REVIEW

Plant photoreceptors and their signalling components in chloroplastic anterograde and retrograde communication.

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Highlight
Phytochrome and cryptochromes photoreceptors are essential for tuning photomorphogenesis and chloroplast functions, yet their integration in the inter-organelar communication cascades for proper environmental responsiveness is just beginning to be addressed.

Abstract
The Red-phytochromes and Blue-cryptochromes plant photoreceptors play essential roles in promoting genome-wide changes in nuclear and chloroplastic gene expression for photomorphogenesis, plastid development, and greening. While their importance in anterograde signalling has been long recognised, the molecular mechanisms involved remain under active investigation. More recently, the intertwining of the light-signalling cascades with the retrograde signals for the optimisation of chloroplast functions has been acknowledged. Advances in the field support the participation of phytochromes, cryptochromes and key light-modulated transcription factors, including HY5 and the PIFs, in the regulation of chloroplastic biochemical pathways that produce retrograde signals, including the tetrapyroles and the chloroplastic MEP-isoprenoids. Interestingly, in a feedback loop, the photoreceptors and their signalling components are targets themselves of these retrograde signals, aimed at optimising photomorphogenesis to the status of the chloroplasts, with GUN proteins functioning at the convergence points. High-light and shade are also conditions where the photoreceptors tune growth responses to chloroplast functions. Interestingly, photoreceptors and retrograde signals also converge in the modulation of dual-localised proteins (chloroplastic/nuclear) including WHIRLY and HEMERA/pTAC12, whose functions are required for the optimisation of photosynthetic activities in changing environments and are proposed to act themselves as retrograde signals.
Keywords
Anterograde signals, Retrograde Signals, Chloroplast, Cryptochrome photoreceptors, GUN Mutants, HY5, MEcPP, Photomorphogenesis, Phytochrome photoreceptors, Plastome, Tetrapyrroles

Abbreviations
Cryptochromes, CRYs
DXP REDUCTOISOMERASE, DXR
DXP SYNTHASE, DXS
EARLY LIGHT INDUCED PROTEIN, ELIP1 and ELIP2
Ferrochelatase 1, FC1
Flavonoid/anthocyanin biosynthesis genes, FAB
GENOMES UNCOUPLED, GUN
GOLDEN2-LIKE protein, GLK
High-light, HL
LIGHT-HARVESTING COMPLEX B genes, LHCB
LONG HYPOCOTYL 5, HY5
Methylerithritol cyclodiphosphate, MEcPP
Methylerithritol phosphate, MEP
Pentatricopeptide domain-containing, PPR
Photosynthesis Associated Plastome Genes, PhAPGs
Photosynthesis-Associated Nuclear Genes, PhANGs
Phytochrome Interacting Factors, PIFs
Phytochromes, phys
Plastid-encoded polymerase, PEP
Plasmid transcriptionally active chromosome, pTAC
Reactive oxygen species, ROS
Ribulose biphosphatase carboxylase small subunit, RBCS-IA
Tetrapyrrole domain-containing, TPR
Whirly1, WHY1
**Introduction**

**Photoreceptor activity is critical to chloroplast development and photosynthetic metabolism.**

Plant photoreceptors utilise light to co-ordinate growth, development, and photosynthetic functions in a changing environment. Mechanistically, both the Red/far Red light sensing phytochromes (phys) and the Blue light sensing cryptochromes (CRYs) are essential in the orchestration of large-scale changes in gene expression to modulate-photomorphogenesis (Franklin and Quail, 2010; Yu \textit{et al.}, 2010). Prominently, their transcriptional cascades facilitate the onset of plastid development, greening, the production of photosynthetic pigments and the set up and maintenance of photosynthetic metabolism, among other light controlled responses (Franklin and Quail, 2010; Yu \textit{et al.}, 2010).

Beyond the photoreceptors’ downstream activation of thousands of nuclear genes whose protein products have a chloroplastic function including in photosynthesis (Chen and Chory, 2011; Ohgishi \textit{et al.}, 2004; Stephenson and Terry, 2008), recent research hints at the involvement of the phytochrome and the cryptochrome photoreceptors in the global transcriptional, post-transcriptional and post-translational modulation of plastid-encoded genes (Chen \textit{et al.}, 2010; Facella \textit{et al.}, 2017; Griffin \textit{et al.}, 2020; Oh and Montgomery, 2014; Yoo \textit{et al.}, 2019). Hence, the light photoreceptors have not only a central role in the anterograde (nucleus to plastid) signalling cascades, but intertwine with the retrograde (plastids to nucleus) signals for the optimisation and maintenance of plastid functions and metabolism.

**Main text**

**Phytochromes and Cryptochromes in anterograde signalling.**

The anterograde signalling pathways are nuclear-to-chloroplast communication channels involved in setting and tuning chloroplast development and functions, circadian responses and photosynthesis (Atkins and Dodd, 2014; Berry \textit{et al.}, 2013; Leister and Kleine, 2016). Anterograde signals became necessary following the ancestral endosymbiotic event that originated the chloroplasts. Through evolution, many of the genes from the chloroplast genome (the plastome) were transferred to the nuclear genome (Garrido \textit{et al.}, 2020), but remained functionally associated to the chloroplast. And by the acquisition of an N-terminal transit peptide, their protein products gained targeting to the chloroplasts after transcription in the nucleus and translation in the cytoplasm (Wollman, 2016).

The tight regulation of these nuclear genes encoding for chloroplast-functioning proteins (globally known as Photosynthesis Associated Nuclear Genes, \textit{PhANGs}) is critical for chloroplast biogenesis and photosynthesis, and the photoreceptors are essential for tuning their transcriptional
responses in changing light environments (Calderon and Strand, 2021; Larkin and Ruckle, 2008; Pogson et al., 2015). Both the phytochromes and the cryptochromes regulate the global light-responsiveness of the PhANGs through the activation or repression of multiple transcription factors including: the bZIP-LONG HYPOCOTYL 5 (HY5) (Osterlund et al., 2000; Toledo-Ortiz et al., 2014); the basic helix-loop-helix Phytochrome Interacting Factors (PIFs) (Franklin and Quail, 2010; Leivar and Quail, 2011); and the GARP proteins GOLDEN2-LIKE 1 and 2 (GLK1, GLK2) (Leister and Kleine, 2016; Waters et al., 2009).

HY5 is a master transcription factor in the control of photomorphogenic responses (Gangappa and Botto, 2016) capable of integrating Red-phys and Blue-CRYs responses. Both photoreceptors tune HY5 abundance in the nucleus by downregulating the COP1-dependent ubiquitination of HY5 and allowing its accumulation in the light (Osterlund et al., 2000). HY5 binds to the promoters of nearly 4000 genes and controls a wide range of developmental processes including the activation of photosynthesis-associated genes (Gangappa and Botto, 2016; Lee et al., 2007a), photopigment and antioxidant accumulation (Lee et al., 2007a; Shin et al., 2007; Toledo-Ortiz et al., 2014), as well as circadian and growth responses (Hajdu et al., 2018; Lee et al., 2007a).

The PIFs are negative modulators of photomorphogenesis that are degraded in the light after the activation of phys and are involved in promoting skotomorphogenesis and shade avoidance responses (Leivar and Quail, 2011; Yoo et al., 2019). While their turn-over and stability is principally regulated by the phytochromes, cryptochromes can repress the transcription of PIF4 without affecting its protein stability (Ma et al., 2016), and may also protect PIF5 from phy-mediated degradation in low Blue light conditions (Pedmale et al., 2016). PIFs promote skotomorphogenesis (Wang et al., 2022) including the down regulation of genes involved in photopigment biosynthesis (Shin et al., 2007; Stephenson et al., 2009), chloroplast development and function (Leivar and Monte, 2014).

The GLK transcription factors target genes involved in light harvesting and chlorophyll biosynthesis through direct binding to their light-sensitive promoters, and are required for chloroplast development (Waters et al., 2009). In addition, GLK1 and GLK2 transcript accumulation is Red-phys and Blue light dependent, and the glk1 glk2 double mutant has reduced accumulation of transcripts for photosynthetic genes and lower chlorophyll content when grown in Blue light (Waters et al., 2009), hinting at their involvement with CRYs signalling cascades leading to greening.

Beyond the important role of the CRYs and phys in the transcriptional response of chloroplast functioning genes, recent research provides with evidence that the phytochromes are also key regulators of ribosome biogenesis and translation during late leaf development, with a global modulation of mRNAs that encode for components of the aminoacyl-tRNA biosynthesis, elongation factors, and ribosomal subunits (Romanowski et al., 2021). Active phyB has also been reported to interact with cytosolic RNA-Binding proteins, including PENTA1 (PTN1), to inhibit the translation of
mRNAs for genes such as protochlorophyllide (PORA) involved in chlorophyll biosynthesis (Paik et al., 2012).

Withal, beyond the activation of the nuclear genome for the production of the chloroplastic proteins encoded by it, chloroplast functions require co-ordination of gene expression with the plastome, wherein essential subunits of the photosynthetic complexes are encoded. As such, part of the anterograde signalling pathways relate to the delivery of information for tuning the chloroplast genome in response to the environment (Facella et al., 2017; Griffin et al., 2020; Oh and Montgomery, 2014). CRY2 over-expression studies in tomato defined a broad contribution to the plastome expression in Long Days (58% of the 114 plastome ORFs), with an up-regulation of Photosystem II (psb), Photosystem I (psa), and cytochrome b6f (pet) transcripts and down-regulation of multiple large and small ribosomal proteins (rps, rpl). In addition, genes encoding for other photosynthetic complexes such the NADH dehydrogenase (ndh) and ATP Synthase (atp) showed a mixed regulation (Facella et al., 2017). A similar analysis in Arabidopsis for phyB mutant in Short Days (SD), revealed an analogous capacity to globally regulate the transcripts of 55 out of 80 plastome encoded genes (Griffin et al., 2020; Michael et al., 2008). While in most cases phyB function was related to transcript up-regulation, down-regulation of key atp (ATP Synthase), ndh (NADH dehydrogenase), psa (Photosystem I), and psb (Photosystem II) transcripts was also detected (Griffin et al., 2020).

Alongside these reports, bioinformatic studies of genomic datasets for Arabidopsis cry1 cry2 and phyabcde revealed a significant contribution of Red-phytochromes and Blue-cryptochromes to the light-dependent expression of nuclear-encoded genes whose protein products are linked to the transcriptional, post-transcriptional, and translational control of the plastome (Griffin et al., 2020). Among the light-modulated gene families identified were the sigma factor transcriptional cofactors required for the activity of the PLASTID-ENCODED POLYMERASE (PEP) (Börner et al., 2015; Oh and Montgomery, 2014); the Pentatricopeptide domain-containing (PPR) and the Tetratricopeptide domain-containing (TPR) families of RNA binding proteins with a role in the plastome post-transcriptional events (Lamb et al., 1995; Ruwe et al., 2011). In addition, for the Blue-cryptochromes, genes encoding for RNA-Recognition Motif (RRM) RNA binding proteins with an annotated role in post-transcription and for the tRNA ligase and Large Ribosomal Protein (RPL) related to translation were identified (Griffin et al., 2020). In this context, HY5 was singled out as a relevant transcription factor delivering light cues to the “plastome-regulatory gene network”. Gene targets include the sigma factors and the PLASMID TRANSCRIPTIONALLY ACTIVE CHROMOSOME class (pTACs), involved in plastome-transcription, and the PPRs and the TPRs likely involved in post-transcriptional processes.
These early studies provide evidence that the photoreceptors and their signalling components, are central in the anterograde signalling cascades to tune the global expression of the plastome in response to environmental signals, but the detailed mechanistic insights remain to be understood.

The chloroplast retrograde signalling pathways.

Retrograde signalling pathways are a second type of inter-organelar communication channels used by the plastids to relay information to the nucleus in response to a range of stresses or external stimuli for the optimisation of growth and for shaping photosynthetic and chloroplast biogenic responses (Hernández-Verdeja and Strand, 2018; Kusnetsov et al., 1996; Leister and Kleine, 2016). Retrograde signalling during chloroplast biogenesis (defined as the transition between etioplasts or proplastids to chloroplasts), germination or early seedling development, is referred to as biogenic signalling (Pogson et al., 2008). Biogenic signalling tunes-up and down- hundreds of nuclear-encoded genes whose protein products function in the chloroplast (Chan et al., 2016). A variety of intermediaries from chloroplastic metabolic pathways, including: tetrapyrroles, methylerythritol phosphate (MEP)-pathway isoprenoids, phosphoadenosines, carbohydrates, carotenoid oxidation products and reactive oxygen species (ROS), have been identified as biogenic signals emitted by the chloroplast to deliver information to the nucleus. The biogenic retrograde signalling pathways have been recently reviewed in detail (Chi et al., 2015; Terry et al., 2019).

The crucial contribution of retrograde signalling to seedling survival has been assessed in mutants with impaired retrograde signalling capabilities, and through pharmacological approaches that induce stress in the chloroplasts (Chan et al., 2016; Pogson et al., 2008). Common retrograde signal activators include Lincomycin (an inhibitor of plastid translation that blocks plastid development) and Norflurazon (an inhibitor of carotenoid biosynthesis that induces photobleached chloroplasts). These chemical agents trigger a reduction in the expression PhANGs, including those encoding for light-harvesting complex proteins (LHCB) and the Rubisco small subunit (RBCS), that are common marker genes for assessing retrograde signal activity (Ruckle et al., 2012; Susek et al., 1993). In Arabidopsis, forward mutagenic screens coupled with the use of Norflurazon identified the gun1 (Genome Uncoupled) mutants with altered accumulation of PhANGs like CAB (Chlorophyll a/b binding protein) (Mochizuki et al., 2001; Susek et al., 1993; Susek and Chory, 1992).

A second type of retrograde signalling involves operational signals that occur after chloroplast biogenesis and in response to stress conditions to induce adjustments in chloroplast homeostasis (Chan et al., 2016; Pogson et al., 2008). Examples of identified operational signalling pathways include the regulation of PSII overexcitation via β-cyclocitral (Ramel et al., 2012), and the methylerythritol cyclodiphosphate (MEcPP) pathway (Jiang and Dehesh, 2021).
This review will focus on the photoreceptors involvement in the regulation of the biogenic and operational pathways, including links to the GUN signalling pathways and MEcPP pathway and novel insights on dual localised proteins in the chloroplast to nuclear signalling (Jiang and Dehesh, 2021; Martín et al., 2016; Qin et al., 2010; Ren et al., 2017).

The intertwining of retrograde signalling and photoreceptor-dependent pathways.

While connections between plastid retrograde signalling and light signalling have been made for decades, most of the mechanisms involved remain elusive (Kusnetsov et al., 1996; Larkin and Ruckle, 2008; Xu et al., 2016). In 1996 Kusnetsov et al. examined the overlap between plastid-derived retrograde signals and light-derived signals on functional promoter sequences of PhANGs. These authors provided with early evidence that chloroplast-derived retrograde signals and light signalling pathways act on the same cis-acting elements (such as L-, I- and G-boxes), and could regulate the same processes, suggesting an intertwining of the pathways. Since then, G-boxes have been characterised as an important Light Responsive Element (LRE) bound by multiple phytochrome and cryptochrome downstream signalling components including HY5 and the PIFs (Chattopadhyay et al., 1998; Leivar and Quail, 2011).

Experimental evidence also supports that the activation of retrograde signalling pathways by Lincomycin and Norflurazon represses or delays plant photoreceptors’ promotion of photomorphogenesis, including chloroplast biogenesis and greening processes (Ruckle et al., 2012; Susek et al., 1993). There is also a clear overlap between the gene targets of the biogenic retrograde signalling pathways and the photomorphogenic cascades initiated by the phys and the CRYs (Ohgishi et al., 2004; Ruckle et al., 2012; Tepperman et al., 2006; Zhao et al., 2019). Examples of common targets include the subunits of the LHCB and RBCS (Mazzella et al.; Reed et al.; Vinti et al., 2005; Woodson et al., 2011). Furthermore, RNA-seq experiments in Norflurazon have provided with evidence that the genes encoding for phyA and for light-modulated transcription factors such as HY5 are up-regulated; and PIF4 and PIF7 are down-regulated upon activation of retrograde signal pathways (Zhao et al., 2019), giving support to the hypothesis that photoreceptors and their signalling components and retrograde signals highly intersect and do not operate independently of each other.

Beyond the chemical activators of retrograde signals, High-light (HL) is also an important trigger (Szechyńska-Hebda and Karpiński, 2013), and photoreceptors are part of the perception and responsiveness to HL (Kreslavski et al., 2020). Reactive Oxygen Species (ROS) including hydrogen peroxide (H$_2$O$_2$), superoxide anions (O$_2^-$) and singlet oxygen (¹O$_2$) are chemical derivatives of O$_2$ produced by metabolic processes in plants (Apel and Hirt, 2004). In HL irradiances chloroplasts increment H$_2$O$_2$ production by Photosystem I and ¹O$_2$ by PSII (Kanervo et al., 2005; Krieger-Liszky, 2005). While H$_2$O$_2$ has been shown to move out of isolated chloroplasts in-vitro, providing it with...
capacity to act as an initiator of retrograde signalling (Mubarakhshina et al., 2010), $^{1}\text{O}_2$ cannot leave the chloroplast due to its short half-life (Gorman and Rodgers, 1992) and therefore secondary messengers yet to be identified must be involved in the transmission of the $^{1}\text{O}_2$ signal to the nucleus.

In addition to ROS, HL-stress also generates 12-oxophytodienoic acid (OPDA), and oxylipins derived retrograde signals (Gollan and Aro, 2020). Among the targets of these retrograde signalling cascades is EARLY LIGHT INDUCIBLE PROTEIN1 (ELIP1) (Gollan and Aro, 2020), a thylakoid protein induced during de-etiolation and in response to HL stress (Rossini et al., 2006). ELIP proteins may participate in enhancing the photoprotective capacity of the plant (Casazza et al., 2005; Rossini et al., 2006) and under HL, CRY1 and HY5 modulate the induction of ELIP1 (Kleine et al., 2007). As part of these cascades, a second cross-regulatory point is the modulation of Heat Shock Protein (HSP) chaperones (including HSP90) which are HY5 targets and participate in the tetrapyrrole mediated plastid signalling to repress PhANGs under oxidative stress (Kindgren et al., 2012).

These examples illustrate that photoreceptors’ activity is crucial for the setup of the protective responses against the HL stress, as well as for the communication channels activated by high-irradiances. Likewise, phytochromes and cryptochromes promote the activation of nuclear genes for the biosynthesis of carotenoids and anthocyanins to deal with excess of light (Kreslavski et al., 2020). Accordingly, the cry1phyAB1 and phyAB1B2 mutants in Solanum lycopersicum, present additive HL stress phenotypes, including reductions in photopigment content, photosynthetic activity and lower transcript accumulation of photosynthesis-associated genes encoded in both the plastome and in the nuclear genome (PhANGs and PhAPGs) (Kreslavski et al., 2020). Furthermore, the more acute HL damage observed for cry1phyAB1 may point to a larger contribution of CRY1 to HL tolerance and responsive mechanisms in tomato plants.

Studies in Arabidopsis further support this primary role of CRY1 in managing photoprotective and HL responses, and single out HY5, whose transcript and protein accumulate in HL, as one of the light signalling components involved (Kleine et al., 2007). In addition to a HL-sensitive phenotype including the photo-inactivation of PSII, the cry1 mutant exhibits at a transcriptomic level mis-regulation of 77 HL-induced genes, with 26 of them also mis-regulated in hy5 (Kleine et al., 2007). Interestingly, further 39 genes showed altered patterns of accumulation in hy5, but not in cry1, indicating that HY5 participates in both HL-CRY1-dependent and HL responsive but CRY-independent pathways.

Additional evidence from studies in emerging rice seedlings grown under high-Blue or high-Red light and Lincomycin support both an integration and a differential contribution of light-quality and photoreceptor activity to seedling photomorphogenesis and non-photochemical quenching mechanisms to tolerate the excess light (Duan et al., 2020). In this context, in high Red-light conditions retrograde signal activators induced photobleaching, but in high Blue-light, enhanced
carotenoid and chlorophyll production contributed to a stronger HL stress tolerance, in a mechanism likely dependent on cryptochromes (Duan et al., 2020; Kleine et al., 2007; Richter et al., 2020).

In summary, HL responses involve both photoreceptors (CRYs, phyS) and light signalling components (such as HY5) capable of sensing and responding to both HL and retrograde signals to tune growth and development with the status of the chloroplast. Current studies also support the conservation of these HL induced-Retrograde signalling cascades between monocots and dicot plants (Duan et al., 2020).

Photoreceptors, HY5, and GUN1 in the convergence of photomorphogenesis and retrograde signalling.

The GENOMES UNCOUPLED (GUN) genes (GUN1-GUN6) were identified in the “gun mutant screens” using Norflurazon to activate retrograde signals (Mochizuki et al., 2001; Susek et al., 1993; Susek and Chory, 1992; Woodson et al., 2011). GUN2-6 play roles in the tetrapyrrole biosynthesis pathway, and while the full functional role of GUN1 remains to be addressed, experimental evidence also supports GUN1 modulation of tetrapyrroles by direct binding to both heme and porphyrins (Shimizu et al., 2019).

Tetrapyrroles, either as bilins or porphyrins, have important functions in multiple biological processes, including respiration and photosynthesis, and are active in light absorption, electron transfer, and oxygen binding (Shimizu et al., 2019). Tetrapyrrole biosynthesis takes place in the plastids and involves two key pathways branching from protoporphyrin IX: the chlorophyll branch, ending in production of chlorophylls \(a\) and \(b\); and the heme branch, ending in phytochromobilin (the chromophore used by the Red and far-Red light phytochrome photoreceptors) (Bae and Choi, 2008; Li et al., 2011). A tight regulation of tetrapyrrole biosynthesis is required to avoid cellular damage by the generation of reactive oxygen species (ROS).

As the gun mutants involve mutations within the tetrapyrroles biosynthetic pathway, the metabolites therein are considered key retrograde signals for chloroplast development (Leister and Kleine, 2016). In the chlorophyll branch of the tetrapyrrole biosynthesis pathway, GUN5 encodes a gene for the H subunit of magnesium chelatase (MgCh), involved in the transition between protoporphyrin IX (Proto) to Magnesium protoporphyrin IX (Mg-ProtoIX) (Mochizuki et al., 2001). GUN4 encodes an activator of Mg-chelatase that also contributes to the accumulation of Mg-Proto IX (Larkin et al., 2003). Mg-ProtoIX has been proposed as one of the important signalling molecules for retrograde signalling (Kindgren et al., 2011), linked to the reduction in transcript levels of PhANGs, including LHCb and RBCS (Shimizu et al., 2019). However, beyond gun4 and gun5, other mutants for genes encoding subunits for Mg-ProtoIX complex do not display a gun phenotype,
making the role of this metabolite in retrograde signalling unclear at present (Mochizuki et al., 2001; Wu and Bock, 2021).

The heme branch of tetrapyrrole synthesis is initiated by GUN6 (also known as plastid FERROCHELATASE 1, FC1) that converts protoporphyrin IX to protoheme by inserting Fe\(^{2+}\). Protoheme is converted first to biliverdin IX by GUN2 (encoding heme oxygenase), and finally to 3Z-phytochromobilin by GUN3 (phytochromobilin synthase). Evidence that heme may function as a second type of retrograde signalling molecule has been provided by the characterisation of gun6-1D, a dominant mutant allele overexpressing FC1, and promoting the flow of tetrapyrroles into the heme branch, with consequent upregulation of PhANG transcripts (Woodson et al., 2011).

While the specific mechanisms through which photoreceptor signalling pathways are involved in the generation, regulation, and response to GUN retrograde signals, have yet to be fully elucidated, tetrapyrrole biosynthesis is induced by light, as previously reviewed (Kobayashi and Masuda, 2016) with the contribution of light-signalling transcription factors including HY5 (Kobayashi et al., 2012a; Kobayashi et al., 2012b; Lee et al., 2007b), the PIFs (Leivar and Quail, 2011; Shin et al., 2009), and GLK1 and GLK2 (Waters et al., 2009).

In particular, GUN1 is a gene of high interest as integratory point for light and retrograde signalling pathways. GUN1 encodes a chloroplast-localised protein containing a pentatricopeptide repeat (PPR) (Koussevitzky et al., 2007). Pentatricopeptide domain-containing proteins are known post-transcriptional regulators of plastid gene expression (Ruwe et al., 2011), but the functional role of GUN1 protein is still under exploration. Of all gun mutants, gun1 exhibits the strongest derepression of PhANGs expression in lincomycin (Koussevitzky et al., 2007) and GUN1 transcript accumulation is light-responsive and dependent on the phytochromes in Red light (Hu et al., 2013).

During de-etiolation, GUN1 is active and involved in cotyledon expansion and hypocotyl elongation (Ruckle et al., 2007; Ruckle and Larkin, 2009) with gun1 also displaying a delayed greening phenotype. As such, GUN1 likely represents a crosstalk point between the photoreceptor signalling cascades and the plastid signals that tune chloroplast greening and growth responses (Mochizuki et al., 1996; Pesaresi and Kim, 2019; Ruckle et al., 2007; Wu and Bock, 2021; Wu et al., 2019).

Further support for this possibility has been provided by additional gun genetic screens, where an allele of cry1 that shares similar phenotypes with gun1-1, including defects in plastid to nucleus signalling affecting LHCB and RBCS transcript accumulation, was identified (Ruckle et al., 2007). Double mutant analysis of gun1-101 cry1 grown in HL showed an additive phenotype for their effects on LHCB accumulation and deficiencies in chlorophyll accumulation, indicating that GUN1 and CRY1 may be partially redundant in modulating LHCB via parallel pathways that converge. A similar phenotype of defective LHCB accumulation was observed for gun1-101 hy5 double mutant, suggesting that this CRY1 dependent pathway requires HY5. Likewise, phyB gun1-1 double mutants
accumulated more LHCB than gun1-1 single mutants when treated lincomycin, providing evidence that phyB may also be a gun mutant, contributing to the repression of LHCB, but only when GUN1 is inactive (Ruckle et al., 2007).

In summary, the light/photoreceptor-dependent modulation of GUN1, together with the additive phenotypes between gun1 and photoreceptor mutants, point at signal integration between the light cascades and the retrograde signals via GUN1, with HY5 as a potential “convergence of signals point” for which full mechanistic insights await full dissection.

**Phytochrome-dependent GLK tuning of PhANGs is antagonized by GUN signalling.**

An additional molecular link identified between the GUN pathways and the photoreceptor signalling cascades during de-etiolation was recently uncovered (Martín et al., 2016). These authors showed that during de-etiolation, the phytochrome photomorphogenic signals and the GUN1 biogenic retrograde signalling pathways converge to antagonistically control photomorphogenesis. Notably, Arabidopsis plants grown in Red or white light with inhibition of chloroplast biogenesis induced by Lincomycin or Norflurazon, showed elongated hypocotyls and unexpanded cotyledons lacking chlorophyll, phenotypes associated to dark-grown seedlings. These observations give support to a retrograde signals-dependent tuning down of light-dependent pathways with suppression of photomorphogenic development.

Interestingly, genomic studies showed that over 343 photomorphogenesis-associated genes involved in de-etiolation and greening are co-repressed by both lincomycin-induced/GUN1-derived retrograde signals and by the PIFs in the dark. This transcriptional effect was further supported by the characterisation of the *pifq* (*pif1 pif3 pif4 pif5*) mutant, for which treatment with lincomycin restored the PIF-repressed genes transcriptomic profile to wild-type levels, indicating a parallel pathway to GUN1 in response to chloroplast dysfunction (Martín et al., 2016). An analysis of the DNA-binding motifs in the promoters of the genes co-repressed by both lincomycin and PIFs identified an enrichment in GLK-binding motifs (Martín et al., 2016). *GLK1* encodes for a transcription factor that is both phytochrome/light-induced and PIF-repressed, and whose down-regulation by retrograde signals in a GUN1/GUN5 dependent manner is reported (Kakizaki et al., 2009; Waters et al., 2009). In addition, characterisation of overexpressing lines for *GLK1* and *GLK2* placed them as gun mutants themselves (Leister and Kleine, 2016). As part of the GUN1/GLK1-mediated responses, the B-Box gene *BBX16* has been identified as a directly induced target of GLK1 for the promotion of photomorphogenesis, and whose transcription is repressed in a GUN1/GLK1-dependent manner upon chloroplast damage, as well as in response to Norflurazon treatment (Veciana et al., 2022; Zhao et al., 2019).
Along with the links between RS and GUN signalling in the light, evidence also suggests that these pathways may operate in darkness, with the involvement of COP1 and the PIFs. Support to this possibility comes from experiments on etiolated Arabidopsis pifq seedlings that, when grown in the presence of lincomycin, show a restoration to phenotypes present in WT-etiolated seedlings, including suppression of cotyledon separation and sustainment of apical hook curvature and of appressed cotyledons (Martín et al., 2016). In addition, lincomycin also reduces the transcript accumulation of photomorphogenesis-associated genes such as LHCB1 in dark-grown cop1, and of 354 transcripts in dark-grown pifq mutants (Martín et al., 2016; Sullivan and Gray, 1999). Also, recent studies of dark grown etioplasts and pro-plastids revealed the presence of GUN1 protein in the dark and transcriptomic studies on dark grown WT and gun1-102 indicate that GUN1 mediated signals regulate nuclear gene expression in the dark with up to 4425 genes, including subunits of the Photosystem I (PSA) and LHCB, differentially expressed in dark gun1-101 compared to WT. These results support a significant role for GUN1 in tuning the expression in the dark of genes involved in the build-up of the photosynthetic apparatus (Hernández-Verdeja et al., 2022).

Therefore, while the molecular connections between the GUN1 retrograde signalling and the phytochrome cascades are only beginning to be addressed, progress in the area points at retrograde signals acting as an antagonistic pathway to suppress phytochrome-induced photomorphogenesis. In this context, GUN1 can integrate retrograde signals downstream COP1 to tune the initiation of photomorphogenesis, including those that modulate the transcriptional responses of transcription factors required for de-etiolation and for chloroplast development such as GLK1, HY5, PIF1, PIF4, PIF5 and PIF8 (Hernández-Verdeja et al., 2022).

Photoreceptors and the MEcPP retrograde signalling pathway.

Along with their roles in initiating greening and tetrapyrrole biosynthesis, phytochromes are downstream targets of the (MEcPP), an isoprenoid derivative of the chloroplastic methylerthritol phosphate (MEP) pathway, and a powerful operational retrograde signalling molecule (de Souza et al., 2017; Jiang and Dehesh, 2021; Jiang et al., 2019) for the expression of nuclear genes involved in stress responses in plastids (de Souza et al., 2017; Xiao et al., 2012). The plastidial accumulation of MEcPP is induced in response to oxidative stress, high light, wounding, high temperature, and heavy metals in plants and eubacteria (Wang et al., 2017; Xiao et al., 2012).

A genetic screen in Arabidopsis to identify genes involved in the regulation of HYDROPEROXIDE LYASE (HPL), a stress-inducible protein in the oxylipin pathway, identified the constitutively expressing HPL (ceh1) mutant (Xiao et al., 2012). ceh1 has a mutation in HMBPP synthase (HDS) that catalyses the conversion of MEcPP to HMBPP (Ostrovsky et al., 1998; Rodríguez-Concepción, 2006; Xiao et al., 2012), and displays short hypocotyls in the light (Jiang et
This phenotype is caused by higher phyB protein levels induced by the over-accumulation of MEcPP (Jiang et al., 2020). Higher phyB levels lead to the repression of PIF4 and PIF5 activity and to an altered accumulation of ethylene and auxin biosynthetic genes such as ACS4, 5, 8, and YUC8 (Jiang et al., 2020; Jiang et al., 2019). Interestingly, the short hypocotyl phenotype of ceh1 mutants was also present in seedlings grown under Blue light, supporting the possibility that Blue light-sensing cryptochromes are also linked to MEcPP accumulation and signalling (Jiang et al., 2019).

While phyB is a downstream target of a MEcPP retrograde signal, phyB and transcription factors acting downstream of phyB are also critical regulators of multiple MEP-pathway genes (e.g. DXP SYNTHASE (DXS), DXP REDUCTOISOMERASE (DXR), HMBPP REDUCTASE (HDR)) from which MEcPP is derived (Chenge-Espinosa et al., 2018). In particular, Red-light signals from phy and HY5, antagonistically transduced by PIFs, are involved in the transcriptional control of DXS and DXR, the genes in the MEP-pathway that are considered rate limiting steps and flux controlling points (Chenge-Espinosa et al., 2018; Wright et al., 2014).

Together, these findings support a cross-regulation between the photoreceptors and the MEcPP retrograde signalling pathways with phyB as both a key target of retrograde signals in Red light as well as a regulator of their generation, in a feedback loop that adjusts photomorphogenic responses to the status of the chloroplast.

HY5 emergence as an important integratory factor for light and multiple retrograde signalling pathways.

HY5 is a master modulator of plant photomorphogenesis, including the control of de-etiolation, photopigment accumulation, hormonal levels, anthocyanin production, and tuning of reactive oxygen stress responses (Gangappa and Botto, 2016; Kobayashi et al., 2012b; Toledo-Ortiz et al., 2014). In the light, several pieces of evidence support the signal integratory capacity of CRYs and phy signals via HY5 with retrograde signalling (Kindgren et al., 2012; Richter et al., 2020; Ruckle et al., 2007). As such, HY5 transcript accumulation increases in response to retrograde signal activators (Zhao et al., 2019), and HY5 has been proposed to alternate between an activator and a repressor of nuclear-encoded gene expression in response to plastid dysfunction (Lee et al., 2007b; Ruckle et al., 2007; Ruckle and Larkin, 2009).

In addition, HY5 mediates the GUN1-triggered rapid light-dependent inhibition of PhANGs, induced by singlet oxygen retrograde signals derived from the photo-excitation of Mg-porphyrins and the accumulation of the chlorophyll intermediate Mg-ProtoIX (Kindgren et al., 2012; Richter et al., 2020; Strand et al., 2003). Mg-ProtoIX interaction with cytosolic HSP90 proteins leads to the
repression or inactivation of nuclear-encoded PhANGs in a HY5-dependent manner (Kindgren et al., 2012). In this pathway, GUN5-HSP90.2-HY5 is emerging as a convergence point for light and retrograde signalling cascades for the modulation of PhANGs. HY5 may also form with GUN1 and HSP90.1 (Wu and Bock, 2021; Wu et al., 2019) a second light-retrograde signals integratory node, whose full biological significance, remains to be investigated.

Farther, together with cryptochromes, HY5 also participates in the co-ordination of light and retrograde signals for anthocyanin and flavonoid accumulation (Richter et al., 2020; Shin et al., 2007; Zhang et al., 2016). In this respect, current evidence shows that in Norflurazon-treated Arabidopsis plants, GUN1/GUN5 retrograde signals can tune down the transcript accumulation of flavonoid/anthocyanin biosynthesis (FAB) genes, including LEUCOANTHOCYANIDIN DIOXYGENASE (LDOX) a gene whose activation depends on CRY1 and HY5 (Richter et al., 2020).

As such, current studies support the participation of CRY1 and HY5 in abiotic-stress triggered retrograde signalling cascades necessary for enabling chloroplasts stress responsiveness, the modulation of photoprotective pigment accumulation, and repression of the expression of the PhANGs.

Another reported link between HY5 and the tetrapyrrole biosynthesis-derived retrograde signalling cascades involves the sigma factors. The sigma transcriptional cofactors are nuclear-encoded genes required for the activity of the PEP (Berry et al., 2013; Börner et al., 2015). In Arabidopsis, there are 6 members of the sigma factor family, with 5 of them (SIGs 1, 2, 3, 5, 6) showing Red-phytochrome, Blue-cryptochrome, or Red/Blue-HY5-dependent transcript accumulation (Griffin et al., 2020; Oh and Montgomery, 2013). For SIG2 and SIG5, links to retrograde signalling are emerging (Oh et al., 2018; Woodson et al., 2013) with SIG2 modulation in the expression of the tRNA-glu, an early step in the tetrapyrrole biosynthesis (Woodson et al., 2013) and a reduced accumulation of PhANGs transcripts (including RBCS and LHCB genes) in sig2, a phenotype that is alleviated by heme-feeding. Transcriptomic studies for SIG2 have also identified under Red-light over 2000 nuclear-encoded mis-regulated genes, some with roles in growth, hormonal cross-talk, stress responses, and photosynthesis (Oh et al., 2018). The enrichment in sig2 of mis-regulated chloroplastic/Red-light responsive genes that are targets of retrograde signals supports an intersection of both pathways for the modulation in particular of chloroplastic acting genes and of genes active during in photomorphogenesis.

A second sigma factor, SIG5, is a light quality and high-light responsive gene that is sensitive to DCMU-dependent retrograde signals (Mellenthin et al., 2014). SIG5 transcript accumulation is CRY1 induced in Blue-light and phy-dependent in Red-light, with HY5 contributing to its transcriptional response in both light qualities (Griffin et al., 2020; Mellenthin et al., 2014). Following DCMU activation of retrograde signals derived from the inhibition of electron flow in Photosystem II
(Mellenthin et al., 2014; Metz et al., 1986), the accumulation of SIG5 is down-regulated. These early studies point at SIG5 capacity to integrate inputs from light and retrograde signals, however the mechanistic insights on signal integration and biological outputs remain to be investigated. Yet, SIG2 and SIG5 as HY5- and retrograde signal-sensitive genes, have a good potential to be part of the anterograde and retrograde pathways to tune the plastid genome and the PhANGs transcriptional responses with the Blue and Red photoreceptors light signals.

**HY5 and phyB in the shade-induced retrograde signalling pathways.**

In addition, HY5’s involvement in retrograde signals to avoid shade and optimise photosynthetic performance has been reported (Bou-Torrent et al., 2015; Cagnola et al., 2012; Ortiz-Alcaide et al., 2019; Roig-Villanova et al., 2007). In this context, HY5 is reported to respond to retrograde signals derived from functional chloroplasts to tune hypocotyl elongation, in a manner similar to its induction by phyA in low Red: far Red conditions to suppress elongation (Bou-Torrent et al., 2015; Ortiz-Alcaide et al., 2019). On the other hand, under shade, signals derived from challenged chloroplasts to de-activate phyB, stimulate the activity of the PIFs to promote hypocotyl elongation and avoid shade (Ortiz-Alcaide et al., 2019).

Studies using norflurazon or lincomycin treatments point at a higher transcript accumulation of HY5 and HY5 protein can be detected in white and in far-Red light enriched environments simulating canopies, but only when retrograde signals derived from functional chloroplasts are active (Ortiz-Alcaide et al., 2019). Interestingly, in the absence of functional chloroplasts, phyB inactivation in response to FR treatments is delayed, with the consequent reduction in the transcripts of shade-induced genes involved in elongation (Ortiz-Alcaide et al., 2019; Roig-Villanova et al., 2007).

In summary, current studies point at antagonistic effects of phyB/PIFs and phyA/HY5 for the proper modulation of elongation responses upon impending competition. Yet, in this setting, chloroplast retrograde signals are also critical for the tuning of light quality/shade perception to the status of the chloroplast.

**Photoreceptors regulate retrograde signalling dependent dual-localised proteins.**

Likewise, there is also evidence to support the involvement of the photoreceptors in the regulation of multiple dual-localised proteins that can communicate information between the nucleus and the chloroplast to tune chloroplast needs and photomorphogenic responses. WHIRLY1 (WHY1) is among such dual-localised proteins with potential to act as a retrograde signal based on a functional
role in chloroplast biogenesis and a capability for translocation from the chloroplast back to the nucleus (Isemer et al., 2012).

WHIRLY proteins are a small family of 3 genes in Arabidopsis, encoding for single-stranded DNA-binding proteins (Desveaux et al., 2002; Krause et al., 2005). WHIRLY1 and WHIRLY3 are targeted to chloroplast, and WHIRLY2 localises to the mitochondria (Krause et al., 2005). WHY1 is involved in the transcriptional modulation of plastid-encoded and nuclear encoded-genes (Desveaux et al., 2002; Desveaux et al., 2005; Isemer et al., 2012). In the chloroplast, WHY1 forms part of the pTAC complexes involved in plastome transcription, and in the nucleus WHY1 stimulates the expression of Pathogen Response (PR) genes by an unknown mechanism (Isemer et al., 2012).

The role of WHY1 as a retrograde signal occurs in response to redox changes in the thylakoid electron transport chain (Foyer et al., 2014). WHY1’s alternate subcellular localisation depends on light via the phyA-dependent regulation of the Calcineurin B-Like-Interacting Protein Kinase14 gene (CIPK14) (Qin et al., 2010), encoding for a protein that phosphorylates and modifies WHY1 binding affinity for different promoters (Ren et al., 2017). Interestingly, CIPK14 transcript accumulation is dependent on multiple light inputs, including transient activation by FR and time dependent modulation by Blue light and Red (Qin et al., 2010). At present, only the response to FR light and the dependence on phyA has been investigated, but based on current studies it can be hypothesised that this phyA-CIPK14-WHY1 regulatory module may be important for the FR blocking of greening response. It remains to be established if the observed Red-light induction of CIPK14 is phyB dependent, but the Blue-light induction of CIPK14 is not dependent on CRY1 CRY2 (Qin et al., 2010).

A second example of the involvement of photoreceptors in the control of nucleo-chloroplastic dual localised proteins include pTAC12/HEMERA (HMR), a member of the pTAC family that regulates the PEP (Chen et al., 2010; Pfalz et al., 2006). HMR transcript accumulation is light-responsive and dependent on the phytochromes in Red and cryptochromes in Blue (Griffin et al., 2020). In the nucleus, HMR acts as a transcriptional co-activator to regulate light-responsive genes, while in the plastids it associates with the PEP to induce plastid-encoded gene expression (Pfalz et al., 2015; Qiu et al., 2015). HMR first localises to the plastids, akin to WHY1 (Grabowski et al., 2008; Isemer et al., 2012), and its relocation to the nucleus is proposed as part of the activation of the retrograde signal cascades (Yoo et al., 2020). Currently this possibility, including the potential cross talk with photoreceptor signalling mechanisms, remains to be fully investigated.

In summary, research supports the involvement of phyB in the modulation of the activity of nuclear-chloroplastic proteins that directly or indirectly impact on the expression of the nuclear and the plastid genomes. At present, only the role of phyB has been studied, but the CRYs integration in
the retrograde signalling pathways that tune photomorphogenesis in Blue light make them interesting candidates to assess for their role in controlling dual-localised proteins that may be retrograde signals.

**Conclusions**

The research highlighted in this review supports an emerging view that the phytochrome and cryptochrome photoreceptors signalling, including through transcription factors such as PIFs and HY5, intertwine with both the anterograde and retrograde signalling pathways. This crosstalk is essential for the tuning of the nuclear and plastid genomes in response to environmental cues (Figure 1).

As part of the anterograde signalling cascades, the photoreceptors and their signalling components contribute to both nuclear and plastid transcription, post-transcription and translational mechanisms. On the other hand, in retrograde signalling, they are not only contributors to the activation of pathways involved in the emission of retrograde signals, such as the tetrapyrrole and MEcPP pathways, but are also targets themselves of the retrograde signals (Figure 1A). These dual functionalities are likely part of their extended capacity to optimise plant growth in response to environmental cues. In particular, phyA and HY5 transcript accumulation and phyB protein abundance increased in response to retrograde signal activators such as Norflurazon and the MEcPP pathway. Additionally, GUN1-signalling tunes CRY1 and HY5 transcript abundance and intersects with the photoreceptors in the control of de-etiolation responses. However, at present, the full reach of these cross-regulations remains to be explored, although the identification of cry1 as a gun mutant hints to a wide involvement of cryptochromes in plastid-to-nucleus signalling (Figure 1C).

CRYs, phys, and HY5 are also part of the chloroplast responsiveness to environmental cues, including the set up and the control of photoprotective mechanisms against the detrimental effects of high-light. HL is emerging as a condition where the crosstalk between photoreceptors and retrograde signals is essential to optimise chloroplasts functions, including the management of stress (Figure 1C). Additionally, as part of the perception of light quality, phys, PIFs, and HY5 participate in the modulation of the Shade Avoidance Syndrome elongation responses that are tuned via retrograde signals to the status of the chloroplast.

Finally, dual-localised proteins with capacity to act as retrograde signals, such as WHY1 and HMR, are also light quality responsive, but the impact of the phys and CRYs on their regulation is just starting to emerge.

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**Author Contribution**

JHCG and GTO designed and wrote the manuscript.

**Conflicts of Interest**

The authors report no conflict of interest.
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**Figure Legend**

**Figure 1.** Phytochromes (phys), Cryptochromes (CRYs) and HY5 integrate light and retrograde signals from the chloroplast to tune nuclear genome responses to a changing environment.

A) MEcPP tuning of phyB-modulated growth responses. Chloroplast stress-induced MEcPP accumulation increases the abundance of phyB-Pr protein. Red-light activated phyB-Pfr translocates to the nucleus to inhibit PIF activity, and target hormonal pathways to halt hypocotyl elongation. In addition to inhibiting PIF activity, phyB promotes HY5 accumulation. In a feedback loop, HY5 and PIFs antagonistically regulate the transcriptional accumulation of *DXS* and *DXR*, two of the rate-limiting steps in the MEP pathway from which MEcPP derives.

B) High light (HL) induced stress responses are dependent on photoreceptor and HY5 activity. HL stress induces damage to the photosynthetic apparatus, triggering the release of retrograde signalling molecules including H$_2$O$_2$ and Oxylipins, which target the phys, CRYs, and HY5-dependent activation of *PhANGs* expression and photoprotective responses including chlorophyll and carotenoid biosynthesis.

C) A GUN1-dependent pathway inhibits *PhANGs* accumulation to halt photomorphogenesis in response to chloroplast stress. GUN1 antagonistically inhibits phy-mediated photomorphogenesis through a GUN1:GLK1 complex that downregulates BBX16-mediated *PhANG*-expression. CRY1 and HY5 also co-target GUN1-dependent *PhANGs* accumulation in a converging pathway, contributing to the *PhANGs*’ responsiveness to chloroplast stress.
A. Oxidative stress, HL, wounding, high temperature, heavy metals

- G3P + Pyruvate
  - DXS
  - HDR
  - HMBPP
- Chloroplast

Light
- PhyB Pr
- PhyB Pfr

PIFs
- HY5
- DXS, DXR
- ACS4, 5, 8
- YUC8

Ethylene
- Auxin

Hypocotyl Elongation

B. HL Stress

- Oversaturation of Photosynthetic Apparatus
  - Retrograde signalling molecules
  - Eg H₂O₂, Oxylipins

Photoprotective response
- Chlorophyll biosynthesis
- Carotenoid biosynthesis
- Chloroplast

Cytoplasm

Nucleus

C. Chloroplast Stress

- GUN1
- GUN1:GLK1
- BBX16
- CRY1
- HY5

Phys
- GLKs
- GLKs

Nucleus

Cytoplasm

Photomorphogenesis
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