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Learning the Languages of the Chloroplast: Retrograde Signaling and Beyond

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Abstract

The chloroplast can act as an environmental sensor, communicating with the cell during biogenesis and operation to change the expression of thousands of proteins. This process, termed retrograde signaling, regulates expression in response to developmental cues and stresses that affect photosynthesis and yield. Recent advances have identified many signals and pathways—including carotenoid derivatives, isoprenes, phosphoadenosines, tetrapyrroles, and heme, together with reactive oxygen species and proteins—that build a communication network to regulate gene expression, RNA turnover, and splicing. However, retrograde signaling pathways have been viewed largely as a means of bilateral communication between organelles and nuclei, ignoring their potential to interact with hormone signaling and the cell as a whole to regulate plant form and function. Here, we discuss new findings on the processes by which organelle communication is initiated, transmitted, and perceived, not only to regulate chloroplastic processes but also to intersect with cellular signaling and alter physiological responses.

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OVERVIEW: THE CHLOROPLAST AS AN ENVIRONMENTAL SENSOR

Retrograde signaling: a process in which a stimulus perturbs plastid homeostasis and gives rise to one or more retrograde signals that alter transcriptional through to posttranslational processes and ultimately change plastid function

Biogenic signaling: retrograde signaling during chloroplast biogenesis that coordinates photosystem assembly and maintenance as well as other processes required for fully functional chloroplasts

Retrograde signaling typically refers to communication from organelles to the nucleus. This phenomenon was first observed nearly four decades ago, when Bradbeer et al. (10) found that repression of plastid protein synthesis perturbed cytosolic protein synthesis of nuclear-encoded genes. In the subsequent decades, and especially in the past few years, tremendous progress has been made in identifying signaling components and pathways involved in forming plastids (biogenic signaling) and altering the plastid homeostasis in response to environmental stimuli (operational signaling) (104). The signals range from tetrapyrroles (133, 158) and phosphoadenosines (26) to carotenoid oxidation products (107, 129), isoprenoid precursors (159), carbohydrate metabolites (42, 151), and hydrogen peroxide (H_2O_2); the signal transduction components include chloroplastic (68, 72) and nuclear (26, 68, 132) proteins as well as cytosolic and mobile proteins (53, 136). The signals and pathways function in different developmental, biogenic, and operational processes and in different tissues and species. Beyond these seemingly disparate individual pathways that regulate different processes, there is an increasing recognition that, because of the nature of the retrograde communication, the chloroplast is by extension an environmental sensor (13).

The plastid is attuned to developmental and environmental cues from the very first stages of the plant life cycle. Within a dormant seed, the cotyledons of the embryo contain proplastids, which are the progenitor organelles of chloroplasts. Upon illumination, chloroplasts form either directly from proplastids or via etioplasts. This biogenic transition is achieved by activation of gene transcription followed by protein synthesis, import, and assembly. Thylakoids and the photosynthetic apparatus are then rapidly synthesized and assembled within hours (51). Thus, nuclear transcription and the related cytosolic protein synthesis are highly sensitive to the requirements of, and signals from, the developing chloroplast.

The mature chloroplast is the site of photosynthesis; various metabolic reactions, including amino acid, lipid, starch, and sulfur metabolism; and production of hormones such as abscisic

acid (ABA), oxylipins [12-oxo-phytodieonic acid and jasmonic acid (JA)], and salicylic acid (SA). Fluctuations in the environment affect these processes: Changes in light quality and/or intensity alter the redox poise and excitation balance between photosystem II (PSII) and photosystem I (PSI) [requiring adjustments in light-harvesting complex II (LHCII) phosphorylation and antenna size, among other processes (8)], and both biotic and abiotic stresses (such as drought, high light, and cold) promote the formation of plastid reactive oxygen species (ROS) and metabolic remodeling (13, 86, 107).

It should therefore be apparent that almost any change in the status of the chloroplast in response to the environment, and the subsequent readjustment(s), will require one or more lines of communication. Coordination across multiple subcellular compartments is also necessary for metabolic remodeling in response to environmental fluctuations and for antioxidant responses to ROS and redox poise in the chloroplast. For instance, the metabolic pathway for glutathione biosynthesis involves isoforms of the same enzyme localized to chloroplasts, cytosol, and/or the mitochondria (14), and cytosolic ascorbate peroxidases are activated by chloroplast redox status (57).

Different chloroplastic processes initiate different transcriptional and posttranscriptional responses, demonstrating the need for multiple signals with discrete and complementary functions. Furthermore, because chloroplasts can have heterogeneous states and respond differently to the same stimulus (74), a stress such as high light is not a single perturbation to chloroplast homeostasis but rather a source of multiple signals that vary temporally and spatially.

In this review, we assess the present state of the field, focusing on recent literature, and identify key avenues of future research by examining the links between plastid signals and hormonal signaling, plant development, and whole-plant function. It is timely to reconsider the nature and roles of retrograde signals. The traditional definition places retrograde signaling in a process of bilateral communication between the plastid and the nucleus, leading to the biogenesis of proteins required by the chloroplast. We propose a broader definition of retrograde signaling as a process in which a stimulus perturbs plastid homeostasis and gives rise to one or more retrograde signals that alter transcriptional through to posttranslational processes and ultimately feed back to plastid function (**Figure 1**). In this definition, the stimulus must be developmentally or environmentally relevant in wild-type chloroplasts. Mutagenic studies of chloroplastic proteins will continue to be informative, but they do not demonstrate the function of a given protein in retrograde signaling in wild-type cells, necessitating complementary lines of evidence. The stimulus must also be sensed in plastids: PSI-sourced H_2O_2 is involved in retrograde signaling, but a plasma membrane burst in response to pathogen attack reflects unrelated secondary messenger functions. The response may involve extraplastidic proteins that are still relevant to plastid homeostasis; for example, Ascorbate Peroxidase 2 (APX2) is cytosolic, but it alters total cellular ROS in response to high light.

In this review, we also consider more complicated issues, such as changes to leaf morphology that optimize light capture within a plastid. We additionally discuss whether a particular chloroplast-derived signal can be considered primarily retrograde, primarily a secondary messenger, some combination of both, or neither. For a summary of the retrograde signaling components, interactors, and targets for regulation by chloroplast signals discussed in the article, see **Supplemental Table 1** (follow the **Supplemental Materials link** from the Annual Reviews home page at **http://www.annualreviews.org**).

Plastid homeostasis: the biochemical and molecular state of a plastid that enables optimal performance under particular developmental and environmental conditions

Operational signaling: retrograde signaling by mature chloroplasts in response to environmental stimuli that perturb plastid function, leading to adjustment of chloroplast homeostasis

H_2O_2: hydrogen peroxide

Ascorbate Peroxidase 2 (APX2): a cytosolic H_2O_2 scavenger encoded by the exemplar plastid redox–associated nuclear gene *APX2* and induced by multiple stresses and plastid redox changes

Supplemental Material

RETROGRADE SIGNALING: A CONTINUOUS PROCESS IN THE LIFE OF A PLANT

Figure 2 and **Table 1** summarize the present state of knowledge on retrograde signaling. Substantial progress has been achieved in the past decade; in 2005, only 4 of the more than 40 components shown in **Figure 2** were known to participate in retrograde signaling.

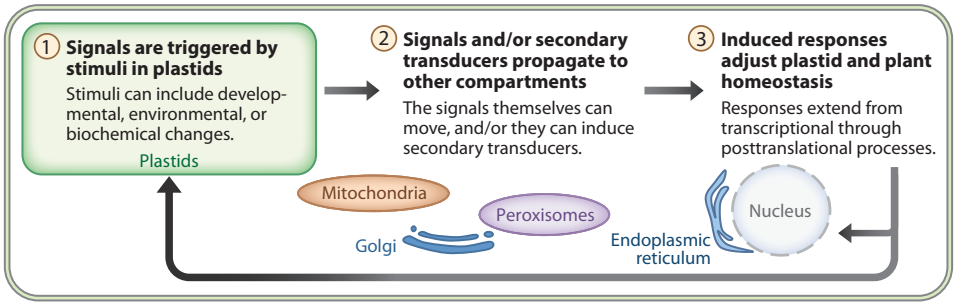

Figure 1

Proposed definition of retrograde signaling, comprising three stages. (①) One or more signals are produced in response to stimuli perceived as changes in plastid homeostasis either during biogenesis (e.g., changes in protein stoichiometry) or following environmental perturbations. (②) The signal or signals propagate to other subcellular compartments, such as the cytosol and nucleus, either directly or via transduction. (③) Transcriptional through to posttranslational responses—largely mediated by the nucleus, although there are a growing number of exceptions—either can be directly targeted to the chloroplast to adjust homeostasis or can involve other subcellular compartments, provided the end result is beneficial to plastid or overall plant function.

Chloroplast Biogenesis

Use of the herbicide norflurazon has provided insights into biogenic signaling pathways during chloroplast development (104). Inhibition of carotenoid biosynthesis by norflurazon or the plastid translational inhibitor lincomycin leads to photobleaching and concomitant repression of photosynthesis-associated nuclear genes (PhANGs) such as those from the chlorophyll-binding *LHCb* gene family (137). Identification of *genomes uncoupled* (*gun*) mutants that exhibit PhANG derepression in response to norflurazon has helped elucidate components of biogenic signaling (1, 69, 83, 133, 136, 137, 157).

Tetrapyrroles as signals. The mutated genes in five of the six *gun* mutants identified thus far map to loci that encode enzymes involved in tetrapyrrole metabolism, demonstrating that this pathway is a key factor in biogenic signaling. GUN5 (83) and GUN4 function in Mg chelation and are thus involved in the biosynthesis of Mg–protoporphyrin IX (Mg-ProtoIX), the first committed precursor of chlorophyll (1, 69). Evidence for a role for Mg-ProtoIX includes (*a*) that 70 of 182 genes downregulated by norflurazon are no longer repressed in *gun5* (133), (*b*) that norflurazon-treated wild-type plants accumulate Mg-ProtoIX (133), and (*c*) that feeding Mg-ProtoIX suppresses PhANG expression (4, 66, 133). Mg-ProtoIX has been proposed to bind to Heat Shock 90 (HSP90)–type proteins (64) and to then interact with the transcription factor Long Hypocotyl 5 (HY5) (66), which regulates PhANGs (71). HY5 acts downstream of photoreceptor signaling, including Cryptochrome 1 (CRY1), mutants of which also exhibit *gun* phenotypes (119). Mg-ProtoIX is also connected to light signaling via interaction with Phytochrome-Associated Protein Phosphatase 5 (PAPP5) (6, 64, 121). However, the role of Mg-ProtoIX has been debated, as other findings suggest a lack of correlation between metabolite levels and gene expression (85, 87, 124). The mechanism for Mg-ProtoIX export from plastids also remains unresolved and strongly debated; thus, the role of GUN4 and GUN5 might instead be to alter tetrapyrrole flux and modulate levels of another tetrapyrrole product, Fe–protoporphyrin IX (heme) (124, 157).

Heme acts as a positive retrograde signal from plastids in algae (152) and in higher plants (157). Three of the six *gun* mutants (*gun2*, *gun3*, and *gun6*) are involved in heme metabolism

Photosynthesis-associated nuclear genes (PhANGs): genes that encode proteins associated with photosystem assembly and maintenance, such as *LHCb* and *CAB*

GUN: Genomes Uncoupled

Heme: Fe–protoporphyrin IX

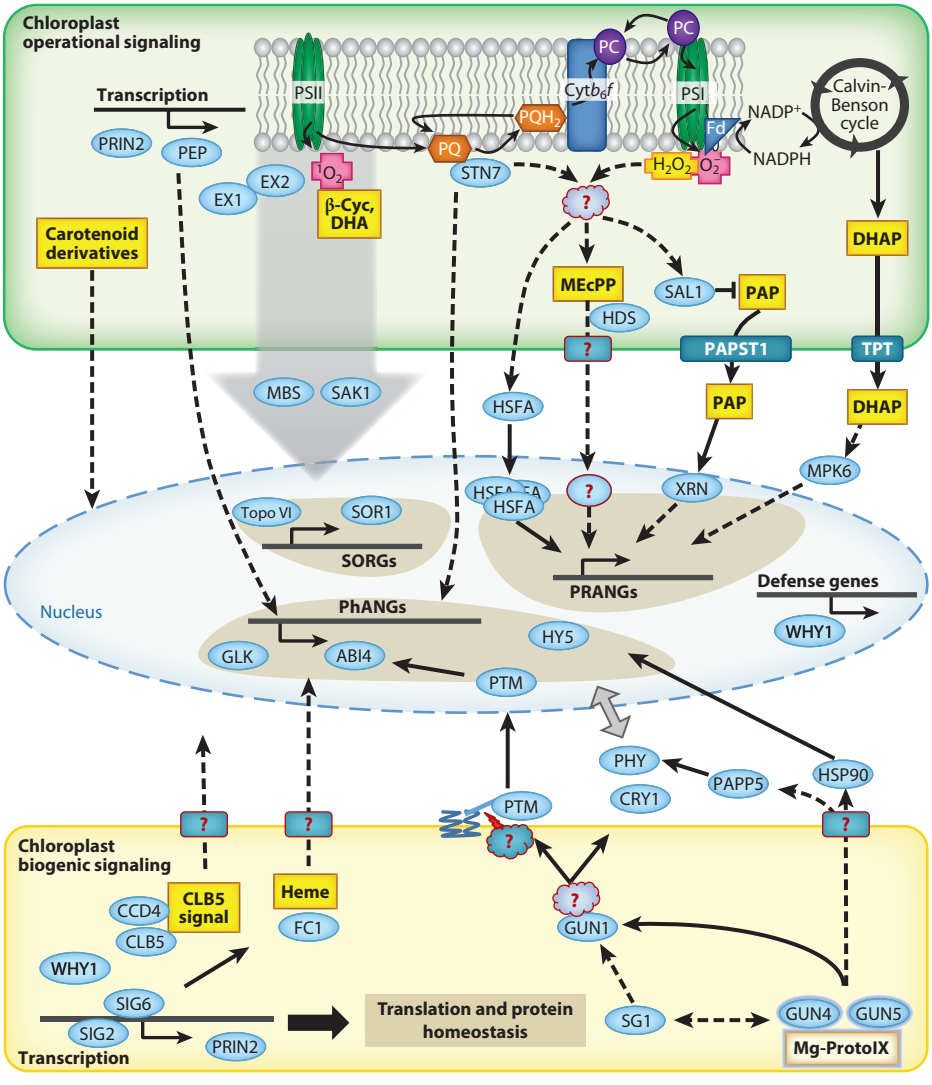

Figure 2

Chloroplast retrograde signaling. Metabolite and protein signals sent from developing chloroplasts (biogenic signaling) and from mature chloroplasts in response to environmental perturbations (operational signaling) regulate genes that can be broadly classified into those associated with photosynthetic protein complexes and function [photosynthesis-associated nuclear genes (PhANGs)], those that respond to singlet oxygen produced from photosystem II [singlet oxygen–responsive genes (SORGs)], and those that respond to general or specific changes in plastid redox poise [plastid redox–associated nuclear genes (PRANGs)]. Dashed and large shadowed arrows indicate components known to regulate nuclear genes via as-yet-undetermined mechanisms or interactions; solid arrows indicate relatively more verified mechanisms. Yellow boxes indicate retrograde signals, and blue ovals and rectangles indicate proteins and transporters, respectively.

Table 1 Summary of known biological processes in which plastid-to-nucleus retrograde communication is active

Purpose of communication	Organelle	Process/stimulus	Retrograde pathway/ components	Nuclear, cellular, or physiological response, with example target genes	Reference(s)
Biogenesis	Chloroplast	Photosystem assembly and maintenance	Heme, tetrapyrroles	Photosynthesis-associated nuclear genes (PhANG) regulation (*LHCb*, *CAB*)	133, 157
			GUN1-PTM-ABI4		68, 136
			PRIN2-PEP	PEP and NEP	65
			GLK		55, 155
Operation		PSII overexcitation ($^1O_2$)	β-Cyclocitral	Programmed cell death (*AAA-ATPase*)	107, 108
			EX1, EX2	Singlet oxygen–responsive genes (SORG) regulation (*GSTU13*, *AAA-ATPase*)	72
		PQ redox state	SAL1-PAP	Plastid redox–associated nuclear genes (PRANG) regulation (*APX2*, *ZAT10*, *ERF*)	26
		PSI overexcitation (H_2O_2, O_2^-)	DHAP-TPT-MPK6		151
			STN7		8
		Plastid redox state[a]	PRIN2	PhANG regulation (*LHCb*, *CAB*)	65
			MEcPP		159
Hormonal crosstalk		SA and biotic stress	MEcPP	Defense (*ICS1*, *HPL*)	159
		Jasmonate synthesis and signaling	SAL1-PAP	JA accumulation (*LOX2*, *OPR3*, *AOC3*)	113
			β-Cyclocitral	Programmed cell death (*AAA-ATPase*)	108
		ABA signaling	SAL1-PAP	Stomatal closure and PRANGs (*APX2*)	116
Development		Shoot development	GUN1	Hypocotyl elongation	119, 137
			SAL1-PAP	Leaf morphology	59, 112
			Apocarotenoids		5
		Fruit development		Chromoplast biogenesis	100
	Nongreen plastids				

[a]Any change in the composition of redox couples (e.g., GSH/GSSG, NADPH/NADP$^+$, or other electrophiles and nucleophiles) that alters the overall redox poise of the plastid.

(83, 157). The dominant *gun6-1D* mutant overexpresses Ferrochelatase 1 (FC1) transcript and protein activity, which derepresses PhANGs (157). The response is specific to FC1 activity, as overexpression of the other chloroplast-localized ferrochelatase, FC2, does not increase PhANG expression. Interestingly, *FC1* overexpression does not alter total heme levels; this finding led Woodson et al. (157) to propose the existence of a specific heme pool, although such a pool has not been experimentally demonstrated. One model places heme as the major tetrapyrrole signal regulating PhANGs, which would adequately explain many phenotypes of *gun* mutants (143). Further research is needed to validate this model, as the transport and metabolism of heme in vivo remain enigmatic (reviewed in 84) and there are indications that 5-aminolevulinic acid (ALA) or flux through tetrapyrroles also contributes to the regulation of PhANGs (21, 124, 158).

Plastid genome expression and signal relay via a mobile transcription factor. GUN1 shares homology with pentatricopeptide repeat (PPR) domain–containing proteins localized to plastids. The *gun1* mutant is unique in exhibiting a *gun* phenotype in response to both norflurazon and lincomycin (68), demonstrating roles for both plastid transcription and translation in the regulation of PhANGs (56, 109, 135). GUN1 signal transduction involves the transcription factor Abscisic Acid Insensitive 4 (ABI4), as the promoters of genes misregulated in *gun1* are enriched for ABA response elements, ABI4 binds to the *LHCb1.2* promoter, and ABI4 overexpression rescues the *gun1* phenotype (68).

The fact that *gun1* reverses nuclear responses to lesions in plastid transcription suggests that GUN1 may relay the status of plastid transcription (65, 141). Impairment of the plastid-encoded polymerase transcriptional complex by *plastid redox insensitive 2* (*prin2*) results in repression of *LHCb* under ambient conditions (65). The repression is released by *gun1*, indicating that GUN1 and PRIN2 may act coordinately in retrograde signaling and plastid transcription (65). However, some PRIN2-specific processes—for instance, transcription of plastid genes and high-light-responsive retrograde signaling—are not dependent on GUN1 (65).

Plastid sigma factors (SIGs) are important components of chloroplast transcription (126). SIG2 and SIG6 regulate PhANGs via partially redundant but distinct pathways (158). Lesions in either gene perturb tetrapyrrole content, most likely because of a deficiency in transcription of $tRNA^{Glu}$, the starting substrate of tetrapyrroles (158). Indeed, the *sig2* phenotype could be rescued by exogenous ALA or by enhancing the flux of the pathway into biosynthesis of the positive signal, heme (158). Significantly, *gun1* complements *sig2* and *sig6*. However, that the complementation was due at least in part to stimulation of tetrapyrrole synthesis in the *gun1* mutant (158) complicates the interpretation because it suggests that, rather than always being a downstream target of tetrapyrroles (68), GUN1 may additionally act upstream (143, 158).

Therefore, the exact biochemical and molecular roles of GUN1 remain enigmatic. As a PPR, it may be expected to contribute to the regulation of plastid mRNA splicing, stability, or translation, but *gun1* does not suffer from loss of chloroplast gene products (7, 158). Nevertheless, a gap in the signal transduction pathway between plastidic GUN1 and nuclear ABI4 was filled by the discovery of a mobile chloroplast membrane-localized plant homeodomain transcription factor called PTM (136). Similar to *gun1*, PhANGs in the *ptm* mutant are derepressed in response to both norflurazon and lincomycin, and GUN1, PTM, and ABI4 are epistatic (136). That is, GUN1, PTM, and ABI4 act in the same signaling pathway, as shown by (*a*) the lack of an additive *gun* phenotype in *gun1 ptm1* and *ptm1 abi4* double mutants compared with the parental mutants and (*b*) the rescue of *gun1* by overexpression of PTM (136). Significantly, a truncated PTM was present in wild-type nuclei, with increased abundance under norflurazon and lincomycin treatments and under high-light conditions via the action of an unknown protease. PTM modulates expression of *ABI4* by directly binding to its promoter (136).

Chloroplastic protein import and homeostasis. Defective chloroplasts in mutants of plastid protein synthesis (99), import (55), and quality control (58) emphasize coordination between chloroplastic protein processing and nuclear transcription. Nuclear control of PhANGs in coordination with protein import involves the related GOLDEN 2–LIKE (GLK) transcription factors GLK1 and GLK2, which are transcriptionally responsive to plastid perturbations (55, 155) and directly bind to the promoters of several PhANGs (155). GLK1 and GLK2 function independently of phytochrome signaling (155), Mg-ProtoIX, and ABI4 (55) but are regulated by GUN1 via distinct, as-yet-unknown mechanisms (55, 155). Hu et al. (47) recently showed that *gun1* partially rescues PhANG repression and defective chloroplast development in *slow greening 1* (*sg1*), a loss-of-function mutant in a tetratricopeptide repeat (TPR) protein proposed to participate in

plastid protein biosynthesis and/or degradation. Intriguingly, both *gun1* and *gun4* rescued the *sg1* phenotypes, but *gun5* did not, suggesting additional distinct roles and signaling interactions (47). With respect to the definition of retrograde communication, the GUN pathway largely fulfills the criteria, although many challenges remain, including determining the actual function of GUN1. In addition, although lesions in GUN4 and GUN5 perturb the pathway, whether they are actually involved in regulating retrograde communication in wild-type plants remains unknown. Finally, although ABI4 is a part of this pathway, not all cellular processes that regulate ABI4 can be considered retrograde communication.

$^1O_2$: singlet oxygen

Plastid redox–associated nuclear genes (PRANGs): genes that respond to chloroplast redox poise fluctuations, especially PQ/PQH_2 and PSI ROS

Oxidative stress: disruption of chloroplast function caused by excess production of ROS and/or other electrophilic or nucleophilic radicals that alter redox poise

Singlet oxygen–responsive (SORGs): genes that are responsive to $^1O_2$ generated in PSII, such as *AAA-ATPase*

Environmental Sensing and Retrograde Signaling in Response to Oxidative Stress

In mature chloroplasts, the purpose of operational retrograde signaling is substantially different from that in biogenic signaling. Rather than coordinating the assembly of chloroplast components, the focus of operational signaling is on adjustments to chloroplast and cellular homeostasis in response to different perturbations in plastid function due to environmental and developmental cues.

Reactive oxygen species generation by photosynthesis. Perturbations in photosynthesis caused by environmental factors such as drought and high light result in formation of the ROS molecules H_2O_2 and O_2^- at PSI and singlet oxygen ($^1O_2$) at PSII (107, 111), which in turn may alter stromal redox state (125) and plastid metabolism (134). The plastid ROS molecules can modify specific proteins (89), are sensed via as-yet-unknown mechanisms, and regulate specific plastid redox–associated nuclear genes (PRANGs): Plastid H_2O_2 production induces nuclear transcriptional changes distinct from H_2O_2 production in other organelles, such as peroxisomes (127). H_2O_2 is a mobile secondary messenger (102) and may itself be a retrograde signal, but this has not been verified. The short half-lives of $^1O_2$ and O_2^- likely prevent these reactive compounds from traversing the cell (61), necessitating signal transduction pathways. Indeed, the majority of operational signals and pathways identified thus far function in transduction of oxidative stress and changes in chloroplast redox poise.

Photosystem II, singlet oxygen, carotenoid oxidation products, and Executer proteins. Production of $^1O_2$ from triplet excited chlorophyll and the concomitant upregulation of singlet oxygen–responsive genes (SORGs) represent one of the early responses to high-light stress in *Arabidopsis* (37). Excess $^1O_2$ under very strong high-light stress (1,400 μmol photons m^{-2} s^{-1} at 7°C) for 1 h leads to increased production of volatile β-carotene-derived oxidation products such as β-cyclocitral (increased 1.5-fold) and dihydroactinidiolide (increased 8-fold) (107, 129). Exogenous β-cyclocitral and dihydroactinidiolide specifically induced genes responsive to $^1O_2$ but not those responsive to H_2O_2 (107, 129). The transcriptome of β-cyclocitral-treated plants (107) has significant similarities to that of the conditional *fluorescent* (*flu*) mutant, which accumulates protochlorophyllide in the dark and suffers a burst of $^1O_2$ upon exposure to light, leading to induction of SORGs (94). Therefore, β-cyclocitral and dihydroactinidiolide appear to be part of a set of $^1O_2$ responses (43, 61, 107, 129). Although the mechanisms of action for β-cyclocitral and dihydroactinidiolide are unknown, plants preincubated with β-cyclocitral and dihydroactinidiolide suffer less lipid peroxidation and deterioration of PSII efficiency upon cold or light stress (107, 129). Similarly, controlled $^1O_2$ accumulation in the $^1O_2$-overproducing *chlorina 1* (*ch1*) mutant induced β-cyclocitral accumulation, transcriptomic reprogramming, and increased tolerance (108).

Related to $^1O_2$ signaling are the plastid-localized Executer (EX) proteins (reviewed in 43, 61). Crossing the *flu* mutant (94) with the *ex1 ex2* double mutant stops programmed cell death from

occurring, despite the $^1O_2$ production (72). Therefore, EX1 and EX2 are involved in $^1O_2$ perception or transduction, most likely via a pathway separate from β-cyclocitral (43, 61, 62, 108). Although β-cyclocitral-mediated $^1O_2$ signaling seems to occur under severe high-light conditions associated with loss of chloroplast integrity, cellular damage, lipid peroxidation, and oxidation of carotenes (62, 108), the rapid induction and subsequent downregulation of EX-regulated SORGs occur normally in wild-type plants, and chloroplasts remain intact (17), indicating that EX participates in $^1O_2$ signaling in intact chloroplasts during mild light stress (61). The chloroplast soluble proteome of high-light-exposed wild-type plants is substantially similar to that of low-light-grown *ex1* and *ex2* mutants (147), suggesting that EX may act partly by repressing plastid defense responses under ambient growth conditions (147).

EX: Executer

No other components of the signal transduction cascade in either the volatile β-cyclocitral or EX protein pathways have been definitively identified. However, Methylene Blue Sensitivity (MBS), a small zinc-finger protein that associates with RNA granules in a stress-dependent manner (128), is required for induction of $^1O_2$-dependent gene expression and stress tolerance in *Chlamydomonas* and *Arabidopsis*. However, direct links between MBS and EX and/or β-cyclocitral remain untested (128). Another *Chlamydomonas* cytosolic protein, Singlet Oxygen Acclimation Knocked-Out 1 (SAK1), is induced and phosphorylated in response to $^1O_2$ and is required for induction of the nuclear response (153).

Nuclear responses to $^1O_2$ involve at least two protein components. In *Arabidopsis*, the topoisomerase VI (Topo VI) complex directly binds to the promoter and activates $^1O_2$-responsive genes such as *AAA-ATPase* (132). Interestingly, Topo VI also regulates H_2O_2-responsive genes but represses their expression (132). In *Chlamydomonas*, the Singlet Oxygen–Resistant 1 (SOR1) basic leucine zipper transcription factor is autoregulated and binds to a conserved palindromic sequence of many $^1O_2$-responsive genes (30). However, no ortholog of this protein has been found in higher plants (30).

Singlet oxygen signaling fulfills the criteria of retrograde communication as defined in this review and exemplifies the need to consider posttranscriptional processes a part of this communication. For instance, MBS alters the response to $^1O_2$ by regulating mRNA (128) but is not a nuclear transcriptional regulator.

The photosynthetic redox state of chloroplasts and PRANG nuclear gene expression. The redox state of the photosynthetic electron transport chain is acutely sensitive to fluctuations in light intensity. In low light, oxidation of plastoquinol (PQH_2) to plastoquinone (PQ) and reduction of thioredoxin are favored; the converse occurs at higher irradiances (11). These redox state changes coincide with state transitions and with PhANG and PRANG expression (11, 28). Indeed, at least 750 nuclear-regulated genes are responsive to the photosynthetic redox state, in particular that of PQ/PQH_2 (53). The protein kinase State Transition 7 (STN7), which phosphorylates LHCII to mediate state transitions, is a posttranslational sensor of PQ/PQH_2 redox state (8, 98). The exact mechanism by which STN7 controls PRANG expression is unknown but may be linked to ROS, because steady-state LHCII phosphorylation by STN7 enables efficient photon capture by PSI for thylakoidal redox balance and ROS homeostasis (145).

Redox cues from photosynthetic electron transport can be coordinated with other retrograde pathways to modulate nuclear transcription (139). In the *prolyl-tRNA synthetase 1-1* (*pros1-1*) mutant, adjustment of light absorption and photosynthetic electron flow through downregulation of PSII antenna size rescues both impaired thylakoidal electron transport and defective organellar protein synthesis and nuclear PhANG regulation (139). Furthermore, plastid-encoded polymerase activity contributes to PhANG regulation (65), as lesions in plastid-encoded polymerase

components such as *prin2* cause PhANG deregulation in response to high-light stress and PQ/PQH_2 redox changes (65).

PAP: 3′-phosphoadenosine 5′-phosphate

SAL1: a 3′(2′),5′-bisphosphate nucleotidase protein that degrades PAP to adenosine monophosphate (AMP)

Nuclear responses to changes in redox couples are mediated by a series of transcription factors, including ZAT10 (118), Rap2.4a (44), and heat shock factors (53); indeed, a substantial proportion of high-light-responsive genes are enriched in heat shock elements (53, 117). The A-type heat shock transcription factors—HSFA1D and, to a lesser extent, HSFA2 and HSFA3—have been identified as key regulators of certain PRANGs (53). HSFA1D is cytosol localized under ambient conditions, but excess light drives disulfide bonding–mediated trimerization and nuclear translocation, wherein it contributes to *APX2* expression (53), indicating that changes to plastid redox homeostasis somehow extend into the cytosol.

The nuclear response to changes in plastid redox state also involves Cyclin-Dependent Kinase E1 (CDKE1) (9), a component of the plant Mediator complex that modulates crosstalk between promoter-bound transcription factors and RNA polymerase II (79). CDKE1 regulates mitochondrial stress-responsive genes such as *Alternative Oxidase 1A* (*AOX1A*) as well as PhANGs such as *LHCb2.4*, but it acts independently of other *AOX1A* regulators, such as ABI4 (9).

Petrillo et al. (101) discovered that PQ/PQH_2 redox signals can regulate alternative splicing, presenting an additional layer of control of chloroplast signaling over activity of nuclear-encoded genes. As this analysis was limited to <100 transcripts, the full extent to which these signals regulate alternative splicing and their specificities will be intriguing subjects for future investigation. Indeed, the signal itself is yet to be identified, although it is thought to be initiated between PSII and PSI.

SAL1-PAP. The accumulation of 3′-phosphoadenosine 5′-phosphate (PAP) as a retrograde signal was identified by screening for misregulation of the exemplar PRANG *APX2* (26, 116, 156). Somewhat enigmatically, the proposed pathway's components do not fit readily with our present conception of a retrograde pathway (such as the above-mentioned β-cyclocitral or ABI4), as it involves a by-product of sulfur assimilation reactions (140), a catabolic enzyme, the 3′(2′),5′-bisphosphate nucleotidase SAL1 (EC 3.1.3.7) (26, 106), and 5′-3′ exoribonucleases (XRNs) (26). Yet constitutive misregulation of the SAL1-PAP pathway accounts for 25% of the high-light transcriptome (26), including transcription factors such as ZAT10 (a regulator of *APX2*) (116); PAP can move between the organelles (26) via a bidirectional transporter (34); *xrn2 xrn3* phenocopies *sal1* in transcriptome changes, growth morphology, and drought tolerance; and PAP increases in response to drought and high-light-induced oxidative stress (26). The transcriptional changes induced by SAL1-PAP signaling correlate with physiological adaptations, including lower ROS, increased osmoprotectants, decreased membrane damage, and oxidative tolerance (26, 77, 116, 156).

PAP likely alters gene expression profiles by inhibiting the RNA-degrading activity of XRNs, thereby altering posttranscriptional gene silencing, mRNA turnover, and transcription (23, 26, 149). Nuclear XRN2 and XRN3 act on uncapped RNAs, such as excised hairpin loops that form part of precursor microRNA transcripts, whereas cytosolic XRN4 is part of the RNA processing stress bodies (82). Uncapped RNAs accumulate in *sal1* (40), *sal1* and *xrn2 xrn3* transcriptomes share significant coregulation (26), and transcription activation of *APX2* and *APX2:luciferase* occurs (26, 116). Hence, the mobile retrograde signal PAP activates expression of the PRANGs and oxidative stress–responsive genes at least in part by repressing XRNs (26). Important areas for further investigation and validation include determining how the pathway senses the chloroplast redox state, establishing the regulation of PAP metabolism (14) and movement, and identifying other potential PAP targets. Aspects of this pathway are consistent with retrograde signaling, and other functions will likely prove to be unrelated, albeit important, cellular processes (discussed below).

Methylerythritol cyclodiphosphate. The isoprenoid precursor methylerythritol cyclodiphosphate (MEcPP) is a retrograde signal that controls the stress-inducible nuclear gene expression of the chloroplast-targeted hydroxyperoxide lyase (HPL) protein (159). During plastid-localized biosynthesis of isoprenoid precursors by the methylerythritol phosphate (MEP) pathway, 1-hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate synthase (HDS) converts MEcPP into hydroxymethylbutenyl diphosphate (HMBPP) (114). Accumulation of MEcPP correlates with induction of *HPL*, upregulation of SA, and enhanced resistance to infection by the biotrophic pathogen *Pseudomonas syringae*. The specific action of MEcPP on HPL and SA production was demonstrated by direct feeding and systematic silencing of all the biosynthetic genes within the MEP pathway (159). MEcPP appears to be a specific operational signal in response to oxidative stress, as its accumulation is induced by wounding and high-light stress in plants (159) and by oxidative stress in bacterial cultures (95). The nuclear response to MEcPP is also distinct from GUN1 and does not include PhANGs (159). The targets of MEcPP in plants are unknown, although it can disrupt the interaction between chlamydial histone-like proteins and DNA. A direct role in regulating chromatin remodeling is therefore possible.

MEcPP: methylerythritol cyclodiphosphate

DHAP: dihydroxyacetone phosphate

The mobile protein WHIRLY1. WHIRLYs are small proteins that bind single-stranded DNA (reviewed in 31) and are required for plastid genome stability (73). Intriguingly, transgenic WHIRLY1 (WHY1) expressed in the plastid genome and translated in chloroplasts can be present in the nucleus without any difference in molecular weight (38, 50), indicating that this protein is intracellularly mobile (50) or actively maintained in two subcellular compartments for different purposes. Indeed, plastid WHY1 mediates nucleoid organization, DNA replication (73), and germination (49), whereas nuclear WHY1 regulates the expression of plant defense genes during biotic stress (22). Whether WHY1 is a genuine retrograde signaling component or a dual-targeted protein with key discrete functions in each organelle is unclear because active translocation of WHY1 to the nucleus in response to perturbation of plastid function has not been demonstrated. Intriguingly, a redox-mediated translocation mechanism has been proposed (31), but this hypothesis still requires validation.

Sugars and mitogen-activated protein kinase phosphorylation cascades. Coordination of chloroplastic and nuclear gene expression may be mediated in part by sensing of sugar levels in the two compartments (42). Indeed, rapid (<10 min) nuclear acclimation to high-light stress, in particular that of Apetala 2/Ethylene Response Factor transcription factors (AP2/ERF-TFs), involves the export of the Calvin-Benson cycle intermediate dihydroxyacetone phosphate (DHAP) from chloroplasts to the cytosol via the action of a triose phosphate translocator (TPT) (151). DHAP export is required for phosphorylation and activation of Mitogen-Activated Protein Kinase 6 (MPK6) as well as regulation of four ERF-TF genes, as these processes are abrogated in the *tpt* mutant. TPT/DHAP action appears to be independent of ROS and sensing of the PQ/PQH_2 redox state by STN7 (151). Hence, the rapid response to high-light stress is mediated by a distinct sugar metabolite–linked pathway. The information conveyed by DHAP export that might be linked to energization and redox state, the mechanism by which cytosolic DHAP activates MPK6, and the subsequent signaling cascade from MPK6 to nuclear transcription of ERF-TFs are among the key areas of future investigation. Identifying direct signaling roles of other sugars will also be critical, as sugar metabolism and signaling exert substantial influence on nuclear gene expression, which is considered in depth elsewhere (42).

Beyond the nucleus: interaction with other organelles. There are points of convergence in chloroplastic and mitochondrial retrograde signaling. The chloroplastic biogenic signaling

component ABI4 (68, 136) is also a regulator of mitochondrial retrograde communication (35). ABI4 mediates induction of the mitochondrial ROS production mediator *AOX1A* in response to mitochondrial oxidative stress (35) and is also a component of sugar signaling (115), which affects both chloroplasts and mitochondria (42, 115). The PAP catabolic enzyme SAL1 is dual targeted to chloroplasts and mitochondria (26), and the transcriptomes of *sal1* and *xrn2 xrn3* show substantial similarity to the transcriptomes of wild-type plants treated with mitochondrial inhibitors and those of mitochondrial mutants (148), but a mitochondrial SAL1-PAP pathway has not been reported to date. Recently, Walley et al. (154) demonstrated that MEcPP is also involved in activation of the unfolded protein response in the endoplasmic reticulum. That is, MEcPP contributes to the induction of specific unfolded protein response genes, even though complete activation of this response still requires the accumulation of unfolded proteins in the endoplasmic reticulum (154). The contribution of chloroplast signaling to the management of endoplasmic reticulum and mitochondrial stress and the presence of nuclear proteins that integrate retrograde signals from both chloroplasts and organelles [such as CDKE1 (9)] highlight the interconnectedness of these organelles beyond biochemical pathways (81) to coordination of acclimatory responses across the cell.

Communication within the cell comprises more than just retrograde signaling cascades; chloroplast biogenesis requires interactions with the peroxisome and cytoskeleton (3), and mature chloroplasts interact with peroxisomes and mitochondria such that adhesions allow efficient shuffling of photorespiratory metabolites (92). An elegant study showed that the chloroplast is biochemically connected to the endoplasmic reticulum, enabling nonpolar metabolite exchange (81). Tubular extensions from plastids called stromules have also been observed to associate with nuclei, plasma membranes, and the endoplasmic reticulum (41) and may facilitate communication, as alterations in photosynthetic redox state and ROS in chloroplasts or sugar metabolism in leucoplasts induce stromule formation (12).

INTERACTION BETWEEN RETROGRADE SIGNALING AND HORMONES

The physiological processes in which biogenic and operational retrograde signals participate are, unsurprisingly, also mediated by hormonal signaling networks. Auxin metabolism and signaling have been linked to chloroplast homeostasis and communication (146), and there is emerging evidence for interactions between specific operational signals and hormones.

Controlled $^1O_2$ production in *ch1* under moderately elevated light intensity not only induced β-cyclocitral accumulation but also concurrently stimulated jasmonate production and suppressed programmed cell death, which is regulated by JA, suggesting an interaction between carotenoid oxidation products and JA signaling in programmed cell death (108). β-cyclocitral is additionally linked to SA in the response to high-light stress (76). Exogenous β-cyclocitral enhanced the Isochorismate Synthase 1 (ICS1)–mediated SA synthesis via Enhanced Disease Susceptibility 1 (EDS1), a key regulator of SA (76). Significantly, SA participates in the repression of ROS production mediated by β-cyclocitral. Enhancement of the nuclear localization of the SA signaling coactivator Nonexpressor of Pathogenesis-Related Genes 1 (NPR1) by β-cyclocitral in turn increased expression of ROS detoxification genes (76). MEcPP has certain similarities to β-cyclocitral, in that MEcPP accumulation is also induced by high-light stress and stimulates a 40-fold increase in SA production (159). However, the induction of some stress-responsive genes by MEcPP is SA independent, and MEcPP does not affect JA (159).

Sensing and communication of plastid redox state appear to be linked to hormonal networks, as ABA is induced by high light (33). Bundle sheath cell expression of certain PRANGs, such as *APX2*,

is also coordinately regulated by a redox retrograde signal from bundle sheath cell chloroplasts and ABA imported from vascular tissues (33). Radical-Induced Cell Death 1/Redox Imbalanced 1 (RCD1/RIMB1), one of the mediators of nuclear responses to chloroplast photooxidative stress (44), is involved in a subset of responses to several plant hormones, including JA as well as ethylene and ABA (2). The interaction with JA and ethylene is especially important, as these hormones act antagonistically via RCD1/RIMB1 in the nuclear regulation of programmed cell death in response to O_2^- (96).

JA and its precursor 12-oxo-phytodieonic acid promote flux into sulfur assimilation (52, 97, 122), and 12-oxo-phytodieonic acid accumulation during oxidative stress (97) may be one of several mechanisms for increasing levels of PAP for retrograde signaling (67). The hypothesized link between PAP and 12-oxo-phytodieonic acid may be feedback regulated via sulfur metabolism, as lesions in SAL1 result in elevated JA (113). JA biosynthetic genes are induced by sulfur starvation (45, 78, 90), which can be invoked by PAP accumulation (70). The high PAP levels in the *sal1* mutant induce upregulation of many ABA-independent genes (26, 112, 156) but also some ABA-dependent genes, including *RD29A* (160), *DREB2A*, and *APX2* (116). The induction of ABA-responsive genes is also enhanced by PAP accumulation (16, 160). However, the exact intersecting points between PAP and hormonal signaling, particularly in stress responses, remain unclear. The extent and evolution of interconnectivity between chloroplast communication and hormonal networks are interesting aspects to explore.

Unicellular photosynthetic organisms such as *Chlamydomonas* can respond to hormones such as ABA (161) but lack some hormones, such as brassinosteroids (153). The *Chlamydomonas* genome also contains a limited number of predicted hormone biosynthesis and signaling genes compared with higher plants (75, 110). Correspondingly, heme and $^1O_2$ retrograde signaling pathways are present in *Chlamydomonas* (30, 152, 153), but it remains unclear whether algae contain other retrograde pathways that interact with hormonal networks in *Arabidopsis*. Most of the retrograde signaling pathways identified in *Arabidopsis* are presumably conserved in higher plants, but key evolutionary differences, such as Brassicaceae-specific glucosinolate metabolism, from which PAP is also sourced (14), may need to be considered. These differences may provide an avenue to probe the intersection between the signaling pathways.

The separation of function between retrograde signaling and hormonal communication needs to be critically assessed for individual components and pathways; for instance, ABI4 functions in ABA signaling separately from *GUN1* (18, 68). Similarly, whether the respective roles of plastid and nuclear WHY1 in ABA (49) and SA (22) signaling represent genuine contributions of retrograde signaling or dual targeting needs to be verified. Therefore, more investigation is needed to determine the extent to which hormonal and developmental phenotypes in each pathway reflect retrograde communication in a broader sense or instead reflect secondary or even unrelated effects. Regardless, it is intriguing that signals from the chloroplast can perform multiple functions and regulate broader processes beyond the transcription of PhANGs, PRANGs, and SORGs.

RETROGRADE PATHWAYS IN PLANT DEVELOPMENT

Lesions in components of chloroplast biogenesis, division, and/or signaling result in large-scale phenotypic effects, including bleached cotyledons (3), variegated leaves with defective palisade differentiation (51, 58), and crumpled leaves and altered rosette morphology (26, 131). These features extend to other tissues, as retrograde signaling is most likely involved in the biogenesis of chromoplasts from chloroplasts during fruit maturation (reviewed in 100) and may indicate that plastid signaling contributes to development. Indeed, retrograde signals can direct plant form and architecture beyond their site of generation in a process known as systemic acquired acclimation

BEYOND SIMPLE METABOLIC PATHWAYS: A CASE STUDY IN CAROTENOIDS

The self-regulation of carotenogenesis carries the hallmarks of metabolic feedback regulation and presents yet another mechanism controlling the expression of *PHYTOENE SYNTHASE* (*PSY*). *PSY* expression is a major rate-limiting step in carotenoid biosynthesis and is regulated heavily by epigenetic and transcriptional regulation of posttranslational modification (163, 164; reviewed in 80). There is growing evidence that apocarotenoids act as signals in both plants and animals. The multitude of apocarotenoids arising from different carotenoid substrates identified via in vitro analysis of plant CCDs suggests that apocarotenoids may be an integral part of the self-regulation of carotenogenesis (48, 130; reviewed in 80). Indeed, Kachanovsky et al. (54) demonstrated that an overaccumulation of neurosporene or prolycopene induces nuclear *PSY1* expression in ripening tomato and speculated that this expression is dependent on CCD activity. In vitro analysis of CCD1A and CCD1B from tomato revealed two apocarotenoids specific to the cleavage of prolycopene by CCD1B as potential signaling candidates (48). Despite the nuclear response, to date such regulatory signals have been observed only after direct impediment of the carotenoid biosynthetic pathway by mutation or targeted transgenic strategies (27, 54), and have not yet been shown to occur in response to environmental or developmental stimuli. Thus, unlike the β-carotene cleavage products β-cyclocitral and dihydroactinidiolide (107, 129), whether other carotenoid derivatives that act to regulate *PSY* and other unassigned processes lie under the umbrella of retrograde signaling remains to be determined.

(138), as shown by the control of alternative splicing in roots via one or more mobile plastid-derived shoot-to-root signals (101).

Seedlings and Leaves

Retrograde signals alter photomorphogenesis and leaves. Although the *gun1* mutant is morphologically indistinguishable from the wild type, the de-etiolation in *gun1* is impaired upon return to light (137). Ruckle et al. (119) demonstrated links between CRY1 (a flavin-type blue light photoreceptor) and GUN1 signaling. Beyond PhANG regulation, *gun1* results in deregulation of hypocotyl elongation and cotyledon expansion (120) as well as altered seedling development in response to exogenous sucrose and ABA (20). Perturbations in plastid transcription or translation are communicated via GUN1 to influence leaf abaxialization and leaf lamina morphology (141). Altered leaf morphology and hypocotyl elongation of *sal1* and *xrn4* could be partly attributable to general alterations of auxin and ethylene signaling (93, 105, 162), most likely unrelated to retrograde functions of PAP; however, there are links between PAP retrograde communication and light signaling, as exemplified by the partial rescue of *sal1* phenotypes when Phytochrome B and HY5 activities are modulated (15, 59).

The fine tuning of leaf development by retrograde signals might be related to light capture and photosynthesis in the chloroplast, as leaf morphology and architecture are linked to factors such as light intensity and gas exchange that impact photosynthesis and the chloroplast as a whole (142). The involvement of retrograde pathways in chloroplast biogenesis, leaf morphology, and regulation of germination and in response to pathogens also suggests that operational signals may be regulated differently and perform different roles depending on the tissue type, developmental stage, and environmental stimuli. In leaves, for instance, PAP accumulation is responsive to chloroplast oxidative stress, but this is probably not the case in seeds and roots (16, 26, 46). We discuss other examples of how nongreen plastids can generate signaling metabolites in the next section and in the sidebar, Beyond Simple Metabolic Pathways: A Case Study in Carotenoids.

Carotenoid Derivatives and Cellular Signaling

A series of advances have demonstrated the unique potential of carotenoid derivatives as signaling molecules (reviewed in 80). In plants, altered accumulation of two undefined carotenoid derivatives (i.e., apocarotenoids) has major implications for plastid, leaf, and root development (5, 150). In the *zeta-carotene desaturase/chloroplast biogenesis 5* (*zds/clb5*) mutant, loss of an early step in carotenoid biosynthesis results in a repression of PhANGs and the chloroplastic Clp protease subunit ClpP1. The phenotype is far more severe than the GUN-mediated suppression and leads to arrested leaf development (5). Interestingly, *clb5* phenocopies the chloroplast transcription mutant *enlarged fil expression domain 2* (*enf2*) in misregulated organelle gene expression and protein synthesis, expression of the abaxial *Filamentous Flower* (*FIL*) gene, and lamina expansion (5, 141). Whether the *clb5* signal involves transduction by GUN1, as it does for *enf2* (141), is unknown. A series of experiments manipulating ABA, strigolactones, and other carotenoid enzymes indicated that the phenotypes were dependent on cleavage of ζ-carotene or phytofluene, forming an unidentified retrograde signal (5). A similar study exploring decreases in lateral root capacity indicated that another uncharacterized carotenoid cleavage product is required for periodic oscillation of gene expression, which is essential for lateral root development (150). However, the apparent preprograming of this carotenoid signaling mechanism, as opposed to induction in response to a perturbation of plastid homeostasis, likely excludes it from the growing list of retrograde signals.

These studies indicate the potential for carotenoid derivatives to play roles in both retrograde signaling and plant development (5, 150) as well as in chloroplastic-nuclear self-regulation of carotenogenesis (54) (see sidebar, Beyond Simple Metabolic Pathways: A Case Study in Carotenoids). Insight into the identity, transport, and perception of these signals is necessary, and apocarotenoid signals in animals may hold some answers.

KEY QUESTIONS IN RETROGRADE SIGNALING

Individual pathways visualized linearly can be compartmentalized into discrete sections, namely (*a*) signal initiation in plastids, (*b*) signal movement or transduction, (*c*) perception in the cytosol and/or transduction to the nucleus, and (*d*) signal perception leading to transcriptional or post-transcriptional responses. For every pathway identified and discussed in this review, at least two out of four of these sections remain unresolved (**Table 2**). We view these black boxes of retrograde signaling as the key questions remaining in this field, along with overarching questions on the connectivity and coordination of different signals, regulation of signal metabolism, the nature of metabolites as signals, and broader roles of chloroplast-derived signals in plant function.

Perception of Altered Homeostasis and Signal Initiation

With the exception of β-cyclocitral generation by $^1O_2$-induced cleavage of β-carotene, there are few clear mechanisms for how each retrograde signal is actually generated in response to altered plastid homeostasis. A shared feature of most operational signals is that the conditions in which they operate invoke perturbations of photosynthesis that alter plastid redox state and generate ROS. Yet in the case of EX proteins, it is not yet clear whether the EX proteins associate with the PSII reaction center to sense $^1O_2$ or $^1O_2$ reaction products (61). Similarly, no generalized receptors for PQ reduction, H_2O_2, and O_2^- produced at PSI have been described that could directly link the perception of oxidative stress to the generation of PRANG retrograde signals. STN7 may act as a PQ/PQH_2 ratio sensor (98) and indeed contains cysteines, which may react with H_2O_2

Table 2 Summary of plastidic, subcellular transport, cytosolic, and nuclear components involved in biogenic and operational signaling pathways that modulate plastid homeostasis in wild-type plants

Pathway components	Plastidic components			Cytosolic components	Nuclear components
	Sensor	Other	Export		
GUN	?	GUN1, CHLH, GUN4 SG1, SIG2, SIG6	PTM protease	HY5, CRY1, PhyB	PTM, ABI4, GLK1, GLK2
Heme	?	FC1	?	?	?
$^1O_2$	β-Carotene	β-Cyclocitral, DHA	Volatile	MBS, SAK1	Topo VI complex, SOR1
	?	EX1, EX2	?		
Redox state and PSI ROS	?	SAL1, PAP	PAPST	PAP	XRN2, XRN3
	?	HDS, MEcPP	?	?	?
	?	STN7, PRIN2, H_2O_2/redox	?	HSFAs	HSFAs, CDKE1, RCD1/RIMB1-Rap2.4a
	?	DHAP	TPT	MPK6	?
?	?	WHY1	?	?	WHY1
?	?	ZDS, clb5 signal, CCD	?	?	?

Black boxes (entirely unknown components) and gray areas (partially known components) indicate interesting areas of research with potential for breakthroughs in understanding retrograde signaling.

(89), but its actual role is under debate (145). Whether any of the metabolic enzymes involved in biosynthesis or degradation of the metabolite-based retrograde signals could themselves be ROS sensors is unknown.

Signal Movement Out of Plastids

For plastid retrograde signals to affect protein expression, they must first traverse the lipid bilayer of the chloroplast membrane. This is particularly true for polar retrograde signals such as PAP, DHAP, and MEcPP. Individual transporters that facilitate the exchange of PAP or DHAP between the chloroplast and cytosol have been identified (34, 151), but corresponding proteins for other retrograde signals have not, despite evidence for export (4, 144). There is evidence of H_2O_2 movement from the apoplast to the symplast in guard cells (39), and thus movement between the plastid and cytosol is logical and feasible, possibly involving aquaporins (39), but this has yet to be conclusively demonstrated. The short half-lives of ROS (61) may preclude movement, but in *Chlamydomonas*, approximately 5–10% of plastid $^1O_2$ may be able to traverse the cell to reach the nucleus (29). Relatively longer-lived oxidation products such as lipid peroxides may also provide means of transmission. Tetrapyrrole movement may utilize carrier proteins, because they are relatively hydrophobic and poorly soluble in aqueous solutions (84), but whether and (if so) how phytotoxic tetrapyrroles are moved intracellularly remain unclear (18, 84). Transport considerations are less pressing for volatile compounds such as β-cyclocitral and dihydroactinidiolide, as they can move by diffusion (107, 129). In principle, the rate of export and import for bidirectional transporters may be a key regulatory step. For instance, rapid ERF-TF activation under high light is repressed when DHAP export by TPT is blocked (151). For GUN1-PTM, the signal does not require traversing the membrane; instead, the unidentified protease cleaving PTM (136) may be rate limiting.

Signal Transduction in the Cytosol

In some instances, a cytosolic receptor and transducer of retrograde signals are not required—for example, membrane-bound PTM can move directly from the chloroplast to the nucleus (136). Receptor protein–independent transduction for ROS signals such as chain reactions of radical formation and decomposition have been proposed for $^1O_2$ (29). These products may allow a degree of unique chemical identity to plastid-sourced ROS. That there is a clear distinction in the nuclear transcriptional profile depending on the subcellular origin of H_2O_2 (127) argues for the presence of specific organellar and cytosolic signal transduction components. Key challenges therefore include identifying all of the cytosolic components that act in transducing signals for the individual pathways as well as the integrators of multiple signals.

Signal Perception and Adjustment of Plastid Homeostasis

At the core of the paradigm of retrograde signaling is the transformation of chloroplast messages into gene expression changes. A degree of specificity is clearly evident; for instance, a comparison of signals emanating from different organelles showed that the respective responses are enriched for transcripts affecting the perturbed organelle (148). A meta-analysis of chloroplastic and mitochondrial retrograde signals demonstrated specificity in relation to operational signaling functions, whereas commonality between chloroplastic and mitochondrial retrograde signals occurs for stress-responsive transcripts (148). A further meta-analysis of a range of perturbations triggering operational retrograde signals also identified a core response module linked to a variety of cellular functions (36). Using *cis*-element analysis and protein-interactome data, this analysis identified significant overlap between the plastid signaling, hormonal regulation, and sugar and light signaling pathways (36).

As discussed above, plastid H_2O_2, O_2^-, and $^1O_2$ induce different nuclear gene subsets (32, 62, 107, 111, 123). However, in some cases even the same stimulus may induce different transcripts. Nuclear genes transcriptionally altered by a low-light PQ reduction signal are enriched for tetrapyrrole metabolism, mitochondrial function, and lipid signaling, as well as a potential redox-responsive *cis*-element (25). These features are absent when PQ is reduced under high light (53), suggesting that the environmental context in which the PQ redox state is sensed can confer specificity (25). The exact mechanisms conferring the specificity of nuclear responses to different retrograde signals still require elucidation, although studies are beginning to identify the components, such as the Topo VI complex in $^1O_2$ and H_2O_2 signaling (132), the ZAT10 and HSFA transcription factors for photosynthetic redox changes during excess light (53, 118), and the RCD1/RIMB1 Rap2.4a transcription factor complex for regulation of chloroplast antioxidant enzymes (44).

Cis-element-binding studies have accounted for transcription factor specificity (44, 53, 132), but in other cases, such as alternative splicing and XRNs in PAP signaling, insights into RNA-directed DNA methylation, splicing regulation, and polymerase function may be required (26). That is, in these two pathways and in the broader context of retrograde signaling per se, the specificity of the nuclear response may include alternative splicing (101), RNA metabolism (26), and epigenetic regulation (25, 136) in addition to orthodox gene regulatory mechanisms that target transcription factors. In some cases, the different mechanisms work in tandem—for instance, during GUN signaling, transcriptional control by ABI4 is in turn regulated by PTM in a manner associated with histone modification (136).

A substantial proportion of nuclear genes transcriptionally altered by retrograde signaling encode chloroplast-targeted proteins (11, 26, 36, 133), in line with the expectation that retrograde

signaling adjusts the homeostasis of chloroplastic processes in response to perturbations in this organelle. Yet numerous concomitant transcriptional changes occur in extraplastidic components such as cytosolic APX2 and multiple cell wall–modifying proteins, which are induced by multiple retrograde pathways responsive to plastid redox state (26, 33, 36, 53, 57). Thus, the anterograde component of chloroplast-nucleus communication may not be directly bilateral. Rather, retrograde signaling invokes multiple adjustments that collectively improve chloroplast function.

Interestingly, some of the proposed cytosolic components of the retrograde pathways, such as MBS and XRN4, associate with RNA granules (82, 128), suggesting that not all retrograde signals function via the nucleus. That is, some of the physiological responses to retrograde signals may be mediated by cytosolic mechanisms that are not transduced to the nucleus for transcription. Furthermore, regulation of cytosolic mRNA turnover, protein synthesis, and posttranslational modifications presents an additional layer of regulation for proteins that are responsive to retrograde signals (91). These findings challenge the concept of a strictly linear retrograde signaling pathway emanating from plastids and terminating in the nucleus, in line with the increasing evidence that retrograde signals play broader roles in plant function.

Connectivity and Coordination of Different Signals

The present paradigm of retrograde communication considers the individual signals and pathways to be distinct from each other, and they are therefore studied mainly in isolation. However, a closer examination shows that the signals are to varying degrees metabolically, spatially, temporally, and physiologically connected (**Figure 3**). The emerging role for MEP and MEP-derived carotenoid signals raises the question of whether plants have evolved to favor certain metabolite signals more than others. One possibility is that interconnected metabolic networks can be coordinately regulated to a degree. The identification of components regulating the metabolic flux in tetrapyrrole and MEP pathways may help to test this speculative hypothesis.

The multiple retrograde signals and pathways may be spatially coordinated by differential localization to different cell types or subplastidic compartments, or they may developmentally differ. For instance, H_2O_2-responsive retrograde signaling during high-light stress may be preferentially invoked in bundle sheath cells, which are the primary site of H_2O_2 production under such conditions (33). Transcriptional analyses also suggest that, in tomato fruit, the biogenic but not operational retrograde pathways function during chromoplast biogenesis (100). Tetrapyrrole- and GUN1-mediated signaling appears to be restricted to young seedlings, in line with their roles in chloroplast development, whereas other pathways, such as PRIN2-mediated plastid transcription retrograde signaling, can function in both developing seedlings and adult rosette leaves (65). The developmental stage of the chloroplast itself may compartmentalize the signals, as developing and fully developed chloroplasts invoke different retrograde signaling pathways in response to the same perturbation (60). The individual retrograde pathways identified thus far have not been exhaustively tested for their roles at different developmental stages and in different tissues. Analysis of spatiotemporal expression of the retrograde signaling components may provide clues in some cases (26), but in others they must be considered in tandem with other factors, such as systemic or volatile signal movement (107, 129) and the lack of correlation between transcript and protein abundance for certain genes (91).

The retrograde signals may also be temporally assigned in a hierarchy. PQ/PQH_2 pools, $^1O_2$, and metabolic products of light reactions such as NADPH and DHAP exert rapid effects within milliseconds to seconds of exposure to high light (24). Intermediate signals and hormones such as H_2O_2, MEcPP, PAP, JA, and ABA act on a timescale of minutes to an hour, whereas other hormones (e.g., SA and auxin) are involved in long-term responses (a timescale of days) (24). The

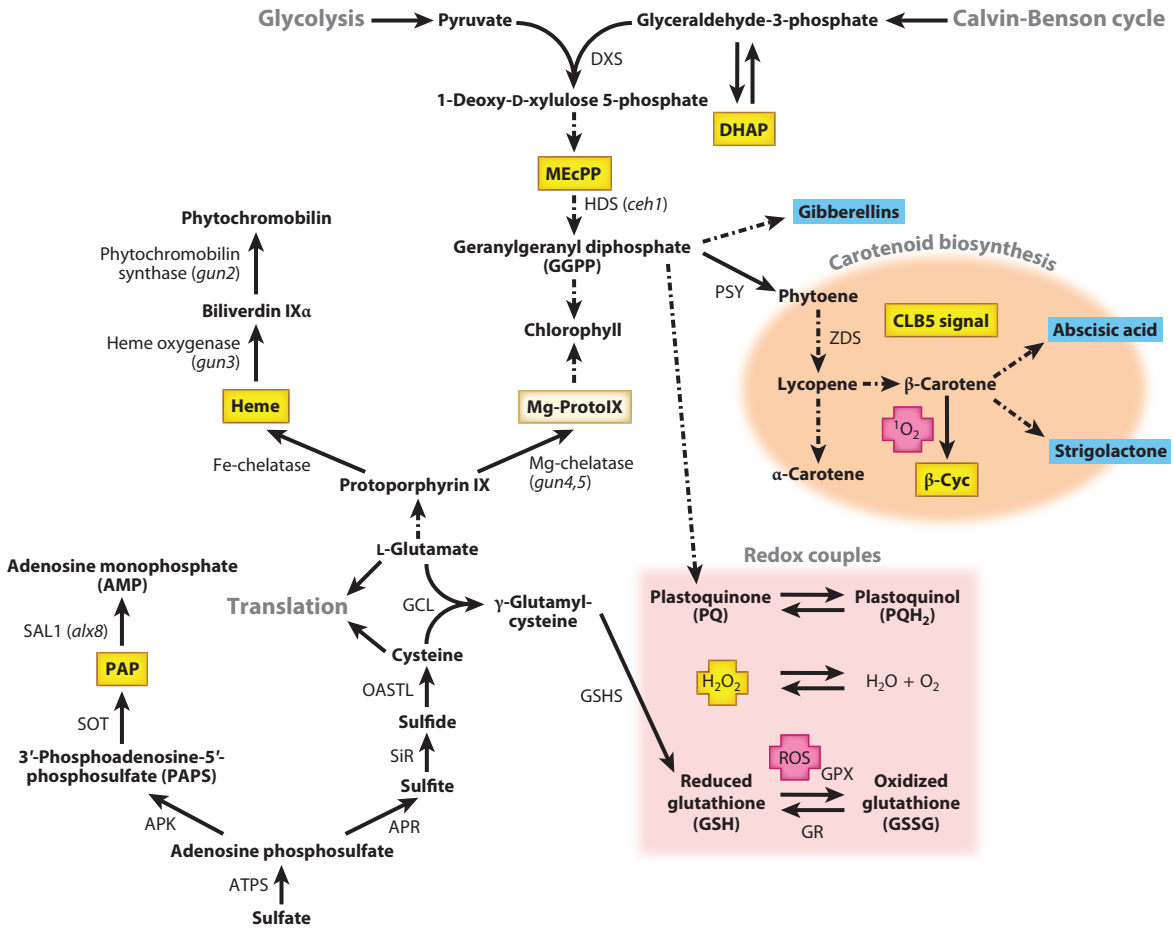

Figure 3

Biochemical pathways of the different metabolite retrograde signals. Known retrograde signals are shown in yellow, and hormones are shown in blue. Solid arrows indicate a single biochemical step, and dash-dotted arrows are simplified representations of more than one biochemical step.

clustering of these signals by timescale can be heterogeneous (24), suggesting that the different retrograde signals and pathways have distinct as well as overlapping roles during chloroplast acclimation to light. Therefore, the acclimation response may be determined by the time- and concentration-dependent composition of the signal mix.

Modulation of Signals and Stress Memory

An underappreciated aspect of retrograde communication is the modulation of the signals after activation of the desired nuclear and physiological responses. Acclimation responses need to be appropriate: For instance, a transient stress may require only a short-term adjustment, whereas a prolonged environmental shift may require a long-term acclimation. The type of response could be mediated by deactivation or amplification of the signal, initiation of repressive signals (103) and transcriptional repressors, or alteration of RNA half-lives (19) and DNA packaging (88). With

respect to metabolite signals, including heme and PAP, catabolism may require transport back into chloroplasts or mitochondria (26, 84), which is feasible for PAP because the transporter is bidirectional (34). Whether carotenoid cleavage dioxygenase (CCD) enzymes regulate the abundance of the *clb5* apocarotenoid signal remains to be seen, but it is conceivable that β-cyclocitral and dihydroactinidiolide signaling is easily turned off owing to the nature of their synthesis by oxidative stress and their dilution by diffusion (107, 129). For protein components, turnover and posttranslational modifications may determine deactivation, and in a possible feedback loop, APX2 may well lower cytosolic redox poise, limiting HSF1A relocation. By contrast, the persistence of high-light acclimation responses even after a recovery period longer than the imposed stress suggests that there is a point of no return for certain retrograde signals (24). Hence, rather than being deactivated, some signals may be propagated or maintained beyond the initial stimulus.

Retrograde Signals: Precursors, Signals, or Secondary Messengers?

Metabolite retrograde signals can have different functions depending on context, location, and concentration. For example, MEcPP is a precursor to hundreds of compounds but can also function as a messenger (159). Mg-ProtoIX is utilized for chlorophyll (63) and might act in signaling (133) but can also be phytotoxic (63). PAP can alter sulfur metabolism (70), activate stress-responsive genes (26), and alter development at high concentrations (16, 26, 59, 112). Each of these examples has parallels with well-studied secondary messengers such as H_2O_2, nitric oxide, and Ca^{2+}, which also play multiple roles depending on dose, flux, and tissue (102). Thus, research defining functions of a retrograde signal will need to consider its abundance, subcellular localization, developmental stage, and cell type.

An examination of secondary messengers involved in hormonal signaling, such as ROS and Ca^{2+}, reveals common characteristics, including separated sites of synthesis and action, alteration of levels in response to hormones, participation in hormonal signaling, and multiple functions in different tissues and/or biological processes (102). Multiple aspects of these features are recapitulated in the different retrograde signals. Hence, there is a need to reexamine the individual retrograde signals and evaluate their function beyond conventional linear pathways of gene expression control to broader roles as secondary messengers. In some instances, further investigation may show that a particular signal is not actually retrograde in particular tissues; in others, it may show that the signal's role is broader than previously thought, encompassing the complexity of cellular signaling in which chloroplast communication is embedded.

SUMMARY POINTS

1. Chemical and protein signals communicate the metabolic, biochemical, and physiological status of the plastid to the nucleus in multiple tissues and developmental stages.
2. There is a large amount of new evidence that retrograde signals play a key role in regulating nuclear gene transcription, thereby altering chloroplast homeostasis to improve adaptation to developmental and environmental stimuli. In the space of a decade, the number of known components has increased from about 4 to more than 40.
3. The retrograde signaling pathways have distinct components but also commonalities, such as reacting to the same stimulus, sharing nuclear regulators of transcriptional responses, and regulating overlapping subsets of genes. Their functions may be spatially, developmentally, or temporally regulated.

4. Beyond bilateral communication, retrograde pathways are more intertwined with hormonal signaling networks than was previously thought, and some may have broader functions as secondary messengers.
5. Observations are emerging that suggest chloroplast communication may not be exclusively bilateral with nuclei.
6. The convergence of chloroplast retrograde signaling with signaling from other organelles and cellular processes suggests that interorganelle and environmental signals can be integrated to coordinate nuclear and cellular responses to plastid stimuli that influence plastid and cellular function.
7. As understanding of the components of retrograde pathways increases, it will be exciting to explore the potential interactions of the pathways with each other and with communications from other organelles, as well as their integration into cellular networks. Integration is key to advancement: As with the Rosetta Stone, languages considered in isolation can be difficult to understand.

DISCLOSURE STATEMENT

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