

1 **Chloroplasts modulate elongation responses to canopy shade by**  
2 **retrograde pathways involving HY5 and ABA**

3

4 Miriam ORTIZ-ALCAIDE<sup>1</sup>, Ernesto LLAMAS<sup>1</sup>, Aurelio GOMEZ-CADENAS<sup>2</sup>, Akira  
5 NAGATANI<sup>3</sup>, Jaime F. MARTINEZ-GARCIA<sup>1,4,\*</sup>, Manuel RODRIGUEZ-  
6 CONCEPCION<sup>1,\*</sup>

7

8

9 1, Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, 08193  
10 Barcelona, Spain.

11 2, Universitat Jaume I, 12071 Castelló de la Plana, Spain

12 3, Kyoto University, 606-8502 Kyoto, Japan

13 4, Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain.

14

15

16 (\*) Corresponding authors:

17 JMG, [jaume.martinez@cragenomica.es](mailto:jaume.martinez@cragenomica.es)

18 MRC, [manuel.rodriquez@cragenomica.es](mailto:manuel.rodriquez@cragenomica.es)

19

20 **Short title:** Interaction of light and retrograde pathways

21

22

23

24 The authors responsible for distribution of materials integral to the findings  
25 presented in this article in accordance with the policy described in the  
26 Instructions for Authors ([www.plantcell.org](http://www.plantcell.org)) are Jaime F. Martinez-Garcia  
27 ([jaume.martinez@cragenomica.es](mailto:jaume.martinez@cragenomica.es)) and Manuel Rodriguez-Concepcion  
28 ([manuel.rodriquez@cragenomica.es](mailto:manuel.rodriquez@cragenomica.es)).

29 **ABSTRACT**

30       Plants use light as energy for photosynthesis but also as a signal of  
31 competing vegetation. By using different concentrations of norflurazon and  
32 lincomycin, we found that the response to canopy shade in *Arabidopsis thaliana*  
33 was repressed even when inhibitors only caused a modest reduction in the level  
34 of photosynthetic pigments. High inhibitor concentrations resulted in albino  
35 seedlings that were unable to elongate when exposed to shade, in part due to  
36 attenuated light perception and signaling via phytochrome B and phytochrome-  
37 interacting factors. The response to shade was further repressed by a GUN1-  
38 independent retrograde network with two separate nodes represented by the  
39 transcription factor HY5 and the carotenoid-derived hormone ABA. The unveiled  
40 connection between chloroplast status, light (shade) signaling, and  
41 developmental responses should contribute to achieve optimal photosynthetic  
42 performance under light-changing conditions.

## 43 INTRODUCTION

44 Life on our planet heavily relies on photosynthesis, i.e. the use of solar  
45 energy (sunlight) to fix carbon into organic matter linked to the production of  
46 oxygen from water. In plants, the quantity and quality of the incoming light  
47 strongly influence growth and development. For example, oxidative stress and  
48 eventual damage can occur if the amount of light exceeds the photosynthetic  
49 capacity of the chloroplast. By contrast, light supply and hence photosynthetic  
50 activity can be compromised by the shading of nearby plants. Under a canopy,  
51 plants might actually be exposed to moments of both excess light (e.g. sunflecks)  
52 and low light (i.e. shading) during the same day. Even in open habitats, plants  
53 are usually found in communities where competition for light might result in  
54 overgrowing and eventual shadowing by neighbors.

55 Light quality is an important signal that informs plants of potential  
56 competitors. Vegetation absorbs light from the visible region (called  
57 photosynthetically active radiation or PAR, 400–700 nm). In particular, it absorbs  
58 red light (R, 600–700 nm) but transmits and reflects far-red light (FR, 700–800  
59 nm), therefore causing a reduction in the R to FR ratio (R/FR). Both PAR (light  
60 quantity) and R/FR (light quality) are greatly reduced under a plant canopy,  
61 whereas the presence of nearby plants (without direct vegetation shading)  
62 involves a more moderate reduction of R/FR without changes in PAR (Casal,  
63 2012; Martinez-Garcia et al., 2014; Fiorucci and Fankhauser, 2017).  
64 Independently of the PAR level, a drop in R/FR acts as a signal that strongly and  
65 differentially affects elongation of shade-avoiding plants such as *Arabidopsis*  
66 *thaliana* and most crops (Martinez-Garcia et al., 2014). Low R/FR signals also  
67 cause a decrease in the levels of photosynthetic pigments (chlorophylls and  
68 carotenoids) in seedlings and adult plants (Roig-Villanova et al., 2007; Patel et  
69 al., 2013; Bou-Torrent et al., 2015; Llorente et al., 2017). These and other  
70 responses triggered by a reduced R/FR are collectively known as the shade  
71 avoidance syndrome (SAS) and aim to overgrow neighboring plants, readjust  
72 photosynthetic metabolism, and eventually launch reproductive development  
73 (Franklin, 2008; Casal, 2012; Gommers et al., 2013; Martinez-Garcia et al.,  
74 2014).

75 Low R/FR signals indicative of shade are perceived by the phytochrome  
76 (phy) family of photoreceptors. Five genes encode the phy family in *Arabidopsis*:

77 phyA to phyE. While phyB is the major phy controlling the responses to shade,  
78 other phy members such as phyD and phyE can also redundantly contribute to  
79 the control of shade-modulated elongation growth or flowering time (Franklin,  
80 2008; Martinez-Garcia et al., 2014). In the case of photolabile phyA, an  
81 antagonistic negative role has been reported for the seedling hypocotyl  
82 elongation response to shade. Thus, the SAS is induced by phyB deactivation  
83 but gradually antagonized by phyA in response to high FR levels characteristic of  
84 plant canopy shade (Casal, 2012; Martinez-Garcia et al., 2014). This intrafamily  
85 photosensory attenuation mechanism might act to suppress excessive elongation  
86 under prolonged direct vegetation shade. It remains unknown whether other SAS  
87 responses, including photosynthetic pigment decrease, are also affected by this  
88 antagonistic regulation by phyA and phyB. In any case, the balance between  
89 positive and negative regulators of the SAS acting downstream phy was found  
90 to be instrumental to regulate not only hypocotyl elongation but also carotenoid  
91 biosynthesis (Franklin, 2008; Casal, 2013; Bou-Torrent et al., 2015). Positive  
92 regulators of the SAS include transcription factors of the basic-helix-loop-helix  
93 (bHLH) (e.g. PIFs, BEEs, BIMs) and homeodomain leucine zipper class II  
94 (ATHB2, ATHB4, HAT1, HAT2 and HAT3) families, whereas the basic leucine  
95 zipper (bZIP) transcription factor HY5 and bHLH family members PIL1, HFR1  
96 and PAR1 have negative roles. Among them, PIFs and HY5 have also been  
97 found to participate in retrograde signaling during deetiolation, i.e. in the  
98 communication between chloroplasts and nucleus when underground seedlings  
99 sense the light and change from skotomorphogenic (i.e. heterotrophic) to  
100 photomorphogenic (i.e. photosynthetic) development (Ruckle et al., 2007; Martin  
101 et al., 2016; Xu et al., 2016). Alterations in the physiological status of the  
102 chloroplast in light-grown plants are also signaled to the nucleus by a variety of  
103 retrograde pathways that readjust nuclear gene expression accordingly (Baier  
104 and Dietz, 2005; Glasser et al., 2014; Chan et al., 2016). Because exposure to  
105 shade causes a decrease in the accumulation of chlorophylls and carotenoids  
106 that can eventually compromise photosynthesis and photoprotection (Roig-  
107 Villanova et al., 2007; Cagnola et al., 2012; Bou-Torrent et al., 2015), we  
108 reasoned that the derived effects on chloroplast homeostasis might not be just a  
109 consequence but influence the response to shade itself (e.g. in terms of

110 elongation) through retrograde signaling. The work reported here aimed to test  
111 this possibility.

112

## 113 **RESULTS AND DISCUSSION**

### 114 **Functional chloroplasts are required for full response to simulated shade.**

115 To initially test whether retrograde signals modulate the elongation  
116 response to plant proximity, we used two kinds of inhibitors of chloroplast  
117 function associated with retrograde signaling: norflurazon (NF), an inhibitor of  
118 carotenoid biosynthesis (Chamovitz et al., 1991) and lincomycin (LIN), an  
119 inhibitor of chloroplast protein synthesis (Mulo et al., 2003). Both inhibitors were  
120 present in the medium used for seed germination and seedling growth (Figure 1).  
121 This medium also contained sucrose to sustain growth even in the absence of  
122 photosynthesis. As expected, a concentration-dependent bleaching was  
123 observed in wild-type (WT) Arabidopsis plants grown under white light (W) with  
124 NF or LIN (Figure 1A). The concentration of inhibitors required to obtain albino  
125 seedlings was adjusted to our experimental conditions. For example, an albino  
126 phenotype was previously observed in WT seedlings grown without sucrose in  
127 the presence of 5  $\mu\text{M}$  NF under 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  or with 50 nM NF under 5  $\mu\text{mol}$   
128  $\text{m}^{-2} \text{s}^{-1}$  (Saini et al., 2011). We used intermediate light intensity conditions (20-24  
129  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and, most importantly, added sucrose in the medium, which  
130 together made it necessary to adjust NF concentration to 200 nM to obtain  
131 completely albino seedlings (Figure 1A).

132 The presence of inhibitors had no significant effect on hypocotyl length  
133 under W (Figure 1B). However, exposure to FR-enriched W (W+FR) to simulate  
134 canopy shade progressively impaired elongation as levels of photosynthetic  
135 pigments (chlorophylls and carotenoids) decreased. Importantly, inhibition of  
136 shade-triggered elongation growth was observed at concentrations of NF or LIN  
137 that only slightly reduced the levels of photosynthetic pigments and had no visual  
138 impact on seedling pigmentation (e.g. 25 nM NF or 5  $\mu\text{M}$  LIN), suggesting that  
139 even moderate alterations in chloroplast function might influence the response to  
140 shade. Hypocotyl elongation in response to shade was completely blocked at  
141 concentrations of NF causing more than a 80% loss of chlorophylls, whereas an  
142 even lower reduction (50%) was required for a lack of response in LIN-treated  
143 seedlings (Figure 1B). In both cases, completely bleached seedlings did not

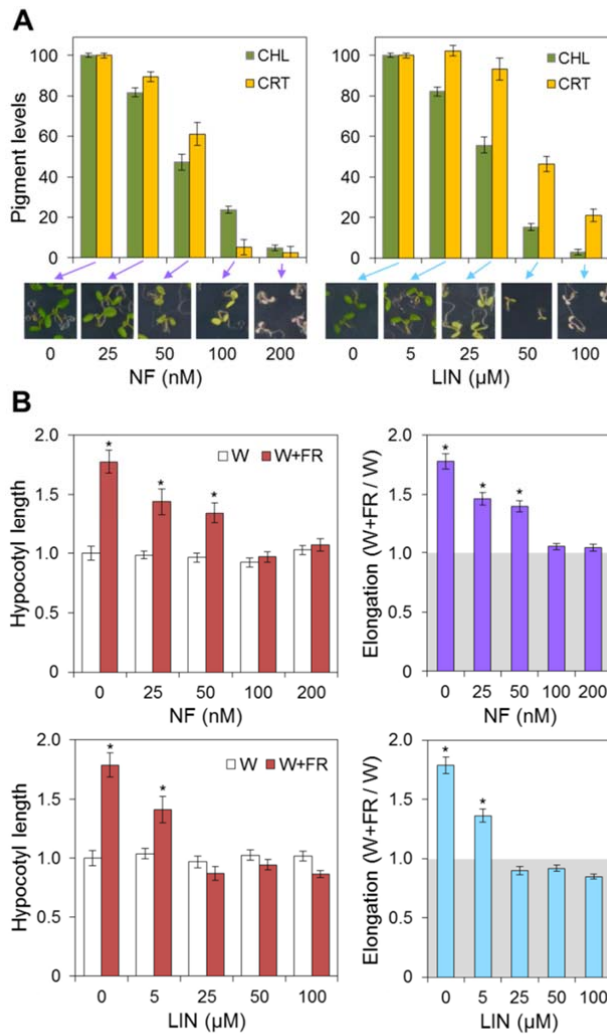


Figure 1. Hypocotyl elongation in response to shade requires functional chloroplasts. (A) WT (Col) plants were germinated and grown under W for 7 days on media with or without the indicated concentrations of NF or LIN. Graphs represent the mean and SEM values of total chlorophyll (CHL) and carotenoid (CRT) contents of at least  $n=8$  independent samples (pools of seedlings) from two different experiments. Pigments were quantified by spectrophotometric methods and represented relative to the levels found in the absence of inhibitors. Pictures show the phenotype of representative seedlings. (B) Hypocotyl elongation in 7-day-old seedlings germinated and grown on media supplemented with the indicated concentrations of NF or LIN under W or exposed to W+FR during the last 5 days. Graphs in the left represent the length of the hypocotyls (mean and SEM of  $n=100$  seedlings grown in different plates in at least 2 independent experiments) relative to the value in samples grown under W in the absence of inhibitors. Graphs in the right represent the elongation response to shade of the same samples. They show the ratio of hypocotyl length under W+FR relative to that under W. A value of 1 means no growth differences between W and W+FR, values above 1 indicate higher growth under W+FR, and values below 1 indicate lower growth under W+FR. Asterisks mark values statistically higher than 1 (T-test,  $p < 0.05$ ), i.e. responsive to shade by increasing hypocotyl elongation.

144 elongate at all when exposed to W+FR compared to W controls (Figure 1B),  
 145 suggesting that functional chloroplasts are required for the elongation response  
 146 to canopy shade.

147 We next aimed to confirm that the disrupted elongation response to W+FR  
 148 observed in bleached seedlings was not due to energetic constraints. If non-  
 149 photosynthetic seedlings lacking chlorophylls maintain an intrinsic capacity to  
 150 grow, it would be expected that their hypocotyls would elongate when treated  
 151 with growth-promoting hormones such as brassinosteroids, auxins, or  
 152 gibberellins. In agreement, seedlings grown in the presence of NF concentrations  
 153 that completely blocked photosynthetic development ( $2 \mu$ M) were able to  
 154 elongate very similarly to control green seedlings when treated with any of these  
 155 hormones (Figure S1). The same hormone treatments caused a similar growth

156 response in the case of mutant *hdr-3* seedlings, which are unable to produce the  
157 precursors for chlorophyll and carotenoid biosynthesis in chloroplasts and hence  
158 display an albino phenotype (Pokhilko et al., 2015). We therefore conclude that  
159 functional chloroplasts are not required for hormone-mediated hypocotyl  
160 elongation (at least in sucrose-supplemented media) but are necessary for  
161 growth in response to shade signals.

162

163 **Defective chloroplast function impairs phytochrome-mediated shade**  
164 **signaling.**

165 Phytochromes are the main photoreceptors involved in shade perception  
166 and signal transduction, with phyB having a predominant role in Arabidopsis  
167 (Casal, 2012; Martinez-Garcia et al., 2014; Fiorucci and Fankhauser, 2017). To  
168 address whether treatment with NF or LIN had an impact on phytochrome  
169 signaling, we used transgenic *35S:PHYB-GFP* plants expressing a biologically  
170 active GFP-tagged version of the phytochrome (Yamaguchi et al., 1999). Under  
171 W, the phyB-GFP fusion protein shows a characteristic distribution in nuclear  
172 speckles, presumably the site where active photoreceptor proteins interact with  
173 other nuclear factors to mediate light signaling (Yamaguchi et al., 1999). We  
174 observed that only minutes after exposing *35S:PHYB-GFP* seedlings to an end-  
175 of-day FR treatment to simulate shade, the green fluorescence associated to the  
176 phyB-GFP reporter became more disperse in the nuclei of epidermal hypocotyl  
177 cells (Figure 2), likely reflecting phyB inactivation. Strikingly, this shade-mediated  
178 inactivation process was clearly delayed in albino seedlings germinated and  
179 grown in the presence of NF (5  $\mu$ M) or LIN (1 mM). While mock (i.e. green)  
180 seedlings displayed evenly distributed nuclear phyB-GFP fluorescence in all  
181 analyzed cells 90 min after the light treatment, inhibitor-grown (i.e. albino)  
182 seedlings still showed nuclear speckles in some cells after 210 min. These  
183 results suggest that functional chloroplasts are required for proper phyB  
184 inactivation in response to shade signals. Consistent with this conclusion, the  
185 shade-triggered and phytochrome-dependent stabilization of photolabile PIF3  
186 was attenuated in NF-bleached seedlings (Figure S2).

187 To further confirm whether phytochrome function was altered in albino  
188 seedlings, we next analyzed the expression of rapidly shade-induced  
189 phytochrome primary target genes in WT plants either treated or not with NF

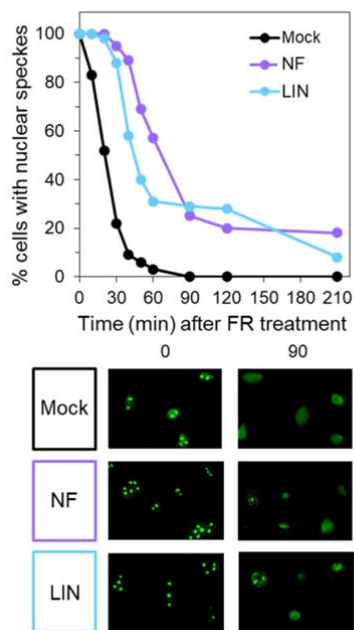


Figure 2. Retrograde signals prevent phyB deactivation in response to shade. Transgenic 35S:PHYB-GFP plants were germinated and grown under W for 7 days on media with or without 5  $\mu$ M NF or 1 mM LIN. Following a 5 min irradiation with FR to simulate shade, the distribution of the phyB-GFP reporter protein in nuclei from epidermal hypocotyl cells was analyzed by confocal laser scanning microscopy. A total of ten seedlings from three independent experiments were observed for each treatment and time point. Graph shows mean and SEM values of the percentage of cells showing nuclear speckles in a total of 90 nuclei per treatment and time point. Lower panels show representative images of nuclei from seedling hypocotyl cells 0 and 90 min after the light treatment.

190 (Figure 3). In particular, we chose genes *PIL1*, *ATHB2*, *HFR1*, *YUCCA8* and  
 191 *PAR1* (Roig-Villanova et al., 2006). WT plants grown under W for 7 days were  
 192 exposed to W+FR for 1h and then samples were collected and used for RNA  
 193 extraction and quantitative RT-PCR (qPCR) analysis. As expected, comparison  
 194 of W-grown controls and shade-exposed (1h W+FR) samples showed that all  
 195 genes analyzed were induced by shade in green seedlings, ranging from 2-fold  
 196 (*PAR1*) to 80-fold (*PIL1*). In NF-grown seedlings, however, the induction was  
 197 much reduced (Figure 3). *HFR1*, *YUCCA8* and *PAR1* gene expression hardly  
 198 changed after W+FR treatment in albino seedlings, whereas *PIL1* induction was  
 199 only 10% compared to that detected in green seedlings and *ATHB2* up-regulation  
 200 was less than half. Together, we conclude that the absence of functional  
 201 chloroplasts somehow prevents normal light (i.e. shade) perception and signal  
 202 transduction by phytochromes.

203 Functional phytochrome holoproteins require the covalent attachment of a  
 204 phytychromobilin (P $\Phi$ B) chromophore to each phytochrome apoprotein monomer  
 205 (Rockwell et al., 2006). The synthesis of P $\Phi$ B occurs in the plastid and the early  
 206 steps are shared with those required to synthesize heme and chlorophylls (Figure  
 207 4). To test whether the observed reduction in shade-triggered phytochrome  
 208 inactivation (and hence hypocotyl elongation) in albino seedlings could result for  
 209 impaired accumulation of P $\Phi$ B, we analyzed the elongation response to shade of



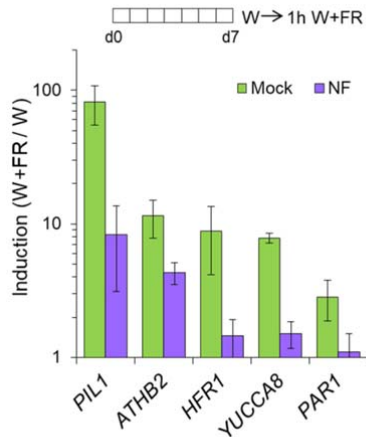


Figure 3. Shade-triggered induction of phytochrome primary target genes is attenuated in bleached seedlings. WT plants were germinated and grown under W for 7 days on media with or without 5  $\mu$ M NF. Before and after 1h of exposure to W+FR, RNA was isolated from seedlings and used to analyze the transcript levels of the indicated genes by RT-qPCR. Graph represents the induction response (transcript levels in W vs. those after exposure to W+FR). Mean and SD of n=3 pools of seedlings from independent experiments are shown.

210 Arabidopsis mutants defective in P $\Phi$ B synthesis (Parks and Quail, 1991). In  
 211 particular, we used the *hy1-1* allele, which was isolated from a fast-neutron  
 212 mutagenized population of Landsberg *erecta* (*Ler*) and carries a short deletion  
 213 that disrupts its function (Davis et al., 1999). As shown in Figure 4, elongation in  
 214 response to W+FR was not repressed but dramatically enhanced in the *hy1-1*  
 215 mutant relative to the corresponding WT (*Ler*). Besides showing a much stronger  
 216 response to shade under normal growth conditions (i.e. in the absence of  
 217 inhibitors), *hy1-1* seedlings were also able to respond to shade and elongate  
 218 when treated with NF (Figure 4B). We therefore conclude that treatment with  
 219 bleaching inhibitors interferes with phytochrome-dependent signaling by  
 220 mechanisms other than defective chromophore availability.

221 Plastid retrograde signaling has been previously shown to interact with  
 222 components of light signaling networks to coordinate chloroplast biogenesis with  
 223 both the light environment and development (Larkin and Ruckle, 2008; Lepisto  
 224 and Rintamaki, 2012; Ruckle et al., 2012; Martin et al., 2016; Xu et al., 2016). In  
 225 fact, mutants defective in the P $\Phi$ B biosynthetic enzymes HY1/GUN2 and  
 226 HY2/GUN3 (Figure 4A) were isolated in a screen for *GENOMES UNCOUPLED*  
 227 (*GUN*) mutants that retained partial expression of genes encoding  
 228 photosynthesis-related plastidial proteins after NF treatment (Mochizuki et al.,  
 229 2001). Other GUN proteins such as GUN5 (Mochizuki et al., 2001) participate in  
 230 a different branch of the tetrapyrrole pathway that leads to the production of  
 231 chlorophylls (Figure 4A). Unlike other GUN proteins, GUN1 is not an enzyme but  
 232 a central integrator of retrograde signaling pathways that was proposed to  
 233 coordinate photomorphogenesis with chloroplast function (Koussevitzky et al.,

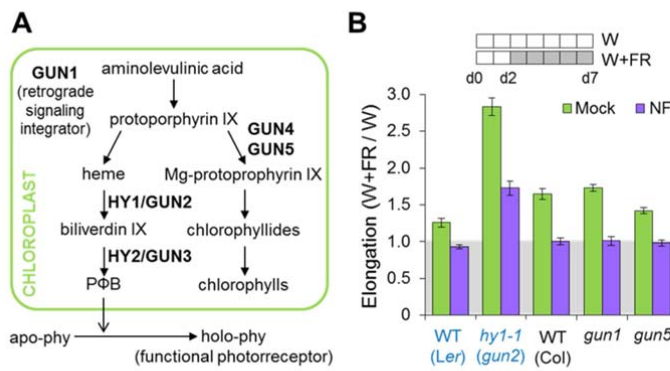


Figure 4. *gun* mutants show different elongation responses to shade. (A) Roles of GUN proteins in retrograde signaling and production of chlorophylls, heme, and the phytochrome chromophore. (B) Elongation responses to shade in mutants defective in some of the GUN proteins represented in (A). Mutants and their respective WT backgrounds (Ler for *hy1/gun2* and Col for the rest) were germinated and grown as indicated with or without 5  $\mu$ M NF. Graph represents the mean and SEM values of at least two independent experiments with  $n \geq 25$  seedlings each.

234 2007; Ruckle et al., 2007; Ruckle and Larkin, 2009). Similar to WT plants,  
 235 mutants *gun1-101* (Ruckle et al., 2007) and *gun5-1* (Mochizuki et al., 2001)  
 236 elongated in response to W+FR under normal growth conditions (i.e. when  
 237 chloroplasts are functional) but not when chloroplast development was blocked  
 238 with NF (Figure 4). Together, the described results suggest that alteration of  
 239 chloroplast function impacts a retrograde signaling pathway independent of GUN  
 240 proteins that modulates the phytochrome-mediated response to shade.

241

### 242 **Retrograde pathways repressing shade-triggered hypocotyl elongation** 243 **involve HY5 but not GUN1.**

244 To identify components of the chloroplast-modulated transduction pathway  
 245 involved in the response to shade, we next tested the possible role of SAS-  
 246 related transcription factors known to be involved in both light and retrograde  
 247 signaling: PIFs (Martin et al., 2016) and HY5 (Ruckle et al., 2007; Xu et al.,  
 248 2016). A role for PIFs as positive regulators of the response to shade (including  
 249 hypocotyl elongation) is well established (Lorrain et al., 2008; Leivar et al., 2012;  
 250 Bou-Torrent et al., 2015). However, under our experimental conditions the  
 251 quadruple *pifQ* mutant defective in PIF1, PIF3, PIF4 and PIF5 showed a WT  
 252 phenotype in terms of shade-triggered hypocotyl elongation in both green and  
 253 albino seedlings (Figure 5). HY5 has been proposed to have a function in the  
 254 adaptation to prolonged shade and the response to sunflecks, i.e. exposure to  
 255 sunlight through gaps in the canopy (Sellaro et al., 2011; Ciolfi et al., 2013). The  
 256 role of this elongation-repressing transcription factor in controlling the shade-  
 257 promoted growth of seedling hypocotyls, however, remains unclear. Our previous

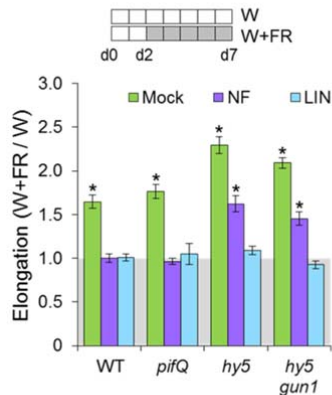


Figure 5. HY5 represses shade-triggered hypocotyl elongation in a GUN1-independent manner. WT (Col) as well as single (*hy5*), double (*hy5 gun1*) and quadruple (*pifQ*) mutant lines were germinated and grown as indicated with or without 5  $\mu$ M NF or 1 mM LIN. Graph represents the mean and SEM elongation values of at least two independent experiments with nh25 seedlings each. Asterisks mark statistically significant responses to shade (T-test,  $p < 0.05$ ).

258 work (Bou-Torrent et al., 2015) showed that complete loss of HY5 activity in the  
 259 null *hy5-2* mutant (referred to as *hy5* from now on) hardly had an impact in the  
 260 elongation of Arabidopsis seedlings exposed to a W+FR treatment mimicking  
 261 vegetation proximity ( $R/FR = 0.05$ ). As shown in Figure 5, however, *hy5*  
 262 seedlings displayed increased hypocotyl elongation compared to the WT when  
 263 illuminated with light of a lower  $R/FR$  (0.02), reminiscent of canopy shade. These  
 264 results suggest that HY5 is a repressor of hypocotyl elongation in green  
 265 seedlings exposed to low or very low  $R/FR$  conditions. Consistently, shade-  
 266 triggered hypocotyl growth was inhibited in transgenic seedlings  
 267 overaccumulating HY5 in a *hy5* background (Figure S3). Similar to WT plants,  
 268 the elongation response to canopy shade of *hy5* seedlings was almost  
 269 completely blocked with LIN (Figure 5). However, the growth response of HY5-  
 270 deficient seedlings was not abolished but just attenuated in NF-supplemented  
 271 medium. Similar results were obtained in medium lacking sucrose, but the effects  
 272 of HY5 gain or loss of function on the elongation response of green or NF-treated  
 273 seedlings, respectively, were much more obvious in the presence of sucrose  
 274 (Figure S3). We therefore kept using sucrose-supplemented media for the rest of  
 275 the work. Double *hy5 gun1-101* mutants were also found to display a partial  
 276 elongation response to shade in NF but not in LIN, similar to that found for the  
 277 single *hy5* mutant (Figure 5). Together, the described results show that HY5 is a  
 278 repressor of canopy shade-triggered hypocotyl elongation. When this negative  
 279 regulator is lost, the elongation response to shade can still be blocked by a  
 280 GUN1-independent retrograde pathway that is active in LIN-treated but not in NF-  
 281 treated albino seedlings.

282 We next analyzed the levels of HY5 transcripts before and after exposure to  
283 our shade conditions (Figure 6). In green WT plants (grown without inhibitors) the  
284 levels of *HY5* transcripts were similar under W and up to 8h of our W+FR  
285 treatment (Figure 6A). In contrast, immunoblot analysis of a HY5-GFP reporter in  
286 complemented *hy5 35S:HY5-GFP* plants showed increased protein levels after  
287 the simulated shade treatment (Figure 6B). Chromatin immunoprecipitation  
288 experiments also detected increased levels of HY5-GFP bound to target  
289 promoters in shade-exposed green seedlings (Figure 6C). Although the  
290 endogenous HY5 protein might not behave exactly as the overexpressed GFP-  
291 tagged version of the protein, our results are in agreement with previous studies  
292 using a different reporter (HY5-myc) that concluded that the low R/FR treatment  
293 stabilizes HY5 (Pacin et al., 2016). Post-transcriptional HY5 accumulation when  
294 R/FR is low or very low in natural environments (such as in deep or canopy  
295 shade) might help to prevent seedlings from exhibiting excessive elongation.

296 Both HY5-encoding transcripts (Figure 6D) and HY5-GFP protein (Figure  
297 6B) were higher in albino seedlings grown with LIN or NF independent of the light  
298 treatment, suggesting that these inhibitors promote HY5 function by increasing  
299 gene expression (or/and transcript stability) and decreasing protein turnover. The  
300 observation that hypocotyl length is not reduced in W-grown seedlings in the  
301 presence of inhibitors (Figure 1B) despite accumulating higher HY5 levels (Figure  
302 6B) suggests that hypocotyl elongation is suppressed to a saturating level by  
303 multiple pathways under W and hence it would not be further repressed by  
304 increasing HY5 function. In response to W+FR, however, enhanced HY5 activity  
305 together with reduced light signaling in bleached WT seedlings would result in no  
306 hypocotyl elongation. Only when the repressor activity of HY5 is removed (i.e. in  
307 HY5-defective mutants), a second pathway that inhibits the elongation response  
308 of albino seedlings becomes apparent in the presence of LIN but not in the  
309 presence of NF (Figure 5).

310

311 **Carotenoid-derived products repress shade-triggered hypocotyl**  
312 **elongation.**

313 The distinct mode of action of LIN and NF and particularly their differential  
314 effect on carotenoid levels is illustrated by their concentration-dependent impact  
315 on photosynthetic pigment accumulation (Figure 1A). HPLC analysis of

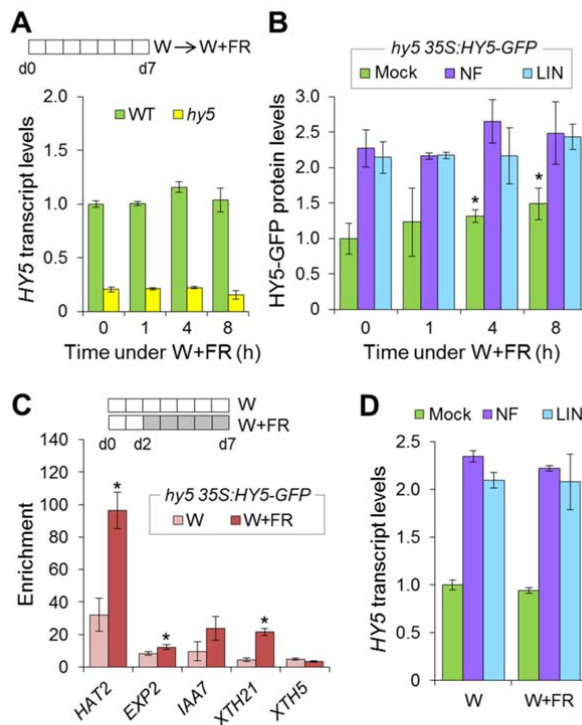


Figure 6. HY5 levels are regulated by shade and retrograde signals. (A) Levels of HY5-encoding transcripts in WT and *hy5* plants germinated and grown under W for 7 days and then exposed to W+FR for the indicated times. Transcript levels were quantified by qPCR and represented relative to those in W-grown WT plants (mean and SEM of n=3 samples corresponding to pools of whole seedlings grown in different experiments). (B) Levels of HY5-GFP protein in *hy5* 35S:HY5-GFP plants germinated and grown under W for 7 days with or without 5  $\mu$ M NF or 1 mM LIN and then exposed to W+FR for the indicated times. Protein levels were quantified from immunoblot analysis with a commercial anti-GFP serum. Mean and SEM values (n=3 samples from pools of whole seedlings grown in different experiments) are represented relative to those in plants grown without inhibitors before exposure to shade. Asterisks mark statistically significant differences relative to the 0h timepoint (T-test, p<0.05). (C) Chromatin immunoprecipitation (ChIP) analysis of HY5-GFP binding to the promoters of the indicated genes. After germinating and growing plants of the *hy5* 35S:HY5-GFP line on media without

inhibitors as indicated, ChIP experiments were done using commercial anti-GFP serum. Chromatin from these samples and from no-antibody controls was then used for qPCR amplification of HY5-binding sites in the promoter of the genes. Enrichment was calculated as the ratio of anti-GFP vs. no-antibody values after normalization with input samples (i.e. before ChIP). Graph shows mean and SEM values of n=2 samples from seedlings grown in different experiments. Asterisks mark statistically significant differences in shade-treated samples (T-test, p<0.05). (D) Levels of HY5-encoding transcripts in WT plants germinated on medium with or without 5  $\mu$ M NF or 1 mM LIN and grown under W for 2 days followed by 5 additional days under W or under W+FR. Transcript levels were quantified by qPCR and represented relative to those in plants grown under W without inhibitors (mean and SEM of n=3 samples corresponding to pools of whole seedlings grown in different experiments).

316 carotenoid contents (Figure S4) confirmed that albino LIN-treated seedlings  
 317 accumulated low but detectable levels of lutein and violaxanthin as well as traces  
 318 of  $\beta$ -carotene and neoxanthin. By contrast, NF blocks the desaturation of  
 319 phytoene, the first committed intermediate of the carotenoid pathway (Figure 7).  
 320 As expected, NF-treated seedlings accumulated phytoene (which is colorless and  
 321 hence not detected in the spectrophotometric assay used in Figure 1A) but were  
 322 virtually devoid of downstream carotenoids (Figure S4). Similar to that observed  
 323 with LIN, other bleaching inhibitors that prevent chloroplast development and  
 324 cause albinism without specifically blocking the production of carotenoids, such  
 325 as the plastid protein synthesis inhibitor chloramphenicol (CAP) or the nitrogen  
 326 assimilation inhibitor phosphinotricin (PPT), were found to prevent shade-  
 327 triggered elongation growth in WT and HY5-defective mutants (Figure 7B). By  
 328 contrast, inhibition of the carotenoid pathway downstream of lycopene by

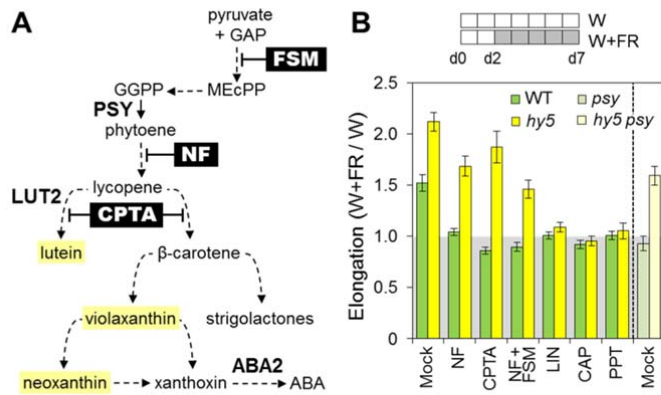


Figure 7. Blockage of the carotenoid pathway derepresses shade-triggered elongation of bleached HY5-defective seedlings. (A) Pathways for the biosynthesis of carotenoids and derived hormones. The steps targeted by NF and other inhibitors and the reactions catalyzed by enzymes that determine metabolic flux to carotenoids (PSY) and ABA (ABA2) are shown. Xanthophylls are boxed in yellow. (B) Elongation responses to shade in WT and mutant plants defective in HY5, PSY, or both. WT and single *hy5* mutant plants were germinated and grown as indicated on media either supplemented or not with concentrations of NF, 2-(4-chlorophenylthio)-triethylamine chloride (CPTA), fosmidomycin FSM), LIN, chloramphenicol (CAP) or phosphinotricin (PPT) producing albino seedlings. Single *psy-1* and double *hy5 psy-1* mutants were only grown without inhibitors. Graph represents the mean and SEM values of nh30 seedlings in a representative experiment.

329 blocking the activity of lycopene cyclases with 2-(4-chlorophenylthio)-  
 330 triethylamine chloride (CPTA) resulted in albino seedlings that were able to  
 331 respond to shade and elongate when HY5 function was lost (Figure 7).

332 To confirm whether the ability to respond to shade of *hy5* seedlings grown  
 333 in the presence of NF or CPTA was specifically due to the blockage of the  
 334 carotenoid pathway, we next used Arabidopsis mutants. The enzyme phytoene  
 335 synthase (PSY) produces phytoene in the first committed step of the carotenoid  
 336 pathway (Figure 7A). Because PSY is encoded by a single gene in Arabidopsis  
 337 (Ruiz-Sola and Rodriguez-Concepcion, 2012), the knock-out mutant *psy-1*  
 338 (Pokhilko et al., 2015) does not produce phytoene and hence cannot feed the  
 339 pathway for the biosynthesis of downstream carotenoids (Figure S4). As a  
 340 consequence, the mutant displays an albino phenotype undistinguishable from  
 341 that observed in WT seedlings treated with NF or CPTA (Pokhilko et al., 2015).  
 342 Similar to that described for WT seedlings grown in the presence of carotenoid  
 343 biosynthesis inhibitors, *psy-1* seedlings were unable to elongate when exposed  
 344 to W+FR (Figure 7B). However, the elongation response was rescued when both  
 345 HY5 and carotenoids were missing in double *hy5 psy-1* mutant seedlings (Figure  
 346 7B).

347 Pharmacological or genetic blockage of the carotenoid pathway prevents  
 348 the biosynthesis of carotenoids and derived products, but it might also cause an  
 349 accumulation of upstream metabolites. Among them, methylerythritol  
 350 cyclodiphosphate (MEcPP), an intermediate of the pathway that supplies the

351 metabolic precursors of carotenoids (Figure 7A), has been shown to act as a  
352 retrograde signal in response to stress (Xiao et al., 2012). Blockage of MEcPP  
353 production with the inhibitor fosmidomycin (Figure 7A), however, did not prevent  
354 the elongation response to shade of NF-treated *hy5* seedlings (Figure 7B). We  
355 therefore conclude that what allows *hy5* seedlings to respond to shade is not the  
356 accumulation of a metabolite upstream PSY but the depletion of a carotenoid-  
357 derived product synthesized after the step blocked by CPTA, i.e. downstream of  
358 lycopene (Figure 7A).

359 As represented in Figure 7A, lycopene cyclization leads to the production of  
360 carotenoids with two  $\beta$  rings ( $\beta,\beta$  carotenoids such as  $\beta$ -carotene and derived  
361 xanthophylls) or with one  $\beta$  and one  $\epsilon$  ring ( $\beta,\epsilon$  carotenoids such as lutein). The  
362 production of  $\beta,\epsilon$  carotenoids in *Arabidopsis* is completely blocked in the green  
363 *lut2* mutant (Figure S4) (Emiliani et al., 2018), which is defective in the only gene  
364 encoding lycopene  $\epsilon$ -cyclase (LCYE/LUT2) in this plant species (Figure 7A)  
365 (Ruiz-Sola and Rodriguez-Concepcion, 2012). Loss of  $\beta,\epsilon$  carotenoids did not  
366 change the elongation response to shade of single *lut2* (vs. WT) or double *hy5*  
367 *lut2* (vs. *hy5*) seedlings (Figure S5). We therefore concluded that the effect  
368 observed with CPTA (Figure 7B) is not due to the absence of  $\beta,\epsilon$  carotenoids but  
369 most likely to defects in the  $\beta$ -carotene branch of the carotenoid pathway (Figure  
370 7A). Considering all these data together, we speculated that unidentified products  
371 derived from  $\beta,\beta$  carotenoids can repress shade-induced elongation growth in  
372 seedlings bleached with LIN and other inhibitors that do not target the carotenoid  
373 biosynthesis pathway. The absence of these products in seedlings treated with  
374 NF or CPTA, or in the *psy-1* mutant, allows hypocotyl elongation in response to  
375 shade but only when the growth-inhibitory effect of HY5 is released.

376

### 377 **ABA represses the elongation response to shade.**

378 Among the biologically active metabolites derived from  $\beta,\beta$  carotenoids  
379 (Hou et al., 2016), we decided to evaluate the role of ABA as this plant hormone  
380 was found to participate in the transduction of chloroplast-derived ROS/redox  
381 signals (Baier and Dietz, 2005; Glasser et al., 2014; Chan et al., 2016), to  
382 modulate hypocotyl growth (Lau and Deng, 2010; Humplik et al., 2017) and to act  
383 together with HY5 in the regulation of several plant cell responses (Chen et al.,  
384 2008; Xu et al., 2014). Furthermore, treatment with low R/FR was reported to

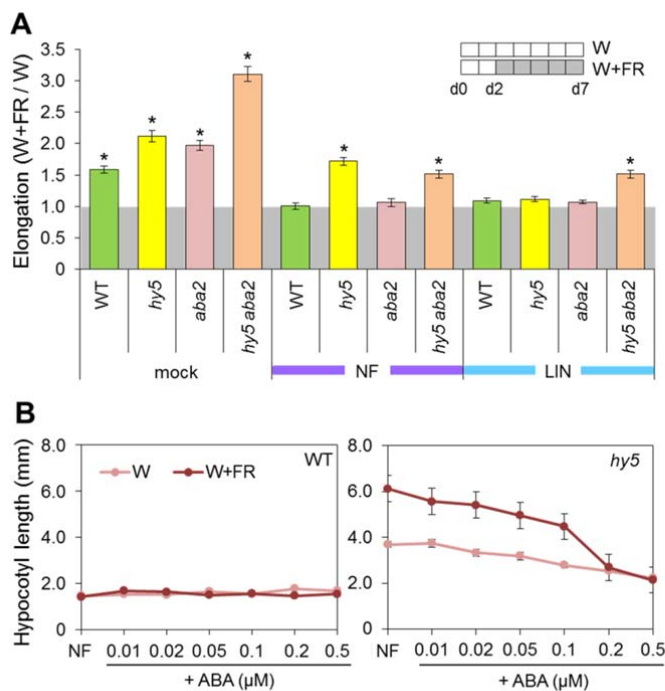


Figure 8. ABA represses shade-triggered hypocotyl elongation independent of HY5. (A) Elongation responses to shade in WT and mutant plants defective in HY5, ABA2, or both. Plants were germinated and grown as indicated with or without 5  $\mu$ M NF or 1 mM LIN. Graph represents the mean and SEM values of a total of nh25 seedlings from at least two independent experiments. Asterisks mark statistically significant responses to shade (T-test,  $p < 0.05$ ). (B) Effect of ABA on the elongation of NF-treated seedling hypocotyls in response to shade. WT and *hy5* plants germinated with or without 5  $\mu$ M NF plus the indicated concentrations of ABA were grown under W for 2 days followed by 5 additional days under W or under W+FR. Graphs represent hypocotyl length (mean and SEM) of nh25 seedlings in a representative experiment.

385 induce ABA production and signaling in tomato and Arabidopsis (Cagnola et al.,  
 386 2012; Gonzalez-Grandio et al., 2013; Holalu and Finlayson, 2017). Indeed, ABA  
 387 contents were also found to slightly increase in green seedlings soon (1h) after  
 388 exposure to our simulated canopy shade conditions, even though the change  
 389 was not statistically significant (Figure S6). As expected, ABA was absent in NF-  
 390 treated seedlings but it could be detected in LIN-grown seedlings (Figure S6). If  
 391 the presence of ABA in LIN-treated *hy5* seedlings contributed to inhibit their  
 392 response to shade, it would be expected that preventing the formation of this  
 393 hormone would be sufficient to rescue their response to shade. In agreement, a  
 394 genetic blockage of the last step of ABA biosynthesis, catalyzed by the ABA2  
 395 protein (Figure 7A), allowed LIN-treated double *hy5 aba2* seedlings to elongate in  
 396 response to shade (Figure 8). Single *hy5* and double *hy5 aba2* seedlings had a  
 397 very similar response to shade in NF-supplemented medium. By contrast, in  
 398 green seedlings grown in the absence of inhibitors (i.e. with functional  
 399 chloroplasts) the double mutant elongated more than single *hy5* seedlings when  
 400 exposed to shade (Figure 8A). We therefore concluded that HY5 and ABA likely  
 401 repress shade-induced hypocotyl elongation by independent pathways. This  
 402 conclusion was confirmed by treating NF-grown WT and *hy5* seedlings with  
 403 increasing concentrations of ABA (Figure 8B). While no effect was observed in



404 the WT, the ability of *hy5* seedlings to elongate in response to W+FR exposure  
405 was progressively repressed as ABA concentration increased. At concentrations  
406 of the hormone of 200 nM or higher, which are within the physiological range  
407 (Waadt et al., 2014), NF-treated *hy5* seedlings did not respond to shade (Figure  
408 8B), similar to that observed with the LIN treatment.

409 Exogenous ABA treatment was also able to repress shade-promoted  
410 hypocotyl elongation in green WT seedlings grown without inhibitors (Figure 9).  
411 We next used this phenotype to identify ABA-related transcription factors  
412 involved in this response. Mutants defective in ABI3 and ABI4 elongated slightly  
413 more than WT seedlings when illuminated with W+FR and this response was not  
414 repressed by ABA. By contrast, ABI5-defective seedlings showed a WT  
415 phenotype in terms of sensitivity of shade-triggered elongation to ABA treatment  
416 (Figure 9A). These results suggest that ABI3 and ABI4 but not ABI5 are required  
417 for ABA to inhibit hypocotyl elongation. If these transcription factors also  
418 transduce the ABA signal in the molecular pathway that blocks elongation in  
419 shade-exposed albino seedlings, it would be expected that double mutants  
420 lacking both HY5 and ABI3 or ABI4 and grown in the presence of LIN would be  
421 able to elongate when exposed to W+FR. Indeed, these double mutants  
422 elongated more than their parental lines when both mock (green) and LIN-treated  
423 (albino) seedlings were grown under simulated shade (Figure 9B). Shade-  
424 triggered elongation of LIN-treated *hy5 abi3* and *hy5 abi4* seedlings, however,  
425 was reduced compared to that of ABA-defective *hy5 aba2* seedlings (Figure 9B).  
426 These results suggest that ABI3 and ABI4 might not participate in the same ABA  
427 signaling pathway eventually repressing hypocotyl elongation but have partially  
428 redundant roles in this process (Figure 10).

429

### 430 **A mechanistic model for the modulation of shade elongation responses by** 431 **plastid-dependent signals.**

432 A model generated based on the described results is shown in Figure 10. In  
433 high plant density environments, like those found in forests, prairies or orchard  
434 communities, a set of R/FR-dependent adaptive responses are unleashed in  
435 shade-avoiding plants. Compared to plant proximity (without direct vegetative  
436 shading), canopy shade in nature involves lower R/FR values associated with a  
437 reduction in the amount of PAR. Although phyB is the major phytochrome

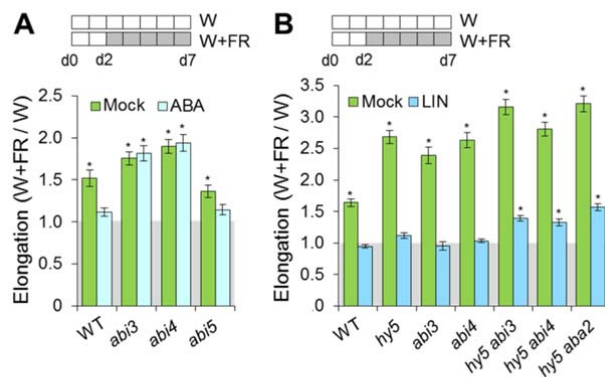


Figure 9. ABI3 and ABI4 but not ABI5 participate in the ABA-mediated repression of shade-induced hypocotyl elongation. (A) Effect of ABA on the elongation responses to shade of WT seedlings and mutants defective in ABI3, ABI4 or ABI5. Plants were germinated and grown as indicated on media with or without 0.2  $\mu$ M ABA. Graph represents the mean and SEM values of a total of n $\approx$ 25 seedlings from two independent experiments. (B) Elongation responses to shade of double mutants defective in HY5 and either ABI3 or ABI4. Plants of the indicated genotypes were germinated and grown as illustrated with or without 1 mM LIN. Graph represents the mean and SEM

values of a total of n $\approx$ 25 seedlings from two independent experiments. Asterisks mark statistically significant responses to shade (T-test, p $\leq$ 0.05).

438 controlling these responses, the photolabile phyA has an antagonistic negative  
 439 role in the shade-mediated regulation of hypocotyl elongation (Ciolfi et al., 2013;  
 440 Martinez-Garcia et al., 2014; Wang et al., 2018; Zhang et al., 2018).  
 441 Independently of the PAR level, phyB is deactivated by shade of intermediate,  
 442 low and very low R/FR, whereas phyA signaling is activated by shade of low and  
 443 very low R/FR. As a result, hypocotyl elongation is derepressed under conditions  
 444 mimicking vegetation proximity (a response aimed at overgrowing neighbors for  
 445 optimal light exposure). Under R/FR values typical of canopy shade, however,  
 446 phyA activation prevents seedlings from exhibiting excessive elongation (Figure  
 447 10). Our results reported here and elsewhere (Bou-Torrent et al., 2015) suggest  
 448 that HY5 represses the hypocotyl elongation response more strongly under  
 449 canopy shade. As previously proposed, HY5 might be principally involved in the  
 450 phyA-dependent pathway (Ciolfi et al., 2013; Wang et al., 2018; Zhang et al.,  
 451 2018) whereas other transcription factors, including growth-promoting PIFs,  
 452 would be mostly associated to the phyB-dependent pathway (Figure 10). These  
 453 antagonistic phyB/PIFs and phyA/HY5 pathways likely provide young seedlings  
 454 with the capacity to rapidly elongate when impending competition is nearby but  
 455 also to attenuate excessive growth when growing under a canopy.

456 During seedling deetiolation, the phyB/PIFs pathway converges with a  
 457 GUN1-dependent retrograde pathway to antagonistically regulate the  
 458 transcriptional photomorphogenic network (Martin et al., 2016). The GUN1-  
 459 mediated retrograde signal involved in this particular process was proposed to  
 460 attenuate photomorphogenesis when chloroplast function is challenged and to be

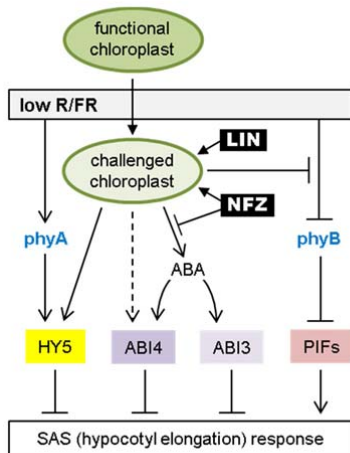


Figure 10. Model for the modulation of shade elongation responses by retrograde signals. In green plants with functional chloroplasts, low R/FR (i.e. canopy shade) signals promote accumulation of growth-promoting PIFs (via phyB deactivation) but also of growth-repressing HY5 (via phyA), likely to prevent an excessive elongation response. Persistent shading or other environmental factors challenging chloroplast function (including exogenous treatment with LIN or NF) can repress phyB inactivation, enhance HY5 expression, and likely promote HY5 stability, eventually resulting in decreased elongation growth. An independent pathway involves ABA, a carotenoid-derived hormone that represses shade-triggered hypocotyl elongation via ABI3 and ABI4. NF (but not LIN) prevents the production of ABA. As a result, loss of both HY5 and ABA in NF-treated *hy5* seedlings allows them to elongate when exposed to low R/FR, whereas this hypocotyl response is blocked by low but detectable levels of ABA in LIN-treated mutants.

461 independent of ABI4 and HY5 (Martin et al., 2016). Our results reported here  
 462 suggest that in shade-exposed seedlings, a completely different retrograde  
 463 network that is independent of GUN1 but does depend on HY5, ABI3 and ABI4  
 464 modulates the antagonistic action of phyA/HY5 and phyB/PIFs signaling  
 465 pathways (Figure 10).

466 Prolonged exposure to shade causes a decrease in the accumulation of  
 467 chlorophylls and carotenoids that can eventually compromise photosynthesis and  
 468 photoprotection (Roig-Villanova et al., 2007; Cagnola et al., 2012; Bou-Torrent et  
 469 al., 2015). Our results suggest that such a challenge to the chloroplast functional  
 470 status might in turn feedback-regulate the response to shade (Figure 10).  
 471 Treatment with low concentrations of NF or LIN (i.e. those causing weak to  
 472 moderate reduction in the level of photosynthetic pigments) was sufficient to  
 473 repress the hypocotyl elongation response to low R/FR (Figure 1), likely due to  
 474 delayed phyB deactivation after a reduction in R/FR (Figure 2). Decreased phyB  
 475 deactivation correlated with impaired PIF accumulation (Figure S2) and  
 476 attenuated gene expression changes (Figure 3). NF or LIN treatments also  
 477 caused an enhanced accumulation of *HY5* transcripts and increased the stability  
 478 of the HY5-GFP reporter protein (Figure 6). Together, our findings suggest that  
 479 retrograde signals inhibit the SAS by repressing the (positive) phyB/PIFs pathway  
 480 and by promoting the (negative) phyA/HY5 pathway (Figure 10).

481 Our work further unveiled ABA as another component of the feedback  
 482 mechanism. This carotenoid-derived hormone was found to repress shade-  
 483 triggered hypocotyl elongation (Figure 8), likely through the action of the  
 484 transcription factors ABI3 and ABI4 (Figure 9). ABI4 has been proposed to

485 participate in GUN1-dependent retrograde signaling (Koussevitzky et al., 2007;  
486 Sun et al., 2011; Guo et al., 2016; Xu et al., 2016). However, the results  
487 supporting this claim have been repeatedly challenged (Kacprzak et al., 2018).  
488 Our data suggest that ABI4 (and ABI3) may act redundantly to transduce the  
489 ABA-dependent signal that represses shade-triggered hypocotyl elongation in  
490 response to chloroplast dysfunction (Figure 9). While HY5 was previously shown  
491 to directly bind and activate the promoter of *ABI5* to promote light-induced  
492 hypocotyl inhibition during deetiolation (Chen et al., 2008; Xu et al., 2014), our  
493 results suggest that this mechanism does not participate in the control of shade-  
494 dependent hypocotyl growth. First, HY5 and ABA appear to repress hypocotyl  
495 growth by independent pathways (Figure 8). And second, *ABI5* is not required to  
496 transduce the ABA signal eventually repressing the response to shade (Figure 9).

497 Arabidopsis mutants defective in *phyB* were found to accumulate greater  
498 amounts of ABA under well-watered conditions and to be less sensitive to  
499 exogenous ABA treatments (Gonzalez et al., 2012). Further supporting a  
500 negative role of light for ABA synthesis, dark treatment of previously light-grown  
501 plants resulted in increased ABA contents (Weatherwax et al., 1996). A shade-  
502 triggered increase in ABA production was reported here (Figure S6) and  
503 elsewhere (Cagnola et al., 2012; Gonzalez-Grandio et al., 2013; Holalu and  
504 Finlayson, 2017). It is possible that W+FR treatment might promote ABA  
505 production to repress the elongation response to shade as part of the mechanism  
506 that prevents a too intense commitment (Figure 10). These results together  
507 support ABA as a central signal connecting the functional status of the  
508 chloroplast with light responses. Interestingly, the plastid-synthesized metabolite  
509 3'-phosphoadenosine 5'-phosphate (PAP), which functions as a retrograde signal  
510 during oxidative stress caused by high light exposure and drought, was recently  
511 shown to act in concert with ABA signaling in guard cells to mediate stomatal  
512 closure and in seeds to mediate dormancy and germination (Pornsiriwong et al.,  
513 2017). PAP accumulates when the *SAL1* phosphatase that normally degrades  
514 this metabolite is inactivated during oxidative stress (Estavillo et al., 2011). *SAL1*-  
515 defective mutants show a short hypocotyl phenotype in the light, indicating that  
516 accumulation of PAP can repress hypocotyl elongation (Kim and von Arnim,  
517 2009; Chen and Xiong, 2011). This phenotype is rescued (at least partially) in  
518 double *sal1 phyB* and *sal1 hy5* mutants (Kim and von Arnim, 2009; Chen and

519 Xiong, 2011), suggesting that functional phyB and HY5 are required for the PAP-  
520 promoted and light-dependent repression of hypocotyl growth. Further  
521 experiments should explore whether PAP is the retrograde signal deduced from  
522 our data to attenuate the response to shade in terms of hypocotyl elongation by  
523 independently inhibiting phyB deactivation, increasing HY5 accumulation, and  
524 promoting ABA signaling (Figure 10).

525 Besides ABA, it is possible that other carotenoid-derived products might  
526 also contribute to the repression of shade-triggered hypocotyl elongation  
527 detected in *hy5* seedlings bleached with LIN, CAP or PPT but not with NF or  
528 CPTA (Figure 7). In particular, strigolactones are hormones derived from  $\beta$ -  
529 carotene (Figure 7A) that inhibit hypocotyl elongation in the light by a mechanism  
530 requiring phytochromes and involving upregulation of *HY5* expression and  
531 protein (Tsuchiya et al., 2010; Jia et al., 2014). Other metabolites produced after  
532 cleavage of carotenoids include  $\beta$ -cyclocitral, and unknown compounds that  
533 modulate developmental and stress responses (Hou et al., 2016). While  $\beta$ -  
534 cyclocitral is a relatively well-established retrograde signal associated to oxidative  
535 stress (Ramel et al., 2012), its contribution to hypocotyl elongation is unknown.  
536 Similarly, no hypocotyl growth alterations have been reported in mutants lacking  
537 carotenoid-derived signals that do have an impact on leaf development (van  
538 Norman et al., 2007; Avendaño-Vazquez et al., 2014). Whether any of these  
539 carotenoid-related metabolites participate in the elongation response to shade  
540 remains to be investigated.

541 Collectively, our data support the notion that chloroplasts are plant cell  
542 compartments with fundamental roles not only for photosynthesis and  
543 metabolism but also for environmental (light) sensing and signaling. Here we  
544 show that HY5 and ABA (via ABI3 and ABI4) are nodes of a plastid-modulated  
545 network that attenuates the response to shade in terms of hypocotyl elongation.  
546 In green plants with functional chloroplasts, light signals associated with canopy  
547 shade rapidly promote hypocotyl elongation via the phyB/PIFs pathway.  
548 Exposure to low R/FR also triggers negative (growth-repressing) circuits involving  
549 the phyA/HY5 pathway and the carotenoid-derived hormone ABA, likely to  
550 prevent an excessive response and facilitate the return to non-shade conditions if  
551 the low R:FR signal disappears (e.g. if a commitment to the shade-avoidance  
552 lifestyle is unnecessary). When maintained, shade further causes a decrease of

553 chlorophyll and carotenoid contents which might eventually disrupt chloroplast  
554 homeostasis. Such situation would be then signaled to feedback-regulate the  
555 response to the light signal by independently inhibiting phyB deactivation,  
556 increasing HY5 accumulation, and promoting ABA signaling. This mechanism  
557 connecting the metabolic status of the chloroplast with light (shade) signaling and  
558 developmental responses likely contributes to achieve optimal photosynthetic  
559 performance.

560

## 561 **MATERIALS AND METHODS**

### 562 **Plant material**

563 All mutants used in this work are listed in Table S1. *Arabidopsis thaliana* lines  
564 used here were in the Columbia (Col) background with the only exception of *hy1-*  
565 *1*, a Landsberg *erecta* (Ler) mutant (Rodriguez-Concepcion et al., 2004). Some  
566 of those lines were already available in our lab and previously used in published  
567 works, including *hdr-3* (Pokhilko et al., 2015), *gun1-101* (Llamas et al., 2017),  
568 *gun5-1* (Llamas et al., 2017), *pifQ* (Toledo-Ortiz et al., 2010), *hy5-2* (Bou-Torrent  
569 et al., 2015), *psy-1* (Pokhilko et al., 2015), *lut2* (Emiliani et al., 2018), *aba2* (Ruiz-  
570 Sola et al., 2014), and *hy5 35S:HA-HY5* (Toledo-Ortiz et al., 2014). Lines *abi3-8*  
571 (Nambara et al., 2002), *abi4-1* (Finkelstein et al., 1998), *abi5-7* (Tamura et al.,  
572 2006), and *35S:GUS-PIF3* (Monte et al., 2004) were requested. For generation of  
573 double mutants, single homozygous plants were crossed and the F2 progeny  
574 was first screened for the characteristic long hypocotyl phenotype associated to  
575 the *hy5* mutation in homozygosis. Long individuals were then PCR-genotyped to  
576 identify homozygous mutants for the second gene and confirm that they were  
577 also homozygous for *hy5*. For the generation of the *35S:HY5-GFP* construct, the  
578 full coding region of the Arabidopsis *HY5* cDNA was PCR-amplified using primers  
579 HY5-attB1-F and HY5-attB2-R (Table S2) and cloned into Gateway pDONR-207.  
580 Cloning into Gateway pGWB405 eventually generated the construct for the 35S  
581 promoter-driven expression of a C-terminal fusion of the sGFP reporter protein to  
582 HY5. This construct was used to transform the *hy5-2* mutant by floral dipping.  
583 The *hy5 35S:HY5-GFP* line used for the experiments reported here was selected  
584 based on complete complementation of the long hypocotyl phenotype associated  
585 with the *hy5* mutation and high levels of nuclear GFP fluorescence. Line

586 *35S:PHYB-GFP* was generated by transforming Col-0 plants with the same  
587 construct previously found to work in an *Arabidopsis phyB* mutant in the Ler  
588 background (Yamaguchi et al., 1999). From the resulting transformants, we  
589 selected for further experiments one of the lines showing a clearer accumulation  
590 of the phyB-GFP protein in nuclear bodies under W.

591

### 592 **Growth conditions and treatments**

593 Seeds were surface-sterilized and germinated on solid Murashige and Skoog  
594 (MS) medium supplemented with 10 mg/ml of sucrose to provide carbon and  
595 energy for albino seedlings to grow. When indicated, the medium was further  
596 supplemented with different concentrations of norflurazon (NF, Zorial), lincomycin  
597 (LIN, Sigma) or abscisic acid (ABA, Sigma). Other chemicals added to the  
598 medium included epibrassinolide (1  $\mu\text{M}$ ), gibberellic acid (10  $\mu\text{M}$ ), picloram (5  
599  $\mu\text{M}$ ), 2-(4-chlorophenylthio)-triethylamine chloride (25 $\mu\text{M}$ ), fosmidomycin (500  
600  $\mu\text{M}$ ), chloramphenicol (50  $\mu\text{M}$ ), or phosphinotricin (100  $\mu\text{M}$ ). When comparing  
601 different lines (e.g. WT vs. mutant), they were grown together on the same plate  
602 instead of growing each line on a different plate. After stratification for at least 3  
603 days at 4°C in the dark, plates were incubated in growth chambers at 22°C under  
604 W of 20-24  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR (R/FR = 1.6). When indicated, W was  
605 supplemented with FR provided by GreenPower LED module HF far-red (Philips)  
606 QB1310CS-670-735 light-emitting diode hybrid lamps (Quantum Devices) to  
607 simulate canopy shade (20-24  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR, R/FR = 0.02). Fluence rates  
608 were measured using a Spectrosense 2 meter associated with a 4-channel  
609 sensor (Skye Instruments Ltd.) as described (Martinez-Garcia et al., 2014).  
610 Grown seedlings were laid out flat on the growth media and digital images were  
611 taken to quantify hypocotyl length using the NIH ImageJ software.

612

### 613 **Microscopy**

614 Whole *35S:PHYB-GFP* seedlings germinated and grown under W for 7 days on  
615 media with or without 5  $\mu\text{M}$  NF or 1 mM LIN were exposed to a 5 min pulse of FR  
616 (735 nm, 60  $\mu\text{mol/m}^2/\text{s}$ ) and then kept in the dark. At different timepoints, treated  
617 seedlings were placed on glass slides under a safety green light and kept in the  
618 dark until observation with an Olympus BX60 FLUOVIEW FV300 microscope.  
619 Confocal laser scan images of the hypocotyl area closer to the cotyledons were

620 obtained at different timepoints in the dark with a combination of 488 nm laser  
621 excitation and 515 nm longpass filter (LP515; Carl Zeiss Jena). For each  
622 timepoint, three sequential images from different focus planes were recorded  
623 automatically.

624

### 625 **Chromatin immunoprecipitation**

626 About 800 µl of seeds from *hy5 35S:HY5-GFP* plants were plated on 8 square  
627 (10 cm x 10 cm) plates of sucrose-supplemented medium. After growth for 2  
628 days under W, 4 plates were left under W and 4 were transferred to W+FR for 5  
629 additional days. For chromatin immunoprecipitation (Moon et al., 2008), each  
630 sample was divided in 3 aliquots after crosslinking and sonication: one input, one  
631 to be incubated with a 1:1000 dilution of anti-GFP antibody (Life Technologies),  
632 and the last one to be processed similarly but without antibody. After DNA  
633 isolation, the three samples were used for qPCR analysis of promoter sequence  
634 abundance with the primers shown in Table S2. After normalization with the  
635 input, enrichment was calculated as the ratio of the signal with vs. without  
636 antibody.

637

### 638 **Gene expression and immunoblot analyses**

639 Total RNA was extracted from whole seedlings and used for qPCR analysis as  
640 described (Llamas et al., 2017) with the gene-specific primers listed in Table S2.  
641 Protein extraction, immunoblot analysis, and quantification of protein abundance  
642 were performed as described (Llamas et al., 2017) using a 1:1000 dilution of anti-  
643 GFP serum (Life Technologies).

644

### 645 **Quantification of metabolite levels**

646 Whole seedlings were frozen in liquid nitrogen, lyophilized, and ground in a  
647 mortar for extraction and quantification of photosynthetic pigments and ABA.  
648 Chlorophyll and carotenoid levels were measured either by spectrometric  
649 methods or by HPLC (Bou-Torrent et al., 2015). ABA content was quantified by  
650 LC/ESI-MS/MS as described (Ruiz-Sola et al., 2014).



**651 ACKNOWLEDGEMENTS**

652 We thank Elena Monte (CRAG) for comments on the manuscript. Technical  
653 support from M. Rosa Rodríguez-Goberna and members of the CRAG core  
654 facilities is greatly appreciated. This work was funded by grants BIO2014-59895-  
655 P, BIO2014-59092-P, BIO2015-71703-REDT, BIO2017-90877-REDT, BIO2017-  
656 85316-R and BIO2017-84041-P from the Spanish Ministry of Science,  
657 Innovation, and Universities (MICINN) and grants 2017SGR-1211 and 2017SGR-  
658 710 from Generalitat de Catalunya to JFMG and MRC. Funding from the Japan  
659 Society for the Promotion of Science (JSPS) KAKENHI grant JP-15H04389 to AN  
660 is also acknowledged. We thank the financial support of the MINECO Severo  
661 Ochoa Programme for Centres of Excellence in R&D 2016-2019 (SEV-2015-  
662 0533) and the Generalitat de Catalunya CERCA Programme to CRAG. MOA and  
663 EL were supported by PhD fellowships from Spanish MINECO (BES-2012-  
664 052597) and Mexican CONACYT (421688 and SEP “beca complemento”),  
665 respectively.

666

**667 AUTHOR CONTRIBUTIONS**

668 MOA, JFMG and MRC designed the research; MOA, EL, and AGC performed  
669 research; AN contributed analytic tools; MOA, EL, AN, JFMG and MRC analyzed  
670 data; JFMG and MRC wrote the paper.



## Parsed Citations

**Avendaño-Vázquez, A.O., Cordoba, E., Llamas, E., San Román, C., Nisar, N., De la Torre, S., Ramos-Vega, M., Gutiérrez-Nava, M.D., Cazzonelli, C.I., Pogson, B.J., and León P. (2014). An Uncharacterized Apocarotenoid-Derived Signal Generated in  $\zeta$ -Carotene Desaturase Mutants Regulates Leaf Development and the Expression of Chloroplast and Nuclear Genes in Arabidopsis. *The Plant cell* 26, 2524-2537.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Baier, M., and Dietz, K.J. (2005). Chloroplasts as source and target of cellular redox regulation: a discussion on chloroplast redox signals in the context of plant physiology. *Journal of experimental botany* 56, 1449-1462.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Bou-Torrent, J., Toledo-Ortiz, G., Ortiz-Alcaide, M., Cifuentes-Esquivel, N., Halliday, K.J., Martinez-Garcia, J.F., and Rodriguez-Concepcion, M. (2015). Regulation of Carotenoid Biosynthesis by Shade Relies on Specific Subsets of Antagonistic Transcription Factors and Cofactors. *Plant physiology* 169, 1584-1594.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Cagnola, J.I., Ploschuk, E., Benech-Arnold, T., Finlayson, S.A., and Casal, J.J. (2012). Stem transcriptome reveals mechanisms to reduce the energetic cost of shade-avoidance responses in tomato. *Plant physiology* 160, 1110-1119.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Casal, J.J. (2012). Shade avoidance. *Arabidopsis Book* 10, e0157.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Casal, J.J. (2013). Photoreceptor signaling networks in plant responses to shade. *Annual review of plant biology* 64, 403-427.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Cioffi, A., Sessa, G., Sassi, M., Possenti, M., Salvucci, S., Carabelli, M., Morelli, G., and Ruberti, I. (2013). Dynamics of the Shade Avoidance Response in *Arabidopsis thaliana*. *Plant physiology*.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Chamovitz, D., Pecker, I., and Hirschberg, J. (1991). The molecular basis of resistance to the herbicide norflurazon. *Plant molecular biology* 16, 967-974.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Chan, K.X., Phua, S.Y., Crisp, P., McQuinn, R., and Pogson, B.J. (2016). Learning the Languages of the Chloroplast: Retrograde Signaling and Beyond. *Annual review of plant biology* 67, 25-53.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Chen, H., Zhang, J., Neff, M.M., Hong, S.W., Zhang, H., Deng, X.W., and Xiong, L. (2008). Integration of light and abscisic acid signaling during seed germination and early seedling development. *Proceedings of the National Academy of Sciences of the United States of America* 105, 4495-4500.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Chen, H. and Xiong, L. (2011). Genetic interaction of two abscisic acid signaling regulators, HY5 and FIERY1, in mediating lateral root formation. *Plant signaling and behavior* 6, 123-125.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Davis, S.J., Kurepa, J., and Vierstra, R.D. (1999). The *Arabidopsis thaliana* HY1 locus, required for phytochrome-chromophore biosynthesis, encodes a protein related to heme oxygenases. *Proceedings of the National Academy of Sciences of the United States of America* 96, 6541-6546.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Emiliani, J., D'Andrea, L., Lorena Falcone Ferreyra, M., Maulion, E., Jose Rodriguez, E., Rodriguez-Concepcion, M., and Casati, P. (2018). A role for beta,beta-xanthophylls in *Arabidopsis* UV-B photoprotection. *Journal of experimental botany* 69, 4921-4933.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Estavillo, G.M., Crisp, P.A., Pornsiriwong, W., Wirtz, M., Collinge, D., Carrie, C., Giraud, E., Whelan, J., David, P., Javot, H., Brearley, C., Hell, R., Marin, E., and Pogson, B.J. (2011). Evidence for a SAL1-PAP chloroplast retrograde pathway that functions in drought and high light signaling in *Arabidopsis*. *The Plant cell* 23, 3992-4012.**

- Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Finkelstein, R.R., Wang, M.L., Lynch, T.J., Rao, S., and Goodman, H.M. (1998).** The Arabidopsis abscisic acid response locus *ABI4* encodes an *APETALA2* domain protein. *The Plant cell* 10, 1043-1054.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Fiorucci, A.S., and Fankhauser, C. (2017).** Plant Strategies for Enhancing Access to Sunlight. *Curr Biol* 27, R931-R940.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Franklin, K.A. (2008).** Shade avoidance. *The New phytologist* 179, 930-944.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Glasser, C., Haberer, G., Finkemeier, I., Pfannschmidt, T., Kleine, T., Leister, D., Dietz, K.J., Hausler, R.E., Grimm, B., and Mayer, K.F. (2014).** Meta-analysis of retrograde signaling in *Arabidopsis thaliana* reveals a core module of genes embedded in complex cellular signaling networks. *Molecular plant* 7, 1167-1190.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Gommers, C.M., Visser, E.J., St Onge, K.R., Voesenek, L.A., and Pierik, R. (2013).** Shade tolerance: when growing tall is not an option. *Trends in plant science* 18, 65-71.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Gonzalez-Grandio, E., Poza-Carrion, C., Sorzano, C.O., and Cubas, P. (2013).** *BRANCHED1* promotes axillary bud dormancy in response to shade in *Arabidopsis*. *The Plant cell* 25, 834-850.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Gonzalez, C.V., Ibarra, S.E., Piccoli, P.N., Botto, J.F., and Boccalandro, H.E. (2012).** Phytochrome B increases drought tolerance by enhancing ABA sensitivity in *Arabidopsis thaliana*. *Plant, cell & environment* 35, 1958-1968.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Guo, H., Feng, P., Chi, W., Sun, X., Xu, X., Li, Y., Ren, D., Lu, C., David Rochaix, J., Leister, D., and Zhang, L. (2016).** Plastid-nucleus communication involves calcium-modulated MAPK signalling. *Nature communications* 7, 12173.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Holalu, S.V., and Finlayson, S.A. (2017).** The ratio of red light to far red light alters *Arabidopsis* axillary bud growth and abscisic acid signalling before stem auxin changes. *Journal of experimental botany* 68, 943-952.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Hou, X., Rivers, J., Leon, P., McQuinn, R.P., and Pogson, B.J. (2016).** Synthesis and Function of Apocarotenoid Signals in Plants. *Trends in plant science* 21, 792-803.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Humplik, J.F., Bergougnoux, V., and Van Volkenburgh, E. (2017).** To Stimulate or Inhibit? That Is the Question for the Function of Abscisic Acid. *Trends in plant science* 22, 830-841.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Jia, K.P., Luo, Q., He, S.B., Lu, X.D., and Yang, H.Q. (2014).** Strigolactone-regulated hypocotyl elongation is dependent on cryptochrome and phytochrome signaling pathways in *Arabidopsis*. *Molecular plant* 7, 528-540.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Kacprzak, S.M., Mochizuki, N., Naranjo, B., Xu, D., Leister, D., Kleine, T., Okamoto, H., and Terry, M.J. (2018).** Plastid-to-nucleus retrograde signalling during chloroplast biogenesis does not require *ABI4*. *Plant physiology*. pii: pp.01047.2018. doi: 10.1104/pp.18.01047  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Kim, B.H. and von Arnim, A.G. (2009).** *FIERY1* regulates light-mediated repression of cell elongation and flowering time via its 3'(2'),5'-bisphosphate nucleotidase activity. *The Plant journal : for cell and molecular biology* 58, 208-219.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Koussevitzky, S., Nott, A., Mockler, T.C., Hong, F., Sachetto-Martins, G., Surpin, M., Lim, J., Mittler, R., and Chory, J. (2007).** Signals from chloroplasts converge to regulate nuclear gene expression. *Science* 316, 715-719.

- Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Larkin, R.M., and Ruckle, M.E. (2008).** Integration of light and plastid signals. *Current opinion in plant biology* 11, 593-599.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Lau, O.S., and Deng, X.W. (2010).** Plant hormone signaling lightens up: integrators of light and hormones. *Current opinion in plant biology* 13, 571-577.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Leivar, P., Tepperman, J.M., Cohn, M.M., Monte, E., Al-Sady, B., Erickson, E., and Quail, P.H. (2012).** Dynamic antagonism between phytochromes and PIF family basic helix-loop-helix factors induces selective reciprocal responses to light and shade in a rapidly responsive transcriptional network in *Arabidopsis*. *The Plant cell* 24, 1398-1419.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Lepisto, A., and Rintamaki, E. (2012).** Coordination of plastid and light signaling pathways upon development of *Arabidopsis* leaves under various photoperiods. *Molecular plant* 5, 799-816.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Lorrain, S., Allen, T., Duek, P.D., Whitlam, G.C., and Fankhauser, C. (2008).** Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. *The Plant journal : for cell and molecular biology* 53, 312-323.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Llamas, E., Pulido, P., and Rodriguez-Concepcion, M. (2017).** Interference with plastome gene expression and Clp protease activity in *Arabidopsis* triggers a chloroplast unfolded protein response to restore protein homeostasis. *PLoS genetics* 13, e1007022.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Llorente, B., Martinez-Garcia, J.F., Stange, C., and Rodriguez-Concepcion, M. (2017).** Illuminating colors: regulation of carotenoid biosynthesis and accumulation by light. *Current opinion in plant biology* 37, 49-55.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Martin, G., Leivar, P., Ludevid, D., Tepperman, J.M., Quail, P.H., and Monte, E. (2016).** Phytochrome and retrograde signalling pathways converge to antagonistically regulate a light-induced transcriptional network. *Nature communications* 7, 11431.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Martinez-Garcia, J.F., Gallemi, M., Molina-Contreras, M.J., Llorente, B., Bevilacqua, M.R., and Quail, P.H. (2014).** The shade avoidance syndrome in *Arabidopsis*: the antagonistic role of phytochrome a and B differentiates vegetation proximity and canopy shade. *PLoS one* 9, e109275.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Mochizuki, N., Brusslan, J.A., Larkin, R., Nagatani, A., and Chory, J. (2001).** *Arabidopsis* genomes uncoupled 5 (GUN5) mutant reveals the involvement of Mg-chelatase H subunit in plastid-to-nucleus signal transduction. *Proceedings of the National Academy of Sciences of the United States of America* 98, 2053-2058.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Monte, E., Tepperman, J.M., Al-Sady, B., Kaczorowski, K.A., Alonso, J.M., Ecker, J.R., Li, X., Zhang, Y., and Quail, P.H. (2004).** The phytochrome-interacting transcription factor, PIF3, acts early, selectively, and positively in light-induced chloroplast development. *Proceedings of the National Academy of Sciences of the United States of America* 101, 16091-16098.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Moon, J., Zhu, L., Shen, H., and Huq, E. (2008).** PIF1 directly and indirectly regulates chlorophyll biosynthesis to optimize the greening process in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America* 105, 9433-9438.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Mulo, P., Pursiheimo, S., Hou, C.X., Tyystjarvi, T., and Aro, E.M. (2003).** Multiple effects of antibiotics on chloroplast and nuclear gene expression. *Funct Plant Biol* 30, 1097-1103.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)
- Nambara, E., Suzuki, M., Abrams, S., McCarty, D.R., Kamiya, Y., and McCourt, P. (2002).** A screen for genes that function in abscisic acid signaling in *Arabidopsis thaliana*. *Genetics* 161, 1247-1255.  
Pubmed: [Author and Title](#)  
Google Scholar: [Author Only Title Only Author and Title](#)

Pacin, M., Semmoloni, M., Legris, M., Finlayson, S.A., and Casal, J.J. (2016). Convergence of CONSTITUTIVE PHOTOMORPHOGENESIS 1 and PHYTOCHROME INTERACTING FACTOR signalling during shade avoidance. *The New phytologist* 211, 967-979.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Parks, B.M., and Quail, P.H. (1991). Phytochrome-Deficient *hy1* and *hy2* Long Hypocotyl Mutants of *Arabidopsis* Are Defective in Phytochrome Chromophore Biosynthesis. *The Plant cell* 3, 1177-1186.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Patel, D., Basu, M., Hayes, S., Majlath, I., Hetherington, F.M., Tschaplinski, T.J., and Franklin, K.A. (2013). Temperature-dependent shade avoidance involves the receptor-like kinase ERECTA. *The Plant journal : for cell and molecular biology* 73, 980-992.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Pornsiriwong, W., Estavillo, G.M., Chan, K.X., Tee, E.E., Ganguly, D., Crisp, P.A., Phua, S.Y., Zhao, C., Qiu, J., Park, J., Yong, M.T., Nisar, N., Yadav, A.K., Schwessinger, B., Rathjen, J., Cazzonelli, C.I., Wilson, P.B., Gilliam, M., Chen, Z.H., and Pogson, B.J. (2017). A chloroplast retrograde signal, 3'-phosphoadenosine 5'-phosphate, acts as a secondary messenger in abscisic acid signaling in stomatal closure and germination. *Elife* 6, e23361.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Pokhilko, A., Bou-Torrent, J., Pulido, P., Rodriguez-Concepcion, M., and Ebenhoeh, O. (2015). Mathematical modelling of the diurnal regulation of the MEP pathway in *Arabidopsis*. *The New phytologist* 206, 1075-1085.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ramel F, Birtic S, Ginies C, Soubigou-Taconnat L, Triantaphylidès C, and Havaux M. (2012). Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. *Proceedings of the National Academy of Sciences of the United States of America* 109, 5535-5540.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Rockwell, N.C., Su, Y.S., and Lagarias, J.C. (2006) Phytochrome structure and signaling mechanisms. *Annual Review of Plant Biology* 57, 837-858.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Rodriguez-Concepcion, M., Fores, O., Martinez-Garcia, J.F., Gonzalez, V., Phillips, M.A, Ferrer, A, and Boronat, A (2004). Distinct light-mediated pathways regulate the biosynthesis and exchange of isoprenoid precursors during *Arabidopsis* seedling development. *The Plant cell* 16, 144-156.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Roig-Villanova, I., Bou, J., Sorin, C., Devlin, P.F., and Martinez-Garcia, J.F. (2006). Identification of primary target genes of phytochrome signaling. Early transcriptional control during shade avoidance responses in *Arabidopsis*. *Plant physiology* 141, 85-96.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Roig-Villanova, I., Bou-Torrent, J., Galstyan, A, Carretero-Paulet, L., Portoles, S., Rodriguez-Concepcion, M., and Martinez-Garcia, J.F. (2007). Interaction of shade avoidance and auxin responses: a role for two novel atypical bHLH proteins. *EMBO J* 26, 4756-4767.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ruckle, M.E., and Larkin, R.M. (2009). Plastid signals that affect photomorphogenesis in *Arabidopsis thaliana* are dependent on GENOMES UNCOUPLED 1 and cryptochrome 1. *The New phytologist* 182, 367-379.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ruckle, M.E., DeMarco, S.M., and Larkin, R.M. (2007). Plastid signals remodel light signaling networks and are essential for efficient chloroplast biogenesis in *Arabidopsis*. *The Plant cell* 19, 3944-3960.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ruckle, M.E., Burgoon, L.D., Lawrence, L.A, Sinkler, C.A, and Larkin, R.M. (2012). Plastids are major regulators of light signaling in *Arabidopsis*. *Plant physiology* 159, 366-390.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ruiz-Sola, M.A, and Rodriguez-Concepcion, M. (2012). Carotenoid biosynthesis in *Arabidopsis*: a colorful pathway. *Arabidopsis Book* 10, e0158.

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Ruiz-Sola, M.A., Arbona, V., Gomez-Cadenas, A., Rodriguez-Concepcion, M., and Rodriguez-Villalon, A. (2014). A root specific induction of carotenoid biosynthesis contributes to ABA production upon salt stress in arabidopsis. PLoS one 9, e90765.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Saini, G., Meskauskiene, R., Pijacka, W., Roszak, P., Sjögren, L.L., Clarke, A.K., Straus, M., and Apel, K. (2011). 'happy on norflurazon' (hon) mutations implicate perturbation of plastid homeostasis with activating stress acclimatization and changing nuclear gene expression in norflurazon-treated seedlings. The Plant journal : for cell and molecular biology 65, 690-702.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Sellaro, R., Yanovsky, M.J., and Casal, J.J. (2011). Repression of shade-avoidance reactions by sunfleck induction of HY5 expression in Arabidopsis. The Plant journal : for cell and molecular biology 68, 919-928.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Sun, X., Feng, P., Xu, X., Guo, H., Ma, J., Chi, W., Lin, R., Lu, C., and Zhang, L. (2011). A chloroplast envelope-bound PHD transcription factor mediates chloroplast signals to the nucleus. Nature communications 2, 477.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Tamura, N., Yoshida, T., Tanaka, A., Sasaki, R., Bando, A., Toh, S., Lepiniec, L., Kawakami, N. (2006). Isolation and characterization of high temperature-resistant germination mutants of Arabidopsis thaliana. Plant cell and physiology 47, 1081-1094.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Toledo-Ortiz, G., Huq, E., and Rodriguez-Concepcion, M. (2010). Direct regulation of phytoene synthase gene expression and carotenoid biosynthesis by phytochrome-interacting factors. Proceedings of the National Academy of Sciences of the United States of America 107, 11626-11631.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Toledo-Ortiz, G., Johansson, H., Lee, K.P., Bou-Torrent, J., Stewart, K., Steel, G., Rodriguez-Concepcion, M., and Halliday, K.J. (2014). The HY5-PIF Regulatory Module Coordinates Light and Temperature Control of Photosynthetic Gene Transcription. PLoS genetics 10, e1004416.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Tsuchiya Y, Vidaurre D, Toh S, Hanada A, Nambara E, Kamiya Y, Yamaguchi S, and McCourt P. (2010). A small-molecule screen identifies new functions for the plant hormone strigolactone. Nature Chemical Biology 6, 741-749.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Van Norman JM, and Sieburth LE. (2007). Dissecting the biosynthetic pathway for the bypass1 root-derived signal. The Plant journal 49, 619-628.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Waadts, R., Hitomi, K., Nishimura, N., Hitomi, C., Adams, S.R., Getzoff, E.D., and Schroeder, J.I. (2014). FRET-based reporters for the direct visualization of abscisic acid concentration changes and distribution in Arabidopsis. Elife 3, e01739.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Wang, F., Wu, N., Zhang, L., Ahammed, G.J., Chen, X., Xiang, X., Zhou, J., Xia, X., Shi, K., Yu, J., Foyer, C.H., and Zhou, Y. (2018). Light Signaling-Dependent Regulation of Photoinhibition and Photoprotection in Tomato. Plant physiology 176, 1311-1326.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Weatherwax, S.C., Ong, M.S., Degenhardt, J., Bray, E.A., and Tobin, E.M. (1996). The interaction of light and abscisic acid in the regulation of plant gene expression. Plant physiology 111, 363-370.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Xiao, Y., Savchenko, T., Baidoo, E.E., Chehab, W.E., Hayden, D.M., Tolstikov, V., Corwin, J.A., Kliebenstein, D.J., Keasling, J.D., and Dehesh, K. (2012). Retrograde signaling by the plastidial metabolite MEcPP regulates expression of nuclear stress-response genes. Cell 149, 1525-1535.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Xu, D., Li, J., Gangappa, S.N., Hettiarachchi, C., Lin, F., Andersson, M.X., Jiang, Y., Deng, X.W., and Holm, M. (2014). Convergence of Light and ABA signaling on the ABI5 promoter. PLoS genetics 10, e1004197.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

---

**Xu, X., Chi, W., Sun, X., Feng, P., Guo, H., Li, J., Lin, R., Lu, C., Wang, H., Leister, D., and Zhang, L. (2016). Convergence of light and chloroplast signals for de-etiolation through ABI4-HY5 and COP1. *Nature plants* 2, 16066.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Yamaguchi, R., Nakamura, M., Mochizuki, N., Kay, S.A., and Nagatani, A. (1999). Light-dependent translocation of a phytochrome B-GFP fusion protein to the nucleus in transgenic *Arabidopsis*. *The Journal of cell biology* 145, 437-445.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

**Zhang, S., Li, C., Zhou, Y., Wang, X., Li, H., Feng, Z., Chen, H., Qin, G., Jin, D., Terzaghi, W., Gu, H., Qu, L.J., Kang, D., Deng, X.W., and Li, J. (2018). TANDEM ZINC-FINGER/PLUS3 Is a Key Component of Phytochrome A Signaling. *The Plant cell* 30, 835-852.**

Pubmed: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)