Intracellular signaling from chloroplast to nucleus followed by a subsequent response in the chloroplast is called retrograde signaling. It not only coordinates the expression of nuclear and chloroplast genes, which is essential for chloroplast biogenesis, but also maintains chloroplast function at optimal levels in response to fluxes in metabolites and changes in environmental conditions. In recent years, several putative retrograde signals have been identified and signaling pathways have been proposed. Here we review retrograde signals derived from tetrpyrroles, carotenoids, nucleotides and isoprene precursors in response to abiotic stresses, including oxidative stress. We discuss the responses that these signals elicit and show that they not only modify chloroplast function but also influence other aspects of plant development and adaptation.

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What is retrograde signaling?
Chloroplasts of higher plants are not only responsible for photosynthesis, but also for the synthesis of many essential compounds, such as fatty acids, amino acids, phytohormones and secondary metabolites. Thus chloroplasts are essential for the viability of plants. Many chloroplast functions are regulated by nuclear factors; this control is referred to as anterograde control or signaling. Conversely, changes in the developmental or metabolic state of chloroplasts can evoke massive changes in the expression of nuclear genes, some of which directly affect chloroplast function. This process is called retrograde signaling [1–3]. Chloroplast retrograde signaling not only coordinates the expression of nuclear and chloroplast genes, which is essential for chloroplast biogenesis, but also maintains chloroplast function at optimal levels in response to fluxes in metabolites and changes in environmental conditions [1–3]. Other signals arising in the chloroplast such as some phytohormones that elicit responses elsewhere in the cell, are usually not considered to be retrograde signals.

Since the concept of chloroplast retrograde signaling was originally conceived nearly 40 years ago [4], different types of retrograde signaling pathways have been defined based on the sources of the corresponding signals. Retrograde signals can largely be grouped into two categories: biogenic control and operational control signals [5]. The biogenic retrograde signals regulate early chloroplast biogenesis as a seedling shifts from a heterotrophic to a photoautotrophic lifestyle. Such knowledge has been derived primarily from studies that used herbicides which inhibit organelle function and development [6]. In contrast, operational signals are important for the normal function of chloroplasts in mature plants. The “operational” retrograde signals from mature photosynthesizing chloroplasts can regulate nuclear genes to alter photosystem stoichiometry or to induce stress responses [5].

Originally, four distinct putative chloroplast retrograde signaling pathways have been recognized based on the sources of the signals. They include tetrpyrrole biosynthesis, chloroplast gene expression, chloroplast redox homeostasis and reactive oxygen species (ROS) [1,2]. In addition to these classical pathways, several novel retrograde signaling pathways have been recently reported. These findings indicate that many metabolic pathways can act as potential sources of retrograde signals during different developmental stages of plants or upon different stress responses. More importantly, unlike the previously identified signals generated during artificial conditions, these novel signaling molecules were identified during physiologically relevant responses to adverse growth conditions such as drought or high light (HL). Here, we will review novel metabolite retrograde signaling pathways defined in recent years and summarize their diverse functions in plant development and adaption.

**Tetrpyrrole signaling**
Support for tetrpyrrole involvement in retrograde signaling came first from studies on the expression of nuclear genes of *Clamydomonas reinhardtii* (*C. reinhardtii*) [7–9]. However, major insights arose from the analysis of genome uncoupled (gun) mutants of *Arabidopsis*. Wild-type plants grown on norflurazon (NF) expressed *Lhcb* (the gene encoding the chlorophyll a/b binding proteins of photosystem II) in lower amounts due to photo-bleaching of chloroplasts whereas *gun* mutants retain high levels of *Lhcb* expression under the same conditions, suggesting
disruption of retrograde signaling [6]. Four mutants (gun2–
gun5) were shown to have lesions in enzymes involved in
tetrapyrrole biosynthesis [10–12]. Analysis of gun2–5 sug-
gested that Mg-Proto IX, the first committed chlorophyll
precursor might act as a negative retrograde signal to
regulate nuclear gene expression [7–9]. However, the
role of Mg-Proto IX in retrograde signaling after NF
treatment was seriously questioned in later reports based
on a lack of correlation between Mg-Proto IX levels and
Lhcb gene expression [13,14].

gun6 is a gain-of-function mutant overexpressing the
conserved chloroplast ferrochelatase1 (FC1, heme
synthase). Increased flux through the heme branch of
the tetrapyrrole biosynthesis pathway increases the ex-
pression of PhANGs (photosynthesis-associated nuclear
genes) in the gun6 mutant, suggesting that heme acts
as a positive retrograde signal to regulate nuclear gene
expression [15*] (Figure 1). Compared to Mg-Proto IX,
heme appears to be a more likely candidate molecule for
retrograde signaling, in part because heme is known to be

Figure 1

Metabolic retrograde signaling pathways. (a) The tetrapyrrole intermediates are involved in retrograde signaling. The plastid ferrochelatase 1 (FC1, heme synthase) is involved in the heme branch of tetrapyrrole biosynthesis. Heme acts as a positive regulator of PhANGs but the exact mechanism by which this compound reaches the nucleus and the nuclear components involved in heme signaling are unknown. In C. reinhardtii, bilins are also generated to regulate nuclear gene expression. GUN5 (H subunit of Mg-chelatase) and GUN4 (binding the substrate and product of the reaction catalyzed by the Mg-chelatase and activating the Mg-chelatase) are involved in the biosynthesis of Mg-ProtoIX from the chlorophyll branch of tetrapyrrole biosynthesis. Mg-ProtoIX might negatively regulate the expression of PhANGs. (b) The carotenoid oxidation products are involved in retrograde signaling. One product of the H2O2 oxidation of carotenoids, β-CC, may act as a second messenger involved in the O2
signaling pathway in plants. Carotenoid oxidation can also occur in vivo through CCD4 to generate apocarotenoids. An unknown apocarotenoid-
derived signal regulates the expression of PhANGs and carotenoid biosynthetic genes. (c) SAL1-PAP signaling pathway. 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 XNR activities, thereby inducing gene expression associated with stress responses. (d) A precursor of isoprenoids, MEP, derived from the MEP pathway for isoprenoid biosynthesis in the chloroplast, is induced by stress and functions as a sensor and communication signal to the nucleus where it induces selected stress-responsive genes through alteration of nuclear architecture and functional dynamics.
exported from chloroplasts, whereas the evidence for Mg-Proto IX export by healthy chloroplasts is not clear [16]. Moreover, Mg-Proto IX is photodynamic and produces toxic ROS in the presence of light, whereas heme is photodynamically inactive [17]. A role for heme as a positive regulator of PhANG expression could provide an alternative explanation for the gun mutant phenotypes: the phenotype of gun2-gun5 mutants might be the results of increased levels of heme instead of decreased amounts of Mg-Proto IX. 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 C. reinhardtii, a portion of heme is converted by heme oxygenase (HMOX) to biliverdin IXa and by phytocromobilin synthase (PCYA) to phytocromobilin, which serves as chromophore of phytochromes [18]. All sequenced chlorophyte genomes lack phytochrome genes, but their genomes encode two HMOXs, HMOX1 and HMOX2, and PCYA [18]. Studies on the functions of HMOXs in C. reinhardtii revealed that bilins might act as a retrograde signal as well as heme [19*] (Figure 1). A bilin-dependent nuclear gene network is critical for C. reinhardtii greening and phototrophic survival. The bilins may be part of a retrograde signaling pathway that evolved in chlorophytes, including C. reinhardtii, for the detoxification of reactive oxygen species generated during the transition from dark to light [19*].

**SAL1-PAP signaling during drought and high light stress**

Nucleotide 3′-phosphoadenosine 5′-phosphate (PAP) was demonstrated to play a role as a retrograde signal in Arabidopsis through the study of the salt mutant [20**] (Figure 1). This mutant was originally isolated in a screen for elevated expression of the antioxidant enzyme ascorbate peroxidase 2 (APX2) under HL. [21]. This mutant is also more tolerant to drought. In salt, 35% of the HL stress-induced genes, including APX2 and early light induced protein 2 were constitutively up-regulated and some metabolites including polyamine putrescines and potential osmoprotectant carbohydrate derivatives were increased significantly, suggesting that SAL1 is a component of HL and drought stress signaling networks [22].

The enzyme SAL1, which is localized in both mitochondria and chloroplasts, regulates PAP levels by dephosphorylating PAP to AMP [23]. Accordingly, PAP accumulates 20-fold in the salt mutant. PAP also increases up to 30-fold in wild-type plants during drought and exhibits a smaller increase in response to HL. In addition, a correlation between total cellular levels of PAP and expression of the nuclear marker gene (APX2) was observed. Moreover, targeting of SAL1 to either the nucleus or chloroplasts in salt mutants reduces total PAP levels and decreases expression of APX2, which indirectly demonstrates that PAP must be able to move between cellular compartments. A plausible mechanism that PAP acts as a retrograde signal molecule was accordingly proposed [20**]. However, how it moves between cellular compartments remains unknown. A 3′-phosphoadenosine 5′-phosphosulfate (PAPS)/PAP chloroplastic antipporter might be responsible for facilitating exchange of PAP between the chloroplast and cytosol [24].

PAP is produced as a byproduct of sulfonation reactions catalyzed by cytosolic sulfo-transferases, whereby sulfate is transferred from PAPS to several metabolic substrates [23]. A key question is the identity of the targets of PAP in the nucleus. As an adenosine phosphate which can bind irreversibly to yeast 5′-3′ exoribonucleases (XRN)s and inhibit their activity, PAP most likely regulates nuclear gene expression by altering RNA metabolism through XRNs, which play an essential role in post-transcriptional regulation of gene expressions [25]. Indeed, SAL1 and the nuclear XRNs modulate the expression of a similar subset of HL- and drought-inducible genes, strongly suggesting that XRNs act as targets of the SAL1-PAP signaling pathway [20**]. However, the mechanism and components of this signaling pathway remain largely unknown. For instance, does SAL1 act directly as a stress sensor or are there additional upstream components?

**Carotenoid-derived retrograde signaling**

Singlet oxygen (1O2) is one ROS that can function as a retrograde signal to activate nuclear gene expression [26]. Because of its high reactivity and short half-life, 1O2 is unlikely to be a signal that is translocated across the chloroplast envelope, but is likely to interact with other chloroplast components close to its site of production and to generate more stable signaling molecules [27]. It has been proposed that β-cyclocitrinal (β-CC), one of the carotenoid oxidation products in chloroplasts, is a stress signal that mediates gene responses to 1O2 in plants [28**] (Figure 1).

Carotenoids are considered to be the main 1O2 quenchers in chloroplasts [29]. Light stress induces the oxidation of the carotenoid β-carotene, leading to the accumulation of different volatile derivatives including β-carotene: β-CC, β-ionone (β-I), and dihydroactinidiolide. The exposure of Arabidopsis plants to low levels of volatile β-CC, leading to internal concentrations close to the levels reached in high-light-treated plants, were found to change the expression of a large set of genes [28**]. Most of the genes affected by β-CC (more than 80%) were identified as 1O2-responsive genes, suggesting that β-CC is an intermediate in the signaling of this ROS. In addition, these effects appear to be specific to β-CC since they were not observed with the related molecule β-I [28**]. The β-CC is a volatile, lipid-soluble compound that should be able to cross lipid membranes and therefore it is a potential candidate for transfer of information out of
the chloroplast. Nevertheless, the precise mechanism of β-CC action remains elusive. Singlet oxygen is able to activate a signaling pathway within chloroplasts that depends on the two plastid-localized proteins EXE-CUTER 1 and 2 (EX1/2) [30]. However, the reprogramming of gene expression by β-CC seems to be independent of the EX-dependent \( \text{O}_2 \) signaling pathway and more work is required to identify the downstream target of β-CC action.

Carotenoid oxidation is not mediated exclusively by ROS, but it can also occur in vivo through the action of specialized enzymes, the carotenoid cleavage dioxygenases (CCDs), which cleave carotenoid molecules at particular positions, generating specific apocarotenoids [31]. A putative apocarotenoid-derived molecule generated in chloroplasts was recently proposed to regulate leaf development and the expression of nuclear genes of chloroplast proteins [32*] (Figure 1). This unidentified molecule originates from phytoflouene and/or carotenoids and its generation requires the activity of CCD4 [28**]. Although this molecule has been conceived as a retrograde signal, further investigations are needed to elucidate its function. For instance, the evidence that it is a signal rather than an essential metabolite is still lacking. The accumulation of this putative signal can lead to severe abnormality of leaf morphology and anatomy, suggesting that retrograde signals in addition to their well-established functions, can clearly affect major developmental processes such as leaf development and function as feedback signal responding to the state of organelle development. This type of retrograde signal not only derives from the carotenoid pathway but also from the chloroplast gene expression system and other unknown sources [33,34*,35,36]. However, the signals for these retrograde signaling pathways have not yet been identified.

**MEcPP signaling in bio-stress response**

The methylenedioxythiol phosphate (MEP) pathway responsible for chloroplast isoprenoid synthesis is critical for plant growth and development [37]. A genetic screen designed to identify genes involved in the regulation of the hydroperoxide lyase gene (HPL), a stress-induced nuclear gene encoding a chloroplast protein in the oxylipin pathway, identified an isoprenoid precursor, methylenedioxy cyclodiphosphate (MEcPP) derived from MEP as a potential retrograde signal from chloroplasts [38**] (Figure 1). A mutant ceh1 (constitutively expressing HPL) was identified that highly and constitutively expresses HPL. CEH1 encodes 1-hydroxy-2-methyl-2-(E)-bute- nyl4-diphosphate synthase, which catalyzes the conversion of MEcPP to hydroxymethylbutenyl diphosphate [38**]. In the ceh1 mutant, MEcPP accumulations and up-regulation of salicylic acid (SA) production confers increased resistance to infection by *Pseudomonas syringae*, a biotrophic pathogen. SA accumulation and induction of *HPL* were due to the accumulation of MEcPP rather than to a general stress syndrome resulting from perturbation of the MEP pathway [38**]. Moreover, feeding experiments demonstrate that MEcPP can directly regulate *HPL*. Thus, it seems that in contrast to the mechanism mediated by chlorophyll precursors where the flux through the pathway is important for retrograde signaling [115*], the induction of *HPL* is specifically triggered by MEcPP, and not by other intermediates of the MEP pathway.

Chloroplasts play a critical role in the plant abiotic stress response because they are the site for the production for SA and jasmonic acid, important mediators of plant immunity [39]. However, the molecular link between chloroplasts and the cytoplasmic-nuclear immune system remains largely unknown. This molecular link may be related to a chloroplast calcium-sensing receptor, which is involved in Pathogen-Associated Molecular Pattern (PAMP)-induced expression of defense genes possibly through \( \text{O}_2 \)-mediated retrograde signaling [40*]. The involvement of MEcPP in retrograde biotic stress signaling opens a way to study the link between chloroplasts and the cytoplasmic-nuclear immune system.

Interestingly, oxidative stress in bacterial cultures also induces MEcPP production [41]. This similarity in the modulation of MEcPP levels in response to abiotic stresses suggests at least some conservation of function of this metabolite as a stress sensor in regulating specific stress-responsive processes in eubacteria and plants. However, how MEcPP is involved in gene expression is still an open question, although it has been suggested that MEcPP could affect chromatin remodeling and gene expression through disruption of the interaction between histone-like proteins and DNA [42]. In addition, direct evidence for MEcPP movement from chloroplasts to the nucleocytoplasmic compartment is still lacking and deserves further investigation.

**Conclusions**

It appears that operational retrograde signaling in response to environmental stresses involves some metabolite-linked pathways in addition to the previously described redox and ROS pathways. Dissecting the metabolite trafficking between chloroplasts and cytoplasm may help in understanding the generation and transmission of metabolite retrograde signals involved in stress responses. For instance, the metabolite export mediated by the triose phosphate/phosphate translocator is proposed to participate in fast retrograde signaling in response to HL [43*]. Nevertheless, the metabolite signal might not act through a linear pathway (generated in and exported from chloroplasts then moving through the cytosol and acting in the nucleus), more novel ideas and scenarios for metabolite retrograde signaling should be explored in the future [44,45]. The nature of the signal molecules from chloroplast gene expression, chloroplast...
redox state and the newly identified chloroplast unfolded protein response pathway still remain elusive [45, 46**]. However, the perturbation of those retrograde signaling pathways may also lead to changes of metabolites in chloroplasts [47]. Therefore unraveling these retrograde signaling pathways via metabolomic approaches may also be useful for elucidating the possible retrograde signals of those pathways [48, 49]. Nevertheless, concentrations of these putative retrograde signals may be too low to detect by current metabolomic technologies and continuous technological developments in the field of metabolomics are expected.

The current studies show that some retrograde signaling pathways converge with other networks such as light signaling [50], immune signaling [40*] and developmental signaling [36]. The elucidation of signaling networks and of the synergistic and antagonistic interactions of diverse retrograde inputs will be necessary for an integrative understanding of multiple retrograde signaling. Several transcription factors participating in retrograde signaling have been identified (Table 1) and dissection of the gene regulatory network mediated by key transcription factors is promising in this regard. This convergence of multiple retrograde signaling pathways is required to optimize chloroplast function to changing physiological conditions and important in adapting plant primary productivity to the environment. The *sall* plants showed markedly increased resistance to drought when water is withheld from soil-grown intact plants [22], raising the possibility that manipulation of metabolite retrograde signaling through genetic approaches might be used for crop improvement in the future.

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### References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


In order to examine the role of bilins in the regulation of chlorophyll synthesis during a dark/light transition, Duanna et al. performed a global comparative transcriptomic analysis with wild-type and hmx01 cells in the presence or absence of biliverdin and identified a bilin-specific network of nuclear gene expression. This study raises the possibility that heme act as retrograde signals in C. reinhardtii.


PAP is a substrate of SAL1 in vivo and PAP accumulates in sal1 mutants during drought or high light stress. SAL1 localizes to both chloroplast and mitochondria. However, overexpression of SAL1 in the nucleus or chloroplasts can complement PAP levels in sal1, suggesting that PAP can move between cellular compartments. The transcriptome analysis of xrn and sal1 mutants indicates that SAL1 and nuclear XRNs coregulate a large subset of genes. This work provides exciting evidence of a SAL1-PAP retrograde signaling pathway regulating the expression of various stress-related nuclear genes.


28. Ramel F, Birtic S, Ginies C, Soubigou-Taconnot L, • Triantaphylidou L, C, Havaux M: Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. Proc Natl Acad Sci USA 2012, 109:5535-5540. The authors demonstrate that one product of β-carotene oxidation, β-cyclocitrinal induces changes in the expression of a large set of genes that have been identified as O2− responsive genes. This study indicates that β-cyclocitrinal is a stress signal produced in high light that is able to induce defense mechanisms and might represent a messenger involved in the O2− signaling pathway in plants.


The accumulation of specific carotenoids in ß-carotene desaturase mutants can lead to profound alternation in leaf morphology and cellular differentiation as well as the alternation of nuclear gene expression. Thus it is proposed that an unidentified apocarotenoid-derived retrograde signal regulates leaf development and nuclear gene expression.


38. Xiao Y, Savchenko T, Baidoo EE, Chehab WE, Hayden DM, • Tolstikov V, Corwin JA, Kleibenstein DJ, Keasling JD, Dehesh K: Retrograde signaling by the plastidial metabolite MEPP regulates expression of nuclear stress-response genes. Cell 2012, 149:1525-1538. A mutation in HDS, a MEP pathway gene leads to constitutive expression of HPL and to high level of salicylic acid. These phenotypes do not reflect a general stress response, but result from the accumulation of MECP. The authors propose that the MEP pathway can function as a stress sensor and coordinator of expression of targeted stress-responsive nuclear genes via modulation of the levels of MECP, a specific and critical retrograde-signaling metabolite.


42. Grieshaber NA, Sager JB, Dooley CA, Hayes SF, Hackstadt T: Regulation of the Chlamydia trachomatis histone H1-Like


A set of AP2/ERF transcription factors rapidly responds to high light. However, this response is compromised in a mutant affected in the triose phosphate/phosphate translocator. The metabolite export mediated by the triose phosphate/phosphate translocator is proposed to be part of fast retrograde signaling in response to high light.


Based on the study on the gene expression of the Chlamydomonas cells in which chloroplast ClpP protease was conditionally depleted, the authors suggest the existence of a chloroplast-to-nucleus signaling pathway that is conceptually similar to the unfolded protein response observed in the endoplasmic reticulum and in mitochondria.


This study shows that PTM, a chloroplast envelope-bound plant homeodomain transcription factor with transmembrane domains, functions in multiple retrograde signaling pathways. The proteolytic cleavage of PTM occurs in response to retrograde signals and amino-terminal PTM accumulates in the nucleus, where it activates ABI4 transcription. It is possible that this mobile protein is involved in retrograde signaling.

