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RESEARCH ARTICLE

The Elongator complex regulates hypocotyl growth in darkness and during photomorphogenesis

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ABSTRACT

The Elongator complex (hereafter Elongator) promotes RNA polymerase II-mediated transcript elongation through epigenetic activities such as histone acetylation. Elongator regulates growth, development, immune response and sensitivity to drought and abscisic acid. We demonstrate that *elo* mutants exhibit defective hypocotyl elongation but have a normal apical hook in darkness and are hyposensitive to light during photomorphogenesis. These *elo* phenotypes are supported by transcriptome changes, including downregulation of circadian clock components, positive regulators of skoto- or photomorphogenesis, hormonal pathways and cell wall biogenesis-related factors. The downregulated genes *LHY*, *HFR1* and *HYH* are selectively targeted by Elongator for histone H3K14 acetylation in darkness. The role of Elongator in early seedling development in darkness and light is supported by hypocotyl phenotypes of mutants defective in components of the gene network regulated by Elongator, and by double mutants between *elo* and mutants in light or darkness signaling components. A model is proposed in which Elongator represses the plant immune response and promotes hypocotyl elongation and photomorphogenesis via transcriptional control of positive photomorphogenesis regulators and a growth-regulatory network that converges on genes involved in cell wall biogenesis and hormone signaling.

KEY WORDS: *Arabidopsis*, Histone acetyl transferase complex, Hypocotyl elongation, Darkness, Light, Transcript elongation

INTRODUCTION

The conserved Elongator complex (hereafter Elongator) is a transcription elongation factor that binds in yeast to CTD-phosphorylated RNA polymerase II (RNAPII) at the coding part of genes and facilitates transcript elongation via histone acetyl transferase (HAT) activity, preferentially targeting lysine 14 of histone H3 (Otero et al., 1999; Woloszynska et al., 2016; Van Lijsebettens and Grasser, 2014). The Elongator complex consists of six subunits, ELP1 to ELP6, and two subcomplexes ELP1 to ELP3 and ELP4 to ELP6, with ELP3 conferring HAT and DNA demethylation activities (Nelissen et al., 2005, 2010; Glatt and Müller, 2013; DeFraia et al., 2013). The ELP4-ELP6 subcomplex plays a role in the modification of uridines at the wobble position in transfer RNAs (Glatt and Müller, 2013). In plants, an epigenetic role for Elongator in transcription and processing of primary microRNAs has been shown (Fang et al., 2015). Analysis of *Arabidopsis* mutants impaired in the expression of Elongator subunits revealed that Elongator regulates growth, development and responses to environmental stimuli (Ding and Mou, 2015). Elongator is expressed in meristematic tissues, which correlates with delayed growth, shortened primary roots, reduced lateral root density, abnormal leaves, defective inflorescence phylotaxis and reduced apical dominance in *elongata* (*elo*) mutants (Nelissen et al., 2010; Skylar et al., 2013; Jia et al., 2015). In addition, *elo* mutants have altered sensitivities to drought and abscisic acid (Chen et al., 2006; Zhou et al., 2009), whereas genes of the plant immune response are down- or upregulated (DeFraia et al., 2010; Wang et al., 2013, 2015). Reduced histone H3K14 acetylation of auxin response-related genes (Nelissen et al., 2010), of genes encoding transcription factors essential for root development (Jia et al., 2015) and of genes coding for salicylic acid, jasmonic acid and ethylene signaling (An et al., 2017; Wang et al., 2013, 2015) correlated with their reduced gene expression and the specific phenotypes in *elo* mutants.

Following germination, seedlings develop according to the skotomorphogenic program, in which hypocotyls elongate (so-called etiolation), apical hooks are closed and cotyledons are folded. When seedlings reach the soil surface, the developmental program switches to photomorphogenesis, resulting in de-etiolation, in which hypocotyl elongation is inhibited, apical hooks open and cotyledons expand. Morphological changes are driven by light-stimulated transcriptional or post-transcriptional shifts in the accumulation of positive skoto- and photomorphogenesis regulators, controlled by photoreceptors and the circadian clock. Interestingly, chromatin modifications modulate the expression of genes encoding regulators of skoto- and photomorphogenesis, such as the phytochrome A (*PHYA*) photoreceptor; the positive photomorphogenesis regulators *ELONGATED HYPOCOTYL 5* (*HY5*) and *HY5-HOMOLOG* (*HYH*) (Cloix and Jenkins, 2008); the positive skotomorphogenesis regulator *SUPPRESSOR OF PHYA-105 1* (*SPA1*) (Bourbousse et al., 2012); the *EARLY LIGHT-INDUCIBLE PROTEIN 1* (*ELIP1*) (Cloix and Jenkins, 2008); and the circadian clock genes *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), *LATE ELONGATED HYPOCOTYL* (*LHY*), *TIMING OF CAB EXPRESSION 1* (*TOC1*), *LUX ARRHYTHMO* (*LUX*), *EARLY FLOWERING 4* (*ELF4*), *PSEUDO RESPONSE REGULATOR 7* (*PRR7*) and *PRR9* (Hemmes et al., 2012; Himanen et al., 2012; Malapeira et al., 2012).

Here, we show that Elongator regulates seedling development in darkness and light via a growth-regulatory network of genes that converge on cell wall biogenesis and positive photomorphogenesis

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factors, some of which are targeted by Elongator HAT activity specifically in darkness, suggesting target gene selection.

RESULTS

Phenotypes of the *elo* seedlings in darkness and light

Narrow, elongated and hyponastic leaves and petioles of *elo* mutants resemble those of photoreceptor mutants (Fig. S1A), suggesting that Elongator plays a role in the light response. Therefore, we investigated the role of Elongator in early *Arabidopsis* development in darkness or light (during etiolation or de-etiolation, respectively) by scoring hypocotyl elongation and apical hook formation, two characteristics of seedling growth that differ between the skoto- and photomorphogenetic developmental programs. Seeds of *elo3-6* and Col-0 (wild type) were sown, stratified for 48 h, illuminated for 6 h in white light to induce germination, and transferred either to darkness or to red, far-red or blue light. Representative seedling phenotypes are shown at 4 days after germination (DAG) (Fig. 1A). The hypocotyl length and seedling morphology was compared between the *elo3-6* mutant and Col-0 control every day between 3 and 7 DAG (Fig. 1A,B; Fig. S1B). Darkness-grown *elo3-6* seedlings had shorter hypocotyls than Col-0 seedlings (Fig. 1B), but cotyledons and apical hooks were similar (Fig. 1A; Fig. S1B), indicating that the mutation affected only hypocotyl growth. The hypocotyl length difference between Col-0 and *elo3-6* seedlings was maximal at 3 DAG (0.55 cm and 0.33 cm, respectively) (Fig. 1B). At 5 DAG, hypocotyl elongation nearly stopped for Col-0, whereas *elo3-6* hypocotyls still elongated, ultimately reaching lengths similar to those of the wild types at 7 DAG (Fig. 1B).

The *elo3-6* seedlings grown in red, far-red or blue light had reduced de-etiolation, visible as longer hypocotyls between 3 and 7 DAG (Fig. 1B), reduced cotyledon expansion and hyponastic growth of the cotyledons (Fig. 1A; Fig. S1B), showing that the mutant is hyposensitive to all light qualities. Light inhibited

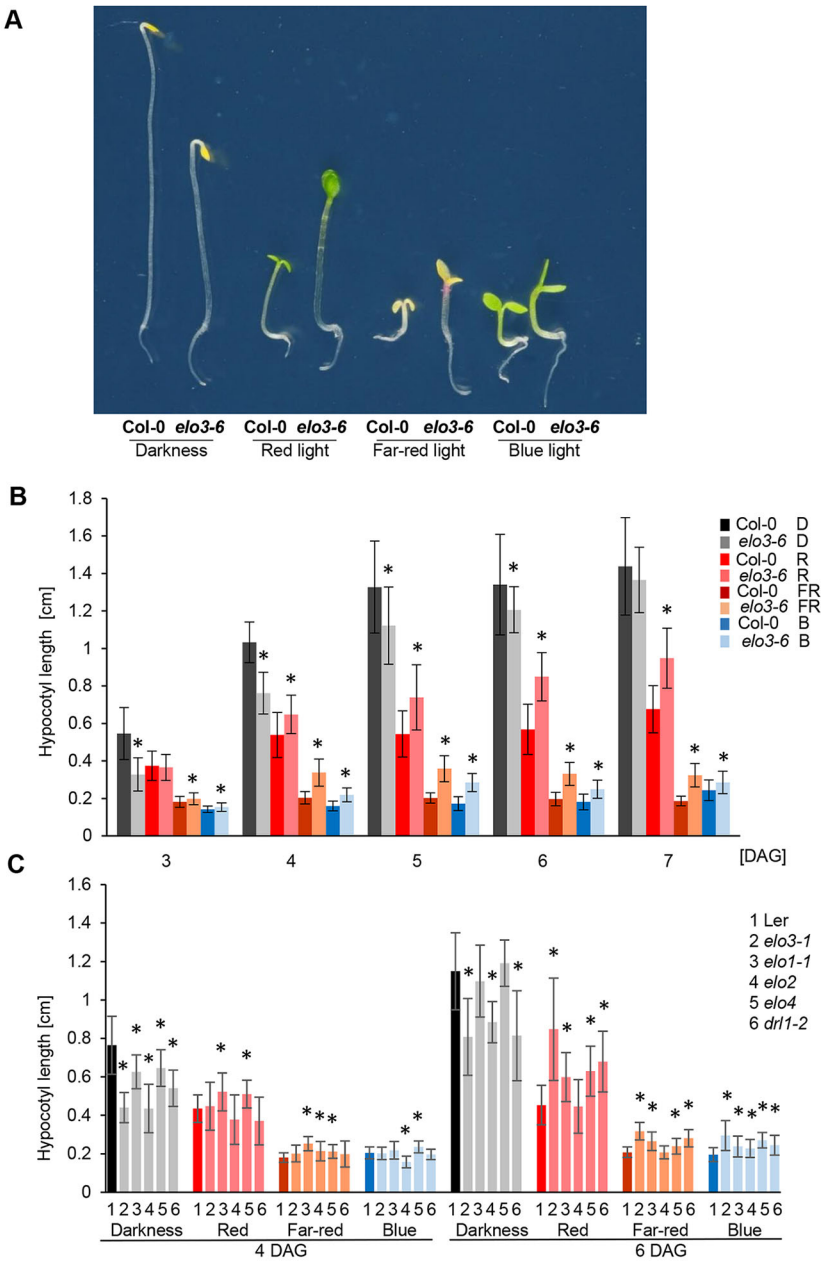

Fig. 1. Phenotypes of *elo* seedlings grown in darkness or under different light conditions. (A) Representative seedlings germinated and grown on half-strength MS medium for 4 days in darkness or under continuous monochromatic light of different wavelengths. (B) Hypocotyl lengths of Col-0 and *elo3-6* seedlings grown in darkness (D) or under continuous red (R), far-red (FR) and blue (B) light. (C) Hypocotyl lengths of mutants of different Elongator subunits grown on half-strength MS medium in darkness or under continuous monochromatic light of different wavelengths. Bars represent mean hypocotyl length of 25 seedlings (mean±s.d.). Differences between mutant and wild type were statistically analyzed with an unpaired two-tailed Student's *t*-test; **P*<0.05.

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hypocotyl elongation in the Col-0 seedlings already at 3 or 4 DAG, whereas hypocotyls elongated until 5 to 7 DAG in the *elo3-6* mutant, depending on the light quality (Fig. 1B).

The seedling phenotypes of the *elo3-1* Landsberg *erecta* (Ler) mutant grown in darkness, red, far-red or blue light were assessed at 4 and 6 DAG relative to the Ler control. Alterations were comparable to those of the *elo3-6* Col-0 allele (Fig. 1C), confirming that ELP3 regulates hypocotyl growth in darkness and in light. Hypocotyl lengths of the *elo1-1* (mutation in the accessory subunit *ELP4* gene), *elo2* (the core subunit *ELP1* gene), *elo4/drl1-4* and *drl1-2* (the Elongator interactor *DRL1/ELO4* gene) mutants, and the wild-type Ler were assayed at 4 and 6 DAG. Results were similar to those obtained for the *elo3-1* and *elo3-6* mutants (Fig. 1C), suggesting that the Elongator as an integral complex regulates hypocotyl elongation in darkness and in different light conditions in *Arabidopsis*.

Genetic interactions between Elongator and light-dependent receptors and regulators for hypocotyl growth

To examine the role of Elongator in the regulation of hypocotyl growth, the *elo3-1* (Ler) or *elo3-6* (Col-0) mutants were used as proxy for the Elongator complex and combined with the *phyB-1*, *phyA-201*, *hfr1-101* and *pif3-3 pif4-1* mutants in light-dependent receptors and regulators. Hypocotyl length was compared between the control, the parental lines and their double or triple mutant combinations grown in darkness or in red or far-red light at 4 and 6 DAG (Fig. 2).

The *phyB-1* (Fig. 2A) and *phyA-201* (Fig. 2B) mutants had significantly longer hypocotyls than the Ler control in darkness and light, because a decrease in active phytochrome molecules results in increased levels of PHYTOCHROME INTERACTING FACTORS (PIFs), which stimulate cell elongation (Leivar et al., 2008a,b). Hypocotyl lengths of double mutants combining *phyB-1* or *phyA-201* with *elo3-1* were significantly longer than those of *elo3-1*, but shorter than those of *phy* single mutants (Fig. 2A,B). This intermediate phenotype probably results from the additive effect of the *phyB-1* or *phyA-201* mutations, leading to increased hypocotyl elongation (comparable to the effect of darkness on the wild type), and the *elo3-1* mutation that disables hypocotyl elongation under such conditions. Therefore, the deficit of Elongator results in two defects leading to opposite changes in hypocotyl growth. First, the *elo3-1* mutant has decreased light sensitivity, resulting in longer hypocotyls in light-grown seedlings and, second, it grows more slowly in conditions of strongly enhanced cell elongation, such as darkness or the *phy* background. These results confirm that Elongator is indispensable for the light response and for the fast growth stimulation that occurs in darkness or upon *phy* mutation.

The hypocotyl length of the *elo3-6* mutant grown in darkness was reduced more than that of the *pif3-3 pif4-2* mutant compared to the Col-0 control (Fig. 2C), indicating that Elongator regulates hypocotyl growth via factors different or additional to PIF3 and PIF4. The combination of *elo3-6* and *pif3-3 pif4-2* mutations in the triple mutant resulted in only slightly shorter hypocotyls than *elo3-6*, suggesting that the PIF pathway positively regulating hypocotyl elongation could already have been downregulated in *elo3-6* in darkness. Therefore, in darkness, Elongator might control hypocotyl elongation via PIFs and other pathways. In red light, the hypocotyl length of *pif3-3 pif4-2* was significantly shorter than that of the Col-0 control, whereas it was intermediate in the *elo3-6 pif3-3 pif4-2* triple mutant compared with its parental lines. This effect was a result of the additive effect of mutations inversely regulating hypocotyl length in red light. These findings suggest that the PIF pathway is not affected by Elongator during growth in red light.

The *hfr1-101* mutant had significantly longer hypocotyls than the Col-0 control in darkness, indicating that HFR1 (LONG

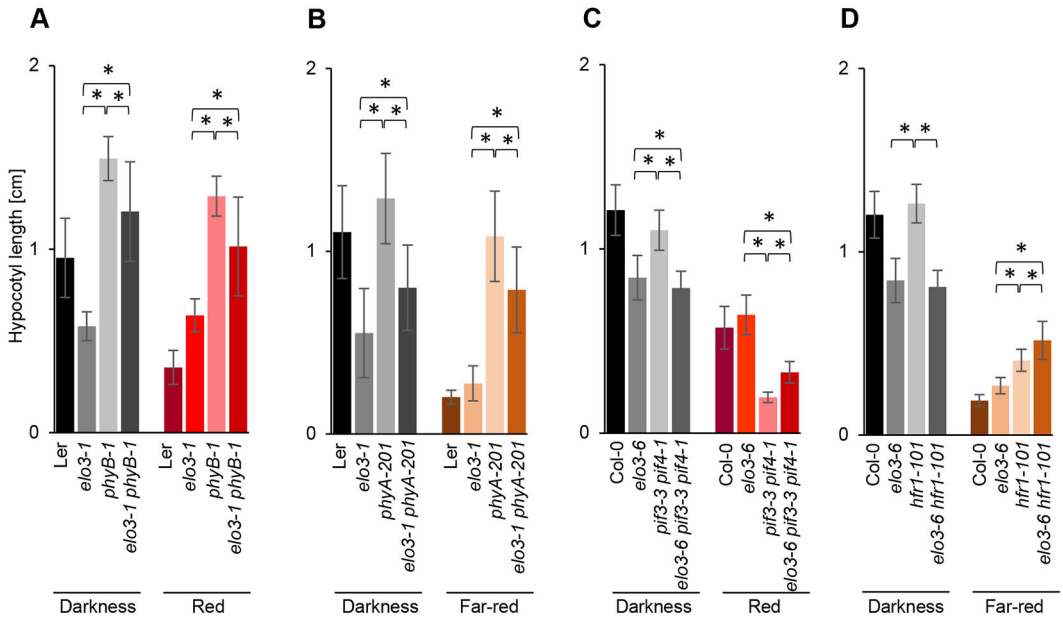

Fig. 2. Genetic interactions for hypocotyl growth between Elongator and phyA, phyB, PIF or HFR1. (A–D) Seedlings of *Arabidopsis* Ler (A,B) or Col-0 (C,D) wild types, and *elo3-1* (A,B), *elo3-6* (C,D), *phyB1* and *elo3-1 phyB-1* (A), *phyA-201* and *elo3-1 phyA-201* (B), *pif3-3 pif4-1* and *elo3-6 pif3-3 pif4-1* (C), and *hfr1-101* and *elo3-6 hfr1-101* (D) mutants were grown for 4 days on half-strength MS medium without sucrose in darkness, continuous red or far-red light. Hypocotyl lengths were quantified. Error bars represent mean values of hypocotyl length of 25 seedlings with standard deviation (mean±s.d.). Differences between genotypes were statistically analyzed with an unpaired two-tailed Student's *t*-test; **P*<0.05. Differences in hypocotyl length between single, double or triple mutants and their respective wild types were always statistically significant and therefore not indicated in the graphs. The experiment was repeated twice.

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HYPOCOTYL IN FAR-RED 1, a positive photomorphogenesis regulator and suppressor of PIF action) is active in the absence of light and counteracts exaggerated hypocotyl elongation (Fig. 2D). The *hfr1-101* mutation did not increase the hypocotyl elongation of *elo3-6* in the *elo3-6 hfr1-101* double mutant in darkness, indicating that Elongator and HFR1 are involved in the same pathway regulating hypocotyl elongation in darkness and that Elongator is located upstream of HFR1. In far-red light, hypocotyls of the *elo3-6* and *hfr1-101* mutants were longer than those of Col-0, and the *elo3-6 hfr1-101* double mutant had hypocotyls longer than those of both parents, indicating a synergistic interaction between Elongator and HFR1 in hypocotyl elongation. This result suggests that in far-red light, in contrast to darkness, the ELO3 and HFR1 activities converge on the same process of hypocotyl elongation, leading to a dramatic elongation of the double-mutant hypocotyl.

In conclusion, double-mutant analyses show that Elongator is required for fast hypocotyl elongation in darkness and that this Elongator function is involved in growth-stimulating mechanisms other than the PIF pathway. Under light conditions, Elongator promotes inhibition of hypocotyl growth by acting in far-red light via an HFR1-interacting pathway.

The *elo3-6* mutant transcriptome in darkness

The gene regulatory network underlying the hypocotyl elongation phenotype of *elo3-6* was compared with that of Col-0 in the microarray dataset of 4-day-old darkness-grown seedlings: 2489 genes were downregulated and 2533 genes were upregulated in the mutant, at $-0.5 \geq \log2FC \geq 0.5$, $P<0.05$ (NCBI, Gene Expression Omnibus, accession number GSE42053).

Upregulated genes in *elo3-6* clustered in two large Gene Ontology (GO) categories (Table S1), i.e. 'Response to stimuli' (defense response genes and genes induced by light, cold, osmotic stress, oxidative stress, water, desiccation, salt, carbohydrates, metal ions, hormones and other organisms) and 'Metabolic process' (genes related to catabolism of carbohydrate, coding for enzymes driving glycolysis, pentose phosphate pathway, TCA cycle, starch breakdown, photorespiration and Calvin cycle, and genes involved in biosynthesis of amino acids, lipids, nucleotides, gibberellins and flavones). The GO category 'Defense response' contains 140 genes, including those encoding important defense regulators and showing moderate (maximally two- to threefold) upregulation. phytoalexin-deficient 4 (PAD4) is a component of basal immunity against virulent pathogens and also contributes to effector-triggered immunity and systemic acquired resistance (Louis et al., 2012). PAD3/CYP71B15 catalyzes biosynthesis of camalexin, determining elicitor-induced resistance against fungal pathogens (Ferrari et al., 2007); its upregulated transcripts are markers for camalexin biosynthesis (Prince et al., 2014). Cytochrome P450s (CYP79B2 and CYP79B3) are involved in tryptophan metabolism and biosynthesis of pathogen defense components. PENETRATION 3 (PEN3) plays a role in the focal immune response and response to fungal and bacterial pathogens and is a marker of plant–pathogen interaction (Xin et al., 2013). Two ELICITOR PEPTIDE PRECURSORs (PROPEP2 and 3) are massively upregulated following pathogen challenges and recognized by PERP1/PERP2 receptors of defense signaling. Upregulation of GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE C SUBUNIT 1 (GAPC1) enhances glycolysis, providing ATP and pyruvate (reactive oxygen species scavenger) for plants undergoing immune response (Henry et al., 2015). Other genes with a confirmed positive effect on plant immunity were also upregulated in *elo3-6*: *AZELAIC ACID INDUCED 1* (*AZI1*), *LONG-CHAIN ACYL-COA SYNTHETASE 2* (*LACS2*), *ENHANCED DISEASE SUSCEPTIBILITY 5* (*EDS5*), *GRETCHEN HAGEN 3.12* (*GH3.12*), *ARABIDOPSIS THALIANA SULFOTRANSFERASE 1* (*ATSOT1*), *ACTIVATED DISEASE RESISTANCE 1* (*ADR1*) and *ADR1-LIKE 1*. Some of the genes involved in carbohydrates catabolism together with genes coding for subunits of the mitochondrial electron transport chain and ATP synthase were grouped in the overrepresented GO category 'Energy derivation by oxidation of organic compounds'. Two smaller GO categories of upregulated genes were identified: 'Cell wall organization or biogenesis', containing genes related to defense and/or cell wall firmness (chitinases, pectin methylesterases), and 'Localization', including the genes coding for transporters of sugars, amino acids, proteins, lipids and metal ions.

In summary, the set of genes upregulated in the *elo3-6* mutant in darkness matches transcriptome profiles typical for the plant response to pathogens (Rojas et al., 2014). The upregulation of defense-related pathways is followed by the upregulation of primary metabolism genes involved in energy production (carbohydrates catabolism, mitochondrial electron transport, nucleotides and amino acid biosynthesis) or synthesis of signaling molecules (carbohydrates and lipids). The upregulation of defense-related genes results in energy deprivation, which activates compensatory downregulation of other pathways and ultimately leads to growth deceleration, as observed in the *elo3-6* mutant in darkness.

GO categories with significantly downregulated genes were 'Response to light stimulus', 'Response to hormone stimulus', 'Cell wall biogenesis', 'Regulation of transcription', 'Regulation of developmental processes' and 'Regulation of cell cycle', with a large proportion of transcription factors within each GO category. From the downregulated GO categories, a growth-controlling network was deduced that consisted of four main hubs: circadian clock, regulators of skoto- and photomorphogenesis, different hormone response pathways, and primary and secondary cell wall biogenesis (Table S2). Downregulated genes encoded both positive upstream regulators and direct downstream effectors of growth, in line with the delayed hypocotyl elongation observed for *elo3-6* seedlings grown in darkness. Some of these pathways were functionally analyzed by means of reporter gene constructs or hypocotyl growth experiments upon treatment.

Circadian clock

The circadian clock is one of the four main hubs of the growth-regulatory network downregulated in *elo3-6* in darkness. Seven genes from this hub (*LHY*, *CCA1*, *RVE8*, *CIR1*, *LCL1/RVE4*, *RVE2* and *PRR8*) showed decreased expression levels in *elo3-6* in darkness (Table S2; Fig. 3A). To check whether downregulation of two key circadian clock components (CCA1 and LHY) contribute to the *elo* phenotype, we assayed the hypocotyl length of the *lhy-21 cca1-11*, *cca1-1lhy RNAi* and *lhy-21* mutants together with their wild type, Wassilewskija (Ws). In darkness, similarly to the *elo* mutants, the hypocotyls of the circadian clock-regulatory mutants were significantly shorter than those of the wild type at 2 and 4 DAG, but the apical hooks remained closed and cotyledons did not expand (Fig. 3B). The effects in the *lhy-21 cca1-11* double and the *lhy-21* single mutants were comparable, indicating that mutation of *LHY* is sufficient to cause decreased hypocotyl length; therefore, lowered expression of the *LHY* gene in the *elo* mutant might contribute to the observed short hypocotyl phenotype in darkness.

Next, the diurnal expression profiles of the *CCA1* and *LHY* genes were examined in wild-type and *elo3-6* mutant plants synchronized under short-day conditions. Samples were taken every 4 h during

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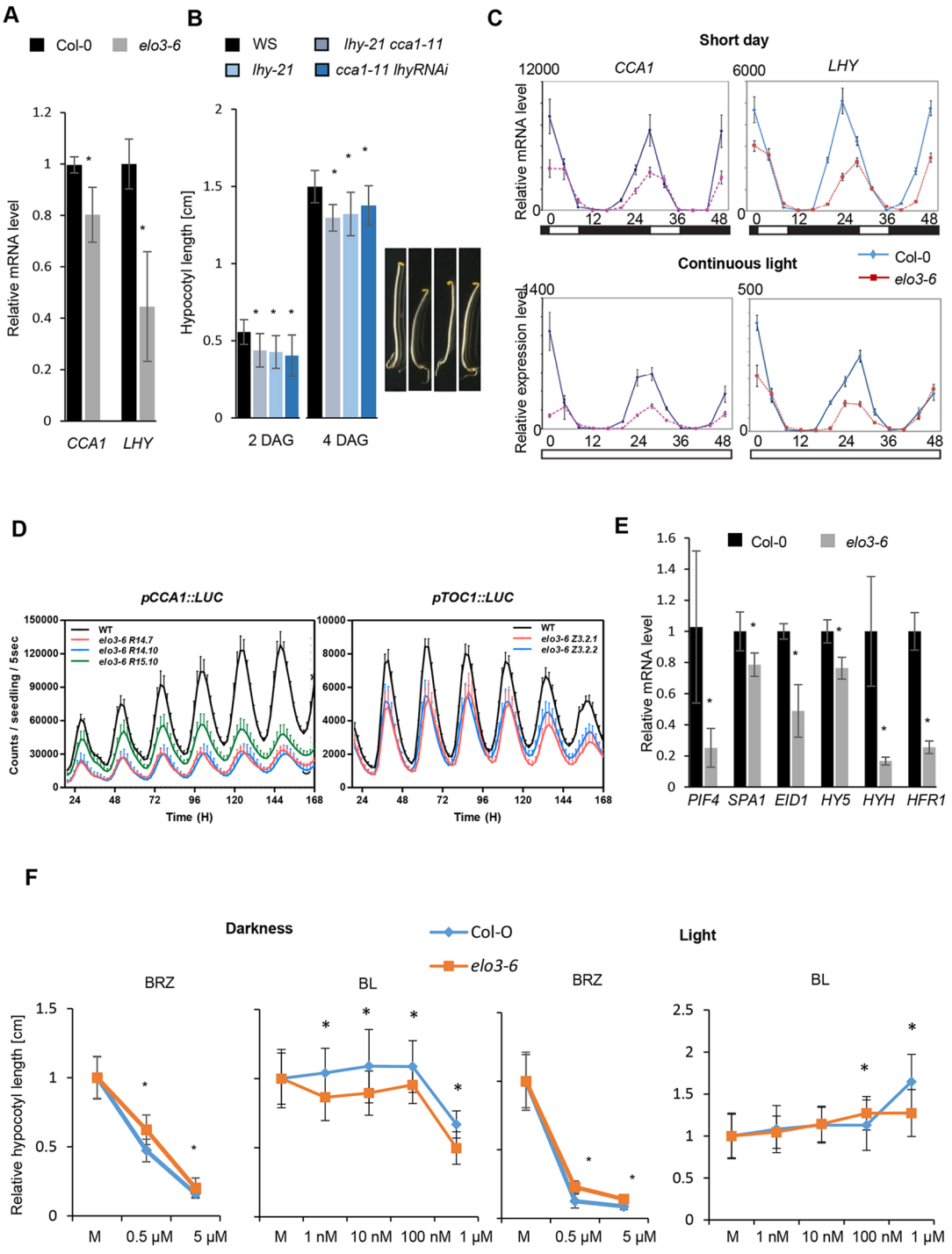

Fig. 3. Expression of circadian clock and skoto- and photomorphogenesis regulatory genes, circadian clock assays and response to BL and BRZ of the *elo3-6* mutant. (A) Relative expression levels of *CCA1* and *LHY* genes in seedlings of *elo3-6* and Col-0 wild type. (B) Hypocotyl length of single and double mutants of *CCA1* and *LHY* genes (*lhy-21*, *lhy-21 cca1-11* and *cca1-11 lhyRNAi*) compared with Ws wild type in darkness. Thirty seedlings were photographed and hypocotyls were measured with ImageJ software. (C) qPCR assessing relative expression levels of *CCA1* and *LHY* genes in the Col-0 and *elo3-6* seedlings grown for 12 days in a short-day photoperiod and analyzed for 48 h in short-day conditions or continuous white light with samples taken every 4 h. White and black boxes below the graphs indicate alternation of light and dark, respectively. (D) Bioluminescence of *pCCA1::LUC* and *pTOC1::LUC* reporter lines measured in the Col-0 wild type and *elo3-6* mutant (R14.7, R14.10 and R15.10 lines for *pCCA1*; Z3.2.1 and Z3.2.2 lines for *pTOC1*) in a time-course analysis under constant white light conditions. (E) Relative expression levels of positive regulators of skotomorphogenesis (*PIF4*, *SPA1* and *EID1*) and positive regulators of photomorphogenesis (*HY5*, *HYH* and *HFR1*) in darkness. (F) Relative hypocotyl lengths of the Col-0 wild type and *elo3-6* seedlings grown in constant darkness or white light in the absence (mock control M) or presence of indicated concentrations of BL or BRZ. In A, E and F, 4-day-old seedlings grown on half-strength MS medium were analyzed. In A and E, the relative expression levels were detected by qPCR with six biological replicates and *PP2A* and *SAND* genes as reference genes (Czechowski et al., 2005). The experiments were repeated twice. Bars represent mean±s.d. In B and F, mean values of hypocotyl length of at least 25 seedlings are presented. Differences between mutant and wild type were statistically analyzed with an unpaired two-tailed Student's *t*-test; *P<0.05. BRZ, brassinazole; BL, brassinolide.

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48 h under short-day or under continuous light conditions following the synchronization. The diurnal fluctuations of the *CCA1* and *LHY* transcripts in the *elo3-6* mutant followed a similar oscillatory trend to that observed in wild-type plants, but mRNA accumulation was clearly reduced in the *elo3-6* mutant under both conditions (Fig. 3C). These results indicate that functionality of ELO3 is important for proper amplitude of *CCA1* and *LHY* expression.

The downregulation of circadian clock components was further examined by monitoring bioluminescence of reporter lines expressing the *LUCIFERASE* (*LUC*) gene fused to the *CCA1* or *TOC1* promoters (*pCCA1::LUC* or *pTOC1::LUC*) in *elo3-6*. Our results show that the amplitude of the circadian activity for both promoters was decreased in the *elo3-6* mutant compared with the wild type and that the circadian period was not affected by the *elo3-6* mutation (Fig. 3D). These results are consistent with the decreased *CCA1* and *LHY* expression observed by quantitative polymerase chain reaction (qPCR) analysis (Fig. 3C) and suggest that altered clock function by mis-expression of oscillator components might contribute to the *elo3* hypocotyl phenotype.

Regulators of skoto- and photomorphogenesis

PHYTOCHROME INTERACTING FACTOR 4 (*PIF4*) and genes encoding other positive skotomorphogenesis regulators, such as *SPA1* and *EMPFINDLICHER IM DUNKELROTEN LICHT 1* (*EID1*) (Fig. 3E), and B-box zinc finger proteins *BBX24* and *BBX25* (Table S2) showed significantly lower expression in the *elo3-6* mutant. Downregulation of such factors reduced hypocotyl elongation, as shown in *pif4* and multiple *pif* mutants (Leivar et al., 2012), *spa1 det1-1* (Nixdorf and Hoecker, 2010), *bbx24 cop1-4* and *bbx25 cop1-4* (Gangappa et al., 2013), and might contribute to the reduced hypocotyl elongation in *elo3-6* in darkness. PIF4 is the key player among factors positively regulating hypocotyl growth. A reduced relative mRNA level of *PIF4* in *elo3-6* in darkness is in line with genetic interactions between PIF4 and Elongator observed in the triple *elo3-6 pif3-3 pif4-2* mutant. Indeed, the genes downregulated in the *elo3-6* transcriptome in darkness largely overlapped with PIF4 targets identified by chromatin immunoprecipitation-sequencing (ChIP-seq) in 5-day-old etiolated seedlings (Oh et al., 2014). There was 41% overlap in the GO category ‘Response to hormones’, 38% in ‘Response to light’, 36% in ‘Secondary cell wall biogenesis’ and 23% in ‘Regulation of transcription’.

In addition to genes of positive skotomorphogenesis regulators (including *PIF4*), the positive photomorphogenesis regulator genes *HY5*, *HYH*, *HFR1* (Fig. 3E) and *HY1* (Table S2) were also downregulated in the *elo3-6* mutant in darkness. Decreased expression of these regulators leads to hypocotyl elongation and prevents opening of the apical hook and cotyledon expansion. Considering that positive regulators of skoto- and photomorphogenesis are known to interact and suppress each other’s phenotypes (Ang and Deng, 1994; Xu et al., 2014; Srivastava et al., 2015), coincidental downregulation of positive regulators of both skoto- and photomorphogenesis in the *elo3-6* mutant could blend into the combinatorial phenotype of a moderately shorter hypocotyl and a closed apical hook. This mechanism is supported by the hypocotyl length of the *elo3-6 hfr1-101* double mutant, which is the same as in *elo3-6*, indicating that introduction of the *hfr1* mutation into *elo3-6* does not result in additional hypocotyl elongation because *hfr1* expression is decreased by the *elo3-6* mutation.

Hormone response

Downregulated genes of the growth-regulatory network are related to hormonal pathways (Table S2), in particular those encoding the brassinosteroid (BR) pathway components. These genes were well represented and included three enzymes crucial for BR synthesis (*CPD*, *DWF4* and *CYP90D1*), the signaling component *BSU1*, and five genes (*VH1*, *MERI5*, *THE1*, *TCH4*, and *IBH1*) encoding response proteins related to control of cell elongation via cell wall modification. To check whether a defective BR pathway contributes to the reduced hypocotyl elongation in *elo3-6*, we tested mutant sensitivity to the BR biosynthesis inhibitor brassinazole (BRZ) and exogenous brassinolide (BL) by means of the hypocotyl elongation assay in darkness. Both Col-0 and *elo3-6* responded with reduced hypocotyl elongation to 0.5 and 5 µM BRZ, but the decrease in hypocotyl length was smaller in the mutant (Fig. 3F). This result hints at BRZ hyposensitivity and reduced activity of BR biosynthesis enzymes, in line with their decreased expression in *elo3*-6 compared with Col-0. BL treatment did not reverse the short hypocotyl phenotype of *elo3-6*, indicating that BR deficiency caused by reduced biosynthesis gene expression is not the primary reason for the short hypocotyl mutant phenotype. This *elo3-6* mutant showed moderate hypersensitivity to BL, with a decreased hypocotyl length even at the lowest BL concentration (1 nM). In the wild type, only the highest concentration of BL (1 µM) decreased the hypocotyl length (Fig. 3F). BRZ hyposensitivity and BL hypersensitivity of *elo3-6* resembled those of the *bzr1-1D* mutant, which contains increased amounts of the BRASSINAZOLE-RESISTANT 1 (BZR1) transcription factor, activated by BRs, which dimerizes with PIF4 to promote cell elongation in etiolated hypocotyls (Wang et al., 2002). Like the *bzr1-1D* mutant, *elo3-6* might also have increased levels of free BZR1 caused by downregulation of PIF4 and, hence, a reduced amount of PIF4-BZR1 dimers and retarded cell elongation. High BZR1 levels in *elo3-6* were suggested by fewer transcripts of BR biosynthesis enzymes, implying feedback inhibition as also detected in *bzr1-1D* (Wang et al., 2002). BRZ and BL sensitivities were modestly affected in *elo3-6*, suggesting that malfunction of the BR pathway contributes only partially to the short *elo3-6* hypocotyls. As indicated by the transcriptome, other growth-related hormonal pathways that might contribute to defective hypocotyl elongation are downregulated in *elo3-6*. For example, downregulation of *PIF4* could affect the auxin responses, because PIF4 stimulates expression of the auxin biosynthetic gene *YUCCA8* (Sun et al., 2012), whose expression is reduced in *elo3-6* (Table S2).

Cell wall biogenesis

Hormone pathways regulate growth by convergence to the cell wall biogenesis pathways. In the *elo3-6* mutant, more than 40 genes related to cell wall formation were downregulated in darkness; these included three genes (*IRX9*, *IRX10*, *IRX14-L*) encoding enzymes of xylan biosynthesis, which are involved in the generation of both primary and secondary cell walls. The *irx9*, *irx10* and *irx14-L* mutants are similar to *elo3-6* in that they have moderately shorter hypocotyls than the wild type in darkness and no opened cotyledons (Faik et al., 2014). In the *elo3-6* mutant, genes regulating secondary cell wall biogenesis are downregulated. These genes include xylem differentiation factors (*ATHB15*, *REV*, *PHV*); NAC and MYB factors (*AtC3H14*, *AtC3H15*, *BLH6*, *MYB42*, *MYB43*, *MYB46*, *MYB52*, *MYB54*, *MYB83*, *MYB85*, *MYB103*, *NAC075*, *XND1*, *SND2*, *VND2* to *VND6*), representing all three tiers of the transcription factor cascade (Hussey et al., 2013); and enzymes of cellulose (*CESA4*, *CESA7*, *CESA8* and *IRX6*/*COBL4*), hemicellulose (*IRX8*, *IRX9*, *IRX10*, *IRX14L*, *FRA8* and *GUX1*) and lignin (*LAC4*, *LAC10* and *LAC17*) synthesis (Table S2).

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H3K14 acetylation activity of Elongator at *LHY*, *HYH* and *HFR1* in darkness

Expression of *CCA1* and *LHY* correlates with the level of the histone H3 modifications, H3K4Me2 and H3K9Ac (Ni et al., 2009). Similarly, some of the light- and/or darkness-related regulatory genes are controlled by histone modifications, suggesting that they might also be direct targets of Elongator HAT activity. Hence, ChIP-qPCR was carried out on chromatin of *elo3-6* and Col-0 4-day-old seedlings germinated in darkness. The analysis used antibodies against acetylated histone H3K14 and primers for promoter and coding regions of the circadian clock genes *CCA1* and *LHY* (Fig. 4A,B), and of the regulatory genes *PIF4* (Fig. 4C), *HYH*, *HFR1* (Fig. 4D,E), *SPA1*, *EID1* and *HY5*. Results were normalized versus both input and the *ACTIN2* gene. To check whether Elongator targets downstream transcription factors related to hormone and cell wall pathways, ChIP-qPCR was done on the *CPD*, *DWF4*, *CYP90D1* and *BSU1* genes from the BR pathway; the *CGA* and *GNC* cytokinin response genes; the secondary cell wall regulator-encoding genes *PHAV*, *REV*, *VND4*, *MYB46*, *MYB83* and *MYB103*; and the structural genes *CESA4*, *CESA7* and *CESA8* (Table S2).

Of the 20 analyzed genes, H3K14 acetylation was only significantly decreased in the coding regions of the *LHY*, *HYH* and *HFR1* genes in *elo3-6* seedlings. The results show that *LHY*, *HYH* and *HFR1* are direct targets of Elongator HAT activity in darkness (Fig. 4B,D,E) and suggest that Elongator provides selective epigenetic control to a few of the highest order transcription factors. Identification of *LHY* as a target for the histone H3K14 acetylating activity of Elongator, together with decreased expression of *LHY* in *elo3-6* and similar hypocotyl phenotypes of *lhy* and *elo3-6* mutants in darkness, indicate that epigenetic control of *LHY* expression via Elongator HAT activity might contribute to hypocotyl growth regulation. Targeting of *HYH* and *HFR1* by Elongator in darkness suggests a fine-tuning mechanism of hypocotyl growth regulation, whereby positive regulators of photomorphogenesis prevent exaggerated elongation. None of the positive skotomorphogenesis regulators showing decreased expression in *elo3-6* was targeted by Elongator HAT activity, as illustrated for *PIF4* (Fig. 4C). These factors might be regulated via other activities of Elongator or via HAT regulation of the higher-order regulators. For example, because *PIF4* is controlled by the circadian clock (Yamashino et al., 2003; Kidokoro et al., 2009), it is possible that the downregulation of *PIF4* in the *elo3-6* mutant is a consequence of the downregulation of *CCA1* and Elongator target *LHY*.

Gene expression in the *elo3-6* mutant in light

Expression levels of genes encoding the main regulators of skoto- and photomorphogenesis, the light response, cell wall-related biosynthesis and brassinosteroid biosynthesis were assayed by qPCR in 4-day-old *elo3-6* and Col-0 seedlings grown in continuous red, far-red or blue light. The genes encoding positive regulators of photomorphogenesis (*HY5*, *HYH* and *HFR1*) and skotomorphogenesis (*EID1*) were downregulated under at least one light condition. By contrast, *PIF4*, which is downregulated in darkness, was upregulated in far-red and blue light (Fig. 5A). The *HY5* gene, encoding the main positive photomorphogenesis regulator, was downregulated in all light qualities, but *HYH* and *HFR1*, encoding two HY5 interactors, were downregulated in red light. *HYH*, which plays an important role in blue light photomorphogenesis, also showed lower transcript levels in blue light. Reduced expression of these regulators, which cooperate in the inhibition of hypocotyl elongation and in the promotion of apical hook opening and cotyledon growth, was consistent with the increased hypocotyl length and unexpanded and hyponastic cotyledons of the light-grown *elo3-6* seedlings. *HY5* downregulation in *elo3-6* coincided with extreme upregulation of *WALL-ASSOCIATED KINASE 1* (*WAK1*), moderate upregulation of *INCREASED SIZE EXCLUSION LIMIT 2* (*ISE1*) (Fig. 5A), and no difference in expression of *ARF2*, *UBP15*, *ATHB-2*, *ATASE2*, *APG3* and *MSL3*, which are all HY5 target genes (Zhang et al., 2011). *WAK1* is negatively regulated by HY5 (Zhang et al., 2011), plays a positive role in cell elongation (Lally et al., 2001) and is the receptor of oligogalacturonides, which are cell wall-integrity signaling components that induce defense responses. High *WAK1* expression

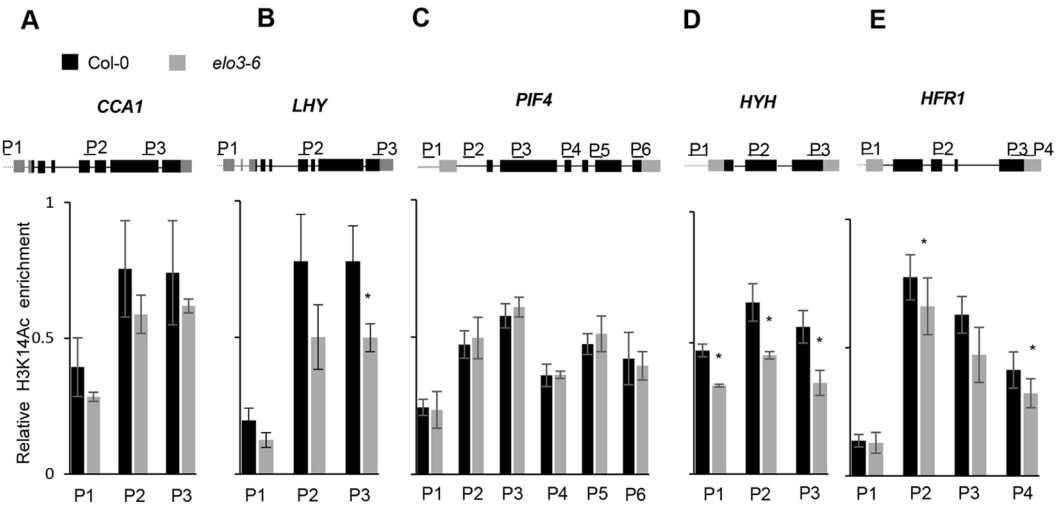

Fig. 4. Histone acetylation of circadian clock and skoto- and photomorphogenesis regulatory genes in the *elo3-6* mutant in darkness. Histone H3K14 acetylation levels in the *CCA1*, *LHY*, *PIF4*, *HYH* and *HFR1* promoter and coding regions. The relative H3K14Ac enrichment was established with antibodies against H3K14Ac for ChIP and primers (P1–P6, Table S5), amplifying fragments of promoter and coding sequences for qPCR. Results were normalized versus input and the actin reference gene. The experiment was repeated four times (*LHY* and *HYH*) or twice (*CCA1*, *PIF4* and *HY5*) with four biological replicates each time. Four-day-old seedlings grown in darkness on half-strength MS medium were analyzed. Bars represent mean±s.d. Differences between mutant and wild type were statistically analyzed with an unpaired two-tailed Student's *t*-test; **P*<0.05.

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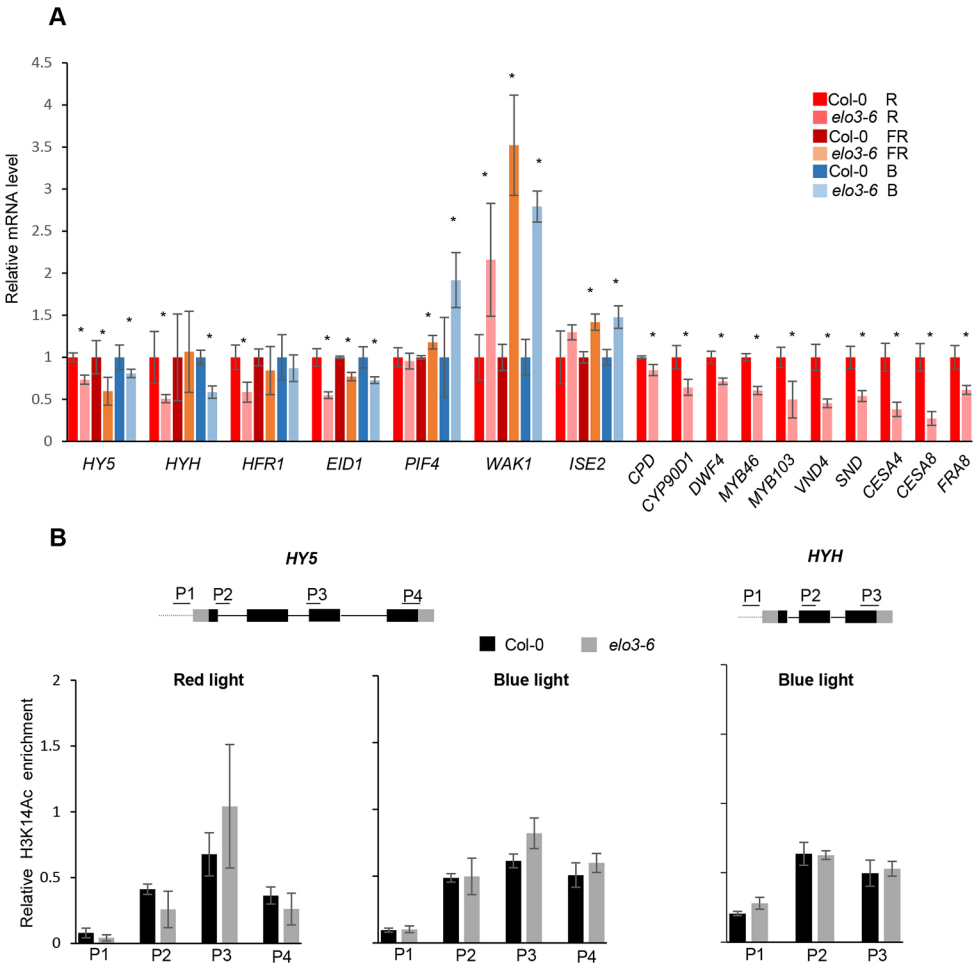

Fig. 5. Expression of genes encoding photomorphogenesis regulators and cell wall biogenesis genes, and histone acetylation of *HY5* and *HYH* in monochromatic light. (A) Relative expression levels of indicated genes determined by qPCR in 4-day-old *elo3-6* and Col-0 seedlings grown under continuous monochromatic light. Expression was normalized using *PP2A* and *SAND* as reference genes. (B) Histone H3K14 acetylation in the *HY5* and *HYH* promoter and coding regions. The relative H3K14Ac enrichment was established with antibodies against H3K14Ac for ChIP and primers (P1–P4, Table S5), amplifying fragments of promoter and coding sequences for qPCR. Results were normalized versus input and the actin reference gene. Average values of six (qPCR) or four (ChIP-qPCR) biological replicates are presented with standard deviation (mean ±s.d.). Differences between mutant and wild type were statistically analyzed with an unpaired two-tailed Student's *t*-test; **P*<0.05.

might contribute to enhanced hypocotyl elongation and/or immune response activation, in line with downregulation of secondary cell wall biogenesis genes under red light (Fig. 5A) (Miedes et al., 2014). Decreased expression of the BR biosynthesis genes *CPD*, *CYP90C11* and *DWFA2* in the *elo3-6* mutant under red light (Fig. 5A) might result from negative feedback regulation by free BZR1 proteins. Free BZR1 might overaccumulate in *elo3-6* because of lower *HY5* levels and, consequently, lower the formation of BZR1/HY5 dimers that suppress hypocotyl elongation (Li and He, 2016). Accordingly, *elo3-6* was hyposensitive to BL and BZR in light (Fig. 3F), confirming that BR signaling was affected in *elo3-6*.

ChIP-qPCR was applied to check whether Elongator promotes photomorphogenesis via histone H3K14 acetylation of the regulatory genes *HY5*, *HYH* and *HFR1* in light. Chromatin isolated from *elo3-6* and Col-0 seedlings grown for 4 days in red, far-red or blue light did not differ in histone acetylation, indicating that Elongator-mediated HAT activity did not target *HY5*, *HYH* (Fig. 5B) or *HFR1* in light. Thus, Elongator is necessary for the expression of *HY5*, *HYH* and *HFR1*, which encode the main photomorphogenesis regulators, and for the downstream pathways controlled by HY5 during photomorphogenesis, but not via Elongator HAT activity.

DISCUSSION

We show that the Elongator complex modulates hypocotyl growth and photomorphogenesis via the regulation of a growth-controlling network consisting of circadian clock regulators, skoto- and photomorphogenesis regulators, hormone pathways and cell wall biogenesis. The regulatory role of Elongator is supported by the hypocotyl phenotypes of *elo3-6* and *elo3-1* and growth-related mutants; identification of the *LHY*, *HYH* and *HFR1* regulators as direct targets of Elongator HAT activity; hormone sensitivity assays; *LUC* reporter gene activity in the *elo3-6* mutant background; and genetic interactions studies with skotomorphogenesis and light response regulators.

Elongator affects early growth in darkness and light through a growth-controlling network

Unlike de-etiolation mutants such as *cop1* and *pif*, which combine short hypocotyls with expanded cotyledons in darkness, *elo3-6* has a short hypocotyl although apical hook and cotyledon folding remain normal. Cotyledons expand in darkness in *cop1* because of high levels of HY5, HYH and/or HFR1; they also expand in multiple *pif* mutants, especially those including mutation in *PIF1*, which is the main cotyledon-folding suppressor in darkness (Leivar et al., 2012). Cotyledons of *elo3-6* do not expand in darkness, because the expression of *HY5*, *HYH* and *HFR1* is lowered and only *PIF4* is downregulated out of all *PIF*s. Hypocotyl phenotypes similar to those of *elo3-6* were observed in *lhy-21*, *lhy-21 cca1-11*, *cca1-11 lhyRNAi* (Fig. 3B), *pif4* (Leivar et al., 2012), and *irx9*, *irx10*, and *irx14-L* (Faik et al., 2014). These plants contain mutations in circadian clock and cell wall biogenesis genes, which are main hubs of the growth-controlling network downregulated in *elo3-6*, indicating that the *elo3-6* hypocotyl

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phenotype is the result of multiple reduced gene activities. This observation is in line with the network topology that consists of upstream regulatory transcription factor pathways converging on cell wall biogenesis and resulting in a cumulative repressing effect on hypocotyl growth. The importance of cell wall biosynthesis for growth and cell elongation has been demonstrated in mutants with impaired cell wall composition (Desnos et al., 1996; McCarthy et al., 2010; Faik et al., 2014). However, growth seems to be reduced in response to cell wall-integrity signaling that activates plant immune responses (Hématy et al., 2007), rather than inhibited directly by a physically weakened cell wall. Mutants defective in the MYB46 regulator of cell wall formation (Ramírez et al., 2011) or in CESA4, CESA7 and CESA8 cellulose synthase subunits required for secondary cell wall synthesis (Hernández-Blanco et al., 2007) activate the plant immune response, leading to growth attenuation (Rojas et al., 2014). Downregulation of over 40 cell wall-related genes (including *MYB46*, *CESA4*, *CESA7* and *CESA8*) and upregulation of defense response genes (including important key regulators) and metabolic genes involved in the plant immune response coincide in *elo3-6*; hence, the hypocotyl growth defects in this mutant might be a result of reduced cell wall biosynthesis and, eventually, activation of the plant immune response (Fig. 6).

Decreased pathogen resistance has been shown for the *elo2* mutant, confirming positive regulation of the plant immune response by Elongator via the targeting of genes encoding important components of the salicylic acid pathway (*NPR1*, *PR2*, *PR5*, *EDS1* and *PAD4*) (Wang et al., 2013) and the jasmonate/ethylene pathway (*WRKY33*, *ORA59* and *PDF1.2*) (Wang et al., 2015) for histone acetylation and/or DNA methylation. Elongator also controls the reactive oxygen species–salicylic acid amplification loop and targets important defense genes for histone acetylation, including the homolog *AtrbohD* that encodes the *Arabidopsis* respiratory burst oxidase, and the salicyclic acid biosynthesis gene *ISOCHORISMATE SYNTHASE1* (An et al., 2017). The incongruences between our data and the results of others (Wang et al., 2013) related to the role of Elongator in the immune response could correspond to different mutants (*elo3* versus *elo2*), diverse developmental stages, or different growth conditions applied in the studies. For example, delayed induction and lower expression of some defense genes (including *PAD4*) in the *elo2* mutant were observed only after pathogen infection, whereas basal

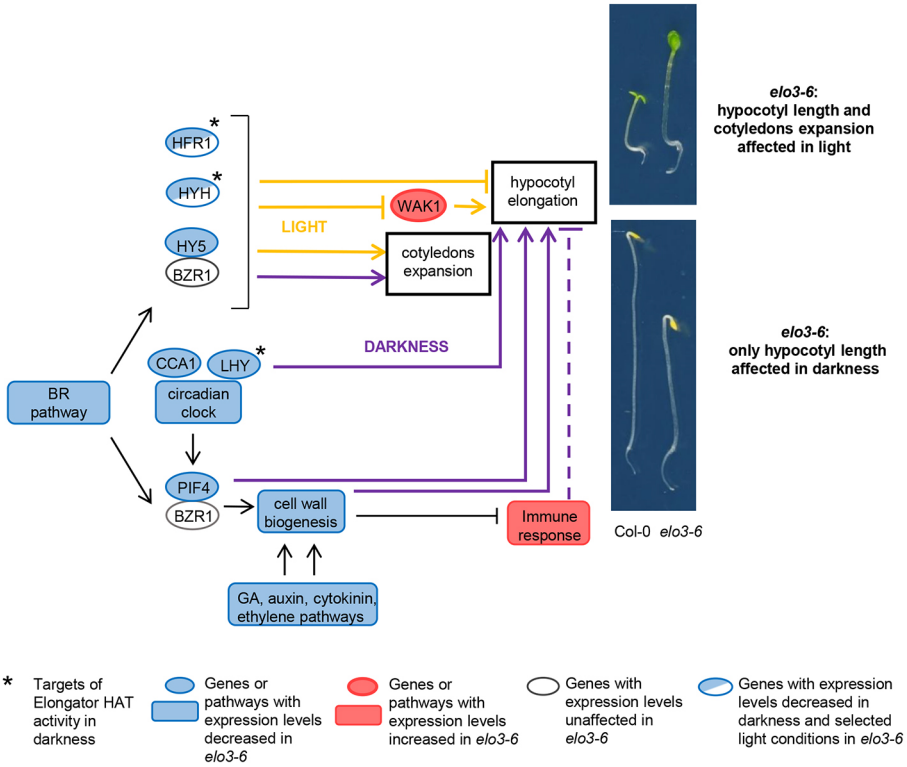

Fig. 6. Model for Elongator transcriptional control of hypocotyl growth in darkness and photomorphogenesis. Elongator controls hypocotyl elongation via several pathways: elongation-suppressing pathways involving positive regulators of photomorphogenesis (HY5, HYH and HFR1) or immune response genes, and elongation-stimulating pathways including circadian clock, PIF4, hormone biosynthesis or signaling, and cell wall biogenesis. In darkness (purple arrows), downregulation of genes in pathways stimulating hypocotyl elongation and upregulation of immune response genes suppressing elongation prevail, resulting in a shorter hypocotyl of the *elo3-6* mutant. In light (yellow arrows), hypocotyl elongation is inhibited very early in the wild type, whereas elongation inhibition fails in the *elo3-6* mutant because of downregulation of positive photomorphogenesis regulators and strong upregulation of WAK1, which stimulates cell elongation and results in a longer hypocotyl. Elongator also regulates cotyledon expansion via positive regulators of photomorphogenesis. The *HY5* gene was downregulated under red, far-red and blue light (blue filling); *HYH* under red and blue light; and *HFR1* under red light only (blue-white filling). Expression of BR pathway and cell wall biogenesis genes was assayed in darkness and red light. Pictures present 4-day-old seedlings grown in darkness (lower panel) or in red light (upper panel). The asterisks indicate targets of Elongator HAT activity in darkness. Blue or red colors indicate, respectively, a lower or higher expression level of the given gene or pathway. Genes half-shaded with blue color have expression levels downregulated in darkness and selected light conditions. The expression level of *BZR1* is unaffected, as indicated by transparent circles. Downregulation of hypocotyl elongation by the immune response is represented by a dashed line because it is not clear whether downregulation of cell wall biogenesis-related genes affects hypocotyl elongation directly or via the immune response, as suggested by higher transcription of genes involved in the immune response in *elo3-6*.

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expression was similar in the mutant and the wild type (Wang et al., 2013). Moderately increased expression of selected immunity pathways in *elo3-6* might result in growth inhibition, but does not necessarily trigger constitutive activation of plant defense pathways, which requires high levels of upregulation (usually in response to pathogen infection) to exceed the defense activation threshold (Kwon et al., 2009). Therefore, in addition to well-established direct positive regulation of the plant immune response, under some conditions Elongator might play an opposite and possibly indirect role as a positive regulator of cell wall-related genes. Elongator might contribute independently and inversely to different immune response pathways, and thus modulate the growth–defense balance (Hématy et al., 2007).

Alternatively, a negative role of Elongator in the plant response to wounding is suggested by the increased levels of jasmonic acid (JA), increased JA biogenesis and responsive gene expression levels (Nelissen et al., 2010), and induction of the jasmonate-controlled MYC2 transcriptional cascade (Wang et al., 2015) reported earlier for the *elo* mutants. The plant response to wounding, similar to the immune response, has a negative JA-mediated effect on growth. However, we did not find JA-related genes among those differentially regulated in *elo3-6* in our microarray dataset. Moreover, JA acts during skotomorphogenesis to reduce hypocotyl length, but also promotes cotyledon opening in etiolated seedlings (Zheng et al., 2017), resulting in the constitutively photomorphogenic phenotype. This is not the case for darkness-grown *elo3* seedlings, which are shorter but have normal apical hooks, arguing against the role of JA and wounding in the *elo3* phenotype.

Transcription-based model of the role of Elongator in early plant development

We propose a model for the role of Elongator in early plant development that elucidates why hypocotyl growth of *elo* mutants is slower in darkness but photomorphogenesis is defective in light, resulting in a longer hypocotyl and unexpanded cotyledons (Fig. 6). Elongator regulates hypocotyl elongation and cotyledon expansion by controlling cell wall biogenesis genes and positive photomorphogenesis regulators. Depending on the light conditions, one of the pathways becomes restrictive and Elongator promotes opposite growth behaviors.

In darkness, expression of the circadian clock regulator *LHY* and of the positive photomorphogenesis regulators *HFR1* and *HYH* is activated by Elongator-mediated, transcript elongation-facilitating histone acetylation. As shown by hypocotyl growth analysis of the *lhy-21*, *lhy-21 cca1-11* and *cca1-11 lhyRNAi* mutants, the circadian clock components LHY and CCA1 positively regulate hypocotyl elongation. One of the possible mechanisms of this regulation involves PIF4, which is controlled by the circadian clock (Nozue et al., 2007) at the transcriptional level and stimulates expression of genes involved in hypocotyl elongation. *LHY*, *CCA1* and *PIF4* genes are downregulated in darkness in *elo3-6* mutants, which affects the expression of many transcription factors, such as components of hormonal and cell wall biosynthesis pathways that partially slow down hypocotyl elongation via activation of the plant immune response (Hématy et al., 2007). A lower level of PIF4 reduces formation of complexes with the BZR1 transcription factor of the BR pathway and compromises induction of cell wall biogenesis genes (Lozano-Durán et al., 2013). In conclusion, in darkness, the *elo3-6* hypocotyl phenotype is determined by the combined effect of decreased levels of cell wall biogenesis genes, reduced expression of clock regulators and decreased expression of *HY5*, *HYH* and *HFR1*, consequently inhibiting hypocotyl elongation. The final phenotype of short hypocotyls indicates that the defect in cell wall biogenesis prevails. Low expression of *HY5*, *HYH* and *HFR1* also prevents cotyledon expansion in *elo3-6*.

Elongator is also required for light responses, because the genes of the major positive photomorphogenesis regulators *HY5*, *HYH* and *HFR1* are downregulated in *elo3-6* although, strikingly, their H3K14Ac levels are unaffected in light. The HAT activity of Elongator might be very dynamic and difficult to capture in a ChIP-qPCR assay using acetylated histone antibodies, which could explain the limited number of genes targeted for Elongator-mediated histone acetylation. In plants, the interaction between Elongator subunits and the SPT4/SPT5 transcript elongation complex (Van Lijsebettens et al., 2014) suggests that Elongator might affect RNAPII transcript elongation indirectly, next to its histone acetylation activity (Antosz et al., 2017). An alternative explanation is that, in light, another epigenetic activity of Elongator such as DNA demethylation (DeFraia et al., 2013; Wang et al., 2013) or processing of primary microRNAs (Fang et al., 2015) might be responsible for decreased expression of *HY5*, *HYH* and *HFR1*. In light, hypocotyl elongation is inhibited very early in wild-type seedlings by diverse factors including HY5, HYH and HFR1, possibly involving suppression of the cell elongation activity of WAK1 (Fig. 6B). In the *elo3-6* mutant, decreased expression of *HY5* leads to a higher accumulation of *WAK1* mRNA and induced hypocotyl elongation. On the other hand, upregulation of *WAK1* might trigger immune responses, as suggested by decreased levels of cell wall biogenesis genes, and might suppress hypocotyl elongation. The two pathways contribute to a final hypocotyl length that is longer in *elo3-6* than in the wild type, indicating that the pathway promoting cell elongation prevails. Lower expression of *HY5*, *HYH* and *HFR1* in the mutant also results in less expanded cotyledons, yielding the phenotype typical of photomorphogenesis defect.

In conclusion, Elongator is known as an enzymatic complex with diverse activities that directly or indirectly, positively or negatively, influence expression of genes located in various pathways. Here, we show that Elongator acts as an interface between growth, immune responses and photomorphogenesis and plays a fine-tuning role in mutual regulatory interactions of those processes at the transcriptional level.

MATERIALS AND METHODS

Plant mutants and reporter lines

The *drl1-2* (Nelissen et al., 2003), *elo1-1*, *elo2-1*, *elo3-1* and *elo4* (Nelissen et al., 2005) mutants corresponding to alleles of *ELP4*, *ELP1*, *ELP3* and *DRL1* genes in Ler and the *elo3-6* mutant in Col-0 (GABI-KAT collection code GABI555_H06, Nelissen et al., 2010) are described previously. *pCCA1::LUC* (Salome and McClung, 2005) and *pTOC1::LUC* (Portolés and Más, 2007) are reporter lines in Col-0. The mutants *phyB-9*, *hfr1-101* and *pif3-3 pif4-2* in Col-0 and *phyA-201*, *phyB-1* and *phyA-201 phyB-5* in Ler were purchased at the Nottingham *Arabidopsis* Stock Centre (NASC). The *lhy-21 cca1-11* (N9380) and *lhy-21* (N9379) mutants in Ws background were also obtained from NASC. The *cca1-11 lhyRNAi* mutant in Ws background was a kind gift from Steve Kay (The Scripps Research Institute, La Jolla, CA, USA). The double or triple mutants *elo3-6 hfr1*, *elo3-6 pif3-3 pif4-2*, *elo3-1 phyB-1* and *elo3-1 phyA-201* were generated by crossing. Homozygous individuals were identified by PCR genotyping with the primers listed in Table S3.

Growth conditions and assays

For hypocotyl assays, seeds were sterilized in 5% (v/v) bleach containing 0.05% (v/v) Tween 20 for 10 min, washed in water, sown on half-strength Murashige

and Skoog (MS) medium (Murashige and Skoog, 1962) without sucrose and stratified at 4°C for 48 h. Seeds were exposed for 6 h to white light (100 µmol m^{-2} s^{-1}) to induce germination. Plants were grown in either darkness, white (cool white fluorescent light; Philips), red [cool white fluorescent light, filtered through red plastic (Rohm and Haas) and red cellophane, (UCB-Sidac, Gent, Belgium)], far-red (incandescent light combined with a 700-nm pass filter) or blue light (dragon tape LEDs, 470 nm; Osram), all at the high fluence rate of 10 µmol m^{-2} s^{-1} for the indicated time at 21°C. Seedlings analyzed for hypocotyl length were put on 1% (w/v) agar and photographed. Hypocotyl length of at least 25 seedlings for each genotype/condition was measured using ImageJ 1.45 software. Significant differences were recovered with the two-tailed Student's *t*-test in Microsoft Excel.

For the hormone assays, BL (24-epibrassinolide; Duchefa-Direct, Cat. E0940.0010) was used at concentrations of 10^{-3}, 10^{-2}, 10^{-1} and 1 µM; BRZ (TCI Europe, Cat. B2829) was used at concentrations of 0.5 and 5 µM.

The clock reporter lines expressing *pCCA1::LUC* and *pTOC1::LUC* were crossed into the *elo3-6* mutant. Lines homozygous for the *elo3-6* mutation and the *pCCA1::LUC* reporter (R14.7, R14.10, R15.10) and lines homozygous for the *elo3-6* mutation and the *pTOC1::LUC* reporter (Z3.2.1 and Z3.2.2) were analyzed by *in vivo* luminescence assays. Plants were stratified for 3 days at 4°C on MS agar medium and grown for 7 days under light/dark cycles (12-h light/12-h dark) with 60 µmol m^{-2} s^{-1} white light at 22°C. Seedlings were subsequently transferred to 96-well plates containing MS agar and 3 mM luciferine (Promega). Luminescence rhythms were monitored using a luminometer LB-960 (Berthold Technologies) and the software MikroWin 2000, version 4.34 (Mikrotek Laborsysteme) for the analysis.

RNA isolation, cDNA synthesis and qPCR

For gene expression analyses, six biological replicates were used. RNA was isolated with the RNeasy Plant Kit (Qiagen) with on-column DNase digestion. The manufacturer's protocol was modified by two additional washes of RNeasy spin columns with RPE buffer. Complementary DNA (cDNA) was synthesized with the SuperScript III First-strand synthesis kit (Life-Invitrogen, CAT. 18080051).

The PCR reactions were performed in technical triplicates with the LightCycler 480 SYBR Green I Master reagent and the Janus robot (PerkinElmer) for pipetting. The LightCycler 480 Real-Time PCR System was used for amplification (95°C for 10 min, 45 cycles of 95°C for 10 s, 60°C for 15 s and 72°C for 30 s, followed by melting curve analysis). The qPCR results were analyzed with the qBase Plus software (Biogazelle). The *PP2A* (At1g13320) and *SAND* (At2g28390) were used as reference genes for gene expression normalization. For the primer sequences, see Table S4.

Microarray analysis

Whole 4-day-old seedlings grown in continuous darkness were harvested. RNA was isolated and analyzed by *Arabidopsis* (V4) Gene Expression Microarray 4×44K (Agilent Technologies). The data are available at NCBI, Gene Expression Omnibus, accession number GSE42053.

ChIP-qPCR

ChIP was carried out with 4-day-old seedlings as described previously (Bowler et al., 2004). The isolated chromatin was sonicated in a SONICS Vibra-cell sonicator with four 15-s pulses at 20% amplitude and immunoprecipitated with 5 µl of anti-acetyl-histone H3 (Lys14) antibodies (Millipore, Cat. no. 7-353). Protein A agarose (Millipore, Cat. No. 16-157) was used to collect immunoprecipitated chromatin. After reverse cross-linking and proteinase K digestion, DNA was purified with the MinElute PCR Purification Kit (Qiagen) and eluted with elution buffer supplemented with RNase A (10 µg/ml). Samples were analyzed by real-time qPCR with primers in the promoter and coding regions of the analyzed genes (Table S5). The amount of immunoprecipitated DNA was calculated relatively to the actin reference gene (At3g18780) and input.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.W., F.V., P.M., D.V.D.S., M.V.L. Methodology: M.W., O.G., S.D.G., L.A.B., P.N., S.L.G., J.F. Formal analysis: M.W., F.V., P.M., D.V.D.S, M.V.L. Writing - original draft: M.W., M.V.L. Writing - review & editing: M.W., M.V.L.

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Supplementary information

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