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Retrograde Signals Navigate the Path to Chloroplast Development[1[OPEN]]

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Light is the main source of energy for life on Earth, and plants and algae are able to convert light energy, through photosynthesis, into chemical energy that can be used by all organisms. The photosynthetic reactions are housed in the chloroplasts, but the chloroplasts also are the site for synthesis of essential compounds like fatty acids, vitamins, amino acids, and tetrapyrroles. Given their essential role, the correct formation and function of chloroplasts is vital for the growth and development of plants and algae, and hence for almost all organisms. Chloroplasts evolved from an endosymbiotic event where a photosynthetic prokaryotic organism was acquired by a proeukaryotic cell. With time, the photosynthetic prokaryote lost or transferred most of its genes to the host genome. As a result, plastid protein complexes, such as the photosynthetic complexes, are encoded by genes of both the nuclear and plastid genomes. This division of genetic information requires a precise coordination between the two genomes to achieve proper plastid development and function. Plastid development and gene expression are under nuclear control, in what is referred to as anterograde control. However, there also is a signaling system originating in the plastids, so-called retrograde signals, transmitting information about the developmental and functional state of the plastids to the nucleus to regulate nuclear gene expression. Retrograde signaling is a complex network of signals that can be divided into "biogenic control," referring to signals generated by the plastid as it develops from a proplastid or etioplast into a chloroplast, and "operational control" signals, including those generated from a mature chloroplast in response to environmental perturbations (Chan et al., 2016).

FROM THE PLASTID SIGNAL TO THE COMPLEX NETWORK OF PLASTID-TO-NUCLEUS SIGNALING

The original idea of a single plastid signal has evolved over the years, and we now know that the retrograde signaling system is a complex network of signals and pathways, most of which are still unknown. To date, four major signals/pathways have been described: (1) signals related to the tetrapyrrole biosynthesis pathway (TBP); (2) signals triggered by plastid gene expression (PGE); (3) reactive oxygen species (ROS) and changes to the activity of the photosynthetic electron transport (PET) chain; and (4) signals deriving from disturbed plastid metabolism, such as accumulation of 3′-phosphoadenosine 5′-phosphate, 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate (MEcPP), or carotenoid derivatives like β-cyclocitral or apocarotenoids (Estavillo et al., 2011; Ramel et al., 2012; Xiao et al., 2012; Avendaño-Vázquez et al., 2014). The signals derived from plastid metabolism are mostly related to stress responses and operational control of the chloroplast. Those pathways have recently been extensively reviewed, and will not be covered in this review (Bobik and Burch-Smith, 2015; Chi et al., 2015; de Souza et al., 2017). Our focus here is on biogenic control and the retrograde signals linked to the early light response and to chloroplast development.

The biogenic signals can be linked to the TBP, PGE, and changes to the PET activity (Fig. 1). Chlorophyll and the other major tetrapyrroles, like heme, siroheme, and phytochromobilin, derive from a common

ADVANCES

- Chloroplast function and integrity impacts leaf and plant development throughout the entire plant life cycle.
- During leaf development, chloroplast differentiation is an important regulator of the simultaneous onset of cell expansion and photosynthesis.
- The light induction of *PhANGs* during chloroplast development occurs in two essential regulatory phases: a photoreceptor-mediated phase and a phase controlled by a plastid signal, where the light signal precedes the plastid signal.
- Two mechanistic modules have been shown to control chloroplast development: the convergence of the PHY-PIFs-dependent light signal and the GUN1-dependent retrograde signal, and the GUN1-PTM-ABI4 retrograde signaling module which antagonizes the COP1-HY5 light signal.

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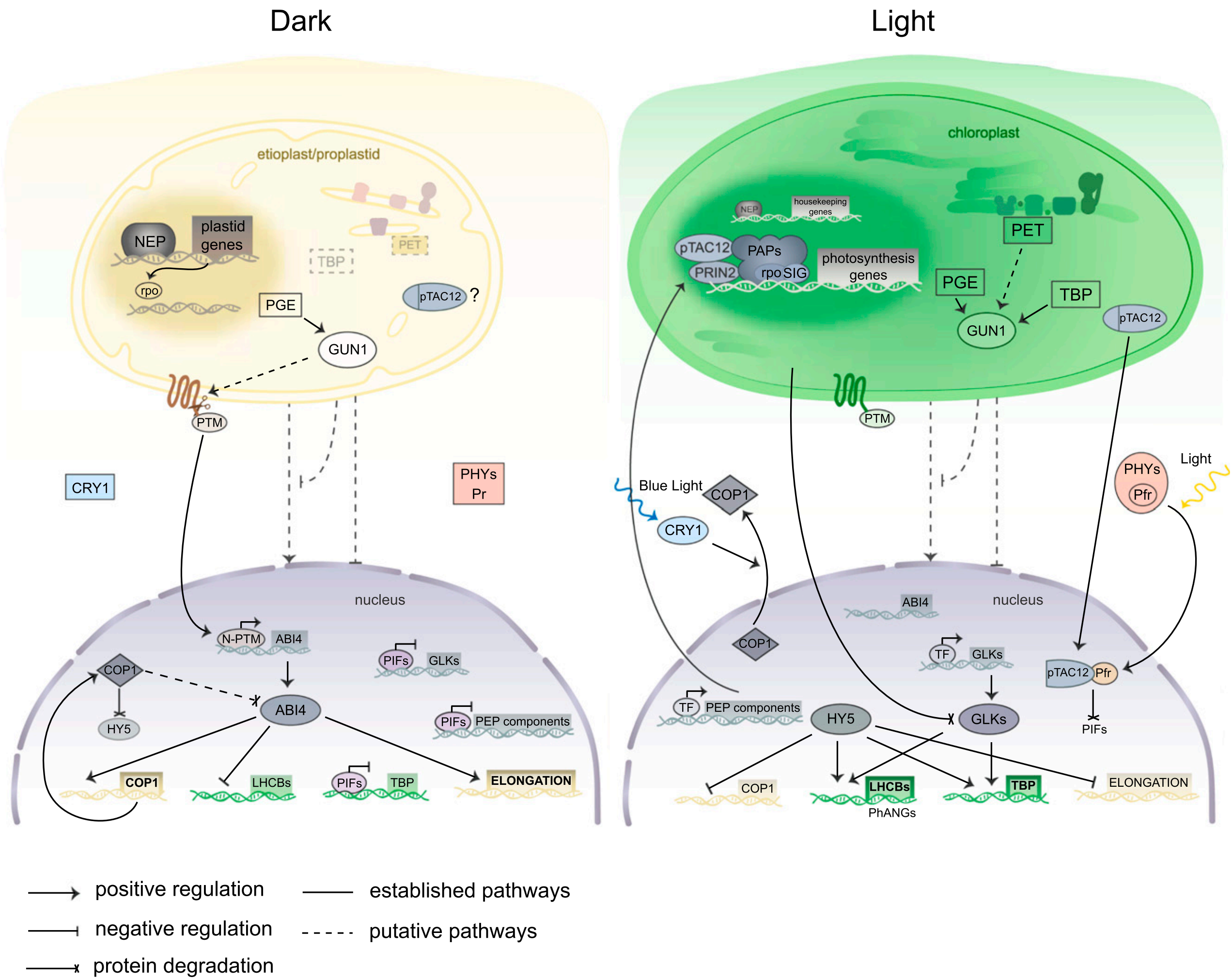

Figure 1. Model of retrograde signaling during chloroplast biogenesis. Chloroplasts develop from etioplasts or proplastids in response to light. In the dark and in the etioplasts/proplastids, the plastid-encoded genes are mainly transcribed by NEP, and PET and TBP are not functional (dashed line boxes). During the greening process, activation of PEP plays a major role. PEP activity requires the rpo core components, SIGMA factors (SIG), PEP-associated proteins (PAPs, like pTAC12), and other proteins located in the nucleoid, like PRIN2. PRIN2 is involved in the light regulation of PEP activity. The disruption of PET, TBP, or PGE generates a signal that is transduced by GUN1. It is to date unknown if the retrograde signal is of a positive nature that is interrupted in the case of chloroplast disruption (for example, by GUN1) or if the disrupted chloroplasts emit a negative signal (that can be mediated by GUN1). In response to chloroplast signals, PTM is cleaved, and the N-PTM form is translocated to the nucleus where it activates *ABI4* expression, inhibiting *LHCB* expression and promoting the expression of *COP1* and genes involved in hypocotyl elongation. In the dark, HY5 is degraded by COP1, which also degrades ABI4. The PIFs repress a set of *PhANG*s, the *GLK*s, and genes encoding the nuclear-encoded PEP components. In the light, the light perception by the photoreceptors CRY1 and PHYs causes exclusion of COP1 from the nucleus and degradation of the PIFs. The chloroplast-localized pTAC12 is an essential part of the PEP complex, but also is processed and translocated to the nucleus. The nuclear pTAC12 interacts with the active form of PHYs (Pfr) and contributes to PIF degradation. The exclusion of COP1 allows for accumulation of HY5. Degradation of PIFs releases the transcription of the *GLK*s and other sets of genes, like the genes encoding the PEP component. HY5 and GLKs induce *PhANG* (*LHCB*s and genes coding TBP enzymes, among others) expression, and repress *COP1* and the elongation-related genes. Figure by Daria Chrobok.

biosynthetic pathway (TBP) located in the plastids. Arabidopsis (*Arabidopsis thaliana*) mutants with impaired communication between the chloroplast and the nucleus, referred to as the *gun* (for *genomes uncoupled*) mutants, were isolated from forward genetic screens (Susek et al., 1993; Mochizuki et al., 2001; Woodson et al., 2011). *GUN2* to *GUN6* all encode components closely associated with tetrapyrrole biosynthesis, and the respective mutants have changes to the flux through TBP (Box 1). The phenotype of the *gun* mutants has been linked to oxidative stress causing perturbations of flux through the TBP

BOX 1. The *genomes uncoupled* Mutants

The Arabidopsis *genomes uncoupled* mutants were identified in a forward genetic screen using the herbicide Norflurazon that inhibits carotenoid biosynthesis and thereby blocks chloroplast biogenesis. The expression of two transgenes under the control of the *chlorophyll a/b-binding protein3* (*CAB3*) promoter was used to screen the mutagenized population for transcription of *CAB3* that was uncoupled from the functional state of the chloroplast (Susek et al., 1993). The screen resulted in the isolation of several *gun* mutants, all of them with mutations in genes encoding chloroplasts-localized proteins:

- *gun1* is defective in a pentatricopeptide-repeat protein that integrates TBP-, PGE-, and PET-derived signals. Additional *gun1* alleles have been isolated in different screens using the *Luciferase* reporter gene under the control of *CAB* promoter. Of the *gun* mutants, *gun1* is the only mutant that shows the *gun* phenotype when grown with Lincomycin, and also in response to high light, clearly indicating a role of GUN1 in the response to disturbed PGE and PET (Koussevitzky et al., 2007).
- *gun2* and *gun3* are defective in heme oxygenase and phytochromobilin synthase, respectively, enzymes that convert Heme to Phytochromobilin. The *gun2* and *gun3* accumulate more Heme but less chlorophyll compared to wild type. The pale phenotypes were explained by a negative regulation of Heme on TBP (Mochizuki et al., 2001).
- *gun4* and *gun5* are defective in a novel Mg-ProtoIX-binding protein and in the H-subunit of Mg-chelatase, respectively. Mg-chelatase is the first specific TBP enzyme of chlorophyll biosynthesis, and both *gun4* and *gun5* accumulate lower levels of chlorophyll compared to wild type (Mochizuki et al., 2001).

In an alternative screen using activation-tagged mutagenesis, an additional *gun* mutant was identified, *gun6*:

- *gun6-1D* is a dominant gain-of-function mutant of Ferrochelatase 1 (FC1), the enzyme upstream of GUN2 and GUN3 in the biosynthesis pathway of Heme (Woodson et al., 2011).

resulting in accumulation of both ROS and specific metabolites (for review, see Larkin, 2016). In addition, the tetrapyrrole heme has been proposed as a plastid signal positively regulating the expression of Photosynthesis Associated Nuclear Genes (*PhANG*s) during chloroplast development (Woodson et al., 2011). During chloroplast development, precise coordination between the synthesis of the light-absorbing pigments and the expression of the nuclear-encoded chlorophyll-binding proteins is required to correctly assemble the antennae-reaction center super complexes of PSII and PSI. Thus, regulation of *PhANG* expression is linked to tetrapyrrole biosynthesis, and it has been suggested that *PhANG* expression is controlled by a balance between light-signaling pathways and a plastid signal triggered by impaired flux through chlorophyll biosynthesis, Mg-ProtoIX and/or heme (Woodson et al., 2011; Barajas-López et al., 2013). Furthermore, $^1O_2$, a known operational control signal, also has been proposed to play a role during chloroplast development by repressing *PhANG*s in response to moderate increases of chlorophyll precursors (Page et al., 2017b).

The status of plastid transcription and translation as a trigger for a retrograde signal has been demonstrated by repression of *PhANG* expression following chemical treatments such as the plastid translational inhibitor lincomycin or rifampicin, which selectively inhibits the plastid-encoded RNA polymerase (PEP; Woodson et al., 2013; Chan et al., 2016). The repression of the *PhANG* expression phenotype also was observed in mutants affected in the plastid transcriptional machinery, including: the RpoTp/SCA3 protein of the nuclear-encoded RNA polymerase (NEP); the sigma factors SIG2 and SIG6 essential for PEP activity; and other proteins required for full expression of the PEP-dependent genes like Polymerase-Associated Protein7 (PAP7), Plastid Redox Insensitive2 (PRIN2), or Yellow Seedlings1 (Hricová et al., 2006; Gao et al., 2011; Kindgren et al., 2012; Woodson et al., 2013; Box 2). The exact nature of the signal generated by PGE is unknown, and different but overlapping pathways have been suggested (Woodson et al., 2013). One of the proposed signaling pathways involves PEP-transcribed $tRNA^{Glu}$, the starting and limiting substrate of TBP, thus linking PGE to TBP-mediated signaling with the regulation of *PhANG* expression during chloroplast biogenesis (Woodson et al., 2013).

In the mature chloroplast, ROS generated by photosynthesis and changes to the redox state of PET act as indicators of environmental fluctuations, and generate signals in the context of operational control (Chan et al., 2016). However, there also are reports where an imbalance of PET is perceived in the frame of biogenic control during the initial stages of plastid development. IMMUTANS (IM) encodes a plastid terminal oxidase (PTOX) required for chloroplast biogenesis, both in seedlings and in adult leaves. IM is a component of a redox pathway that desaturates phytoene, where electrons are transferred from phytoene to plastoquinone (PQ) via phytoene desaturase and from PQ to oxygen via IM. Thus, in the *ptox* mutant, this pathway is inhibited and carotene biosynthesis is blocked at the phytoene desaturase step. The lack of protective carotenoids results in photooxidation of plastid components, which halts plastid development (Kambakam et al., 2016; Pogorelko et al., 2016). Transcriptomic analyses of etiolated and de-etiolating *im* seedlings revealed altered expression of nuclear transcription factors that control *PhANG* expression (Kambakam et al., 2016). The retrograde signal or signals triggered by the imbalance of PET during chloroplast development could potentially coordinate chlorophyll with carotenoid biosynthesis during the early light response.

BOX 2. The Plastid Transcription Complexes, NEP and PEP.

Chloroplast genes are transcribed by two types of RNA polymerases; the nuclear-encoded plastid RNA polymerase (NEP) and the plastid-encoded RNA polymerase (PEP). Essential for proper chloroplast development is a shift in the usage of the major RNA polymerase from NEP to PEP. NEP is a nuclear-encoded phage-type single subunit polymerase that is encoded by the *RNA polymerase of the phage T3/T7 type* (*RPOT*) genes, while PEP is a multisubunit RNA polymerase inherited from the cyanobacterial ancestor. The PEP core subunits are encoded by the *rpo* plastid genes, but PEP requires the nuclear-encoded sigma factors for promoter recognition and a large number of other proteins referred to as PEP-associated proteins (PAPs) or Transcriptionally Active Chromosome proteins (pTACs), for its function. Several mutants have been reported in NEP, PEP, or PEP-associated proteins and those mutations trigger the PGE retrograde signal:

- *scabra3 (sca3)* is defective in the exclusively plastid-localized RPOTp of NEP. SCA3 is essential for chloroplast biogenesis, and the mutants have pale or yellowish cotyledons and leaves (Hricová et al., 2006).
- *sigma2* and *sigma6 (sig2* and *sig6)* are defective in the SIGMA2 and -6 proteins that are essential for the DNA sequence recognition of PEP during chloroplasts development and the mutant seedlings are pale (Woodson et al., 2013).
- *ptac12/hemera* is defective in the dual-localized protein pTAC12/HMR that is associated with PEP. The protein is essential for PEP activity and chloroplast development in the light, which is reflected in the albino phenotype of the mutant. In addition to the role of pTAC12/HMR in the plastids, the protein could potentially act as a plastid signal transducer as it has also been identified in the nucleus associated with Phy (Chen et al., 2010; Galvão et al., 2012; Nevarez et al., 2017).
- *pap7/ptac14* is defective in PAP7 protein that is associated with PEP. *pap7* mutant seedlings are albino and defective in PEP-dependent transcription (Gao et al., 2011)
- *prin2* is defective in Plastid Redox Insensitive2 (PRIN2), a novel protein that interacts with TRXz and is essential for light induced activation of PEP. *prin2* mutants are pale and have impaired chloroplasts (Kindgren et al., 2012; Kremnev and Strand, 2014; Díaz et al., 2017).

FROM THE CHLOROPLAST TO THE NUCLEUS: TRANSDUCERS OF PLASTID SIGNALS

The different signals originating in the plastids need to be transduced to the nucleus and connected to transcriptional regulators that control the transcription of *PhANG*s. GUN1 seems to act as a central hub in the chloroplast, integrating different retrograde signals. Similar to GUN5, GUN1 plays a role in the tetrapyrrole-mediated pathway (Koussevitzky et al., 2007), and genetic analyses revealed that GUN1 also is implicated in the PGE-triggered retrograde signal, as supported by the *gun1* mutation reverting the altered *PhANG* expression phenotype caused by mutations in SIG2, SIG6, and PRIN2 (Kindgren et al., 2012; Woodson et al., 2013). GUN1 is a nuclear-encoded, plastid-localized protein with homology to pentatricopeptide repeat (PPR) domain-containing proteins, but the exact molecular role(s) of GUN1 remains unknown. PPR proteins are involved in RNA processing, and initially a fragment of GUN1 including the PPR domain was shown to bind DNA. However, no interaction was detected between GUN1 and nucleic acids when the entire GUN1 protein was used (Koussevitzky et al., 2007; Tadini et al., 2016). Proteomic analyses have identified a large number of GUN1-interacting proteins representing a wide range of functions, including ribosomal proteins and factors involved in ribosome biogenesis, enzymes in tetrapyrrole biosynthesis, and protein chaperones involved in protein import to the chloroplast, assembly of proteins, and protein degradation (Tadini et al., 2016). However, the identified interactions were weak, suggesting that GUN1 could transiently associate with different protein complexes (Tadini et al., 2016). In addition, given the diversity of proteins identified, the proteomic efforts have so far generated few clues about the true function(s) of GUN1. Possibly, in vivo interaction studies would generate more relevant results.

A plant homeodomain transcription factor with transmembrane domains located to the chloroplast envelope, named PTM, was identified as a potential transduction component of the GUN1-mediated signaling pathway (Sun et al., 2011). Following treatments with lincomycin or norflurazon, a truncated form of PTM (N-PTM) accumulated in the nucleus in a Ser protease-dependent manner. Furthermore, the cleaved N-PTM form was shown to directly control the expression of transcription factors, such as Abscisic Acid Insensitive4 (ABI4), in the nucleus (Sun et al., 2011). The *gun* phenotype of the *ptm* mutant was recently challenged after analysis of three *ptm* mutant alleles (Page et al., 2017a), and alternative pathways for the transduction of the plastid signal to the nucleus were suggested. An alternative for the control of ABI4 activity in response to a retrograde signal is a calcium-dependent, three-component MAPK system. Perturbations of chloroplast function, following norflurazon and/or lincomycin treatments, cause transient increases in cytosolic Ca^{2+} that activates the MAPK cascade involving the calcium-binding protein 14-3-3ω. The activated MPK3 or MPK6 phosphorylates and activates ABI4 in the nucleus (Guo et al., 2016). In addition, an as yet unknown mechanism transduces retrograde signals to control the protein levels of the nuclear transcription factor Golden2-like1 (GLK1), in a GUN1-independent way (Tokumaru et al., 2017).

Overall, very few transcriptional regulators have been assigned a role in retrograde signaling during the process of chloroplast development. ABI4 acts downstream of GUN1 and the calcium signal, and in response to chloroplast dysfunction, the GUN1-PTM module activates the transcription of *ABI4*, whereas

the Ca^{2+}-activated MPK3/MPK6 phosphorylates ABI4, which will then bind the promoters of *PhANG*s and thus repress their transcription (Koussevitzky et al., 2007; Sun et al., 2011; Guo et al., 2016). The GLK1 and GLK2 transcription factors are major players in chloroplast biogenesis, integrating retrograde and light signals (Waters et al., 2009; Martín et al., 2016). GLK1 and GLK2 promote chloroplast development by directly activating the expression of nuclear genes encoding proteins involved in chlorophyll biosynthesis, light harvesting, and electron transport (Waters et al., 2009). The important role of the GLKs is reinforced by the pale *glk1glk2* double mutant with partially developed chloroplasts and a, albeit weak, *gun* phenotype (Fitter et al., 2002; Waters et al., 2009). Although genetic analyses indicated that GLK1 and GLK2 are redundant, recent work has revealed specificity that is regulated at transcriptional and posttranscriptional levels (Powell et al., 2012; Kobayashi et al., 2013; Wang et al., 2013; Martín et al., 2016; Tokumaru et al., 2017).

INTERACTION OF PLASTID AND LIGHT SIGNALS DURING CHLOROPLAST DEVELOPMENT

Chloroplast biogenesis is a complex process, and the mechanisms involved differ not only between different plant species but also between different organs within the individual plant. Numerous studies have revealed the significance of proper chloroplast development during all stages of plant growth and the necessity for coordination between growth and chloroplast development (Pogson et al., 2015; Chan et al., 2016). Much of our current knowledge of the molecular mechanisms controlling chloroplast biogenesis comes from studies using the angiosperm model species Arabidopsis. Angiosperms germinate in darkness (e.g. covered by soil) and develop following a dark-adapted program named skotomorphogenesis, which is characterized by long hypocotyls and etiolated cotyledons that contain a form of plastid referred to as etioplasts. Following light exposure, the developmental program changes into photomorphogenesis, hypocotyl elongation is inhibited, and the cotyledons turn green and develop functional chloroplasts (Pogson et al., 2015). In adult leaves, mature chloroplasts develop from proplastids in the stem cells of the apical meristem. Proplastids start to differentiate and form the first extended thylakoid membranes in specific layers of the meristem and in the leaf primordia. Developmental gradients can be observed from the base to the tip (most matured chloroplasts) and from the margin to the midrib within a given leaf in Arabidopsis (Pogson et al., 2015; Gügel and Soll, 2017). However, the spatial (base-to-tip) developmental gradient is more easily detected in monocotyledonous leaves (Pogson et al., 2015).

In angiosperms, chloroplast biogenesis is dependent on light. Exposure to light leads to extensive transcriptional reprogramming, involving up to one-third of the nuclear-encoded genes that are either induced or repressed in response to light signals (Ma et al., 2001; Jiao et al., 2005; Dubreuil et al., 2018). A significant number of the induced genes encode *PhANG*s. Light and plastid signals regulate expression of the same group of photosynthesis-related genes, and it has been reported that light and retrograde signals also are mediated by cis-elements found in close proximity (Koussevitzky et al., 2007; Chi et al., 2013). Recent studies revealed that nuclear genes encoding photosynthesis-associated genes, such as genes encoding light-harvesting antenna proteins and TBP enzymes, are enriched in G-box elements in their promoters and could be regulated directly by ELONGATED HYPOCOTYL5 (HY5) and indirectly by GLKs, which are central regulators of light and chloroplast development signaling, respectively (Lee et al., 2007; Waters et al., 2009). This suggests a close interaction between these two signaling pathways that we have recently begun to untangle. However, these two pathways also can act independently, as retrograde signals from the plastid also were shown to regulate *PhANG* expression in the dark (Sullivan and Gray, 1999; Larkin, 2014). Interestingly, this specific feature of plastid signaling is observed in many gymnosperms where the seedlings green in the dark, and recent work from pine suggested that activation of gymnosperm *PhANG*s in the dark may be regulated by a plastid-to-nucleus signal (Hills et al., 2015).

The first regulatory step leading to the development of functional chloroplasts is the perception of light by a set of photoreceptors, phytochromes (PHY) and cryptochromes (CRY), which undergo conformational changes to interact with downstream proteins and initiate intracellular signaling pathways (Jiao et al., 2007; Waters and Langdale, 2009). Genetic data revealed that the blue light receptor CRY1 is involved in chloroplast development during blue light induction and that the transcription factor HY5 also is involved in this pathway (Ruckle et al., 2007; Fig. 1). The red and far-red light receptors, the PHYs, also regulate HY5 upon light activation, but PHY signaling mainly proceeds through the Phytochrome-Interacting Proteins (PIFs), which are transcriptional repressors of chloroplast biogenesis (Jiao et al., 2007). A recent quantitative mathematical model, validated in vivo, established a direct link between light input via PHYB-PIF3 and the initiation of chloroplast development. The light-activated form of PHYB degrades PIF3, which represses the nuclear-encoded components of the plastid transcriptional machinery required for transcription of the plastid-encoded photosynthesis genes (Dubreuil et al., 2017; Fig. 1). pTAC12/HMR, one of the components associated with the PEP, was shown to be dually targeted to the plastid and the nucleus. In the nucleus, pTAC12/HMR interacts with the photoactivated PHYs, and PHY-pTAC12/HMR participates in the degradation of PIFs in the light, thereby promoting photomorphogenesis (Chen et al., 2010; Galvão et al., 2012; Fig. 1). The newly synthetized pTAC12/HMR has

a plastid transit peptide and is transported to the plastids where it is processed for the subsequent nuclear localization. However, the mechanisms and regulation of this translocation from the plastids to the nucleus is unknown (Nevarez et al., 2017). Detailed investigations of the timing of pTAC12/HMR localization during early light response and chloroplast development could confirm the exciting proposition that pTAC12/HMR might be involved in the coordination of photosynthetic gene expression in both the nucleus and the chloroplast in response to light.

Two molecular pathways for the interaction of light and retrograde signals have recently been described during the transition from skotomorphogenesis to photomorphogenesis in Arabidopsis seedlings (Fig. 1). One of the pathways involves the GUN1-PTM-ABI4 retrograde signaling module, which in this model antagonizes the COP1-HY5 light signal. During de-etiolation of seedlings, ABI4 and HY5 integrate both signals by directly controlling the expression of a set of genes involved in hypocotyl elongation and chloroplast development (Xu et al., 2016). Moreover, the proposed pathway involves HY5 or ABI4 degradation by COP1. Conversely, HY5 and ABI4 both regulate the expression of *COP1*: ABI4 stimulates and HY5 inhibits expression (Fig. 1). Thus, the balance between these three components provides the means to fine-tune the response to light (Xu et al., 2016).

The second pathway describes the convergence of PHY-PIF-dependent light signal and the GUN1-dependent retrograde signal. The interaction point is downstream of the PIFs and antagonistically regulates the transcriptional photomorphogenic network (Martín et al., 2016). This pathway is independent of ABI4; instead, the GUN1- and the PIF-dependent signals converge on *GLK1*, repressing or inducing its expression, respectively. This transcriptional control over *GLK1* is both ABI4- and HY5-independent, and the existing evidence suggests that *GLK1* is a direct target of PIF-mediated repression in the dark (Oh et al., 2012; Leivar and Monte, 2014; Martín et al., 2016). In the model that has been proposed, PIFs directly inhibit *GLK1* expression in the dark to support skotomorphogenesis. In the light, *GLK1* expression is released and allows photomorphogenesis to proceed. When the plastid is dysfunctional or damaged, a GUN1-mediated retrograde signal represses *GLK1* and attenuates photomorphogenesis (Martín et al., 2016; Fig. 1).

Clearly, there is a close interaction between light and plastid signals, but the timing of these two signals during the normal progression of chloroplast biogenesis has been unclear. Early research provided strong indications that a plastid retrograde signal is required for full expression of the nuclear-encoded photosynthetic genes (Sullivan and Gray, 1999). Blocking PEP-driven plastid transcription with rifampicin or using mutants of various PEP components blocked *PhANG* expression in a light-independent way (Woodson et al., 2013). However, a recent comparative transcriptomic analysis of seedlings grown in light or dark, and the albino *pap7* mutant, revealed that altered PGE and blocked plastid development does not affect global *PhANG* expression (Grübler et al., 2017). On the other hand, a new model where the light response has been investigated in an Arabidopsis cell culture with very high temporal resolution of the chloroplast developmental process suggests that the light signal precedes a plastid signal (Dubreuil et al., 2018). Two phases were clearly observed in the expression profile of the *PhANG*s in the cell culture. The first phase is dependent on light and triggers changes that will initiate chloroplast development, and more importantly initiates expression of the PEP components. The second phase is dependent on the activation of the chloroplast as the second phase of *PhANG* induction was absent when chloroplast development was blocked (Dubreuil et al., 2018).

The nature of the plastid signal, whether it is a negative or a positive signal, is another open question, and different hypotheses have been proposed over the years (Pfannschmidt, 2010; Terry and Smith, 2013). However, recent experimental work goes some way to clarifying this question. Supporting the early study by Sullivan and Gray (1999), the work of Martín et al. (2016) demonstrated that the inhibitory effect of lincomycin blocks the photomorphogenic responses early and rapidly, indicating that the retrograde signal is already present in the proplastids/etioplasts or is developed rapidly in response to defective chloroplasts to prevent normal development in light. The results from the experiments by Martín et al. (2016) can be interpreted in two ways: (1) a positive signal from intact plastids that is necessary for *GLK1* expression is interrupted by plastid dysfunction in a GUN1-dependent manner; or (2) functional chloroplasts do not emit signals, whereas dysfunctional chloroplasts generate a GUN1-dependent negative signal that represses *GLK1* expression. Support for the positive nature of the plastid signal comes from analyses of *LHCB* expression in pine, where *LHCB*s are expressed in the dark and thus *PhANG* expression was shown to be independent of light but dependent on plastid-to-nucleus signals (Hills et al., 2015). These authors proposed an evolutionary model in which angiosperms recruited light-signaling repressors to suppress the response of *PhANG*s to a positive plastid signal also present in the dark (Hills et al., 2015). Furthermore, modeling of expression data from the plastid and the nuclear genomes strongly suggests that the plastid signal required for full *PhANG* induction is positive in nature (Dubreuil et al., 2018). In addition, it was recently shown using the *prin2* mutant that *LHCB* expression was directly correlated with the recovery of PEP activity in the *prin2* mutant complemented with different PRIN2 variants. It was further suggested that a positive signal is generated by PEP activity in the plastids that stimulates *LHCB* expression in the nucleus (Díaz et al., 2018). On the other hand, the transcriptomic results using *pap7* seedlings have identified different sets of genes regulated by either healthy chloroplast or arrested plastids, respectively, suggesting the existence of both positive and negative signals (Grübler et al., 2017).

HIGHER-ORDER RESPONSES IN THE NUCLEUS TO RETROGRADE SIGNALS

Light has been shown to induce changes in splicing activity (Petrillo et al., 2014; Shikata et al., 2014; Hernando et al., 2017), and the splicing pattern of specific splicing factors involved in dark-to-light shifts is regulated in response to the redox status of PET. Proper splicing of these factors was proposed to play a role in the adaptation of the photosynthetic machinery to changes in the light conditions (Petrillo et al., 2014). However, more experimental work is required to identify the components that regulate splicing in response to retrograde signals, the genes affected, and how those changes affect plant development and photosynthetic activity.

Light has been shown to induce changes to nuclear size and architecture that correlate with transcriptional changes (Bourbousse et al., 2015; Perrella and Kaiserli, 2016). A specific light-regulated locus, CAB, was shown to relocate to the nuclear periphery just before transcriptional induction in a PHYA, PHYB, PIF, COP1, and DET1-dependent manner (Feng et al., 2014). A change to the nuclear position was also observed for the photosynthesis-related genes *RBCS1A, GUN5*, and *PC*, supporting the model of repositioning of loci to the nuclear periphery as a mechanism to activate gene expression in response to light (Feng et al., 2014). So far, neither the repositioning of the gene loci nor the changes to nuclear architecture have been linked to retrograde signals, but due to the close relationship between light and plastid signals, a detailed analysis of a potential contribution of plastid signals to those interesting features would be worth pursuing.

In addition, the proportion of heterochromatin was shown to change in light-exposed cotyledons in a CRY1/CRY2-DET1/COP1-HY5-dependent manner (Bourbousse et al., 2015), and RNA-seq, in combination with DNase I hypersensitive site sequencing, revealed that the level of chromatin condensation in darkness is correlated with blocked expression of light- and photosynthesis-related genes. The genes affected by changes in chromatin condensation are enriched in plastid signaling-related genes and their promoters frequently contain the GLK1 binding elements (Liu et al., 2017), but at this point no clear link between retrograde signal(s) and chromatin changes has been established. Currently, much research attention is directed toward the connection between metabolism and chromatin dynamics in animal systems. Thus, exploring the dynamics of global chromatin organization during chloroplast development is a critical future direction for plant sciences.

THE IMPACT OF RETROGRADE SIGNALS ON WHOLE-PLANT PHYSIOLOGY AND DEVELOPMENT

Chloroplast function and integrity are important not only for seedling development during the transition to photomorphogenesis, but also for leaf and plant development throughout the entire plant life cycle. The importance of chloroplast activity during seed and embryo development is reflected in the large number of essential proteins localized to the chloroplast, many of them involved in PGE. The complete loss of SIG5 or RUG2/BSM results in embryo-lethal phenotypes (Hsu et al., 2010; Babiychuk et al., 2011; seedgene.org). The significant role of PEP activity and therefore PGE-triggered retrograde signaling during embryo development was further supported by the embryo pigment-defective phenotype of mutants in PEP components, like pTAC3 or MurE, or with altered PEP activity, like PRIN2 (Garcia et al., 2008; Kremnev and Strand, 2014; seedgene.org). Similarly, the complete loss of essential components of the plastid ribosomal complex, like ClpP proteins or Plastid Ribosomal Protein S5 (Scabra1), also results in embryo lethality (Pogson and Albrecht, 2011; Mateo-Bonmatí et al., 2015). Furthermore, the NEP-defective *sca3* mutant demonstrated reticulate patterns with perturbed mesophyll cell differentiation (Hricová et al., 2006). In addition, several *anu* (for *angulata*) mutants with abnormal leaf development are affected in genes encoding chloroplast proteins (Casanova-Sáez et al., 2014, Muñoz-Nortes et al., 2017). ANU7 is involved in the regulation of PGE and *PhANG* expression and interacts genetically with GUN1, indicating a role for retrograde signals in the development of leaf lamina and mesophyll cells (Muñoz-Nortes et al., 2017). PGE was specifically shown to regulate leaf abaxial-adaxial pattern in a GUN1-dependent manner, affecting the expression of specific genes that control asymmetric abaxial-adaxial differentiation (Tameshige et al., 2013). Thus, mutants with impaired plastid development have revealed the close relationship between plastid integrity and leaf development (Aluru et al., 2006; Casanova-Sáez et al., 2014; Lundquist et al., 2014; Van Dingenen et al., 2016; Muñoz-Nortes et al., 2017).

The classic variegated Arabidopsis mutant *im* is defective in PTOX, and the hypothesis for the formation of the white and green sectors is based on a threshold model where the variegation is dependent on the redox and excitation pressures during the early stages of chloroplast biogenesis when the thylakoid membranes are being formed. The green sectors in the *im* mutants emerge from plastids that managed to escape the overreduction of the membrane by the action of compensating factors that affect the excitation pressure threshold (Kambakam et al., 2016; Pogorelko et al., 2016). Using detailed kinematic and gene expression studies, it was shown that the leaf becomes photosynthetically active at the same time as it shifts from primary to secondary morphogenesis (Andriankaja et al., 2012). Thus, chloroplast differentiation is an important regulator of the simultaneous onset of cell expansion and photosynthesis. Furthermore, it was suggested that retrograde signaling through tetrapyrroles plays a role in the shift to cell expansion and activation of photosynthesis (Andriankaja et al., 2012).

OUTSTANDING QUESTIONS

- What is the nature of the retrograde signal(s) during seedling establishment? Is it a positive signal from functional chloroplasts or a negative signal from disrupted chloroplasts, or both?
- What is the molecular mechanism behind the function of the key player GUN1 and what is the timing of GUN1 action?
- Which are the cytosolic transducers of plastid signals to the nucleus?
- What is the impact of retrograde signals on chromatin modifications and nuclear architecture?

Regulation of flowering time in response to plastid signals was indicated by the flowering phenotypes of mutants like *crd* (which overaccumulates MgProto-IX and MgProto-IX-ME), mutants with impaired NEP activity, and the *sco1* mutant that is defective in the plastid elongation factor G (Baba et al., 2004; Albrecht et al., 2006; Barajas-López et al., 2013). A model has been proposed where chloroplasts act as stress sensors that transmit information to the nucleus to regulate flowering-related gene expression and thereby also the timing of the plant life cycle. MEcPP, a plastid metabolite that plays a role in a stress-induced retrograde signaling pathway, also regulates flowering time through the regulation of the transcription factor BBX19, which interacts with CO to regulate FT expression (Wang et al., 2014). In addition, the cleaved N-PTM transcription factor was shown to be involved in the repression of the flowering time regulator FLC by recruiting FVE and interacting with the promoter of *FLC* (Feng et al., 2016).

The GLK transcription factors have been shown to be implicated in fruit development, for example, through the pale siliques of the Arabidopsis *glk2* mutant or the identification of *GLK2* as the locus affected in the *uniform ripening* mutation that results in uniformly green tomatoes (*Solanum lycopersicum*; Waters et al., 2008; Powell et al., 2012; Nguyen et al., 2014). Similarly, a correlation was reported between CaGLK2 and variation in chlorophyll content and color in pepper (*Capsicum annuum*) fruit (Brand et al., 2014). However, a clear involvement of a retrograde signal controlling *GLK2* expression and/or activity during fruit development remains to be demonstrated. A theoretical model referred to as "degradational control" has been proposed where the degradation of Rubisco during senescence, as a source of nitrogen, and its export out of the chloroplast constitute a potential retrograde signal (Pfannschmidt, 2010). Senescence is a highly controlled light- and/or age-dependent program that is accompanied by a transcriptional reprogramming. The light-dependent signaling pathway (PHYB-PIFs-GLKs) involved in senescence is equivalent to the light-signaling pathway regulating chloroplast biogenesis, but the outcome is repression rather than induction of the *GLK*s and *PhANG*s (Liebsch and Keech, 2016). However, as is the case for fruit development, the involvement and nature of a plastid retrograde signal in the senescence process has yet to be experimentally demonstrated.

CONCLUDING REMARKS

The field of plastid-to-nucleus signaling has been very dynamic over the last few years, and there have been several major breakthroughs leading to a much more advanced understanding of the mechanisms involved in the communication between the plastids and the nucleus. These recent findings, as highlighted in this review, have made it clear that chloroplast development is controlled by a delicate interplay between light and plastid signaling pathways. In addition, a number of key players involved in this interplay are now identified (Advances box). Critical future directions for this field of plant sciences include efforts to finally understand the mechanism of the elusive key signaling component GUN1, to identify cytosolic components acting as signal transducers from the chloroplast to the nucleus, and to explore the dynamics of global chromatin organization in response to retrograde signals and the establishment of photosynthesis (Outstanding Questions box).

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