

1 **REVIEW**

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

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11 **Highlight**

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

15
16 **Abstract**

17 The Red-phytochromes and Blue-cryptochromes plant photoreceptors play essential roles in
18 promoting genome-wide changes in nuclear and chloroplastic gene expression for
19 photomorphogenesis, plastid development, and greening. While their importance in anterograde
20 signalling has been long recognised, the molecular mechanisms involved remain under active
21 investigation. More recently, the intertwining of the light-signalling cascades with the retrograde
22 signals for the optimisation of chloroplast functions has been acknowledged. Advances in the field
23 support the participation of phytochromes, cryptochromes and key light-modulated transcription
24 factors, including HY5 and the PIFs, in the regulation of chloroplastic biochemical pathways that
25 produce retrograde signals, including the tetrapyrroles and the chloroplastic MEP-isoprenoids.
26 Interestingly, in a feedback loop, the photoreceptors and their signalling components are targets
27 themselves of these retrograde signals, aimed at optimising photomorphogenesis to the status of the
28 chloroplasts, with GUN proteins functioning at the convergence points. High-light and shade are also
29 conditions where the photoreceptors tune growth responses to chloroplast functions. Interestingly,
30 photoreceptors and retrograde signals also converge in the modulation of dual-localised proteins
31 (chloroplastic/nuclear) including WHIRLY and HEMERA/pTAC12, whose functions are required for
32 the optimisation of photosynthetic activities in changing environments and are proposed to act
33 themselves as retrograde signals.

35 **Keywords**

36 Anterograde signals, Retrograde Signals, Chloroplast, Cryptochrome photoreceptors, GUN Mutants,
37 HY5, MEcPP, Photomorphogenesis, Phytochrome photoreceptors, Plastome, Tetrapyrroles

38

39 **Abbreviations**

| | | | |
|----|---|----|---|
| 40 | Cryptochromes, CRYs | 56 | PENTRATRICOPEPTIDE DOMAIN- |
| 41 | <i>DXP REDUCTOISOMERASE, DXR</i> | 57 | CONTAINING, PPR |
| 42 | <i>DXP SYNTHASE, DXS</i> | 58 | Photosynthesis Associated Plastome Genes, |
| 43 | <i>EARLY LIGHT INDUCED PROTEIN, ELIP1</i> | 59 | <i>PhAPGs</i> |
| 44 | and <i>ELIP2</i> | 60 | Photosynthesis-Associated Nuclear Genes, |
| 45 | FERROCHELATASE 1, FC1 | 61 | <i>PhANGs</i> |
| 46 | Flavonoid/anthocyanin biosynthesis genes, | 62 | Phytochrome Interacting Factors, PIFs |
| 47 | <i>FAB</i> | 63 | Phytochromes, phys |
| 48 | <i>GENOMES UNCOUPLED, GUN</i> | 64 | PLASMID TRANSCRIPTIONALLY |
| 49 | GOLDEN2-LIKE protein, GLK | 65 | ACTIVE CHROMOSOME, pTAC |
| 50 | High-light, HL | 66 | PLASTID-ENCODED POLYMERASE, PEP |
| 51 | <i>LIGHT-HARVESTING COMPLEX B</i> genes, | 67 | pTAC12/HEMERA, HMR |
| 52 | <i>LHCB</i> | 68 | Reactive oxygen species, ROS |
| 53 | LONG HYPOCOTYL 5, HY5 | 69 | <i>RIBULOSE BIPHOSPHATE CARBOXYLASE</i> |
| 54 | Methylerythritol cyclodiphosphate, MEcPP | 70 | <i>SMALL SUBUNIT, RBCS-1A</i> |
| 55 | Methylerythritol phosphate, MEP | 71 | TETRATRICOPEPTIDE DOMAIN- |
| | | 72 | CONTAINING, TPR |
| | | 73 | WHIRLY1, WHY1 |

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77 **Introduction**

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

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

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

96

97 **Main text**

98 **Phytochromes and Cryptochromes in anterograde signalling.**

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

108 The tight regulation of these nuclear genes encoding for chloroplast-functioning proteins
109 (globally known as Photosynthesis Associated Nuclear Genes, *PhANGs*) is critical for chloroplast
110 biogenesis and photosynthesis, and the photoreceptors are essential for tuning their transcriptional

111 responses in changing light environments (Calderon and Strand, 2021; Larkin and Ruckle, 2008;
112 Pogson *et al.*, 2015). Both the phytochromes and the cryptochromes regulate the global light-
113 responsiveness of the *PhANGs* through the activation or repression of multiple transcription factors
114 including: the bZIP-LONG HYPOCOTYL 5 (HY5) (Osterlund *et al.*, 2000; Toledo-Ortiz *et al.*,
115 2014); the basic helix-loop-helix Phytochrome Interacting Factors (PIFs) (Franklin and Quail, 2010;
116 Leivar and Quail, 2011); and the GARP proteins GOLDEN2-LIKE 1 and 2 (GLK1, GLK2) (Leister
117 and Kleine, 2016; Waters *et al.*, 2009).

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

126 The PIFs are negative modulators of photomorphogenesis that are degraded in the light after
127 the activation of phys and are involved in promoting skotomorphogenesis and shade avoidance
128 responses (Leivar and Quail, 2011; Yoo *et al.*, 2019). While their turn-over and stability is principally
129 regulated by the phytochromes, cryptochromes can repress the transcription of *PIF4* without affecting
130 its protein stability (Ma *et al.*, 2016), and may also protect PIF5 from phy-mediated degradation in
131 low Blue light conditions (Pedmale *et al.*, 2016). PIFs promote skotomorphogenesis (Wang *et al.*,
132 2022) including the down regulation of genes involved in photopigment biosynthesis (Shin *et al.*,
133 2007; Stephenson *et al.*, 2009), chloroplast development and function (Leivar and Monte, 2014).

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

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

146 mRNAs for genes such as protochlorophyllide (PORA) involved in chlorophyll biosynthesis (Paik *et*
147 *al.*, 2012).

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

164 Alongside these reports, bioinformatic studies of genomic datasets for Arabidopsis *cry1 cry2*
165 and *phyabcde* revealed a significant contribution of Red-phytochromes and Blue-cryptochromes to the
166 light-dependent expression of nuclear-encoded genes whose protein products are linked to the
167 transcriptional, post-transcriptional, and translational control of the plastome (Griffin *et al.*, 2020).
168 Among the light-modulated gene families identified were the sigma factor transcriptional cofactors
169 required for the activity of the PLASTID-ENCODED POLYMERASE (PEP) (Börner *et al.*, 2015; Oh
170 and Montgomery, 2014); the Pentatricopeptide domain-containing (PPR) and the Tetratricopeptide
171 domain-containing (TPR) families of RNA binding proteins with a role in the plastome post-
172 transcriptional events (Lamb *et al.*, 1995; Ruwe *et al.*, 2011). In addition, for the Blue-cryptochromes,
173 genes encoding for RNA-Recognition Motif (RRM) RNA binding proteins with an annotated role in
174 post-transcription and for the tRNA ligase and Large Ribosomal Protein (RPL) related to translation
175 were identified (Griffin *et al.*, 2020). In this context, HY5 was singled out as a relevant transcription
176 factor delivering light cues to the “plastome-regulatory gene network”. Gene targets include the sigma
177 factors and the PLASMID TRANSCRIPTIONALLY ACTIVE CHROMOSOME class (pTACs),
178 involved in plastome-transcription, and the PPRs and the TPRs likely involved in post-transcriptional
179 processes.

180 These early studies provide with evidence that the photoreceptors and their signalling
181 components, are central in the anterograde signalling cascades to tune the global expression of the
182 plastome in response to environmental signals, but the detailed mechanistic insights remain to be
183 understood.

184

185 **The chloroplast retrograde signalling pathways.**

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

199 The crucial contribution of retrograde signalling to seedling survival has been assessed in
200 mutants with impaired retrograde signalling capabilities, and through pharmacological approaches
201 that induce stress in the chloroplasts (Chan *et al.*, 2016; Pogson *et al.*, 2008). Common retrograde
202 signal activators include Lincomycin (an inhibitor of plastid translation that blocks plastid
203 development) and Norflurazon (an inhibitor of carotenoid biosynthesis that induces photobleached
204 chloroplasts). These chemical agents trigger a reduction in the expression *PhANGs*, including those
205 encoding for light-harvesting complex proteins (*LHCB*) and the Rubisco small subunit (*RBCS*), that
206 are common marker genes for assessing retrograde signal activity (Ruckle *et al.*, 2012; Susek *et al.*,
207 1993). In *Arabidopsis*, forward mutagenic screens coupled with the use of Norflurazon identified the
208 *gun1* (Genome Uncoupled) mutants with altered accumulation of *PhANGs* like *CAB* (Chlorophyll a/b
209 binding protein) (Mochizuki *et al.*, 2001; Susek *et al.*, 1993; Susek and Chory, 1992).

210 A second type of retrograde signalling involves operational signals that occur after
211 chloroplast biogenesis and in response to stress conditions to induce adjustments in chloroplast
212 homeostasis (Chan *et al.*, 2016; Pogson *et al.*, 2008). Examples of identified operational signalling
213 pathways include the regulation of PSII overexcitation via β -cyclocitral (Ramel *et al.*, 2012), and the
214 methylerythritol cyclodiphosphate (MEcPP) pathway (Jiang and Dehesh, 2021).

215 This review will focus on the photoreceptors involvement in the regulation of the biogenic
216 and operational pathways, including links to the GUN signalling pathways and MEcPP pathway and
217 novel insights on dual localised proteins in the chloroplast to nuclear signalling (Jiang and Dehesh,
218 2021; Martín *et al.*, 2016; Qin *et al.*, 2010; Ren *et al.*, 2017).

219

220 **The intertwining of retrograde signalling and photoreceptor-dependent pathways.**

221 While connections between plastid retrograde signalling and light signalling have been made
222 for decades, most of the mechanisms involved remain elusive (Kusnetsov *et al.*, 1996; Larkin and
223 Ruckle, 2008; Xu *et al.*, 2016). In 1996 Kusnetsov *et al.* examined the overlap between plastid-
224 derived retrograde signals and light-derived signals on functional promoter sequences of *PhANGs*.
225 These authors provided with early evidence that chloroplast-derived retrograde signals and light
226 signalling pathways act on the same cis-acting elements (such as L-, I- and G-boxes), and could
227 regulate the same processes, suggesting an intertwining of the pathways. Since then, G-boxes have
228 been characterised as an important Light Responsive Element (LRE) bound by multiple phytochrome
229 and cryptochrome downstream signalling components including HY5 and the PIFs (Chattopadhyay *et al.*,
230 1998; Leivar and Quail, 2011).

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

243 Beyond the chemical activators of retrograde signals, High-light (HL) is also an important
244 trigger (Szechyńska-Hebda and Karpiński, 2013), and photoreceptors are part of the perception and
245 responsiveness to HL (Kreslavski *et al.*, 2020). Reactive Oxygen Species (ROS) including hydrogen
246 peroxide (H₂O₂), superoxide anions (O²⁻) and singlet oxygen (¹O₂) are chemical derivatives of O₂
247 produced by metabolic processes in plants (Apel and Hirt, 2004). In HL irradiances chloroplasts
248 increment H₂O₂ production by Photosystem I and ¹O₂ by PSII (Kanervo *et al.*, 2005; Krieger-Liszakay,
249 2005). While H₂O₂ has been shown to move out of isolated chloroplasts *in-vitro*, providing it with

250 capacity to act as an initiator of retrograde signalling (Mubarakshina *et al.*, 2010), $^1\text{O}_2$ cannot leave the
251 chloroplast due to its short half-life (Gorman and Rodgers, 1992) and therefore secondary messengers
252 yet to be identified must be involved in the transmission of the $^1\text{O}_2$ signal to the nucleus.

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

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

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

280 Additional evidence from studies in emerging rice seedlings grown under high-Blue or high-
281 Red light and Lincomycin support both an integration and a differential contribution of light-quality
282 and photoreceptor activity to seedling photomorphogenesis and non-photochemical quenching
283 mechanisms to tolerate the excess light (Duan *et al.*, 2020). In this context, in high Red-light
284 conditions retrograde signal activators induced photobleaching, but in high Blue-light, enhanced

285 carotenoid and chlorophyll production contributed to a stronger HL stress tolerance, in a mechanism
286 likely dependent on cryptochromes (Duan *et al.*, 2020; Kleine *et al.*, 2007; Richter *et al.*, 2020).

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

292

293 **Photoreceptors, HY5, and GUN1 in the convergence of photomorphogenesis and retrograde** 294 **signalling.**

295 The *GENOMES UNCOUPLED* (GUN) genes (*GUN1-GUN6*) were identified in the “gun
296 mutant screens” using Norflurazon to activate retrograde signals (Mochizuki *et al.*, 2001; Susek *et al.*,
297 1993; Susek and Chory, 1992; Woodson *et al.*, 2011). GUN2-6 play roles in the tetrapyrrole
298 biosynthesis pathway, and while the full functional role of GUN1 remains to be addressed,
299 experimental evidence also supports GUN1 modulation of tetrapyrroles by direct binding to both
300 heme and porphyrins (Shimizu *et al.*, 2019).

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

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

319 making the role of this metabolite in retrograde signalling unclear at present (Mochizuki *et al.*, 2001;
320 Wu and Bock, 2021).

321 The heme branch of tetrapyrrole synthesis is initiated by GUN6 (also known as plastid
322 FERROCHELATASE 1, FC1) that converts protoporphyrin IX to protoheme by inserting Fe²⁺.
323 Protoheme is converted first to biliverdin IX by GUN2 (encoding heme oxygenase), and finally to 3Z-
324 phytychromobilin by GUN3 (phytychromobilin synthase). Evidence that heme may function as a
325 second type of retrograde signalling molecule has been provided by the characterisation of *gun6-1D*, a
326 dominant mutant allele overexpressing *FC1*, and promoting the flow of tetrapyrroles into the heme
327 branch, with consequent upregulation of *PhANG* transcripts (Woodson *et al.*, 2011).

328 While the specific mechanisms through which photoreceptor signalling pathways are
329 involved in the generation, regulation, and response to GUN retrograde signals, have yet to be fully
330 elucidated, tetrapyrrole biosynthesis is induced by light, as previously reviewed (Kobayashi and
331 Masuda, 2016) with the contribution of light-signalling transcription factors including HY5
332 (Kobayashi *et al.*, 2012a; Kobayashi *et al.*, 2012b; Lee *et al.*, 2007b), the PIFs (Leivar and Quail,
333 2011; Shin *et al.*, 2009), and GLK1 and GLK2 (Waters *et al.*, 2009).

334 In particular, *GUN1* is a gene of high interest as integratory point for light and retrograde
335 signalling pathways. *GUN1* encodes a chloroplast-localised protein containing a pentatricopeptide
336 repeat (PPR) (Koussevitzky *et al.*, 2007). Pentatricopeptide domain-containing proteins are known
337 post-transcriptional regulators of plastid gene expression (Ruwe *et al.*, 2011), but the functional role
338 of GUN1 protein is still under exploration. Of all *gun* mutants, *gun1* exhibits the strongest de-
339 repression of *PhANGs* expression in lincomycin (Koussevitzky *et al.*, 2007) and *GUN1* transcript
340 accumulation is light-responsive and dependent on the phytychromes in Red light (Hu *et al.*, 2013).
341 During de-etiolation, GUN1 is active and involved in cotyledon expansion and hypocotyl elongation
342 (Ruckle *et al.*, 2007; Ruckle and Larkin, 2009) with *gun1* also displaying a delayed greening
343 phenotype. As such, GUN1 likely represents a crosstalk point between the photoreceptor signalling
344 cascades and the plastid signals that tune chloroplast greening and growth responses (Mochizuki *et al.*,
345 1996; Pesaresi and Kim, 2019; Ruckle *et al.*, 2007; Wu and Bock, 2021; Wu *et al.*, 2019).

346 Further support for this possibility has been provided by additional *gun* genetic screens,
347 where an allele of *cry1* that shares similar phenotypes with *gun1-1*, including defects in plastid to
348 nucleus signalling affecting *LHCB* and *RBCS* transcript accumulation, was identified (Ruckle *et al.*,
349 2007). Double mutant analysis of *gun1-101 cry1* grown in HL showed an additive phenotype for their
350 effects on *LHCB* accumulation and deficiencies in chlorophyll accumulation, indicating that GUN1
351 and CRY1 may be partially redundant in modulating *LHCB* via parallel pathways that converge. A
352 similar phenotype of defective *LHCB* accumulation was observed for *gun1-101 hy5* double mutant,
353 suggesting that this CRY1 dependent pathway requires HY5. Likewise, *phyB gun1-1* double mutants

354 accumulated more *LHCB* than *gun1-1* single mutants when treated lincomycin, providing evidence
355 that phyB may also be a *gun* mutant, contributing to the repression of *LHCB*, but only when GUN1 is
356 inactive (Ruckle *et al.*, 2007).

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

361

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

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

372 Interestingly, genomic studies showed that over 343 photomorphogenesis-associated genes
373 involved in de-etiolation and greening are co-repressed by both lincomycin-induced/GUN1-derived
374 retrograde signals and by the PIFs in the dark. This transcriptional effect was further supported by the
375 characterisation of the *pifq* (*pif1 pif3 pif4 pif5*) mutant, for which treatment with lincomycin restored
376 the PIF-repressed genes transcriptomic profile to wild-type levels, indicating a parallel pathway to
377 GUN1 in response to chloroplast dysfunction (Martín *et al.*, 2016). An analysis of the DNA-binding
378 motifs in the promoters of the genes co-repressed by both lincomycin and PIFs identified an
379 enrichment in GLK-binding motifs (Martín *et al.*, 2016). *GLK1* encodes for a transcription factor that
380 is both phytochrome/light-induced and PIF-repressed, and whose down-regulation by retrograde
381 signals in a GUN1/GUN5 dependent manner is reported (Kakizaki *et al.*, 2009; Waters *et al.*, 2009).
382 In addition, characterisation of overexpressing lines for *GLK1* and *GLK2* placed them as *gun* mutants
383 themselves (Leister and Kleine, 2016). As part of the GUN1/GLK1-mediated responses, the B-Box
384 gene *BBX16* has been identified as a directly induced target of GLK1 for the promotion of
385 photomorphogenesis, and whose transcription is repressed in a GUN1/GLK1-dependent manner upon
386 chloroplast damage, as well as in response to Norflurazon treatment (Veciana *et al.*, 2022; Zhao *et al.*,
387 2019).

388 Along with the links between RS and GUN signalling in the light, evidence also suggests that
389 these pathways may operate in darkness, with the involvement of COP1 and the PIFs. Support to this
390 possibility comes from experiments on etiolated *Arabidopsis pifq* seedlings that, when grown in the
391 presence of lincomycin, show a restoration to phenotypes present in WT-etiolated seedlings, including
392 suppression of cotyledon separation and sustainment of apical hook curvature and of appressed
393 cotyledons (Martín *et al.*, 2016). In addition, lincomycin also reduces the transcript accumulation of
394 photomorphogenesis-associated genes such as *LHCBI* in dark-grown *cop1*, and of 354 transcripts in
395 dark-grown *pifq* mutants (Martín *et al.*, 2016; Sullivan and Gray, 1999). Also, recent studies of dark
396 grown etioplasts and pro-plastids revealed the presence of GUN1 protein in the dark and
397 transcriptomic studies on dark grown WT and *gun1-102* indicate that GUN1 mediated signals regulate
398 nuclear gene expression in the dark with up to 4425 genes, including subunits of the Photosystem I
399 (*PSA*) and *LHCB*, differentially expressed in dark *gun1-101* compared to WT. These results support a
400 significant role for GUN1 in tuning the expression in the dark of genes involved in the build-up of the
401 photosynthetic apparatus (Hernandez-Verdeja *et al.*, 2022).

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

409 410 **Photoreceptors and the MEcPP retrograde signalling pathway.**

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

418 A genetic screen in *Arabidopsis* to identify genes involved in the regulation of
419 HYDROPEROXIDE LYASE (*HPL*), a stress-inducible protein in the oxylipin pathway, identified the
420 *constitutively expressing HPL* (*ceh1*) mutant (Xiao *et al.*, 2012). *ceh1* has a mutation in HMBPP
421 synthase (*HDS*) that catalyses the conversion of MEcPP to HMBPP (Ostrovsky *et al.*, 1998;
422 Rodríguez-Concepción, 2006; Xiao *et al.*, 2012), and displays short hypocotyls in the light (Jiang *et*

423 *al.*, 2020; Jiang *et al.*, 2019). This phenotype is caused by higher phyB protein levels induced by the
424 over-accumulation of MEcPP (Jiang *et al.*, 2020). Higher phyB levels lead to the repression of PIF4
425 and PIF5 activity and to an altered accumulation of ethylene and auxin biosynthetic genes such as
426 *ACS4*, *5*, *8*, and *YUC8* (Jiang *et al.*, 2020; Jiang *et al.*, 2019). Interestingly, the short hypocotyl
427 phenotype of *ceh1* mutants was also present in seedlings grown under Blue light, supporting the
428 possibility that Blue light-sensing cryptochromes are also linked to MEcPP accumulation and
429 signalling (Jiang *et al.*, 2019).

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

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

441

442 **HY5 emergence as an important integratory factor for light and multiple retrograde signalling** 443 **pathways.**

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

453 In addition, *HY5* mediates the *GUN1*-triggered rapid light-dependent inhibition of *PhANGs*,
454 induced by singlet oxygen retrograde signals derived from the photo-excitation of Mg-porphyrins and
455 the accumulation of the chlorophyll intermediate Mg-ProtoIX (Kindgren *et al.*, 2012; Richter *et al.*,
456 2020; Strand *et al.*, 2003). Mg-ProtoIX interaction with cytosolic HSP90 proteins leads to the

457 repression or inactivation of nuclear-encoded *PhANGs* in a HY5-dependent manner (Kindgren *et al.*,
458 2012). In this pathway, GUN5-HSP90.2-HY5 is emerging as a convergence point for light and
459 retrograde signalling cascades for the modulation of *PhANGs*. HY5 may also form with GUN1 and
460 HSP90.1 (Wu and Bock, 2021; Wu *et al.*, 2019) a second light-retrograde signals integratory node,
461 whose full biological significance, remains to be investigated.

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

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

472 Another reported link between HY5 and the tetrapyrrole biosynthesis-derived retrograde
473 signalling cascades involves the sigma factors. The sigma transcriptional cofactors are nuclear-
474 encoded genes required for the activity of the PEP (Berry *et al.*, 2013; Börner *et al.*, 2015). In
475 *Arabidopsis*, there are 6 members of the sigma factor family, with 5 of them (*SIGs 1, 2, 3, 5, 6*)
476 showing Red-phytochrome, Blue-cryptochrome, or Red/Blue-HY5-dependent transcript accumulation
477 (Griffin *et al.*, 2020; Oh and Montgomery, 2013). For SIG2 and SIG5, links to retrograde signalling
478 are emerging (Oh *et al.*, 2018; Woodson *et al.*, 2013) with SIG2 modulation in the expression of the
479 tRNA-glu, an early step in the tetrapyrrole biosynthesis (Woodson *et al.*, 2013) and a reduced
480 accumulation of *PhANGs* transcripts (including *RBCS* and *LHCB* genes) in *sig2*, a phenotype that is
481 alleviated by heme-feeding. Transcriptomic studies for SIG2 have also identified under Red-light over
482 2000 nuclear-encoded mis-regulated genes, some with roles in growth, hormonal cross-talk, stress
483 responses, and photosynthesis (Oh *et al.*, 2018). The enrichment in *sig2* of mis-regulated
484 chloroplastic/Red-light responsive genes that are targets of retrograde signals supports an intersection
485 of both pathways for the modulation in particular of chloroplastic acting genes and of genes active
486 during in photomorphogenesis.

487 A second sigma factor, SIG5, is a light quality and high-light responsive gene that is sensitive
488 to DCMU-dependent retrograde signals (Mellenthin *et al.*, 2014). *SIG5* transcript accumulation is
489 CRY1 induced in Blue-light and phy-dependent in Red-light, with HY5 contributing to its
490 transcriptional response in both light qualities (Griffin *et al.*, 2020; Mellenthin *et al.*, 2014). Following
491 DCMU activation of retrograde signals derived from the inhibition of electron flow in Photosystem II

492 (Mellenthin *et al.*, 2014; Metz *et al.*, 1986), the accumulation of *SIG5* is down-regulated. These early
493 studies point at *SIG5* capacity to integrate inputs from light and retrograde signals, however the
494 mechanistic insights on signal integration and biological outputs remain to be investigated. Yet, *SIG2*
495 and *SIG5* as *HY5*- and retrograde signal-sensitive genes, have a good potential to be part of the
496 anterograde and retrograde pathways to tune the plastid genome and the *PhANGs* transcriptional
497 responses with the Blue and Red photoreceptors light signals.

498

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

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

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

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

518

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

520 Likewise, there is also evidence to support the involvement of the photoreceptors in the
521 regulation of multiple dual-localised proteins that can communicate information between the nucleus
522 and the chloroplast to tune chloroplast needs and photomorphogenic responses. *WHIRLY1* (*WHY1*)
523 is among such dual-localised proteins with potential to act as a retrograde signal based on a functional

524 role in in chloroplast biogenesis and a capability for translocation from the chloroplast back to the
525 nucleus (Isemer *et al.*, 2012).

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

533 The role of WHY1 as a retrograde signal occurs in response to redox changes in the thylakoid
534 electron transport chain (Foyer *et al.*, 2014). WHY1's alternate subcellular localisation depends on
535 light via the phyA-dependent regulation of the Calcineurin B-Like-Interacting Protein Kinase14 gene
536 (*CIPK14*) (Qin *et al.*, 2010), encoding for a protein that phosphorylates and modifies WHY1 binding
537 affinity for different promoters (Ren *et al.*, 2017). Interestingly, *CIPK14* transcript accumulation is
538 dependent on multiple light inputs, including transient activation by FR and time dependent
539 modulation by Blue light and Red (Qin *et al.*, 2010). At present, only the response to FR light and the
540 dependence on phyA has been investigated, but based on current studies it can be hypothesised that
541 this phyA-CIPK14-WHY1 regulatory module may be important for the FR blocking of greening
542 response. It remains to be established if the observed Red-light induction of *CIPK14* is phyB
543 dependent, but the Blue-light induction of *CIPK14* is not dependent on CRY1 CRY2 (Qin *et al.*,
544 2010).

545 A second example of the involvement of photoreceptors in the control of nucleo-chloroplastic
546 dual localised proteins include pTAC12/HEMERA (HMR), a member of the pTAC family that
547 regulates the PEP (Chen *et al.*, 2010; Pfalz *et al.*, 2006). *HMR* transcript accumulation is light-
548 responsive and dependent on the phytochromes in Red and cryptochromes in Blue (Griffin *et al.*,
549 2020). In the nucleus, HMR acts as a transcriptional co-activator to regulate light-responsive genes,
550 while in the plastids it associates with the PEP to induce plastid-encoded gene expression (Pfalz *et al.*,
551 2015; Qiu *et al.*, 2015). HMR first localises to the plastids, akin to WHY1 (Grabowski *et al.*, 2008;
552 Isemer *et al.*, 2012), and its relocation to the nucleus is proposed as part of the activation of the
553 retrograde signal cascades (Yoo *et al.*, 2020). Currently this possibility, including the potential cross
554 talk with photoreceptor signalling mechanisms, remains to be fully investigated.

555 In summary, research supports the involvement of phys in the modulation of the activity of
556 nuclear-chloroplastic proteins that directly or indirectly impact on the expression of the nuclear and
557 the plastid genomes. At present, only the role of phys has been studied, but the CRYs integration in

558 the retrograde signalling pathways that tune photomorphogenesis in Blue light make them interesting
559 candidates to assess for their role in controlling dual-localised proteins that may be retrograde signals.

560

561 Conclusions

562 The research highlighted in this review supports an emerging view that the phytochrome and
563 cryptochrome photoreceptors signalling, including through transcription factors such as PIFs and
564 HY5, intertwine with both the anterograde and retrograde signalling pathways. This crosstalk is
565 essential for the tuning of the nuclear and plastid genomes in response to environmental cues (Figure
566 1).

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

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

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

589

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592

593 **Author Contribution**

594 JHCG and GTO designed and wrote the manuscript.

595

596 **Conflicts of Interest**

597 The authors report no conflict of interest.

598

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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 H₂O₂ 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.

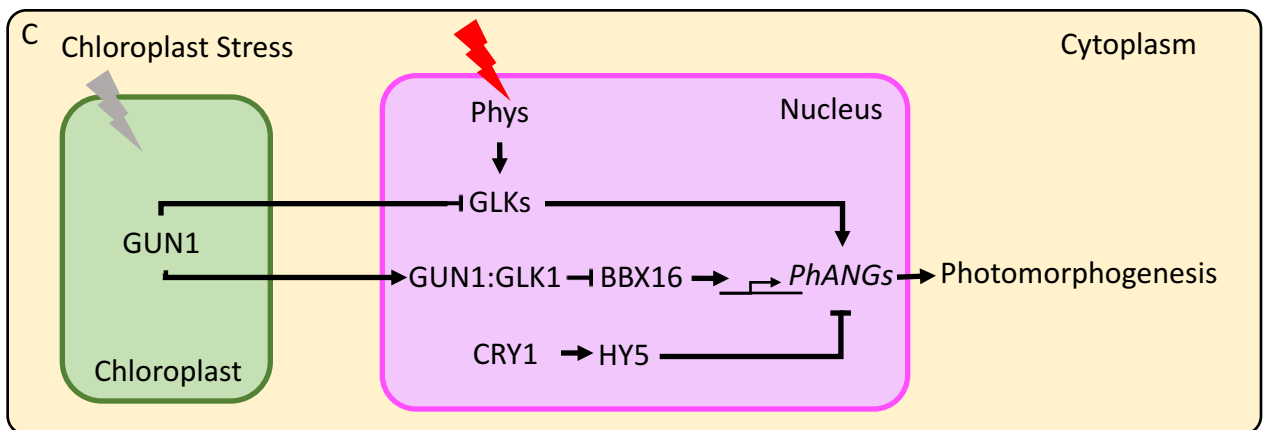
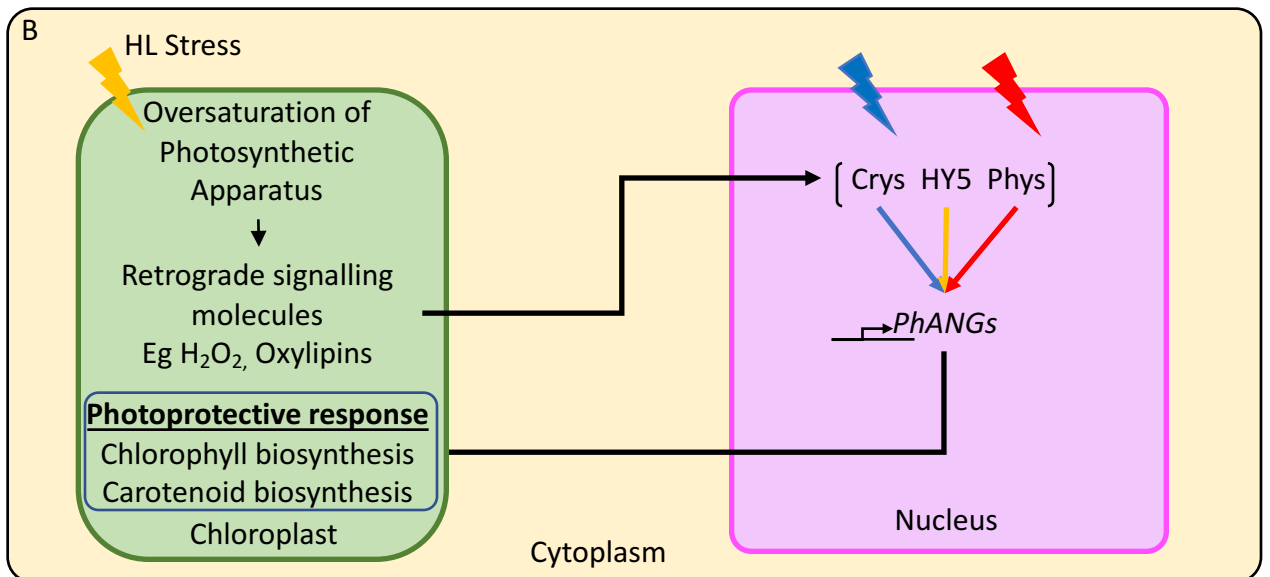
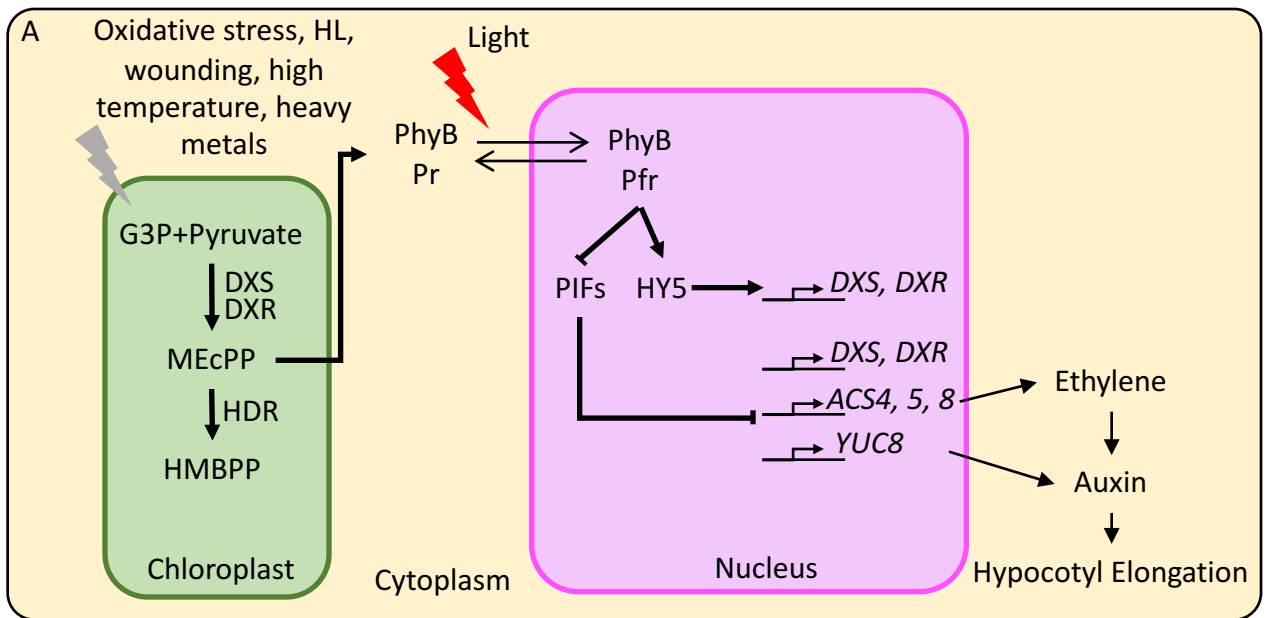


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