ZINC-FINGER interactions mediate transcriptional regulation of hypocotyl growth in *Arabidopsis*

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Integration of environmental signals and interactions among photoreceptors and transcriptional regulators is key in shaping plant development. TANDEM ZINC-FINGER PLUS3 (TZP) is an integrator of light and photoperiodic signaling that promotes flowering in *Arabidopsis thaliana*. Here we elucidate the molecular role of TZP as a positive regulator of hypocotyl elongation. We identify an interacting partner for TZP, the transcription factor ZINC-FINGER HOMEODOMAIN 10 (ZFHD10), and characterize its function in coregulating the expression of blue-light–dependent transcriptional regulators and growth-promoting genes. By employing a genome-wide approach, we reveal that ZFHD10 and TZP coassociate with promoter targets enriched in light-regulated elements. Furthermore, using a targeted approach, we show that ZFHD10 recruits TZP to the promoters of key coregulated genes. Our findings not only unveil the mechanism of TZP action in promoting hypocotyl elongation at the transcriptional level but also assign a function to an uncharacterized member of the ZFHD transcription factor family in promoting plant growth.

light | transcription | *Arabidopsis* | zinc-finger | development

Deetiolation and flowering provide great examples in which environmental and endogenous signal integration is required to mediate major transitions and morphological changes during early and late plant development. Light, temperature, photoperiod, clock, and hormone pathways share signaling components that coordinate physiological responses such as hypocotyl elongation and flowering (1–3). Genome-wide transcript analysis, proteomic, imaging, and genetic studies indicate that photoreceptors and circadian and hormone signaling components converge in the nucleus and act on gene expression at the level of transcription (1, 2, 4–11). Direct interactions among plant photoreceptors, key transcription factors (TFs) such as the bHLH family of PHYTOCHROME INTERACTING FACTORS (PIFs), BRASSINAZOLE RESISTANT 1 (BZR1), and TIMING OF CAB EXPRESSION (TOC1), as well as transcriptional regulators, such as HISTONE DEACETYLASES (HDA15, HDA19), TOPLESS (TPL), and scaffold proteins such as EARLY FLOWERING 3 (ELF3), have been shown to play important roles in controlling the expression of growth-promoting genes (2, 6–8, 12–17). Furthermore, recent reports have identified protein components that contribute to the repertoire of signal integrators of light and circadian regulation of hypocotyl elongation and photoperiodic flowering (7, 8, 18).

TANDEM ZINC-FINGER PLUS3 (TZP) was originally identified as a regulator of morning-specific hypocotyl growth based on a Quantitative Trait Locus study on recombinant-inbred lines of two natural *Arabidopsis* ecotypes (*Shahdara* and *Bay-0*) (19). Microarray analyses showed that TZP up-regulates the expression of growth-promoting and auxin-related genes in a blue-light and dawn-specific manner; however, the molecular mechanism of its action remains unknown (19). Recent studies have revealed a dual role for TZP in regulating plant development. In addition to its role in blue-light-induced photomorphogenesis, TZP was recently shown to integrate light and photoperiodic signaling by interacting with the red-light receptor phytochrome B (phyB) in transcriptional nuclear photobodies (8). More specifically, ChIP assays and gene expression analyses revealed that TZP regulates the expression of the flower inducer *FLOWERING LOCUS T* (*FT*) to promote flowering initiation in long days (8). An independent study employing a proteomic approach showed that TZP is part of an in planta multiprotein complex consisting of known and novel light signaling and circadian components including phytochromes A-E, ELF3, and PHOTOPERIODIC CONTROL OF HYPOCOTYL1 (PCH1), placing TZP at the crossroads of light, clock, and photoperiodic pathways (7, 20, 21).

Here we uncover the molecular mechanism of TZP action in regulating blue-light-induced hypocotyl elongation. TZP acts at the transcriptional level by recruiting a member of the plant-specific

Significance

Light coordinates energy production, growth, and survival throughout plant development. In *Arabidopsis*, light stimulates transcriptional reprogramming during developmental transitions such as photomorphogenesis and flowering through the action of photoreceptors, transcription factors, and signaling components. Here we assign a function to a member of the zinc-finger homeodomain (ZFHD) transcription factor family in regulating light-induced development. Our findings reveal ZFHD10 to be a missing link in understanding how the recently discovered integrator of light and photoperiodic flowering, TANDEM ZINC-FINGER PLUS3 (TZP), controls the expression of growth-promoting transcriptional regulators via direct association with light-regulated promoter elements. Elucidating how such novel protein complexes coordinate gene expression will allow scientists and breeders to optimize plant growth and development in response to unfavorable environmental conditions.

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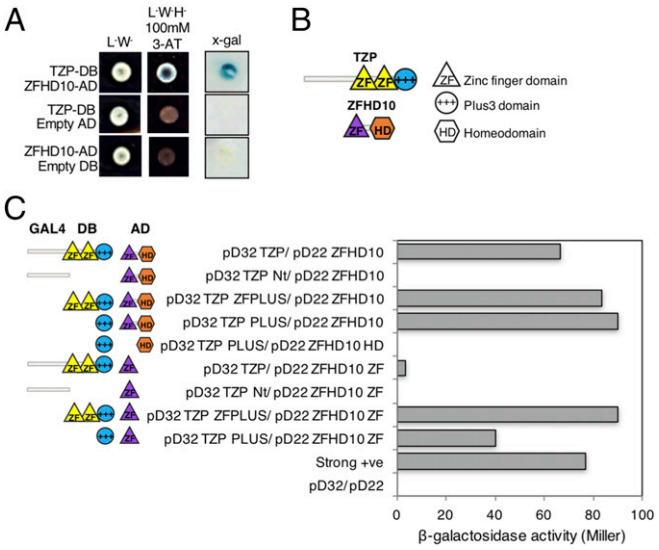

Fig. 1. Identification of ZFHD10 as an interacting partner for TZP, using a genome-wide *Arabidopsis* TF library. (*A*) Yeast-two-hybrid analysis of pDEST32-TZP (GAL4BD-bait) and pDEST22-ZFHD10 (GAL4AD-prey) interactions by assessing growth on nonselective media (L^-W^-), selective media ($L^-W^-H^-$ 100 mM 3-AT), or the x-galactosidase assay. pD22, pDEST22; pD32, pDEST32; 3-AT, 3-amino-1,2,4-triazole. (*B*) Schematic representation of TZP and ZFHD10 domain composition as a guide for the deletion constructs used for the interaction studies shown in Fig. 1*C* and *SI Appendix*, Fig. S1 *E* and *F*. (*C*) Deletion analysis of the interaction between TZP and ZFHD10. Yeast-two-hybrid analysis of pDEST32-TZP, pDEST32-TZP^{Nt}, pDEST32-TZP^{ZFPLUS}, pDEST32-TZP^{PLUS} (GAL4BD-bait), pDEST22-ZFHD10, pDEST22-ZFHD10^{ZF}, pDEST22-ZFHD10^{HD} (GAL4AD-prey) interaction, using the quantitative β-galactosidase assay. Data shown are representative of three independent experimental repeats.

zinc-finger homeodomain (ZFHD) TF family, ZFHD10. *Arabidopsis* contains 14 ZFHD family members with limited information on their molecular function in shaping plant growth, patterning, and embryo development, contrary to the well-established role of the closely related HD-ZIP TFs (22–25). Our genome-wide ChIP studies and quantitative transcript analysis reveal that TZP and ZFHD10 associate with common promoter elements of transcriptional regulators, light-responsive and growth-promoting genes required for hypocotyl elongation. Moreover, our study assigns a role for ZFHD TFs in light-regulated plant development via a direct interaction with TZP.

Results

TZP Interacts with a Member of the ZFHD Transcription Factor Family. TZP contains two ZF domains and a PLUS3 domain, all of which have potential nucleic acid binding activity; however, there is no evidence suggesting that these domains confer transcriptional activation or repression activity (8, 19, 26–28). To investigate the molecular role of TZP in regulating transcriptional control of gene expression, a large-scale directed yeast–two-hybrid screen was performed using a gold standard TF ORFeome library (29) with TZP as the bait (*SI Appendix*, Fig. S1*B*). Nine putative TZP-interacting TFs were identified and verified, seven of which were members of the TEOSINTE BRANCHED 1/CYCLOIDEA/PROLIFERATING CELL NUCLEAR ANTIGEN FACTOR (TCP) TF family, one abscisic-acid-responsive NAC TF (ANAC), and one ZFHD TF (*SI Appendix*, Fig. S1 *B* and *C*). No autoactivation was observed for either the bait (TZP) or the positive prey TFs (*SI Appendix*, Fig. S1 *A* and *D*). The strongest interaction was observed between TZP and ZFHD10 (At5g39760), a member of the ZFHD TF family, which became the central focus of this study (Fig. 1*A* and *SI Appendix*, Fig. S1 *B* and *C*).

Deletion analysis revealed that the ZF of ZFHD10 is sufficient to associate with the ZFPLUS3 and PLUS3 domains of TZP (Fig. 1 *B* and *C* and *SI Appendix*, Fig. S1*E*). ZFHD10 showed homodimerization properties through the ZF (CHCC3H2) motif consistent with a previous report (*SI Appendix*, Fig. S1*F*) (22). No interaction was observed between TZP and ZFHD9, the closest ZFHD family member to ZFHD10 or the homeodomain-leucine zipper (HD-ZIP), ATHB23, recently shown to interact with phyB (*SI Appendix*, Fig. S1*G*) (30).

To validate the interaction between TZP and ZFHD10 in planta, we used bimolecular fluorescence complementation (31, 32). C-terminal translational fusions of ZFHD10-nYFP (spyNe) and TZP-cYFP (spyCe) showed reconstitution of YFP fluorescence in the nucleus when coexpressed transiently in *Nicotiana benthamiana* epidermal cells, whereas no signal was detected for the expression of TZP-cYFP or ZFHD10-nYFP with the empty vector controls (spyNe and spyCe, respectively; Fig. 2*A* and *SI Appendix*, Fig. S2*C*). Colocalization studies also confirmed that TZP-mCherry and ZFHD10-GFP reside in the nucleus in light-grown plants (Fig. 2*B*). In planta coimmunoprecipitation (co-IP) was also performed to further verify the interaction between TZP-GFP and ZFHD10-RFP (Fig. 2*C* and *SI Appendix*, Fig. S2*D*). Overall, these data demonstrate that TZP and ZFHD10 interact in yeast and in planta.

TZP and ZFHD10 Promote Hypocotyl Elongation. qRT-PCR showed that *ZFHD10* is abundant in seedlings primarily when grown in blue light, which correlates with the expression pattern of *TZP*, whereas no correlation was observed for *ZFHD9* (*SI Appendix*, Fig. S3 *D*–*G*). No significant diurnal regulation was observed for *ZFHD10* transcript or protein abundance in transgenic lines expressing $35S_{pro}$ZFHD10-GFP/Col-0 (OXZFHD10) (*SI Appendix*, Fig. S3*C*) (33, 34). A closer look at seedlings revealed that *TZP* and *ZFHD10* are present in the cotyledons as well as the hypocotyl, with an increase in abundance in the hypocotyl apex (*SI Appendix*, Fig. S3*I* and refs. 35 and 36). *TZP* is also highly expressed in roots (*SI Appendix*, Fig. S3 *F* and *H* and ref. 37), suggesting a potential role in other tissues.

To further explore the physiological significance of TZP-ZFHD10 interactions, we examined the photomorphogenic phenotypes of knockout and overexpressing lines for ZFHD10 and TZP (Fig. 3 and *SI Appendix*, Figs. S3 *A* and *B*, S4, and S5 *A* and *B*).

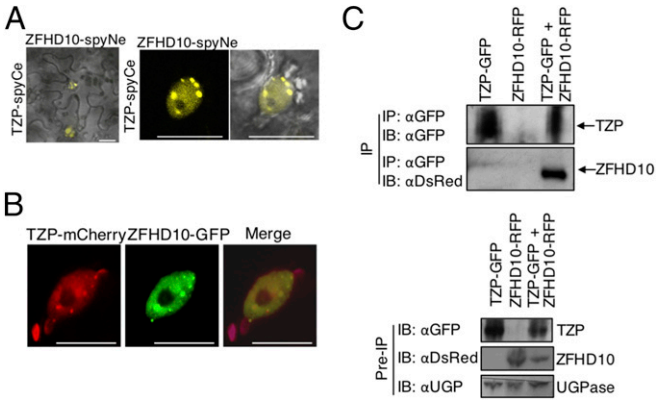

Fig. 2. TZP colocalizes and interacts with ZFHD10 in planta. (*A*) Bimolecular fluorescence complementation assay shows YFP reconstitution between TZP-spyCe and ZFHD10-spyNe when coexpressed transiently in *N. benthamiana* leaves. (Negative and positive controls are shown in *SI Appendix*, Fig. S2*C*.) (*B*) Representative images of *N. benthamiana* leaves coexpressing TZP-mCherry and ZFHD10-GFP. (*C*) Coimmunoprecipitation of TZP-GFP and ZFHD10-RFP coexpressed transiently in *N. benthamiana*. Single infiltration of TZP-GFP or ZFHD10-RFP were used as negative controls. Plants were grown in white light before examination using confocal microscopy and coimmunoprecipitation. Data shown are representative of three independent experimental repeats. (Scale bars, 20 μm.)

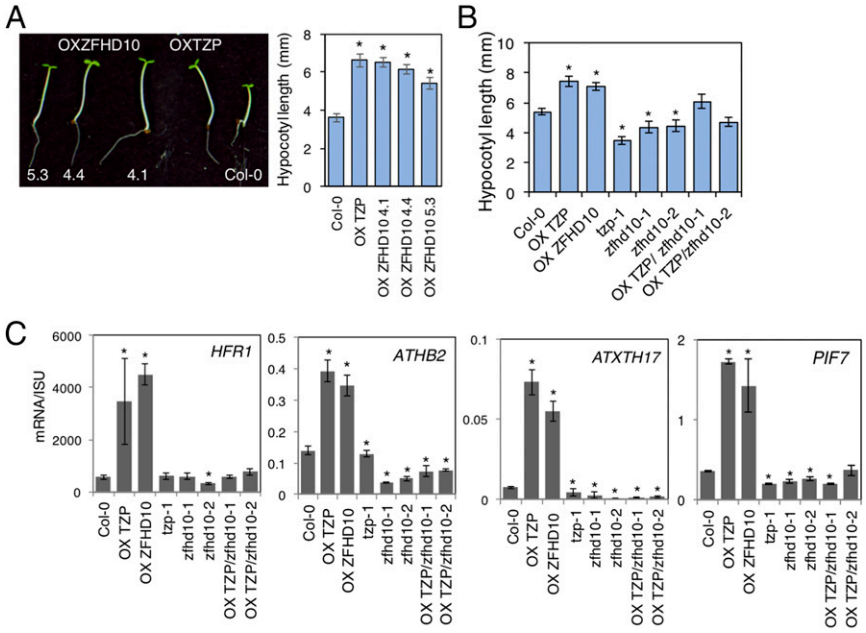

Fig. 3. ZFHD10 and TZP promote hypocotyl elongation. (*A*) Hypocotyl measurements and representative images of homozygous transgenic *Arabidopsis* lines overexpressing ZFHD10 or TZP in Col-0. Plants were grown for 7 d in blue light (1 μmol $m^{-2}·s^{-1}$). (*B*) Hypocotyl measurements of the indicated genotypes. Plants were grown for 7 d in blue light (1 μmol $m^{-2}·s^{-1}$). Col-0 was used as the wild-type control. Representative images are shown in *SI Appendix*, Fig. S5*A*. The data presented are mean ± SE ($n = 15$ seedlings). (C) qRT-PCR analysis of *HFR1*, *ATHB2*, *ATXTH17*, and *PIF7* mRNA normalized to housekeeping gene *ISU1* of the indicated genotypes. Seedlings were grown in continuous blue light (1 μmol $m^{-2}·s^{-1}$) for 7 d. Bars are means ± SE ($n = 4$ technical replicates). Graphs are representative of three independent experimental repeats. Asterisks indicate difference to Col-0 at $P < 0.05$. An independent biological repeat is shown in *SI Appendix*, Fig. S5*C*.

It has previously been shown that overexpression of TZP promotes hypocotyl elongation, whereas a premature stop codon within the *TZP* locus in Bay-0 results in shorter hypocotyls primarily in response to blue light (19). Hypocotyl elongation measurements showed that OXZFHD10 phenocopies OXTZP in response to blue-light irradiation, whereas *tzp* and *zfhd10* knockout/knockdown mutants exhibited shorter hypocotyls relative to the wild-type (Col-0), OXTZP, and OXZFHD10, primarily under low fluence rate blue light (Fig. 3 *A* and *B* and *SI Appendix*, Fig. S5 *A* and *B*) (19). We also generated and examined transgenic lines overexpressing TZP in the *zfhd10* background, and although they showed partial rescue of the mutant *zfhd10* phenotype, they never reached the level of elongation demonstrated by OXTZP/Col-0 (Fig. 3*B*). Overexpression of ZFHD10 in the absence of functional TZP (OXZFHD10/Bay-0) showed rescue of the short hypocotyl Bay-0 phenotype (*SI Appendix*, Fig. S6). These observations suggest that TZP requires, at least in part, ZFHD10 for function and positions ZFHD10 downstream of TZP action with regard to blue-light-regulated hypocotyl elongation.

ZFHD10 and TZP Regulate Common Growth-Promoting Gene Targets. TZP is known to regulate the expression of growth-promoting genes based on microarray analysis (19). At this time, there is no report of transcriptional targets for ZFHD10. Because ZFHD10 interacts with TZP and regulates hypocotyl elongation, we were interested in examining whether ZFHD10 controls the expression of TZP-regulated growth-promoting genes (19). Indeed, ZFHD10 induces the expression of *ATHB2*, *LONG HYPOCOTYL IN FAR-RED* (*HFR1*), *XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE* 17 (*ATXTH17*), and *PIF7*, as shown by qRT-PCR analysis performed on 7-day-old seedlings irradiated with low blue light (Fig. 3*C* and *SI Appendix*, Fig. S5*C*). ATHB2 is an HD-ZIP TF that promotes hypocotyl growth under low-light conditions and in response to auxin (38–40). HFR1 is a non-DNA-binding bHLH TF that associates with PIF TFs to regulate stem elongation in response to far-red, blue, and shade (38, 41–43). ATXTH17 is a light-induced plant cell wall enzyme essential for growth promotion in response to light and hormones (44, 45). PIF7 is known to accumulate in response to light and interacts with phyB to promote plant growth in response to shade (4, 46, 47). An increase in the expression of these genes and the function of the proteins they encode has been associated with light-regulated hypocotyl elongation (19).

OXTZP and OXZFHD10 show increased transcript levels of the aforementioned genes, whereas *tzp* and *zfhd10* mutants show partial reduction in their expression (Fig. 3*C* and *SI Appendix*, Fig. S5*C*). Collectively, TZP and ZFHD10 show a similar trend in the regulation of the expression of well-characterized genes, which directly correlate with the hypocotyl phenotypes described earlier.

To test whether TZP and ZFHD10 could directly control transcription by associating with the promoter regions of the genes they coregulate, we performed ChIP assays followed by qPCR. TZP associates preferentially with the transcriptional start site (TSS) of *ATHB2*, *ATXTH17*, and *PIF7* and with the G-box (CACGTG), a well-characterized light-regulated element, of *HFR1* (Fig. 4 and *SI Appendix*, Fig. S5*D*). No significant enrichment was observed for the 3′ untranslated region, the *IAA1* promoter, or Col-0 (Fig. 4). ZFHD10 showed a similar pattern of preferential binding to the TSS of *ATHB2*, *PIF7*, as well as the first G-box and TSS of *HFR1* (Fig. 4 *A*, *B*, and *D* and *SI Appendix*, Fig. S5*D*). ZFHD10 showed no enrichment on two canonical ZFHD binding sites on the *ATHB2* promoter, whereas binding was observed on the HUD element (Hormone Up at Dawn) of $ATXTH17_{pro}$ (Fig. 4 *B* and *C* and *SI Appendix*, Fig. S5*D*) (19, 22, 33, 48).

We previously showed that TZP^{PLUS3} is sufficient for binding nonspecific ssDNA in vitro, so we were interested in investigating whether the interaction of TZP with ZFHD10 confers sequence specificity and guides TZP to specific promoter sequences (8). ChIP-qPCR on transgenic plants expressing equal protein levels of TZP-GFP in Col-0 and *zfhd10* showed a considerable decrease in the recruitment of TZP on *HFR1*, *ATHB2*, and *ATXTH17* promoters (Fig. 5 and *SI Appendix*, Fig. S5*E*). These results

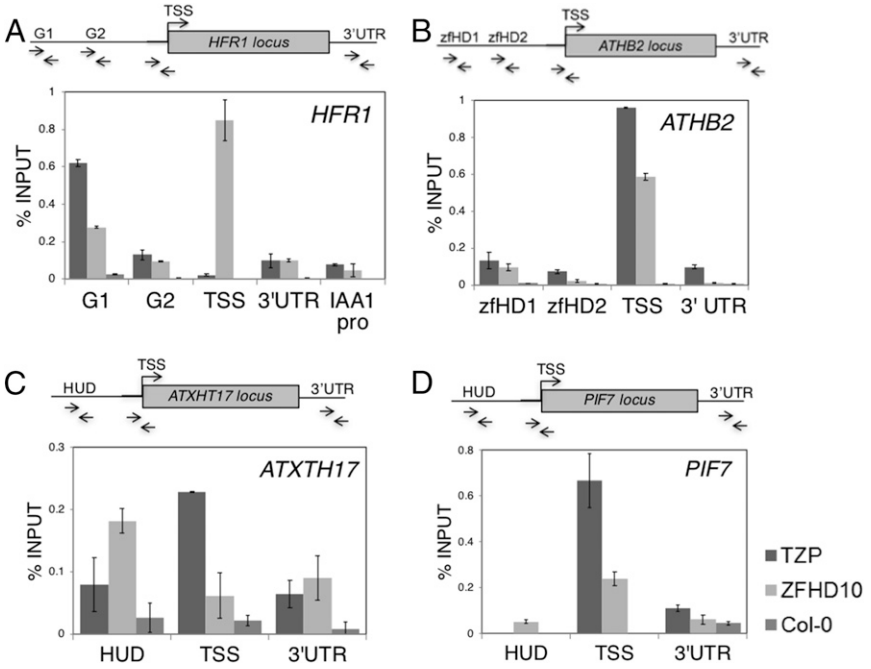

Fig. 4. TZP and ZFHD10 associate with common genomic regions of growth-promoting genes. (*A–D*) Relative enrichment of TZP and ZFHD10 *on HFR1*, *ATHB2*, *ATXTH17*, and *PIF7* loci. Col-0, a region in the 3′ untranslated region of each locus, and the *IAA1* promoter were used as negative controls. Seedlings were grown for 7 d under blue light (1 μmol m^{-2}·s^{-1}). Bars are means ± SE ($n = 4$ technical replicates). Graphs shown are representative of three independent experimental repeats. An independent experimental repeat is shown in *SI Appendix*, Fig. S5*D*. G, G-box promoter element; HUD, Hormone Up at Dawn promoter element (CACATG); ZfHD, Zinc finger Homeo-Domain binding site TAAATTG.

suggest that ZFHD10 is important for recruiting TZP on specific promoter sequences.

TZP and ZFHD10 Associate with Common Chromatin Regions Enriched in Light-Responsive Elements. In addition to examining whether TZP and ZFHD10 regulate and bind to promoters of genes known to be involved in light-regulated hypocotyl elongation, an unbiased genome-wide approach was also performed using ChIP coupled to next-generation sequencing on 7-day-old GFP-tagged transgenic lines irradiated with low blue light. Bioinformatics analysis on data obtained by ChIP sequencing revealed that TZP and ZFHD10 preferentially associate with promoter regions, as

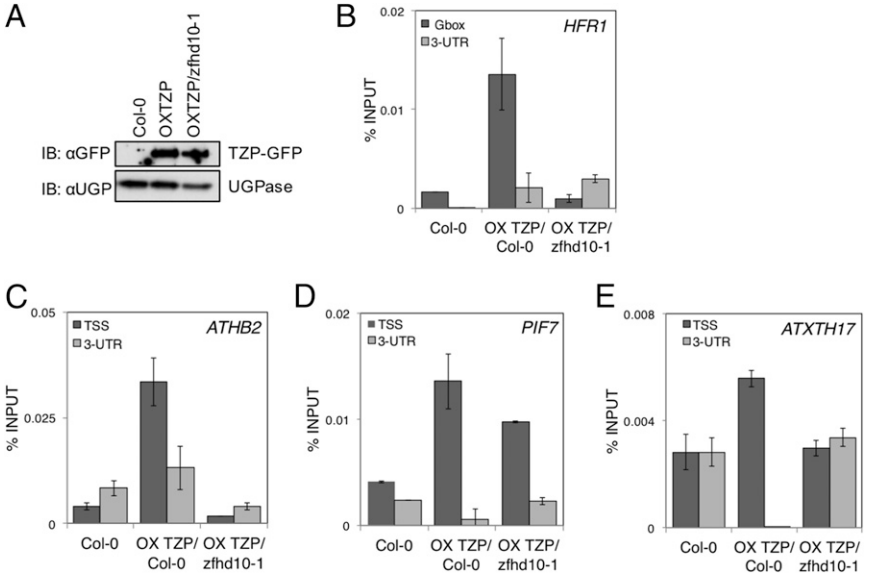

Fig. 5. ZFHD10 is important for the recruitment of TZP on light-regulated promoters. (*A*) Western blot analysis of TZP protein levels in OXTZP/Col-0 and OXTZP/*zfhd10-1* transgenic lines. Col-0 was used as a negative control for the anti-GFP antibody, and UGPase was used a loading control. Relative enrichment of TZP on *HFR1* (*B*), *ATHB2* (*C*), *PIF7* (*D*), and *ATXTH17* (*E*) loci when expressed in Col-0 or *zfhd10* mutant background. Col-0 and the 3′ untranslated region were used as negative controls. Seedlings were grown for 7 d under blue light (1 μmol m^{-2}·s^{-1}). Bars are means ± SE ($n = 4$ technical replicates). Graphs shown are representative of two independent experimental repeats. An independent experimental repeat is shown in *SI Appendix*, Fig. S5*E*. G, G-box.

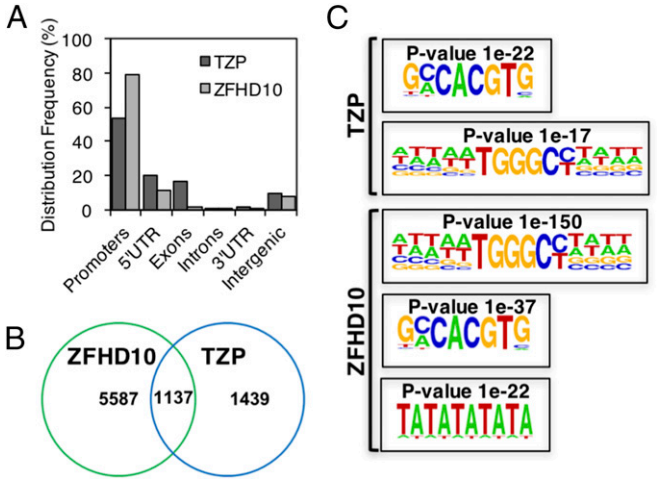

Fig. 6. TZP and ZFHD10 bind to common genomic regions. (*A*) Relative binding-peak distribution of TZP and ZFHD10 across genomic regions. Values were normalized to those of Col-0. (*B*) Venn diagram depicting the overlap between TZP and ZFHD10 targets (promoters and TSS) determined by whole-genome ChIP sequencing analysis. (*C*) Enriched motifs identified within 1 kb of the surrounding peak summits for overlapping TZP and ZFHD10 ChIP-seq peaks.

80% and 50% of ZFHD10 and TZP genomic binding sites, respectively, were within 1 kb of the TSS of gene loci (Fig. 6*A*). Data analysis using stringent parameters revealed a significant amount of promoter regions corresponding to 1,137 genes bound by both TZP and ZFHD10 (Fig. 6*B* and Dataset S2). The number of targets bound exclusively by ZFHD10 (5,587) was higher than the number identified for TZP (1,439), possibly because ZFHD10 has a canonical DNA-binding domain (HD), whereas TZP may associate with specific promoters indirectly via binding to TFs (Fig. 6*B*).

De novo analysis of consensus motifs enriched within the promoter-binding peaks of TZP and ZFHD10 identified well-characterized light-, temperature-, and circadian-regulated elements such as the G-box (CACGTG), which is a variation of the E-box, and the SORLIP2 (Sequence Over-represented in Light-Induced Promoters; TGGGCC) (Fig. 6*C*) (3, 49–51). Furthermore, peaks specifically bound by ZFHD10 showed over-representation of the transcriptional initiator TATA-box (TATATATA). Recent studies have highlighted the significance of interactions between the TATA-box and cis-regulatory light-responsive promoter elements in activating gene expression in plants (52).

To verify the results obtained by ChIP-seq (Fig. 7 *A*, *D*, and *G*), we performed ChIP-qPCR on selected genomic targets that showed binding by both TZP and ZFHD10 (Fig. 7 *B*, *E*, and *H*). ChIP of seedlings expressing TZP-GFP and ZFHD10-GFP exposed to low blue light showed enrichment in the promoter regions of *ARABIDOPSIS AUXIN RESPONSE FACTOR* (*ARF6*), the histone-lysine *N*-methyltransferase *SU(VAR)3–9* (*SUVR1*), and *EARLY FLOWERING 4-LIKE* (*EFL4*; Fig. 7 *B*, *E*, and *H*).

In *Arabidopsis*, SUVR1 promotes transcriptional gene silencing by forming a complex with SNF2-related chromatin-remodeling proteins that modify nucleosome positioning at RNA-directed DNA methylation-dependent and independent loci (53). EFL4 has 44% sequence identity to ELF4, a protein that regulates phyB-mediated seedling deetiolation in response to red light as well as diurnal regulation and flowering time (6, 54). ARF6 is involved in regulating hypocotyl elongation and flower development (55–57). Recent studies have shown that ARF6 interacts with PIF4 and BZR1 and coregulates genes that promote hypocotyl elongation in response to light, hormones, and temperature (55). Overexpression of TZP leads to a higher induction of *ARF6*, *SUVR1*, and *EFL4* transcripts than OXZFHD10, even though ZFHD10 showed a greater enrichment on these loci (Fig. 7 *B*, *C*, *E*, *F*, *H*, and *I*). ChIP experiments on TZP/*zfhd10* lines showed a reduction in TZP enrichment on *SUVR1* and *EFL4* promoters (*SI Appendix*, Fig. S8 *B* and *C*). However, no difference in TZP binding is observed for *ARF6* (*SI Appendix*, Fig. S8*A*), suggesting this target could be bound by separate TZP and ZFHD10 protein complexes. Furthermore, *tzp* and *zfhd10* mutants showed down-regulation of *SUVR1* and, to a lesser extent, *EFL4* and *ARF6* transcripts (Fig. 7 *C*, *F*, and *I*). These observations could be a result of additional TZP-interacting proteins and photoreceptor pathways regulating the expression of these genes.

Gene ontology analysis of common promoter targets of TZP and ZFHD10 showed enrichment in TFs (LFY, CCA1, members of the AGAMOUS-like, Dof ZF, WRKY, TCP, ZFHD, and HD-ZIP TF families), transcriptional regulators (ORIGIN OF REPLICATION COMPLEX 1B, NUCLEAR RNA POLYMERASE 1), chromatin remodeling enzymes [HISTONE DEACETYLASE 2 and 19 (HDA2, HDA19), HISTONE MONOUBIQUITINATION 1 (HUB1)], F-box and RNA-binding proteins (splicing factor, poly-A binding proteins, RAD5, MEDIATOR 7), and hormone response factors (ETHYLENE RESPONSE FACTOR 10, EIN3-BINDING F-BOX), suggesting an active role in the transcriptional regulation of gene expression in response to environmental, biotic, abiotic, and endogenous stimuli (*SI Appendix*, Fig. S9*A* and Dataset S3).

Promoter regions exclusively associated by TZP show a huge over-representation in proteins involved in transcription such as C2H2-ZF, WRKY, bHLH, and MYB TFs, as well as TOPLESS-related, brassinosteroid (BZR1 and BZR-like homolog), clock (PRR9 and PRR5), flowering (VIN3-like, FLD, SPATULA), and light signaling components (PIF4, PIL6, PAP2, phyE, cry2, CIB1, SPA4), reflecting its involvement in regulating gene expression in response to environmental stimuli such as light and temperature (*SI Appendix*, Fig. S9*B*). On the contrary, ZFHD10-specific targets were enriched in RNA processing factors as well as proteins involved in salt stress and ABA signaling, in addition to embryo development (*SI Appendix*, Fig. S9*C*). Overall, our findings show that TZP and ZFHD10 coassociate with and regulate the expression of not only light-regulated loci but also transcriptional regulators to shape plant development in response to environmental stimuli.

Discussion

Plant growth and development are coordinated through the action of multiple light, hormone, and photoperiodic pathways. This study focuses on uncovering the molecular role of TZP, a signal integrating component, in promoting hypocotyl elongation in response to low blue light.

To elucidate how TZP promotes growth and regulates gene expression during the early stages of plant development, we screened for TF-interacting partners for TZP, using a large-scale, directed Y2H approach. Nine proteins exhibited positive interactions of various strength with TZP, seven of which were members of the TCP TF family (TCP8, TCP9, TCP12, TCP15, TCP20, TCP22, TCP23) and one ANAC TF (ANAC87; *SI Appendix*, Fig. S1*B*). TCP TFs regulate many aspects of plant development, including circadian rhythms and photoperiodic flowering (58–60). However, in this study, we focus on the characterization and functional significance of the strongest TZP interactor, which is a member of the ZFHD family (Fig. 1).

Homeobox TFs have key functions in shaping animal and plant development (25). In *Arabidopsis*, there are more than 100 proteins that contain DNA-binding HDs. The HD-ZIP class of TFs is one of the most well-characterized classes of HD-containing proteins with major roles in hormone and light-regulated developmental responses (24, 39). A unique combination of ZF and HD modules has given rise to the plant-specific ZFHD class of TFs originally discovered in the C4 plant *Flaveria*

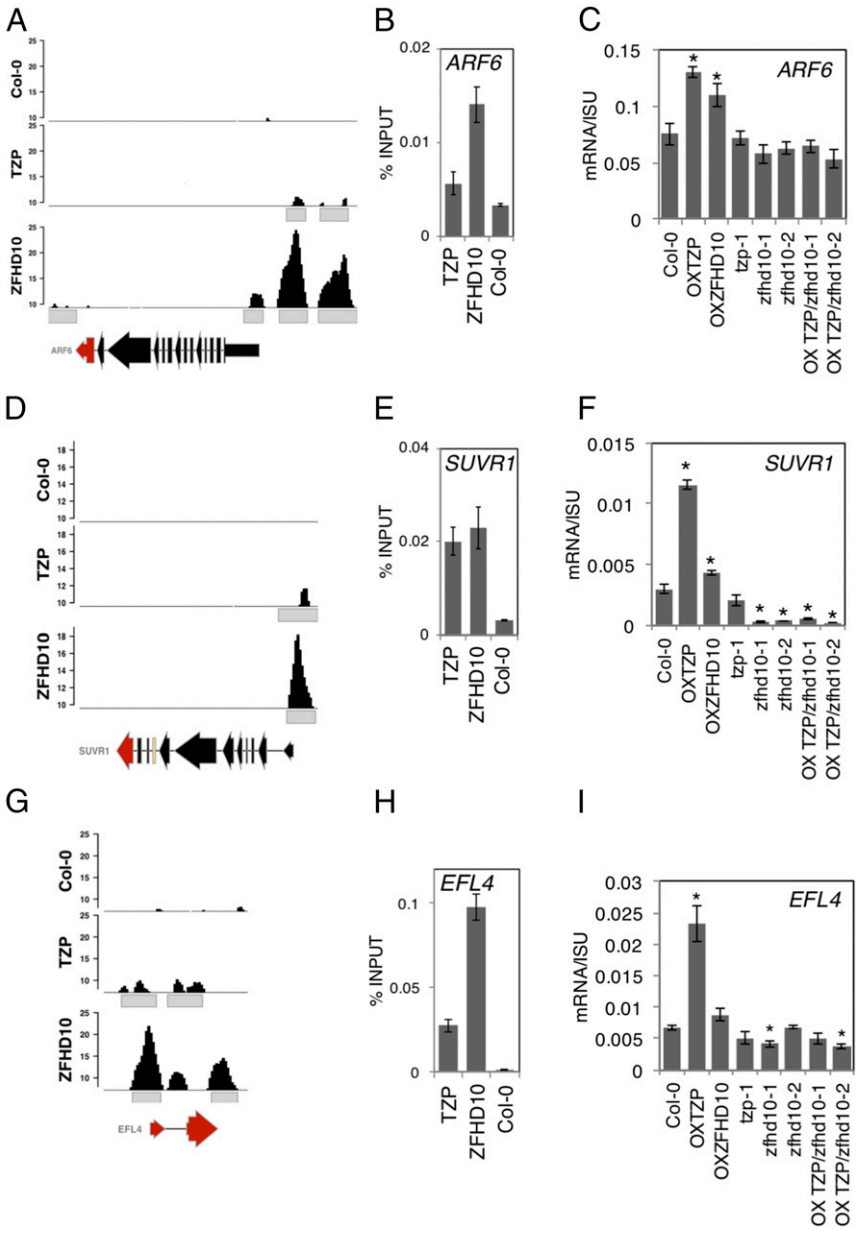

Fig. 7. ChIP sequencing identifies overlapping targets for TZP and ZFHD10. (*A*, *D*, and *G*) Visualization of ChIP-seq data in the genomic regions encompassing three representative genes, *ARF6*, *SUVR1*, and *EFL4*. The ChIP tracks show the pile-up distribution of the raw reads from three pooled biological replicates of ChIP-seq data. Gray bars underneath the peaks indicate differences identified by SICER between TZP vs. Col-0 and ZFHD10 vs. Col-0 (TZP peaks ≥ 1.5 and ZFHD10 ≥ twofold change to Col-0). Gene regions are represented as arrow-shaped boxes, which shows transcriptional orientation. (*B*, *E*, and *H*) Relative enrichment of TZP and ZFHD10 on *ARF6*, *SUVR1*, and *EFL4* promoter regions identified by SICER. Col-0 was used as a negative control. Bars are means ± SE (n = 4 technical replicates). (*C*, *F*, and *I*) qRT-PCR analysis of absolute *ARF6, SUVR1*, and *EFL4* mRNA levels normalized to housekeeping gene *ISU1* of the above-indicated genotypes. For all the experiments, seedlings were grown in continuous blue light (1 μmol $m^{-2}{\cdot}s^{-1}$) for 7 d. Bars are means ± SE (n = 4 technical replicates). Graphs are representative of two independent experimental replicates.

(61). Seminal reports on specific *Arabidopsis* ZFHDs clearly support their action as transcriptional regulators of drought tolerance and flower development via direct DNA binding through the HD (22, 48). However, there is no current study characterizing the function of ZFHD10 or the role of ZFHDs in light signaling and photomorphogenesis.

ZF domains are highly conserved and act as sites for protein or nucleic acid association (28). In the case of ZFHD10 and TZP, deletion analyses showed association through ZFPLUS3 (TZP)–ZF(ZFHD10) interactions (Fig. 1 *B* and *C*). There is no evidence suggesting that ZFHDs bind DNA as dimers or monomers. It would be interesting to further investigate whether the interaction of TZP with ZFHD10 interferes with the homodimerization status of ZFHD10 or its heterodimerization with other ZFHD TFs.

Our in planta studies (colocalization, co-IP, hypocotyl measurements) verify that the interaction between TZP and ZFHD10 is physiologically significant and reveal a function for ZFHD10 as a positive regulator of hypocotyl growth in response to low blue light (Figs. 2 and 3 *A* and *B* and *SI Appendix*, Fig. S5*B*). To dissect the molecular mechanism of TZP and ZFHD10 function, we performed gene expression analysis and discovered coregulation of key genes involved in light-induced hypocotyl elongation and showed that ZFHD10 guides TZP to light-regulated promoter sequences (Figs. 3*C*, 4, and 5 *B*–*E* and *SI Appendix*,

Fig. S5 *D* and *E*). Furthermore, targeted as well as genome-wide chromatin association studies clearly showed that TZP and ZFHD10 bind to common genomic loci enriched in promoter regions of light-, temperature-, and hormone-regulated growth-promoting genes (Figs. 4, 6, and 7 and Dataset S2).

Research on how transcriptional regulators and chromatin remodeling factors control plant development is currently flourishing; however, there is limited information on how the expression of chromatin-modifying enzymes is regulated by environmental stimuli. Our ChIP sequencing analysis shows that TZP and ZFHD10 associate with the promoters of histone deacetylases and methyltransferases and an E3 ubiquitin ligase that monoubiquitinates H2B. These findings will therefore open avenues in investigating a potential role for TZP and ZFHD10 in controlling the transcript levels of these enzymes and further understanding the role of chromatin remodeling in fine-tuning light-regulated plant growth and development. A putative EAR (Ethylene-responsive element binding factor-associated Amphiphilic Repression) (LxLxL) motif within the TZP PLUS3 domain indicates that TZP could also recruit and directly modulate the action of transcriptional corepressors and histone remodeling enzymes at the protein level (17, 62, 63).

Motif analysis of the TZP-ZFHD10 promoter targets revealed a clear over-representation of light-responsive elements such as the G-box: CACGTG and the SORLIP2 previously associated with cryptochrome and phytochrome regulated gene expression (Fig. 6*C*) (49–51).

The importance of signal integration among blue (cry1 and cry2) and red/far-red (phyA and phyB) light receptors in regulating plant development, growth, and photoprotection is well established (4, 43, 64–69). Our data show that there is a decrease in the blue-light-dependent induction of *TZP* in *cry1cry2*, *cry1*, and *phyA* compared with Col-0, suggesting that cry1 and phyA have a redundant role in regulating blue-light-induced *TZP* expression (*SI Appendix*, Fig. S7*A*). Furthermore, ChIP analysis revealed a reduction in the enrichment of TZP on the promoters of blue-light-regulated loci indicating that cry1, cry2, and phyA are important for recruiting TZP to light-regulated genomic regions (*SI Appendix*, Fig. S7*C*). Our ChIP and physiological analysis suggests that TZP regulates blue-light-induced hypocotyl elongation through the action of cry1, cry2, and phyA (*SI Appendix*, Fig. S7). Whether cry1, cry2, and/or phyA can positively or negatively regulate TZP or vice versa through direct interactions or via the action of other TFs requires further investigation.

At this time, we have no evidence suggesting a role for the interaction between TZP and phyB in regulating blue-light-mediated hypocotyl growth (7, 8, 20). ZFHD10 is recruited to nuclear bodies (NBs) only when coexpressed with TZP in *N. benthamiana* exposed to white light, possibly because of the phyB-induced recruitment of TZP (and its partners) to NBs. Neither TZP nor ZFHD10 form NBs in *Arabidopsis* or *N. benthamiana* leaves when exposed to blue light (8). These observations suggest that the blue-light regulation of gene expression conferred by TZP and ZFHD10 is independent of phyB-mediated signaling or red-light-induced NB formation. It would be interesting to investigate whether TZP and ZFHD10 regulate gene expression by interacting or interfering with the action of key bHLH PIF4 and PIF5 TFs in response to low levels of blue light (4, 43).

Future studies are necessary to establish whether TZP and ZFHD10 operate through a PIF-dependent or independent pathway. Employing imaging tools that have recently been developed to study cell-type-specific interactions among key transcriptional regulators of cell fate in *Arabidopsis* roots would provide invaluable insights in understanding how nuclear signal integration influences plant growth and development (70).

The significance of our findings is multivalent. First, we elucidate the molecular and physiological role of TZP as a positive regulator of hypocotyl growth by associating with the promoters of chromatin remodeling enzymes, transcriptional regulators, and well-characterized light-responsive genes. Second, we identify ZFHD10 as the TF that directly associates and acts in concert with TZP to regulate the expression of genes required for hypocotyl elongation in response to low blue light. Last and very important, we report a function for a member of the unique family of ZFHD TFs in light-induced plant growth. Our data position TZP and ZFHD10 downstream of the clock and the photoreceptors with direct links to light, temperature, and hormone signal integration, which is essential for coordinating optimal plant growth and development in response to a constantly changing environment (Fig. 8).

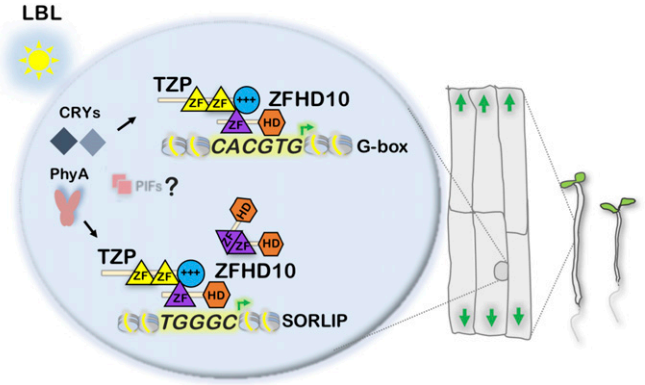

Fig. 8. The role of TZP and ZFHD10 in regulating hypocotyl elongation. TZP acts as a positive regulator of growth downstream of the clock and the photoreceptors. TZP and ZFHD10 directly interact through their ZFPLUS3 and ZF domains, respectively. ZFHD10 recruits TZP to light-regulated promoter elements (G-box, SORLIP), and they act in concert to control the expression of growth-promoting transcriptional regulators in response to blue light (LBL). Whether TZP and ZFHD10 act cooperatively or independent of the PIF TFs remains to be investigated.

Materials and Methods

Plant material and growth conditions are described in *SI Appendix, SI Materials and Methods*. The ProQuest Two-Hybrid System (Invitrogen) was used to identify and verify direct interactions between TZP (bait, pDEST32) and a library of TFs (prey, pDEST22) by sequential transformation of the yeast strain MaV203. Construction of the pDEST22-TF library screened has been described previously (29). For more information, please see *SI Appendix, SI Materials and Methods*. Protein extraction and Western blot analysis are described in *SI Appendix, SI Materials and Methods*. Transient protein expression in *N. benthamiana* using Agrobacterium-mediated infiltration was performed as previously described (8). Coimmunoprecipitation was performed using the μMACS GFP Tag Protein Isolation Kit (Miltenyi Biotech), using 500 μg total protein extracted as described for *Arabidopsis* (6, 8). Confocal microscopy was performed with Leica SP2 and SP8 inverted microscopes, and image analysis was performed as described previously (8). Representative images from three independent biological repeats are shown in this study. Transient expression and imaging in *N. benthamiana* was performed as described previously (32). Cloning and genotyping strategies are described in *SI Appendix, SI Materials and Methods*. Quantitative RT-PCR and ChIP assays were performed as described previously, with minor modifications (8, 71). *SI Appendix, SI Materials and Methods*. Next-generation sequencing and analysis of the ChIP DNA was carried out in the Glasgow Polyomics Facility (University of Glasgow). Raw data, analysis, and gene ontology studies can be accessed in *SI Appendix, SI Materials and Methods* (Datasets S2 and S3). A complete list of primers used for genotyping, qRT-PCR, cloning, and ChIP-qPCR are listed in *SI Appendix, SI Materials and Methods* and Dataset S1. Accession numbers of *Arabidopsis* genes that are the main focus of this study are At5g43630 (TZP) and At5g39760 (ZFHD10).

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