Arabidopsis DET1 degrades HFR1 but stabilizes PIF1 to precisely regulate seed germination

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Seed is an essential propagation organ and a critical strategy adopted by terrestrial flowering plants to colonize the land. The ability of seeds to accurately respond to light is vital for plant survival. However, the underlying mechanism is largely unknown. In this study, we reveal a circuit of triple feed-forward loops adopted by *Arabidopsis* seeds to exclusively repress germination in dark conditions and precisely initiate germination under diverse light conditions. We identify that de-etiolated 1 (DET1), an evolutionarily conserved protein, is a central repressor of light-induced seed germination. Genetic analysis demonstrates that DET1 functions upstream of long hypocotyl in far-red 1 (HFR1) and phytochrome interacting factor 1 (PIF1), the key positive and negative transcription regulators in seed germination. We further find that DET1 and constitutive photomorphogenic 10 (COP10) target HFR1 for protein degradation by assembling a COP10–DET1–damaged DNA binding protein 1–cullin4 E3 ligase complex. Moreover, DET1 and COP10 directly interact with and promote the protein stability of PIF1. Computational modeling reveals that phytochrome B (phyB)–DET1–HFR1–PIF1 and phyB–DET1–Protease–PIF1 are new signaling pathways, independent of the previously identified phyB-PIF1 pathway, respectively mediating the rapid and time-lapse responses to light irradiation. The model-simulated results are highly consistent with their experimental validations, suggesting that our mathematical model captures the essence of *Arabidopsis* seed germination networks. Taken together, this study provides a comprehensive molecular framework for light-regulated seed germination, improving our understanding of how plants respond to changeable environments.

seed germination | DET1-COP10 | CDD-CUL4 | HFR1-PIF1 | phyB

Seed germination is controlled by a wide range of environmental factors to ensure that plants start a new lifecycle in favorable conditions. Among them, light plays a major role in initiating seed germination (1–4). Plants perceive light signals through distinct families of photoreceptors, in which the red light photoreceptor phytochrome B (phyB) mediates the initial phase of light-induced seed germination (3, 5–8). Previous studies showed that in seeds, phyB modulates downstream regulatory networks through one of its interacting factors, phytochrome interacting factor 1 (PIF1) (9–11). PIF1 is a basic helix–loop–helix (bHLH) transcription factor that plays a primary role in repressing seed germination, and PIF1 proteins are highly accumulated in dark-incubated seeds (9, 10, 12). Under light irradiation, the light-activated phyB interacts with PIF1 to induce PIF1 phosphorylation and degradation via the 26S proteasome (12–16). Our recent study identified long hypocotyl in far-red 1 (HFR1) as a core transcription regulator in seed germination (17). HFR1 positively regulates seed germination by forming heterodimers with PIF1 to sequester PIF1 from binding to its target genes (17). The HFR1–PIF1 pair governs the transcriptional networks of light-initiated seed germination (17). However, how light signals modify the HFR1–PIF1 transcriptional module to control seed germination remains unknown.

Here we report that de-etiolated 1 (DET1) and constitutive photomorphogenic 10 (COP10) function as the substrate receptor of COP10–DET1–damaged DNA binding protein 1 (DDB1)–cullin4 (CDD–CUL4) E3 ligase to target HFR1 for degradation in the dark-incubated seeds. Moreover, DET1 and COP10 directly interact with PIF1 to maintain PIF1 accumulation. These biochemical results are supported by the genetic evidence that DET1 acts upstream of both HFR1 and PIF1 to predominantly repress seed germination. Our mathematically simulated results further indicate that two feed-forward loops linked by DET1, cooperating with a direct inhibition from phyB to PIF1, constitute a core machinery for seeds to exclusively repress germination in the dark and precisely initiate germination under various light irradiations.

Results

DET1 Predominantly Represses Seed Germination in the Dark. To investigate previously unidentified components in regulating light-induced seed germination, we first examined the germination phenotypes of light signaling-related mutant seeds. In the seed germination assay, moist seeds were first exposed to white light for 1 h (1 h WL), followed by the pulse illumination of far-red light (FR) for 5 min to inactivate phyB for the true dark (D) condition (12, 18). Then the seeds were incubated in the dark for 5 d before the germination frequency was counted (Fig. 1*A*). As COP1 was previously reported to target HFR1 for degradation in seedlings (19–21), we first examined the *cop1-4* mutant but found no visible difference in germination phenotype from Columbia-0 (Col-0) (wild type, WT) (Fig. 1*A*). We further investigated the seed germination phenotypes of other mutants. Strikingly,

Significance

How organisms respond to environment changes is a fundamental and intriguing question in biology. Light is the energy resource and a crucial environmental cue for plant major developmental switches, such as seed germination. Studying the underlying mechanism is important for us to understand the basic principles of plant development and improve crop productions. Here we identify DET1 as a novel central repressor of seed germination. We further reveal that seeds use a multilevel regulatory circuit of triple feed-forward loops to sensitively and precisely mediate light-regulated germination. This study provides a comprehensive framework of how light regulates seed germination.

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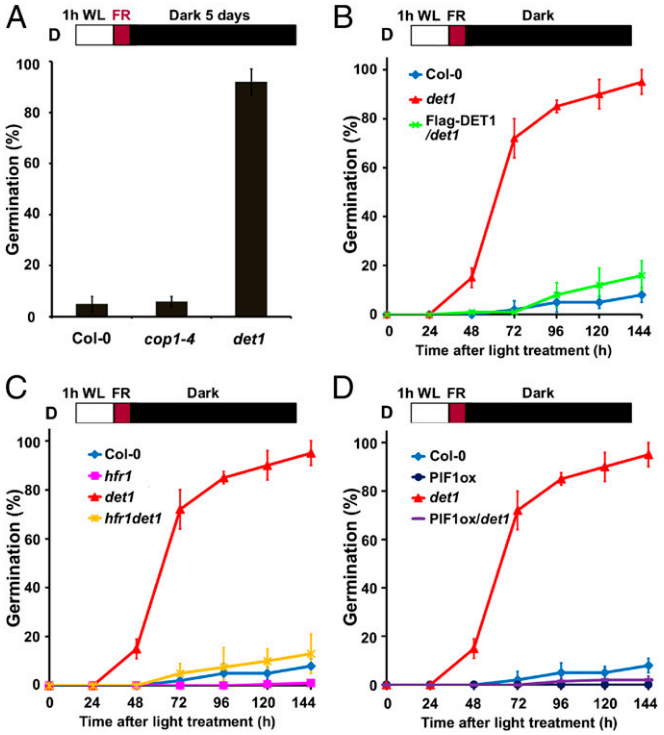

Fig. 1. DET1 acts upstream of HFR1 and PIF1 to predominantly repress seed germination in the dark. (*A–D*) Germination frequencies of imbibed seeds in the true dark condition (D condition). In the assay, seeds were irradiated with 5 min of far-red light (FR) to inactivate phyB and then incubated in the dark for 5 d and the germination frequencies were counted (*A*); or the imbibed seeds were incubated in the dark for indicated length of time, and their germination frequencies were recorded every 24 h after far-red light treatment (*B–D*). All of the seeds used in Fig. 1 *B–D* were prepared together and their germination frequencies were examined side by side, and the results are presented in separate panels with the same controls (Col-0 and *det1*) to address different questions. Mean ± SD, $n = 3$.

although the *det1* mutant displays similar constitutive photomorphogenic phenotypes in etiolated seedlings to those of *cop1-4* (22, 23), almost all of the *det1* seeds constitutively germinated in the dark (Fig. 1*A*). We also found that the *det1* seeds started to germinate after 2 d (48 h) in the dark, and the germination frequency increased with extended incubation time to reach 100% for 6 d (Fig. 1*B*). Furthermore, the repression of seed germination in the dark was completely restored in the *det1* mutant by the DET1-Flag transgene, which overexpressed the Flag epitope-tagged full-length DET1 driven by the constitutive 35S promoter of cauliflower mosaic virus (Fig. 1*B*). These results indicate that DET1 represses seed germination in the dark.

DET1 Genetically Acts Upstream of HFR1 and PIF1 in Repressing Seed Germination. Next, we analyzed the epistatic relationships between DET1 and the transcription regulators of seed germination. HFR1 is the key positive transcription regulator in promoting light-induced seed germination (17). By examining the germination phenotypes of Col-0, *hfr1*, *det1*, and *hfr1det1* in the dark, we found that *hfr1* showed no difference from Col-0, whereas the *det1* seeds constitutively germinated (Fig. 1*C*). The double-mutant *hfr1det1* exhibited similar germination frequency to that of *hfr1*, suppressing the constitutive germination phenotype of *det1* (Fig. 1*C*). These pieces of genetic evidence suggest that DET1 functions upstream of HFR1 to repress seed germination in the dark.

In addition to HFR1, PIF1 is a crucial transcription factor of light-induced seed germination, but in a negative way (9, 10, 12). Our results showed that the *pif1* mutant constitutively germinated in the dark and DET1 overexpression (DET1ox) seeds did not germinate, whereas DET1ox/*pif1* displayed constitutive germination phenotypes similar to those of *pif1* (Fig. S1). Conversely, although PIF1 overexpression (PIF1ox) did not germinate and *det1* germinated independent of light, the homozygote of PIF1ox/*det1* displayed similar phenotypes to those of PIF1ox, fully suppressing the constitutive germination phenotypes of dark-incubated *det1* seeds (Fig. 1*D*). Taken together, these genetic analyses demonstrate that DET1 functions upstream of both HFR1 and PIF1 to repress seed germination in the dark.

HFR1 Is Docked to CDD-CUL4 E3 Ligase via Direct Interaction with DET1 and COP10. It has been known that DET1 forms a stable protein complex with COP10 and DDB1, termed the CDD complex (24). The CDD complex binds to CUL4 and forms a CUL4-based multimeric E3 ligase complex in plants (25), but the substrate of the complex remains unknown. We further found that the *cop10-4* mutant partially germinated in the dark, consistent with *det1* phenotypes (Fig. S2 and Fig. 1*A*). In addition, overexpression of COP10 did not germinate even under the red light (R) condition (with an additional 5 min of red light irradiation before dark incubation) (Fig. S2), indicating the crucial roles of the CDD complex in regulating seed germination.

Then we investigated the biochemical relationship of the CDD–CUL4 complex and HFR1 protein. In yeast two-hybrid assays, COP10 bound a region of DET1 from the 26th to the 391st aa residues, whereas the HFR1 protein was found to specifically interact with a small fragment of DET1 from the 26th to the 87th aa in the DET1 N-terminal region (DET1N) (Fig. 2*A*). To map the interacting domain of HFR1, we performed yeast two-hybrid assays by using a series of deletion constructs of HFR1 with DET1N and COP10. Our results showed that either N-terminal or C-terminal portions of HFR1 including the HLH domain were capable of interacting with DET1N and COP10 (Fig. 2*B*). However, the HLH domain of HFR1 alone did not interact with DET1N or COP10, whereas the truncated HFR1 without the HLH domain showed strong interaction with DET1N and COP10 (Fig. 2*B*). These results suggest that the flanking sequences of the HLH domain are the core fragments required for HFR1 to interact with DET1 and COP10.

To investigate the in vivo interactions of HFR1 with DET1 and COP10 in plants, we carried out transient bimolecular fluorescence complementation (BiFC) and firefly luciferase complementation imaging (LCI) assays in tobacco leaves. In the BiFC assay, full-length HFR1 fused with the N-terminal region of YFP (HFR1–YFP^n) was transiently coexpressed with full-length DET1 or COP10 fused with the C-terminal region of YFP (DET1–YFP^c or COP10–YFP^c), respectively. Our results showed that HFR1–YFP^n reconstituted strong YFP fluorescence signals with either DET1–YFP^c or COP10–YFP^c in the nucleus (Fig. 2*C*), indicating that HFR1 interacts with both DET1 and COP10 in the plant nucleus. The LCI results further showed that coexpression of the HFR1-fused C terminus of luciferase (cLUC) and the DET1N- or COP10-fused N terminus of luciferase (nLUC) in tobacco leaves could reconstitute a high luciferase activity (Fig. 2 *D* and *E*), confirming the strong interaction between HFR1 and DET1 or COP10 in vivo. These results demonstrate that DET1 and COP10 directly interact with HFR1 in the nucleus of plant cells.

Because DET1 and COP10 were found to directly interact with HFR1 in plants, we wanted to know whether HFR1 could associate with the CDD–CUL4 complex. Coimmunoprecipitation results showed that HFR1 pulled down DET1 as well as CUL4 (Fig. 2*F*). Moreover, with the elevated HFR1 protein levels by the 26S proteasome-specific inhibitor MG132 treatment, the interactions between HFR1 and CUL4 or DET1 were both accordingly enhanced (Fig. 2*F*). Taken together, these results suggest that HFR1 is docked to the CDD–CUL4 complex through the physical interaction with DET1 and COP10, and DET1–

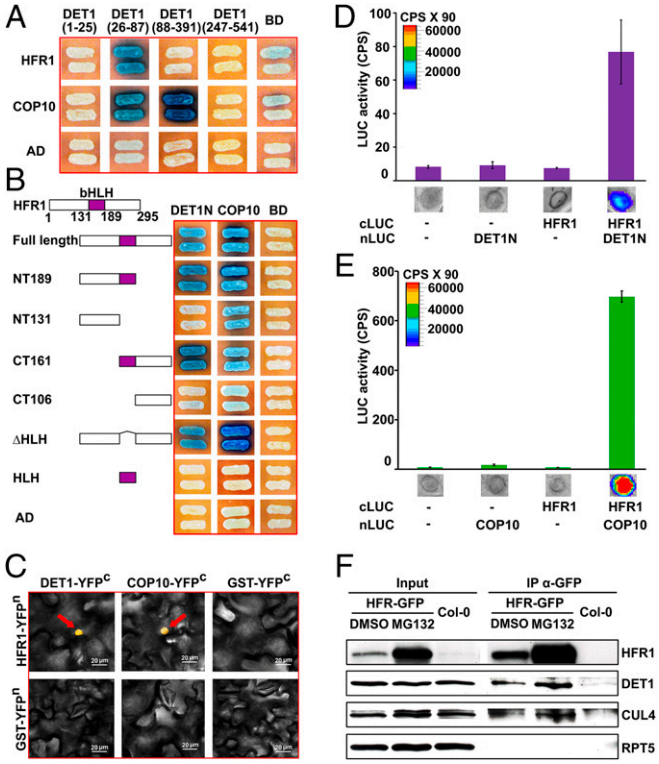

Fig. 2. DET1–COP10 directly interacts with HFR1 and recruits HFR1 to the CDD–CUL4 E3 ligase. (*A*) Yeast two-hybrid assays for interaction between HFR1, COP10, and the deletion series of DET1. The various fragments of DET1 fused with the LexA DNA-binding domain (BD) were the prey constructs. Full-length HFR1 and COP10 fused with the activation domain (AD) were used as the baits. Empty vectors (BD or AD) were the negative controls. The numbers indicate the amino acid residues in DET1. (*B*) Yeast two-hybrid analysis defines the interaction domains of HFR1 with DET1 and COP10. (*Left*) The bait constructs encoding AD alone (negative control) and AD-fused full-length HFR1 and its fragments. A BD-fused DET1N (26–87 aa), full-length COP10, and BD alone (negative control) were the prey constructs. The numbers indicate the amino acid residues in HFR1. (*C*) Bimolecular fluorescence complementation (BiFC) assay for in vivo interaction between HFR1 and DET1/COP10. Red arrow indicates the position of YFP speckle. (Scale bar, 20 μm.) (*D* and *E*) Firefly luciferase complementation imaging (LCI) analysis for the in vivo interaction between HFR1 and DET1N (*D*) or HFR1 and COP10 (*E*). CPS, counts of luciferase activities per second. Mean ± SD, $n = 5$. (*F*) Coimmunoprecipitation assay shows that HFR1 associates with the CDD–CUL4 complex in plants. Total proteins were extracted from 4-d-old etiolated seedlings of transgenic plants HFR1-GFP/*hfr1-201* and Col-0. Anti-GFP antibody was used for immunoprecipitation and anti-DET1, -CUL4, and -RPT5 antibodies were used for immunoblotting detection.

COP10 might serve as the substrate receptor of CDD–CUL4 E3 ligase to target HFR1 in plants.

HFR1 Is Targeted by CDD–CUL4 E3 Ligase for Protein Degradation. Next we examined the HFR1 protein levels in imbibed seeds. After 4 h incubation, we found that compared with the true dark condition (D4 condition), the HFR1 proteins were notably elevated in the red light R4 condition (with an additional 5 min red light irradiation and then incubated in the dark for 4 h) (Fig. S3*A*). To further illustrate the biochemical dynamics of the HFR1 protein in seeds, we performed a cell-free degradation assay in which purified HFR1-His proteins were added into the cell extracts of seeds under different incubation conditions. When added into the D4-condition seed extract, the HFR1 protein was rapidly degraded within 2 h and the degradation was largely prevented by MG132 (Fig. S3*B*). In contrast, when incubated in the R4-condition seed extract, the HFR1 protein was much more stable and the degradation was notably repressed to a lower rate comparable to that of the MG132-treated seed extract (Fig. S3*B*). These results suggest that the HFR1 protein is stabilized by light in imbibed seeds via inhibiting the 26S proteasome-mediated degradation.

Given the association of HFR1 with the CDD–CUL4 complex, we then examined whether CDD–CUL4 E3 ligase mediates HFR1 protein degradation. To investigate the effects of DET1 and COP10 on the HFR1 protein levels in imbibed seeds, we introduced the HFR1-GFP transgene into *det1-1* (*det1*) and *cop10-4* (*cop10*) mutant backgrounds by crossing HFR1-GFP/*hfr1* with the mutants. We obtained a homozygote of HFR1-GFP/*cop10hfr1*, but the adult plant of the HFR1-GFP/*det1hfr1* homozygote is sterile, forcing us to propagate the seeds in the heterozygous state for the *det1* background. Fluorescence microscope results showed that in the dark-incubated seeds (D4 condition), the HFR-GFP protein accumulation was barely observed in the *hfr1* background (control) (Fig. 3*A*). Whereas in the red light-irradiated seeds (R4 condition), the HFR1 protein was notably elevated in the control (Fig. 3*A*), consistent with the immunoblot results (Fig. S3*A*). However, in the backgrounds with mutations of DET1 (*det1*) and COP10 (*cop10*), the HFR1 proteins were highly accumulated in the dark-incubated seeds to a comparable level to that of light-irradiated HFR1-GFP/*hfr1* (control) seeds (Fig. 3*A*). In addition, light did not further stabilize the HFR1 protein in the seeds with *det1* or *cop10* backgrounds (Fig. 3*A*). These results indicate that degradation of HFR1 in the dark requires DET1–COP10, and light stabilizes HFR1 by repressing the action of DET1–COP10.

In addition to that in imbibed seeds, we also examined whether DET1 and COP10 regulate HFR1 protein degradation in seedlings. Similarly, fluorescence microscopic examination of the root cells of 4-d-old dark-grown seedlings showed that the HFR1-GFP protein levels were dramatically accumulated with the *DET1* and *COP10* mutations (Fig. 3*B*). Immunoblot analysis of dark-grown seedlings revealed that many more HFR-GFP proteins were accumulated in the *det1* and *cop10* mutant backgrounds than the control (Fig. 3*C*). Notably, higher molecular weight HFR1-GFP bands were detected in a large amount in dark-grown *det1* and *cop10* mutants, whereas they were largely decreased under light conditions (Fig. 3*C*), suggesting that the higher bands were probably modified HFR1-GFP proteins in the dark. Taken together, these results demonstrate that DET1 and

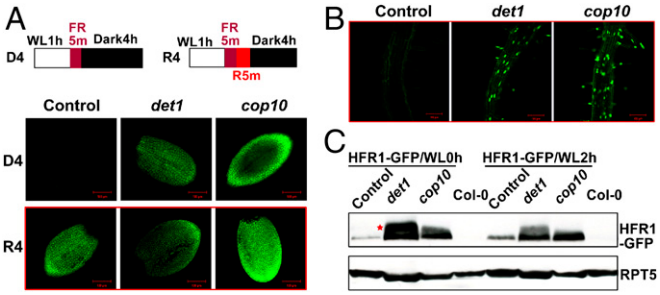

Fig. 3. DET1 and COP10 promote HFR1 protein degradation. (*A* and *B*) Fluorescence microscopic analysis of the HFR1-GFP levels in the imbibed seeds (*A*) or etiolated seedlings (*B*) of transgenic plants expressing HFR1-GFP in *hfr1-201* (control), *det1hfr1* (*det1*), and *cop10hfr1* (*cop10*) backgrounds. In *A*, *Top* diagrams indicate the light irradiation treatments used in the experiment. After 4 h dark incubation, the seed coats were removed and the photographs were taken under a fluorescence microscope. In *B*, the seedlings were grown in the dark for 4 d, and the HFR1-GFP accumulation in the root cells was examined. (Scale bar, 100 μm.) (*C*) Immunoblot analysis of the protein levels of HFR1-GFP. The seedlings were grown in the dark for 4 d without (WL0h) or with an additional 2 h of white light irradiation (WL2h). Star indicates the possibly modified higher band of HFR1-GFP proteins. Col-0 was used as a negative control, and anti-RPT5 was used as a sample loading control.

COP10 in CDD–CUL4 E3 ligase act as substrate receptors to directly target HFR1 for its protein degradation.

DET1 and COP10 Interact with PIF1 to Suppress PIF1 Degradation. As both DET1 and PIF1 repress seed germination and DET1 acts upstream of PIF1, we were intrigued about whether and how DET1 regulates PIF1 proteins in seeds. Yeast two-hybrid results showed that both DET1 and COP10 directly interacted with PIF1 (Fig. 4*A*). Interestingly, the interacting region of DET1 with PIF1 was located in the fragment of the 26th to 87th aa in DET1's N-terminal region, the same region of interaction between DET1 and HFR1 (Figs. 4*A* and 2*A*). We further confirmed the interaction of DET1 and COP10 with PIF1 *in planta* by using BiFC and LCI assays. In the BiFC assay, PIF1 was found to interact strongly with DET1 and COP10 in the nucleus of plant cells (Fig. 4*B*). Also, both DET1N and COP10 were able to interact with PIF1 to reconstitute a high luciferase activity in the LCI assay (Fig. 4*C* and Fig. S4). These results show that DET1 and COP10 directly interact with PIF1.

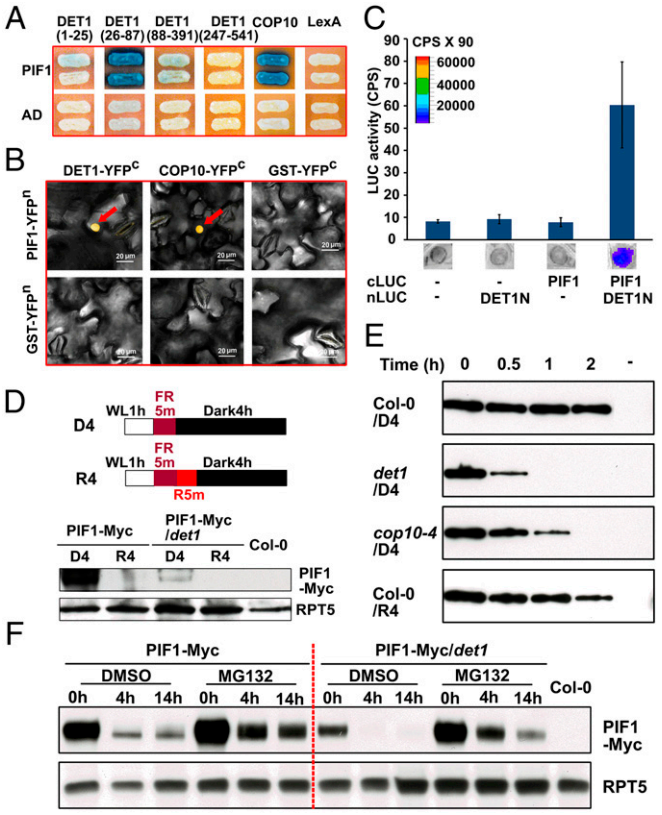

Fig. 4. DET1–COP10 directly interacts with PIF1 and represses protease-mediated PIF1 protein degradation. (*A*) Yeast two-hybrid analysis of the direct interaction between PIF1 and DET1–COP10. The fragments of DET1 and full-length COP10 were fused with LexA (BD), and PIF1 was fused to AD for the activation assay in yeast. (*B*) BiFC assay for the interaction of PIF1 and DET1–COP10 in plants. Red arrow indicates the position of YFP speckle. (Scale bar, 20 μm.) (*C*) LCI assay for the interaction of PIF1 with DET1N in tobacco leaf cells. Mean ± SD, $n = 5$. CPS, luciferase activity counts per second. (*D*) Immunoblot analyses of seed PIF1-Myc proteins in Col-0 and *det1* backgrounds. (*E*) Cell-free degradation of recombinant PIF1-His proteins in imbibed seeds. Equal amounts of purified PIF1-His proteins were incubated in the cell extracts of D4 or R4 seeds for the indicated time, and then they were probed with an anti-His antibody. "-", the mock control. (*F*) Immunoblot analysis of the PIF1-Myc protein levels. The seedlings were grown in the dark for 3.5 d, and 50 μM MG132 or DMSO was added to pretreat the seedlings for an additional 12 h. After that, the etiolated seedlings were transferred to red light for the indicated time. Col-0 was used as a negative control, and RPT5 was used as a sample loading control. The D4 and R4 diagrams indicate the light irradiation treatments used in the experiment.

Moreover, we found that the PIF1 proteins were highly accumulated in the D condition but barely detected in the R condition of imbibed seeds (Fig. 4*D*), consistent with the previous reports that light induces PIF1 degradation (12, 14–16). Strikingly, in the *det1* background, the PIF1 proteins were dramatically decreased to a very low level even in the dark-incubated seeds (Fig. 4*D*), and the PIF1 protein level further declined to an undetectable state in the R-condition seeds (Fig. 4*D*). Consistently, a recent study showed that DET1 interacted with and stabilized the PIF1 protein in etiolated seedlings (26). To illustrate the degradation dynamics of the PIF1 protein, we further performed a cell-free degradation assay. When purified PIF1-His proteins were added into the cell extracts of WT seeds in the true dark condition (D4 condition), the PIF1 proteins were stable and no obvious degradation was observed within 2 h (Fig. 4*E*). In contrast, the PIF1 proteins were rapidly degraded to an undetectable level when added into the cell extracts of true dark condition *det1* or *cop10-4* mutant (D4 *det1* or *cop10-4*) seeds (Fig. 4*E*). The degradation rate of PIF1 proteins in the dark-incubated *det1* or *cop10-4* mutant seeds was even faster than in the red-light–treated WT (R4 Col-0) seeds (Fig. 4*E*). Moreover, MG132 treatments suppressed light-induced PIF1 degradation and rescued the PIF1 protein accumulation in the dark-grown *det1* mutant to a comparable level to that of WT (Fig. 4*F*). Taken together, these results suggest that DET1 plays an essential role in preventing the 26S proteasome-mediated PIF1 degradation, therefore effectively stabilizing PIF1 in the dark, opposite to its regulation of HFR1.

Computational Modeling Reveals a Circuit of Feed-Forward Loops Precisely Controlling Seed Germination Under Dark and Diverse Light Conditions. It has been known that phyB is synthesized as an inactive Pr form in the dark and is activated by red light very quickly (within minutes), whereas the dark reversion to the inactive form requires several hours (27–30). Previous studies reported that red light-activated phyB directly interacts with PIF1 to induce PIF1 degradation, constituting the signaling pathway for light-induced seed germination (9, 12, 14–16). However, some light-induced germination behaviors cannot be well explained by this phyB–PIF1 direct inhibition model. For example, all of the WT germinated but the *hfr1* mutant and PIF1ox scarcely germinated when phyB was fully activated by 5 min of red light exposure (9, 17). More importantly, the germination frequency of the *hfr1* mutant and PIF1ox progressively increased with extended red light irradiation (17). These results indicate that there are other signaling pathways besides the known phyB–PIF1 direct inhibitory way, conducting the rapid and time-lapse germination response to diverse light irradiations. In this study, we identified that DET1 is a core component in light-induced seed germination. DET1 was further found to directly degrade HFR1 and stabilize PIF1 to act as a central repressor. To specify the roles of DET1–HFR1–PIF1 and DET1–Protease–PIF1 pathways with the known phyB–PIF1 pathway in regulating light-induced seed germination, we qualitatively analyzed the experimental results in this and previous studies (*Materials and Methods*) and built a mathematical model to simulate the seed germination network (Fig. 5*A*).

Based on previously reported results, a phyB transformation model is adopted to describe the sensing of light irradiation (27, 29–31). Then by combining the germination frequencies of WT and *hfr1* seeds under increasing periods of light irradiation (17) and the protein regulation results in this and previous studies (12, 14, 15), we formulated a "triple feed-forward loop model" for precisely controlling seed germination under dark and various light conditions (Fig. 5*A*). This model consists of two tandem linked coherent feed-forward loops: An upper loop links with a lower loop through the DET1 protein and a parallel direct

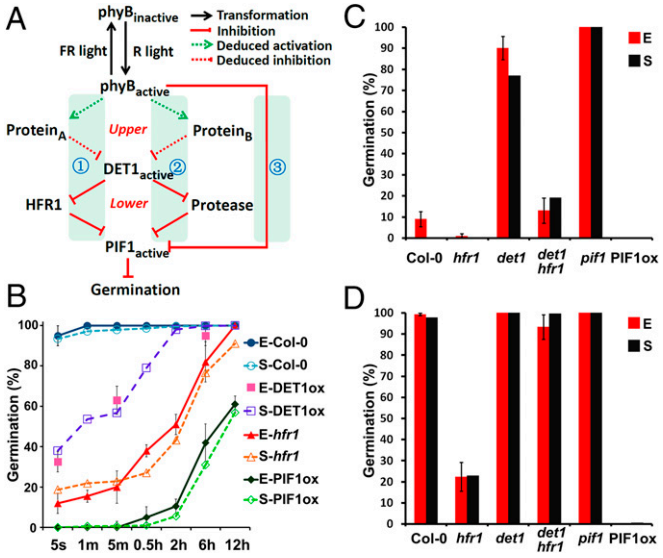

Fig. 5. Computational simulation indicates that a triple feed-forward loop circuit precisely controls seed germination under dark and diverse light conditions. (*A*) Mathematically simulated regulatory network of light-induced seed germination. The network is mainly composed of two DET1-linked coherent feed-forward loops and a direct phyB–PIF1 inhibition. The upper loop functions to precisely control the activity of DET1, and the lower loop is to forcefully regulate seed germination. In the dark, DET1 exclusively represses seed germination through the lower loop, whereas light initiates seed germination by turning off DET1 through the upper loop. phyB–PIF1 directly inhibits PIF1 to a moderate level under light conditions. (*B*) Germination response to different illumination periods of light is reproduced by the mathematical model. Comparisons of germination frequencies between experimental (E) and simulated (S) results are shown. (*C* and *D*) Experimental validation of model predictions. Comparisons of germination frequencies between experimental (E) and predicted simulation (S) results are shown. The predicted results of setting the light period parameter in the model as 0 min were experimentally validated by examining the germination of true dark condition-incubated seeds (*C*), and the predicted results of setting the light period parameter in the model as 5 min were experimentally validated by examining the germination of red light condition-treated seeds (*D*). An extrinsic noise of 20% coefficient of variation is applied to $PIF1_{\text{threshold}}$.

inhibition from phyB to PIF1, forming another feed-forward loop with the tandem loops. The upper loop accomplishes the inhibitory effect posed on the DET1 protein by light-activated phyB, whereas the regulation from active DET1 protein to PIF1 via HFR1 and Protease pathways forms the lower loop. To fit the experimental results, we predicted that there are at least two components, termed Protein_A and Protein_B, mediating light's repression on DET1. Protein_A is proposed to conduct a rapid response to red light by initiating HFR1's function, as WT seeds achieved almost full germination under 5 s or 1 min of light exposure whereas the *hfr1* mutant scarcely germinated (Fig. 5*B*). Protein_B is supposed to accumulate with extended red light irradiation to further inhibit DET1 activity, allowing the *hfr1* mutant to progressively germinate under prolonged light exposure (Fig. 5*B*). A Protein_B missing model could not work, because a quick-responding protein (Protein_A) could not achieve time-lapse response simultaneously, given the slow reversion rate of Pfr-Pfr form phyB in the dark (27, 29, 30). The lower loop was mainly based on direct experimental results, with the unknown protein in mediating light-independent PIF1 degradation marked as Protease. The previously identified phyB–PIF1 direct pathway cooperatively works with the DET1-linked tandem loops to rapidly inhibit PIF1's function and facilitate seed germination in response to light.

Then we used the mathematical model to simulate seed germination of WT and mutants under different periods of red light irradiation. Each predicted germination frequency was obtained by counting the germination events from 10,000 runs of simulation. As described in *Materials and Methods*, only the germination data of WT and *hfr1* were used for fitting the parameters, and thus all of the other simulation results served as validation of our model. We first compared the simulated and experimental germination results of WT and *hfr1*, and got a very good consistency (Fig. 5*B*). By adjusting only the protein amount parameter of PIF1 or DET1, respectively, in the model (parameters in *Materials and Methods*), we successfully simulated the germination patterns of PIF1ox and DET1ox in response to different light irradiations (Fig. 5*B*). To further test the model, we set the light fluence rate parameter in the model as 0 to simulate the true dark condition (D condition). As shown in Fig. 5*C*, the experimental results of all of the seeds in different backgrounds, including double mutants, were very similar to the predicted ones. We also used the model to predict the germination frequencies under the red light condition (R condition) and found that all experimental and simulation results were highly consistent (Fig. 5*D*). To test whether our simulation results were artificially influenced by applying the threshold variation to germination criteria ($PIF1_{\text{threshold}}$), we simulated germination frequencies by our model with a deterministic threshold ($PIF1_{\text{threshold}} = 15.6$, no noise). Still, all simulation and experimental results showed a very good consistency (Fig. S5). The conformity between experimental and simulation results suggests that our model captures the essence of light-regulated seed germination networks, and the "triple feed-forward loops" assemble a central machinery for precisely regulating seed germination under dark and diverse light conditions.

Discussion

Light is the energy resource and a critical environmental cue for plant development and growth (5, 32–35). The ability of seeds to rapidly and precisely respond to light is vital for plant survival. If the seed germinates in deep ground darkness, the stored nutrients might run out before it penetrates soil to reach light. On the contrary, if the seed does not properly respond to dim light changes and fails to germinate, it will miss the opportunity to start a new life cycle. Therefore, the plant seeds have to be equipped with elaborate molecular mechanisms to monitor and respond to light signals sensitively and robustly, deciding whether the condition is favorable for plant growth and when to germinate.

Previous studies showed that PIF1 is a key transcription factor in repressing seed germination in darkness (9, 10). Our recent study identified HFR1 as a positive regulator of light-induced seed germination (17). In this study, we further revealed that DET1 is a central repressor of light-induced seed germination. Our results showed that DET1 functions genetically upstream of HFR1 and PIF1, controlling the protein stability of PIF1 and HFR1 in an opposite way. Acting in the form of the CDD–CUL4 complex, DET1 maximizes PIF1's action by both removing PIF1's transcriptional repressor HFR1 and protecting PIF1 from proteasome-mediated degradation. As a result, in the dark-incubated seeds, PIF1 is highly accumulated and free from the sequestration of HFR1 and therefore exerts maximized activity to turn off seed germination. Under light conditions, the abundance of PIF1 is rapidly reduced via phyB–PIF1 direct interaction. At the same time, light inactivates DET1, elevating HFR1 to sequester PIF1's suppression on seed germination. Further inactivation of DET1 under extended light irradiation would eliminate DET1's protection on PIF1 to cause PIF1 degradation. As a result, the germination program is robustly launched. The DET1, HFR1, and PIF1 proteins use multilevels of regulation and form a coherent feed-forward loop. Fully active DET1 in the dark through this mechanism effectively turns off seed germination, whereas light suppresses DET1 to rapidly turn on seed germination. Therefore, DET1 functions as a molecular switch to control the process of seed germination in response to light signals.

To better illustrate the architecture of the regulation network, we represent the germination circuit topology of our mathematic model with pathway notations in Fig. 5*A*. The inhibition exerted from phyB to PIF1 via $Protein_A$, DET1, and HFR1 is denoted as pathway ① and the route via $Protein_B$, DET1, and Protease as pathway ②. The direct inhibitory interaction from phyB to PIF1 is denoted as pathway ③. Judging from the germination results of WT and *hfr1* seeds in response to various periods of light irradiation, the phyB–PIF1 direct inhibitory pathway (pathway ③) alone is insufficient to initiate seed germination. For example, under 5 min red light irradiation where pathway ③ was fully activated, most of the *hfr1* seeds (without pathway ①) still did not germinate (Fig. 5 *B* and *D*), indicating that pathway ① plays an additive role with pathway ③ in rapidly initiating seed germination under short light exposure. With increasing red light irradiation (more than 5 min), *hfr1* seeds exhibited progressive germination (Fig. 5*B*), suggesting that pathway ② is independent of the other two pathways and mediates the time-lapse seed germination response under prolonged light exposure conditions.

Therefore, pathways ①–③ form multiple levels of regulation to precisely initiate seed germination under various light conditions. The indirect inhibition via DET1 (pathways ① and ②) consists of two complementary pathways. Pathway ① achieves rapid response to light, enabling the seeds to sensitively germinate in response to a short period of light irradiation. Whereas pathway ② that further removes DET1's protection on PIF1 stability would ultimately ensure a full germination under extended light irradiation. The phyB–PIF1 direct inhibition pathway ③ reduces PIF1 actions to a moderate level under light conditions, allowing the other two pathways to precisely regulate and initiate seed germination in response to various light conditions. Taken together, these pathways compose a rigorous system in controlling seed germination under dark and diverse light environments. This model reconciles the new data and the previous concept, resulting in a comprehensive network of light-regulated seed germination. The successful validations indicate that our model is reliable and might be useful in designing future studies.

Materials and Methods

Arabidopsis thaliana Col-0 was used as the wild-type control in this study. Details of plant materials and growth conditions are described in *SI Materials and Methods*.

For the germination assay, plants were grown side by side and the seeds were kept at room temperature for 6–8 wk after harvesting. Then the seeds were surface sterilized and plated on Murashige and Skoog (MS) medium (4.4 g/L MS salts, 1% sucrose, pH 5.7, and 8 g/L agar). Starting from surface sterilization and plating, seeds were exposed to white light (about 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 1 h. After that, the seeds were irradiated by far-red light for 5 min to inactivate phyB as the true dark condition (D condition). For the light condition, the seeds were additionally irradiated with indicated period lengths of red light (about 10–15 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to activate phyB. After light irradiation, the seeds were then incubated in darkness at 22 °C for the indicated time. Germination frequencies were counted after dark incubations. At least 80 seeds were used for each experimental set and at least three biological replicates were performed for the statistical analysis.

The experimental procedures of yeast two-hybrid assay, BiFC assay, firefly LCI assay, coimmunoprecipitation (co-IP), immunoblot analysis, cell-free degradation assay, mathematical modeling, and germination simulation are provided in *SI Materials and Methods*.

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