Processing bodies control the selective translation for optimal development of Arabidopsis young seedlings

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Germinated plant seeds buried in soil undergo skotomorphogenic development before emergence to reach the light environment. Young seedlings transitioning from dark to light undergo photomorphogenic development. During photomorphogenesis, light alters the transcriptome and enhances the translation of thousands of mRNAs during the dark-to-light transition in *Arabidopsis* young seedlings. About 1,500 of these mRNAs have comparable abundance before and after light treatment, which implies widespread translational repression in dark-grown seedlings. Processing bodies (p-bodies), the cytoplasmic granules found in diverse organisms, can balance the storage, degradation, and translation of mRNAs. However, the function of p-bodies in translation control remains largely unknown in plants. Here we found that an *Arabidopsis* mutant defective in p-body formation (Decapping 5; *dcp5-1*) showed reduced fitness under both dark and light conditions. Comparative transcriptome and translatome analyses of wild-type and *dcp5-1* seedlings revealed that p-bodies can attenuate the premature translation of specific mRNAs in the dark, including those encoding enzymes for protochlorophyllide synthesis and PIN-LIKES3 for auxin-dependent apical hook opening. When the seedlings protrude from soil, light perception by photoreceptors triggers a reduced accumulation of p-bodies to release the translationally stalled mRNAs for active translation of mRNAs encoding proteins needed for photomorphogenesis. Our data support a key role for p-bodies in translation repression, an essential mechanism for proper skotomorphogenesis and timely photomorphogenesis in seedlings.

photomorphogenesis | skotomorphogenesis | translation | p-bodies | light

Plant seeds are often buried in soil. After germination, a young seedling elongates to protrude from the soil and reach sunlight for photomorphogenic development. Photomorphogenesis is an essential developmental process transforming a young seedling from heterotrophic to autotrophic growth. Successful photomorphogenic development depends on an intricate coordination of massive transcriptional reprogramming and selective protein degradation (1–3). Light treatment additionally enhances a global translation by increasing both ribosome occupancy and density on mRNAs in de-etiolating *Arabidopsis* (4, 5). Nearly 40% of the mRNAs with light-enhanced translation show no increase in transcript levels after illumination (4). These mRNAs may preexist but are translationally inert in dark-grown seedlings and become actively translated only with light treatment.

RNA granules, the cytoplasmic foci composed of mRNA–ribonucleoprotein complexes, are modulators of mRNA translation and decay (6–8). RNA granules are classified as processing bodies (p-bodies), stress granules, neuronal granules, germ cell granules, and so on, according to their composition and presence in different cell types (9). P-bodies are detected at various developmental stages or under stress conditions in yeast and mammals (10). In plants, p-body components are required for postembryonic development and responses to heat and osmotic stress (11–16). P-bodies are often considered the site for mRNA decay because of their association with the decapping complexes (17). However, increasing evidence from studies in yeast and human cells suggests that p-bodies also temporarily store translationally silenced mRNAs that may reenter translation (18–20).

Here, we demonstrate that p-bodies are required to stall the translation of thousands of mRNAs in dark-grown *Arabidopsis* seedlings. Light triggers the reduced accumulation of p-bodies in young seedlings, which then release the mRNAs for active translation to produce proteins essential for photomorphogenic development. We also provide evidence linking the premature translation of key mRNAs and compromised growth and developmental fitness of an *Arabidopsis* mutant defective in p-body formation. This study offers biological significance and the mechanistic insights of selective translation mediated by p-bodies to ensure plants' adequate adaptation to dark and light environments during early seedling development.

Results

The P-Body Component DCP5 Negatively Regulates Photomorphogenesis. Light enhances the translation of thousands of transcripts including those encoding proteins for photosynthesis and the translation apparatus essential for de-etiolation (4). The role of p-bodies in translational repression (12, 18) prompted us to examine whether p-bodies also regulate translation in de-etiolating *Arabidopsis* seedlings. If they do, then *Arabidopsis* mutants defective in p-body formation would exhibit abnormal photomorphogenic development. Because available knockout mutants of p-body core components exhibit

Significance

Photomorphogenic development allows young plant seedlings to acquire photosynthetic activities and growth competitiveness when they emerge from soil. More than 1,000 mRNAs are translationally inert in dark-grown seedlings but quickly engage in translation when seedlings are first exposed to light signals. However, why some mRNAs undergo selective translation, how the selective translation is regulated, and why it is important remain unknown. Our work fills in this missing gap by demonstrating that processing bodies function as mRNA reservoirs in dark-grown seedlings. Photoreceptors negate the function of a negative regulator CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), thereby reducing the accumulation of processing bodies in light-grown seedlings to release thousands of mRNAs for translation, which explains in part the light-enhanced global translation in photomorphogenic seedlings.

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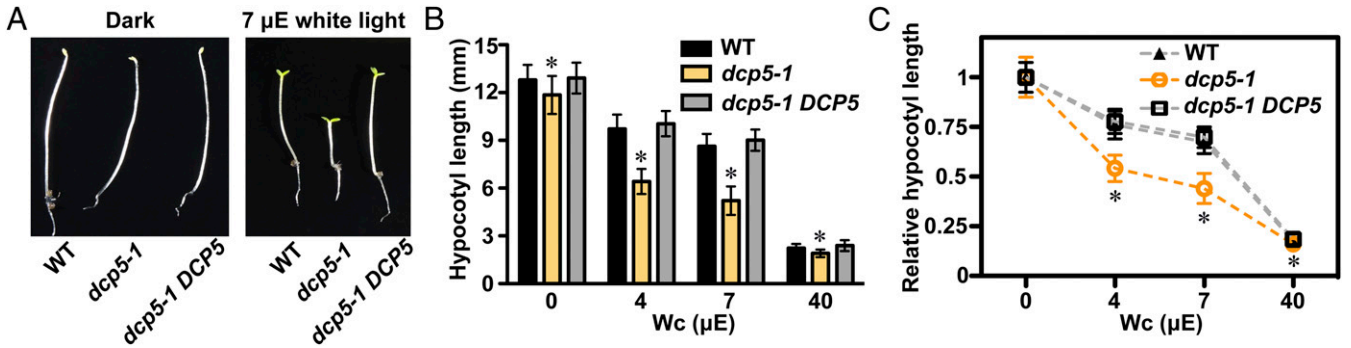

Fig. 1. *dcp5-1* mutant is hypersensitive to light. (*A*) Representative images of 4-d-old WT, *dcp5-1*, and *dcp5-1 DCP5* complementation line grown under the dark or 7 μE white light. (*B*) Fluence response curves of 4-d-old WT, *dcp5-1*, and *dcp5-1 DCP5* complementation line under dark, 4 μE, 7 μE, and 40 μE continuous white light (Wc). (*C*) Fluence response curves of normalized hypocotyl length to that of corresponding genotype grown under the dark condition. Asterisks in *B* and *C* indicate shorter hypocotyl length for *dcp5-1* than the WT (Student's *t* test; $*P < 0.001$). Data are mean ± SD from one representative experiment ($n \geq 30$). Similar results were observed in three independent experiments.

postembryonic lethality, we used *dcp5-1*, a weak p-body mutant with reduced expression of *DECAPPING 5* (*DCP5*). DCP5 is an *Arabidopsis* p-body component critical for p-body formation (12).

In the photomorphogenic development of *Arabidopsis*, light inhibits hypocotyl elongation in a fluence-dependent manner. Four-day-old *dcp5-1* seedlings had shorter hypocotyls than wild-type (WT) seedlings under all fluences of white light (Wc) examined (Fig. 1 *A* and *B*). The fluence rate response curves shown in Fig. 1*C* clearly indicate that the exaggerated inhibition of hypocotyl elongation in *dcp5-1* was light-dependent. *dcp5-1* seedlings were also hypersensitive to monochromatic blue, red, and far-red light (*SI Appendix*, Fig. S1). The light-hypersensitivity of *dcp5-1* could be complemented by the expression of a genomic fragment containing *DCP5* driven by its native promoter (*dcp5-1 DCP5* in Fig. 1).

These results indicate a negative role of DCP5 in conveying light signals for photomorphogenic development. Because DCP5 plays a pivotal role in p-body formation, we next investigated whether p-bodies are regulated by light.

Light Triggers Reduced Accumulation of P-Bodies in De-Etiolating Seedlings. To evaluate how light regulates p-body dynamics, we first checked the transcript levels of genes encoding p-body components during de-etiolation: *DCP1*, *DCP2*, *VARICOSE* (*VCS*), *DCP5*, *EXORIBONUCLEASE 4* (*XRN4*), and the RNA-binding protein CCCH tandem zinc finger protein 1 (*TZF1*) (21, 22). With the exception of *DCP2* and *TZF1* showing a reduced expression, the expression of *DCP1*, *DCP5*, *VCS*, and *XRN4* remained largely unchanged during the de-etiolation process (*SI Appendix*, Fig. S2). These genes also express ubiquitously throughout the developmental stages when their expression data were queried in the *Arabidopsis* eFP browser (bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi).

By expressing fluorescent protein tagged DCP1, DCP2, or VCS in transgenic *Arabidopsis*, p-bodies could be visualized in roots and epidermal cells of inflorescence stem (11–14, 16). Heat stress was found to stimulate the assembly of p-bodies composed of both DCP1 and DCP2, whereas only DCP1-containing p-bodies will form in response to cold stress (16). RNA granules have also been microscopically detected in etiolated seedlings (22). We examined whether p-bodies represented these RNA granules by monitoring yellow fluorescent protein (YFP)-tagged DCP2, an evolutionarily conserved p-body component (11), in transgenic *Arabidopsis*. A construct harboring *DCP2-YFP* driven by a constitutive 35S promoter could complement the *dcp2-1* postembryonic lethality phenotype, which confirms that DCP2-YFP was biologically functional (*SI Appendix*, Fig. S3).

P-bodies were clearly observed in cotyledons of etiolated seedlings (Fig. 2*A*). These florescent foci were likely bona fide p-bodies because their number was markedly reduced with cycloheximide treatment (*SI Appendix*, Fig. S4*A*). Cycloheximide is a translation inhibitor commonly used to stall translation by trapping mRNAs on polysomes, thus reducing the number of mRNAs destined for p-bodies for triage and leading to a decreased number of p-bodies (17). The number of p-bodies was also decreased in *dcp5-1* (*SI Appendix*, Fig. S4*B*), which is consistent with DCP5's role in p-body formation (12).

After 4 h light illumination (L4h), both the number and size of p-bodies in de-etiolating WT seedlings were reduced (Fig. 2 *A* and *B*), even though the protein level of DCP2-YFP driven by a 35S promoter was comparable in seedlings before and after light treatment (Fig. 2*C*). A dynamic decrease in p-body number, but not protein level, in response to light was also observed in transgenic plants expressing a YFP-tagged *DCP2* genomic fragment under the control of its native promoter (*SI Appendix*, Fig. S5). Therefore, the reduced *DCP2* mRNA level (*SI Appendix*, Fig. S2) did not lead to a lower DCP2 protein level. Also, the reduced p-body accumulation on light treatment shown in Fig. 2*B* did not result from decreased DCP2 protein level.

Light-Mediated P-Body Reduction Depends on Light Perception and Signaling. We next addressed whether light perception and signaling are involved in light-mediated reduction of p-body accumulation. DCP2-YFP was introduced into *Arabidopsis* mutants impaired in *HY2*, which encodes phytochromobilin synthase that catalyzes the formation of phytochrome chromophores (23). The covalent binding of phytochromobilins with phytochrome apoproteins allows phytochromes to become red and far-red light photoreceptors. With much-reduced accumulation of photoactive phytochromes, the *hy2-106* mutant is less sensitive to light and exhibits a long hypocotyl phenotype under light (23). The number of DCP2-YFP fluorescent foci was comparable in *hy2-106* seedlings with or without L4h (Fig. 2 *D* and *E*), which implies that phytochromes are the primary photoreceptors for the light-mediated reduction of p-body accumulation.

For the role of light signaling, we investigated DCP2-YFP cytoplasmic foci dynamics in an *Arabidopsis* mutant defective in CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), an E3 ubiquitin ligase and a negative regulator of photomorphogenesis (24–26). Dark-grown *cop1-6* mutants have open cotyledons and short hypocotyls, resembling light-grown WT seedlings (27). P-bodies labeled by DCP2-YFP were barely detectable in etiolated *cop1-6* seedlings, which was consistent with its photomorphogenic phenotype of light-grown seedlings (Fig. 2*F*). We also confirmed that the different patterns of p-body foci

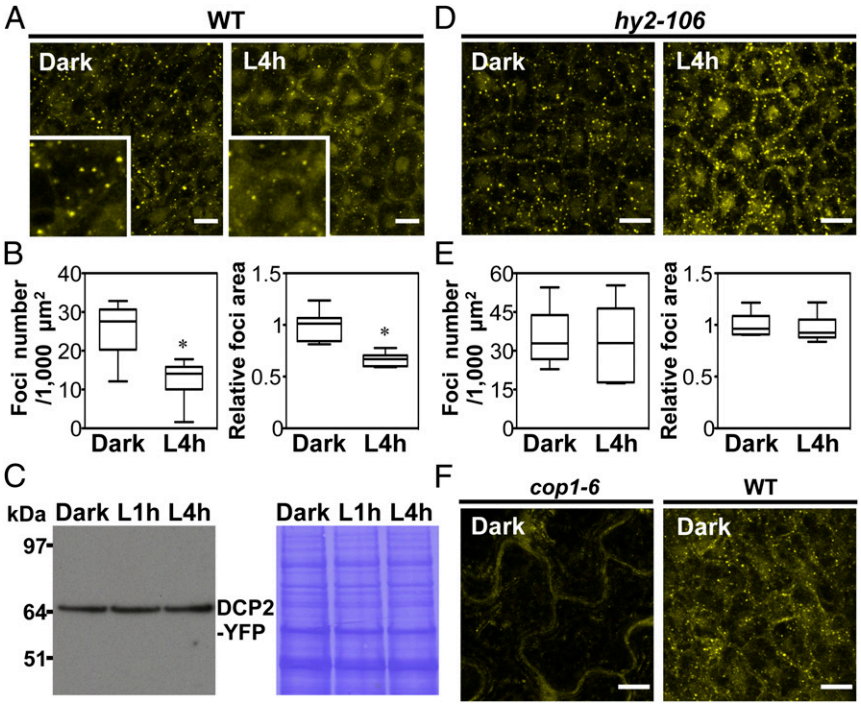

Fig. 2. Light reduces p-body accumulation. (*A* and *B*) Representative images of p-bodies observed in cotyledons of WT expressing DCP2-YFP. Enlarged images were shown in *Insets* at bottom left corners. DCP2-YFP foci number and relative foci area (normalized to the mean of Dark samples) were calculated as described in *SI Appendix, SI Materials and Methods*. Box and whisker plots from one representative experiment are shown [n = 7 (Dark); *n* = 6 (L4h)]. The top, middle, and bottom of the box represent the 25th, 50th and 75th percentiles, respectively. The whiskers are minimum and maximum. Similar results were observed in three independent experiments. Asterisk indicates significantly different number and size for p-bodies in L4h vs. the Dark samples (Student's *t* test; $*P < 0.005$). (*C*, *Left*) shows protein level of DCP2-YFP in 4-d-old WT seedlings before and after light treatment for 1 or 4 h. (*C*, *Right*) shows Coomassie blue-stained blot as a protein loading control. (*D* and *E*) Representative photographs and plots of DCP2-YFP in *hy2-106* under Dark and L4h (*n* = 6). (*F*) DCP2-YFP in etiolated *cop1-6* mutant and WT. (Scale bar, 10 μm.)

among WT, *cop1-6*, and *hy2-106* seedlings were not caused by changes in DCP2-YFP or DCP5 protein level (*SI Appendix*, Fig. S6).

These results together indicate that the negative regulator COP1 is needed for the accumulation of p-bodies in the dark-grown seedlings, and that the reduced number of p-bodies in light-grown seedlings is largely modulated by the photoreceptor phytochromes, possibly by repressing the action of COP1.

P-Bodies Repress the Translation of Thousands of mRNAs in Etiolated Seedlings. We previously showed that the translation of more than 1,000 mRNAs was enhanced by light, even though the abundance of these mRNAs remained comparable before and after light treatment (4). The mRNA storage capacity of p-bodies prompted us to hypothesize that these preexisting mRNAs are stored in p-bodies in etiolated seedlings, and that light triggers a reduction in the number of p-bodies to release stored mRNAs for

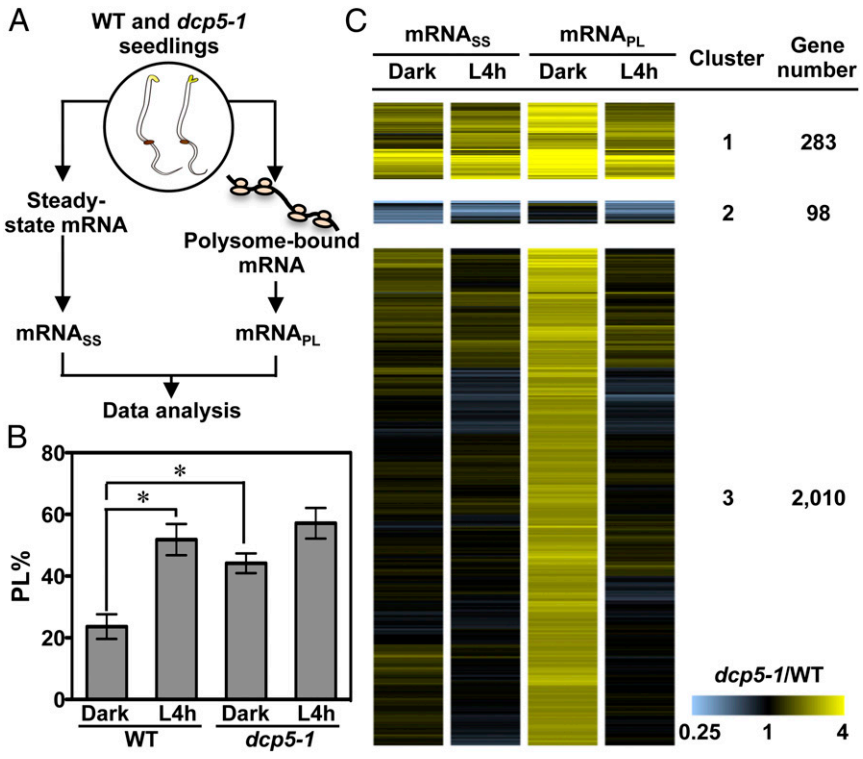

Fig. 3. P-bodies repress massive translation in etiolated seedlings. (*A*) An illustration of the experimental design. Steady-state mRNAs ($mRNA_{SS}$) and polysome-bound ($mRNA_{PL}$) were isolated in parallel and hybridized to Affymetrix ATH1 GeneChips for transcriptomic profiling analyses. (*B*) PL% in WT and *dcp5-1* under Dark and L4h conditions. Data are mean ± SEM from four biological replicates. Spike-in RNA (*DAP*) was used for data normalization. Asterisk indicates translation efficiency of conditions statistically different from that of 4-d-old etiolated WT seedlings (Student's *t* test; $*P < 0.05$). (*C*) Classification of DCP5-regulated genes at $mRNA_{SS}$ or $mRNA_{PL}$ level in *dcp5-1* normalized to the WT. K-means clustering was used to classify the 2,391 genes regulated by DCP5. Extreme yellow and blue colors indicate fourfold up-regulation and down-regulation, respectively.

active translation. To test this hypothesis, the mRNA populations of polysome-bound ($mRNA_{PL}$) and steady-state mRNA ($mRNA_{SS}$) from 4-d-old etiolated *dcp5-1* and WT seedlings before (Dark) and after L4h were individually profiled and compared (Fig. 3*A*). The fraction of $mRNA_{PL}$ in total RNA (including both nonpolysome and polysome-bound RNAs, representative profiles shown in *SI Appendix*, Fig. S7) was designated PL%, representing the proportion of total RNA committed to active translation (4). Clearly, in the dark, more active translation occurred in *dcp5-1* than WT seedlings (Fig. 3*B*), which suggests that p-bodies function to repress translation in etiolated seedlings.

P-bodies are associated with both mRNA decay and translation (10, 17). To determine the potential effect of p-bodies on the fate of mRNAs, we profiled and compared transcriptomes ($mRNA_{SS}$) and translatomes (inferred from $mRNA_{PL}$) of *dcp5-1* and WT seedlings with the criteria shown in *SI Appendix*, Fig. S8. Compared with the WT, in etiolated *dcp5-1* plants, 2,391 genes were identified as DCP5-regulated genes with statistically significant changes at the $mRNA_{SS}$ or $mRNA_{PL}$ level (Dataset). These genes were subjected to *k*-means clustering analysis to determine the extent and type of regulation by DCP5. mRNAs in Cluster 1 (283 genes) showed a concordant increase in $mRNA_{SS}$ and $mRNA_{PL}$ levels in *dcp5-1* under both Dark and L4h (Fig. 3*C*). The mRNAs in cluster 1 are likely targets of de-capping and RNA degradation by p-bodies. mRNAs in Cluster 2 (98 genes) showed a slight reduction in $mRNA_{SS}$ or $mRNA_{PL}$ level in *dcp5-1*, so p-bodies are required to maintain the steady-state level or translation efficiency of these mRNAs. Cluster 3, representing the largest group of mRNAs (2,010 genes), showed increased translation but negligible changes in $mRNA_{SS}$ level in *dcp5-1* under the Dark condition. More than 80% of DCP5-regulated genes are in Cluster 3. *SI Appendix*, Table S1 shows that these genes are overrepresented in encoding proteins for translation machinery and photosynthesis. P-bodies likely govern the translation repression of Cluster 3 mRNAs in dark-grown seedlings.

qRT-PCR was used to confirm the transcriptome data for two to three selected genes in each Cluster (*SI Appendix*, Fig. S9). Also, for proteins with available antisera, we validated the increased $mRNA_{PL}$ levels for *psbO1*, encoding oxygen-evolving complex protein OE33 (28, 29), and GENOMES UNCOUPLED 5 (GUN5) (30) in Cluster 3, which indeed showed increased OE33 and GUN5 protein levels in etiolated *dcp5-1* than in WT seedlings (*SI Appendix*, Fig. S10).

A compromised de-capping activity was previously observed in *dcp5-1* (12). We next assessed whether the increased translation in *dcp5-1* mutant was an indirect consequence of its reduced de-capping activity. We adopted a modified RNA Ligase Mediated-Rapid Amplification of cDNA Ends procedure (detailed in *SI Appendix*, *SI Material and Method*) for a semiquantitative measurement of capped and de-capped mRNAs, separately, in both WT and *dcp5-1* (*SI Appendix*, Fig. S11 *A* and *B*). For *AT3G22620*, a Cluster 1 gene, the proportion of de-capped mRNA decreased from more than 30% to less than 10% of the total mRNA population, and the $mRNA_{SS}$ level increased in etiolated *dcp5-1* seedlings (*SI Appendix*, Fig. S11*C*), which is consistent with the transcriptome and qRT-PCR results (Fig. 3*C*

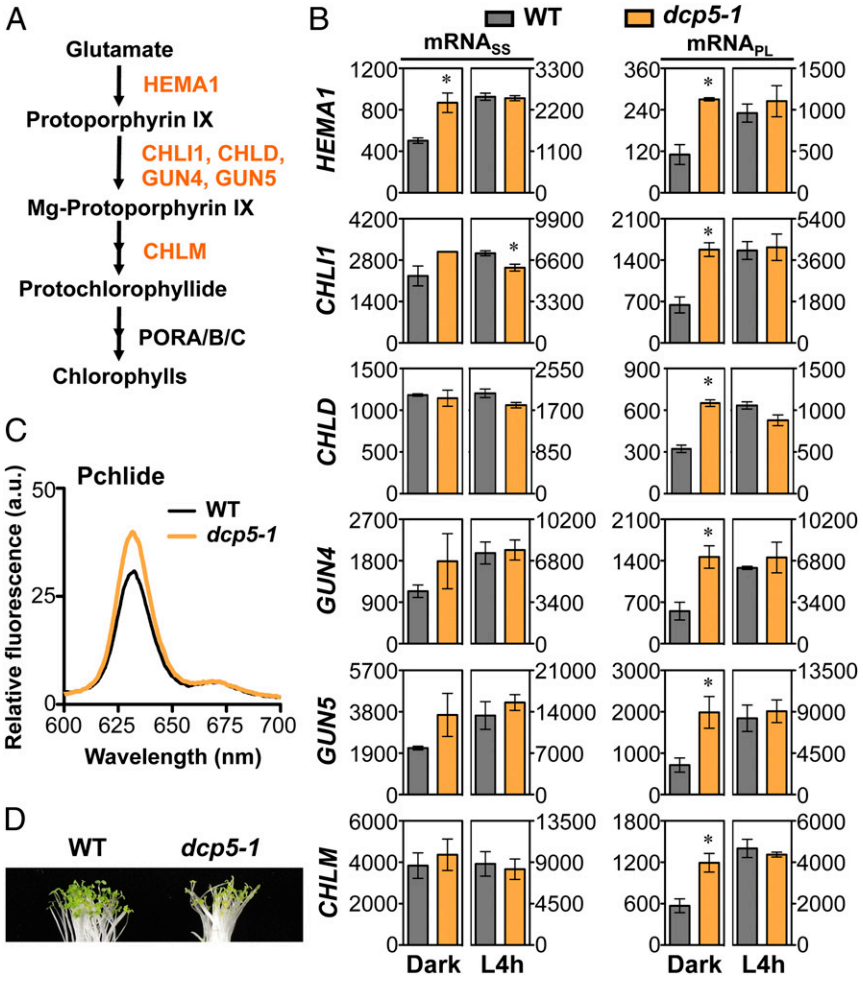

Fig. 4. DCP5 regulates the translation of genes encoding the chlorophyll biosynthetic enzymes. (*A*) A diagram of chlorophyll biosynthesis pathway. Genes with translation regulated by DCP5 are in orange. (*B*) Expression of $mRNA_{SS}$ and $mRNA_{PL}$ in *dcp5-1* or WT plants under Dark or L4h. Data are mean ± SEM from three biological replicates. Asterisk indicates that the level of a given gene significantly differs in *dcp5-1* vs. the WT (Student's *t* test; $*P < 0.05$). (*C*) Pchlide level in etiolated WT and *dcp5-1* seedlings. *y* axis marks relative fluorescence with arbitrary units (a.u.). (*D*) The representative photograph showing photobleaching phenotype was only observed in *dcp5-1* seedlings.

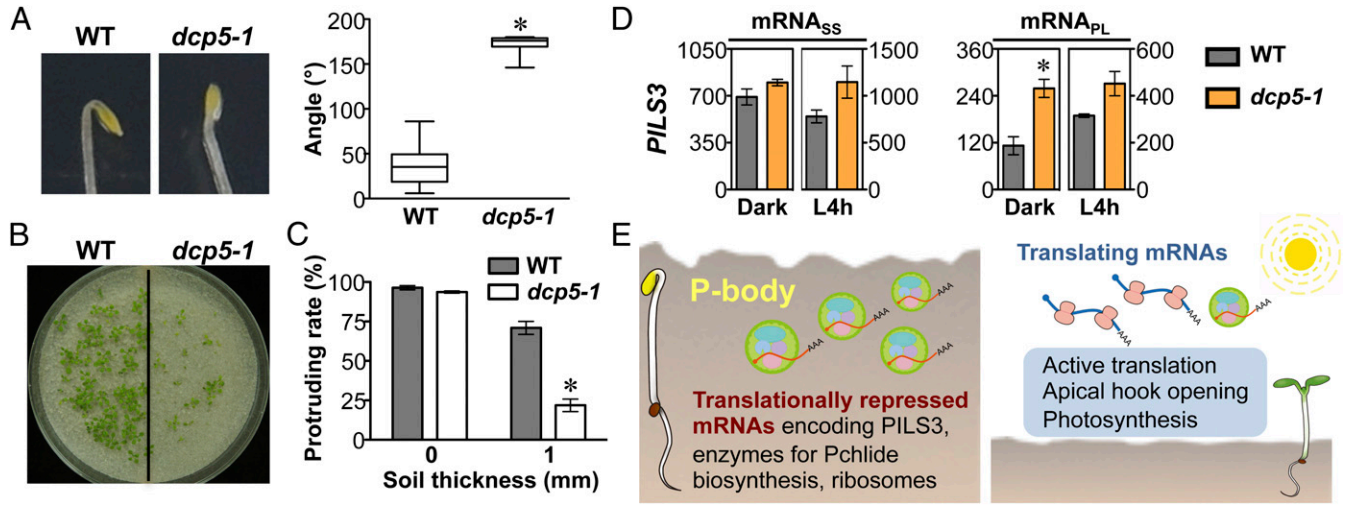

Fig. 5. DCP5 is required for skotomorphogenic development. (*A*) Representative photograph and box-and-whisker plot of apical hook angle of 4-d-old etiolated WT and *dcp5-1* seedlings (n = 38–44). Asterisk indicates that the hook angle is larger for *dcp5-1* than the WT (Student's t test; *$P < 0.001$). (*B*) Representative photograph of WT and *dcp5-1* after germination under 1 mm sand. (*C*) Protruding rate of WT and *dcp5-1* seedlings grown with or without coverage of 1 mm sand. Data are mean ± SEM from three biological replicates (n = 98–126). Asterisk represents a much-reduced protrusion rate for *dcp5-1* than the WT (Student's t test; *$P < 0.001$). (*D*) Expression of *PILS3* $mRNA_{SS}$ and $mRNA_{PL}$ in *dcp5-1* or WT under Dark or L4h. Data are mean ± SEM from three biological replicates. Asterisk indicates that the level of *PILS3* in *dcp5-1* is statistically different from that in WT (Student's t test; *$P < 0.05$). (*E*) A working model for translational regulation by p-bodies during de-etiolation. Image courtesy of Hsuan Pai (Academia Sinica, Taipei, Taiwan).

and *SI Appendix*, Fig. S9). However, most of the mRNAs for a Cluster 3 gene, *psbO1*, were capped in both WT and *dcp5-1* (*SI Appendix*, Fig. S11*D*). No noticeable increase of de-capped *psbO1* mRNA was observed in *dcp5-1*. Thus, *psbO1* mRNA was not a target of de-capping activity associated with p-bodies and explained the comparable *psbO1* mRNA levels in the WT and *dcp5-1* (Fig. 3*C* and *SI Appendix*, Fig. S9). These results imply that for most DCP5-regulated mRNAs (those in Cluster 3), p-bodies primarily contribute to translation repression rather than de-capping and mRNA decay during photomorphogenic development in *Arabidopsis*.

P-Bodies Attenuate the Translation of Protochlorophyllide Biosynthesis Genes. Among genes with translation regulated by DCP5, genes in the protochlorophyllide (Pchlide) biosynthesis pathway caught our attention (Dataset, Cluster 3). Pchlide is the precursor of chlorophyll (Fig. 4*A*). Etiolated seedlings with abnormally high levels of Pchlide are easily bleached by light (31–33). Therefore, the accumulation of Pchlide must be strictly controlled in etiolated seedlings to avoid photobleaching on light illumination (34, 35). Etiolated *dcp5-1* seedlings showed enhanced translation of genes encoding Pchlide biosynthetic enzymes, including glutamyl tRNA reductase (HEMA1), CHLI subunit of magnesium chelatase (CHLI1), GUN4, GUN5, Mg-chelatase D subunit (CHLD), and Mg protoporphyrin IX methyltransferase (CHLM; Fig. 4*B*). Except for a modest increase in *HEMA1* mRNA (1.75 ± 0.27-fold) in etiolated *dcp5*-1 compared with the WT, all other genes had negligible changes at the $mRNA_{SS}$ level between the WT and *dcp5-1* (Fig. 4*B*).

Consistent with the increased translation of these mRNAs, Pchlide level was increased in etiolated *dcp5-1* seedlings (Fig. 4*C*), and *dcp5-1* seedlings were more prone to be photobleached (Fig. 4*D*). Our data support that p-bodies function to prevent overproduction of Pchlide in etiolated seedlings by attenuating the translation of Pchlide biosynthetic genes. This mechanism reduces the risk of de-etiolating seedlings being photobleached when first exposed to light.

P-Bodies Optimize Skotomorphogenic Development. Etiolated *dcp5-1* plants showed increased translation of thousands of mRNAs and phenotypes mimicking weak photomorphogenic development, including slightly shorter hypocotyls (Fig. 1*B*), increased accumulation of anthocyanin, and increased expression of *CAB2* (*SI Appendix*, Fig. S12). Compared with WT seedlings, *dcp5-1* plants also had a partially open apical hook with an increased angle between the cotyledon and hypocotyl (Fig. 5*A*). The premature opening of the apical hook also rendered a significantly reduced emergence rate for *dcp5-1* mutants when germinated under a 1-mm layer of sand (Fig. 5 *B* and *C*).

An auxin-dependent asymmetric growth of the upper hypocotyl can contribute to the apical hook formation (36). Under the light condition, PIN-LIKES (PILS), putative auxin carriers, function to reduce nuclear auxin signaling, which leads to apical hook opening (37). The translation of *PILS3* mRNA was significantly increased in dark-grown *dcp5-1* (Fig. 5*D*). Hence, in dark-grown seedlings, p-bodies may contribute to maintaining apical hooks by repressing the translation of *PILS3* to ensure the growth fitness of young seedlings in penetrating the soil.

Discussion

Environmental light signals markedly shape the transcriptome and translatome of young plant seedlings. Recent studies have elegantly showed that light can impose gene expression regulation at various levels of central dogma, including alternative promoter selection, alternative splicing, and translation (5, 38–40). Our mechanistic study elucidated that p-bodies control the selective translation of mRNAs to ensure a highly coordinated and successful development of both etiolated and de-etiolating young seedlings (Fig. 5*E*). Without p-bodies, the growth fitness of both etiolated and de-etiolating seedlings is significantly compromised, including impeded protrusion from the soil by the partially open cotyledons (Fig. 5*A*), increased risk of photobleaching (Fig. 4 *C* and *D*), and dwarf plant stature (Fig. 1).

Only limited studies have highlighted the roles of p-bodies in translation control in plants. Two previous reports showed that p-bodies could regulate the translation of *EIN3-BINDING F-BOX 1* and *BINDING F-BOX 2* (*EBF1* and *EBF2*) mRNAs (41, 42). On perceiving the ethylene signal, *EBF1* and *EBF2* mRNAs are targeted to p-bodies, where their translation is inhibited to negate their inhibitory roles in ethylene responses

(41, 42). In addition, the translation of mRNAs encoding seed storage proteins was found to be enhanced in the germinated *dcp5-1* mutant, which suggests that p-bodies negatively regulate the translation of these mRNAs (12). Our current report indicates that p-bodies could halt the translation of nearly 20% of the expressed mRNAs in dark-grown seedlings, which significantly increases the repertoire of mRNAs whose translation is under the control of p-bodies. A recent study of purifying p-bodies of mammalian cells also demonstrated that thousands of mRNAs accumulated in p-bodies with translational repression, but not degradation (20). These findings support our model that p-bodies can serve as mRNA reservoirs in regulating the homeostasis of mRNA translation.

In mammals, both the Argonaute proteins in the RNA-induced silencing complex and mRNAs targeted by microRNAs (miRNAs) were found to localize in p-bodies, which suggests a link between miRNA function and p-bodies (43, 44). In plants, *Arabidopsis* Argonaute 1 also colocalizes with p-bodies (22). Hundreds of expressed mRNAs carrying miRNA target sites possessed lower translation efficiency in etiolated seedlings than those not targeted by miRNAs (5). Among these miRNA targets, 600 were considered expression and 93 were in Cluster 3 ($P = 0.27$, two-tailed Fisher exact test), which suggests that only a small fraction of the miRNA-mediated translation repression was mediated by DCP5 or p-bodies (Dataset).

P-bodies are not the only RNA granules that function to orchestrate translation control. Poorly translated mRNAs were found to associate with stress granules in plants grown under hypoxia stress (45). Hence, RNA granules of different origins could exert translation control in plants undergoing developmental transition and also responding to environmental fluctuations. Future studies of the underlying mechanisms used by different RNA granules to sequester selected mRNAs would reveal a deeper understanding of the regulatory network of posttranscriptional and translational control.

Materials and Methods

Detailed description of plant materials, plant growth conditions, and methods for the phenotype analyses, p-bodies observation, immunoblot analysis, RNA isolation, microarray analyses, and detection of capped and decapped mRNAs can be found in *SI Appendix, SI Material and Method*.

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