Arabidopsis Chromatin Remodeling Factor PICKLE Interacts with Transcription Factor HY5 to Regulate Hypocotyl Cell Elongation[C][W]

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Photomorphogenesis is a critical plant developmental process that involves light-mediated transcriptome changes, histone modifications, and inhibition of hypocotyl growth. However, the chromatin-based regulatory mechanism underlying this process remains largely unknown. Here, we identify ENHANCED PHOTOMORPHOGENIC1 (EPP1), previously known as PICKLE (PKL), an ATP-dependent chromatin remodeling factor of the chromodomain/helicase/DNA binding family, as a repressor of photomorphogenesis in *Arabidopsis thaliana*. We show that *PKL/EPP1* expression is repressed by light in the hypocotyls in a photoreceptor-dependent manner. Furthermore, we reveal that the transcription factor ELONGATED HYPOCOTYL5 (HY5) binds to the promoters of cell elongation–related genes and recruits PKL/EPP1 through their physical interaction. PKL/EPP1 in turn negatively regulates HY5 by repressing trimethylation of histone H3 Lys 27 at the target loci, thereby regulating the expression of these genes and, thus, hypocotyl elongation. We also show that HY5 possesses transcriptional repression activity. Our study reveals a crucial role for a chromatin remodeling factor in repressing photomorphogenesis and demonstrates that transcription factor–mediated recruitment of chromatin-remodeling machinery is important for plant development in response to changing light environments.

INTRODUCTION

Unlike animals, plants respond to the surrounding environments by shaping their growth and development. A developmental transition from heterotrophic growth in subterranean darkness to autotrophic growth upon reaching the light is a critical event in the life cycle of plants. Dark-grown seedlings undergoing skotomorphogenesis (etiolation) are characterized by elongated hypocotyls, apical hooks with unexpanded cotyledons, undifferentiated chloroplasts, and repression of light-regulated genes. Light triggers photomorphogenesis, resulting in seedlings with deetiolated morphologies (i.e., short hypocotyls and open cotyledons) that contain differentiated chloroplasts and undergo chlorophyll and anthocyanin biosynthesis (Von Arnim and Deng, 1996). Plants have evolved an array of photoreceptors, including the red and far-red light-absorbing phytochromes (phyA to phyE) and the blue/UV-A light-absorbing cryptochromes (cry1 and cry2), to perceive and transduce light signals that ultimately modulate the transcriptome and trigger photomorphogenic growth and development (Chen et al., 2004).

Numerous studies demonstrate that phytochromes and cryptochromes suppress two main branches of light signaling during photomorphogenesis (Lau and Deng, 2010). A group of CONSTITUTIVE PHOTOMORPHOGENIC (COP)/DEETIOLATED (DET)/FUSCA proteins acts as central repressors downstream of the photoreceptors (Wei and Deng, 1996). Among them, COP1 functions as a RING finger E3 ubiquitin ligase that targets a number of positive factors, such as ELONGATED HYPO-COTYL5 (HY5), for degradation, and thus desensitizes light signaling (reviewed in Henriques et al., 2009; Lau and Deng, 2012). HY5 is a basic domain/Leucine zipper transcription factor that plays a key role in promoting photomorphogenesis in all light conditions by regulating the transcription of a wide range of genes, largely by binding to consensus sequences, such as the G-box (Oyama et al., 1997; Lee et al., 2007). HY5 is stabilized by light, and its abundance correlates inversely with the extent of hypocotyl growth (Osterlund et al., 2000). HY5 and its close homolog HYH can act as heterodimers and homodimers, thus mediating light-regulated expression of overlapping as well as distinct target genes (Holm et al., 2002). Furthermore, a group of basic helix-loop-helix transcription factors, named PHYTOCHROME-INTERACTING FACTORS (PIFs), is active in darkness and thus regulates gene expression to promote the skotomorphogenic response (Leivar et al., 2008). Under light, PIFs interact with photoactivated phytochromes and result in their phosphorylation and subsequent degradation (Leivar and Quail, 2011). Extensive studies have also identified dozens of intermediates in the light signaling pathway and revealed the importance of transcriptional regulatory networks in controlling photomorphogenesis (Jiao et al., 2007; Chory, 2010).

Chromatin remodeling plays a central role in establishing specific gene expression patterns and maintaining transcriptional states in eukaryotes (Jarillo et al., 2009; Ho and Crabtree, 2010). Accumulating evidence suggests that light-mediated gene expression and responses also require chromatin reorganization (Fisher and Franklin, 2011). The first piece of evidence came from the observation that increased acetylation of histone H3 and H4 of a pea (*Pisum sativum*) plastocyanin promoter correlates with light-induced transcription (Chua et al., 2003). It was shown that DET1, a repressor of photomorphogenesis, binds to unacetylated histone tails, consistent with its role in transcriptional repression (Benvenuto et al., 2002). Furthermore, histone acetylation, which is mediated by histone acetyltransferase and deacetylase, is required for regulating light-responsive gene expression and seedling growth (Bertrand et al., 2005; Benhamed et al., 2006). This notion is further supported by a study that showed that rapid light-mediated changes in *phyA* transcript levels are correlated with alterations of specific histone modifications (Jang et al., 2011). Two recent genome-wide analyses suggest the existence of a chromatin-based program that regulates photomorphogenesis (Guo et al., 2008; Charron et al., 2009). However, the mechanism by which chromatin configuration controls light-regulated gene expression and photomorphogenic responses is largely unknown.

ATP-dependent chromatin remodeling enzymes use the energy released by ATP hydrolysis to alter histone-DNA contacts and, thus, the accessibility of genomic regions to the transcriptional machinery or transcription factors. Although ATP-dependent chromatin remodeling enzymes are well characterized in animals, only a few have been functionally studied in plants (Clapier and Cairns, 2009). The SWITCH/SUCROSE NONFERMENTING (SWI/SNF) family of ATP-dependent chromatin remodelers has been studied extensively. Two members of this family, BRAHMA (BRM) and SPLAYED (SYD), are involved in regulating diverse developmental programs, such as seed maturation, root development, floral development, and biotic stress (Farrona et al., 2004, 2011; Hurtado et al., 2006; Tang et al., 2008; Walley et al., 2008). The CHD (for chromodomain, helicase/ATPase, and DNA binding domain) proteins belong to the SNF2-like family of ATPases and have been identified in a variety of organisms (Hall and Georgel, 2007; Hargreaves and Crabtree, 2011). *Arabidopsis thaliana* PICKLE (PKL; also named GYMNOS/Suppressor of *slr2* [SSL2]/CYTOKININ-HYPERSENSITIVE2) is a member of the CHD3 subfamily that shares similarity with Mi-2 in fly and human genomes (Clapier and Cairns, 2009) and possesses nucleosome-stimulated ATPase activity (Ho et al., 2012). PKL regulates multiple plant developmental processes, including embryonic development, seed germination, and root meristem activity (Ogas et al., 1999; Fukaki et al., 2006; Perruc et al., 2007; Aichinger et al., 2011).

Through forward genetic screening, we identify the *enhanced photomorphogenic1* (*epp1*) mutant and reveal that *EPP1* encodes PKL and acts as an important negative regulator of photomorphogenesis. We demonstrate that PKL/EPP1 physically interacts with HY5 to directly regulate hypocotyl cell elongation by repressing trimethylation of histone H3 Lys 27 (H3K27me3) on the regulatory regions of several cell elongation–related genes in response to changing light conditions.

RESULTS

EPP1 Is a Repressor of Photomorphogenesis

To identify novel components of the light signaling pathway, we performed a genetic screen for *Arabidopsis* mutants with an *epp* phenotype in a T-DNA mutant pool (Weigel et al., 2000). Mutants exhibiting short hypocotyls and/or large cotyledons under red light were chosen and the phenotypes were further confirmed by analyzing the next generation of mutants under red, far-red, and blue light. One of the mutants, referred to as *epp1-1*, exhibited shorter hypocotyls and more expanded cotyledons in far-red, red, and blue light conditions than the Columbia (Col) wild type (Figures 1A and 1B; see Supplemental Figure 1A online). Remarkably, the *epp1-1* seedlings had hypocotyls that were slightly reduced in length, opened cotyledons, and unfolded apical hooks in darkness (Figures 1C and 1D). In addition, *epp1-1* accumulated increased levels of chlorophyll and anthocyanin compared with the wild type (see Supplemental Figures 1B and 1C online). Furthermore, the expression of two light-inducible genes, *CHS* (encoding chalcone synthase) and *CAB2* (encoding chlorophyll *a/b* binding protein), was increased in *epp1-1* in both light and darkness (see Supplemental Figure 1D online). Together, these results suggest that *epp1-1* is a weak *cop*-like mutant.

Next, we generated double mutants and tested for genetic interaction between *epp1-1* and the photoreceptor mutants. The *epp1 phyA*, *epp1 phyB*, and *epp1 cry1* double mutants had slightly shorter hypocotyls than the photoreceptor parent mutants under far-red, red, and blue light conditions, respectively (see Supplemental Figures 2A and 2B online), indicating that EPP1 is a positive regulator of hypocotyl growth that acts downstream of both phytochromes and cryptochromes. Because COP1 acts as a central repressor in the light signaling pathway, we then examined the relationship between *epp1* and *cop1*. The *epp1 cop1* double mutant had much shorter hypocotyls and more expanded cotyledons and accumulated more anthocyanin than either of its parent mutants (Figures 1C to 1E; see Supplemental Figure 2C online), suggesting that EPP1 functions in parallel with COP1.

EPP1 Encodes PKL, an ATP-Dependent Chromatin Remodeling Factor

Using inverse PCR, we identified a single T-DNA insertion in the last exon of At2g25170 in the *epp1-1* mutant (Figure 1F). At2g25170 encodes PKL, an ATP-dependent chromatin remodeling factor exhibiting a high level of similarity to human Mi2/CHD4 and CHD3 (Ogas et al., 1999). A 4.3-kb region of genomic DNA containing the entire open reading frame of *PKL/SSL2* driven by its own promoter (Fukaki et al., 2006) was able to complement the short hypocotyl phenotype of *epp1-1* (see Supplemental Figure 1E online). Next, we examined other *epp1* alleles, including *pkl-1*, *epp1-2*, and *epp1-3* (Figure 1F). These mutants did not accumulate PKL; an immunoblotting analysis using antibodies that detected the C-terminal region of PKL (residues 1178 to 1384 amino acids) recognized a specific endogenous band of around 170 kD only in the wild type, although a weak band of 150 kD was also present (Figure 1G). *pkl-1*, *epp1-2*, and *epp1-3* phenocopied
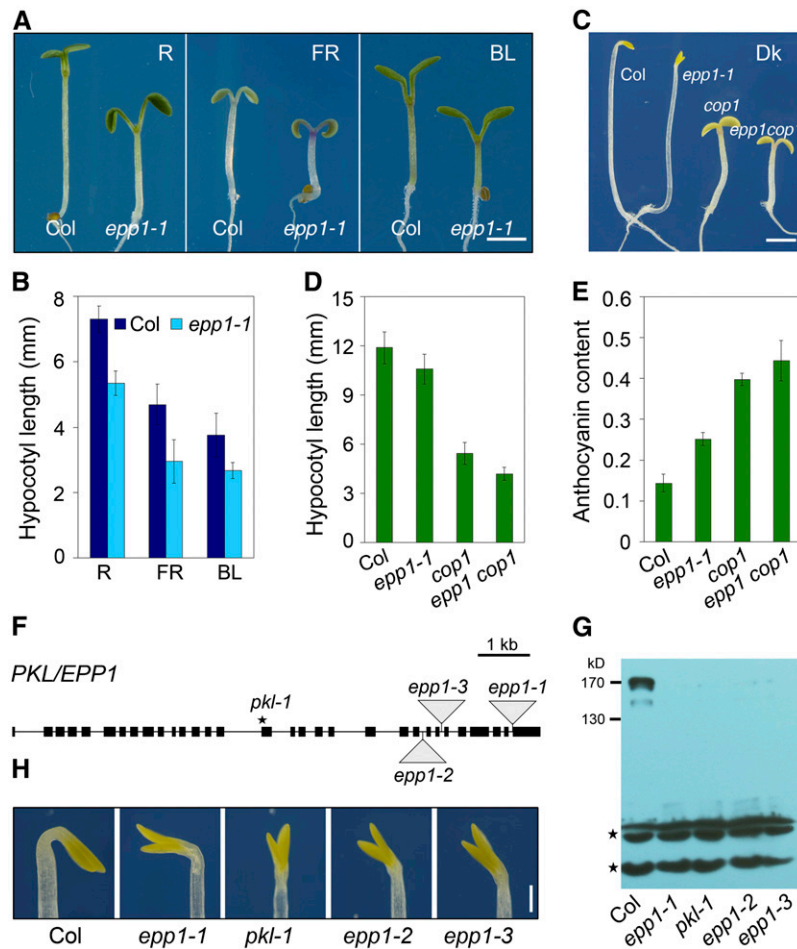
Figure 1. Loss of *EPP1*, Encoding PKL, Triggers a Constitutive Photomorphogenic-Like Response.

(A) The *epp1-1* mutant is hyposensitive to red (R), far-red (FR), and blue (BL) light compared with the Col wild type. Bar = 2 mm.

(B) Hypocotyl length of *epp1-1* and wild-type seedlings under various light conditions, as shown in **(A)**. Data are mean ± SD of 30 seedlings.

(C) Phenotypes of Col, *epp1-1*, *cop1*, and *epp1 cop1* seedlings in darkness (Dk). Bar = 2 mm.

(D) Hypocotyl length of wild-type and mutant seedlings in darkness. Data represent the mean ± SD of 30 seedlings.

(E) Anthocyanin content [$(A_{530} - 0.25 \times A_{657})/100$ seedlings] of wild-type and mutant seedlings in far-red light. Data represent the mean ± SD of triplicate assays.

(F) Diagram of *PKL/EPP1* and positions of the mutations. Black boxes represent exons, and lines between the boxes indicate introns. Triangles denote T-DNA insertions, and the star indicates a point mutation.

(G) Immunoblot analysis showing the presence of PKL in the wild type but not in the *epp1* mutants. Stars indicate cross-reacting bands used for a loading control.

(H) Multiple *epp1/pkl* mutant alleles displaying open cotyledons without an apical hook under dark conditions. Bar = 0.5 mm.

All seedlings in **(A)** to **(E)**, **(G)**, and **(H)** were grown in the light or dark conditions for 5 d.

[See online article for color version of this figure.]

epp1-1 in terms of hypocotyl length and cotyledon angle (Figure 1H; see Supplemental Figures 1F and 1G online). Therefore, these data confirm that *EPP1* is indeed *PKL*.

Light Represses *PKL* Expression in the Hypocotyls in a Photoreceptor-Dependent Manner

To further reveal how light influences *PKL* expression, we first compared the mRNA levels of *PKL* in 5-d-old Col seedlings grown under dark, far-red, red, and blue light by quantitative real-time PCR with reverse transcription (qRT-PCR). As shown in Figure 2A, *PKL* expression levels were dramatically lower in the light conditions tested than in darkness. Furthermore, *PKL* transcript levels were decreased within 12 h of transfer from dark to light, although the difference was not evident during the first six hours (Figure 2A). In addition, compared with those in the wild type, the *PKL* transcripts were greatly upregulated in the *phyB*, *phyA*, and *cry1* mutants in red, far-red, and blue light, respectively (Figure 2B), suggesting that the light-mediated repression of *PKL* is facilitated by phytochrome and cryptochrome photoreceptors.
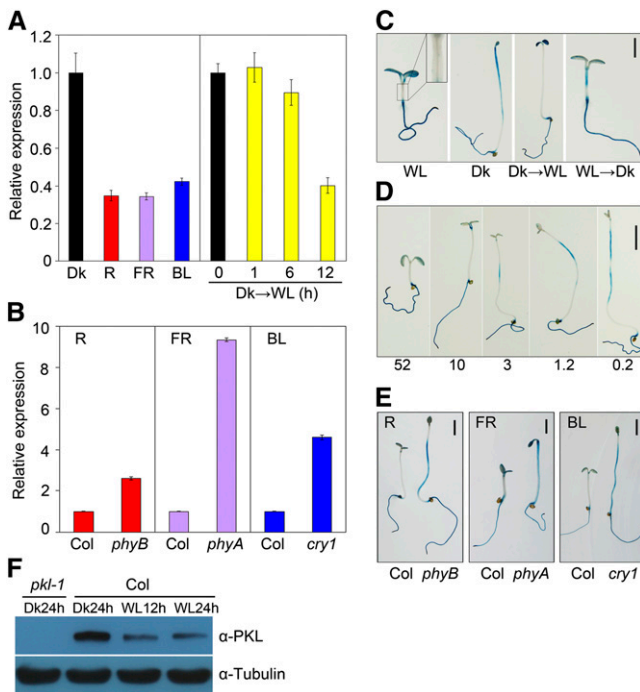
Figure 2. *PKL* Expression in the Hypocotyls Is Repressed by Light.

(A) A qRT-PCR assay showing *PKL* transcript levels in wild-type seedlings subjected to red (R), far-red (FR), or blue (BL) light or darkness (Dk) for 5 d or in 5-d-old dark-grown seedlings transferred to white light (WL) for the indicated periods of time (0 to 12 h). Data represent the mean $\pm$ SD of three biological replicates.

(B) qRT-PCR assay showing increased *PKL* expression, relative to the Col wild type, in *phyB*, *phyA*, and *cry1* photoreceptor mutants grown in red, far-red, and blue light conditions, respectively, for 5 d. For **(A)** and **(B)**, relative expression was normalized to that of *UBQ1*, and data represent the mean $\pm$ SD of three biological replicates.

(C) GUS staining of *ProPKL:GUS* transgenic seedlings. Seedlings were grown in white light or darkness for 5 d, 4-d-old dark-grown seedlings were exposed to white light for an additional 1 d (Dk→WL), or 4-d-old light-grown seedlings were transferred to darkness for an additional 1 d (WL→Dk). The inset in the WL panel is an enlargement of the hypocotyl. Bar = 2 mm.

(D) GUS staining gradually increased in the hypocotyls of seedlings grown in a decreasing series of light intensities (μmol m^{-2} s^{-1}; indicated below) for 5 d. Bar = 2 mm.

(E) GUS staining in the hypocotyls was significantly enhanced in the photoreceptor mutants compared with the Col wild type. Seedlings harboring the *ProPKL:GUS* reporter were grown under the indicated light conditions for 5 d. Bar = 2 mm.

(F) Immunoblotting of PKL protein. Seedlings were grown in long-day conditions (16 h day/8 h night) for 3 d and were then transferred to darkness or white light for the indicated period of time at the end of the day. Hypocotyls and cotyledons were detached and harvested for protein isolation. Immunoblotting against the tubulin antibody served as a loading control.

PKL was previously shown to be ubiquitously expressed in different tissues of the adult plant (Ogas et al., 1999). We decided to examine whether *PKL* undergoes tissue-specific regulation in response to light. To this end, transgenic plants expressing *ProPKL:GUS* (a β-glucuronidase reporter gene

driven by a 2.0-kb promoter fragment of *PKL*) were generated. The *GUS* reporter was expressed in the cotyledons and roots regardless of the type of light treatments tested (Figure 2C). Interestingly, *GUS* was strongly expressed in the uppermost regions of hypocotyls in darkness and disappeared upon exposure to light. However, *GUS* expression was weak in light but became stronger when transferred to darkness (Figure 2C). In addition, GUS activity in the hypocotyls increased with the light intensity decreased (Figure 2D), indicating that *PKL* expression correlates positively with the extent of hypocotyl elongation. Next, we introduced *ProPKL:GUS* into the *phyB*, *phyA*, and *cry1* mutants and tested the expression of the transgene in red, far-red, and blue light conditions, respectively. As shown in Figure 2E, the photoreceptor mutants displayed significantly enhanced *GUS* expression compared with the wild-type control in the region of the hypocotyls but not in the cotyledons and roots. Together, these observations indicate that, through phytochromes and cryptochromes, light represses *PKL* expression specifically in the hypocotyls, reinforcing a role for PKL in promoting hypocotyl growth.

To investigate whether light regulates PKL abundance, Col wild-type seedlings were grown in long-day conditions for 3 d. At the end of the light period, seedlings were kept in darkness or exposed to white light for an additional 12 or 24 h. We then performed an immunoblotting assay with PKL antibody and found that PKL protein level was drastically decreased in seedlings exposed to light compared with those in darkness (Figure 2F), suggesting that light also represses PKL at the protein level.

PKL Directly Activates Cell Elongation–Related Genes

Plant growth involves both cell elongation and proliferation. To assess the contribution of defects in cell elongation and/or proliferation to the short hypocotyls of *epp1/pkl* mutants, we examined cell length and cell number along the vertical axes of the hypocotyls. The average cell length of both *epp1-1* and *pkl-1* hypocotyls was significantly decreased to 60% of the wild type, correlating with the decrease in overall hypocotyl length, whereas the number of cells did not differ significantly between *epp1/pkl* and the wild type (Figure 3A). This finding indicates that PKL predominantly regulates cell elongation, but not cell proliferation, during hypocotyl growth.

To further explore the molecular mechanism by which PKL regulates hypocotyl cell elongation, we attempted to identify its putative direct target genes from the public data available. Candidate genes were selected based on the following criteria: (1) they should encode cell wall loosening or hydrolytic enzymes that are implicated directly in regulating cell elongation (Cosgrove, 2005); (2) they should encode components involved in the biosynthesis or signaling of brassinosteroids or auxin (Sun et al., 2010; Chapman et al., 2012); and (3) if the genes satisfy criteria (1) and (2), expression of the genes should be repressed by light in a manner similar to *EPP1/PKL* (Ma et al., 2005). Accordingly, six genes, including *EXTENSIN3* (*EXT3*), *EXPANSIN2* (*EXP2*), *XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE17* (*XTH17*), *XYLOGLUCAN ENDOTRANSGLYCOSYLASE6* (*XTR6*), *DWARF4* (*DWF4*), and *INDOLE-3-ACETIC ACID INDUCIBLE19*
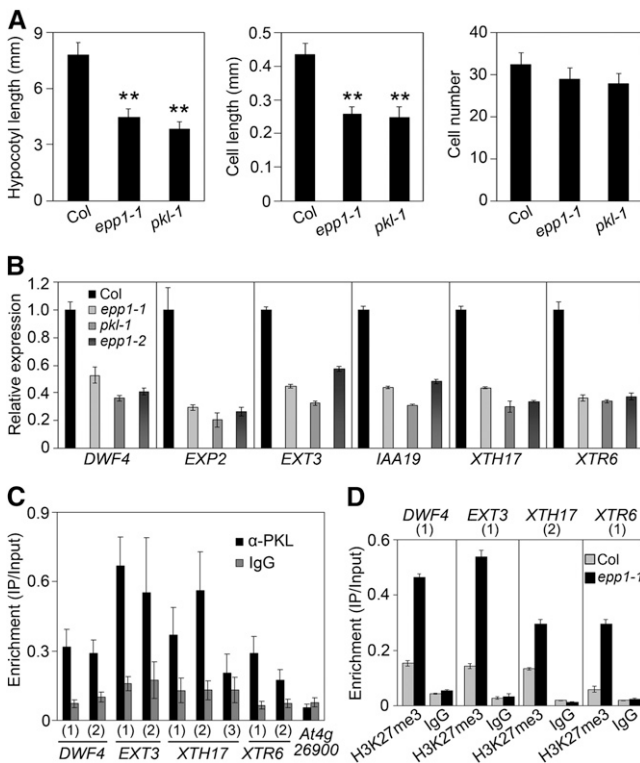
Figure 3. PKL Directly Promotes the Expression of Cell Elongation Genes.

(A) The short hypocotyls of the *epp1* mutants correlate with a reduction in cell elongation but not in cell number. Seedlings were grown in white light for 5 d. Data represent the mean ± SD of 30 seedlings. Asterisks indicate significant difference from the wild type at P < 0.01 using Student's *t* test.

(B) qRT-PCR assay showing reduced expression of various genes involved in cell elongation in 5-d-old white light–grown *epp1* mutants relative to the Col wild type. Relative expression was normalized to that of *UBQ1*. Data represent the mean ± SD of biological triplicates.

(C) ChIP-qPCR assay showing enrichment of various cell elongation genes in DNA samples pulled down by PKL antibody or IgG control. Numbers in parentheses indicate regions for amplification, as shown in Supplemental Figure 3A online. The Col wild-type seedlings were grown under white light for 5 d. At4g26900 served as a negative control. Data represent the mean ± SD of triplicates.

(D) ChIP-qPCR assay using the H3K27me3 antibody, showing relatively high enrichment of *DWF4*, *EXT3*, *XTH17*, and *XTR6* in the *epp1-1* mutant compared with the Col wild type. Numbers in parentheses indicate regions for amplification, as shown in Supplemental Figure 3A online. Precipitation by IgG preimmune serum served as a control. IP, immunoprecipitation. Data represent the mean ± SD of triplicates.

(*IAA19*) were selected for further analysis. We performed qRT-PCR analysis to determine whether these genes are regulated by PKL. As shown in Figure 3B, the transcript levels of these genes were drastically decreased in the *epp1-1*, *pkl-1*, and *epp1-2* mutants compared with the wild type, suggesting that PKL does indeed contribute to the activation of these genes.

To test whether PKL activates the expression of *EXT3*, *EXP2*, *XTH17*, *XTR6*, *DWF4*, and *IAA19* directly, we performed chromatin

immunoprecipitation with quantitative PCR (ChIP-qPCR) using DNA isolated from Col wild type and the PKL antibody. Indeed, the promoter and coding regions of *DWF4*, *EXT3*, *XTH17*, and *XTR6* was greatly enriched in DNA precipitated using the PKL antibody, but not in DNA pulled down with the IgG serum control (Figure 3C; see Supplemental Figure 3A online; *EXP2* and *IAA19* were analyzed below in detail). As a negative control, the enrichment of the At4g26900 promoter (Lee et al., 2007) was very low and indistinguishable between the PKL antibody and IgG. These data indicate that PKL directly associates with the genomic sequences of these cell elongation–related genes. In support of our finding that these genes are downstream targets of PKL, loss-of-function mutants of *EXP2* and *DWF4* also displayed short hypocotyls when grown in light (see Supplemental Figure 4 online). Furthermore, *IAA19* expression in the hypocotyls was repressed by light in a manner similar to *PKL* (Tatematsu et al., 2004) and is also involved in hypocotyl cell elongation (Oh et al., 2012).

Previous studies documented that H3K27me3 plays an important role in PKL-mediated transcriptional regulation (Zhang et al., 2008, 2012; Aichinger et al., 2009). A ChIP assay using the H3K27me3 antibody followed by quantitative PCR revealed that *EXT3*, *XTH17*, *XTR6*, *DWF4*, *EXP2*, and *IAA19* were all marked by H3K27me3; however, H3K27me3 levels were substantially increased in the *epp1-1* mutant (Figure 3D; *EXP2* and *IAA19* were analyzed below). Taken together, these results demonstrate that PKL promotes cell elongation likely by inhibiting H3K27me3 of regions of chromatin that are specifically associated with the target genes involved in cell elongation.

HY5 Physically and Genetically Interacts with PKL

In mammals, the PKL homolog Mi-2b (CHD4) acts as an integral subunit of different complexes and interacts with transcription factors to modulate gene expression (Denslow and Wade, 2007). We reasoned that PKL might also have interacting partners involved in the regulation of light signaling. Given that PKL represses photomorphogenesis under all light conditions and that HY5 is a central transcription factor in the light signaling pathway, HY5 was a good candidate partner. To investigate whether PKL and HY5 interact with each other, we generated LexA fusions of full-length PKL and of the N-terminal (D1) and C-terminal (D2) fragments of PKL for yeast two-hybrid analysis with B42 activation domain–tagged HY5 (AD-HY5) (Figure 4A). However, neither full-length PKL (LexA-PKL) nor the C-terminal domain (LexA-D2) showed a positive interaction with AD-HY5. Notably, coexpression of LexA-D1 (including the PHD domain, chromodomains, and ATPase domain) and AD-HY5 activated the *LacZ* reporter gene (Figure 4B). To further determine which domain(s) of PKL is required for binding to HY5, a series of deletion constructs was generated (Figure 4A). As shown in Figure 4B, deletion of the ATPase domain (D3) or of both the ATPase domain and the chromodomain (D4) abolished the PKL-HY5 interaction. Intriguingly, the ATPase domain (D6) was necessary and sufficient for the interaction with HY5 and also with HYH, the close homolog of HY5 (see Supplemental Figure 5 online). However, a point mutation (Lys changed to Ala) of an evolutionarily conserved amino acid, Lys-304, which is predicted
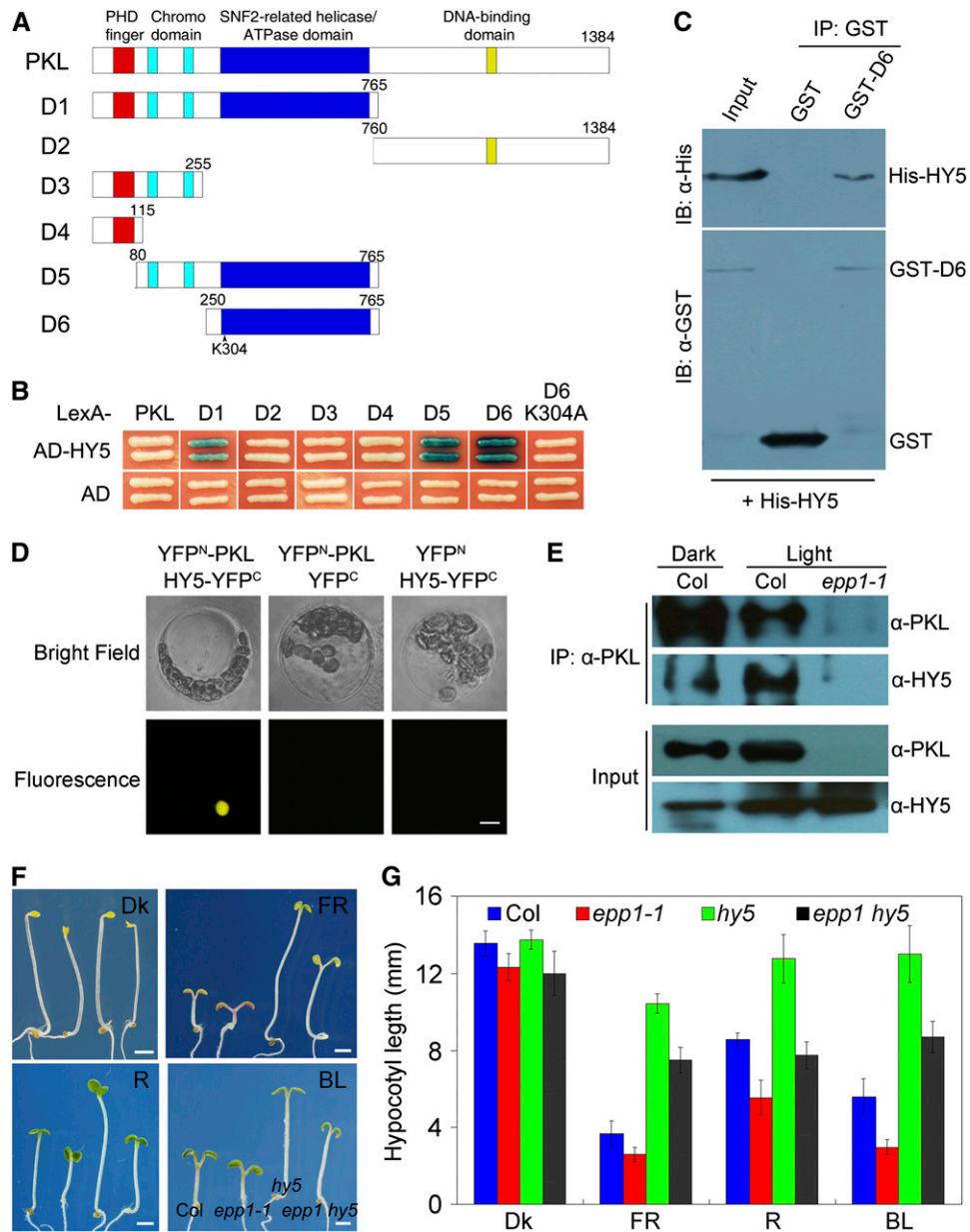
Figure 4. PKL and HY5 Interact with Each Other.

(A) Diagram of the domain structures of PKL and various PKL deletions (D1-6).

(B) Yeast two-hybrid assay between various fragments or mutant forms of PKL shown in **(A)** fused to the LexA DNA binding domain and AD-tagged HY5 (AD-HY5) or AD alone.

(C) Pull-down assay showing direct interaction between GST-PKL-D5 and His-HY5 fusion proteins in vitro. IB, immunoblotting; IP, immunoprecipitation.

(D) BiFC assay showing that YFP^N-PKL and HY5-YFP^C interact to form a functional YFP in the nucleus. Bar = 5 μm.

(E) Coimmunoprecipitation assay showing that the PKL antibody could precipitate HY5 in 5-d-old Col wild-type seedlings grown in both white light (1.5 μmol m^{-2} s^{-1}) and darkness.

(F) Seedling phenotypes of *hy5*, *epp1-1*, and *epp1 hy5* mutants and the Col wild type after exposure to red (R), far-red (FR), blue light (BL), or darkness (Dk) for 5 d. The seedlings are arranged in identical order in each panel. Bars = 2 mm.

(G) Quantification of hypocotyl length of the wild-type and mutant seedlings shown in **(F)**. Data represent the mean ± sd of 30 seedlings.

to bind ATP within the ATPase domain, abolished the interaction (see Supplemental Figure 6 online; Figure 4B). Next, we performed a pull-down assay and found that glutathione *S*-transferase (GST)-D6 successfully precipitated His-tagged HY5 (Figure 4C). Together, these results demonstrate that PKL physically interacts with HY5 through the ATPase domain.

To confirm the PKL-HY5 interaction in vivo, we performed a bimolecular fluorescence complementation (BiFC) assay in *Arabidopsis* protoplasts and found that coexpression of PKL-YFP[N] (fused with the N-terminal half of yellow fluorescent protein) and HY5-YFP[C] (fused with C-terminal half of YFP) reconstituted a functional YFP in the nucleus, whereas the controls did not (Figure 4D). Furthermore, coimmunoprecipitation analysis showed that PKL was able to precipitate HY5 in Col seedlings grown in either light or dark conditions but not in the *epp1-1* mutant (Figure 4E). Collectively, these results indicate that PKL constitutively interacts with HY5 in the nucleus, regardless of the type of light treatment.

To study the functional relationship between PKL and HY5, we constructed an *epp1 hy5* double mutant by crossing *epp1-1* with *hy5-215* (Oyama et al., 1997). The *epp1 hy5* seedlings displayed intermediate hypocotyl elongation in all light conditions and were indistinguishable from the Col wild type in red light (Figures 4F and 4G), suggesting that PKL and HY5 antagonistically regulate hypocotyl growth.

PKL Negatively Regulates HY5 by Repressing H3K27me3 on Target Loci

A previous study reported that HY5 directly binds target genes with ACGT-containing elements or a G-box (CACGTG) in their promoter sequences (Lee et al., 2007). Promoter analysis revealed that *EXT3*, *EXP2*, *XTH17*, *XTR6*, *DWF4*, and *IAA19* contain a putative G-box or ACGT-containing elements within their regulatory regions and coding regions close to translational start site (Figure 5A; see Supplemental Figure 3A online). ChIP-qPCR assay showed that these genes were highly enriched in DNA samples precipitated with HY5 antibody (see Supplemental Figure 3B online; Figure 5B), suggesting that, similar to PKL, HY5 associates with the DNA sequences of these cell elongation–related genes. In this study, we selected *EXP2* and *IAA19* as examples for further detailed molecular analysis.

Yeast one-hybrid assays showed that AD-HY5 fusion proteins, but not AD alone, bound to the promoters (1.0 and 1.7 kb upstream from the ATG start site, respectively) or to a 39-bp fragment containing the G-box motif of *EXP2* and *IAA19* (wild type) and strongly activated the expression of the *LacZ* reporter gene (Figure 5B). However, point mutations in the G-box (m, CACGTG changed to TTTTTG) of both genes abolished *LacZ* activation (Figure 5B). These observations indicate that HY5 specifically binds to the regulatory regions of *IAA19* and *EXP2* via the G-box motif.

ChIP-qPCR was performed to investigate the in vivo binding of *IAA19* and *EXP2* to HY5 and PKL in light- and dark-grown Col seedlings. Relative to the IgG serum, HY5 antibody markedly pulled down the G-box–containing fragments of *EXP2* (E2) and *IAA19* (I2) in both darkness and light and even bound to the coding sequence of *IAA19* (I3) but did not pull down the *actin2*

negative control (Figure 5C). Intriguingly, these pulled-down fragments were also drastically enriched in samples precipitated by antibodies against PKL. In addition, there was a slight increase in samples pulled down by the HY5 antibody in seedlings grown in light versus darkness, and the opposite was true for PKL (Figure 5C). We further found that the ability of PKL to bind to target loci was compromised in the *hy5 hyh* double mutant, as it was in *epp1-1*, but not in the *hy5* single mutant (Figure 5D). These results together suggest that PKL and HY5 (and HYH) likely associate with the cell elongation–related gene loci at the same time during light-mediated seedling development. The recruitment of PKL requires the presence of both HY5 and HYH, consistent with their redundant function in regulating hypocotyl growth (Holm et al., 2002).

The interaction between PKL and HY5 as well as their association with the target chromatin prompted us to analyze how *EXP2* and *IAA19* expression was coregulated by these proteins. As shown in Figure 5E, the expression of *EXP2* and *IAA19* was dramatically decreased after the transition from darkness to light. Remarkably, *EXP2* and *IAA19* expression was stronger in *hy5* but weaker in *epp1* compared with the Col wild type. Loss of HY5 activity caused a substantial induction of *EXP2* and *IAA19* expression in the *epp1* mutant background (Figure 5E). Thus, PKL and HY5 act antagonistically to regulate downstream gene expression.

This antagonistic regulation led us to further investigate the levels of H3K27me3 on *IAA19* and *EXP2* loci. ChIP-qPCR assays using the H3K27me3 antibody were performed in *hy5* and/or *epp1* mutants and also in the Col wild type. Mutation of *PKL* caused a remarkable increase in H3K27me3 levels at critical *IAA19* and *EXP2* regulatory regions, as was observed for other cell elongation–related genes (Figure 3D). However, H3K27me3 levels were reduced by more than 50% in the *hy5-215* mutant compared with the wild type (Figure 5F). Strikingly, in the *epp1 hy5* double mutant, similar amounts of DNA were pulled down by H3K27me3 antibody as in the wild type (Figure 5F). These data suggest that PKL antagonizes with HY5 in regulating the level of H3K27me3 at the cell elongation gene loci, whereas HY5 promoters H3K27me3 at these loci, correlating with their antagonistic role in modulating gene expression. Consistent with the expression pattern of *EXP2* and *IAA19*, the H3K27me3 repressive mark was markedly recruited to these loci in light conditions but was low in the dark (see Supplemental Figure 7A online). It is noteworthy that the transcript level of *EXP2*, but not of *IAA19*, was drastically inhibited in *epp1-1* compared with the wild type in darkness (Figure 5E). Accordingly, a ChIP assay showed that relatively more H3K27me3 marks were deposited on *EXP2* locus, but not on *IAA19*, in dark-grown *epp1-1* than those were in the wild type (see Supplemental Figure 7B online).

HY5 Possesses Transcriptional Repression Activity

The fact that HY5 directly binds to the promoters of *EXP2* and *IAA19* and that it represses their expression prompted us to investigate whether HY5 has transcriptional repression activity. Li et al. (2010) failed to detect any transcriptional repression activity of HY5 in yeast and plant cells. We thus fused HY5 with the VP16 activation domain and tested the activity in a transient luciferase (LUC) expression system (Figure 6A). As shown in
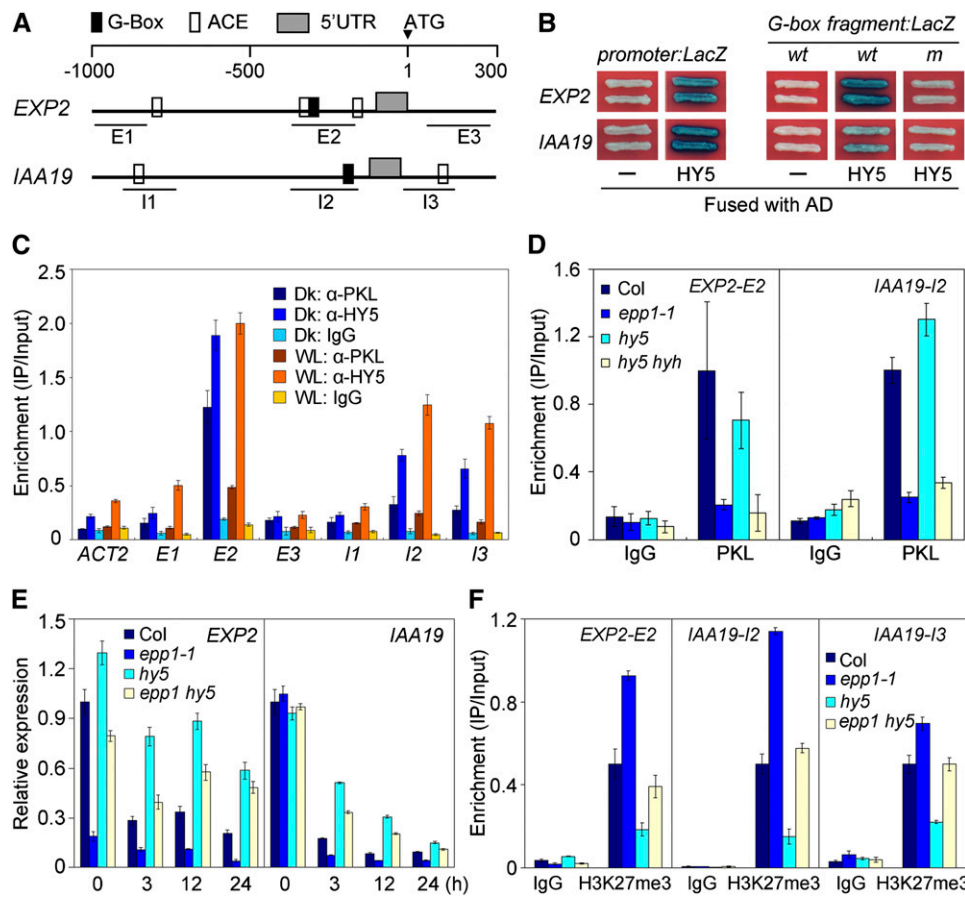
Figure 5. PKL Negatively Regulates HY5 by Inhibiting H3K27me3 of Target Loci.

(A) Diagram of promoter structures of *IAA19* and *EXP2*. ACE, ACGT-containing *cis*-element; UTR, untranslated region. E1 to E3 and I1 to I3 indicate fragments for ChIP-qPCR amplification.

(B) Yeast one-hybrid assay showing that AD-HY5 binds to the promoter regions of *IAA19* and *EXP2* via the G-box. wt and m indicate wild-type and mutant forms of the G-box–containing fragments, respectively. "–" means empty AD fusion.

(C) ChIP assay showing the relative enrichment of *IAA19* (I1 to I3) and *EXP2* (E1 to E3) genomic fragments upon precipitation with PKL or HY5 antibodies in light- or dark-grown seedlings. Dk, dark; WL, white light.

(D) ChIP assay showing that binding of PKL to the regulatory regions of *IAA19* and *EXP2* was compromised in the *hy5 hyh* double mutant, as in the *epp1-1* mutant. DNA samples were pulled down with PKL antibody or the IgG sera control.

(E) Relative gene expression of *EXP2* and *IAA19* in *hy5*, *epp1*, and *epp1 hy5* mutants and the Col wild type following transition from darkness to light for the indicated number of hours.

(F) ChIP assay with DNA isolated from Col, *epp1-1*, *hy5*, and *epp1 hy5* seedlings using anti-H3K27me3 antibody or the IgG control. In **(C)** to **(F)**, data represent the mean ± sp of triplicates.

Figure 6B, BD-HY5 (fused with GAL4 DNA binding domain) did not affect transcription of the *ProGAL4:LUC* reporter gene. BD-VP16 strongly activated the LUC reporter gene; however, this activity was drastically inhibited by HY5 (BD-HY5-VP16), in a manner similar to the function of the repression domain of ERF3 (Ohta et al., 2001). Therefore, this result suggests that HY5 indeed has intrinsic transcriptional repression activity in vivo.

DISCUSSION

Extensive studies have established a light signaling framework composed of photoreceptors, key repressors (e.g., COP1), and downstream transcription regulators (Lau and Deng, 2010). Our

study identifies the CHD3 chromatin remodeling factor PKL/EPP1 as an important repressor of light signaling. Although both COP1 and PKL/EPP1 interact with HY5 and regulate HY5 activity to repress photomorphogenesis, their underlying molecular mechanisms are distinct. COP1 is known to be an E3 ubiquitin ligase that targets positive regulators of photomorphogenesis, such as HY5, HYH, LONG AFTER FAR-RED LIGHT1, and LONG HYPOCOTYL IN FAR RED1, for degradation at the post-translational level (Lau and Deng, 2012). However, PKL/EPP1 acts as an ATP-dependent chromatin remodeling factor that alters the pattern of histone marks on HY5 target genes involved in cell elongation. The additive phenotype of the *epp1 cop1* double mutant supports the notion that PKL/EPP1 and COP1
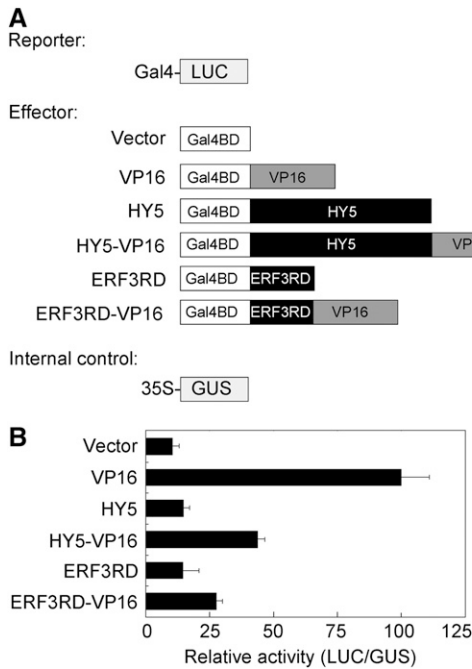
A

Reporter:

Gal4- LUC

Effector:

Vector   Gal4BD

VP16   Gal4BD VP16

HY5   Gal4BD HY5

HY5-VP16   Gal4BD HY5 VP16

ERF3RD   Gal4BD ERF3RD

ERF3RD-VP16   Gal4BD ERF3RD VP16

Internal control:

35S- GUS

B

Figure 6. HY5 Has Transcriptional Repression Activity.

(A) Diagram of various constructs used in this assay.
(B) Plasmid combinations of LUC reporter, GUS internal control, and effectors were cotransformed into *Arabidopsis* protoplasts. The protoplasts were incubated in weak light for 16 h, and relative activity was expressed as the ratio of LUC to GUS activity. Data represent the mean ± SD of three biological replicates.

have additive effects. Using the molecular and biochemical evidence presented in this study, we therefore propose a model in which HY5 (and HYH) constitutively binds to the proximal regulatory regions of cell elongation–related genes and recruits PKL/EPP1 through direct interaction. PKL/EPP1 in turn represses the H3K27me3 repressive mark, resulting in the activation of target genes and the promotion of hypocotyl elongation in the dark. Light stabilizes HY5 and decreases the transcript and protein levels of *PKL/EPP1*, leading to the recruitment of more H3K27me3 mark at cell elongation loci and the reduction of hypocotyl elongation (Figure 7).

The biochemical functions of HY5 and PKL/EPP1 are opposite; whereas HY5 promotes H3K27me3 deposition on cell elongation target loci and represses their gene expression, PKL represses this mark on cell elongation target loci and activates gene expression. Surprisingly, full-length PKL exhibited transcriptional repression activity in a protoplast transient expression system (see Supplemental Figure 8 online). The other transcription activator(s) might be required to induce the expression of target genes. Since HY5 is a strong, positive master regulator of the light signaling pathway, the recruitment of PKL/EPP1 might dampen HY5 activity and thereby prevent inadvertent entry into photomorphogenesis, providing an additional regulatory mechanism to fine-tune the pathway at the epigenetic level in response to environmental light intensity. Therefore, compared with the strong and pleiotropic effects of

the *cop1* mutation, the phenotype of *epp1/pkl* plants is mild under dark condition. In support of this model, the H3K27me3 repressive mark is strongly associated with *EXP2* and *IAA19* in light conditions, but only weakly in the dark (see Supplemental Figure 7A online). Furthermore, more than 30% of genes that exhibited significantly decreased transcript levels in *pkl* plants were enriched for H3K27me3 (Zhang et al., 2012). In addition, the protein-coding genes exhibited higher levels of H3K27me3 after the dark-to-light transition than in darkness (Charron et al., 2009). Our study provides strong evidence that chromatin remodeling factor–mediated histone methylation plays a crucial role in determining cell elongation and plant growth.

Phylogenetic analysis revealed that PICKLE RELATED1 (PKR1), PKR2, and CHROMATIN REMODELING5 (CHR5) are close homologs of PKL (Shaked et al., 2006).The mild phenotype of *epp1/pkl* mutants may also be caused by redundancy with PKL homologs. Mutants lacking *PKR1*, *PKR2*, or *CHR5* were indistinguishable from the wild type in terms of hypocotyl elongation and cotyledon opening. Strikingly, the *pkl pkr1*, *pkl pkr2*, and *pkl chr5* double mutants exhibited slightly but repeatable shorter hypocotyls than the *pkl* single mutant in both light and darkness, suggesting that PKR1, PKR2, and CHR5 may play redundant roles with PKL in regulating hypocotyl growth (see Supplemental Figure 9 online). However, the cotyledon phenotype of *pkl* was not enhanced in these double mutants. Previous studies also documented that PKL and PKR2 redundantly regulate root cell identity and root growth (Aichinger et al.,
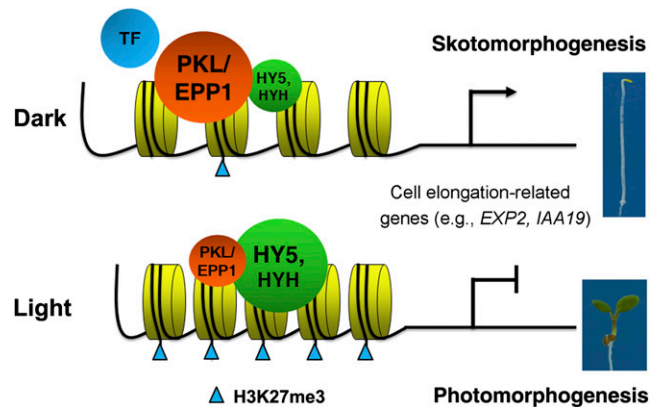

Figure 7. A Proposed Model of the Action of PKL/EPP1 and HY5 in Regulating *Arabidopsis* Hypocotyl Growth.

In darkness, HY5 (and HYH) is largely degraded by the 26S proteasome–mediated pathway, whereas *PKL/EPP1* is strongly expressed in the hypocotyl. A small pool of HY5 binds to the proximal promoter of cell elongation–related genes, including *IAA19* and *EXP2*, which enables the recruitment of the chromatin remodeling factor PKL/EPP1 to these target loci through physical interaction. This largely prevents H3K27me3 formation and thereby activates cell elongation genes, leading to the promotion of hypocotyl growth. Other transcription activator(s) (indicated as TF) might also be involved in the activation of gene expression. Light triggers the stabilization of HY5 but reduces the level of PKL/EPP1. This allows the recruitment of more H3K27me3 marks on histones at cell elongation–related loci, leading to their repression and the inhibition of hypocotyl elongation.

2009, 2011). Further studies using higher order mutants are needed to elucidate the redundant functions of PKL and its homologs.

Although HY5 functions as a master transcription factor that acts downstream in the photomorphogenic pathway and the *hy5* null mutant displays elongated hypocotyls under light conditions, the cellular mechanism by which HY5 mediates hypocotyl growth remains unknown (Oyama et al., 1997; Lau and Deng, 2010). This study uncovers a direct link between HY5-mediated repression of cell elongation genes and hypocotyl growth in the light. This notion is further supported by the observation that HY5 possesses intrinsic transcriptional repression activity and that loss of *HY5* reduces H3K27me3 at the cell elongation genes whose expression is inhibited by light. Conversely, numerous photosynthesis-related light-induced HY5 targets are marked by histone acetylation, the active mark, during seedling deetiolation (Guo et al., 2008; Charron et al., 2009). Thus, histone modifications are essential for differentially determining the transcriptional activity of HY5 targets.

Chromatin remodeling plays an important role in specifying gene expression states and patterns. Compared with extensive research in animals, only a small body of evidence has documented the role of chromatin-remodeling factors in regulating plant growth and development (Clapier and Cairns, 2009; Jarillo et al., 2009). For instance, the SWI2/SNF2 ATP-dependent chromatin-remodeling enzyme BRM plays crucial roles in vegetative, embryonic, and reproductive plant development by repressing specific target genes (Farrona et al., 2004, 2011; Kwon et al., 2006; Tang et al., 2008). SYD is required for shoot apical meristem maintenance, flower patterning, and biotic stress signaling (Kwon et al., 2005, 2006; Walley et al., 2008; Wu et al., 2012). Previous studies on PKL mostly focused on its involvement in regulating embryonic identity and root meristem activity (Ogas et al., 1999; Henderson et al., 2004; Li et al., 2005; Fukaki et al., 2006; Perruc et al., 2007; Aichinger et al., 2011). However, its role in the hypocotyl/shoot remains obscure. Here, we demonstrate that PKL/EPP1 participates in the repression of photomorphogenesis by directly promoting hypocotyl cell elongation, thus elucidating an important function for CHD chromatin-remodeling enzymes in regulating cell elongation that does not involve cell differentiation and proliferation (Hall and Georgel, 2007; Ho and Crabtree, 2010). Consistent with this, a genome-wide analysis revealed that genes exhibiting hypocotyl-specific expression are overrepresented among the genes that display decreased levels in the *pkl* mutant (Zhang et al., 2012). Although PKL targets H3K27me3-enriched loci, such as LEAFY COTYLEDON1 (LEC1), LEC2, and PHERES1, and acts as a transcriptional corepressor to inhibit their gene expression during germination (Zhang et al., 2008, 2012), our accumulated data strongly suggest that this protein represses H3K27me3 modification and activates the expression of target genes involved in cell elongation.

Consistently, studies also showed that PKL reduces the level of H3K27me3 on root stem cell and meristem marker genes and promotes their expression (Aichinger et al., 2009, 2011). Thus, PKL either promotes or represses H3K27me3 and acts as a transcriptional corepressor or coactivator, respectively. These opposing roles of PKL might be determined

by sequence-specific transcription factors that it associates with and their targets and/or by the recruitment of different histone modification enzymes that catalyze methylation or demethylation. PKL function is probably also affected by endogenous and exogenous cues associated with particular tissues and developmental processes. It would be of great interest to uncover the underlying mechanisms for chromatin remodeling factors that mediate a specific biological function. The PHD superfamily of proteins is proposed to function as readers of histone methylation (Liu et al., 2010). In agreement with this, a rice (*Oryza sativa*) CHD3 protein, CHR729, can interact with H3K27me3 and H3K4me2 via its PHD finger and chromodomains, respectively (Hu et al., 2012). PKL likely acts as a reader that recognizes histone marks.

Our study points to an example of a member of plant CHD-type chromatin remodeling factors, PKL/EPP1, directly interacting with a key transcription factor, HY5, to control cell growth. Although HY5 is largely degraded in darkness, a small pool of less active phosphorylated HY5 might be responsible for interacting with PKL/EPP1 (Hardtke et al., 2000). The SNF2-related helicase/ATPase domain of PKL/EPP1 is necessary and sufficient for the interaction with HY5, suggesting that this domain mediates protein–protein interactions besides its function in ATP hydrolysis. Interestingly, full-length PKL/EPP1 did not interact with HY5, and the N-terminal fraction (D1) interacted less strongly with HY5 than did the D5 and D6 fragments in the yeast two-hybrid assay (Figure 4B). This is likely because the yeast two-hybrid assay is a transcription-based method, and full-length PKL may repress the transcriptional activity in yeast cells. Similar interactions have been observed for animal CHD3 chromatin remodelers. For instance, *Drosophila melanogaster* Mi-2 physically interacts with the transcription factor dDREF to regulate cell development (Hirose et al., 2002). A recent study also documented that SYD and BRM interact with LEAFY and SEPALLATA3 transcription factors to regulate floral organ identity (Wu et al., 2012). It is thus speculated that chromatin remodeling factors might determine the target and regulatory specificity by interacting with specific transcription factors in a spatial and temporal manner, underlining the fundamental importance of chromatin configuration during development. Consistent with this, *PKL/EPP1* expression is repressed by light specifically in the hypocotyl regions in a photoreceptor-dependent manner and negatively correlates with the extent of photomorphogenesis. Similarly, HY5 abundance peaks in early seedling development and is positively correlated with photomorphogenesis (Hardtke et al., 2000; Osterlund et al., 2000). Both components act together for the fine regulation of downstream genes in response to light. Future studies should investigate how photoreceptors inhibit *PKL/EPP1* expression in the hypocotyls. It is possible that PKL modulates transcriptional activities in a range of processes (e.g., embryo and root meristem development) by interacting with other transcription factors as well.

The Polycomb-repressive Complex2 (PRC2) catalyzes the trimethylation of H3K27 and acts as a global repressor of transcription (Schubert et al., 2006). Since the level of H3K27me3 on the regulatory regions of several cell elongation–related genes is enhanced by the *epp1* mutation, it is tempting to speculate that

PKL antagonizes the function of PRC2 components to repress H3K27me3 and mediate light signaling. This possibility is supported by the antagonistic regulation of root meristem activity and floral organ identity by CURLY LEAF (a PRC2 component) and PKL or SYD, respectively (Aichinger et al., 2011; Wu et al., 2012). Besides preventing methylation, PKL could also promote the demethylation of genes involved in cell elongation and thereby activate them. Future studies are required to distinguish between these possibilities. Additionally, PKL homologs, Mi-2b (CHD4) and CHD3, belong to the integral subunits of the Mi-2/NuRD (for nucleosome-remodeling and histone deacetylase) complex that functions in transcription regulation (Denslow and Wade, 2007). *Arabidopsis* histone deacetylase HD1 is closely related to HDAC1 and HDAC2, two NuRD components in human. A previous study provided evidence that HD1 is required for histone H3 and H4 Lys deacetylation in several light-responsive genes and that the *hd1* mutant displayed hyposensitivity to all light treatments, just like the *epp1* mutant (Benhamed et al., 2006). These observations argue for a possible relationship between HD1 and PKL/EPP1 in the regulation of histone modification and light signaling, although PKL is unlikely to be a member of the plant equivalent NuRD complex (Ho et al., 2012). Nevertheless, the identification of more factors involved in chromatin modification will enable us to better understand the chromatin remodeling–mediated epigenetic control of plant plasticity in response to ambient light conditions.

METHODS

Plant Materials and Growth Conditions

The *epp1-1* mutant was originally isolated from activation-tagging T-DNA insertion stocks (Weigel et al., 2000). *pkl-1* (Ogas et al., 1999), *epp1-2* (SAIL_73_H08), *epp1-3* (Salk_033554C), *exp2-1* (Salk_137972C), *dwf4-11* (SAIL_713_F05), *phyA-211* (Reed et al., 1994), *phyB-9* (Reed et al., 1993), *cry1-304* (Mockler et al., 1999), *hy5-215* (Oyama et al., 1997), *hyh* (CS849765), *cop1-4* (McNellis et al., 1994), *pkr1-1*, *pkr2-2* (Aichinger et al., 2009), *chr5-1* (Salk_087282), and *ProPKL:PKL-4Myc* (Fukaki et al., 2006) are of the Col ecotype. The T-DNA mutants from the Arabidopsis Biological Resource Center were confirmed by PCR genotyping and sequencing. Double mutants were generated by genetic crossing and were verified by phenotype inspection, antibiotic selection, PCR genotyping, and/or sequencing. After sterilization, seeds were sown onto Murashige and Skoog (MS) medium containing 1% Suc and 0.8% agar and were incubated at 4°C in darkness for 3 d, followed by various light treatments. Far-red (12 μmol m^{-2} s^{-1}), red (10 μmol m^{-2} s^{-1}), and blue (14 μmol m^{-2} s^{-1}) light were supplied by light-emitting diode light sources, or the light intensities were otherwise indicated. White light (60 μmol m^{-2} s^{-1}) was supplied by cool white fluorescent lamps.

Phenotypic Analysis

After growth under appropriate light conditions, the seedlings were transferred to MS plates and were photographed with a digital camera (Olympus). Cell length and number were determined by inspection under a microscope. The hypocotyl length, cotyledon angle, and cell length were measured using NIH Image J software (http://rsbweb.nih.gov/ij/).

Anthocyanin and Chlorophyll Measurements

Anthocyanin and chlorophyll measurements were conducted as previously described (Lin et al., 2007). Anthocyanin content was expressed as $(A_{530} - 0.25 \times A_{657})$ per 100 seedlings. Chlorophyll content was expressed as micrograms per 100 seedlings. All experiments were performed in triplicate.

Inverse PCR Cloning

The T-DNA insertion site in *epp1-1* was identified by inverse PCR analysis. Genomic DNA was extracted from the *epp1-1* seedlings and digested with *Bam*HI or *Spe*I. After self-ligation with T4 DNA ligase, PCR was performed to amplify the T-DNA flanking sequence using T3 and pSKI-RB primers. The resulting PCR product was cloned into the pEASY vector (TransGen) and subjected to DNA sequencing with the T3 primer.

Plasmid Construction

To obtain the open reading frame (ORF), first-strand cDNA was reverse transcribed using oligo(dT)$_{18}$ primer from total RNA extracted from Col wild-type seedlings. The ORFs of *PKL* and *HY5* were amplified using high fidelity *Pfu* DNA polymerase (Invitrogen) and cloned into the pEASY vector, resulting in pEASY-PKL and pEASY-HY5, respectively. To facilitate the follow-up cloning, two restriction sites within the PKL ORF, *Mfe*I and *Xho*I, were mutagenized without changing the amino acid sequence by site-directed mutagenesis (Takara). The resulting plasmid was designated pEASY-PKLm. To generate a series of PKL deletions, D1, D2, D3, D4, D5, and D6 fragments, as shown in Figure 4A, were amplified using the pEASY-PKLm plasmid as template and the corresponding primer pairs and cloned into the pEASY vector, resulting in pEASY-D1/D2/D3/D4/D5/D6, respectively. To generate a point mutation derivative within the D6 fragment, D6^{K304A}, site-directed mutagenesis was performed using pEASY-D6 as template. The resulting vector was named pEASY-D6^{K304A}.

To construct vectors for the yeast two-hybrid assay, the pEASY-PKLm/D1/D2/D3/D4/D5/D6/D6^{K304A} plasmids were cut with *Mfe*I and *Xho*I, and the corresponding fragments were ligated into *Eco*RI/*Xho*I-digested pEG202 vector (Clontech) to give rise to pLexA-PKL/D1/D2/D3/D4/D5/D6/D6^{K304A}, respectively. The *HY5* fragment was released from *Eco*RI/*Xho*I-cut pEASY-HY5 and ligated into the pJG4-5 vector digested with the same enzymes to generate pAD-HY5.

To prepare constructs for the BiFC assay, the fragment liberated from *Mfe*I/*Xho*I-cut pEASY-PKLm was cloned into the pUC-SPYNE vector (Walter et al., 2004) digested with *Eco*RI and *Xho*I to generate pYFP^N-PKL. pEASY-HY5 plasmid was cut with *Eco*RI and *Xho*I, and the *HY5* fragment was cloned into the pUC-SPYCE vector digested with *Eco*RI and *Xho*I to give rise to pYFP^C-HY5.

To generate bacterial expression vectors, the pEASY-D6 plasmid was digested with *Mfe*I and *Xho*I, and the D6 fragment was inserted into the pGEX-5X-1 vector (GE Healthcare) digested with *Eco*RI and *Xho*I, resulting in pGEX-D6. pEASY-HY5 plasmids were cut with *Eco*RI and *Xho*I, and the *HY5* ORF was ligated into the pET-28a vector (Novagen) digested with the same enzymes to generate pHis-HY5. To make the construct for generating PKL antibody, a DNA fragment corresponding to amino acids 1178 to 1384 was amplified and cloned into pEASY, resulting in pEASY-PKL-CT. pEASY-PKL-CT was then digested with *Bam*HI and *Xho*I, and the PKL-CT fragment was inserted into pET-28a cut with *Bam*HI and *Xho*I to give rise to pHis-PKL-CT.

To prepare constructs for the yeast one-hybrid assay, 1.0- and 1.7-kb fragments of the *EXP2* and *IAA19* promoters, respectively, were PCR amplified from Col genomic DNA and ligated into the pEASY vector to give rise to pEASY-EXP2p and pEASY-IAA19p, respectively. The *EXP2* and *IAA19* promoter fragments were released from pEASY-EXP2p (cut with *Eco*RI/*Xho*I) and pEASY-IAA19p (cut with *Mfe*I/*Xho*I) and inserted into pLacZi2μ (Lin et al., 2007) to generate *ProEXP2:LacZ* and *ProIAA19:LacZ*, respectively. To construct *LacZ* reporter genes driven by the subfragments of the *EXP2* and *IAA19* promoters (including the wild-type and mutant form of the G-box), oligonucleotides were synthesized as two

complementary oligo primers with an *Eco*RI site overhang at the 5′-end and an *Xho*I site overhang at the 3′-end. The oligo primers were annealed and the double-stranded oligonucleotides were ligated into the *Eco*RI-*Xho*I sites of pLacZi2μ to produce *EXP2wt:LacZ*, *EXP2m:LacZ*, *IAA19wt*: *LacZ*, and *IAA19m:LacZ*, respectively.

To generate constructs for the transient expression assay, a fragment encoding the ERF3 repression domain (ERF3RD) was amplified from Col DNA, and a fragment encoding the VP16 activation domain was amplified from pGEM-VP16A (Lin et al., 2007). These genes were cloned into pEASY to generate pEASY-ERF3RD and pEASY-VP16, respectively. The GAL4 DNA binding domain was amplified from the pGBKT7 vector (Clontech). The fragment was then digested with *Nco*I and *Eco*RI and ligated into the pSAT vector (Tzfira et al., 2005) cut with the same enzymes, resulting in pSAT-GAL4BD. The *HY5* and *ERF3RD* fragments were released from pEASY-HY5 and pEASY-ERF3RD digested with *Eco*RI and *Xho*I and inserted into the *Eco*RI-*Sal*I sites of pSAT-GAL4BD to generate pGAL4DB-HY5 and pGAL4DB-ERF3RD, respectively. pEASY-VP16 was cut with *Bam*HI and *Sal*I to release VP16, which was then inserted into the *Bam*HI-*Sal*I sites of pSAT-GAL4BD, resulting in pGAD4BD-VP16. pEASY-HY5 and pEASY-ERF3RD plasmids were cut with *Eco*RI and *Xho*I, and the fragments were then inserted into *Eco*RI-*Sal*I–cut pGAD4BD-VP16 to generate pGAD4BD-HY5-VP16 and pGAD4BD-ERF3RD-VP16, respectively. VP16 was amplified and digested with *Kpn*I and *Bam*HI, and the fragment was inserted into pSAT-GAL4BD cut with the same enzymes to generate pGAL4BD-VP16-2. pEASY-PKLm was cut with *Mfe*I and *Xho*I and the *PKL* fragment was cloned into *Eco*RI-*Sal*I–cut pSAT-GAL4BD and pGAL4BD-VP16-2, resulting in pGAL4DB-PKL and pGAL4DB-PKL-VP16, respectively.

To construct *ProPKL:GUS*, a fragment spanning the region 2 kb upstream of the ATG start site of the *PKL* coding sequence was amplified by PCR and cloned into pEASY, resulting in pEASY-PKLp. pEASY-PKLp plasmid was digested with *Sal*I and *Bam*HI to release the *PKL* promoter, which was then ligated into the pRI101-AN vector (Takara) digested with the same enzymes to generate *ProPKL:GUS*. The primers used for cloning are listed in Supplemental Table 1 online. All amplified fragments were validated by sequencing.

The binary constructs were electroporated into *Agrobacterium tumefaciens* strain GV3101 and then introduced into destination plants via the floral dip method (Clough and Bent, 1998). Transgenic plants were selected on MS plates in the presence of 50 mg/L kanamycin.

Gene Expression Analysis

Seedling growth conditions are described in the text. Plant total RNA was isolated by RNA extraction kit (Tiangen), and the first-strand cDNA was synthesized by reverse transcriptase (Invitrogen). Real-time PCR was performed with the SYBR Premix ExTaq kit (Takara) following the manufacturer's instructions. Primers are listed in Supplemental Table 1 online. Three biological replicates were performed for each sample and the expression level was normalized to that of a *UBQ* control.

GUS Histochemical Assay

Seedlings of the *ProPKL:GUS* transgenic line were harvested and incubated overnight in 0.1 M sodium phosphate buffer containing 50 mM $K_3Fe(CN)_6$, 50 mM $K_4Fe(CN)_6$, and 1 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronide at 37°C. GUS expression was examined under a dissecting microscope (Olympus), and images were captured by a digital camera (Olympus).

LUC Activity Assay

The LUC activity assay was performed as previously reported (Tang et al., 2012). LUC reporter activity was detected with a luminescence kit using the LUC assay substrate (Promega). Relative reporter gene expression levels are expressed as the ratio of LUC to GUS.

Purification of Recombinant Protein and Antibody Preparation

GST, GST-D6, His-PKL-CT, and His-HY5 recombinant fusion proteins were induced by isopropyl β-D-1-thiogalactopyranoside and expressed in the *Escherichia coli* BL21 (DE3) strain. The proteins were then purified using Glutathione Sepharose 4B beads (GE Healthcare; for GST fusions) or Ni-NTA agarose (Qiagen; for His fusions) following the manufacturer's instructions. His-PKL-CT and His-HY5 were then used to raise PKL and HY5 polyclonal antibodies in rabbits, respectively. For tubulin antibody, a synthetic peptide (EEVGAEGGDDEDDEGEEY) derived from amino acids 433 to 450 of tubulin 4 was conjugated with KLH (Cali-Bio) and used for injecting rabbit.

ChIP Assays

ChIP assays were performed as described previously (Tang et al., 2012). Chromatin complexes were incubated with anti-PKL, anti-HY5, anti-H3 (Millipore 07-690), or anti-H3K27me3 (Millipore 07-449) polyclonal antibodies or IgG serum. The precipitated DNA fragments were recovered and quantified by quantitative PCR with the primers shown in Supplemental Table 1 online. Relative enrichment is expressed as the ratio of DNA amount after immunoprecipitation to that in input.

Pull-Down and Coimmunoprecipitation

The procedures used for pull-down, coimmunoprecipitation, and immunoblot assays were described previously (Tang et al., 2012). For the pull-down assay, ~2 μg of purified recombinant bait protein (GST-D6 or GST) and 2 μg of prey protein (His-HY5) were incubated in binding buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 0.6% Triton X-100) for 2 h at 4°C. Glutathione Sepharose 4B beads (Roche) were added and reactions were incubated for 1 h. After washing with binding buffer, precipitated proteins were eluted in 2× SDS loading buffer. The proteins were then size fractionated on a 10% SDS-PAGE gel and immunoblotted with anti-His or anti-GST antibodies (Abcam ab19256). For the coimmunoprecipitation assay, seedlings were grown in the light for 4 d. Total proteins were extracted with extraction buffer and incubated with 2 μg anti-PKL antibody for 2 to 3 h at 4°C. Fifty microliters of protein G-Sepharose beads (Roche) was added and the reaction was incubated for a further 2 to 3 h. The beads were washed three times with coimmunoprecipitation buffer, and the precipitated proteins were eluted in 2× SDS loading buffer by boiling for 10 min. The proteins were separated on a 6 or 8% SDS-PAGE gel and detected by immunoblotting with anti-PKL and anti-HY5 antibodies.

BiFC Assays

For BiFC assays, plasmids of N- and C-terminal fusions of YFP were cotransformed into *Arabidopsis thaliana* protoplasts as previously described (Walter et al., 2004). The protoplasts were incubated under weak light for 12 to 16 h before observation. The YFP fluorescence was determined using a confocal microscope (Olympus).

Yeast Interaction Assays

Yeast one-hybrid and two-hybrid assays were performed according to the Yeast Protocols Handbook (Clontech). Briefly, for the yeast one-hybrid assay, the AD fusion constructs were cotransformed with various *LacZ* reporter plasmids into yeast strain EGY48. Transformants were grown on SD/-Trp-Ura dropout plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) for blue color development. For yeast two-hybrid analysis, the AD fusion constructs were transformed into the strain Ym4271, and LexA-fusion plasmids were cotransformed with a *LexAop*: *LacZ* (Clontech) reporter into the strain EGY48. After mating, transformants

were grown on SD/-Trp-Ura-His dropout plates with X-gal for blue color development.

Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL data libraries under the following accession numbers: *PKL/EPP1* (At2g25170), *HY5* (At5g11260), *HYH* (At3g17609), *EXP2* (At5g05290), *IAA19* (At3g15540), *DWF4* (At3g50660), *EXT3* (At1g21310), *XTH17* (At1g65310), *XTR6* (At4g25810), *CHS* (At5g13930), *CAB2* (At1g29920), *PKR1* (At5g44800), *PKR2* (At4g31900), *CHR5* (At2g13370), *ERF3* (AT1G50640), *UBQ1* (At3g52590), *ACT2* (At3g18780), and *TUA4* (At1g04820). Mutants investigated in this study are listed in Methods.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure 1. Characterization of *epp1* Mutants.

Supplemental Figure 2. Genetic Relationship between PKL/EPP1 and the Photoreceptors and COP1.

Supplemental Figure 3. ChIP-qPCR Assay Using HY5 Antibody.

Supplemental Figure 4. Photomorphogenic Phenotype of the *exp2* and *dwf4* Loss-of-Function Mutants.

Supplemental Figure 5. HYH Interacts with PKL in Yeast Cells.

Supplemental Figure 6. Amino Acid Alignment of Truncated ATP Binding/Helicase Domains of CHD3/CHD4.

Supplemental Figure 7. ChIP Assay Using H3K27me3 Antibody of Samples Grown under Light and Dark Conditions.

Supplemental Figure 8. Transient Transcriptional Activity Assay of PKL.

Supplemental Figure 9. Phenotype of *pkr1*, *pkr2*, and *chr5* Single Mutants and Their Double Mutants with *pkl*.

Supplemental Table 1. List of Primers Used in This Study.

AUTHOR CONTRIBUTIONS

Y.J. and R.L. designed the research. Y.J., D.Z., X.W., W.T., W.W., J.H., G.X., D.C., and Y.L. performed the research. Y.J. and R.L. analyzed the data and wrote the article.

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