynthesis and increases the levels of ALA, Mg–proto IX, and Mg–proto IX methyl ester (75). Tetrapyrrole involvement in plastid signaling of vascular plants has been resolved in more depth from the analysis of *genomes uncoupled* (*gun*) mutants of *Arabidopsis*. Wild-type plants grown on NF have low expression of *Lhcb* owing to photobleaching of plastids, whereas *gun* mutants retain high levels of *Lhcb* expression under the same conditions, suggesting the disruption of plastid signaling (135). The *gun2–gun5* mutants were shown to have lesions in enzymes involved in tetrapyrrole biosynthesis (73, 88, 131), whereas *gun6* is a gain-of-function mutant overexpressing the conserved plastid ferrochelatase 1 (FC1, heme synthase) (149).

The *gun2* [allelic to *long hypocotyl 1* (*hy1*)] and *gun3* (allelic to *hy2*) mutants have mutations in the genes encoding heme oxygenase and phytochromobilin synthase, respectively (88) (**Figure 1**). The biosynthesis of phytochromobilin is blocked in these two mutants, leading to heme accumulation. Mg–proto IX accumulation could be reduced owing to feedback inhibition of glutamyl-tRNA reductase by heme when *gun2* and *gun3* mutants are treated with NF (111, 131, 139). Instead, *gun4* and *gun5* are directly associated with Mg–proto IX biosynthesis (**Figure 1**). The *gun5* mutant has a leaky point mutation in the *ChlH* gene (encoding the H subunit of Mg-chelatase), which is responsible for the last step of Mg–proto IX biosynthesis (88). *GUN4* encodes an Mg-proto-binding protein that can significantly activate Mg-chelatase in vitro (3, 73). Characterization of the *gun2–gun5* mutants raises the possibility that Mg–proto IX acts as a negative factor in retrograde signaling (**Figure 1**). Indeed, plastid retrograde signaling is impaired by lesions in other enzymes leading up to the biosynthesis of Mg–proto IX, including porphobilinogen deaminase (PBD); the D, L, and M subunits of Mg-chelatase (CHLD, -L, and -M, respectively); and coproporphyrinogen oxidase (LIN2) (45, 131). In contrast to Mg–proto IX, heme may be a positive retrograde signal in plants, as increased flux through the heme branch of the tetrapyrrole biosynthesis pathway increases the expression of PhANGs in the *gun6* mutant (149) (**Figure 1**; see below for details).

**GUN:** genomes uncoupled

**The plastid gene expression pathway.** The PGE pathway for retrograde signaling was revealed by the finding that treatment with inhibitors of PGE, such as LIN, decreases PhANG expression (114, 132) (**Figure 1**). The retrograde signal derived from the PGE pathway seems to be light independent because treatment with LIN in the dark can still repress *Lhcb1.2* expression in two constitutively photomorphogenic mutants: *lip1* in pea (*Pisum sativum*) and *cop1-4* in *Arabidopsis* (in which *Lhcb1.2* accumulates significantly in the dark) (132).

Initially, it was proposed that this retrograde signaling pathway is active only in young mustard seedlings (96). Later characterization of the *Arabidopsis prors1* mutant indicated that the PGE pathway may also persist in mature leaf tissues (102). This mutant is defective in the prolyl-tRNA synthetase found in both plastids and mitochondria and exhibits decreased protein synthesis in both organelles. A specific light-independent downregulation of PhANGs is found in mature *prors1* plants (102). This downregulation is not observed in *Arabidopsis prpl11* or *mrpl11* mutants (which are impaired in plastid and mitochondrial ribosomal L11 protein, respectively) (104) but is found in the *prpl11 mrpl11* double mutant. This confirms that plastid and mitochondrial translation act together to regulate PhANG expression (102). Thus, it appears that both plastids and mitochondria send retrograde signals when protein synthesis is impaired, and that their signals are synergistic.

**Plastoquinone (PQ):** a quinone molecule involved in the electron-transport chain in the light-dependent reactions of photosynthesis

**Singlet oxygen ($^1O_2$):** the common name for an electronically excited state of molecular oxygen

**Plastid redox state and the reactive oxygen species pathway.** The plastid redox signals are proposed to derive from multiple sources, including the plastoquinone (PQ) pool, the acceptor availability of photosystem I (PSI), and the thioredoxin system (7, 103) (**Figure 2**). Several nuclear genes are under the control of plastid redox signals, but different genes respond to signals originating from different sources. For instance, the expression of *petE*, *Lhcb*, and *apx* is regulated by the redox state of the PQ pool (28, 54, 107, 128), whereas the expression of *psaD*, *psaF*, *ferredoxin*, and *nitrate reductase* are regulated by components in the electron-transport chain, distinct from the PQ pool (105, 108, 128).

The redox signal seems to modulate PhANG expression in response to fluctuating light conditions. The redox signal originating from the PQ pool plays an important role in the long-term adaptation of plants to changes in light quality (103), whereas the modulation of PhANG expression in response to short-term changes in illumination depends mainly on the redox state of the PSI acceptor site, especially during early phases of signaling (108). In addition to responses to excess light, the PQ redox signal may be involved in crosstalk between light acclimation and immunity via LESION SIMULATING DISEASE 1 (LSD1) (92).

Little is known about the mechanism of redox retrograde signaling. At present, the best candidate for sensing PQ redox signals is STN7, a thylakoid light-harvesting complex II (LHCII) membrane protein kinase involved in state transitions and photosynthetic acclimation (14, 101). The *Arabidopsis stn7* mutant exhibits differential expression of PhANGs compared with the wild type, suggesting that STN7 may participate in transduction of the redox signal from plastids to the nucleus (101). Six redox-imbalanced *Arabidopsis* mutants (*rimb*), in which the expression of the nuclear gene encoding the antioxidant enzyme 2-Cys-peroxiredoxin is uncoupled from the redox state of the PSI acceptor site, have been isolated to identify components with a defined role in redox signaling (40). Nevertheless, none of these *RIMB* genes have been cloned. Kindgren et al. (58) recently identified a chloroplast component, PLASTID REDOX INSENSITIVE 2 (PRIN2), involved in redox-mediated retrograde signaling (**Figure 2**). PRIN2 is a nucleoid protein associated with the plastid-encoded RNA polymerase (PEP) machinery. The *Arabidopsis prin2* mutants show misregulation of PhANGs in response to excess light and inhibition of photosynthetic electron transport, suggesting a direct role for PEP activity in redox-mediated retrograde signaling. Indeed, similar to *prin2*, another mutant with impaired PEP activity also demonstrates reduced *Lhcb* repression (58).

When plant cells are under environmental stress, several chemically distinct ROS molecules are simultaneously generated in various intracellular compartments (5). Plastids are one of the main production sites of several ROS molecules, including singlet oxygen ($^1O_2$), the superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical ($HO^{-\bullet}$) (6) (**Figure 1**). In plastids, ROS molecules can be generated during photosynthesis when $O_2$ rather than $NADP^+$ accepts either energy or an electron from the electron-transport chain (6). Excess excitation can result in the production of $^1O_2$ from triplet chlorophyll at photosystem II (PSII) and from $O_2^{-\bullet}$ and $H_2O_2$ at PSI. ROS molecules derived from plastids are involved in retrograde signaling (5, 35, 81); however, the ROS signaling pathway might be used primarily for stress signaling rather than genome coordination.

Because several chemically distinct ROS molecules are simultaneously generated in plastids under stress, it is difficult to link a particular stress response to a specific ROS. However, a specific function for $^1O_2$ in retrograde signaling was discovered in the conditional fluorescent *Arabidopsis* mutant *flu*, which allows the induction of only $^1O_2$ within plastids in a noninvasive and controlled manner (86) (**Figure 2**). Global gene expression studies on *flu* plants indicated that $^1O_2$ activates primarily genes encoding

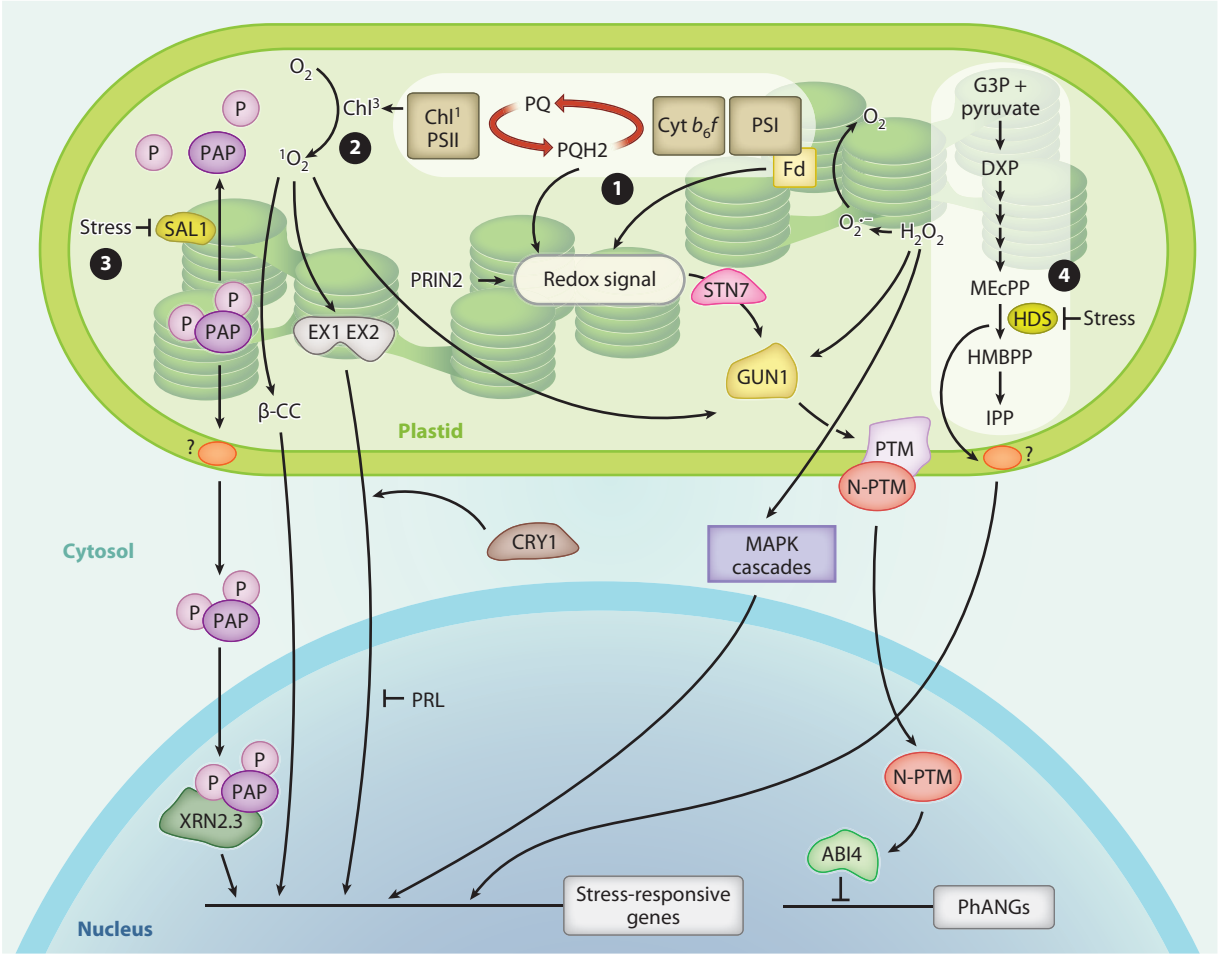


**Figure 2**

Plastid retrograde signaling is involved in stress responses of vascular plants. The following distinct retrograde signals are known to be involved in stress responses of vascular plants. ① Redox signals are generated through the reduced state of plastoquinone (PQ) or via elements on the reducing side of photosystem I (PSI). STN7 may be involved in transmitting the changes in chloroplast redox status to the nucleus. PRIN2 contributes to the production of plastid redox signals. ② $H_2O_2$ and $^1O_2$ accumulate during exposure to excess light and activate distinct signaling pathways. The $^1O_2$ accumulated in the plastid is sensed or transmitted to the nucleus via the concerted action of two chloroplast proteins, EXECUTER 1 and EXECUTER 2 (EX1 and EX2). In the cytosol/nucleus, the blue-light photoreceptor CRY1 is involved in $^1O_2$-mediated stress responses, but PRL may act as a general negative regulator of $^1O_2$ signaling. One product of the $^1O_2$ oxidation of carotenoids, β-cyclocitral (β-CC), may act as a second messenger involved in the $^1O_2$ signaling pathway in plants. Reactive oxygen species and redox signaling may also converge on the GUN1-PTM-ABI4 pathway. ③ Plant stress triggered by drought or high light inhibits the activity of SAL1 and enhances the accumulation of PAP in the plastid. PAP is transported to the nucleus by unknown mechanisms and inhibits XNR2 and XNR3 activities, thereby inducing gene expression associated with stress responses. ④ MEcPP, an isoprenoid precursor derived from the MEP pathway for isoprenoid biosynthesis in the plastid, is induced by stress and functions as a sensor and a communication signal to the nucleus that induces selected stress-responsive genes through alteration of nuclear architecture and functional dynamics.

components involved in cell death, and fewer than 15% of the $^1O_2$-responsive genes are predicted to encode plastid proteins, suggesting that $^1O_2$ has nonplastid functions (98). Extensive genetic screens to identify constituents involved in $^1O_2$-mediated plastid-to-nucleus signaling have found two plastid-localized proteins, EXECUTER 1 and EXECUTER 2, that act as sensors and/or mediators of $^1O_2$ accumulation (76, 145) (**Figure 2**). In contrast, the screens failed to identify extraplastidic signaling components, suggesting that once $^1O_2$-mediated signals have been transferred from the plastid to the surrounding cytoplasm, they are translocated to the nucleus not through a single linear pathway but rather through a complex signaling network (76). However, a nuclear WD40 repeat protein involved in this signaling network, PLEIOTROPIC RESPONSE LOCUS 1 (PRL1), was recently identified in *Arabidopsis* via a novel genetic screening approach (10) (**Figure 2**). PRL1 may act as a general negative regulator of $^1O_2$ signaling: Inactivation of PRL1 leads to constitutive upregulation of $^1O_2$-responsive genes. In *Chlamydomonas*, the basic leucine zipper (bZIP) transcription factor SINGLET OXYGEN RESISTANT 1 (SOR1) was recently shown to be involved in the signal transduction and activation of $^1O_2$-mediated response (33). SOR1 regulates the expression of various oxidative stresses and reactive electrophile species (RES) detoxification genes in response to RES signals. This study indicated that RES-stimulated and SOR1-mediated responses of detoxification genes are part of the $^1O_2$-induced acclimation process in *Chlamydomonas* (33).

Although different ROS molecules activate distinct signaling pathways, these signaling pathways do not always operate independently but rather merge into a complex signaling network that integrates various cues. It appears that $^1O_2$-mediated signaling interacts with $H_2O_2$-mediated signaling, as overexpression of an $H_2O_2$ scavenger enhances the intensity of $^1O_2$-mediated stress responses in the *flu* mutant (71). In addition, the network of ROS retrograde signaling appears to be more complex than expected. For instance, disruption of plastid protein homeostasis by attenuating protein synthesis in *Arabidopsis* affects $^1O_2$-dependent retrograde signaling, although the molecular mechanism for this remains unknown (87, 124).

**Novel plastid retrograde signaling pathways.** Besides the above intensively studied retrograde signaling pathways, a novel SAL1-PAP retrograde signaling pathway that functions in drought and high-light responses was proposed based on characterization of the *Arabidopsis altered apx2 expression 8* (*alx8*) mutant (29) (**Figure 2**). The *alx8* mutant is drought tolerant (119, 147). However, it exhibits constitutive upregulation of 25% of the high-light-regulated transcriptome, including *ZAT10*, *DREB2A*, *ELIP2*, and *APX2* (119, 147). ALX8 is a phosphatase that modulates PAP levels, which may be a retrograde signal that regulates nuclear gene expression by inhibiting nuclear exoribonucleases that target certain stress-responsive genes (29).

The methylerythritol phosphate (MEP) pathway, which is responsible for isoprenoid biosynthesis, represents another retrograde signaling pathway in plant adaptation to stresses (150) (**Figure 2**). Isoprenoids are the most diverse group of plant metabolites reported to date and participate in a wide range of physiological processes, including photosynthesis (chlorophyll and carotenoids) and seed germination [gibberellic acid and abscisic acid (ABA)] (144). A mutation in the *Arabidopsis HDS* gene of the MEP pathway leads to constitutive expression of *HPL*, a stress-inducible nuclear gene encoding the hydroperoxide lyase plastidic enzyme in the HPL branch of the oxylipin pathway (20). HDS functions in the conversion of MEcPP to hydroxymethylbutenyl diphosphate (HMBPP) (117). MEcPP therefore accumulates in this mutant plant as well as increasing under stresses such as high light and wounding (150). MEcPP is proposed to serve as a retrograde signaling metabolite that induces expression of selected stress-responsive genes but does not

coordinate PhANG expression (150). In *Chlamydia trachomatis*, a small molecule produced by the MEP pathway, 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate, has been shown to be directly involved in chromatin remodeling through disruption of the association of histone-like proteins with DNA (39). The MEcPP of plants has been proposed to have a similar function (150); however, the actual mechanism of its participation in gene expression and stress response remains to be investigated.

## Convergence of Multiple Retrograde Pathways

Among the original *gun* mutants, *gun1* is unique because it is the only one to respond similarly to both NF and LIN treatments (88). Earlier studies had shown that *gun1 gun4* and *gun1 gun5* double mutants exhibit enhanced *gun* phenotypes and that the transcriptomes of *gun1* and *gun5* mutants have little similarity to each other, suggesting that GUN1 is unrelated to the tetrapyrrole signaling pathway (88). However, a subsequent study with a stronger allele found that *gun1-9* displays altered responses to PET-dependent, tetrapyrrole-mediated, and PGE-mediated retrograde signals (64). *Lhcb* derepression was enhanced in NF-treated *gun1-9 gun5* plants, and similar expression profiles upon treatment with NF were found in *gun1-9* and *gun5* plants (64). It seems that multiple signal transduction pathways are integrated within the plastids upstream of GUN1 (**Figures 1** and **2**). Transcriptomic and genetic analysis identified the nuclear-localized APETALA 2 (AP2)–type transcription factor ABSCISIC ACID–INSENSITIVE 4 (ABI4) as being downstream of GUN1 in the same pathway (64). A model has therefore been proposed in which GUN1 integrates several signals within the plastid and acts as a master switch that generates or transmits a signal that in turn induces ABI4 to bind to promoter sequences and block the expression of photosynthetic genes in the nucleus, perhaps by inhibiting the access of transcription factors such as LONG HYPOCOTYL 5 (HY5) to the G box (64) (**Figures 1** and **2**; see below for details). However, this model does not involve the export of Mg–proto IX from plastids, as suggested by Ankele et al. (4).

GUN1 encodes a plastid-localized protein with pentatricopeptide repeat (PPR) domains (64). Such domains comprise tandem repeats of 35 residues and are thought to mediate sequence-specific binding to nucleic acids, in particular to RNA (126). Some PPR proteins are involved in posttranscriptional processes such as RNA splicing, editing, processing, and translation in plastids and mitochondria (126). How such a protein integrates multiple signals in plastids remains an open question. So far, the nature of the secondary signal generated by the common pathway has not been revealed. Moreover, not all GUN1-regulated promoters have an ABI4-binding motif overlapping or in close proximity to a G box (64), and ABI4 has no effect on the plastid regulation of reporter genes driven by particular *RbcS* promoters (2). Thus, the data suggest that GUN1-dependent plastid signals may also regulate nuclear gene expression through other mechanisms, which still need to be addressed.

## Possible Plastid Signaling Molecules

The nature of the plastid signaling molecules remains a mystery, although the distinct retrograde signaling pathways have been revealed. Mg–proto IX has long been regarded as the top candidate retrograde signaling molecule. Several pieces of evidence from the *gun2–gun5* mutants support this hypothesis. Wild-type *Arabidopsis* seedlings grown on NF have approximately 15-fold more Mg–proto IX than do untreated controls, whereas *gun2* and *gun5* seedlings grown on NF accumulate much less (131). Direct feeding of Mg–proto IX to leaf protoplasts is sufficient to repress *Lhcb* expression, whereas feeding of other tetrapyrrole intermediates such as porphobilinogen, heme, or proto IX does not repress the expression of a luciferase-encoding gene driven by the *Lhcb* promoter (131). This suggests that

**ABSCISIC ACID–INSENSITIVE 4 (ABI4):** an AP2-type transcription factor of *Arabidopsis*

**LONG HYPOCOTYL 5 (HY5):** a bZIP transcription factor of *Arabidopsis*

Mg–proto IX accumulation is both necessary and sufficient to regulate PhANG expression. Confocal laser scanning microscopy analysis showed that Mg–proto IX accumulates in both the plastids and the cytosol during stress (4). This raises the possibility that the signaling metabolite Mg–proto IX is exported from the plastid, transmitting a plastid signal to the cytosol (4). However, the mechanism of Mg–proto IX transport across the plastid envelope membranes has not been determined.

Although the evidence might seem compelling, the role of Mg–proto IX in plastid signaling after NF treatment was critically reevaluated in two complementary reports (90, 91). Both studies investigated Mg–proto IX accumulation following NF treatment, using either a liquid chromatography–mass spectrometry system or conventional high-performance liquid chromatography with fluorescence detection, and concluded that changes in Mg–proto IX accumulation do not correlate with changes in *Lhcb* expression (90, 91). Thus, doubts about Mg–proto IX functioning as a mobile signaling molecule have emerged. However, there are some possible explanations for the present conflicts in the data. One is that local or transient concentrations of Mg–proto IX could be more important than total steady-state levels in regulating PhANG expression (89, 149), but they are difficult to detect (89). Another possibility is that the tetrapyrrole-generated signal may be directly or indirectly derived from ROS and/or redox signals because impaired tetrapyrrole biosynthesis may cause localized ROS generation or changes in the plastid's redox status (90, 91), which might trigger retrograde signaling. Alternatively, ChlH may be involved in retrograde signaling (88).

Heme, another tetrapyrrole intermediate, has also been implicated in retrograde control of nuclear gene expression. Yeast (*Saccharomyces cerevisiae*) has been shown to use heme synthesized in the mitochondria to regulate transcription of nuclear genes encoding mitochondrial proteins (85, 152). In *Chlamydomonas*, a shift from dark to light results in a transient reduction in heme levels, and heme feeding to cultures in the dark activates *HSP70A*, suggesting that heme may also serve as a plastid signal to regulate the expression of nuclear genes (141). Heme molecules appear to play evolutionarily conserved roles in interorganellar communication. The identification of heme as a retrograde signaling molecule in vascular plants was confirmed in the *gun6* gain-of-function mutant of *Arabidopsis* (149). This mutant overexpresses FC1, an enzyme that inserts iron into proto IX to form heme. Genetic and biochemical approaches demonstrated that heme but not proto IX/Mg–proto IX synthesis is primarily responsible for the regulation of PhANG expression in *gun6*, although a role for the latter has not been completely ruled out (149).

Heme could be considered a more likely candidate than Mg–proto IX to be the retrograde signaling molecule, in part because heme is known to be exported from plastids, whereas the evidence for Mg–proto IX export by healthy plastids is less convincing (140). Moreover, Mg–proto IX is photodynamic and produces toxic ROS in the presence of light, whereas heme is photodynamically inactive (89, 137). If heme really acts as a positive retrograde signal in plastids, then the retrograde signaling mechanism of *gun* mutants needs to be reevaluated. A role for heme as a positive regulator of PhANG expression could provide an alternative explanation for the *gun* mutant phenotypes: The phenotypes of *gun2–gun5* mutants might result from increased heme levels rather than decreased Mg–proto IX levels. Indeed, the introduction of the *fc1* allele into *gun5* partially repressed PhANG expression, suggesting that *gun5* mutants may increase FC1-produced heme by blocking the chlorophyll branch of the tetrapyrrole biosynthesis pathway (149). Nevertheless, tetrapyrrole metabolism is complex and regulated at multiple levels; further examination of tetrapyrroles as signaling molecules requires more intensive work with novel approaches.

In addition to tetrapyrroles, two small metabolites, PAP and MEcPP, are synthesized in plastids and thought to serve as signals to communicate perturbations in the subcellular

environment to the nucleus. PAP was originally regarded as a by-product with no physiological role in plant cells, but it accumulates under drought or exposure to excess light in *Arabidopsis* (29). To reach the nucleus, PAP must exit the plastid and enter the nucleus. A line of genetic evidence supports the movement of PAP between cellular compartments: PAP is assumed to be synthesized in plastids, but the expression of SAL1 (which dephosphorylates PAP to AMP) in the nucleus lowers the total PAP contents (29). However, in vivo movement of PAP between organelles has not yet been observed. Similarly to PAP, the potential involvement of MEcPP in plastid retrograde signaling not only provides new insights into the communication between plastids and the nucleus but also raises new questions (150). For instance, how MEcPP might sense abiotic stresses and how it could be transported to the nucleus remain a mystery. Discovering the underlying gene regulatory mechanisms triggered by the MEcPP signaling pathway is an exciting project for the future.

Based on current data, metabolites are the most likely candidates to be the retrograde signaling molecules, although at present it is difficult to unambiguously verify that any specific metabolite is a signaling molecule (77). The idea of a metabolite signal seems reasonable because the plastid could continuously communicate its metabolic state to the cytosol via the exchange of various metabolites, and changes in the metabolic profile could be sensed by cytosolic or nuclear receptors and lead to the alteration of nuclear gene expression (77, 106). Several metabolites, such as carbohydrates and redox valves, have therefore been regarded as potential candidates for retrograde signaling molecules (60). Nevertheless, the assignment of a metabolite as a retrograde signaling molecule should be more cautious (106). For example, alteration of the plastids' redox state results in a transient increase in intracellular sucrose concentration in *Arabidopsis*; however, this transient accumulation has a different biological role and seems to not be a retrograde signal (16).

ROS molecules are also considered candidate retrograde signaling molecules. An important characteristic of a signaling molecule is the ability to move across subcellular compartments. In this regard, $H_2O_2$ is the best candidate. It has high stability and can move between cellular compartments, whereas $^1O_2$ is thought to react close to its production site and is unlikely to serve as a long-distance signal because of its high reactivity, short half-life, and limited diffusion ability (63, 66, 93).

Nevertheless, ROS molecules are produced at different sites in the cell and respond to various stimuli. Therefore, a significant task is to envision how plastid-generated ROS could specifically participate in the communication between the nucleus and plastids. The toxicity of ROS molecules, which causes the oxidation of biomolecules, should also be considered. One product of the $^1O_2$ oxidation of carotenoids, β-cyclocitral, has been identified as a likely messenger involved in the $^1O_2$ signaling pathway in *Arabidopsis*, as it could induce changes in the expression of a large set of genes that have been identified as responsive to $^1O_2$ (113) (**Figure 2**). β-cyclocitral is a volatile derivative; thus, it has the potential ability to traverse the cytosol to regulate gene expression in the nucleus. It could therefore function as a retrograde signal that acts as a downstream messenger of or proxy for rising $^1O_2$ levels (113).

## NUCLEAR RESPONSES TO PLASTID RETROGRADE SIGNALS

### Nuclear Genes Regulated by Plastid Signals

The expression of a subset of nuclear genes encoding plastid-localized proteins, such as *Lhcb* genes, is frequently analyzed to dissect the response of nuclear genes to plastid retrograde signals (135). Recent studies using large-scale analysis of transcript profiles have revealed that the retrograde signals induce massive reprogramming in the transcriptome (31, 64, 69, 98, 143). Large sets of nuclear genes are

possible targets of the retrograde signaling. These include not only PhANGs but also genes involved in many different processes, such as stress responses, cell death, and cellular metabolism (16, 98, 143).

The first study of transcriptome responses to plastid retrograde signaling was carried out on the *Arabidopsis ppi1* mutant, which lacks the chloroplast protein import receptor AtToc33 (69). Transcriptomic analysis of nuclear genes encoding plastid proteins in *ppi1* indicated that the mutation in *PPI1* specifically causes the downregulation of PhANGs but not of genes in other functional categories, suggesting the existence of retrograde signaling networks coordinating plastid protein import and PhANG expression (69). Microarray analyses identified more than 1,000 nuclear genes that were differentially expressed in *gun1* or *gun5* compared with the wild type grown on NF (64). Approximately 500 of these genes were repressed by NF treatment in the wild type, of which 330 were expressed in both *gun1* and *gun5*, suggesting that the retrograde signaling regulates a large number of nuclear genes with diverse functions (64). In *Chlamydomonas*, global changes of gene expression in response to heme and Mg–proto IX feeding indicate that the expression levels of almost 1,000 genes change transiently but significantly (143). Among them were only a few genes for photosynthetic proteins, suggesting that both tetrapyrroles play signaling roles as secondary messengers for adaptive responses affecting the entire cell (143).

The transcriptomic responses to redox and ROS retrograde signals have also been analyzed in great detail. Shifting conditions between PSI light and PSII light changes the plastid redox state, and 286 genes among 3,292 probe spots specifically respond to the reduced plastid redox state in *Arabidopsis* (31). Within 30 min of the release of $^1O_2$ in plastids, approximately 300 nuclear genes are rapidly upregulated at least threefold in *flu* plants compared with the wild type (98). Many of those genes have been previously shown to be under the control of phytohormones, in particular ABA, ethylene, methyl jasmonate, and salicylic acid. Bräutigam et al. (16) dissected the time-dependent impact of redox signals on the transcriptome and metabolome of *Arabidopsis*. They found that plastid redox signaling is highly dynamic and induces differential and specific expression patterns for genes associated with photosynthesis and metabolism. It appears that the redox signal coordinates photosynthesis, gene expression, and metabolism in the long-term response of the plants (16).

There are different layers of retrograde control over the transcriptional expression of nuclear genes encoding plastid proteins, and two distinct mechanisms are probably involved in this control (77). One is a proposed "master switch" that induces or represses the same large set of nuclear genes in a binary manner (12, 116). The other is coregulation of nuclear and plastid genes for photosynthetic and plastid components (12, 82), which seems to be widely conserved in eukaryotes (125). Indeed, large-scale analysis of the transcript profiles of plastid- and mitochondrion-related genes in *Arabidopsis* showed that the activity of gene sets involved in organellar energy production or organellar gene expression in each of the organelles and the nucleus is highly coordinated (78).

## Possible *Cis*-Elements Responsive to Retrograde Signals

The regulation of nuclear gene expression by plastid signals occurs mainly at the transcriptional level, and *cis*-elements within the promoters of the nuclear genes are involved in this process. Several studies have identified *cis*-elements required for retrograde regulation. Interestingly, these *cis*-elements are either identical to or largely overlapping with light-responsive elements. In every case that has been examined, the light- and plastid-responsive elements (PREs) are inseparable (13, 70, 129, 142). In *Chlamydomonas*, a PRE with qualities of an enhancer was identified in the *HSP70A* promoter (141). PRE mediates induction of *HSP70A* by heme, Mg–proto IX, and light. Thus, these signals are proposed to converge onto one pathway involving PRE (141).

In vascular plants, G-box and GATA elements are found in many nuclear genes and respond to both light and plastid retrograde signals. Within the promoters of *Lhcb* genes, two light-responsive elements were identified: a GATA element and the *cab* upstream factor 1 (CUF1) element, which is similar to a G box (112). Luciferase accumulation under the control of an *Lhcb1* promoter with a mutated CUF1 element was reduced to wild-type levels in *gun5* seedlings grown on NF, confirming the involvement of CUF1 in retrograde signaling (131). Regarding the *Rbcs* promoter, CMA5—a light-responsive unit that not only positively responds to light signals but also negatively responds to sugars and ABA—was identified (2). CMA5 contains a G box and a putative ABI4-binding site (named the S box) that is closely associated with the G box (2). Close association or even overlap of the ABI4-binding site with the G box has been found not only in *Rbcs* promoters but also in the promoters of several *Lhcb* and plastocyanin-encoding genes (2). Based on this evidence, Rook et al. (118) proposed a model for a negative regulatory mechanism involving competition for binding between activators and repressors. As light- and plastid-specific signals appear to act on the same *cis*-element, this mechanism could also be suitable for plastid signaling responses. Indeed, analysis of the promoters of 330 genes that are expressed in both *gun1* and *gun5* grown on NF indicated that the association of the G box and the ABI4-binding site is common to most of them but that this ABI4-binding site is significantly smaller than previously described ABI4-binding sites (64).

## Transcription Factors Involved in Plastid Retrograde Signaling Pathways

The G box, which commonly exists in light-regulated promoters, is essential for plastid retrograde signaling–controlled transcriptional activity. Several transcription factors have been identified through screens for G-box binding proteins (84, 136). Two of them, HY5 and GLK, were found to act downstream of plastid retrograde signaling.

*GLK*, defined as *GOLDEN 2* in maize (*Zea mays*), encodes a GARP nuclear transcription factor (120). *GLK* is present in diverse groups of vascular plants, and each species contains at least two *GLK* genes (34, 151). In *Arabidopsis* and moss (*Physcomitrella patens*), each gene acts largely redundantly to promote PhANG expression in all photosynthetic cell types (34, 151). GLK proteins regulate a large set of genes encoding photosynthetic thylakoid membrane proteins, and this regulation may depend on its direct binding to the promoters of genes such as *Lhcb* (136, 146). *GLK* genes are sensitive to plastid-derived retrograde signals, at least one of which is term-InText*GUN1*term-InText independent (146). GLK1 may act as a positive regulator in the plastid-to-nucleus signaling pathway that coordinates plastid protein import and nuclear gene expression in response to the functional state of plastids, but this pathway is not associated with ABI4 activation (53).

HY5 is a bZIP transcription factor that acts downstream of several photoreceptors, including CRY1, to induce PhANG expression (49). CRY1 is involved in the $^1O_2$-mediated regulation of nuclear gene expression and programmed cell death response, suggesting its role in plastid-to-nucleus signaling (23) (**Figure 2**). Genetic screens have identified four mutants with subtle *gun* phenotypes, which turned out to be allelic to the *cry1* mutant (122). The *cry1* mutants treated with LIN accumulate approximately 5–10% of the *Lhcb* mRNA levels that accumulate in wild-type seedlings untreated with LIN, in contrast to the 2–3% that accumulates in LIN-treated wild-type seedlings. The *cry1 gun1* double mutant shows much stronger derepression of *Lhcb* when grown on LIN than did either single mutant (122). These results indicate that CRY1 acts as a negative factor to repress *Lhcb* expression when plastid biogenesis is blocked. In addition, CRY1 and GUN1 act synergistically to repress most, if not all, *Lhcb* repression under blue light (122). Like *cry1*, *hy5* is a subtle *gun* mutant, but a *hy5 cry1* double mutant is indistinguishable from either single mutant in blue light, suggesting that CRY1 and HY5 operate in the

same pathway. A follow-up experiment showed that the conversion of CRY1 from a positive to a negative regulator of *Lhcb* results largely from the conversion of HY5 from a positive to a negative regulator of *Lhcb*, possibly assisted by an unknown factor (74, 122).

The CRY1-HY5 model seems to conflict with the previous GUN1-ABI4 model, in which HY5 may act as a positive factor (64). It appears that ABI4 and HY5 are simultaneously required for plastid-dependent repression of *Lhcb* or that at least one of these factors represses *Lhcb* by an indirect mechanism (74). Future studies aimed at detecting ABI4 or HY5 binding to the *Lhcb* promoter in vivo in seedlings treated with inhibitors of plastid function will be helpful to address this question. The integration of light and plastid signals is not surprising (74, 80, 121–123), as plastid signals and light signals can regulate the expression of PhANGs using common or adjacent promoter elements (13, 64, 70, 129, 141, 142). Such integration appears to be essential not only for chloroplast biogenesis but also for chloroplast maintenance under stress conditions (74, 121, 122).

The ABI4 protein is a nuclear-localized AP2-type transcription factor that was originally identified as playing a role in ABA signaling (32). Dijkwel et al. (26) fused the promoter of the nuclear-encoded plastocyanin gene to the luciferase reporter gene and screened for mutants defective in the sugar-responsive repression of the transgene. One of these *sucrose uncoupled* (*sun*) mutants, *sun6*, is allelic to *abi4*, suggesting an additional role for ABI4 in sugar signaling (46). ABI4 has been found to be associated with plastid retrograde signaling in several cases. PET-induced gene expression is inhibited by sugar, and this inhibition requires ABI4, as evidenced by the finding that in the *sun6* (*abi4*) mutant, exogenous sucrose does not inhibit PhANG expression (100). The wide distribution of ABI4-binding sites in the promoters of plastid signaling–responsive genes suggests that it may play a general role in plastid retrograde signaling (64). In agreement with this, it is supposed based on *gun1* mutant phenotypes that ABI4 may be a component of the previously proposed master switch required for the regulation of nuclear genes in response to developmental cues and environmental signals integrated within plastids (64).

Although ABI4 is involved in plastid retrograde signaling, such involvement does not appear to be associated with its role in ABA signaling. Unlike *abi4*, other *abi* mutants do not show *gun* phenotypes (64). Moreover, the addition of ABA, with or without NF, inhibits the accumulation of *Lhcb* transcripts in wild-type and *gun1* seedlings but has no effect on *abi4* seedlings. It seems that ABA might not be a plastid-derived retrograde signal (64). However, the involvement of ABI4 in plastid retrograde signaling is likely to be associated with its role in sugar signaling, as an obscure connection between sugar and retrograde signaling has been reported (22, 64, 100). For instance, 7% glucose significantly reduces *Lhcb* expression when applied to three-day-old wild-type seedlings but not when applied to *gun1* seedlings (64). Sucrose and LIN have an additive effect in repressing *Lhcb* expression in wild-type seedlings, whereas LIN partially releases *Lhcb* from sucrose repression in *gun1* seedlings (22). In addition, ABI4 is a regulator of mitochondrial retrograde signaling (36) and therefore likely serves as an integration point for organellar retrograde signals and sugar signals.

## RETROGRADE SIGNAL TRANSDUCTION FROM PLASTIDS TO THE NUCLEUS

Retrograde signals are produced in plastids, but how retrograde signals are perceived in the cytosol and communicated to the nucleus remains largely unknown. Retrograde signals involved in stress responses are presumed to interact with stress signaling pathways at some level. Therefore, it appears reasonable that plastid retrograde signals could utilize known cytosolic components of stress signaling pathways. However, there are also specific signal transduction pathways for plastid retrograde signaling.

## Protein Movement from Plastids to the Nucleus

The GUN1-derived plastid retrograde signal raises the question of how this signal is transmitted to nuclear-localized ABI4 to repress nuclear gene expression. A study by Sun et al. (133) provided genetic and biochemical evidence that PTM, a plastid envelope–bound plant homeodomain (PHD) transcription factor, functions in this process (**Figures 1** and **2**). PTM acts downstream of GUN1 and has a role in transmitting multiple chloroplast-derived signals to the nucleus, similar to ABI4 and GUN1. The PTM protein has N-terminal DNA-binding homeobox and different transcription factors (DDT) and PHD domains with highly conserved secondary and tertiary structures and four transmembrane domains at its C terminus. Full-length PTM was detected exclusively in the plastid outer envelope membrane, whereas an N-terminal fragment (N-PTM) without transmembrane domains was detected in nuclear fractions (133). Treatments with NF and LIN, as well as exposure to high light, increased the detection of the N-terminal fragment in the nuclear fractions. In vitro experiments with cycloheximide and protease inhibitors confirmed that N-PTM is derived from full-length PTM, which can be processed by an unidentified protease. The proteolytic cleavage of PTM occurs in response to retrograde signals, and the N-PTM release to the nucleus is dependent on plastid signals (133).

The *ptm* mutant showed a *gun* phenotype when treated with NF and LIN. In addition, when exposed to high light, the *ptm* mutant maintained expression of *Lhcb* compared with the wild type. PTM may therefore have a role in multiple plastid-derived signals to the nucleus, similar to ABI4 and GUN1. Analysis of *ptm gun1* and *ptm abi4* double mutants showed that PTM, GUN1, and ABI4 are involved in the same signaling pathway. The *gun1* phenotype can be suppressed by constitutive expression of N-PTM, and the level of processed N-PTM in *gun1* is lower under stress compared with the wild type, suggesting that PTM acts downstream of GUN1 (133).

*ABI4* expression is reduced in the *ptm* mutant. In addition, in transgenic plants expressing N-PTM proteins fused with a glucocorticoid receptor to control its nuclear localization, the expression of *ABI4* is recovered only in the presence of dexamethasone (133). These results indicate that the nuclear localization of PTM is required for normal *ABI4* expression. Further experiments indicated that N-PTM can activate *ABI4* transcription in a PHD-dependent manner associated with histone modification, which is necessary for the suppression of term-InText*Lhcb*term-InText in response to retrograde signals. Thus, it is clear that PTM plays a critical role in mediating retrograde signals from plastids to the nucleus.

PTM belongs to a family of membrane-bound transcription factors (MTFs) that have been shown to regulate diverse cellular functions through an intriguing regulated proteolytic activation mechanism (21, 44, 127). In *Arabidopsis*, MTFs characterized to date belong to either the bZIP or the NAC family, and most of them are associated with the endoplasmic reticulum and plasma membranes (21, 127). The relocalization of an MTF to associate with plastids was first demonstrated for a plastid envelope DNA-binding protein, PEND (138). However, the functions of most MTFs associated with plastid envelope membranes remain elusive. Studies on PTM raise the possibility of a novel mechanism for plastid-to-nucleus signal transduction via the relocalization of transcription factors. Indeed, the relocalization of regulatory factors may represent a general mechanism in signal transduction. For instance, the cytoplasmic and nuclear relocalization of NRIP1 (a protein that directly interacts with the 50-kDa helicase domain of tobacco mosaic virus) from plastids is necessary for pathogen recognition (17). Another example is the Whirly1 protein, which is translocated from the plastid to the nucleus, where it affects the expression of *PR*

genes such as *PR1* (24). However, Whirly1 appears to not behave like other MTF proteins, because it is the same size in both plastids and the nucleus (37). Further analysis regarding how PTM senses retrograde signals within the plastids and how the PTM transcription factor is activated will be of great interest in understanding retrograde signaling in plants.

## The Tetrapyrrole-HSP Complex

Although the tetrapyrrole intermediates have been revealed to be involved retrograde signaling, it is not clear how they regulate the expression of nuclear photosynthetic genes. One possibility is that they directly interact with cytosolic and/or nuclear transcription factors, as in yeast and humans. In yeast, heme acts as an important retrograde signal resulting from dysfunctional mitochondria via stringent controls of the transcription factor HAP1 (85, 152). In the cytosol, newly synthesized HAP1 forms a complex with chaperone proteins, including HSP90, HSP70, Sro9, and Ydj1 (43, 72). However, the association of HSP90 with this complex is transient (43, 72). As this complex binds to heme, which is released from dysfunctional mitochondria, the association of HSP90 with the HAP1 multichaperone complex is enhanced and leads to the activation of HAP1 (72). Recent studies have demonstrated the interaction between Mg–proto IX and three cytosolic HSP90 proteins in vitro (57, 59). Based on genetic analysis, a regulatory Mg–proto IX–HSP90–HY5 complex, analogous to HSP70-HSP90-HAP1 in yeast, was proposed, although no direct interaction was found between HSP90 and HY5 (59). Nevertheless, it appears likely that HSP90 and HY5 are involved in the regulation of PhANGs in response to perturbations of tetrapyrrole biosynthesis (**Figure 1**).

## Cytoplasmic Kinases and Phosphatases

Several pieces of evidence indicate that cytoplasmic kinases and phosphatases may be involved in the transduction of redox retrograde signals from plastids. With regard to vascular plants, both kinase and phosphatase inhibitors affect GUS reporter gene expression driven by the spinach *PsaF* promoter, which is regulated by the PET redox signal (19). In the green alga *Dunaliella tertiolecta*, the phosphatase inhibitor okadaic acid can block the low-light accumulation of cellular chlorophyll controlled by the redox state of the PQ pool (28). In addition, *Lhcb* and *CAO* induction after a shift from high light to low light is blocked by a protein kinase inhibitor (83). A model that involves the action of cytosolic kinase activities in the control of *Lhcb* expression has been proposed for *D. tertiolecta* (28). Protein phosphorylation may also be involved in ROS-mediated plastid-to-nucleus signal transduction (**Figure 2**). $H_2O_2$ is assumed to activate a mitogen-activated protein kinase (MAPK) cascade in the cytosol that subsequently affects gene expression in the nucleus (5, 65), which would represent an example of ROS retrograde signal transduction.

## CONCLUSION AND PERSPECTIVES

Our understanding of the mechanisms involved in plastid-to-nucleus signaling has advanced greatly over the past few years. One of the major breakthroughs is the identification of more candidate signal molecules. However, along with that, more open questions and/or subjects of debate have been raised. PGE and the plastid redox state have been considered to be sources of retrograde signals for several years, but we still know nothing about the nature of the signal(s) they produce. The involvement of tetrapyrrole intermediates in plastid retrograde signaling has also been challenged. Nevertheless, it is clear that perturbation of tetrapyrrole biosynthesis alters PhANG expression. Whether this is due to a direct effect of the mutations or a secondary effect should be investigated further. Besides the role of tetrapyrrole intermediates, roles of other metabolites in plastid-to-nucleus signaling are also emerging. However, how they act as

retrograde signals to coordinate communication between plastids and the nucleus is still obscure. For theoretically ideal signals like metabolites, dynamic correlations between the signal at the molecular level and nuclear gene expression should be established. At present, this appears to be very difficult owing to technical limitations, but systematic biology approaches may open novel ways to overcome them (8, 52).

How retrograde signals are transduced from plastids to the nucleus also remains largely unknown. An idealized route for retrograde signal transduction could be that signal molecules are produced in and exported from plastids and then enter the nucleus to regulate gene expression. This route seems perfect but might be too simplistic because no in vivo movement of any retrograde signal molecule across the plastid, cytosol, and nucleus has yet been detected. Some retrograde signal molecules, like $^1O_2$, may be restricted largely to plastids and transfer information to the cytosol and nucleus via downstream messengers and/or a complex signaling network, representing another route for retrograde signal transduction. A third route for retrograde signal transduction may occur through the movement of proteins, especially transcription factors like PTM, PEND, and Whirly1, from plastids to the nucleus. It seems that PTM is in a position where distinct retrograde signals converge and plays an important role in transmitting common signals from plastids to the nucleus. Further study on the molecular mechanism of PTM action will be of great interest to enhance our understanding of retrograde signal transduction.

At present, distinct retrograde signaling pathways are proposed based on research using different approaches and different experimental models. Therefore, reconstruction of signaling networks and the synergistic and antagonistic integration of diverse retrograde inputs will be a significant challenge for the near future. In this regard, dissection of the gene regulatory network mediated by key transcription factors is promising. ABI4 has been shown to be involved in the integration of three plastid retrograde signals as well as mitochondrial retrograde signals. Another transcription factor, AP2/EREBP, was assigned a novel role in the integration of metabolic, hormonal, and environmental signals in stress acclimation and retrograde signaling (25). Whereas this function is established, many details of signal integration remain elusive (25). In addition to regulating the expression of nuclear genes, plastid-to-nucleus signaling is involved in other cellular processes, ranging from the coordination of organelle and nuclear DNA replication (61, 62) to amyloplast differentiation (27). The underlying signal transduction for the coordination of these processes appears to be intrinsically associated with that involved in the coordination of nuclear gene expression in various contexts. Thus, further elucidation of the integration of distinct signal transduction pathways will be helpful to understand plastid retrograde signaling more comprehensively.

## SUMMARY POINTS

1. In addition to Mg–proto IX, heme is a possible retrograde signal that positively regulates nuclear gene expression.
2. PAP and MEcPP are two novel possible retrograde signals involved in stress responses.
3. Plastid-to-nucleus retrograde signaling involves multiple and partially redundant signaling pathways, but they might converge to regulate nuclear genes.
4. Nuclear responses to plastid retrograde signals involve several layers of transcriptional controls mediated by key transcription factors and *cis*-elements of nuclear gene promoters.

5. The retrograde signal transduction from plastids to the nucleus involves distinct routes, one of which is protein movement from plastids to the nucleus.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

## ACKNOWLEDGMENTS

Our research was supported by the Major State Basic Research Development Program (2009CB118500) and the National Natural Science Foundation of China (30725003).

## LITERATURE CITED

1. Abdallah F, Salamini F, Leister D. 2000. A prediction of the size and evolutionary origin of the proteome of chloroplasts of *Arabidopsis*. *Trends Plant Sci.* 5:141–42
2. Acevedo-Hernandez GJ, Leon P, Herrera-Estrella LR. 2005. Sugar and ABA responsiveness of a minimal RBCS light-responsive unit is mediated by direct binding of ABI4. *Plant J.* 43:506–19
3. Adhikari ND, Froehlich JE, Strand DD, Buck SM, Kramer DM, Larkin RM. 2011. GUN4-porphyrin complexes bind the ChlH/GUN5 subunit of Mg-chelatase and promote chlorophyll biosynthesis in *Arabidopsis*. *Plant Cell* 23:1449–67
4. Ankele E, Kindgren P, Pesquet E, Strand Å. 2007. In vivo visualization of Mg-protoporphyrin IX, a coordinator of photosynthetic gene expression in the nucleus and the chloroplast. *Plant Cell* 19:1964–79
5. Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55:373–99
6. Asada K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiol.* 141:391–96
7. Baier M, Dietz K-J. 2005. Chloroplasts as source and target of cellular redox regulation: a discussion on chloroplast redox signals in the context of plant physiology. *J. Exp. Bot.* 56:1449–62
8. Baldazzi V, Bertin N, de Jong H, Génard M. 2012. Towards multiscale plant models: integrating cellular networks. *Trends Plant Sci.* 17:728–36
9. Barajas-López JD, Blanco NE, Strand Å. 2013. Plastid-to-nucleus communication, signals controlling the running of the plant cell. *Biochim. Biophys. Acta* 1833:425–37
10. Baruah A, Simkova K, Hincha DK, Apel K, Laloi C. 2009. Modulation of $^1O_2$-mediated retrograde signaling by the PLEIOTROPIC RESPONSE LOCUS 1 (PRL1) protein, a central integrator of stress and energy signaling. *Plant J.* 60:22–32
11. Beck CF. 2005. Signaling pathways from the chloroplast to the nucleus. *Planta* 222:743–56
12. Biehl A, Richly E, Noutsos C, Salamini F, Leister D. 2005. Analysis of 101 nuclear transcriptomes reveals 23 distinct regulons and their relationship to metabolism, chromosomal gene distribution and co-ordination of nuclear and plastid gene expression. *Gene* 344:33–41
13. Bolle C, Kusnetsov VV, Herrmann RG, Oelmüller R. 1996. The spinach *AtpC* and *AtpD* genes contain elements for light-regulated, plastid-dependent and organ-specific expression in the vicinity of the transcription start sites. *Plant J.* 9:21–30
14. Bonardi V, Pesaresi P, Becker T, Schleiff E, Wagner R, et al. 2005. Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases. *Nature* 437:1179–82
15. Bradbeer JW, Atkinson YE, Borner T, Hagemann R. 1979. Cytoplasmic synthesis of plastid polypeptides may be controlled by plastid-synthesized RNA. *Nature* 279:816–17
16. Bräutigam K, Dietzel L, Kleine T, Ströher E, Wormuth D, et al. 2009. Dynamic plastid redox signals integrate gene expression and metabolism to induce distinct metabolic states in photosynthetic acclimation in *Arabidopsis*. *Plant Cell* 21:2715–32

17. Caplan JL, Mamillapalli P, Burch-Smith TM, Czymmek K, Dinesh-Kumar SP. 2008. Chloroplastic protein NRIP1 mediates innate immune receptor recognition of a viral effector. *Cell* 132:449–62
18. Chan KX, Crisp PA, Estavillo GM, Pogson BJ. 2010. Chloroplast-to-nucleus communication: current knowledge, experimental strategies and relationship to drought stress signaling. *Plant Signal. Behav.* 5:1575–82
19. Chandok MR, Sopory SK, Oelmüller R. 2001. Cytoplasmic kinase and phosphatase activities can induce *PsaF* gene expression in the absence of functional plastids: evidence that phosphorylation/dephosphorylation events are involved in interorganellar crosstalk. *Mol. Gen. Genet.* 264:819–26
20. Chehab EW, Raman G, Walley JW, Perea JV, Banu G, et al. 2006. Rice HYDROPEROXIDE LYASES with unique expression patterns generate distinct aldehyde signatures in *Arabidopsis*. *Plant Physiol.* 141:121–34
21. Chen YN, Slabaugh E, Brandizzi F. 2008. Membrane-tethered transcription factors in *Arabidopsis thaliana*: novel regulators in stress response and development. *Curr. Opin. Plant Biol.* 11:695–701
22. Cottage A, Mott EK, Kempster JA, Gray JC. 2010. The *Arabidopsis* plastid-signalling mutant *gun1* (*genomes uncoupled1*) shows altered sensitivity to sucrose and abscisic acid and alterations in early seedling development. *J. Exp. Bot.* 61:3773–86
23. Danon A, Coll NS, Apel K. 2006. Cryptochrome-1-dependent execution of programmed cell death induced by singlet oxygen in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 103:17036–41
24. Desveaux D, Subramaniam R, Despres C, Mess JN, Levesque C, et al. 2004. A "Whirly" transcription factor is required for salicylic acid-dependent disease resistance in *Arabidopsis*. *Dev. Cell* 6:229–40
25. Dietz KJ, Vogel MO, Viehhauser A. 2010. AP2/EREBP transcription factors are part of gene regulatory networks and integrate metabolic, hormonal and environmental signals in stress acclimation and retrograde signalling. *Protoplasma* 245:3–14
26. Dijkwel PP, Huijser C, Weisbeek PJ, Chua N-H, Smeekens SCM. 1997. Sucrose control of phytochrome A signaling in *Arabidopsis*. *Plant Cell* 9:583–95
27. Enami K, Ozawa T, Motohashi N, Nakamura M, Tanaka K, Hanaoka M. 2011. Plastid-to-nucleus retrograde signals are essential for the expression of nuclear starch biosynthesis genes during amyloplast differentiation in tobacco BY-2 cultured cells. *Plant Physiol.* 157:518–30
28. Escoubas JM, Lomas M, Laroche J, Falkowski PG. 1995. Light-intensity regulation of *cab* gene-transcription is signaled by the redox state of the plastoquinone pool. *Proc. Natl. Acad. Sci. USA* 92:10237–41
**29. Estavillo GM, Crisp PA, Pornsiriwong W, Wirtz M, Collinge D, et al. 2011. Evidence for a SAL1-PAP chloroplast retrograde pathway that functions in drought and high light signaling in *Arabidopsis*. *Plant Cell* 23:3992–4012**
30. Fernández AP, Strand Å. 2008. Retrograde signaling and plant stress: plastid signals initiate cellular stress responses. *Curr. Opin. Plant Biol.* 11:509–13
31. Fey V, Wagner R, Brautigam K, Wirtz M, Hell R, et al. 2005. Retrograde plastid redox signals in the expression of nuclear genes for chloroplast proteins of *Arabidopsis thaliana*. *J. Biol. Chem.* 280:5318–28
32. Finkelstein RR, Wang ML, Lynch TJ, Rao S, Goodman HM. 1998. The *Arabidopsis* abscisic acid response locus *ABI4* encodes an APETALA2 domain protein. *Plant Cell* 10:1043–54
33. Fischer BB, Ledford HK, Wakao S, Huang SG, Casero D, et al. 2012. *SINGLET OXYGEN RESISTANT 1* links reactive electrophile signaling to singlet oxygen acclimation in *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. USA* 109:E1302–11
34. Fitter DW, Martin DJ, Copley MJ, Scotland RW, Langdale JA. 2002. *GLK* gene pairs regulate chloroplast development in diverse plant species. *Plant J.* 31:713–27
35. Galvez-Valdivieso G, Mullineaux PM. 2010. The role of reactive oxygen species in signalling from chloroplasts to the nucleus. *Physiol. Plant.* 138:430–39
36. Giraud E, Van Aken O, Ho LH, Whelan J. 2009. The transcription factor ABI4 is a regulator of mitochondrial retrograde expression of *ALTERNATIVE OXIDASE1a*. *Plant Physiol.* 150:1286–96
37. Grabowski E, Miao Y, Mulisch M, Krupinska K. 2008. Single-stranded DNA-binding protein Whirly1 in barley leaves is located in plastids and the nucleus of the same cell. *Plant Physiol.* 147:1800–4
38. Gray JC, Sullivan JA, Wang JH, Jerome CA, MacLean D. 2003. Coordination of plastid and nuclear gene expression. *Philos. Trans. R. Soc. Lond. B* 358:135–44

**29. Supplies evidence that PAP may act as a retrograde signal molecule and function in drought and high-light signaling in *Arabidopsis*.**

39. Grieshaber NA, Fischer ER, Mead DJ, Dooley CA, Hackstadt T. 2004. *Chlamydial* histone-DNA interactions are disrupted by a metabolite in the methylerythritol phosphate pathway of isoprenoid biosynthesis. *Proc. Natl. Acad. Sci. USA* 101: 7451–56
40. Heiber I, Stroher E, Raatz B, Busse I, Kahmann U, et al. 2007. The redox imbalanced mutants of *Arabidopsis* differentiate signaling pathways for redox regulation of chloroplast antioxidant enzymes. *Plant Physiol.* 143:1774–88
41. Hess WR, Müller A, Nagy F, Börner T. 1994. Ribosome-deficient plastids affect transcription of light-induced nuclear genes: genetic evidence for a plastid-derived signal. *Mol. Gen. Genet.* 242:305–12
42. Hess WR, Schendel R, Börner T, Rüdiger W. 1991. Reduction of mRNA level for two nuclear encoded light regulated genes in the barley mutant *albostrians* is not correlated with phytochrome content and activity. *J. Plant Physiol.* 138:292–98
43. Hon T, Lee HC, Hach A, Johnson JL, Craig EA, et al. 2001. The Hsp70-Ydj1l molecular chaperone represses the activity of the heme activator protein Hap1 in the absence of heme. *Mol. Cell. Biol.* 21:7923–32
44. Hoppe T, Rape M, Jentsch S. 2001. Membrane-bound transcription factors: regulated release by RIP or RUP. *Curr. Opin. Cell Biol.* 13:344–48
45. Huang YS, Li H. 2009. *Arabidopsis* CHLI2 can substitute for CHLI1. *Plant Physiol.* 150:636–45
46. Huijser C, Kortstee A, Pego J, Weisbeek P, Wisman E, Smeekens S. 2000. The *Arabidopsis SUCROSE UNCOUPLED-6* gene is identical to *ABSCISIC ACID INSENSITIVE-4*: involvement of abscisic acid in sugar responses. *Plant J.* 23:577–85
47. Inaba T, Yazu F, Ito-Inaba Y, Kakizaki T, Nakayama K. 2011. Retrograde signaling pathway from plastid to nucleus. *Int. Rev. Cell Mol. Biol.* 290:167–204
48. Jasper F, Quednau B, Kortenjann M, Johanningmeier U. 1991. Control of *cab* gene expression in synchronized *Chlamydomonas reinhardtii* cells. *J. Photochem. Photobiol. B* 11:139–50
49. Jiao Y, Lau OS, Deng XW. 2007. Light-regulated transcriptional networks in higher plants. *Nat. Rev. Genet.* 8:217–30
50. Johanningmeier U. 1988. Possible control of transcript levels by chlorophyll precursors in *Chlamydomonas*. *Eur. J. Biochem.* 177:417–24
51. Johanningmeier U, Howell SH. 1984. Regulation of light-harvesting chlorophyll-binding protein messenger-RNA accumulation in *Chlamydomonas reinhardi*: possible involvement of chlorophyll synthesis precursors. *J. Biol. Chem.* 259:3541–49
52. Jung HS, Chory J. 2010. Signaling between chloroplasts and the nucleus: Can a systems biology approach bring clarity to a complex and highly regulated pathway? *Plant Physiol.* 152:453–59
53. Kakizaki T, Matsumura H, Nakayama K, Che FS, Terauchi R, Inaba T. 2009. Coordination of plastid protein import and nuclear gene expression by plastid-to-nucleus retrograde signaling. *Plant Physiol.* 151:1339–53
54. Karpinski S, Escobar C, Karpinska B, Creissen G, Mullineaux PM. 1997. Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in *Arabidopsis* during excess light stress. *Plant Cell* 9:627–40
55. Karpinski S, Reynolds H, Karpinska B, Wingsle G, Creissen G, Mullineaux P. 1999. Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. *Science* 284:654–57
56. Keeling PJ. 2010. The endosymbiotic origin, diversification and fate of plastids. *Philos. Trans. R. Soc. Lond. B* 365:729–48
57. Kindgren P, Eriksson MJ, Benedict C, Mohapatra A, Gough SP, et al. 2011. A novel proteomic approach reveals a role for Mg-protoporphyrin IX in response to oxidative stress. *Physiol. Plant.* 141:310–20
58. Kindgren P, Kremnev D, Blanco NE, Barajas-López JD, Fernández AP, et al. 2012. The *plastid redox insensitive 2* mutant of *Arabidopsis* is impaired in PEP activity and high light-dependent plastid redox signalling to the nucleus. *Plant J.* 70:279–91
59. Kindgren P, Norén L, Barajas-López JD, Shaikhali J, Strand Å. 2012. Interplay between HEAT SHOCK PROTEIN 90 and HY5 controls *PhANG* expression in response to the GUN5 plastid signal. *Mol. Plant* 5:901–13
60. Kleine T, Voigt C, Leister D. 2009. Plastid signalling to the nucleus: messengers still lost in the mists? *Trends. Genet.* 25:185–90

61. Kobayashi Y, Imamura S, Hanaoka M, Tanaka K. 2011. A tetrapyrrole-regulated ubiquitin ligase controls algal nuclear DNA replication. *Nat. Cell Biol.* 13:483–87
62. Kobayashi Y, Kanesaki Y, Tanaka A, Kuroiwa H, Kuroiwa T, Tanaka K. 2009. Tetrapyrrole signal as a cell-cycle coordinator from organelle to nuclear DNA replication in plant cells. *Proc. Natl. Acad. Sci. USA* 106:803–7
63. Kochevar IE. 2004. Singlet oxygen signaling: from intimate to global. *Sci. STKE* 221:pe7
64. **Koussevitzky S, Nott A, Mockler TC, Hong F, Sachetto-Martins G, et al. 2007. Signals from chloroplasts converge to regulate nuclear gene expression. *Science* 316:715–19**
65. Kovtun Y, Chiu W-L, Tena G, Sheen J. 2000. Functional analysis of oxidative stress activated mitogen-activated protein kinase cascade in plants. *Proc. Natl. Acad. Sci. USA* 97:2940–45
66. Krieger-Liszkay A. 2005. Singlet oxygen production in photosynthesis. *J. Exp. Bot.* 56:337–46
67. Kropat J, Oster U, Rüdiger W, Beck CF. 1997. Chlorophyll precursors are signals of chloroplast origin involved in light induction of nuclear heat-shock genes. *Proc. Natl. Acad. Sci. USA* 94:14168–72
68. Kropat J, Oster U, Rüdiger W, Beck CF. 2000. Chloroplast signalling in the light induction of nuclear *HSP70* genes requires the accumulation of chlorophyll precursors and their accessibility to cytoplasm/nucleus. *Plant J.* 24:523–31
69. Kubis S, Baldwin A, Patel R, Razzaq A, Dupree P, et al. 2003. The *Arabidopsis ppi1* mutant is specifically defective in the expression, chloroplast import, and accumulation of photosynthetic proteins. *Plant Cell* 15:1859–71
70. Kusnetsov V, Bolle C, Lubberstedt T, Sopory S, Herrmann RG, Oelmüller R. 1996. Evidence that the plastid signal and light operate via the same *cis*-acting elements in the promoters of nuclear genes for plastid proteins. *Mol. Gen. Genet.* 252:631–39
71. Laloi C, Stachowiak M, Pers-Kamczyc E, Warzych E, Murgia I, Apel K. 2007. Cross-talk between singlet oxygen- and hydrogen peroxide-dependent signaling of stress responses in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 104:672–77
72. Lan C, Lee HC, Tang S, Zhang L. 2004. A novel mode of chaperone action: heme activation of Hap1 by enhanced association of Hsp90 with the repressed Hsp70-Hap1 complex. *J. Biol. Chem.* 279:27607–12
73. Larkin RM, Alonso JM, Ecker JR, Chory J. 2003. GUN4, a regulator of chlorophyll synthesis and intracellular signaling. *Science* 299:902–6
74. Larkin RM, Ruckle ME. 2008. Integration of light and plastid signals. *Curr. Opin. Plant Biol.* 11:593–99
75. La Rocca N, Rascio N, Oster U, Rüdiger W. 2001. Amitrole treatment of etiolated barley seedlings leads to deregulation of tetrapyrrole synthesis and to reduced expression of *Lhc* and *RbcS* genes. *Planta* 213:101–8
76. **Lee KP, Kim C, Landgraf F, Apel K. 2007. EXECUTER1- and EXECUTER2-dependent transfer of stress-related signals from the plastid to the nucleus of *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 104:10270–75**
77. Leister D. 2012. Retrograde signaling in plants: from simple to complex scenarios. *Front. Plant Sci.* 3:135
78. Leister D, Wang X, Haberer G, Mayer KF, Kleine T. 2011. Intracompartmental and intercompartmental transcriptional networks coordinate the expression of genes for organellar functions. *Plant Physiol.* 157:386–404
79. Leon P, Arroyo A, Mackenzie S. 1998. Nuclear control of plastid and mitochondrial development in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49:453–80
80. Lepistö A, Rintamäki E. 2012. Coordination of plastid and light signaling pathways upon development of *Arabidopsis* leaves under various photoperiods. *Mol. Plant* 5:799–816
81. Li Z, Wakao S, Fischer BB, Niyogi KK. 2009. Sensing and responding to excess light. *Annu. Rev. Plant Biol.* 60:239–60
82. Maclean D, Jerome CA, Brown AP, Gray JC. 2008. Co-regulation of nuclear genes encoding plastid ribosomal proteins by light and plastid signals during seedling development in tobacco and *Arabidopsis*. *Plant Mol. Biol.* 66:475–90
83. Masuda T, Tanaka A, Melis A. 2003. Chlorophyll antenna size adjustments by irradiance in *Dunaliella salina* involve coordinate regulation of chlorophyll *a* oxygenase (*CAO*) and *Lhcb* gene expression. *Plant Mol. Biol.* 51:757–71


**64. Provides genetic evidence that multiple retrograde signals converge within the chloroplasts; GUN1 and ABI4 are involved in this process.**

**76. Proposes that $^1O_2$-mediated signals are translocated to the nucleus by a complex signaling network rather than a single linear pathway.**

84. Meier I, Gruissem W. 1994. Novel conserved sequence motifs in plant G-box binding proteins and implications for interactive domains. *Nucleic Acids Res.* 22:470–78
85. Mense SM, Zhang L. 2006. Heme: a versatile signaling molecule controlling the activities of diverse regulators ranging from transcription factors to MAP kinases. *Cell Res.* 16:681–92
86. Meskauskiene R, Nater M, Goslings D, Kessler F, den Camp RO, Apel K. 2001. FLU: a negative regulator of chlorophyll biosynthesis in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 98:12826–31
87. Meskauskiene R, Würsch M, Laloi C, Vidi PA, Coll NS, et al. 2009. A mutation in the *Arabidopsis* mTERF-related plastid protein SOLDAT10 activates retrograde signaling and suppresses $^1O_2$-induced cell death. *Plant J.* 60:399–410
88. Mochizuki N, Brusslan JA, Larkin R, Nagatani A, Chory J. 2001. *Arabidopsis genomes uncoupled 5* (*GUN5*) mutant reveals the involvement of Mg-chelatase H subunit in plastid-to-nucleus signal transduction. *Proc. Natl. Acad. Sci. USA* 98:2053–58
89. Mochizuki N, Tanaka R, Grimm B, Masuda T, Moulin M, et al. 2010. The cell biology of tetrapyrroles: a life and death struggle. *Trends Plant Sci.* 15:488–98
90. Mochizuki N, Tanaka R, Tanaka A, Masuda T, Nagatani A. 2008. The steady-state level of Mg-protoporphyrin IX is not a determinant of plastid-to-nucleus signaling in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 105:15184–89

**91. Along with Ref. 90, casts doubt on the most popular candidate signaling molecule, Mg–proto IX.**

**91. Moulin M, McCormac AC, Terry MJ, Smith AG. 2008. Tetrapyrrole profiling in *Arabidopsis* seedlings reveals that retrograde plastid nuclear signaling is not due to Mg-protoporphyrin IX accumulation. *Proc. Natl. Acad. Sci. USA* 105:15178–83**

92. Muhlenbock P, Szechynska-Hebda M, Plaszczyca M, Baudo M, Mullineaux PM, et al. 2008. Chloroplast signaling and *LESION SIMULATING DISEASE1* regulate crosstalk between light acclimation and immunity in *Arabidopsis*. *Plant Cell* 20:2339–56
93. Mullineaux PM, Karpinski S, Baker NR. 2006. Spatial dependence for hydrogen peroxide-directed signaling in light-stressed plants. *Plant Physiol.* 141:346–50
94. Neuhaus HE, Emes MJ. 2000. Nonphotosynthetic metabolism in plastids. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51:111–40
95. Nott A, Jung HS, Koussevitzky S, Chory J. 2006. Plastid-to-nucleus retrograde signaling. *Annu. Rev. Plant Biol.* 57:739–59
96. Oelmüller R, Levitan I, Bergfeld R, Rajasekhar VK, Mohr H. 1986. Expression of nuclear genes as affected by treatments acting on the plastids. *Planta* 168:482–92
97. Oelmüller R, Mohr H. 1986. Photooxidative destruction of chloroplasts and its consequences for expression of nuclear genes. *Planta* 167:106–13
98. op den Camp RGL, Przybyla D, Ochsenbein C, Laloi C, Kim C, et al. 2003. Rapid induction of distinct stress responses after the release of singlet oxygen in *Arabidopsis*. *Plant Cell* 15:2320–32
99. Oster U, Brunner H, Rüdiger W. 1996. The greening process in cress seedlings. V. Possible interference of chlorophyll precursors, accumulated after thujaplicin treatment, with light-regulated expression of *Lhc* genes. *J. Photochem. Photobiol. B* 36:255–61
100. Oswald O, Martin T, Dominy PJ, Graham IA. 2001. Plastid redox state and sugars: interactive regulators of nuclear-encoded photosynthetic gene expression. *Proc. Natl. Acad. Sci. USA* 98:2047–52
101. Pesaresi P, Hertle A, Pribil M, Kleine T, Wagner R, et al. 2009. *Arabidopsis* STN7 kinase provides a link between short- and long-term photosynthetic acclimation. *Plant Cell* 21:2402–23

**102. Demonstrates that the PGE pathway may also persist in mature leaf tissues.**

**102. Pesaresi P, Masiero S, Eubel H, Braun HP, Bhushan S, et al. 2006. Nuclear photosynthetic gene expression is synergistically modulated by rates of protein synthesis in chloroplasts and mitochondria. *Plant Cell* 18:970–91**

103. Pesaresi P, Schneider A, Kleine T, Leister D. 2007. Interorganellar communication. *Curr. Opin. Plant Biol.* 10:600–6
104. Pesaresi P, Varotto C, Meurer J, Jahns P, Salamini F, Leister D. 2001. Knock-out of the plastid ribosomal protein L11 in *Arabidopsis*: effects on mRNA translation and photosynthesis. *Plant J.* 27:179–89
105. Petracek ME, Dickey LF, Nguyen TT, Gatz C, Sowinski DA, et al. 1998. Ferredoxin-1 mRNA is destabilized by changes in photosynthetic electron transport. *Proc. Natl. Acad. Sci. USA* 95:9009–13
106. Pfannschmidt T. 2010. Plastidial retrograde signaling—a true plastid factor or just metabolite signatures? *Trends Plant Sci.* 15:427–35

107. Pfannschmidt T, Schutze K, Brost M, Oelmüller R. 2001. A novel mechanism of nuclear photosynthesis gene regulation by redox signals from the chloroplast during photosystem stoichiometry adjustment. *J. Biol. Chem.* 276:36125–30
108. Piippo M, Allahverdiyeva Y, Paakkarinen V, Suoranta UM, Battchikova N, Aro EM. 2006. Chloroplast-mediated regulation of nuclear genes in *Arabidopsis thaliana* in the absence of light stress. *Physiol. Genomics* 25:142–52
109. Pogson BJ, Albrecht V. 2011. Genetic dissection of chloroplast biogenesis and development: an overview. *Plant Physiol.* 155:1545–51
110. Pogson BJ, Woo NS, Forster B, Small ID. 2008. Plastid signalling to the nucleus and beyond. *Trends Plant Sci.* 13:602–9
111. Pontoppidan B, Kannangara CG. 1994. Purification and partial characterisation of barley glutamyl-tRNA(Glu) reductase, the enzyme that directs glutamate to chlorophyll biosynthesis. *Eur. J. Biochem.* 225:529–37
112. Puente P, Wei N, Deng XW. 1996. Combinatorial interplay of promoter elements constitutes the minimal determinants for light and developmental control of gene expression in *Arabidopsis*. *EMBO J.* 15:3732–43
113. Ramel F, Birtic S, Ginies C, Soubigou-Taconnat L, Triantaphylidés C, et al. 2012. Carotenoid oxidation products are stress signals that mediate gene responses to single to oxygen in plants. *Proc. Natl. Acad. Sci. USA* 109:5535–40
114. Rapp JC, Mullet JE. 1991. Chloroplast transcription is required to express the nuclear genes *Rbcs* and *Cab* plastid DNA copy number is regulated independently. *Plant Mol. Biol.* 17:813–23
115. Reyes-Prieto A, Weber AP, Bhattacharya D. 2007. The origin and establishment of the plastid in algae and plants. *Annu. Rev. Genet.* 41:147–68
116. Richly E, Dietzmann A, Biehl A, Kurth J, Laloi C, et al. 2003. Covariations in the nuclear chloroplast transcriptome reveal a regulatory master-switch. *EMBO Rep.* 4:491–98
117. Rodríguez-Concepción M. 2006. Early steps in isoprenoid biosynthesis: multilevel regulation of the supply of common precursors in plant cells. *Phytochem. Rev.* 5:1–15
118. Rook F, Hadingham SA, Li Y, Bevan MW. 2006. Sugar and ABA response pathways and the control of gene expression. *Plant Cell Environ.* 29:426–34
119. Rossel JB, Walter PB, Hendrickson L, Chow WS, Poole A, et al. 2006. A mutation affecting *ASCORBATE PEROXIDASE 2* gene expression reveals a link between responses to high light and drought tolerance. *Plant Cell Environ.* 29:269–81
120. Rossini L, Cribb L, Martin DJ, Langdale JA. 2001. The maize *Golden2* gene defines a novel class of transcriptional regulators in plants. *Plant Cell* 13:1231–44
121. Ruckle ME, Burgoon LD, Lawrence LA, Sinkler CA, Larkin RM. 2012. Plastids are major regulators of light signaling in *Arabidopsis*. *Plant Physiol.* 159:366–90
**122. Ruckle ME, DeMarco SM, Larkin RM. 2007. Plastid signals remodel light signaling networks and are essential for efficient chloroplast biogenesis in *Arabidopsis*. *Plant Cell* 19:3944–60**
123. Ruckle ME, Larkin RM. 2009. Plastid signals that affect photomorphogenesis in *Arabidopsis thaliana* are dependent on GENOMES UNCOUPLED 1 and cryptochrome 1. *New Phytol.* 182:367–79
124. Saini G, Meskauskiene R, Pijacka W, Roszak P, Sjögren LL, et al. 2011. "*happy on norflurazon*" (*hon*) mutations implicate perturbance of plastid homeostasis with activating stress acclimatization and changing nuclear gene expression in norflurazon-treated seedlings. *Plant J.* 65:690–702
125. Sardiello M, Tripoli G, Romito A, Minervini C, Viggiano L, et al. 2005. Energy biogenesis: one key for coordinating two genomes. *Trends Genet.* 21:12–16
126. Schmitz-Linneweber C, Small I. 2008. Pentatricopeptide repeat proteins: a socket set for organelle gene expression. *Trends Plant Sci.* 13:663–70
127. Seo PJ, Kim SG, Park CM. 2008. Membrane-bound transcription factors in plants. *Trends Plant Sci.* 13:550–56
128. Sherameti I, Sopory SK, Trebicka A, Pfannschmidt T, Oelmüller R. 2002. Photosynthetic electron transport determines nitrate reductase gene expression and activity in higher plants. *J Biol. Chem.* 277:46594–600

**122. Provides genetic evidence that light and plastid retrograde signals interact and that this interaction contributes to efficient chloroplast biogenesis.**

129. Simpson J, Van Montagu M, Herrera-Estrella L. 1986. Photosynthesis-associated gene families: differences in response to tissue-specific and environmental factors. *Science* 233:34–38
130. Strand Å. 2004. Plastid-to-nucleus signalling. *Curr. Opin. Plant Biol.* 7:621–25
131. Strand Å, Asami T, Alonso J, Ecker JR, Chory J. 2003. Chloroplast to nucleus communication triggered by accumulation of Mg-protoporphyrinIX. *Nature* 421:79–83
132. Sullivan JA, Gray JC. 1999. Plastid translation is required for the expression of nuclear photosynthesis genes in the dark and in roots of the pea *lip1* mutant. *Plant Cell* 11:901–10

133. Provides the first evidence for movement of PTM, an MTF, in transmitting plastid signals to the nucleus.

**133. Sun X, Feng P, Xu X, Guo H, Ma J, et al. 2011. Chloroplast envelope-bound PHD transcription factor mediates chloroplast signals to the nucleus. *Nat. Commun.* 2:477**

134. Surpin M, Larkin RM, Chory J. 2002. Signal transduction between the chloroplast and the nucleus. *Plant Cell* 14:S327–38
135. Susek RE, Ausubel FM, Chory J. 1993. Signal-transduction mutants of *Arabidopsis* uncouple nuclear *CAB* and *RBCS* gene-expression from chloroplast development. *Cell* 74:787–99
136. Tamai H, Iwabuchi M, Meshi T. 2002. *Arabidopsis* GARP transcriptional activators interact with the Pro-rich activation domain shared by G-box binding bZIP factors. *Plant Cell Physiol.* 43:99–107
137. Tanaka R, Tanaka A. 2007. Tetrapyrrole biosynthesis in higher plants. *Annu. Rev. Plant Biol.* 58:321–46
138. Terasawa K, Sato N. 2009. Plastid localization of the PEND protein is mediated by a noncanonical transit peptide. *FEBS J.* 276:1709–19
139. Terry MJ, Kendrick RE. 1999. Feedback inhibition of chlorophyll synthesis in the phytochrome chromophore-deficient *aurea* and *yellow-green-2* mutants of tomato. *Plant Physiol.* 119:143–52
140. Thomas J, Weinstein JD. 1990. Measurement of heme efflux and heme content in isolated developing chloroplasts. *Plant Physiol.* 94:1414–23
141. von Gromoff ED, Alawady A, Meinecke L, Grimm B, Beck CF. 2008. Heme, a plastid-derived regulator of nuclear gene expression in *Chlamydomonas*. *Plant Cell* 20:552–67
142. Vorst O, Kock P, Lever A, Weterings B, Weisbeek P, Smeekens S. 1993. The promoter of the *Arabidopsis thaliana* plastocyanin gene contains a far upstream enhancer-like element involved in chloroplast-dependent expression. *Plant J.* 4:933–45
143. Voss B, Meinecke L, Kurz T, Al-Babili S, Beck CF, Hess WR. 2011. Hemin and magnesium-protoporphyrin IX induce global changes in gene expression in *Chlamydomonas reinhardtii*. *Plant Physiol.* 155:892–905
144. Vranová E, Coman D, Gruissem W. 2012. Structure and dynamics of the isoprenoid pathway network. *Mol. Plant* 5:318–33
145. Wagner D, Przybyla D, op den Camp R, Kim C, Landgraf F, et al. 2004. The genetic basis of singlet oxygen-induced stress responses of *Arabidopsis thaliana*. *Science* 306:183–85

149. Along with Ref. 141, provides genetic evidence demonstrating the roles of heme in plastid retrograde signaling.

146. Waters MT, Wang P, Korkaric M, Capper RG, Saunders NJ, Langdale JA. 2009. GLK transcription factors co-ordinate expression of the photosynthetic apparatus in *Arabidopsis*. *Plant Cell* 21:1109–28
147. Wilson PB, Estavillo GM, Field KJ, Pornsiriwong W, Carroll AJ, et al. 2009. The nucleotidase/phosphatase SAL1 is a negative regulator of drought tolerance in *Arabidopsis*. *Plant J.* 58:299–317
148. Woodson JD, Chory J. 2008. Coordination of gene expression between organellar and nuclear genomes. *Nat. Rev. Genet.* 9:383–95

**149. Woodson JD, Perez-Ruiz JM, Chory J. 2011. Heme synthesis by plastid ferrochelatase I regulates nuclear gene expression in plants. *Curr. Biol.* 21:897–903**

150. Proposes that the isoprenoid intermediate MEcPP is a plastid retrograde signaling metabolite coordinating stress-response pathways.

**150. Xiao Y, Savchenko T, Baidoo EE, Chehab WE, Hayden DM, et al. 2012. Retrograde signaling by the plastidial metabolite MEcPP regulates expression of nuclear stress-response genes. *Cell* 149:1525–35**

151. Yasumura Y, Moylan EC, Langdale JA. 2005. A conserved transcription factor mediates nuclear control of organelle biogenesis in anciently diverged land plants. *Plant Cell* 17:1894–907
152. Zhang L, Hach A. 1999. Molecular mechanism of heme signaling in yeast: The transcriptional activator Hap1 serves as the key mediator. *Cell. Mol. Life Sci.* 56:415–26