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# Light triggers the miRNA-biogenetic inconsistency for de-etiolated seedling survivability in *Arabidopsis thaliana*

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**Running Title:** Light triggers the miRNA-biogenetic inconsistency

## SHORT SUMMARY

During the de-etiolation process, light triggers the accumulation of the core microprocessor components and pri-miRNAs, but not miRNAs. Light-induced suppression of DCL1 activity and SDN1-induction modulate this phenomenon, called miRNA-biogenetic inconsistency, and that is essential for the survival of de-etiolated seedlings after long-term skotomorphogenesis.

## 1 **ABSTRACT**

2  
3 The shift of dark-grown seedlings into light causes enormous transcriptome changes  
4 followed by a dramatic developmental transition. Here, we show that miRNA biogenesis also  
5 undergoes regulatory changes during de-etiolation. Etiolated seedlings maintain low levels of  
6 primary-miRNAs (pri-miRNAs) and miRNA processing core proteins, such as Dicer-like 1  
7 (DCL1), SERRATE (SE) and HYPONASTIC LEAVES 1 (HYL1), whereas during de-  
8 etiolation, both pri-miRNAs and the processing components accumulated to high levels.  
9 However, most miRNA levels did not notably increase in response to light. To reconcile this  
10 inconsistency, we demonstrate that an unknown suppressor decreases miRNA-processing  
11 activity and light-induced SMALL RNA DEGRADING NUCLEASE 1 (SDN1) shortens the  
12 half-life of several miRNAs in de-etiolated seedlings. Taken together, we suggest a novel  
13 mechanism, miRNA-biogenetic inconsistency, which accounts for the intricacy of miRNA  
14 biogenesis during de-etiolation. This mechanism is essential for the survival of de-etiolated  
15 seedlings after long-term skotomorphogenesis and their optimal adaptation to ever-changing  
16 light conditions.

## 17 18 **INTRODUCTION**

19  
20 Light regulates multiple developmental processes in plants, including seed  
21 germination, seedling de-etiolation, shade avoidance, phototropism, flowering time,  
22 photoperiod, and the circadian rhythm (Casal, 2012; Deng and Quail, 1999; Li et al., 2011; Lin,  
23 2000; Lin, 2002; Neff et al., 2000; Roux et al., 1994). Among the light-induced developmental  
24 processes, seedling de-etiolation is the most remarkable. Photoreceptors perceive light signals  
25 and, via interacting transcription factors, rapidly change the expression of numerous  
26 downstream target genes. Such orchestration of photoreceptor signaling induces dramatic  
27 transcriptome shifts, characteristic of de-etiolation (Labuz et al., 2012; Nelson et al., 2000;  
28 Reed et al., 1994; Tepperman et al., 2001; Wang et al., 2016; Yang et al., 2000). Light-  
29 dependent signaling governed by photoreceptors alters the expression of many transcription  
30 factors and induces transcriptional cascades, thereby rapidly changing the expression profiles  
31 of numerous down-stream genes (Duek and Fankhauser, 2003; Hong et al., 2008; Jiao et al.,  
32 2003; Kang et al., 2005; Tepperman et al., 2001).

33 MicroRNAs (miRNAs) are small non-coding RNAs that function in mRNA

34 degradation or translational repression (Chen, 2005; Huntzinger and Izaurralde, 2011; Pillai,  
35 2005). The processing of plant miRNAs, from primary miRNA transcripts (pri-miRNA)  
36 requires the type III RNase, DICER-LIKE1 (DCL1), and two RNA-binding proteins—  
37 SERRATE (SE), and HYPONASTIC LEAVES1 (HYL1), known as the core microprocessor  
38 (Kurihara et al., 2006; Kurihara and Watanabe, 2004; Yang et al., 2006; Yang et al., 2010).  
39 Mature miRNAs are methylated by HUA ENHANCER 1 (HEN1) for stabilization (Li et al.,  
40 2005), and known to be exported to the cytoplasm by HASTY, and loaded onto ARGONAUTE  
41 1 (AGO1) (Bartel, 2004; Park et al., 2005). However, a recent study showed that miRNA  
42 translocation and functionality entail the nuclear-cytoplasmic shuttling of AGO1 (Bologna et  
43 al., 2018). Furthermore, many recent discoveries witnessed the diverse functions of AGO1 (Ma  
44 and Zhang, 2018). Core microprocessor activity is regulated negatively or positively by many  
45 proteins such as C-TERMINAL DOMAIN PHOSPHATASE-LIKE 1 (CPL1), SNF1-  
46 RELATED PROTEIN KINASE 2 (SnRK2), NOT2, KARYOPHERIN ENABLING THE  
47 TRANSPORT OF THE CYTOPLASMIC HYL1 (KETCH1), RECEPTOR FOR  
48 ACTIVATED C KINASE 1 (RACK1), PROTEIN PHOSPHATASE 4 (PP4), SUPPRESSOR  
49 OF MEK1 (SMEK1), RNA DEBRANCHING ENZYME 1 (DBR1), TOUGH (TGH),  
50 DAWDLE (DDL), and CHROMATIN REMODELING FACTOR 2 (CHR2) (Li et al., 2016;  
51 Manavella et al., 2012; Ren et al., 2012; Speth et al., 2013; Su et al., 2017; Wang et al., 2013;  
52 Wang et al., 2018; Yan et al., 2017; Yu et al., 2008; Zhang et al., 2017). Many components  
53 such as STABILIZED 1 (STA1), INCREASED LEVEL OF POLYPLOIDY1-1D (ILP1) and  
54 NTC-RELATED PROTEIN 1 (NTR1) are also involved in miRNA biogenesis by removing  
55 introns of intron-harboring pri-miRNAs, promoting, and facilitating transcriptional elongation  
56 of *MIRNA* (*MIR*) genes, respectively (Ben Chaabane et al., 2013; Wang et al., 2019a).  
57 Furthermore, MOS4-ASSOCIATED COMPLEX (MAC) plays roles in modulating miRNA  
58 levels through adjusting pri-miRNA transcription, processing, and stability (Jia et al., 2017).

59 Light mediated changes in *MIRNA* gene expression has been observed in different  
60 plant species (Li et al., 2014; Mancini et al., 2016; Qiao et al., 2017; Shikata et al., 2014; Sun  
61 et al., 2015; Xie et al., 2017; Xu et al., 2017; Zhang et al., 2011). In *Arabidopsis*, white-light  
62 pulse treatment of etiolated seedlings reportedly increased the expression of *MIR157*, *MIR163*,  
63 and *MIR398*, but reduced that of *MIR408*, *MIR822*, and *MIR834* (Mancini et al., 2016). Red-  
64 light altered the expression of *MIR163*, *MIR156c*, *MIR157c*, *MIR169l*, and *MIR824a* (Shikata  
65 et al., 2014). A genome-wide mapping study revealed that ELONGATED HYPOCOTYL 5  
66 (HY5) recognizes the promoter regions of at least eight *MIRNA* genes (Zhang et al., 2011).

67 PHYTOCHROME INTERACTING FACTORS (PIFs) bind to the promoters of five *MIR156*  
68 genes to suppress their expression, showing that light can directly control *MIRNA* gene  
69 expression (Xie et al., 2017). Furthermore, light can affect the expression and localization of  
70 HYL1; CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) protects HYL1 from an  
71 unidentified cytoplasmic protease in a light-dependent manner (Cho et al., 2014). Moreover,  
72 the nuclear localization of HYL1 is maintained by phosphorylation under darkness (Achkar et  
73 al., 2018). Light-induced expression of *HEN1* is mediated by *HY5* and its homolog *HYH* (Tsai  
74 et al., 2014). However, it is increasingly evident that not only does light modulate miRNA  
75 processing, but light-induced development itself is affected by miRNA-biogenetic components  
76 and miRNAs. For example, deficiency of *DCL1*, *AGO1*, *HYL1*, *HASTY*, or *HEN1* results in  
77 shorter hypocotyls than those in wild-type seedlings under dark and light conditions (Lu and  
78 Fedoroff, 2000; Sorin et al., 2005; Sun et al., 2018; Tsai et al., 2014). The light-responsive  
79 miRNAs, miR157 and miR319 promote the degradation of *HY5* and *TCP24* transcripts, key  
80 positive and negative transcription factors of photomorphogenesis, respectively (Tsai et al.,  
81 2014). Compared to the wild-type, *mir390b* and *mir160b* mutants display long hypocotyl  
82 phenotypes, whereas the *mir167b* and *mir848* mutants have short hypocotyl phenotypes,  
83 indicating either a positive or negative role for specific miRNAs in photomorphogenesis (Sun  
84 et al., 2018).

85 However, the detailed crosstalk between light signaling and miRNA biogenesis during  
86 the de-etiolation process remains elusive. In the present study, we show how light adjusts the  
87 functionality of microprocessor components and subsequently the miRNAome profiles to drive  
88 a light-responsive transcriptome shift, and thereby the survivability of de-etiolated seedlings.

89

## 90 **RESULTS**

### 91 **Light induces accumulation of core microprocessor components**

92

93 We previously showed that HYL1 is a short-lived protein and is stabilized by light  
94 (Cho et al., 2014). This prompted us to test whether other core microprocessor proteins such  
95 as DCL1 and SE are also regulated by light. Therefore, five-day-old dark-grown (hereafter  
96 referred to as 5D) seedlings were transferred to continuous white light. After three days, the  
97 abundance of DCL1, SE, and HYL1 was determined and found to be low in etiolated seedlings.  
98 Upon exposure to white light, all tested proteins accumulated to higher levels each day (Figure

99 1). Plants detect and respond to specific wavelengths of light such as blue, red and far-red. So,  
100 we also monitored the levels of DCL1, SE and HYL1 proteins in seedlings exposed to  
101 monochromatic irradiation. Levels of DCL1, SE and HYL1 increased after all three light  
102 treatments as compared to de-etiolated seedlings. However, the degree of induction was  
103 consistently higher in response to blue and red light than in response to the far-red light  
104 treatment (Figures 1A-1C). DCL1 protein abundance rapidly decreased in four-day-old dark-  
105 grown (hereafter referred to as 4D) seedlings irradiated with red light for 3-5 h (Sun et al.,  
106 2018). This result contradicts our findings because we observed that all the three proteins  
107 accumulated in response to each monochromatic light treatment (Figure 1D). To further  
108 validate the light-induced accumulation of DCL1, we created transgenic plants expressing  
109 DCL1-6myc protein under the control of the constitutive 35S promoter. DCL1-6myc protein  
110 accumulated to high levels under white light and monochromatic irradiation (Figures S1A-  
111 S1C). Moreover, we observed a similar increase in DCL1-6myc levels after a few hours of  
112 irradiation of 4D seedlings (Figure S1D). These data support our conclusion that light increases  
113 DCL1 as well as SE and HYL1 protein levels (Figures S1A-S1C). In addition, we tested the  
114 effect of light to dark transition using two-week-old plantlets grown under continuous white  
115 light. When the plantlets were transferred to darkness for 12 h, the levels of DCL1 and HYL1  
116 decreased notably, while that of SE remained unaltered (Figure 1E). This finding is consistent  
117 with our previous report (Cho et al., 2014) and implied that the SE level is differentially  
118 regulated in plantlets grown under constant light condition. To elucidate whether the  
119 expression of microprocessor components was modulated at the level of mRNA or protein,  
120 we examined the levels of *DCL1*, *SE*, and *HYL1* transcripts in etiolated and de-etiolated  
121 seedlings using quantitative reverse transcription polymerase chain reaction (qRT-PCR).  
122 We found that *DCL1*, *SE*, and *HYL1* transcripts were unaltered after light exposure for one  
123 day. However, all the tested transcripts were up-regulated by at least 2-fold in the extended  
124 light treatments over two days (Figure 1F). We speculated that light affected the whole  
125 transcriptome including many well-known internal reference genes. Thus, we selected  
126 *UBQ10* as a reference gene having the least fluctuation to determine fold-differences in the  
127 expression of target genes (Figures S2A-S2C; see methods). Next, we treated 4D seedlings  
128 with carbobenzoxy-Leu-Leu-leucinal (MG132) or protease inhibitor cocktail (PIs) to  
129 examine the role of possible proteolytic degradation of the microprocessor components. We  
130 detected accumulation of DCL1, SE, and HYL1 in response to these chemical treatments,  
131 indicating the degradation of microprocessor components in darkness (Figure 1G).

132 Furthermore, we applied these chemicals to 4D Col-0/35S:*DCL1-6Myc* transgenic  
133 seedlings, resulting in identical patterns of protein accumulation (Figure S1E). Based on  
134 these results, we speculated that DCL1 could be degraded by protease or proteases (Cho et  
135 al., 2014). In addition, we also found that the core microprocessor proteins are synthesized  
136 *de novo* after germination (Figure S1F). In darkness, the levels of the components gradually  
137 increased for 4 days after germination and dropped after 5 days (Figure S1F). Conclusively,  
138 our results indicated that irradiation increases the abundance of these microprocessor  
139 components via altered transcriptional regulation and modulation of protein half-life.

140

## 141 **Light signaling pathways mediate the accumulation of microprocessor** 142 **proteins**

143

144 Photoreceptors are key light sensing molecules that mediate light signaling in plants.  
145 Therefore, we investigated which photoreceptors are involved in the light-induced  
146 accumulation of microprocessor proteins. We grew *phyA/phyB* and *cry1/cry2* double mutant  
147 plants in darkness for 5 days and shifted them to white light for 1-3 days or alternatively for  
148 17-days under continuous white light (CL). Under white-light irradiation, DCL1, SE and HYL1  
149 accumulated in the tested mutants (Figures 2A, 2B, S2D and S2E). Next, we tested whether  
150 the accumulation of the microprocessor proteins occurs under monochromatic irradiation with  
151 different light sources. DCL1, SE, and HYL1 did not accumulate to high levels in *phyA/phyB*  
152 mutants under red or far-red light (Figures 2C and 2D). Likewise, the core microprocessor  
153 components did not accumulate notably in the *cry1/cry2* mutant under blue light (Figure 2E).  
154 We also germinated *phyA/phyB* mutants under red or far-red light and the *cry1/cry2* mutant  
155 under blue light. The four-day irradiation with each light type did not result in the accumulation  
156 of the microprocessor components in those mutant backgrounds (Figure 2F). These  
157 observations suggest that the light-induced accumulation of core microprocessor components  
158 is modulated by phytochromes A and B under red, and far-red, whereas cryptochromes 1 and  
159 2 governed the photoreceptor-mediated pathways under blue light. These show that  
160 monochromatic light treatments - blue, red, and far-red light - can independently trigger the  
161 accumulation of the microprocessor components through these photoreceptors.

162

## 163 **Primary-miRNAs are highly up-regulated during de-etiolation process**

164

165 Even though up- and down-regulation of *MIRNA* genes in response to various  
166 environmental changes have been reported (Li and Zhang, 2016; May et al., 2013; Sunkar et  
167 al., 2012), the expression profiles of pri-miRNAs in response to light are not entirely clear. We  
168 investigated the expression profiles of known pri-miRNAs in etiolated seedlings that were  
169 irradiated with white light. Using qRT-PCR, we found that the transcript levels of these pri-  
170 miRNAs increased at least 3-fold in de-etiolated seedlings compared to that in the five-day-old  
171 etiolated seedlings grown in the dark. For instance, *pri-miR163* and *pri-miR159* transcripts  
172 were dramatically up-regulated over 400-fold and 40-fold in de-etiolated seedlings,  
173 respectively. *Pri-miR157*, *pri-miR160*, *pri-miR165*, *pri-miR390* transcripts increased over 15-  
174 fold in response to light. In the case of *pri-miR164*, *pri-miR166*, *pri-miR168*, *pri-miR171*, *pri-*  
175 *miR172*, *pri-miR319*, *pri-miR393*, *pri-miR396* and *pri-miR403* transcript levels increased 3 to  
176 10-fold (Figure 3A). These pri-miRNAs showed light-responsive accumulation patterns.  
177 Therefore, in line with the light-responsive increment of microprocessor components, we  
178 hypothesized that light may accelerate miRNA production during photomorphogenesis.

179

## 180 **MiRNAome selectively shifts during the early stage of de-etiolation**

181

182 To test the hypothesis that the miRNAome shift occurs during de-etiolation, we  
183 investigated the steady-state-levels of mature miRNAs in de-etiolated seedlings and compared  
184 them to that in the etiolated seedlings. We performed two independent sets of miRNA  
185 sequencing experiments on samples of five-day-old etiolated (5D) seedlings and of 5D  
186 seedlings that were irradiated with white light for one day (hereafter referred to as 5D+1L)  
187 or for three days (hereafter referred to as 5D+3L). We found that the median expression  
188 level of miRNAs was slightly altered in 5D+1L seedlings (change-ratio:  $\log_2\Delta$ TPTM value  
189 of 0.13 for set-1; 0.18 for set-2) and in 5D+3L seedlings (the change ratio of -0.01 for set-  
190 1; 0.07 for set-2) (Figure S3A). Of the 151 miRNAs that had a total expression of at least  
191 twenty transcripts per 10 million (TPTM), nearly two-thirds of the miRNAs had higher  
192 expression in 5D+1L seedlings (60% for set-1; 63% for set-2) and in 5D+3L light-treated  
193 seedlings (60% for set-1; 62% for set-2) (Figures S3B and S3C). However, we noted that  
194 most of the miRNAs whose expression increased in all the light-treated seedlings  
195 (43%~46%) had increased to less than 0.5 (change-ratio) as compared to that in the 5D



196 seedlings. Likewise, most of the reduced miRNAs (32%~34%) decreased between 0 and 0.5  
197 (change-ratio) (Figure S3C). When we filtered the miRNAs for those with read-counts over  
198 100 reads, only 25 miRNAs (17%) from set-1 and 26 miRNAs (17%) from set-2 increased  
199 above 0.5 in both, 5D+1L and 5D+3L seedlings (Figure 3B). Among these, miR157 (3.9 for  
200 both set-1 and set-2 in 5D+1L seedlings; 4.8 for both set-1 and set-2 in 5D+3L seedlings)  
201 and miR163 (7.4 for both set-1 and set-2 in 5D+1L seedlings; 7.7 for both set-1 and set-2 in  
202 5D+3L seedlings) were most remarkably up-regulated by light. Besides, only 8 miRNAs  
203 (5%) for set-1 and 7 miRNAs (5%) for set-2 decreased below -0.5, and miR406 (-1.2 for  
204 both sets in 5D+1L seedlings; -2.4 for both sets in 5D+3L seedlings) and miR8176 (-3.1 for  
205 set-1 and -1.9 for set-2 in 5D+1L seedlings; -1.0 for both set-1 and set-2 in 5D+3L seedlings)  
206 decreased after both light treatments (Figure 3C). In addition to these miRNAs, 22 miRNAs  
207 from both, sets-1 and 2, were specifically up-regulated ( $\log_2\Delta\text{TPTM} > 0.5$ ), while 5 miRNAs  
208 from set-1 and 6 miRNAs from set-2 were notably down-regulated ( $\log_2\Delta\text{TPTM} < 0.5$ ) in  
209 5D+3L seedlings. In particular, miR397, miR8175, miR399, and miR408 notably increased  
210 in 5D+3L seedlings. Conversely, miR845 and miR858 specifically decreased in 5D+3L  
211 seedlings (Figure S4A). Given that we analyzed miRNAs based on a high change-ratio in  
212 response to light, several miRNAs without notable changes ( $\log_2\Delta\text{TPTM} < 0.5$  or  $> -0.5$ )  
213 were excluded in the analysis, regardless of their abundance. Furthermore, many miRNAs  
214 shown in Figure 3B and 3C are less-defined in their functions. Therefore, we further  
215 analyzed well-defined and abundant miRNAs with read-counts over 20,000. We found that  
216 miR162, miR173, and miR408 significantly increased, while miR156, miR159, miR165,  
217 and miR396 consistently decreased with the light treatments (Figure S4B). We monitored  
218 the change in number of reads ( $\Delta\text{Tprm}$ ) to confirm the actual number of miRNAs.  
219 Interestingly, we found that miR158 ( $\Delta\text{Tprm} = \sim 400,000$  to  $\sim 800,000$  reads) had a greater  
220 increase than did miR163 ( $\Delta\text{Tprm} = \sim 100,000$  reads) and miR157 ( $\Delta\text{Tprm} = \sim 100,000$   
221 reads) (Figure S4C). This result implied that miR158 could be an important light-responsive  
222 miRNA even though its change-ratio is less than 0.5. In the same context, we found that the  
223 greatest decrease in  $\Delta\text{Tprm}$  occurred for miR156 and miR159 (Figure S4D). The duration  
224 of the light exposure differently regulated several miRNAs. For instance, miR166, miR319,  
225 and miR168 slightly increased in 5D+1L seedlings and then reduced in 5D+3L seedlings. In  
226 contrast, miR167, miR161, and miR403 reduced in 5D+1L seedlings, and then increased in  
227 5D+3L seedlings (Figure S4E). Using northern blot analysis, we further confirmed the light-

228 responsiveness of selected miRNAs. Consistent with the sequencing results, miR156 and  
229 miR159 decreased, miR166 fluctuated, and miR157 increased (Figure 3D). Lastly, we  
230 performed miRNA sequencing analysis using the third set of samples, which were  
231 independently treated with white light on 5D seedlings for a day (5D+1L). We obtained a  
232 similar expression pattern as presented in Figure S3C (Figure S4F). Taken together, we found  
233 that most miRNAs are not notably altered during skoto- to photo-morphogenic developmental  
234 transition and our hypothesis mentioned above is improbable.

235

### 236 **Primary miRNAs and the core microprocessor components further** 237 **accumulated in response to prolonged light treatment**

238

239 The miRNAome shift in the continuous light-grown plants compared to that in the  
240 etiolated seedlings prompted us to test the levels of miRNA precursors and microprocessor  
241 proteins under prolonged irradiation after the skoto- to photo-morphogenic transition.  
242 Therefore, we investigated the miRNA biogenesis in de-etiolated seedlings exposed to  
243 prolonged light for 12 days (5D+12L) and in CL seedlings. To test the light-induced  
244 accumulation of pri-miRNAs and their correlation to mature miRNAs, we performed northern  
245 blot analyses and tested the expression levels of *pri-miR156a-j*, *pri-miR159a-c*, *pri-miR163*,  
246 *pri-miR166a-f*, and *pri-miR319a-c* transcripts by using qRT-PCR. We observed that all the  
247 tested pri-miRNAs increased after 3-12 days of WL irradiation in 5D seedlings. The levels of  
248 pri-miRNAs in 5D+12L seedlings were similar to those observed in CL plantlets (Figure 4A-  
249 4E). Despite the dramatic increase in miRNA precursors, the amount of mature miR156,  
250 miR159, and miR166 decreased and the levels of miR163 and miR319 were not notably altered  
251 after 9-12 days of irradiation or in the CL plantlets as compared to that observed in 5D+1L  
252 seedlings (Figure 4F). These results corroborate the miRNAome analysis (Figure 4G and S6).  
253 We further tested whether the expression of microprocessor components was altered by such  
254 prolonged light conditions. Transcripts of the components were not further increased after two  
255 or three days of light treatments (Figure 4H). Moreover, the levels of all the microprocessor  
256 proteins gradually increased when the light treatments were extended (Figure 4I). The reduced  
257 or unaltered miRNAs in the de-etiolated seedlings and the light-grown plantlets were not easy  
258 to comprehend considering the levels of microprocessor and pri-miRNAs amassed: hereafter,  
259 this phenomenon is referred to as the miRNA-biogenetic inconsistency.

260

## 261 **Light lowers pri-miRNA processing activity of the microprocessor**

262

263 We hypothesized that the miRNA-biogenetic inconsistency might be caused by unknown  
264 negative regulatory pathways during photomorphogenesis, light adaptation, and  
265 photomorphogenic growth under constant light. To verify the existence of the unknown  
266 negative regulatory pathways, we formulated three possible scenarios: 1) the enzyme activity  
267 of the core microprocessor could be different between etiolated and de-etiolated seedlings, 2)  
268 the presence of a light-induced repressor of the core microprocessor, or 3) differential  
269 regulation of miRNA turnover. To test the first scenario, we investigated the enzyme activity  
270 of the core microprocessor using Col-0/35S:*DCL1-6Myc* transgenic seedlings. First, we  
271 purified the core microprocessor complex using  $\alpha$ -myc antibody from 5D and 5D+1L  
272 transgenic seedlings. Then, the immunoprecipitated microprocessor complex was incubated  
273 with  $^{32}\alpha$ -UTP isotope incorporated pri-miR166c as a substrate (Figure 5A). We observed that  
274 four intermediate fragments and a miRNA-like fragment only in the reaction using  
275 immunoprecipitates from Col-0/35S:*DCL1-6Myc* transgenic seedlings (Figure 5B). Next, to  
276 confirm whether the observed lower band having the size of a miRNA is really miR166, we  
277 used non-labeled pri-miR166c substrate for the enzymatic assay and performed PAGE before  
278 subsequent blotting and hybridization with  $^{32}\gamma$ -ATP labeled miR166-specific probe or non-  
279 miR166 probe as a negative control. We detected a specific band which has the same size as  
280 the synthetic miR166 and was recognized by only by the radioactively labelled miR166-  
281 specific probe (Figure 5C). Based on these control experiments, we further examined the DCL1  
282 enzyme activity between dark-grown 5D and 5D+1L de-etiolated seedlings. We observed that  
283 the cleavage activity of the purified microprocessor in 5D+1L seedlings was approximately  
284 similar to that of 5D seedlings, even though the protein level of microprocessor was over 10-  
285 fold higher in 5D+1L seedlings (Figure 5D). Thus, we equilibrated the amount of DCL1-6myc,  
286 to compare the unit activity between the two samples, and found that the processing activity  
287 was much higher in 5D seedlings (Figure 5E). As shown in Figure 3, *pri-miR166a* and *pri-*  
288 *miR390a* transcripts accumulation was highly induced by light (about 4.5- and 25-fold,  
289 respectively). To search for the reason of this phenomenon, we tested the promoter activity of  
290 *MIR166a* and *MIR390a* under these circumstances. Although the expression levels of the  
291 examined reporter genes were not influenced by light (Figure S7), we cannot rule out that other

292 *MIR* genes can be specifically light-regulated (Chung et al., 2016). This result suggests that not  
293 gene expression changes but decreased microprocessor activity triggered by light could lead to  
294 the accumulation of many pri-miRNAs.

295 To further examine this scenario, we adopted the micro-Protein-DCL1 (miP-DCL1) system  
296 that ectopically expresses DCL1-PAZ domain, producing more miRNAs, possibly by titrating  
297 a yet unidentified potential negative regulator (Dolde et al., 2018) (Figure 6A). Using 17-day-  
298 old light-grown Col-0/35S:*miP-DCL1* transgenic plants, we showed the increased amounts of  
299 miR156, miR159 and miR319 and the notable reduction of *pri-miRNA* transcripts as compared  
300 to wild-type plants, thus confirming the positive role of miP-DCL1 in miRNA biogenesis  
301 (Dolde et al., 2018)(Figures 6B and 6C). Next, we tested whether the production of miRNAs  
302 is influenced by overexpressing miP-DCL1 during the de-etiolation process. We found that the  
303 light-reduced miRNAs - miR156, miR159, miR166, and miR319 - accumulated to higher levels  
304 in miP-DCL1 seedlings compared with wild-type plantlets after 1-3 days of light treatments  
305 (Figures 6D - 6F) indicating miP-DCL1 impact on miRNA levels during de-etiolation.  
306 Likewise, the production of light-increased miRNAs such as miR157 and miR163 (Figures 3B  
307 and S5C) were more distinctively elevated in miP-DCL1 seedlings than in wild-type seedlings  
308 (Figures 6D-6F). This result though intriguing, implied that this unknown negative regulator  
309 also suppressed the production of light-induced miRNAs to a certain degree. Considering the  
310 similar levels of tested miRNAs in the 5D seedlings of miP-DCL1 and wild-type, the light-  
311 driven miRNA-biogenetic inconsistency could be caused by reduced microprocessor activity.  
312 With these results, we speculated that the first and second scenarios are plausible to account  
313 for light-induced miRNA-biogenetic inconsistency. Furthermore, we found that the up-  
314 regulated miRNA levels in Col-0/35S:*miP-DCL1* transgenic plants led to proportional defects  
315 in cotyledon opening (Figure 6G). In sugar-free medium, 5D+1L seedlings of miP-DCL1  
316 showed a delay in cotyledon opening (25-30%), while 5D+1L seedlings of Col-0 had fully-  
317 opened cotyledons (100%). Moreover, when 6D seedlings of miP-DCL1 - in which the  
318 endosperm could be depleted - were irradiated with white light for 5 days, only 50-55% of the  
319 seedlings survived via photomorphogenesis, while 90% of 6D+5L seedlings of Col-0 survived  
320 (Figures 6G and 6H). Light-grown seedlings of miP-DCL1 had defects in leaf development,  
321 flowering time, and growth (Figures 6I and S8). These results indicated the down-regulation  
322 of microprocessor activity by light, which is important for normal photomorphogenesis and  
323 development.

324

## 325 **The half-life of miRNAs is differentially regulated during de-etiolation**

326

327 While it was not possible to rule out the activity of miRNA-decaying RNases such as  
328 SDN1, SDN2, and SDN3 that can modulate the half-life of miRNAs, possibly leading to  
329 miRNA-biogenetic inconsistency, we found that *SDN1* expression is highly up-regulated  
330 during photomorphogenesis (Figures 7A and 7B). RNA polymerase II is responsible for the  
331 *MIRNA* gene expression, and  $\alpha$ -amanitin is a specific inhibitor (Rajjou et al., 2004). Thus, we  
332 monitored the half-life of miRNA by treating 5D+2L seedlings and 5D seedlings with  $\alpha$ -  
333 amanitin for 6 hours (Figure 7C). Under these experimental conditions, several miRNAs such  
334 as miR156, miR157, miR158, miR159, miR160, miR169, and miR319 decayed, while miR163,  
335 miR166, miR167, miR168, miR171, miR390, and miR393 were either unaltered or increased  
336 in the 5D+2L seedlings. By contrast, only miR157 and miR158 notably decreased in the 5D  
337 seedlings, implying that the third scenario is also applicable. This result indicated that miRNAs  
338 could have different turnover rates during the de-etiolation process. The outcome of  $\alpha$ -  
339 amanitin-chase assay was in concordance with the expression profiles of several miRNAs. For  
340 instance, miR156 and miR159 rapidly decayed; therefore, their levels notably decreased,  
341 whereas miR163 was stable; accordingly, its levels increased with light irradiation treatments  
342 (Figure 4G). Even though several miRNAs have a short half-life, their amounts did not  
343 decrease; instead, miR157 (16-fold) and miR158 (1-fold) increased, while miR160 and  
344 miR319 were less altered by light exposure (Figures 3B, 7C, S4E and 4G). To account for the  
345 different expression levels of these rapidly-decaying miRNAs, we hypothesized the varied  
346 abundance of miRNA precursors for these fast-decaying miRNAs. Using a statistical approach  
347 to the Droplet Digital PCR (ddPCR) method, we compared the absolute sum of the miRNA  
348 precursors in 5D and 5D+2L seedlings. In the tested samples, *pri-miR157* and *pri-miR158* had  
349 57 and 46.5 copies per 0.02 ng of total RNA, respectively, *pri-miR160* and *pri-miR319* had  
350 30.9 and 38 copies, while *pri-miR159* had a much lower expression level with 23.9 copies  
351 (Figure 7D). This result indicated that the levels of *pri-miRNA* could also determine the  
352 production rate of a short-lived miRNA. We also found that *pri-miR163* had 827 copies, which  
353 was remarkably higher than the copy number of the other *pri-miRNAs* observed (Figure 7D).  
354 Moreover, the stability of miR163 could be attributed to the 192-fold increment of miR163 by  
355 light treatments (Figures 3B, 7C and 7D). These results indicated the balance between the  
356 degree of *MIR* gene expression (the level of *pri-miRNA* transcripts), microprocessor activity,

357 and miRNA-decay rate that determines the expression levels of miRNAs during  
358 photomorphogenesis (Table S1).

359

## 360 **DISCUSSION**

361

362 We demonstrated that the transition from skoto- to photo-morphogenic development  
363 triggers the accumulation of the elements of core microprocessor and miRNA precursors. Light  
364 immediately stabilizes DCL1, SE, and HYL1 via post-translational regulation, and  
365 simultaneously up-regulates the expression of these genes. With this two-track regulation, de-  
366 etiolated seedlings dramatically increase the levels of the core microprocessor proteins in  
367 response to white, blue, red, and far-red light (Figure 1). These observations contradict a recent  
368 study that suggests destabilization of DCL1 and HYL1 during dark to red light transition (Sun  
369 et al., 2018). We speculate that the contradiction could be caused by incognizant differences in  
370 the experimental conditions between the two studies. However, to ensure our experimental  
371 conditions, using two different types of anti-DCL1 antibodies (see Methods), we confirmed  
372 that both endogenous DCL1 and heterologous DCL1-6myc with expected molecular weights  
373 of ~215 kDa and ~250 kDa, respectively, are stabilized by light (Figures 1, S1 and S9). Despite  
374 the light-mediated accumulation of the core microprocessor components and up-regulation of  
375 pri-miRNAs, most miRNAs displayed minor fluctuations in their amounts upon skoto- to  
376 photomorphogenic transition. To reconcile this discordance, we showed that the processing  
377 activity of the core microprocessor is down-regulated by light exposure, possibly due to a light-  
378 induced suppressor. This mechanism is enough to account for minor fluctuations of miRNAs  
379 such as miR160 and miR319. However, the low processing activity of the microprocessor is  
380 insufficient to explain the other notably down-regulated miRNAs such as miR156 and miR159,  
381 particularly, considering the up-regulation of their pri-miRNA transcripts. The reduction of  
382 these miRNAs by light could be explained by accounting for another variable—miRNA  
383 turnover. Therefore, SDN1 could be concomitantly involved in the modulation of miRNAs  
384 (Figure 7). The light-increased SDN1 accounts for the diminished levels of miR156 and  
385 miR159, each of which has a relatively short half-life. In addition to these two negative  
386 regulations, the degree of *MIRNA* gene expression could be another determinant for some  
387 miRNAs, such as miR157 and miR158. As seen in the increments of their pri-miRNA transcript  
388 levels, the transcriptional up-regulation of some *MIRNA* genes could countervail both negative

389 regulations during de-etiolation (Figure 7D). In the same context, the stability of miR163 and  
390 the high expression level of pri-miR163 could result in a significant increase of miR163  
391 (Figures 3A, 3B and 4G). By contrast, the light-induced suppressor activity and SDN1 activity  
392 could influence the production of miR159 under light because such miRNA decays rapidly and  
393 the level of its pri-miRNA is relatively low (Figures 7C, 3C, 4B, and S5D). However, the levels  
394 of some miRNAs are unexplained by these three regulatory notions. For instance, the notable  
395 reduction of miR166 in CL seedlings as compared to 5D seedlings (Figures 4G and S6A) is  
396 mysterious not only because all the *pri-miR166* transcripts accumulated (Figure 4D), but also  
397 the half-life of miR166 was longer (Figure 7C). In plants, pri-miRNAs vary in size and shape,  
398 and are processed by different processing modes (Moro et al., 2018). Thus, one plausible  
399 speculation is that the regulation of the microprocessor's accessibility to specific pri-miRNAs  
400 via co-factors (Li et al., 2017; Ren et al., 2012; Wang et al., 2019b) might be differentially  
401 modulated by light, but there is no evidence to substantiate. To elucidate this puzzle, light-  
402 induced reduction of specific miRNAs should be investigated to clarify if their half-life is  
403 differentially-modulated during de-etiolation, day-and-night shift, shading, and continuous  
404 light irradiation, and if some of the specific sequences are more susceptible to the RNases.  
405 Above all, to clearly understand the miRNA-biogenetic inconsistency observed during  
406 photomorphogenesis, it is essential to identify the unknown suppressor and/or possible light-  
407 induced post-translational modifications of microprocessor components (e.g., possible  
408 phosphorylation-mediated regulation of DCL1). *CHR2* could also be a possible suppressor, as  
409 its negative regulatory function on miRNA biogenesis has been demonstrated (Wang et al.,  
410 2018). However, *CHR2* does not seem to be induced by light at the transcriptional level (Figure  
411 S10).

412 Light provides important cues for the distinctive transcriptome shifts that define  
413 photomorphogenesis. Our findings here suggest a novel mechanism, the miRNA-biogenetic  
414 inconsistency, at play describing the regulatory gearshift in miRNA biogenesis during  
415 photomorphogenesis. Through this mechanism, de-etiolated seedlings could modulate the  
416 balance between light-induced transcriptome and miRNA-mediated gene silencing in the early  
417 stages of photomorphogenesis (Figure 7E). Without such a mechanism, de-etiolated seedlings  
418 could possibly experience internal conflicts between miRNAs and light-responsive  
419 transcripts—most of them are transcription factors, and that eventually result in pleiotropic  
420 defects in the growth and development of plants or risk seedling survivability (Figures 6H and  
421 6I). The miRNA-biogenetic inconsistency could be essential for seedlings buried deep in the

422 soil. For such seedlings, reaching the surface with an elongated hypocotyl expends most of  
423 their endosperm. Barely emerged seedlings could improve their survivability by conditionally  
424 suppressing miRNA biogenesis to promote expression of favorable genes, which may enable  
425 energy-saving while facilitating adapting to ever-changing light conditions (Figures 6G and  
426 6H). When the seedlings survive and receive optimal light, they could increase the expression  
427 of additional microprocessor components to increase miRNAs, as what was observed in the  
428 prolonged light-grown seedlings (Figure 4). However, to further validate this speculation,  
429 investigation of the detailed networks between miRNAs biogenesis and light signaling is  
430 warranted. We emphasize that the challenges should include at least these three questions; i)  
431 whether miRNA-biogenetic components are directly involved in the light signaling pathway,  
432 regardless of their function in miRNA biogenesis; ii) whether microprocessor components are  
433 differently regulated during the developmental transition from skoto- to photomorphogenesis,  
434 light adaptation, shade avoidance, and photoperiodic changes; and iii) how light influences the  
435 post-translational regulation of microprocessor components such as proteolysis (Cho et al.,  
436 2014), and phosphorylation (Achkar et al., 2018). Furthermore, a study recently reported  
437 intriguing relationship between pri-miRNAs and corresponding miRNAs under differential  
438 abiotic stresses that partially resemble the miRNA-biogenetic inconsistency what we described  
439 (Barciszewska-Pacak et al., 2015). Likewise, extensive post-transcriptional regulations of  
440 miRNAs implicate for cancers that also resemble the miRNA-biogenetic inconsistency  
441 (Thomson et al., 2006). These observations suggest that the miRNA-biogenetic inconsistency  
442 can be triggered by different environmental stimuli and developmental regulations thus the  
443 underlying mechanism should be further investigated focusing on the functionality of miRNAs  
444 during these responses in plants and animals.

445

## 446 **METHODS**

### 447 **Plant material and growth conditions**

448 Arabidopsis plants in the Columbia background were used. The *cry1-304/cry2-1*, *phyA-*  
449 *211/phyB-9* and *35S:miP-DCL1* transgenic lines were obtained from previous studies (Dolde  
450 et al., 2018; Lascève et al., 1999; Reed et al., 1994). The *35S:DCL1-6Myc* and *35S:2B8-DCL1-*  
451 *2xFlag* constructs were introduced in plants by *Agrobacterium tumefaciens* (GV3101 strain)-  
452 mediated infiltration using the floral dip method. Arabidopsis seeds were surface-sterilized  
453 and plated onto half-strength Murashige & Skoog (MS) solid media including 1% sucrose



454 and 2 mM MES, pH 5.7. After plating, the seeds were stratified for 3 days at 4 °C, and then  
455 exposed to 6 h of white fluorescent light ( $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) at 22 °C to initiate germination.  
456 After this, the plates were wrapped in three layers of aluminum foil and incubated at 22 °C  
457 for further five days. Subsequently, irradiation with blue (470 nm,  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), red  
458 (660 nm,  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and far-red (740 nm,  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) light-emitting diodes  
459 (LEDs) or white light at 22 °C was performed for the indicated time periods.

460

### 461 **RNA extraction and analyses**

462 Total RNA was extracted from Arabidopsis seedlings using RNeasy Plant Mini kit (Qiagen).  
463 Reverse transcription was performed using iScript™ cDNA Synthesis Kit (Bio-Rad) according  
464 to the manufacturer's instructions. Primers for pri-miRNAs, *HYL1*, *SERRATE*, *DCLI*, and  
465 *UBQ10* are listed in Supplementary Table S1. qRT-PCR was carried out using Thermo  
466 Scientific™ PikoReal™ Real-Time PCR System. Relative amounts of transcripts were  
467 obtained by calibrating threshold cycles of target genes with that of UBQ10 reference gene.  
468 Calculations were performed using the formula  $2^{(-\Delta\Delta C_T)}$ , where  $C_T$  is the cycle number at which  
469 the fluorescence reaches the threshold point for detection. The experiments were performed  
470 with three independent biological replicates.

471

### 472 **Small RNA isolation and northern blot analysis**

473 Total RNA was isolated from etiolated or de-etiolated seedlings using TRIzol reagent  
474 (Invitrogen). The extracted aqueous phase was precipitated with 2-propanol twice (100 and  
475 75%) and dissolved in 50% formamide. Purified RNA was resolved using 5-15% denaturing  
476 polyacrylamide gel (National Diagnostics) before transferring to a nylon membrane  
477 (Amersham). The 5'-end-labelled DNA probes were used for hybridization of membrane blots  
478 for 12 h (Ambion). Blots were washed twice with washing buffer ( $2\times$  SSC and 0.1% SDS) for  
479 20 min each. Hybridization signals were detected using a phosphor-imager scanner (BAS-2500,  
480 Fujifilm).

481

### 482 **Deep sequencing and analysis of small RNAs**

483 We performed two independent sets of miRNA sequencing experiments using the Illumina  
484 platforms of 5D seedlings, 5D+1L seedlings, and 5D+3L seedlings: one was performed in  
485 Korea (Set-1), and another one in Denmark (Set-2). We obtained 42, 42, and 48 million  
486 clean reads for set-1 and 39, 42, and 46 million clean reads for set-2 that were aligned with

487 the Arabidopsis genome. We compared the normalized counts of mature miRNAs in 5D+1L  
488 seedlings and 5D+3L seedlings versus 5D seedlings. Construction of small RNA libraries  
489 with 5D, 5D+1L, and 5D+3L samples, deep sequencing, and analysis of small RNAs were  
490 performed by Macrogen (Belgium) or LAS Inc. (Korea). The expression levels of miRNAs  
491 (transcripts per 10 million, TPTM) in the indicated samples were calculated by normalizing the  
492 miRNA counts with the total number of clean reads in the small RNA libraries. Furthermore,  
493 a part of the analysis was additionally tested in Argentina (Figure S3F, Dataset S3).

494

#### 495 **Dark to light transition assay**

496 For the dark to light transient assay, etiolated five-day-old wild-type, *cry1-304/cry2-1*, *phyA-*  
497 *211phy/B-9* and Col-0/35S:*DCL1-6Myc* transgenic seedlings were transferred to continuous  
498 blue, red, and far-red LED illumination boxes or a white light growth chamber at 22°C for  
499 the indicated time periods. Samples were harvested under safe green LED light in a dark  
500 room.

501

#### 502 **Production of antibodies**

503 We generated rabbit polyclonal  $\alpha$ -DCL1 antibody using GST-DCL1-N-terminal fragment as  
504 an antigen and mouse polyclonal  $\alpha$ -DCL1 antibody using synthetic oligopeptides that match  
505 the DUF283 domain of DCL1. Specific  $\alpha$ -DCL1 antibodies were purified from rabbit and  
506 mouse serum using protein A agarose resin.

507

#### 508 **Protein gel blot analyses**

509 A frozen powder of the samples (100 mg) was directly mixed with 5 × SDS sample buffer for  
510 10 min, and 10  $\mu$ g of total proteins were resolved using 8-12% SDS-PAGE after boiling at  
511 100°C. The proteins were transferred to a PVDF membrane. Blots were hybridized with  $\alpha$ -  
512 DCL1 antibody (dilution 1:3,000, this study),  $\alpha$ -SERRATE antibody (dilution 1:5,000, Cho et  
513 al., 2014),  $\alpha$ -HYL1 antibody (dilution 1:20,000, Yang et al., 2010),  $\alpha$ -ACTIN antibody  
514 (dilution 1:3,000, Agrisera, AS13 2640),  $\alpha$ -Histone H3 antibody (dilution 1:10,000, Agrisera  
515 AS10 710),  $\alpha$ -2B8 epitope antibody (dilution 1:10,000, S.-H. Bhoo provided), and  $\alpha$ -SDN1  
516 antibody (dilution 1:1000, Abmart, X-A3KPE8 –C) respectively.

517

#### 518 ***In vitro* transcription of RNA**

519 RNA substrates were *in vitro* transcribed under the T7 promoter using PCR-generated

520 templates. The templates and primers used for PCRs and the synthetic pri-miRNAs are as listed  
521 in a previous study (Zhu et al., 2013). The *in vitro* transcription of RNAs was carried out in a  
522 20- $\mu$ L reaction incubated at 37 °C for 2 h or at 30 °C overnight as follows: DNA template (200  
523 ng), 4  $\mu$ L of 5 $\times$  transcription buffer (400 mM HEPES, pH 7.5, 10 mM spermidine, 200 mM  
524 DTT, 125 mM MgCl<sub>2</sub> and 20 mM of each NTP), 1  $\mu$ L of RNase inhibitor (Ambion), 2  $\mu$ L of  
525 T7 RNA polymerase and up to 20  $\mu$ L of water. RNA was fractionated on 5% polyacrylamide  
526 and 6 M urea gel (denaturing gel), and eluted using a nucleotide removal kit (Qiagen, Cat#  
527 28304). For internal labeling, [ $\alpha$ -<sup>32</sup>P]-UTP (PerkinElmer) was included in the NTP mixture (20  
528 mM CTP, 20 mM ATP, 20 mM GTP and 4 mM UTP) for *in vitro* transcription as described  
529 above. All the labeled RNAs were resolved using 5% denaturing gel and eluted from the  
530 resolved gel slice. Labeled RNAs were folded by heating to 95 °C for 2 min, slowly cooled to  
531 room temperature and normalized to  $\sim 2 \times 10^3$  c.p.m.  $\mu$ L<sup>-1</sup>.

532

### 533 **Immunoprecipitation and *in vitro* pri-miRNA processing assay**

534 For the *in vitro* experiment processing assay, the core microprocessor complex was purified  
535 using  $\alpha$ -Myc antibody from five-day-old etiolated and two-day-old de-etiolated Col-  
536 0/35S:DCL1-6Myc seedlings. Immunoprecipitation was performed as described (Zhu et al.,  
537 2011) with some modifications. Arabidopsis samples were ground in liquid nitrogen, and  
538 protein-sRNA complexes were extracted using immunoprecipitation buffer (40 mM Tris-HCl,  
539 pH 7.5, 300 mM KCl, 5 mM MgCl<sub>2</sub>, 5 mM DTT, 0.2 mM EDTA, pH 8, 0.2% Triton X-100, 1  
540 mM PMSF, 2% glycerol and 0.3% (vol/vol) proteinase inhibitor cocktail (Sigma)) and 1 tablet  
541 of EDTA-free protease inhibitor cocktail (Roche) per 25 mL immunoprecipitation buffer. After  
542 removal of insoluble materials by centrifugation twice at 15000  $\times$ g for 15 min at 4 °C, extracts  
543 were incubated with  $\alpha$ -Myc Antibody (GeneScript, A00704) for 3 h at 4 °C. The mixtures were  
544 incubated for 3 h at 4 °C with SureBeads™ Protein A Magnetic Beads (BioRad, #1614013).  
545 The beads were washed three times with immunoprecipitation buffer and then three times with  
546 washing buffer (20 mM Tris-HCl, pH 7.5, 1 mM DTT, 4 mM MgCl<sub>2</sub> and 100 mM KCl). Briefly,  
547 *in vitro* DCL1 cleavage assays were performed in a total volume of 30  $\mu$ L in 20 mM Tris-HCl,  
548 pH 7.5, 53 mM KCl, 4 mM MgCl<sub>2</sub>, 1 mM DTT, 7.5 mM ATP, 1 mM GTP and 1  $\mu$ L<sup>-1</sup> RNase  
549 Inhibitor (Ambion), including 1  $\mu$ L of [ $\alpha$ -<sup>32</sup>P]-UTP labeled RNA ( $\sim 2 \times 10^3$  c.p.m.) and 15  $\mu$ L  
550 of the immunopurified magnetic beads in washing buffer. The reaction mixture was incubated  
551 at 37 °C for 0.5-3 h. After extraction with phenol-chloroform and ethanol, the processing  
552 products were fractionated using 5-15% denaturing gels. The RNA marker used was

553 synthesized miR166 that was 5' end-labeled according to the manufacturer's protocol with [ $\alpha$ -  
554  $^{32}\text{P}$ ]-ATP (PerkinElmer). The denaturing gel was dried in a Gel Dryer (Bio-Rad) at 65 °C for  
555 2 h. The processed RNA products were detected after exposure overnight to a phosphor plate  
556 and signals were detected with a phosphorimager scanner (BAS-2500, Fujifilm)

557

### 558 **Amanitin-chase assay**

559 5D and 5D+2L seedlings were mock-treated or incubated with 10 mM of  $\alpha$ -amanitin (Sigma,  
560 A2223) for 6 h. Total RNA was isolated using TRIzol reagent (Invitrogen) and was used for  
561 northern blot analyses.

562

### 563 **Droplet Digital PCR**

564 The QX200<sup>TM</sup> Droplet Digital<sup>TM</sup> PCR System (Bio-Rad) was used in this study according to the  
565 manufacturer's instructions. Droplets were generated by a Droplet Generator (DG). The  
566 prepared droplets were transferred to a 96-well PCR plate. The PCR plate was subsequently  
567 heat-sealed and amplified in a C1000 Touch<sup>TM</sup> deep-well thermal cycler (Bio-Rad). The  
568 thermocycling protocol was: initial denaturation at 95 °C for 5 min, then 40 cycles of  
569 denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s (temperature ramp 2 °C/s) and, final  
570 incubation at 98 °C for 10 min and storage at 4 °C. After cycling, the 96-well plate was fixed  
571 to a plate holder and placed in the Droplet Reader (Bio-Rad). Primers used for Droplet Digital  
572 PCR analyses are listed in the Supplementary Table 2.

573

### 574 **Author contributions**

575 SWY, SKC, FN and AV conceived this study, wrote the manuscripts, critically reviewed. AM,  
576 SW, and PM edited; SKC, SWC, HJJ, GMK, and AV performed most of the molecular and  
577 biochemical analyses; MYR, ALA, NPA and PM conducted the informatics activity; SWC,  
578 SW, and UD conducted the phenotype analyses of transgenic plants.

579

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590

591

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774

775

## 776 **Figure legends**

777

### 778 **Figure 1. Light augments the core microprocessor components DCL1, SE, and HYL1**

779 **A.** Expression levels of the core microprocessor components in five-day-old etiolated seedlings  
780 (5D) were increased by white or blue light treatments. **B.** Expression levels of the core  
781 microprocessor components in 5D seedlings were increased by white or red light treatments.

782 **C.** Expression levels of the core microprocessor components in 5D seedlings were increased  
783 by white or far-red light treatments. **D.** Expression levels of the core microprocessor  
784 components in four-day-old etiolated seedlings were increased by blue, red, or far-red (FR)  
785 irradiation for 3 h and 5 h. **E.** Expression levels of DCL1 and HYL1 in 2-week-old light-grown  
786 plantlets (L) were diminished after 12 h darkness (D). **F.** qRT-PCR analysis revealed that  
787 transcripts of the core microprocessor components increased slightly after two days of light  
788 treatment. Data are plotted as the average value of four biological replicates, error bars  
789 indicate  $\pm$ s.d. ( $n=12$ ). *UBQ10* transcript level was used as the calibration reference. **G.**

790 Effects of proteolysis inhibitors on the core microprocessor components. Four-day-old  
791 etiolated seedlings were treated with Mock, MG132 (10  $\mu$ M), and PIs (0.2 $\times$ ) for 2 h or 6 h.  
792 In panels A-C: seedlings were grown in darkness for 5-8 days (5D-8D). 5D seedlings were



793 irradiated with white or different monochromatic light for 1-3 days (5D+1L, 5D+2L,  
794 5D+3L). In panels A-E and G, the levels of endogenous DCL1, SE, and HYL1 were  
795 determined with  $\alpha$ -DCL1 (Agrisera),  $\alpha$ -DCL1-N (mouse polyclonal),  $\alpha$ -DCL1-Rb (rabbit  
796 polyclonal),  $\alpha$ -SE, or  $\alpha$ -HYL1 antibodies, respectively. Uniform loading of samples was  
797 confirmed with  $\alpha$ -actin or/and  $\alpha$ -histone antibodies.

798

799 **Figure 2. Photoreceptors are important for mediating the light-induced accumulation**  
800 **of the core microprocessor components.**

801 **A.** Expression levels of the core microprocessor components in *phyA/phyB* double mutant  
802 background under white light. **B.** Expression levels of the core microprocessor components  
803 in *cry1/cry2* double mutant background under white light. **C.** Expression levels of the core  
804 microprocessor components in *phyA/phyB* double mutant background under red light. **D.**  
805 Expression levels of the core microprocessor components in *phyA/phyB* double mutant  
806 background under far-red light. **E.** Expression levels of the core microprocessor components  
807 in *cry1/cry2* double mutant background under blue light. In panels a-e: Seedlings were  
808 grown in darkness for 5-8 days (5D-8D). Five-day-old etiolated (5D) seedlings were  
809 irradiated with white or different monochromatic light for 1-3 days (5D+1L, 5D+2L,  
810 5D+3L). Plants were also grown in constant light for 17 days (CL). **F.** Expression levels of  
811 the core microprocessor components in *Col-0*, *phyA/phyB*, and *cry1/cry2* mutants were  
812 grown for the indicated period with the corresponding light treatment. In all panels, the  
813 levels of endogenous DCL1, SE, and HYL1 were determined using  $\alpha$ -DCL1,  $\alpha$ -SE, or  $\alpha$ -  
814 HYL1 antibodies, respectively. Uniform loading of samples was confirmed with  $\alpha$ -actin  
815 or/and  $\alpha$ -histone antibodies.

816

817 **Figure 3. Primary miRNAs and small RNA sequencing analysis in five-day old etiolated**  
818 **(5D) seedlings and de-etiolated seedlings treated with white-light for 1 and 3 days.**

819 **A.** qRT-PCR analysis of *pri-miRNA* transcripts in 5-day-old etiolated seedlings (5D) that  
820 were irradiated with white light for 1 day (5D+1L) 2 days (5D+2L) or 3 days (5D+3L). Data  
821 are plotted as the average value of four biological replicates, error bars indicate  $\pm$ s.d. ( $n=12$ ).  
822 *UBQ10* transcripts were used as a calibration reference. **B.** Highly up-regulated miRNAs  
823 with reading frequencies  $>100$ . 17% of miRNAs increased with change-ratio  $>0.5$   
824 ( $\log_2$ DTPTM  $> 0.5$ ). In panels A-E: The results of two independent small RNA sequencing  
825 experiments (SET1 and SET2) are presented in this figure. **C.** Highly down-regulated

826 miRNAs with reading frequencies >100. 5% of miRNAs decreased with change-ratio <-0.5  
827 ( $\log_2\text{DTPTM} < -0.5$ ). **D.** Northern blot analysis of selected up- and down-regulated miRNAs  
828 upon exposure to white light. The loading control used is 5.8S rRNA.

829

830 **Figure 4. Accumulation of core microprocessor components and pri-miRNAs under**  
831 **prolonged light conditions**

832 **A-E.** qRT-PCR analysis of *pri-miRNA* transcripts in etiolated and de-etiolated seedlings  
833 irradiated with white light for 3 (5D+3L) or 12 (5d+12L) days. CL: 17-day-old light-grown  
834 plants. The relative amount of pri-miRNAs was calculated with  $\pm$ s.d. from three biological  
835 repeats ( $*P < 0.05$ ;  $**P < 0.005$ ; unpaired, two-tailed Student's *t*-test). **F.** Northern blot  
836 analysis of selected up- and down-regulated mature miRNAs in etiolated and de-etiolated  
837 seedlings under extended light treatments. Total RNA samples were resolved by gradient-  
838 PAGE (5-15%) and hybridized with specific radioisotope labeled probes. 5.8S rRNA is used  
839 as loading control, CL: 17-day-old light-grown plants. **G.** Comparison of the expression of  
840 tested miRNAs from two independent small RNA sequencing experiments (SET 1 and SET  
841 2). **H.** qRT-PCR analysis of *DCL1*, *SE*, and *HYL1* transcripts in 5D seedlings subjected to  
842 prolonged light conditions (1-12 days) and in plants grown for 17 days in constant light (CL)  
843 seedlings. In all the qRT-PCR analyses, data plotted is average of four biological replicates.  
844 Error bars indicate  $\pm$ s.d. ( $n=14$ ). *UBQ10* transcripts were used as the calibration reference.  
845 **I.** The protein levels of DCL1, SE, and HYL1 in etiolated seedlings under prolonged light  
846 conditions are shown. The protein level of actin is presented as the loading control. RuBisCo  
847 is used as a control for light-growth period.

848

849 **Figure 5. Deceleration of core microprocessor processing activity during transition**  
850 **from skoto- to photomorphogenic development**

851 **A.** Schematic depicting the use of artificial  $^{32}\alpha$ -UTP incorporated pri-mR166c substrate for  
852 the *in vitro* enzymatic assay. **B.** *In vitro* pri-miRNA processing assay. Immunoprecipitated  
853 DCL1-6Myc isolated from five-day-old etiolated (5D) Col-0 seedlings, 5D Col-  
854 0/35S:*DCL1-6Myc* seedlings, and 5D Col-0/35S:*DCL1-6Myc* seedlings irradiated with white  
855 light for one-day (5D+1L) were incubated with  $^{32}\alpha$ -UTP incorporated pri-mR166c substrate  
856 for three hours. Red arrowheads indicate the processed fragments. The red asterisk marks a  
857 band corresponding to mature miRNA size. **C.** *In vitro* pri-miRNA processing assay. Cold  
858 pri-miR166c substrates were incubated with immunoprecipitated DCL1-6Myc isolated from

859 Col-0/35S:*DCL1-6Myc* seedlings grown under 5D or 5D+1L light regimes. PAGE-resolved  
860 cleavage products were blotted with <sup>32</sup>γ-ATP-labeled miR166 probe or non-miR166 probe.  
861 The lane marked as SM contains non-radioactive synthetic 22-nt miR166 as a positive  
862 control. **D.** *In vitro* pri-miRNA processing assay. Upper panel shows the levels of  
863 immunoprecipitated DCL1-6Myc isolated from 5D and 5D+1L Col-0/35S:*DCL1-6Myc*  
864 seedlings. **E.** *In vitro* pri-miRNA processing assay. Upper panel shows the  
865 immunoprecipitated DCL1-6Myc adjusted to an equal level, isolated from isolated from 5D  
866 and 5D+1L Col-0/35S:*DCL1-6Myc* seedlings. Bottom panels of panels **D** and **E** show the  
867 cleaved pri-miR166c intermediates and mature miR166 from *in vitro* pri-miRNA processing  
868 assay. The asterisk denotes the processed miR166 from the artificial pri-miR166c substrate.  
869 SM means size maker of <sup>32</sup>γ-ATP-labeled synthetic 22-nt miR166. Red arrowhead indicates  
870 the processed fragments.

871

872 **Figure 6. Overexpression of DCL1-PAZ domain as a miR-DCL1 in Col-0 increases the**  
873 **functionality of microprocessor**

874 **A.** Schematic drawing of the microProtein(miP)-DCL1 system (Dolde et al., 2018). **B.**  
875 Tested miRNAs were up-regulated in 17-day-old light-grown (CL) miP-DCL1 over-  
876 expressing transgenic plantlets as compared to that in the wild-type plantlets. **C.** qRT-PCR  
877 analysis of *pri-miRNA* transcripts in CL Col-0 and CL miP-DCL1 over-expressing  
878 transgenic plantlets. The relative amount of pri-miRNAs was calculated with ±s.d. from  
879 three biological repeats (\**P* < 0.05; \*\**P* < 0.005; unpaired, two-tailed Student's *t*-test). **D-**  
880 **F.** Northern blot analyses show restoration of light-induced reduction of microprocessor  
881 activity in the miP-DCL1 system. Overexpression of miP-DCL1 maintains or further  
882 increases miRNA production in 5D seedlings and 5D seedlings that were irradiated for 1, 2  
883 or 3 days (5D+1L, 5D+2L, 5D+3L) with white light. The loading control was 5.8S rRNA. **G.**  
884 5D+1L seedlings of miP-DCL1 grown in sugar-free MS medium showing proportional  
885 reduction in hypocotyl opening. **H.** 6D+5L seedling of miP-DCL1 transgenic plants grown  
886 in sugar-free MS medium with a significantly low survival rate during photomorphogenesis.  
887 The data are average of six biological samples with ±s.d. (*n*=180). **I.** Two-week-old plants  
888 expression miP-DCL1 show slight defects in rosette development.

889

890 **Figure 7. Light induces the accumulation of SDN1 transcripts and proteins**

891 **A.** qRT-PCR analysis of *SDN1*, *SDN2*, and *SDN3* transcripts in etiolated seedlings (5 to 8-

892 days old) or in five-day old etiolated (5D) seedlings irradiated with white light for 1-3 days  
893 (5D+1L - 5D+3L). The data are average of four biological samples with  $\pm$ s.d. ( $n=12$ ).  
894 *UBQ10* transcripts were used as the calibration reference. **B.** Expression profile of SDN1  
895 protein in etiolated seedlings (5 to 8-days old) or in 5D seedlings irradiated with white light  
896 for 1-3 days (5D+1L - 5D+3L). The levels of endogenous SDN1 were determined with  $\alpha$ -  
897 SDN1 antibody. In all tests, uniform sample loading was ascertained with  $\alpha$ -actin and  $\alpha$ -  
898 histone antibodies. **C.** Amanitin-chase assay. 5D+2L seedlings and 5D seedlings were  
899 treated with  $\alpha$ -amanitin (10 mM) for 6 h to block *MIR* gene transcription and miRNA decay  
900 monitored (upper panel). Levels of miRNA were determined by northern blot analysis. The  
901 loading control was 5.8S rRNA (lower panel). Red asterisks denote rapidly-decaying miRNAs.  
902 **D.** The quantification of pri-miRNA levels in 5D+2L seedlings based on droplet digital PCR  
903 analysis. The ordinate scales indicate the fluorescent amplitude. Pink line indicates the  
904 threshold, above which, blue dots are positive droplets containing at least one copy of the target  
905 cDNA, and below which, gray dots indicate negative droplets without the target cDNA (upper  
906 panel). The bar graph shows the total read counts of each pri-miRNA in the 5D+2L sample  
907 (lower panel). The data are values of the PoissonConfMax/Min that normalize for the  
908 high/low error bar of the droplet Poisson distribution for the 95% confidence interval. **E.**  
909 Graphical abstract of the miRNA-biogenetic inconsistency