PLANT SCIENCES

A PIF-SAUR module safeguards hypocotyl elongation from ABA inhibition in the dark

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Driven by cell elongation, hypocotyl growth is tightly controlled by light and responds to the stress signaling hormone abscisic acid (ABA). However, the molecular connections between ABA and light to control cell elongation are poorly understood. Here, we show that, in Arabidopsis, ABA inhibits hypocotyl elongation in the light but not in the dark. In the dark, hypocotyl sensitivity to ABA is restored in *pifq* and *cop1-4* mutants, suggesting that an active light signaling pathway is necessary for hypocotyl responsiveness to ABA. Through an RNA sequencing subtractive approach, we identified ABA differentially expressed genes that correlate with ABA inhibition of hypocotyl elongation, including several *SAUR* genes. The abrogation of PP2C.D2-SAUR interaction in the *pp2c.d2*/*PP2C.D2*^{M331K}*-GFP* line restored ABA sensitivity in the dark, suggesting that SAUR proteins are key to maintain hypocotyls insensitive to ABA. This hypocotyl-specific mechanism enables growth toward the light, overriding ABA inhibition of cell elongation, ensuring subsequent seedling establishment and survival.

INTRODUCTION

Immediately after underground germination, seed plants like Arabidopsis have a brief window of time to reach the soil surface, perceive light, and establish themselves as autotrophic organisms. In this soil-buried phase, the young seedling, fed by the seed reserves, rapidly elongates its hypocotyl in search for light, a primordial process for seedling establishment and survival, that is driven by cell elongation (*1*, *2*). Upon light perception, hypocotyl elongation is reduced, the small cotyledons expand and turn green, and photosynthetic competency is established. Under this photomorphogenic developmental program, plants grow and develop full competencies to perceive, transduce, and respond to abiotic and biotic signals that allow further adaptation to the environment (*3*).

The bHLH (basic helix-loop-helix)–type transcription factors (TFs) PHYTOCHROME-INTERACTING FACTORS (PIFs) play an active role controlling hypocotyl elongation in the dark. Darkness releases PIFs from the light-activated phytochrome inhibition, allowing these TFs to bind to the promoters of genes involved in hypocotyl cell elongation (*4*–*6*). In the dark, the quadruple *pif* mutant (*pifq*) lacking PIF1, PIF3, PIF4, and PIF5 displays short hypocotyls and expanded cotyledons characteristic of a photomorphogenic development (*7*, *8*). Transcriptional activated genes by PIFs include auxin biosynthetic genes and the induction of a number of genes concomitantly regulated by auxins, as the *SMALL AUXIN UP-RNA* (*SAUR*) (*9*, *10*). *SAURs* are a family of 81 genes in Arabidopsis, with different levels of tissue specificity that, in most cases, regulate cell expansion (*9*, *11*). The overaccumulation of SAUR proteins such as SAUR19 and SAUR63 are sufficient to trigger hypocotyl elongation (*12*–*15*). SAURs activate plasma membrane (PM) H^+-ATPase by directly binding and inactivating PROTEIN PHOSPHATASES TYPE 2C (PP2C)-D subfamily of type 2C (PP2C.D), which can inactivate PM H^+-ATPases by dephosphorylating their penultimate threonine (pThr) residue (*14*, *16*).

Considered a key stress signaling hormone, abscisic acid (ABA) regulates many physiologic responses as seed dormancy, resistance to drought, stomatal closure, and cell elongation (*17*, *18*). ABA binds to PYRABACTIN RESISTANCE 1 (PYR1)/PYR1-LIKE (PYL)/REGULATORY COMPONENTS OF THE ABA RECEPTOR (RCAR) protein family members (PYR/PYL/RCAR) (*19*–*22*), which strongly increases their affinity for the clade A PP2Cs, such as ABA-INSENSITIVE 1 (ABI1), ABI2, HYPERSENSITIVE TO ABA1 (HAB1), and PP2CA (*23*), leading to their inactivation. As a consequence, SUCROSE NON–FERMENTING-1–RELATED PROTEIN KINASES 2 (SnRK2s), which act as positive regulators in the ABA signal cascade, i.e., SnRK2.2, SnRK2.3, and OPEN STOMATA 1 (OST1)/SnRK2.6 (*20*, *22*), are released to phosphorylate downstream targets, including many TFs, such as the ABA RESPONSIVE TRANSCRIPTION FACTORS (ABF)/ABRE-binding (AREB) to activate ABA-responsive genes (*24*, *25*). In addition, SnRKs directly activate various types of water and ion channels [as SLOW ANION CHANNEL-ASSOCIATED 1 (SLAC1) and K^+ channel (KAT1)] that determine turgor of guard cells and stomatal aperture (*26*, *27*). Thus, ABA can induce stress responses in a transcription-dependent and transcription-independent manner (*28*, *29*).

Two distinct roles for ABA in the regulation of cell elongation have been proposed. Endogenous ABA is required for growth and hypocotyl elongation, as evidenced by the short hypocotyl phenotype observed in ABA-deficient mutants, such as *sitiens* (*sit*) and *notabilis* (*not*) in tomato, and *aba1* in Arabidopsis (*30*, *31*). On the contrary, exogenously applied ABA has long been reported to reduce etiolated hypocotyls elongation in squash (*32*). In Arabidopsis, ABA represses hypocotyl elongation by reducing PM H^+-ATPase Thr^{947} phosphorylation in an ABI1-dependent manner (*33*). In addition, Lorrai *et al.* (*34*) demonstrated that ABA negatively controls hypocotyl elongation either in etiolated or de-etiolated hypocotyls by acting on gibberellin (GA) metabolism, inducing the accumulation of the DELLA proteins, affecting GA signaling, and repressing auxin biosynthetic genes.

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Here, we show that etiolated Arabidopsis hypocotyls are insensitive to ABA whereas roots respond to increasing ABA concentrations either under light or dark conditions. The existence of a constitutively active light pathway in the dark, as in *pifq* or in *cop1* mutants, is sufficient to confer hypocotyl responsiveness to ABA. Transcriptional data and mutant analysis revealed that the PIF-SAUR module impairs hypocotyl sensitivity to ABA in the dark. This hypocotyl-specific mechanism prioritizes cell elongation during the critical dark growth phase, essential for seedling establishment and survival.

RESULTS

Etiolated hypocotyls elongation is insensitive to exogenous ABA

The interplay between ABA and light signaling in shoots has been a subject of limited studies. With the aim of assessing the role of exogenous ABA application in hypocotyl elongation, we developed a system to avoid the inhibitory effect of ABA on seed germination (fig. S1). In this system, seedlings germinated on a nylon mesh, under dark or light conditions, were rapidly bulk transferred to a new plate after root and hypocotyl emergence. Using this strategy, wild-type (WT) seedlings that germinated in the dark or under white light (100 μmol m^{-2} s^{-1}) were transferred to plates containing 10 μM ABA or ethanol (mock) and maintained for 4 or 5 days, respectively, in the dark or under low light (10 μmol m^{-2} s^{-1}). Light-grown seedlings exhibit root and hypocotyl sensitivity to ABA, whereas, unexpectedly, in dark-grown seedlings, only the roots showed sensitivity to ABA, and the hypocotyls remained unaffected (Fig. 1A).

When a gradient of ABA concentrations was tested, ranging from 1 to 50 μM, root elongation was progressively impaired in plants growing under low light or dark conditions (Fig. 1B). Similarly, seedlings germinated under low light showed a progressive reduction in hypocotyl's length with increasing ABA concentrations, contrasting with the notable insensitivity of etiolated seedlings' hypocotyls to growth-inhibitory effect of ABA at concentrations up to 50 μM. We also detected that, in the dark, treatment with ABA concentrations of about 5 to 10 μM tends to induce a small increase in hypocotyl length, suggesting that, at low concentrations, ABA can have a very minor effect in increasing seedling elongation in the dark (Fig. 1B).

Often used to address drought stress responses, ABA, salt stress (NaCl), and osmotic stress inducers, such as polyethylene glycol (PEG), mannitol, or hard agar (HA), elicit both common and distinct transcriptional and physiological responses (*35*, *36*). To test the effect of these stressors on etiolated hypocotyl elongation, after

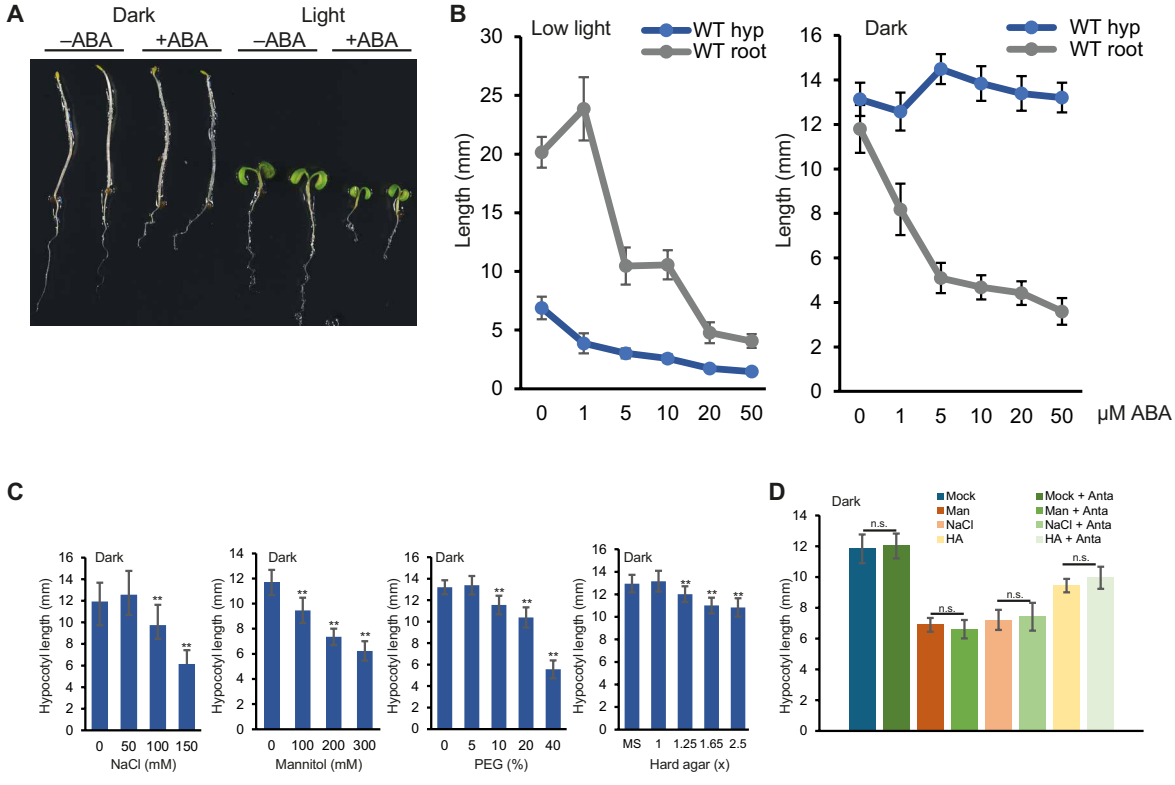

Fig. 1. ABA inhibits hypocotyl elongation in the light but not in the dark. (**A**) Representative images showing ABA-induced hypocotyl growth inhibition in light-grown seedlings but not in etiolated seedlings, with roots displaying sensitivity to ABA under both conditions. Seedlings were germinated in media without ABA for 62 hours (dark) or 72 hours (light) and then transferred to media with or without ABA (10 μM) for 4 days (dark-grown) or 5 days (light-grown) in low light (10 μm m^{-2} s^{-1}). (**B**) Hypocotyl and root length of seedlings transferred to up to 50 μM ABA. Roots are progressively responsive to increasing ABA concentrations, independently of light condition, whereas hypocotyl sensitivity to ABA is light dependent. Lines represent a dose-response with the average ($n > 20$), and error bars represent SD. (**C**) Etiolated Arabidopsis hypocotyls exhibit sensitivity to increasing concentrations of salt (NaCl), mannitol, PEG, and hard agar (HA). Hypocotyl length (mm) was measured in seedlings grown in the dark for 62 hours and then transferred to plates containing NaCl, mannitol, PEG, or HA for 4 days. Data are represented as mean ($n > 30$) ± SD. (**D**) Etiolated hypocotyls 4 days after transfer to 200 mM mannitol (man), 150 mM NaCl, or 2.5x HA with or without 100 μM antabactin (Anta). Data are represented as mean ($n > 25$) ± SD. Asterisks indicate significant differences from the respective controls (**$P < 0.01$), and n.s. means not significant in one-way analysis of variance (ANOVA) with Tukey post hoc test.

germination, seedlings were transferred to a medium containing increasing concentrations of NaCl (up to 150 mM), mannitol (up to 300 mM), PEG (up to 40%), or HA (up to 2.5 x). Despite transfer to mild salt (50 mM), mild PEG (5%), and mild HA (1x) conditions has little effect on hypocotyl elongation, the exposure to increasingly higher salt, mannitol, PEG, and HA, to a lesser extent, leads to a proportional reduction of hypocotyl elongation after 4 days in the dark (Fig. 1C). Thus, etiolated hypocotyl sensitivity to salt and high osmotic stress contrasts with the insensitivity to high ABA concentrations, suggesting that salt and high osmotic stresses might operate by an ABA-independent mechanism to control hypocotyl elongation in the dark. To further test this possibility, we used the ABA-receptor antagonist antabactin that blocks ABA responses at the receptor level (*37*). The combined use of 100 μM antabactin and salt, mannitol, or HA showed that antabactin did not prevent the growth-inhibitory effect of these stressors, supporting the idea that they act independently of ABA perception by PYR/PYL receptors and thus independently of a canonical ABA signaling (Fig. 1D).

To test whether ABA-insensitivity of etiolated hypocotyls occurs in other species, we further tested the responsiveness to ABA of etiolated hypocotyls of *Lactuca sativa* (lettuce) and *Cardamine hirsuta* (Cardamine), a Brassicaceae, phylogenetically close to Arabidopsis. In both species, hypocotyls from light-grown plants display higher sensitivity to ABA than those grown in the dark. However, etiolated lettuce hypocotyls are very sensitive to ABA whereas etiolated Cardamine hypocotyls show only a mild sensitivity (fig. S2). Overall, these experiments show that, in terms of elongation, the etiolated lettuce and Cardamine hypocotyls are less sensitive to ABA than the de-etiolated ones, suggesting that, in all cases, darkness clearly attenuates hypocotyl sensitivity to ABA. The level of insensitization in the dark is a variable feature across species, going from complete insensitization in Arabidopis to mild insensitization in lettuce (fig. S2).

Hypocotyl sensitivity to ABA requires an active light signaling pathway

Because light is essential for hypocotyl responsiveness to ABA, we tested the ABA effect on hypocotyl growth in mutants where a light signaling pathway is constitutively active by using *cop1-4* and the *quadruple pif* mutant *pifq* (*pif1 pif3 pif4 pif5*) (*8*, *38*). Under low light conditions, *pifq* and *cop1-4* plants are proportionally less responsive to ABA than WT plants, suggesting that PIFs and COP1 are positive regulators of ABA responsiveness in the light (Fig. 2, A and B).

In the dark, both *pifq* and *cop1-4* mutants showed reduced hypocotyl length when transferred to plates with 10 μM ABA in the dark (Fig. 2, A and B) contrasting to WT plants in which hypocotyl length remained unaffected by ABA, indicating that an active light signaling pathway, downstream COP1 and PIFs, is required for hypocotyl sensitivity to ABA. Notably, *pifq* or *cop1-4* neither accumulate chlorophyll nor perform photosynthesis in the dark (*38*, *39*), suggesting that photosynthetic capacity is not necessary for sensitivity to ABA. The removal of sucrose from the culture media rendered *pifq* and *cop1-4* mutants with shorter hypocotyls that also significantly respond to ABA, whereas WT hypocotyls do not (fig. S3), indicating that our observation of ABA insensitivity of WT dark-grown hypocotyls is valid independently of the presence of sucrose in the media.

Both GAs and brassinosteroids (BRs) are necessary for hypocotyl elongation in the dark (*40*–*42*). GA inhibits DELLA proteins that repress PIF function (*43*, *44*), and BRs stimulate hypocotyl elongation through BES1 and BZR1, which work as PIF counterparts, and by BIN2-dependent dephosphorylation and activation of PIF4 in the dark (*45*–*47*). Because GA, BRs, and ABA effect on hypocotyl depend on PIFs we treated seedlings grown in the dark with paclobutrazol (Paclo), an inhibitor of GA biosynthesis, and with brassinazole (BRZ) an inhibitor of BRs biosynthesis. Both treatments led to a reduction in hypocotyl elongation as expected, both in WT and *pifq* (Fig. 2C). When dark-grown seedlings were transferred to plates containing ABA and Paclo, or ABA and BRZ, the WT hypocotyl length was reduced by ABA compared to plant controls treated solely with Paclo or BRZ. Thus, impairment in GA and BR synthesis is sufficient to elicit ABA responsiveness in the dark, which might be due to a reduction of PIF activity. In *pifq* mutants, the hypocotyl sensitivity to ABA is still present but attenuated in plants treated with BZR or Paclo, suggesting that other factors besides PIF1,3-5 are involved in ABA responsiveness. These factors, regulated by BRs and or GAs, might be other PIF proteins or additional players still to be uncovered. Together, our data support the idea that PIFs are key proteins in hypocotyl responsiveness to ABA; in the dark, PIFs act as repressors of the ABA-mediated inhibition of hypocotyl elongation.

NPA renders light-grown seedlings' hypocotyls insensitive to ABA

It has been reported that auxin transport [blocked by *N*-1-naphthylphthalamic acid (NPA)] is required for hypocotyl elongation in the light but not in the dark (*48*). Given our observation of differential ABA effects on hypocotyl growth under light versus dark conditions, we investigated whether NPA treatment could alter the plant's responsiveness to ABA. Thus, we tested the responsiveness to ABA in light- and dark-grown Arabidopsis hypocotyls, in the presence or absence of 0,5 μM NPA. In the dark, NPA treatment did not affect WT or *pifq* hypocotyl elongation. Under these conditions, NPA did not affect the insensitivity of etiolated WT plants to ABA or *pifq* sensitivity to ABA, suggesting that NPA-inhibited auxin transport does not mediate hypocotyls sensitivity to ABA in the dark, even in the absence of PIFs (Fig. 2D, left panel).

In light-grown seedlings, the presence of NPA resulted in shorter hypocotyls and suppressed hypocotyl sensitivity to ABA, either in WT plants or in *pifq* mutants (Fig. 2D, right panel). These results show that ABA inhibition of hypocotyl elongation in the light is dependent on a functional auxin transport, which impairment abrogates responsiveness to ABA, and that works downstream of PIFs. Thus, ABA-dependent inhibition of hypocotyl elongation in the light and in mutant plants de-etiolated in the dark operates by different mechanisms, suggesting that ABA regulation of auxin transport is elicited upon light perception.

Mutants partially blind to light display hypocotyl hypersensitivity to ABA

As darkness confers hypocotyl insensitivity to ABA, we then tested whether photoreceptor mutants that display partial blindness to specific light qualities *phyA* (far-red), *phyB* (red), and *cry1*, *cry2*, *cry1cry2* (blue) show some degree of insensitivity to ABA. We found that, upon growing in 10 μM ABA for 6 days, *phyA* and *cry2* mutants displayed a reduction of hypocotyl elongation similar to that of WT (Fig. 3A). Unexpectedly, elongated hypocotyl mutants such as *phyB*, *cry1*, *cry1cry2*, and *hy5* display a reduction by more than half of hypocotyl elongation when treated with ABA (Fig. 3B).

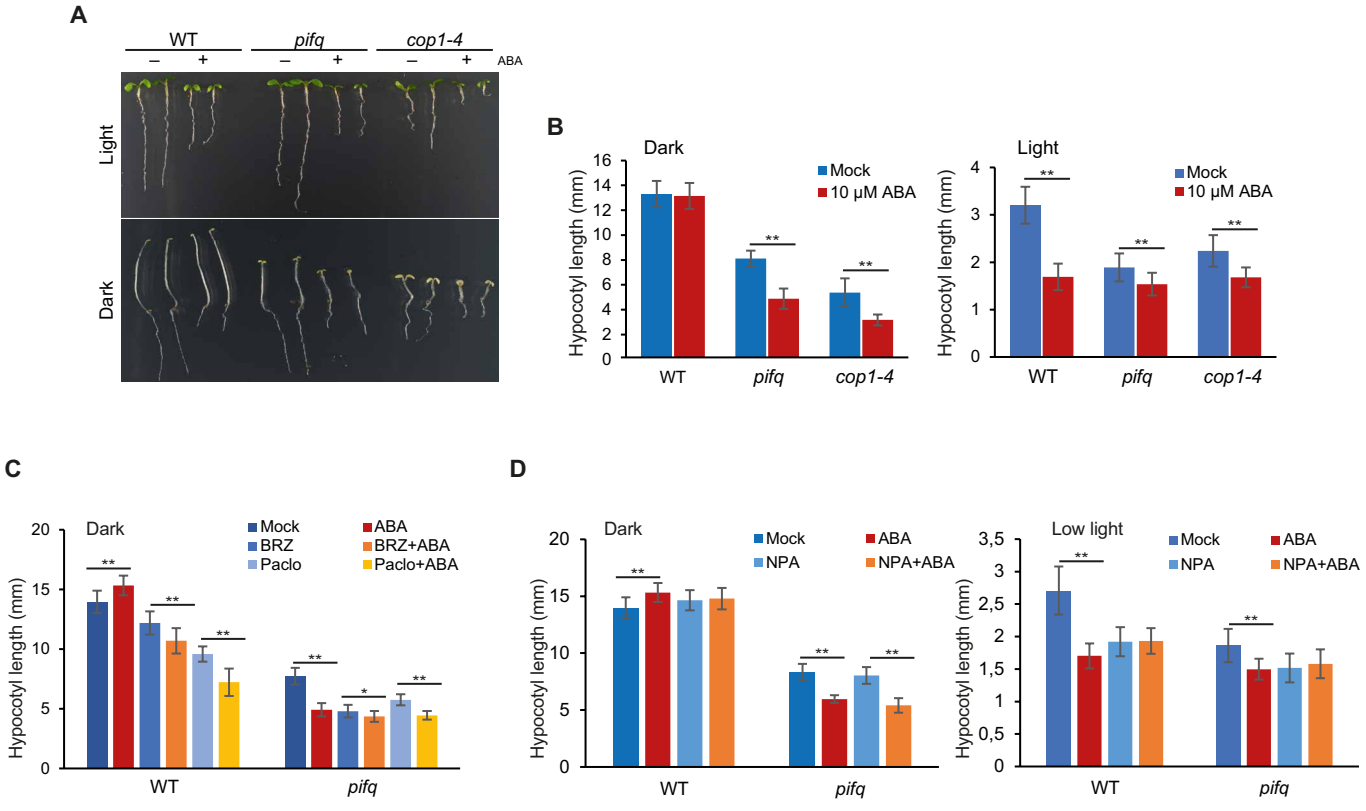

Fig. 2. An active light signaling pathway is necessary for hypocotyls responsiveness to ABA. (**A**) Representative image showing the inhibition of hypocotyl elongation in de-etiolated *pifq* and *cop1-4* mutants grown in the dark or light and with or without ABA treatment. (**B**) Hypocotyl length measurements corresponding to the experiments shown in (A). Seedlings were germinated in media without ABA for 62 hours (dark) or 72 hours (light) and transferred to media containing 10 μM ABA or mock for 4 days (dark-grown) or 5 days (light-grown). (**C**) Hypocotyl length of WT and *pifq* seedlings treated as described in (B) and transferred to a medium supplied with mock, BZR, or Paclo, with or without ABA (10 μM). (**D**) ABA repression of hypocotyl elongation in the light requires auxin transport. Hypocotyl length of WT and *pifq* seedlings grown under dark or low light conditions. Seedlings were germinated in media without ABA for 62 hours (dark) or 72 hours (light) and transferred to media supplemented with ABA (10 μM) or NPA (0.5 μM) for 4 days (dark) or 5 days (light). Data are presented as mean ($n > 20$) ± SD, with asterisks indicating significant differences from the mock control (*$P < 0.05$; **$P < 0.01$) in one-way ANOVA with Tukey post hoc test.

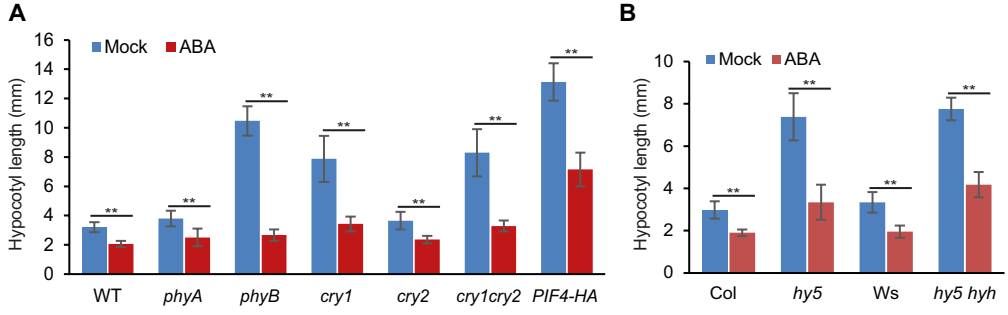

Fig. 3. Elongated hypocotyl mutants display sensitivity to ABA. (**A**) Hypocotyl length of light-grown WT, photoreceptor mutants (*phyA*, *phyB*, *cry1*, *cry2*, and *cry1cry2*), and *35S:PIF4-HA* line treated with ABA or mock. (**B**) Hypocotyl length of *hy5* and *hy5 hyh* mutants and their respective control backgrounds Columbia (Col) or Wassilewskija (Ws) when treated with ABA. Seedlings were germinated in media without ABA for 72 hours and transferred to media with or without ABA (10 μM) for 4 days. Columns represent average ($n > 20$), and error bars represent SD. Asterisks represent significantly different samples (**$P < 0.01$) regarding the mock control in one-way ANOVA with Tukey post hoc test.

Thus, partial blindness to light does not decrease hypocotyl responsivity to ABA but, on the contrary, results in ABA hypersensitivity, suggesting that PhyB and cryptochromes repress hypocotyl sensitivity to ABA in the light. In addition, the *35S:PIF4-HA* line, which displays very long hypocotyls by ectopically accumulating high levels of PIF4, displays ABA responsiveness in the light similar to that of WT (Fig. 3A). Because, in the *phyB* mutant, PIFs are stabilized and more active, it is notable that the PIF4 overexpression line is less sensitive to ABA than *phyB*. This relative attenuation of ABA sensitivity might be due to the ectopic overaccumulation of PIF4 that is not, however, sufficient to mimic in the light the etiolated hypocotyls insensitivity to ABA. However, we cannot rule out that

phyB hypersensitivity to ABA is due to a regulatory mechanism independent from PIFs.

Overall, these results suggest that, once light perception occurs, additional molecular pathways are enabled, allowing the inhibition of hypocotyl growth by ABA through a mechanism that does not occur in the dark. As the NPA experiments indicate, this mechanism depends on auxin transport and occurs downstream of PIFs.

ABA-mediated transcriptional regulation occurs in etiolated hypocotyls

Light regulates the expression of ABA biosynthesis and signaling genes (*49*, *50*). To test whether, in the dark, a lack of expression of ABA positive upstream signaling components, as receptors and *SnRKs*, could explain ABA insensitivity, we tested by quantitative reverse transcription polymerase chain reaction (qRT-PCR) the expression of 10 *PYR/PYL* genes (*PYR1* and *PYL1* to *PYL9*) and *SnRKs2.2* and *2.3* in a transition from dark to light conditions up to 48 hours. For most of the *PYL* genes tested (except *PYL7* and *PYL8*), transcripts accumulated gradually with light exposure, with *PYL2* and *PYL6* being induced 10-fold after 48 hours, contrasting with the transcript accumulation of *SnRK2.2* and *SnRK2.3* that showed a slight reduction after 48 hours of light exposure (fig. S4). These data suggest that full expression of the ABA receptors is achieved under light (perhaps due to the onset of tissue specialization) but can hardly justify hypocotyl insensitivity in the dark.

To test whether an active ABA signaling pathway is operating in the dark in WT and *pifq* mutants in a similar way as it is operating in the light, we analyzed by qRT-PCR the accumulation of transcripts from ABA-responsive genes. Transcripts for *ABI1*, *ABI2*, and *RD29A* were induced in plants transferred to ABA either under dark or light conditions (Fig. 4A). This induction was also observed in *cop1-4* and *pifq* mutants independently of the presence of light (Fig. 4A), suggesting that, once seedlings are treated with ABA, signal transduction is activated in the dark and in the light. This signal transduction does not depend on light or the presence of PIF1,3,4,5 or COP1 proteins.

We further tested whether this ABA-induced activation of genes also occurs in the hypocotyl cells in which ABA does inhibit expansion in the dark. For this, we obtained a cotyledon-enriched fraction (cot), a hypocotyl-containing fraction (hyp), and a root-enriched fraction (root) from WT and *pifq* plants grown in the dark, being especially careful to keep hypocotyl fraction free from root or cotyledon tissue. In these plant materials, the accumulation of *ABI1* and *RD29B* transcripts was fully induced by ABA, including the WT etiolated hypocotyls that does not stop elongating in response to ABA (Fig. 4B). Considering that hypocotyls of WT and *pifq* display similar ABA-induced gene expression but different cell elongation responses, we conclude that hypocotyl growth inhibition by ABA and induction of ABA-responsive genes are regulated by divergent molecular paths in the dark.

ABA-sensitivity in *pifq* depends on a canonical ABA signaling

To test whether the same canonical ABA response pathway known to trigger the ABA transcriptional response also controls hypocotyl

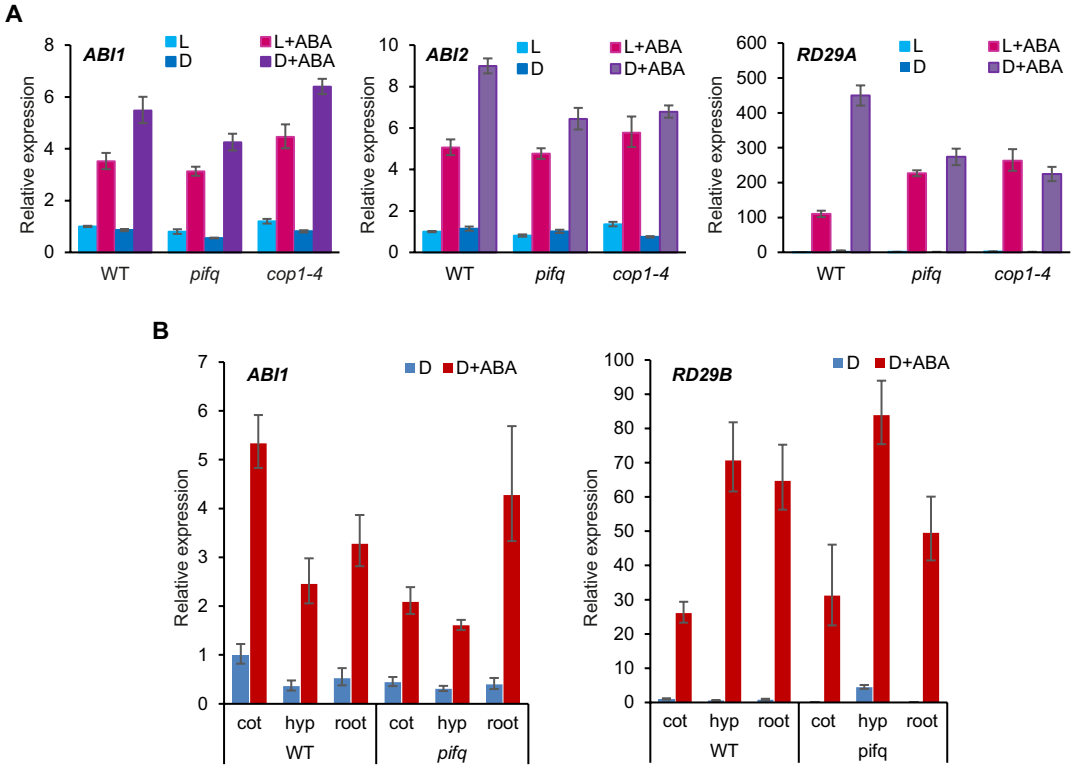

Fig. 4. Canonical ABA-responsive genes are induced by ABA in the dark as in the light. (**A**) Relative expression of ABA-responsive genes obtained by qRT-PCR. *ABI1*, *ABI2*, and *RD29A* were induced by ABA treatment either under dark or light conditions in WT and *pifq* or *cop1-4* mutants. (**B**) qRT-PCR analysis of gene expression in different tissue samples: cotyledon enriched (cot), hypocotyl (hyp), and root enriched (root). *ABI1* and *RD29B* expression is induced by ABA in all the tissues sampled. The data represent the average of four replicates, with error bars indicating the SD.

elongation in photomorphogenic seedlings, we used mutants in positive regulators in different steps in the ABA signaling pathway. We treated the *pyl112458* sextuple mutant (*6xpyl*), *snrk2.2/2.3*, and *arebQ* (impaired in *ABF1*, *ABF3*, *AREB1*/*ABF2*, and *AREB2*/*ABF4*) with 10 μM ABA. The *6xpyl* mutant showed slight hypocotyl insensitivity to ABA, whereas *snrk2.2/2.3* and *arebQ* behave as WT (Fig. 5A). Given the strong functional redundancy across the ABA signaling pathway, especially at the receptor level, these results suggest that PYL receptors are involved in hypocotyl responsiveness to ABA in the light and that the sensitivity to ABA in the *6xpyl* mutant might be due to the activity of the remaining PYL proteins. In the case of the SnRKs and ABF/AREB, it might be that other SnRKs and other TFs might play major roles in hypocotyl responsiveness to ABA. It can also be that the inhibition of hypocotyl elongation by ABA relies in a transcription-independent pathway. In agreement with these results and accounting for a canonical ABA signaling being involved in hypocotyl responsiveness, the quadruple *pp2c* mutants *Qhai1-1* and *Qabi2-2* (*51*), which lack four of the type A PP2C proteins, display hypersensitivity to ABA (Fig. 5B). These mutants display longer hypocotyls in the light (Fig. 5B) and larger roots (*52*), a phenomenon that, in roots, was explained by endogenous ABA capacity to repress the activity of PM H^+-ATPase (*52*).

We further tested hypocotyl sensitivity of *6xpyl* and *arebQ* mutants to ABA in the dark where no changes regarding the WT were detected (Fig. 5C). Unexpectedly, the etiolated *Qhai1-1* and *Qabi2-2* mutants are sensitive to ABA (Fig. 5D). These results agree with the ABA hypersensitive phenotypes previously described (*51*) and suggest a subjacent activity for these PP2C proteins in the ABA-mediated regulation of hypocotyl elongation. However, the molecular path behind this phenotype is still unclear and requires further research.

Last, we tested whether *pifq* responsiveness to ABA, in the dark, depends on a canonical ABA perception by using antabactin to block ABA perception at the receptor level. In a dose-dependent manner, antabactin could overcome *pifq* sensitivity to ABA at concentrations between 50 and 100 μM, showing that *pifq* sensitivity in the dark is fully dependent on the PYL/PYR receptors (Fig. 5E). Together, our data show that, in the dark, a path for hypocotyl repression depending on PYL receptors and on PP2C clade A phosphatases is functional and that this path is being repressed by active PIFs.

Transcriptional changes associated with hypocotyl elongation responsiveness to ABA

Because we identified that an active ABA signaling pathway in the dark triggers the induction of ABA-responsive genes but is not inhibiting hypocotyl elongation, we performed an RNA sequencing (RNA-seq) analysis to compare the transcriptome response to ABA in hypocotyl responsive seedlings WT in low light (WT L) and *pifq* in the dark (*pifq* D) and in unresponsive seedlings [WT in the dark (WT D); fig. S5A].

We first selected the genes being up-regulated or down-regulated by twofold by ABA [Log_2 fold change (Log_2FC) > 1 and false discovery rate (FDR) < 0.01] in WT and *pifq* plants grown under dark or light conditions. Because the hypocotyls of WT dark-grown seedlings are insensitive to ABA, and ABA-induced stress responses are active in hypocotyls as in roots, independently of light conditions, we considered all the differentially expressed genes (DEGs) in WT dark-grown plants treated with ABA to be unrelated to hypocotyl elongation. Thus, to identify ABA-responsive genes responsible for the reduction in hypocotyl growth under an active light signaling pathway, these genes were subtracted from the ABA-induced and ABA-repressed genes found in *pifq* D, *pifq* L, and WT L. To ensure that the resulting lists of genes of interest were thoroughly flushed of all the ABA-responsive genes in WT D, we applied a less stringent threshold of $|Log_2FC| > 0$ to WT D DEGs (Fig. 6A). This unveiled that several hundreds of genes respond to ABA specifically in the context of an activated light signaling pathway (i.e., in WT L and *pifq* D or L). Specifically, we found 384 and 709 genes induced and repressed, respectively, by ABA in WT L but not in WT D, and 402 and 668 genes induced and repressed, respectively, by ABA in *pifq* D but not in WT D (Fig. 6B).

Next, we compared those sets of ABA-regulated genes specifically in WT L or in *pifq* D but not in WT D. This showed that most of these genes were specific to WT L *or pifq* D as only 138 were common between the 384 and 402 ABA up-regulated genes and 311 of the 709 and 668 ABA down-regulated genes (Fig. 6B and data S1). Accordingly, genes were further separated in three subgroups for up-regulated and for down-regulated genes: "*WT Light only*" (246 and 398 genes, respectively), "*pifq Dark only*" (264 and 357 genes, respectively), and "*WT Light & pifq Dark*" (138 and 311 genes, respectively). The core genes that mediate hypocotyl responsiveness whenever a light signaling pathway is activated fall in the "*WT Light & pifq Dark*" category. The heatmap representation of Log_2FC of expression from the different pairwise RNA-seq comparisons within the six gene categories defined in Fig. 6B shows the efficiency of the gene selection process with genes regulated in "WT light only" categories showing no response in WT D or *pifq* D and genes in the "*pifq* dark only" categories showing no response in WT D or L (Fig. 6C). The response in *pifq* L largely follows the response in WT L, in agreement with the low levels of PIFs in this condition due to their degradation in the light.

To gain insight into biological processes involved in the differential responses to ABA, we analyzed the Gene Ontology (GO) term enrichment of the same six categories of genes showing a response in WT L and *pifq* L but not WT D. For down-regulated genes, GO terms related to cell wall organization and biogenesis, response to auxin, and developmental processes were significantly enriched for all the three ABA down-regulated classes. The GO terms cell division, cell cycle, and carbohydrate metabolic process are enriched in the "*WT light & pifq Dark*" category, suggesting that these processes are regulated in both photomorphogenic contexts. The GO terms response to light is enriched in the classes "*WT light & pifq Dark*" and "*pifq Dark only*," supporting a cross-talk between PIF-dependent light signaling and ABA response (Fig. 6D). For up-regulated genes, GO terms that are enriched in "*WT Light & pifq Dark*" are responsive to ABA, secondary metabolism, suberin biosynthesis (connecting ABA with the development of secondary cell wall), and flavonoid biosynthesis. Together, this analysis shows that many genes involved in stress, secondary metabolism, cell wall remodeling, cell division, and response to auxin are regulated by ABA in the light or photomorphogenic seedlings but not in etiolated dark-grown seedlings, suggesting that these classes are important for ABA hypocotyl elongation responsiveness in the light versus dark.

ABA DEGs related to cell elongation

It is known that hypocotyl elongation and its inhibition by ABA depend on the activity of PM-associated proteins (*33*, *53*). Therefore, we looked among our datasets for genes that have been previously linked to hypocotyl elongation either by acting directly at the level

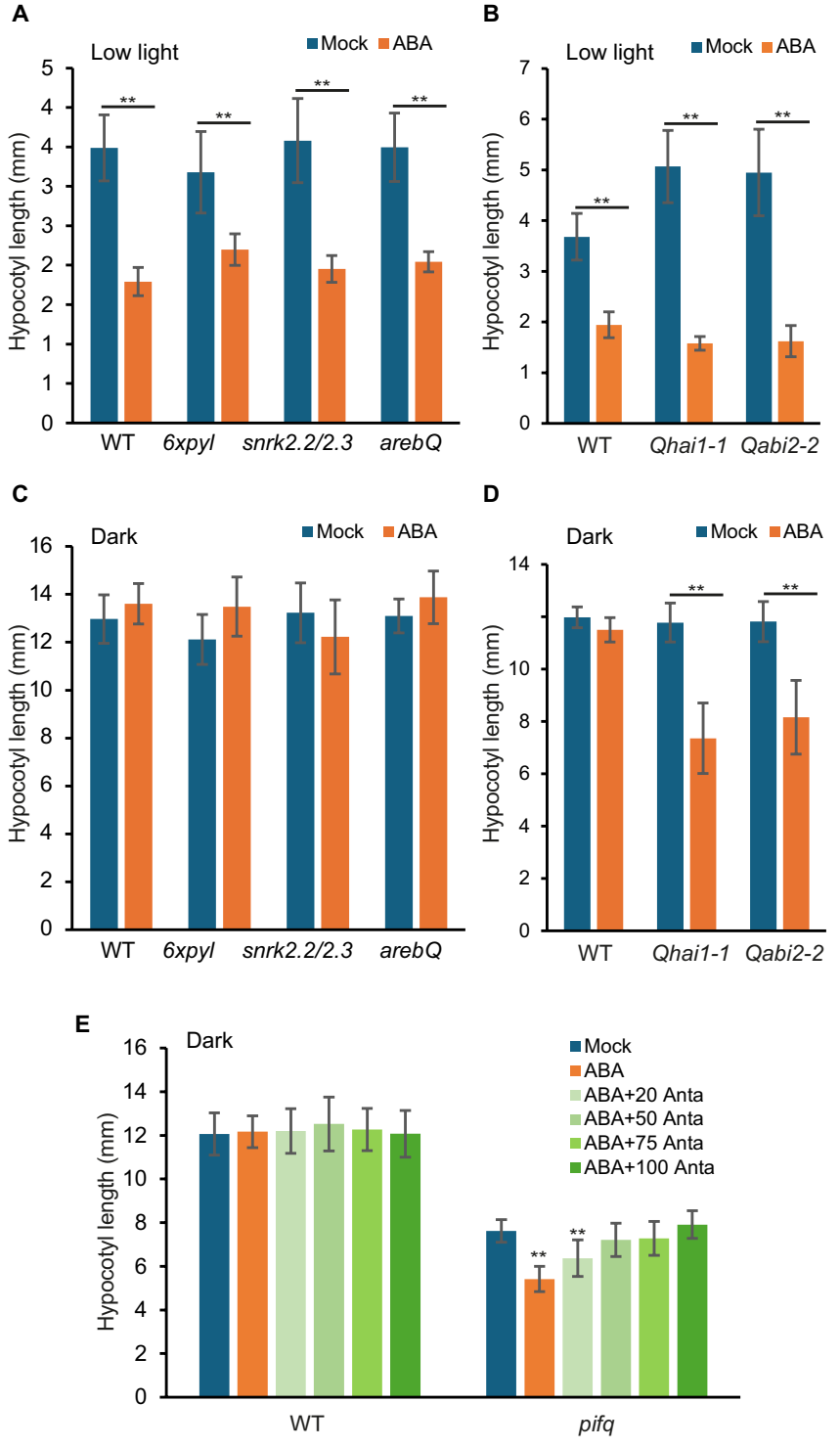

Fig. 5. ABA-responsive genes are induced by ABA in the dark as in the light. (**A** to **D**) Hypocotyl length of WTs and mutants impaired in ABA signaling grown in low light. Seedlings were germinated in low light for 72 hours (A) or dark for 62 hours (C) and transferred to media with or without ABA (10 μM) for 5 days. For *Qhai1-1* mutant and *Qabi2-2* mutants, with delayed germination, seedlings were transferred to ABA at 96 hours in the light (B) and at 86 hours in the dark (D). (**E**) Hypocotyl length (mm) of WTs and *pifq* treated with mock or ABA in the presence or not of increasing concentrations (20, 50, 75, or 100 μM) of antabactin, an antagonist of ABA at the receptor level. Seedlings were germinated in low light for 72 hours and transferred to media with or without ABA (10 μM) for 5 days. The data represent the average ($n > 26$), with error bars indicating the SD. Asterisks represent significantly different samples (**$P < 0.01$) regarding the mock control in one-way ANOVA with Tukey post hoc test.

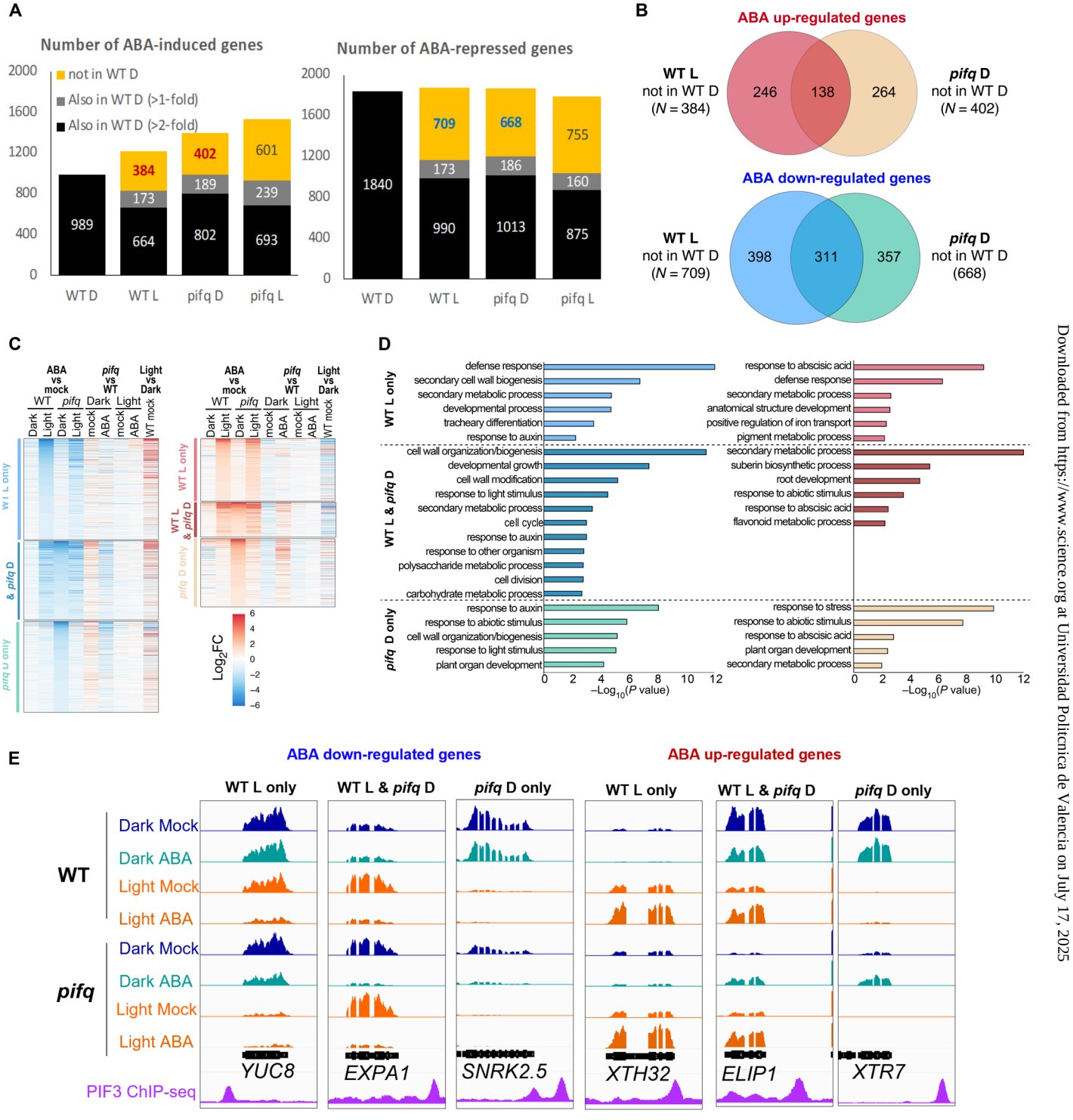

Fig. 6. Effect of light and *pifq* mutant background on ABA-induced changes in gene expression. (**A**) Number of genes up-regulated or down-regulated by ABA in WT and *pifq* seedlings grown in the dark (D) or light (L) (FDR < 0.01 and $Log_2FC > 1$). The number of overlapping genes with WT D is displayed within the WT L, *pifq* D, and *pifq* L categories, allowing us to select the set of genes specifically down-regulated or up-regulated in that categories but not in WT D. (**B**) Comparisons of the selected genes according to (A) between WT L and *pifq* D. (**C**) Heatmap representing the Log_2FC changes in expression in the different pairwise comparisons on the six gene categories from (B). (**D**) GO analysis on the six gene categories from (B). (**E**) Genome browser view of the RNA-seq data on selected example gene from the six gene categories from (B) (average of three biological replicates). The profile of PIF3 in WT D seedlings and HY5 in WT L seedlings and the position of G-box motifs have been retrieved from (*69*).

of the PM, on the regulation of transporters or channels, that can be connected to the activity of PM H^+-ATPase (the final driver of cell elongation), or are involved in auxin synthesis, transport, or metabolism, thus having final repercussion on cell elongation. In addition, we considered enzymes involved in cell wall modifications as they might work as a consequence and as drivers of cell elongation (*54*).

SAUR gene expression is known to be regulated by PIFs and auxins, and a number of them have been associated with hypocotyl elongation (*9*, *13*, *14*, *55*, *56*). We found eight *SAUR* genes (*SAURs 9*, *10*, *11*, *16*, *17*, *34*, *51*, and *63*) to be down-regulated by ABA specifically in *pifq* D, six *SAUR* genes (*SAURs 1*, *3*, *5*, *12*, *28*, and *62*) being repressed by ABA in *pifq* D and WT L, and nine *SAUR* genes (*SAURs 20*, *21*, *22*, *25*, *26*, *29*, *54*, *56*, and *68*) being down-regulated by ABA exclusively in WT L. In addition, five *SAURs* (*SAURs 32*, *35*, *59*, *71*, and *72*) are significantly induced by ABA only in WT L. In total, 28 members of a family of 81 members (*57*) are DEGs (mostly down-regulated) upon ABA treatment in the light or *pifq* in the dark but not in the WT D (fig. S5C and data S1), correlating with the hypocotyl inhibition caused by ABA. Thus, SAURs might play an important role in ABA-mediated inhibition of hypocotyl elongation.

Other genes, related to auxin-driven hypocotyl elongation, were among all the three categories of ABA down-regulated genes: *IAA5* being down-regulated in "*pifq Dark only*," *IAA6/SHY1* being down-regulated by ABA in "*WT L and pifq D*," and *IAA19*, together with genes involved in auxin biosynthesis as *TAA1*, *YUC3*, *YUC8*, and *YUC9* being down-regulated by ABA in "*WT L only*," suggesting that ABA down-regulates auxin signaling and biosynthesis under light.

We also found a number of ABC transporters to be down-regulated by ABA. *ABCB19*, a protein involved in auxin transport, that has been recently shown to be a BR transporter (*58*) is among the ABA down-regulated genes specifically regulated by ABA in *pifq* D (fig. S5C and data S1). In addition, three ABC-2 type transporters (*ABCG4*, *ABCG42*, and *ABCG8*) are down-regulated in "*WT Light & pifq Dark*" and seven ABC transporters (*ABCB3*, *ABCB4*, *ABCB14*, *ABCC9*, *ABCG13*, *ABCG18*, and *ABCG6*) are specifically down-regulated by ABA in "*WT Light*." Of these, ABCB4 and ABCB14 are involved in auxin transport (*59*–*61*).

Among the DEGs that regulate processes at the PM level, we spotted a high number of ion exchangers, solute transporters, or channels (data S1) that, by regulating ion homeostasis, can directly or indirectly contribute to the control of cell expansion. In particular, we identified the down-regulation of *KAT1* and *KAT2* in the PIF-dependent categories ("*pifq D only*" and "*WT Light & pifq Dark*," respectively) as genes for which ABA regulation in the darkness is blocked by PIFs (fig. S4C and data S1). Both genes have been described as inward rectifying channels to compensate for the PM H^+-ATPase activity (*62*–*65*). In addition, the gene encoding AKT2, a photosynthate- and light-dependent inward rectifying potassium channel, gated by Ca^{2+} that can coassemble with KAT1 (*66*) is up-regulated by ABA (fig. S5C and data S1).

Among the genes associated with cell elongation, we found several, belonging to cell wall remodeling families to be DEGs in response to ABA. Among the down-regulated, we found seven expansins (*EXPA1*, *EXPA5*, *EXPA6*, *EXPA8*, *EXPA10*, *EXPA15*, and *EXPB1*), extensins (*EXT23* and *EXT33*), XTHs (*XTH4*, *XTH8*, and *XTH19*), and 18 genes involved in pectin modifications. Among the up-regulated, *EXPA7*, *EXPA18*, *EXT7*, eight XTHs (*XTH9*, *XTH13*, *XTH14*, *XTH16*, *XTH15/XTR7*, *XTH22*, *XTH23*, and *XTH32*), and eight genes involved in pectin modifications were found (fig. S5C and data S1). These DEGs highlight the tremendous changes triggered by ABA at the level of cell wall reorganization that are essential to change the velocity of cell expansion.

Because our RNA-seq was performed with whole-seedling and the ABA DEGs related to hypocotyl elongation were obtained by subtractive analysis, we compared the ABA up-regulated and down-regulated genes "not in WT D" (Fig. 6B) with published transcriptomic data obtained specifically from hypocotyl tissues in response to light, either in dark-to-light transitions (*9*, *67*), or in response to shade (*68*). This analysis revealed that 44% of ABA down-regulated genes in photomorphogenic plants are down-regulated by light and/or up-regulated by shade [467/1066; representation factor (RF) 10.3 $P < 1.854 \times 10^{-127}$] against 25% up by light and/or down by shade (250/1066; RF 1 $P < 0.264$, as expected randomly). Twenty-three percent of ABA up-regulated genes in photomorphogenic plants are down by light and/or up by shade (172/648; RF 1, $P < 0.435$, as expected randomly) against 30% up by light and/or down by shade (190/648; RF 1.2, $P < 0.002$, as expected randomly) (fig. S6 and data S2). Thus, the ABA down-regulated genes "not in WT D" are highly enriched in genes down-regulated by light in hypocotyls in dark-to-light transitions or either up-regulated in shaded light, a suboptimal light condition where hypocotyls elongate. To further confirm the deregulation of a subset of these genes in hypocotyls in response to ABA, we analyzed by qRT-PCR the accumulation of transcripts in response to ABA in WT, *pifq*, and *cop1-4* hypocotyls of dark-grown plants. For all the tested genes, there is a significant down-regulation in hypocotyls in response to ABA in *pifq* and *cop1-4* but not in WT (fig. S7), confirming that the down-regulation of these genes by ABA occurs in hypocotyls and only in de-etiolated/photomorphogenic seedlings.

The expression of direct PIF3 and PIF4 targets is regulated by ABA

To further inspect the cross-talk between light and ABA signaling, we further searched for the presence of direct PIF3 (*69*) or PIF4 targets (*56*) among the ABA DEGs. We identified 74 direct PIF3 targets and 243 PIF4 targets among the ABA down-regulated genes and 50 PIF3 targets and 144 PIF4 targets among the ABA up-regulated genes. Among the total number of DEGs, PIF3 and PIF4 targets are homogeneously distributed across the six categories and are significantly enriched regarding the expectancy for the total genome (fig. S5B). Among the direct PIF4 targets, several genes related to auxin synthesis, and signaling, as *YUC8*, *TAA1*, *IAA19*, *IAA29*, *AXR3*, and several *SAURs*. The *KAT1* channel, *ABA-INSENSITIVE PROTEIN KINASE 1* (*AIK1*), or the *RECEPTOR-LIKE PROTEIN KINASE 1* (*RPK1*) are among other target genes previously associated with ABA responses (fig. S5C). Among the direct PIF3 targets are several *SAURs*, *IAA19*, *YUC8 EXPA1*, *SnRK2.5*, and several light and cell wall–related genes (Fig. 6E and data S1).

In the dark, SAUR repression of PP2C.D2 render plants insensitive to ABA

Because SAUR overexpression (i.e., *SAUR19* or *SAUR63*) is sufficient to promote hypocotyl elongation (*13*, *14*) and the SAUR family is highly represented among of the ABA down-regulated genes that are up-regulated by PIFs, we used multiple *saur* CRISPR-Cas9 mutants (*70*) and overexpression lines to investigate the role of SAUR proteins in ABA responses. The multiple *saur* mutants *saur19/21/23/24*, *saur19/20/21/22/23/24* (*saur19-24*), *saur61/62/63/64/65/66/67/68/75* (*saur61-68/75*), *saur19/20/21/22/23/24/61/62/63/64/65/66/67/68/75*

(*saur19-24/61-68/75*), *saur19/20/21/22/23/24/26/27/29/61/62/63/64/65/66/67/68/73/75* (*saur19-24/26/27/29/61-68/73/75*), and *saur9/16/19/20/21/22/23/24/26/27/29/61/62/63/64/65/66/67/68/73/75* (*saur9-16/19-24/26/27/29/61-68/73/75*), all behave similar to WT, responding to ABA in the light and failing to respond in the dark (Fig. 7, A and B), maybe due to the strong redundancy among the large SAUR family.

However, *SAUR19* overexpression (*SAUR19OX*) line show, in the light, a reduced sensitivity to ABA compared to WT, suggesting a role for SAURs in hypocotyl responsiveness to ABA (Fig. 7B). Because SAUR proteins directly interact and inhibit the PP2C.D phosphatases proteins, which dephosphorylate the PM H^+-ATPase thereby inhibiting cell elongation, we tested the relevance of this relationship in the sensitivity to ABA. The triple *pp2c.d2/5/6* [*pp2c.d2/pp2c.d5/pp2c.d6*; (*71*)] mutant displays elongated hypocotyls in the light compatible with a less repressed and thus more active ATPase. It showed no ABA sensitivity under dark conditions but was, however, strongly responsive to ABA in the light, compatible with the fact that the SAUR pathway is not the only one used by ABA in the light (Fig. 7, A and B). Last, to overcome SAUR redundancy and block the action of SAUR over PP2C.D, we used the *pp2c.d2*/PP2C.D2^{M331K}-GFP line, where a mutated version of PP2C.D2, unable to bind SAURs, is expressed, being constitutively repressing the PM H^+-ATPase (*72*). In the dark, despite displaying short hypocotyls, these seedlings are sensitive to ABA (Fig. 7, C and D), suggesting that blocking SAUR inhibitory effect on PP2C.D2 is sufficient for hypocotyl sensitivity to ABA in etiolated plants. Thus, by constantly dephosphorylating PM H^+-ATPase, PP2C.D2 elicits ABA responsiveness in etiolated hypocotyls. To further test hypocotyl responsiveness to ABA under conditions of altered PM H^+-ATPase function, we measured hypocotyl responsiveness of *aha2-4* and *ost2-2D* mutants (AHA2 loss and AHA1 gain of function mutants, respectively). Whereas, in the light, *ost2-2D* displays long hypocotyls and is hypersensitive to ABA, in the dark, we found no differences compared to the WT in etiolated plants (fig. S8, A and B). To overcome the strong functional redundancy among PM H^+-ATPase, we tested benzyl isothiocyanate (BITC), a Brassicales-specific metabolite and potent inhibitor of PM H^+-ATPase phosphorylation (*73*). At 50 μM, BITC reduces hypocotyl

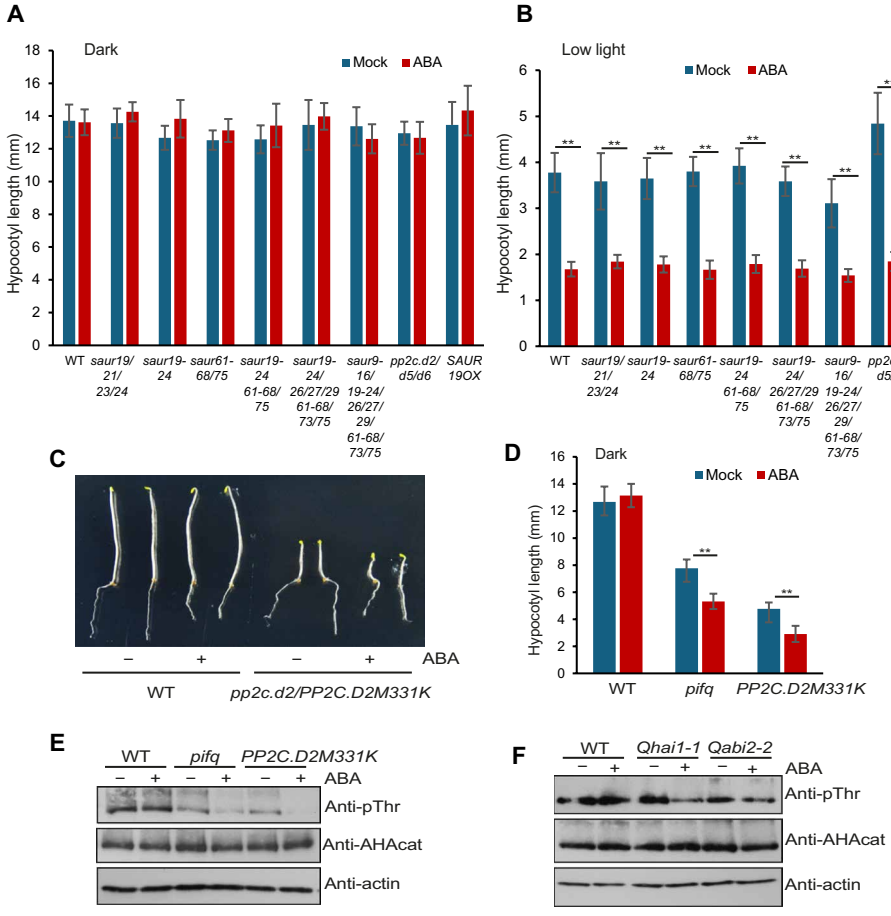

Fig. 7. Insensitivity to SAURs renders hypocotyl elongation sensitive to ABA in the dark. (**A** and **B**) Hypocotyl length of *saur* and *pp2c.d* multiple mutants treated with ABA or mock. Seedlings were germinated in media without ABA for 62 hours (dark) (A) or 72 hours (light) (B) and transferred to media supplemented with ABA (10 μM) or mock for 4 days (dark) or 5 days (light). Columns represent average ($n > 25$), and error bars represent SD. (**C**) The *pp2c.d2/PP2C.D2M331K-GFP* seedlings in which the mutated PP2C.D2 version is impaired in SAUR binding renders dark-grown plants sensitive to ABA. (**D**) Hypocotyl length of WT, *pifq*, and *pp2c.d2/PP2C.D2M331K-GFP* seedlings treated as mentioned above. Columns represent average ($n > 30$), and error bars represent SD. Asterisks represent significantly different samples (**$P < 0.01$) regarding the mock control in one-way ANOVA with Tukey post hoc test. (**E**) Immunoblot using WT, *pifq*, or *pp2c.d2/PP2C.D2M331K-GFP* hypocotyls from plants transferred to ABA or mock in the dark. (**F**) Immunoblot of WT, *Qhai1-1*, and *Qabi2-2* hypocotyls transferred to ABA or mock in the dark. Anti-phosphorylated penultimate Thr (anti-pThr), anti-AHAcat, for total ATPase levels, and anti-actin for loading control were used.

elongation in WT etiolated seedlings, an effect that is enhanced by the application of 5 and 10 μM ABA, suggesting that, in the dark, a dephosphorylated PM H^+-ATPase is responsive to ABA, mimicking the results obtained with the *pp2c.d2*/PP2C.D2^{M331K}-GFP line (fig. S8).

The analysis of the PM H^+-ATPase AHA2 phosphorylation levels in dark-grown *pifq* and *pp2c.d2*/*PP2C.D2^{M331K}-GFP* in response to ABA showed reduced levels of Thr^{947} (the pThr in AHA2) phosphorylation upon ABA treatment, whereas no changes were detected in WT plants (Fig. 7E). These results are compatible with ABA leading to an inhibition of AHA2 due to a reduction of pThr phosphorylation, a mechanism repressed by the PIF-SAUR module that actively operates in etiolated hypocotyls in the dark. In agreement with this idea, we further detected lower AHA phosphorylation levels in the *Qhai1-1* and *Qabi2-2* etiolated plants treated with ABA, suggesting that, in the dark, PP2C type A phosphatases, central repressors of ABA signaling, might be important for the regulation of PM H^+-ATPase phosphorylation levels in response to ABA (Fig. 7F).

DISCUSSION

In nature, for many seed plants, hypocotyl elongation, at early stages of seed development, allows the emergence from soil and the onset of the photoautotrophic growth. Being driven by cell elongation, hypocotyl growth has been used as a fast-responsive and hormone-sensitive system to study molecular interactions subjacent to cell elongation. The output of hypocotyl elongation rates is the result of the integration of exogenous stimuli into central pathways that directly control growth and that allow the adaptation to changing environmental conditions.

Regarded as an abiotic stress signaling hormone, ABA's effect on growth stunting is well known. However, the number of studies on ABA effect on controlling hypocotyl elongation is still limited (*32*–*34*). Here, we report that exogenous ABA inhibits hypocotyl elongation in a light-dependent manner. Despite ABA effectively reducing hypocotyl elongation in the light, in etiolated seedlings, PIFs drive SAUR expression and counteract the inhibitory effect of ABA. This mechanism of organ-specific insensitization to ABA favors skotomorphogenic growth as a mechanism to maximize the chances of reaching light, even under stressful conditions, and thus autotrophic establishment.

We report that hypocotyls of light-grown plants are sensitive to ABA, whereas dark-grown etiolated plants display hypocotyl growth insensitivity to ABA. This behavior is different from that of roots, whose growth is inhibited by ABA independently of the presence of light. As ABA concentration can affect plant development and growth in different ways, with low ABA concentrations promoting growth and high ABA repressing it (*74*, *75*), we tested whether high concentrations of ABA can inhibit hypocotyl growth, a phenotype that we could not observe even at the high 50 μM ABA concentration. Our results differ from those obtained by other groups, which could detect ABA inhibition of etiolated hypocotyls (*32*–*34*). This might be due to the different experimental setups developed, including the fact that, in these previous works, plants were manipulated under dim red light (*33*) or have been subjected to higher times of exposure to light at the initial step of seed synchronization (*34*).

The idea of a light-dependent sensitivity to ABA was reinforced by the finding that the constitutively photomorphogenic mutants *pifq* and *cop1-4* grown in the dark display hypocotyl sensitivity to ABA, reproducing the ABA-sensitive phenotype of light-grown plants (Fig. 2, A and B). It also suggests that the molecular components for a regulatory path from ABA perception to the regulation of cell size exist in the dark and is being down-regulated in a PIF-dependent manner. Similarly, ABA induction of ABA-responsive genes occurs in hypocotyls in the dark, suggesting that the ABA signaling pathway is intact in etiolated hypocotyls despite the insensitivity to ABA in relation to hypocotyl growth. It also discards the possibility of ABA transport from roots to shoots being impaired in the dark; otherwise, the induction of these genes could not occur. In addition, the sensitivity of *pifq* to ABA in the dark was impaired by antabactin, an ABA-receptor antagonist, in a dose-dependent manner. Together, our data show that canonical ABA perception and signaling occur in the dark, with PIFs inhibiting a subset of ABA responses associated with hypocotyl elongation through a hypocotyl specific mechanism (Fig. 8).

The transcriptomic analysis of WT and *pifq* plants treated with ABA in the dark allowed the identification of genes being up-regulated or down-regulated by ABA in photomorphogenic plants (WT L and *pifq* D), but for which deregulation is prevented in WT plants in the dark, where PIFs are active. Among the ABA down-regulated genes, we found numerous genes encoding proteins that might play a role in controlling cell elongation directly at the plasma membrane. Among these, there are 23 SAUR proteins, including SAUR9, which can directly interact and inactivate members of the D-clade PP2Cs (*76*) and SAUR63 that can directly promote growth at the PM, by antagonizing PP2C.D5 phosphatase and increasing PM H^+-ATPase activity (*71*). The activation of PM H^+-ATPase has been shown to be sufficient to trigger growth (*77*), and the regulation of the phosphorylation of some of its residues is key in the control of its activation (*52*, *53*). Although SAURs are transcriptionally down-regulated by ABA, the *pp2c.d2*/PP2C.D2^{M331K}-GFP expressing plants are sensitive to ABA in the dark. As the M331K substitution impairs PP2C.D2 binding to SAURs (*72*), this PP2C.D2 mutated form that is constitutively repressing PM H^+-ATPase renders plants responsive to ABA (Fig. 8), meaning that (i) SAURs are key for the inhibition of hypocotyls sensitivity to ABA; (ii) the transcriptional regulation of *SAURs* is not the main path by which ABA inhibits hypocotyl elongation. Instead, it may serve as an additional reinforcing mechanism once the inhibitory effect is initiated; (iii) keeping PM H^+-ATPase in a dephosphorylated state turns hypocotyls responsive to ABA, an effect mimicked by the application of BITC (fig. S8C).

Our transcriptomic analysis reveals that, together with *SAURs*, a large number of DEGs that act at the PM (hormone and ion transporters and channels) or at the cell wall level (EXPs, XTHs, and PMEs) correlate with hypocotyl responsiveness and might play a role in cell elongation homeostasis. Some of these genes are direct targets of PIFs that are coregulated by ABA. This is the case for the potassium channels KAT1 and KAT2, which work as inward rectifying K^+ channels for PM H^+-ATPase activity and are involved in auxin-induced hypocotyl elongation of etiolated seedlings (*78*). Moreover, *KAT1* has been shown to be a direct PIF3 target, playing a role on the PIF control of stomata opening at the onset of day periods (*79*). Another inward rectifying channel, AKT2, regulated by Ca^{2+} that can associate with KAT1 (*80*), is up-regulated by ABA, suggesting that the control of K^+ channels might play a role in hypocotyl responsiveness to ABA. However, this does not appear to be the main regulatory mechanism as *KAT1OX in* WT or in *pifq* does not affect responsiveness to ABA regarding the respective backgrounds (fig. S9).

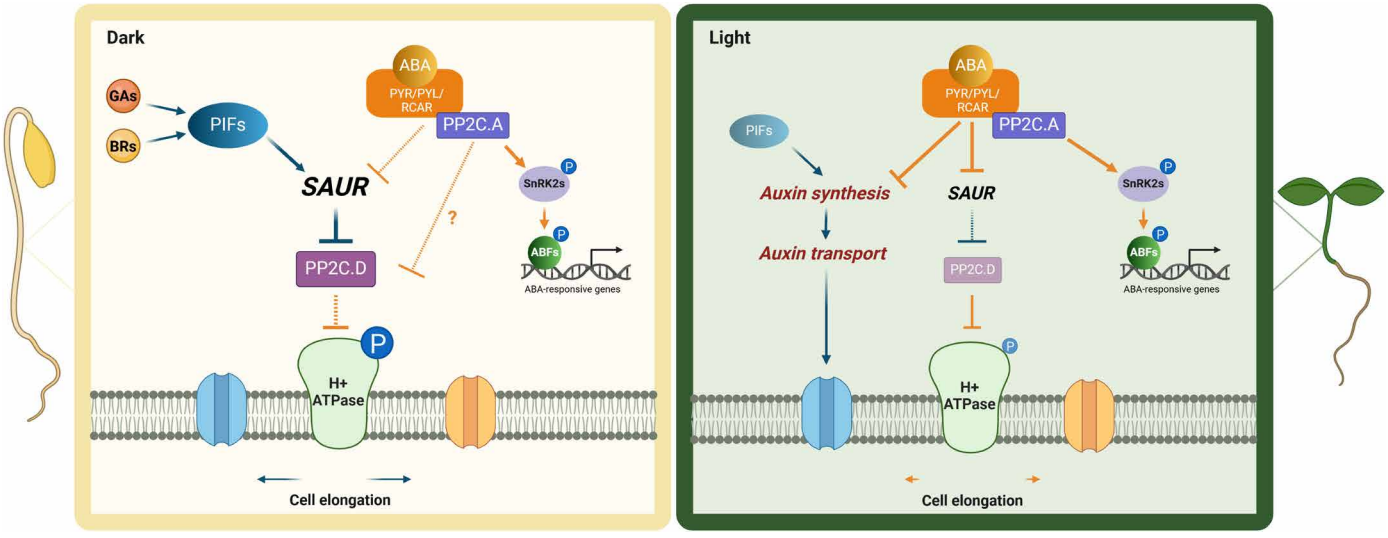

Fig. 8. Proposed model for ABA-induced inhibition of hypocotyl elongation. In the dark, hypocotyls are insensitive to ABA, despite an intact ABA signaling pathway being present and ABA-responsive genes being induced. The accumulation of PIFs in the dark transcriptionally induces *SAUR* gene expression. SAURs interact with PP2C.D, preventing them to interact with and dephosphorylate the PM H^+-ATPase (*14*, *76*). Under these conditions, hypocotyls are insensitive to ABA. In the absence of PIFs, the level of SAURs is reduced and PP2CD.2 can dephosphorylate the PM H^+-ATPase, rendering hypocotyls sensitive to ABA. The mutation of PP2C.$D2^{M331K}$ makes the phosphatase unable to be inhibited by SAURs, restoring hypocotyl responsiveness to ABA, as in *pifq* mutants. The hypocotyl responses to ABA in the dark involve a canonical ABA perception by the PYL receptors and PP2C.A-type phosphatases. In the light, ABA down-regulates auxin synthesis and signaling, which is also regulated by PIFs. ABA inhibition of hypocotyl elongation depends on auxin transport. Blue lines represent a path or step that promotes cell elongation, and orange lines represent inhibition or regulation by ABA. Dashed lines represent connections or mechanisms that are not active or are not important for cell elongation in the WT plants in the represented condition (dark or light).

Previous works have shown that ABA suppresses hypocotyl elongation, in part by promoting the dephosphorylation of PM H^+-ATPase (Thr^{947} residue), in which the PP2C-type phosphatase ABI1 plays a role (*33*). Recently, it was shown that ABI1 can directly bind to PM H^+-ATPases and inhibit its activity by promoting the dephosphorylation of Thr^{947}, controlling root elongation (*52*). Although this mechanism could account for the elongated hypocotyl phenotype displayed by the *Qhai1-1* and *Qabi2-2* mutants in the light (Fig. 5), it can hardly explain how the lack of these type A PP2Cs in the dark results in reduced phosphorylated PM H^+-ATPase Thr^{947} levels and reduced hypocotyl elongation. Further research is needed to understand the role of type A PP2C in hypocotyl responsiveness to ABA in the dark.

Under light conditions, ABA controls hypocotyl elongation through a different pathway. Contrary to what would be expected, hypophotomorphogenic mutants that display elongated hypocotyls as *phyB* and *cry1cry2* display enhanced sensitivity to ABA compared to WT or with *35S:PIF4-HA* lines. Although further experiments are needed, it might be that PhyB controls ABA responsiveness trough PIF-dependent and PIF-independent pathways or that, in the absence of PhyB, other photoreceptors might trigger compensatory responses. It might also be that the ectopically overexpression of PIF4 generates a constant and deregulated accumulation of PIF4 and of its downstream targets. However, the ectopic overaccumulation of PIF4 is not sufficient to trigger light insensitivity to ABA, suggesting that, in the light, ABA uses other mechanisms that do not depend on PIF repression (via SAURs) to inhibit hypocotyl growth. This suggests that, upon light perception, a different mechanism by which ABA can reduce hypocotyl elongation is enabled.

NPA, a traditionally used auxin transport inhibitor, has been shown to compete with auxin for the PIN1 auxin transporter binding pocket (*81*). In our assays, in the dark, NPA does not reduce hypocotyl elongation or block the effect of ABA on hypocotyl elongation in *pifq* mutants, showing that no NPA-dependent auxin transport is needed for the regulation of hypocotyl size in the dark, even in a constitutively photomorphogenic *pifq* genetic background. Because auxin transport is required for hypocotyl elongation in seedlings grown in the light but not in seedlings grown in the dark (*48*), ABA unresponsiveness is not affected by NPA treatment in the dark.

In the light, NPA renders WT and *pifq* plants insensitive to ABA, suggesting that ABA requires an NPA-repressible auxin transport to inhibit hypocotyl elongation in the light, a mechanism that occurs downstream of PIFs. This supports the idea that NPA blocks the effect of ABA on hypocotyl elongation in light-grown plants, advocating for a mechanism of ABA control of hypocotyl elongation in the light that does not exist in the dark. In addition, in the light, but not in the dark, ABA promotes the down-regulation of auxin biosynthetic genes as *TAA1* and *YUCCA*s (*YUC3*, *YUC8*, and *YUC9*), which might account for specific relations between ABA and auxins in hypocotyls in the light. These genes are direct PIF targets and account for the PIF regulation of shade avoidance responses (*56*, *69*, *82*, *83*). Thus, ABA and PIFs converge on the regulation of auxin biosynthesis, which, to have effective consequences in cell elongation, requires auxin transport (Fig. 8). On the other hand, it has been reported that auxin regulation of hypocotyl elongation in the dark requires ABA biosynthesis (*84*), suggesting that the ABA and auxin relations under light are complex and might be finely tuned to adjust developmental responses to environmental stress circumstances.

Response to abiotic stresses induced by ABA triggers a transcriptional reprograming that allows plant to adapt to environmental changes, by adapting their morphology and metabolic responses.

The control of growth by elongation is part of these responses. Here, we show how the inhibition of hypocotyl elongation by ABA occurs in the light but not in dark-growing seedlings. These responses might reflect an adaptative mechanism by which the germinating seedling prioritizes reaching light for survival and insensitizes hypocotyl cells to the ABA signal, preventing the reduction of the elongation rate, ensuring to quickly reach the soil surface even under stressful conditions. In this way, for some species, a sudden shortage of soil water content due to weak water retention, just after germination, would not reduce the possibilities to reach soil surface and perceive light.

MATERIALS AND METHODS

Plant materials

Arabidopsis WT and mutant plants used in this study were all in the Columbia-0 (Col-0) ecotypes with the exception of the *hy5 hyh* double mutant (*85*), which is in Wassilewskija (WS) ecotype. The mutants and lines used are *phyB* (*86*), *phyA* (*87*), and *pifq* (*8*); *35S:PIF-HA* (*43*), *cop1-4* (*88*), *cry1* (*89*), *cry2* (*90*), *cry1 cry2* (*91*), and *hy5-215* (*92*); sextuple *pyl* mutant *pyl112458* [*pyr1 pyl1 pyl2 pyl4 pyl5 pyl8*; (*93*)]; quadruple *pp2c Qabi2-2* and *Qhai1-1* (*51*); and *areb1 areb2 abf3 abf1* (*arebQ*) seeds (*94*). In addition, we used multiple saur mutants, *saur19/21/23/24*, *saur19/20/21/22/23/24* (*saur19-24*), *saur61/62/63/64/65/66/67/ 68/75* (*saur61-68/75*), *saur19/20/21/22/23/24/61/62/63/64/65/66/67/68/75* (*saur19-24/61-68/75*), *saur19/20/21/22/23/24/26/27/29/61/62/63/64/65/66/67/68/73/75* (*saur19-24/26/27/29/61-68/73/75*), and *saur9/16/19/20/21/22/23/24/26/27/29/61/62/63/64/65/66 /67/68/73/75* (*saur9-16/19-24/26/27/29/61-68/73/75*) (*70*), the triple *pp2c.d2/pp2c.d5/pp2c.d6 mutant* (*95*), and the *pp2c.d2*/PP2C.D2^{M331K}-GFP line (*72*). The *KAT1OX* (*96*) and *pifqKAT1OX* lines (*79*) *ost2-2D* (*97*) and *aha2-4* (*98*) were also used in this study.

Plant growth conditions

Arabidopsis seedlings were sterilized with a solution of 75% sodium hypochlorite and 0.1% Tween 20 and stratified at 4°C during 2 days in the dark. Seedlings were seeded on a 350-μm pore nylon mesh (Distribuciones Quimebora S.L., Madrid, Spain) placed over solid Murashige and Skoog (MS) medium with 1% sucrose and 0.6% agar. For experiments in the dark, seeds were exposed to light for 2 hours to synchronize germination, wrapped in aluminum foil, and, after 62 hours, transferred on the top of the mesh to plates containing ethanol (mock) or ABA (Sigma-Aldrich) and kept in the dark for four additional days, unless otherwise stated. For experiments under low light, seeds were placed under fluorescent white light (100 μmol m^{-2} s^{-1}) with a 16-hour light/8-hour dark period and transferred to mock- or ABA-containing plates after 72 hours and kept under low light (10 μmol m^{-2} s^{-1}) for 5 days, unless otherwise stated. All the plants were grown at 22°C. For the hormone inhibitors, 0.5 μM NPA (Duchefa Biochemie), 0.5 μM BZR (MedChemExpress), and 0.1 μM Paclo (Duchefa Biochemie) were used. BITC is from Sigma-Aldrich, and antabactin is from Kerafast. The use of a 350-μm pore mesh reproduced initial results obtained by transferring seedlings to ABA- or mock-containing media very gently with the help of sterilized forceps. For osmotic stress assays, PEG plates were prepared using PEG, molecular weight 6000 as described by van der Weele *et al.* (*99*) and HA plates as described by Gonzalez *et al.* (*36*).

Lettuce (*L. sativa*, var. capitata) and Cardamine (*C. hirsuta*. Accesion Oxford) seeds were sterilized with a solution of 75% sodium hypochlorite and 0.1% Tween 20 and stratified at 4°C during 2 and 7 days, respectively, in the dark. Seeds were seeded over solid MS medium with 1% sucrose and 0.7% agar. For experiments in the dark, lettuce seedlings were transferred carefully with forceps after 48 hours to plates containing ethanol (mock) or ABA and kept in the dark for three additional days. For experiments under low light, lettuce seeds were germinated under white light and transferred to mock- or ABA-containing plates after 48 hours and kept under low light for 4 days. Cardamine seedlings germinated under light or dark conditions were transferred after 96 hours to plates containing mock or ABA and kept in the light or dark, respectively, for four additional days.

Hypocotyl measurements

For hypocotyl measurements, light- or dark-grown plants were disposed on agar plates and photographed and hypocotyls were measured using ImageJ software (https://imagej.net). At least three biological replicates, each consisting of measurements of at least 20 seedlings grown at different times, were analyzed with similar results.

Quantitative RT-PCR

For RT-qPCR assays, 2 μg of total RNA was extracted from 7-day-old seedlings with the Favorprep Plant Total RNA Purification Mini kit (Favorgen) was used for cDNA synthesis with using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) with DNAse I treatment (Roche). Quantitative PCR was carried out using 5x PyroTaq qPCR mix Plus EvaGreen (CMB Cultek Molecular Bioline) in a QuantStudio5 machine (Applied Biosystems). Transcripts were amplified, and results were normalized to *PP2A* transcript levels. Primers used for qRT-PCR are represented in table S1.

RNA-seq analyses

For RNA-seq, 2 μg of total RNA was extracted from three independent biological replicates of WT and *pifq* seedlings, grown under light (for 72 hours) or dark conditions (for 62 hours), and transferred to ABA or mock, as described above in the "Plant growth conditions" section. After 4 days for the dark-grown plants or 5 days for the low light-grown plants, total RNA was extracted with the Favorprep Plant Total RNA Purification Mini kit (Favorgen). Messenger (polyA+) RNAs were purified from total RNA using oligo(dT). Stranded libraries were prepared using DNB-seq (BGI). A 100–base pair (bp) paired read end sequencing was performed on the DNBSEQ-G400 system (BGI). Reads were quality checked with FASTQC (*100*) and mapped on TAIR10 genome assembly of *Arabidopsis thaliana* genome using STAR (*101*) with relevant options "--alignIntronMin 20 --alignIntronMax 5000 --alignMatesGapMax 10000." Duplicated alignments were removed using samtools markdup -r (*102*). Browser tracks were generated using deepTools function bamCoverage with options "--binSize 10 --normalizeUsing RPKM" and visualized using the IGV browser (*103*). Read counts were retrieved using QoRTs (*104*) using gene coordinates obtained from file Arabidopsis_thaliana.TAIR10.42.gtf. Differential expression was analyzed with DESeq2 (*105*) using default options. DEGs for each comparison were selected by adjusted P value < 0.01 and $|FC| > 1$. Heatmaps and GO enrichment analysis was performed as in (*106*).

Protein extraction and immunoblot analysis

Shoots of dark-grown seedlings were dissected in the dark (under green light) and frozen in liquid nitrogen. Total protein was extracted according to Hayashi *et al.* (*107*) in SDS buffer containing 3% (w/v) SDS, 30 mM tris-HCl (pH 8.0), 10 mM EDTA, 10 mM NaF, and 30% (w/v) sucrose and incubated at 60°C for 5 min. After centrifugation,

the supernatants were quantified for total protein and loaded in an SDS–polyacrylamide gel electrophoresis gel. For the immunoblot, the antibodies anti-AHAcat and anti-pen-Thr (*107*) were used in a 1/3000 dilution. Anti-actin (Sigma-Aldrich, no. A04080) was used as a total protein loading control.

Supplementary Materials

The PDF file includes:
Figs. S1 to S9
Table S1
Legends for datasets S1 and S2

Other Supplementary Material for this manuscript includes the following:
Datasets S1 and S2

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