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Periodic root branching is influenced by light through a HY1-HY5-auxin pathway

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Summary

The spacing of lateral roots (LRs) along the main root in plants is driven by an oscillatory signal, often referred to as the “root clock” that represents a pre-patterning mechanism which can be influenced by environmental signals. Light is an important environmental factor that has been previously reported to be capable in modulating the root clock although the effect of light signaling on the LR pre-patterning has not yet been fully investigated. In this study, we reveal that light can activate the transcription of a photomorphogenic gene *HY1* to maintain high frequency and amplitude of the oscillation signal, leading to the repetitive formation of prebranch sites. By grafting and tissue-specific complementation experiments, we demonstrated that HY1 generated in the shoot or locally in xylem pole pericycle cells was sufficient to regulate LR branching. We further found that HY1 can induce the expression of HY5 and its homologue HYH, and act as a signalosome to modulate the intracellular localization and expression of auxin transporters, in turn promoting auxin accumulation in the oscillation zone to stimulate LR branching. These fundamental mechanistic insights improve our understanding of the molecular basis of light-controlled LR formation and provide a genetic interconnection between shoot- and root-derived signals in regulating periodic LR branching.

Introduction

Plant development is largely influenced by environmental stimuli. As an important environmental factor, light drives photosynthesis to produce energy sources (i.e. sugars) for promoting photomorphogenic development. Light quality and exposure time can drive major developmental changes of plant shoots, such as photomorphogenesis, flowering time, phototropism, and shade avoidance.[1–3] Recent studies have revealed a significant role of light as an important player in regulating underground root system architecture. For instance, light signaling can lead to production and accumulation of the plant hormone auxin or sugars in the shoot, which subsequently are transported to the roots to stimulate primary root (PR) elongation.[4,5] *ELONGATED HYPOCOTYL 5* (*HY5*), a long-distance mobile transcription factor, is also induced by light and moves from shoot to root to regulate PR elongation thereby optimizing nutrient uptake.[6] In addition to its effect on the PR, light is also essential for the formation of lateral roots (LRs) along the primary root. LR formation is increased in concert with higher intensities of light irradiance in the shoot, whereas it is completely blocked in dark-grown plants.[7] Light also positively regulates gravitropic responses of LRs through the activation of a HY5-LAZY4 signaling module in the root.[8] In contrast, high UV-B and far-red light negatively affects lateral root primordium (LRP) development and emergence by interfering with polar auxin transport (PAT) and auxin signaling pathways.[9,10]

LR founder cells are specified in a subset of xylem pole pericycle cells that were previously primed by an oscillatory signal. LR founder cells, once specified,

immediately undergo cell division to develop into a LRP which further emerges out of the PR as a LR.[11]. The upstream oscillatory signal is demonstrated by the fluctuation of the *DR5:Luciferase* (*DR5:LUC*) auxin-reporter in a zone of the root proximal to the elongation zone, referred to as the oscillation zone (OZ) and is synchronized with the expression of wide range of oscillating genes.[12] This recurrent signal seems to trigger the repetitive formation of prebranch sites in which the founder cells become specified and develop into actively dividing LPRs. While not every prebranch site develops into a lateral root, every lateral root derives from a prebranch site, suggesting that the decision to make a prebranch site is a rate-limiting factor of root branching. The periodic formation of prebranch sites is thus thought to be instructive for the control over lateral root spacing and can be regarded as a pre-patterning mechanism.[11]

The plant hormone auxin has been implicated in all developmental steps of LR formation, and also plays a predominate role in regulating DR5 oscillations.[13,14] TIR1-mediated auxin signaling and lateral root cap (LRC)-derived auxin biosynthesis are both essential for maintaining the oscillation amplitude,[15] whilst cyclic programmed cell death of LRC cells contribute to the pool of auxin that is transported to and periodically accumulating in the OZ to stir prebranch site formation.[16] The oscillation signal can also be influenced by environmental stimuli, such as the heavy metal cadmium and water deficits, which both repress the oscillation signal and subsequent prebranch site formation.[17,18] A recent elegant study has shown a substantially reduction in prebranch site formation in dark-grown Arabidopsis seedlings which convincingly argues for a possible regulation of light in oscillation.[7]

Long hypocotyl1 (*HY1*) encodes a heme oxygenase, a key regulator in phytochrome chromophore biosynthesis and mediates photomorphogenesis in higher plants.[19,20] It catalyzes the oxygenation of heme to carbon monoxide (CO), Fe^{2+} and biliverdin Ixα (BV).[21] BV is further converted to phytochromobilin, the precursor of a group of phytochromes that are required for the activity of HY5, HY5 homologue (HYH), and B-BOX CONTAINING PROTEINs (BBXs) in regulating photomorphogenesis.[22,23] Plants lacking the functional HY1 display defects in LR development and adventitious root formation,[24,25] but the role of HY1 in mediating root development and the possible mechanism have been rarely studied.

Incentivized by the previously reported effects of light on lateral root formation and the genetic evidence for the functional involvement of HY1, we decided to assess the role of HY1 during the pre-patterning mechanism of lateral root formation. We found that light is essential for maintaining the oscillation periodicity and amplitude, and promotes periodic LR branching in an HY1-dependent manner. Furthermore, we provide genetic evidence that shoot- and root-derived HY1 are localized in plastids, and both contribute to its function in regulating LR branching. HY1 induces HY5 expression and stimulates shoot-to-root auxin transport, leading to the accumulation of auxin at the OZ, thereby promoting LR branching. Our study therefore provides novel insights into the molecular mechanism underlying light-regulated LR branching and highlights a HY1-HY5-auxin module in mediating oscillation and LR response to light signaling.

Results

HY1 is required for light-induced LR branching and PR elongation

Previous studies have shown that light signal is crucial for root development in Arabidopsis.[4,7] Consistently, both lateral root (LR) formation and primary root (PR) elongation of wild type (WT) seedlings were severely reduced when 5-day-old seedlings were grown in the dark or transferred from light to dark for 5 days, while LR formation and PR growth could be restored when dark-grown seedlings were re-supplied with light for 5 days (Figures 1A and 1B). To investigate *HY1* dependency of light-regulated root development, two independent null mutants of *HY1*, *hy1-100* and *GK_034B01*, were analyzed. Interestingly, LR number, PR length, and LR density were significantly reduced in light-grown *hy1-100* by 49.2% ($P < 0.001$), 26.3% ($P < 0.001$), and 30.8% ($P < 0.001$) of light-grown WT respectively, whereas dark-grown seedlings of *hy1-100* exhibited identical root phenotypes as dark-grown WT seedlings (Figures 1A and 1B). A similar root phenotype was also observed in the *GK_034B01* mutant (Figures S1A and S1B). The defective root phenotype of *hy1-100* mutants can be fully recovered by complementing the *hy1-100* mutant with the HY1-VENUS protein under the control of its native promoter (*HY1-VENUS hy1-100*) (Figures 1A and 1B). These data suggest light-induced root growth requires HY1/HO1 function.

The role of *HY1* in the regulation of root response to light was further confirmed under natural soil conditions by using an Ara-rhizotron system.[26] In agreement with above results, LR formation and PR elongation were reduced in light-grown *hy1-100* seedlings compared to WT in soil, and they became severely impaired to an equal level

in dark-grown *hy1-100* and WT (Figures 1C and 1D). All these results demonstrate that the light-promoted LR branching and PR elongation are largely dependent on the photomorphogenic gene *HY1*/*HO1* in Arabidopsis.

Sucrose is the main energy source produced by plant leaves in light conditions and can promote root development.[4] However, *hy1-100* exhibited reduced LR number, PR length, and LR density compared to WT in the sucrose-free medium under both light and dark conditions (Figures S1C and S1D), while addition of 3% sucrose also did not rescue the root defect in *hy1-100* under both conditions. These results indicate that the HY1-mediated root responses to light is independent of sucrose.

HY1 regulates LR branching through promoting pre-branch site formation

To probe the mechanism underlying HY1-regulated LR formation, we first performed lateral root primordium (LRP) staging in *hy1-100* mutants. Compared to WT, *hy1-100* seedlings showed a significant decrease of LRP at stage I and in the number of emerged lateral roots whilst the numbers of LRP at stages II to VII were equal to those of WT (Figure S2A). Meanwhile, the morphology of LRPR was not altered in the *hy1-100* mutants (Figure S2B). Hence, *HY1* might play a role in promoting the initiation of LRP.

LRP is formed in a pre-branch site that became earlier induced by gene oscillation in the OZ,[11,12] and light was previously shown to have a dramatic effect on maintaining DR5 oscillation in OZ and subsequent pre-branch site formation.[7] To access if *HY1* mediates light-induced oscillations and prebranch site formation, the specific reporter line *DR5:LUC* was introduced in *hy1-100* and its dynamic expression was quantified.

In dark-grown WT and *hy1-100* seedlings, *DR5* oscillation and prebranch site production were severely suppressed (Figures 2A-F). When light is given, the *DR5:LUC* oscillation signal in the OZ and prebranch site formation were reinstalled in WT, whilst *hy1-100* seedlings showed weaker signal amplitude of *DR5:LUC* (86.3% of WT, $P < 0.05$) and a longer time interval between *DR5:LUC* oscillations (147.6% of WT, $P < 0.01$) (Figures 2C-F and Movie S1), in concert with less prebranch site formation in *hy1-100* than WT (Figures 2A and 2B). These results indicate *HY1* acts on *DR5* oscillation to promote prebranch site formation.

The *DR5* oscillation is activated by a cyclic programmed cell death (PCD) signal in the distal LRC cells,[16] we thus investigated if the LRC-PCD is also affected by HY1. At the distal LRC cells of light-grown WT and *hy1-100* seedlings, the PCD marker *pPASPA3:NLS-tdTOMATO* exhibited a striped-like expression pattern, but the *PASPA3* signal in the LRC was much weaker in *hy1-100* than in WT while the PASPA3 signal was almost absent in the LRC cells of both dark-grown WT and *hy1-100* (Figures 2G and 2H). Real-time imaging of *pPASPA3:NLS_tdTOMATO* expression in the root tip showed that the time interval of the disappearance between consecutive PASPA3 stripes was delayed in *hy1-100* compared to the WT in the presence of light, whilst there is no difference between dark-grown WT and *hy1-100* (Figures 2G, 2H and Movie S2). Light-promoted LR branching might therefore be mediated by HY1 through maintaining cyclic PCD of LRC cells and in turn activating DR5 oscillation signal at OZ to trigger prebranch site formation.

HY1 is required for light-activated root meristem division activity

Since *hy1-100* mutant produces a shorter PR compared to WT in the light (Figures 1A and 1B), we next investigated whether HY1 affects division activity of the root meristem and subsequent cell elongation, two main factors regulating PR elongation. Our results showed that light-grown WT seedlings have increased meristem cortex cell numbers, and longer meristems and elongation zones than dark-grown WT seedlings, suggesting light promotes meristem cell division activity and cell elongation (Figures 3A-D). However, compared to WT, knock-out of HY1 reduces the cortical cell numbers of the meristem and the elongation zone but increases the cell length in the elongation zone under light conditions, resulting in a shorter meristem but in an elongation zone of the same length (Figures 3C, 3D and S2C). Noticeably, the LRC number was correspondingly reduced in the light-grown *hy1-100* or dark-grown WT and *hy1-100*, which might explain the delay of LRC-PCD in these lines (Figure 3E). The meristem and LRC defect of light grown *hy1-100* could be fully rescued by functional *HY1-VENUS* (Figures 3A, 3C-E), implying that the HY1 exerts a critical role in mediating light-promoted meristem cell division.

Staining of *pCYCB1:GUS*, a marker for the G2-M phase transition of cell cycle progression,[27] showed that the cell division activity at the root meristem of *hy1-100* was relatively weaker than that of WT upon light activation, but was substantially reduced in *hy1-100* and WT root meristem under dark conditions (Figure 3B). Taken together, the results demonstrate HY1 activity is involved in light-mediated root meristem activity and PR elongation.

_HY1_ is expressed in LRPs and LRC cells and its expression is up-regulated by light

To explore the mechanism that underlies the regulatory role of HY1 for LR branching and root meristem activity, we first analyzed the expression pattern of *HY1* in roots by generating a transcriptional reporter line of *HY1* (*pHY1:GUS/GFP*). Being a gene involved in light signaling, *HY1* exhibited strong expression in vascular tissues of leaves and hypocotyls (Figure S3A). Interestingly, both GUS staining and confocal imaging also showed a specific expression of HY1 in root tissues, i.e. columella cells, distal LRC cells, xylem-pole pericycle (XPP) cells, and developing LRP at stage I to VII (Figures 4A and 4C). A translational fusion of HY1-VENUS under its native promoter (*pHY1:HY1-VENUS*) also exhibited identical tissue-localization of the HY1 protein in the root (Figures 4B and 4D). The specific expression of *HY1* in LRP and LRC further supports its function in regulating LR branching. At the cellular level, the HY1-VENUS signal is localized in plastids in the root, cotyledon, and hypocotyl (Figures 4B and S3B), in accordance with a previous report.[20] Noticeably, in the distal LRC cells, the HY1-VENUS signal was detected in the nucleus and cytoplasm rather than in the plastids (Figures 4B, S3C and S3D).

We next investigated if the expression of *HY1* in root tissues is responsive to light. When dark-grown seedlings were transferred from dark to light, *HY1* expression in the root meristem and oscillation zone is quickly induced after 6 h of light exposure (Figure 4E). On the contrary, *HY1* expression was repressed when the seedlings were transferred from light to dark (Figure 4E). The light-induced *HY1* expression in the root

was also confirmed by RT-qPCR (Figure 4F). Additionally, we also found that the expression pattern of *HY1* with respect to light was not altered by sucrose supply, in accordance with the observed root responses of *hy1-100* mutants to sucrose (Figure S1E) and further supporting the idea that HY1 expression is regulated by light in a sucrose-independent manner.

Shoot- and root-derived HY1 both contribute to light-mediated LR development

In plants, light-responsive mobile signals can move from shoot to root through the stele to regulate root development.[6] HY1 expression is detected in the shoot as well as in root tissues involved in LR formation, raising the question whether shoot- or root-derived HY1 is essential for light-mediated LR branching.

To further probe whether HY1 is a mobile signal, we performed reciprocal grafting among *hy1-100*, WT and *hy1-100* complemented (*HY1-VENUS hy1-100*) plants. Grafting scions of WT or *HY1-VENUS hy1-100* onto the root stock of *hy1-100* mutant fully rescued the LR formation and PR elongation, while the rootstocks of WT or *hy1-100* complemented line could also partially restore the LR formation and PR elongation (Figures 5A and 5B). These results suggest mainly shoot- but also root-derived HY1 are responsible for HY1-mediated LR formation and PR elongation. However, we noticed that the HY1-VENUS signal was not detected in the *hy1-100* rootstock when it was grafted with the scions of the *hy1-100* complemented line and *vice versa* (Figure 5C). Western blot analyses further confirmed that HY1-VENUS was only detected in tissues of the donor explants[28] (Figure 5D). Hence, HY1 is not a mobile signal between

shoot and root, but plays a role in a shoot-to-root transmissible signaling for LR branching and PR elongation.

Because *HY1* exhibited specific expression in LRC and XPP cell layers, both of which are associated with LR branching, we further determined whether these root tissues are contributing to HY1 function in regulating root development. A functional VENUS-tagged HY1 protein was specifically targeted to respective LRC or XPP tissue domain of *hy1-100* mutant by using promoters of *SMB* (root cap specific),[29] *GATA23* and *MAKR4* (XPP and LRP specific).[15,30] As a positive control, expressing *HY1-VENUS* under the control of its native promoter could fully rescue the PR elongation and LR formation of *hy1-100* mutant to WT level (Figures 1A, 1B, 5F and 5G). The expression of *HY1-VENUS* driven by *GATA23* and *MAKR4* promoters are detected in the plastids in XPP cells, and could also largely rescue LR branching and PR elongation in *hy1-100* (Figures 5E-G). Detailed analysis also showed that the division activity of the root meristem was recovered in these two lines (Figure S3E). By contrast, expressing *HY1-VENUS* in the root cap domain failed to rescue the root growth suppression by *hy1-100* (Figures 5E-G). These data suggest that XPP-localized *HY1* contributes to its function in mediating LR branching.

Genetic interaction of HY1 with HY5 and its homologue HYH in promoting root growth triggered by light signaling

HY1 encodes a haem oxygenase that can degrade haem into CO gas, BV and Fe^{2+}, while BV is quickly degraded to bilirubin (BR) *in vivo*.[31] We tested whether these metabolites

are responsible for or involved in the HY1 effect on root development. Treatments of BR, CORM-2 (a CO donor), and their combination only slightly increased the number of LRs of *hy1-100* without affecting the PR elongation (26.7%, 27.8%, and 34.4% of Mock level, respectively), while $FeSO_4$ treatment did not affect the root growth of *hy1-100* (Figure S4A-C). Our results suggest that instead of the known haem metabolites, HY1 might activate other signaling molecules to regulate root development.

To screen for the potential components involved in HY1-mediated root responses to light signaling, we compared the transcriptomes of root tips of WT and *hy1-100* that were either transferred from dark to light (LRs activated by light) or from light to dark (LRs repressed by dark) (See Materials and Methods, and Figure 6A). Considering a significant change of *HY1* expression at the root tip by light observed after 6 h and the emergence of clear root responses after 24 h (Figures 4E, 4F and S1E), root tips were sampled 6 h and 24 h after treatment. Differentially expressed genes (DEGs) analysis showed that 3211 and 1677 genes were transcriptional regulated by light and dark treatment in a HY1-dependent manner, respectively (log_2fold change > 1.5, *P*-value < 0.05; Figure 6B, and Table S1). 337 DEGs were found to overlap in both light- and dark-regulated datasets with an opposite gene expression pattern, strengthening their potential invovlement in light-mediated root responses downstream of HY1.

Among these genes, *HY5* and its homologue *HYH* which have been shown to act as a light-responsive local or systemic signals to regulate root growth,[6,9,32] were identified. RT-qPCR results confirmed that the light-accumulated *HY5* and *HYH* expressions at the root tip requires functional HY1 (Figure 6C). *HY5* and *HYH* are

highly expressed in root meristems and the elongation zone,[33] and similar to *hy1-100* mutant, *hy5hyh* mutant displayed reduced numbers of LRP and LR, and shorter PR, in relation to WT (Figures 6D, 6E, and S5A). These results suggest that HY1 genetically interacts with HY5 and HYH to regulate root growth in response to light.

We next checked whether the expression of *HY5* and *HYH* can be activated by shoot-derived or root-derived HY1. Therefore, expression of *HY5* and *HYH* in the root tip of WT and *hy1-100* reciprocal grafted seedlings was determined. Compared to the self-grafted *hy1-100* seedlings, grafting Col-0 scions onto *hy1-100* rootstocks strongly induced the expressions of *HY5* and *HYH* in the root, which were less induced in the rootstock of Col-0 grafted with *hy1-100* scion (Figure S5B). In a next grafting experiment, we found that the LR branching and PR elongation defect of *hy5hyh* mutants could be restored by grafting Col-0 scions on the rootstocks but not by using *hy1-100* mutant scions, whilst grafting of *hy5hyh* scions with *hy1-100* mutant rootstocks showed an identical root defect as self-grafted *hy1-100* seedlings (Figures 6F and 6G). Together, these results suggest that HY1 is governing *HY5* and *HYH* expression to regulate root growth in response to light signaling.

HY1 stimulates the auxin accumulation in the OZ for LR branching through manipulating polar auxin transport

We further overlapped the HY1-dependent light-regulated core 337 DEGs with Visual LRTC, a compendium of LR initiation-associated transcriptome datasets ,[34] to screen for genes specifically involved in HY1-regulated LR branching (See Supplemental

Methods and Table S1). From this comparative analysis, two auxin inducible genes emerged, i.e. *IAA29*, an AUX/IAA protein encoding gene and *GH3.6/DFL1,* a gene encoding an IAA-amido synthetase which conjugates excess of free IAA *in vivo*.[35] RT-qPCR analysis showed that *DFL1* and *IAA29* expression were significantly induced by light and repressed by dark at the root tip respectively, in a HY1-dependent manner, confirming the regulation of *HY1* in their expression in the root (Figure 6C). Induction of auxin-related genes indicates HY1 might stimulate auxin accumulation and signaling at the root. Because HY5 and HYH can also regulate expression of major auxin signaling genes (Figure S5C),[36–38] we hypothesized that auxin might act downstream of HY1 and HY5 to regulate root responses to light signaling.

To confirm this hypothesis, the expression of the auxin response reporter *DR5* in the root tip of *hy1-100* was examined under light and dark conditions. Compared to WT, *hy1-100* showed a significant reduction of *DR5:GUS* and *DR5rev:VENUS* expression at LRC and the vascular tissue in the root meristem and OZ under light conditions; the *DR5* signals were both substantially decreased in these tissues to a level comparable to that found in dark-grown WT and *hy1-100* roots (Figures 7A-C). These results further corroborate an essential role of HY1 in sustaining light-induced auxin accumulation in the OZ.

Auxin accumulation at the root meristem and OZ is required for PR elongation and prebranch site formation, and it is mediated by the polar auxin transport (PAT) machinery driven by auxin influx and efflux carriers.[16,39,40] A previous study also pinpointed that light could promote shoot-to-root PAT by sustaining the intracellular

localization and stability of auxin efflux carriers PIN1 and PIN2.[5] To determine whether PAT is involved in HY1-regulated auxin accumulation in the OZ, we analyzed the expression pattern of auxin flux carriers *pAUX1:AUX1-YFP*, *pPIN1:PIN1-GFP*, *pPIN2:PIN2-GFP*, *pPIN3:PIN3-GFP*, *pPIN7:PIN7-GFP* in *hy1-100*. PIN1 is localized at the basal plasma membranes (PM) of the cells in the stele and mediates shoot-to-root PAT.[39] In light-grown *hy1-100* seedlings, PIN1 abundance was reduced in the root stele and its PM localization was also disturbed, similar to that observed in dark-grown WT and *hy1-100* seedlings (Figures 7D and 7F). Meanwhile, the AUX1-YFP and PIN2-GFP signals at PM were also attenuated in the root epidermis of light-grown *hy1-100* seedlings as compared to WT, and largely reduced in dark-grown WT and *hy1-100* roots (Figures 7E, 7G, and 7H). PM localization of AUX1 and PIN2 was not altered in *hy1-100* (Figures 7E and 7H). Furthermore, PIN3 and PIN7 expression at the root meristem was slightly affected in *hy1-100* as compared to WT under either light or dark (Figure S6). Thus, our results suggest that the HY1 is essential for maintaining the intracellular localization and expression of auxin flux carriers at the root, which promotes auxin accumulation in the meristem and OZ for root growth.

Given the reduced levels of PIN transcripts and proteins in *HY1* knock-out mutants, we hypothesized that this could be causative for decreased auxin levels in the root tip and consequently the observed LR defect. In such a scenario, exogenous application of the auxin analogue 1-NAA might alleviate the root defects of the *hy1-100* mutants. Indeed, upon light activation, the numbers of prebranch site and LR of *hy1-100* seedlings were both significantly increased by NAA treatment, approximately to those

of WT seedlings. NAA treatment also partially restored the prebranch site and LR formation in dark-grown WT and *hy1-100* seedlings (Figure S7). These results highlight the requirement of auxin accumulation for HY1-mediated LR branching in response to the light signaling.

Discussion

Light is essential for plants as it activates photosynthesis and provides energy source supporting plant shoot and root growth.[3] Through our study, we found that light perceived by the shoot plays an important role in regulating LR branching through maintaining high levels in oscillation amplitude and frequency, guaranteeing the recurrent production of prebranch sites and LRs along the primary root axis. Previous studies have shown that light can affect LRP initiation and outgrowth,[7] here we could more precisely situate the effect of light-activated signaling during the very early steps of lateral root formation that occur even upstream of LRP initiation. Oscillation and prebanch site formation have been previously suggested to be modulated by root-derived auxin,[15,16] carotenoid signals,[41] peptides,[42,43] and vesicle trafficking,[44] our results further pinpointed a critical role of shoot-perceived light signaling activating oscillation signal for prebranch site formation.

The regulation of light in oscillation and periodic LR branching was identified to mediated by a HY1-HY5-auxin signal module. In plants, HY1 is required for phytochrome chromophore biosynthesis, and has been reported to regulate LR development in plants.[45,46] We showed that *HY1* expression is transiently induced at the OZ by light and genetic evidence demonstrated that HY1 mediates the impact of light signaling on the oscillation signal and prebranch site formation, as well as root meristem activity and PR elongation (Figures 2, and 3). However, it should be taken into account that PR elongation is interconnected with the periodic LR branching as a fast cell division and elongation rate at the OZ sustains the high levels of amplitude and

frequency of DR5 oscillation in OZ for prebranch site formation.[12] However, the reduced LR density in *hy1-100* mutant and dark-grown WT seedlings indicates that the regulation of light and HY1 in oscillation and prebranch site is not due to the shorter PR.

It is a general understanding that shoot-derived signals (e.g. HY5) perceives light signaling and subsequently moves to the root to activate downstream signaling components to regulate root development.[6,32] However, grafting and tissue-specific complementation experiments suggested that HY1 is not a mobile signal (Figure 5), and shoot- and root-derived HY1 are able to activate LR formation. We further identified HY5 and its homologue HYH as signaling components downstream of HY1. HY5 has been previously identified as a shoot-to-root mobile signal to promote root growth,[6] while local HY5 activity at LRP can also be induced by phytochromes to repress LRP emergence under far-red light conditions.[9] Furthermore, HY5 and the PHYTOCHROME INTERACTING FACTORs (PIFs) exert a central function in maintaining auxin levels controlling root growth responses to high ambient temperature.[32] In addition, HY1 has been found to functionally interact with HY5 during seedlings development.[47] Here, we confirmed the light-induced HY5 and HYH expression in root relied on HY1 activity, and HY5 and HYH are required for HY1 function in regulating LR branching (Figures 6D-G, S5A and S5B).

HY5 can regulate LRP development by modulating local auxin signaling,[9] and it can also trans-activate the expression of *DFL1* and *SLR/IAA14*, key regulators of auxin signaling and LR initiation (Figure S5C).[38,48] HY1 showed identical effects as HY5 in

DFL1 expression and auxin signaling in the OZ (Figure 6C), in line with its effects in LR branching. Auxin has been hypothesized to act as light-responsive mobile signal to mediate light-triggered root elongation, by the aid of auxin transporters that orchestrate accumulation of auxin at specific tissues to trigger plant organogenesis.[5] We further demonstrated that HY1 is involved in the maintenance of expression and intracellular localization of auxin transporters AUX1, PIN1 and PIN2 (Figures 7D-H), which facilitate PAT to generate an auxin maximum gradient in the root meristem and OZ for root development.[7,9,49] Exogenous NAA can largely recover the auxin signaling and LR branching in *hy1-100* mutant under both light and dark conditions (Figure S7), emphasizing the essential role of auxin for HY1 function in LR branching. Based on these findings, we proposed a HY1-HY5-auxin signal module that mediates light-regulated LR branching. In this model, light responsive HY1 induces the expression of HY5 and HYH to modulate auxin homeostasis at the OZ through the auxin transporter-mediated PAT, in turn activating the oscillation signal resulting in periodic LR branching (Figure 7I).

However, we cannot exclude the involvement of other molecules for this process. Sucrose, a major product during photosynthesis, is suggested to be necessary and sufficient for the regulation of LRP and PR development by light.[4,7] However, sucrose supply was unable to rescue LR branching and PR elongation in *hy1-100* mutant, thus is not accounted for HY1 function in LR branching. Interestingly, HY1 is a localized to plastids (Figures 4B, 4D and S3B) and also a regulator of reactive oxygen species (ROS) and redox signals in plants.[50] Recently, a metabolic study searching for light-induced

systemic signaling has identified a cluster of ROS-associated molecules, that change in concert with the light-mediated photomorgenic responses[51]. ROS has been suggested to act downstream of auxin to trigger LR formation,[52] and two regulator of redox signal, Glutathione Reductase 2 and NADPH-thioredoxin Reductase C, are also localized to plastid and essential for root development.[53,54] GR2 function is also required for maintenance of auxin signaling and PIN expression in root meristem.[53] However, little information is available on the interplay between HY1 and ROS signal during LR formation, and future studies will be required to characterize the role of these metabolites in HY1-mediated root responses.

Root branching and elongation is critical for seedlings to explore nutrient and water in the soil. Under nature condition, the germinating seedlings are covered by soil in dark or limited light conditions, and root growth is only activated to branch and elongate when the shoot perceives light signal. Our study revealed a molecular mechanism how shoot-derived signals transduce external light signals to the pre-pattern signal for repetitive prebranch site formation, and identified a critical role of *HY1* in mediating light-promoted oscillation signal and prebranch site formation. We provide genetic evidence of its tissue-specific action in regulating LR branching, and further suggest a "HY1-HY5-auxin" signal cascade that coordinates shoot- and root-derived signals to regulate periodic LR branching. In this module, auxin most likely acts as the shoot-to-root mobile signal that transmits the photoreceptors-mediated light signaling to a development signal thereby fine-tuning the photomorphogenic responses.

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Supplemental information

Supplemental information includes Supplemental Methods, ten figures, two table, and two movies.

Author contributions

W.X., W.S., and X.D. designed the experiments and wrote the manuscript. X.D. performed most of the experiments and analysis. S.X. helped with vector construction, Y.X. helped with imaging, L.L. helped with grafting experiment, W.Q. helped with phenotyping analysis, B.P. helped with transcriptome analysis. T.C. performed western-blot experiments. All authors discussed the results and contributed in the finalization of the manuscript.

Data availability

All the RNA-seq data presented in this paper, including the raw data, are available in the NCBI with the accession number PRJNA673323 (https://submit.ncbi.nlm.nih.gov/subs/sra/).

Competing interests

The authors declare no competing interest.

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Figure legends

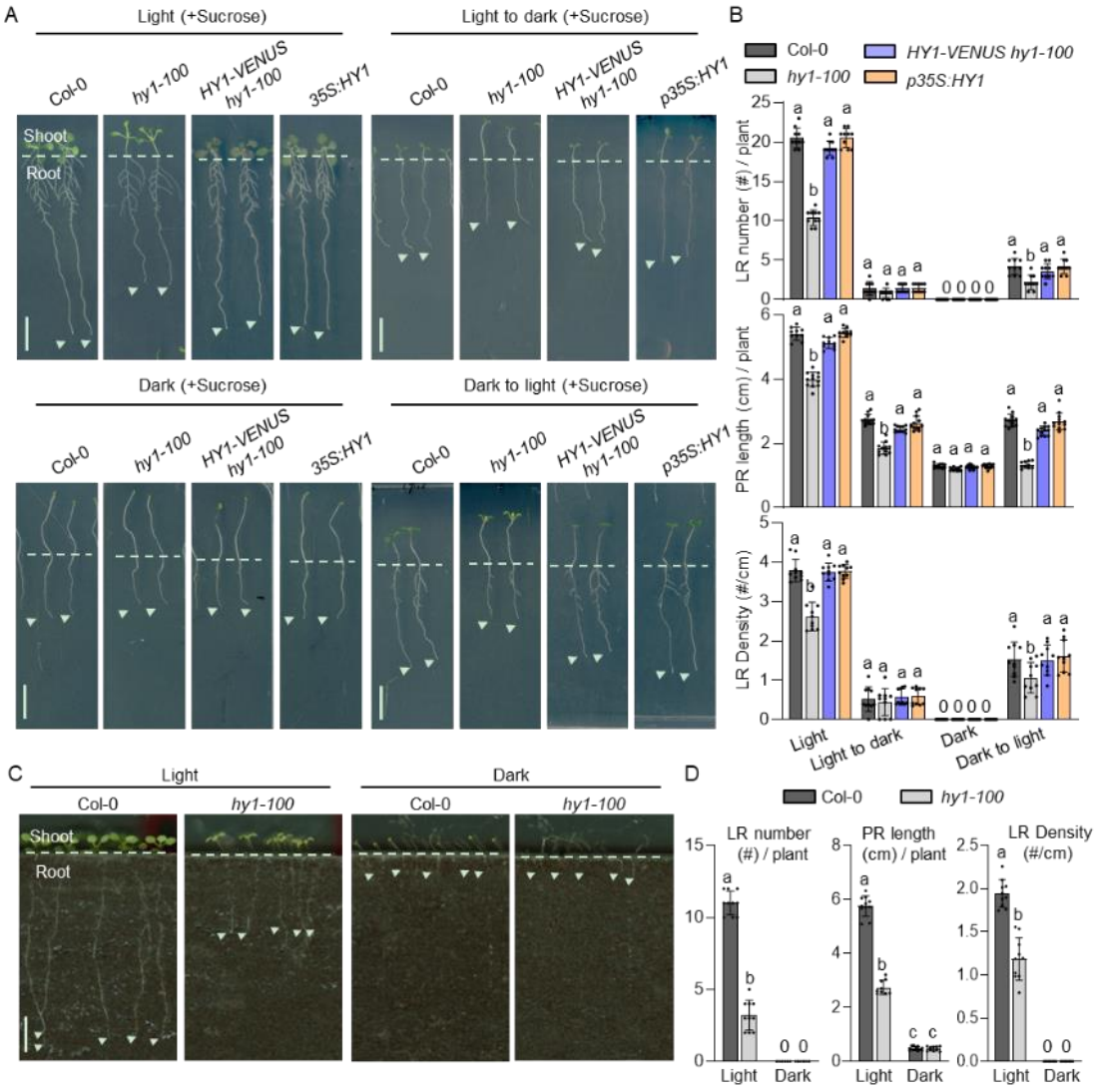

Figure 1. HY1-dependent light signaling is required for LR branching and PR elongation. (A and B) Root phenotype (A) and quantification of LR number, PR length and LR density (#/cm) (B) of Col-0, *hy1-100*, *HY1-VENUS hy1-100* and *p35S:HY1* seedlings grown with sucrose under continuous light or darkness for 5 days and then subjected to light, or light to dark, or darkness, or dark to light transition for another 5 days. (C) Root morphology of wild type and *hy1-100* mutant seedlings grown in rhizotrons for 10 days under light or dark condition. (D) Quantification of LR number, PR length and LR density of Col-0 and *hy1-100* seedlings after 10 days germination in soil. The arrowheads indicate the root tips. The dash lines indicate the shoot-root

junction. 0 stands for Not detected. Error bars represent SD. Different letters represent a significant difference ($P < 0.05$) determined by one-way ANOVA with post hoc Tukey test. Letter code labels the significant differences between the genotypes. (n >10). Scale bar, 1 cm.

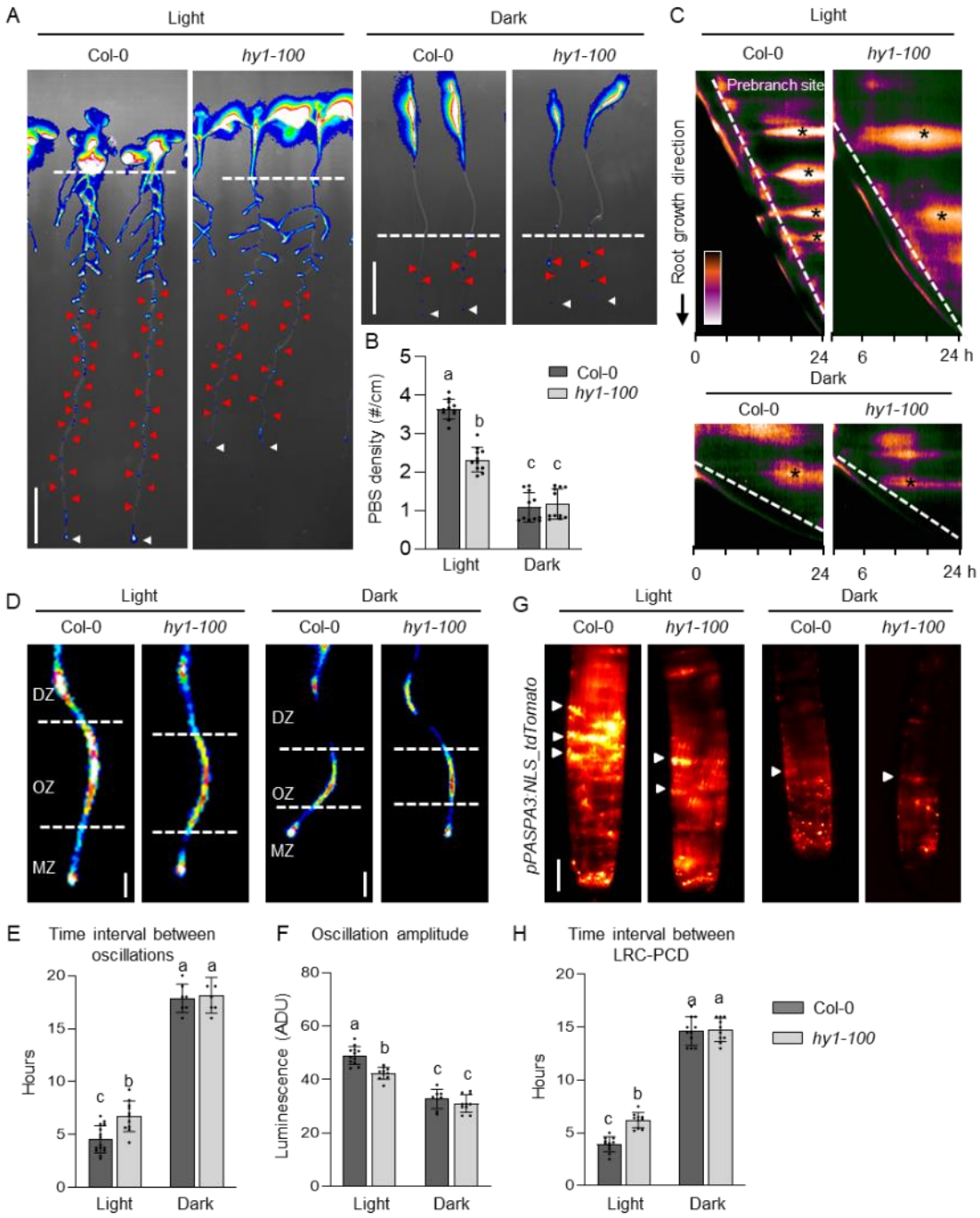

Figure 2. HY1 is involved in the regulation of light signal in oscillation signal and prebranch site formation. (A and B) Analysis of *DR5:Luciferase* expression and quantification of the number of prebranch site in Col-0 and *hy1-100* mutant seedlings after 10 days germination (n >10). Images were overlaid with bright-field images. Asterisks indicate prebranch sites. Scale bar, 1 cm. (C) Kymograph of *DR5:Luciferase*

intensity along the primary root of 3-day-old transgenic seedlings with or without light treatment over 24 hours. DR5 luminescence intensity is color coded (see color code in the bottom left corner of the panels) and plotted following the primary root elongation (y axis) and time (x axis). Dashed lines highlight the position of the oscillation zone. Red arrow indicates prebranch site revealed by persistent *DR5:Luciferase* signal. (D) Expression pattern of *DR5:Luciferase* in the OZ of 3-day-old seedling under light or darkness. MZ, meristem zone; OZ, oscillation zone; DZ, differentiation zone. Scale bar, 100 μm. (E and F) Quantification of the oscillation frequency and the prebranch site number of *DR5:Luciferase* in 3-day-old seedlings (n >10). (G) Stereomicroscopy of *pPASPA3:NLS-tdTomato* expression in the root tips of 3-day-old seedlings under light or darkness. White arrows indicate the *pPASPA3:NLS-tdTomato* stripes in the most-distal LRC. Scale bar, 50 μm. (H) Quantification of the time interval between the consecutive disappearance of *pPASPA3:NLS-tdTomato* stripes in the most-distal LRC (n >8). Error bars represent SD. Different letters represent a significant difference ($P < 0.05$) determined by one-way ANOVA with post hoc Tukey test. Letter code labels the significant differences between the genotypes.

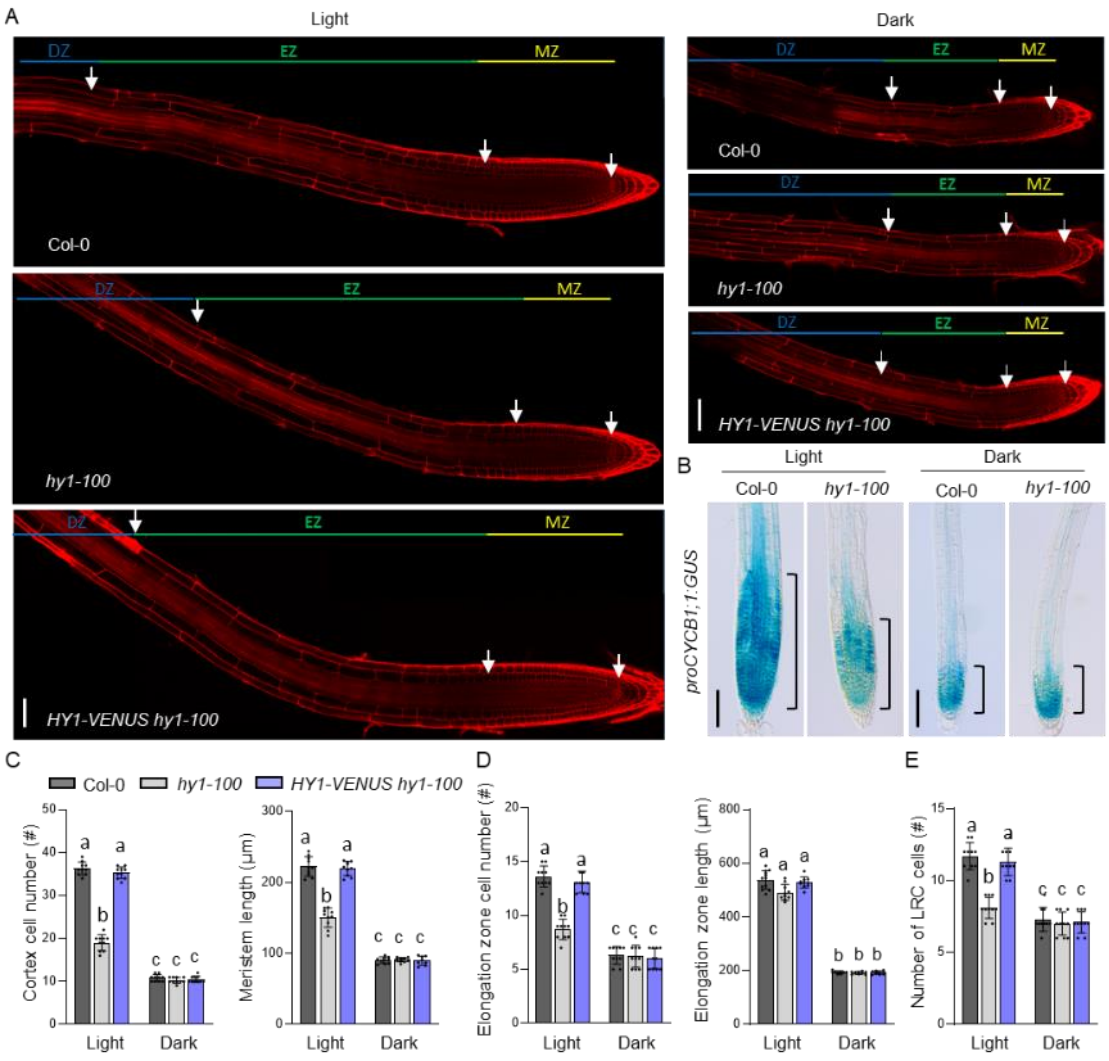

Figure 3. Light-activated root meristem division and cell elongation requires HY1 function. (A) Confocal images of the root elongation and meristem zones in Col-0, *hy1-100*, and *HY1-VENUS hy1-100* seedlings upon light and dark treatments. MZ, meristem zone (yellow line); EZ, elongation zone (green line); and DZ, differentiation zone (blue line). White arrows indicate the position of the stem cells, the first elongated cell in the cortex and the first cell initiating root hair formation. Each image was made by joining several photographs of the same root (n >10). (B) The expression of *proCYCB1;1:GUS* in the wild-type or *hy1-100* root tips with and without light treatment. Brackets indicate a zone of active cell division as highlighted by

proCYCB1;1:GUS expression. (C, D and E) Quantification of cell number and length in the cortex cells, elongation zone cells, and lateral root cap (LRC) in 5-day-old seedlings (n >10). Error bars represent standard deviation (SD). Different letters represent a significant difference ($P < 0.05$) determined by one-way ANOVA with post hoc Tukey test. Letter code labels the significant differences between the genotypes. Scale bar, 50 μm.

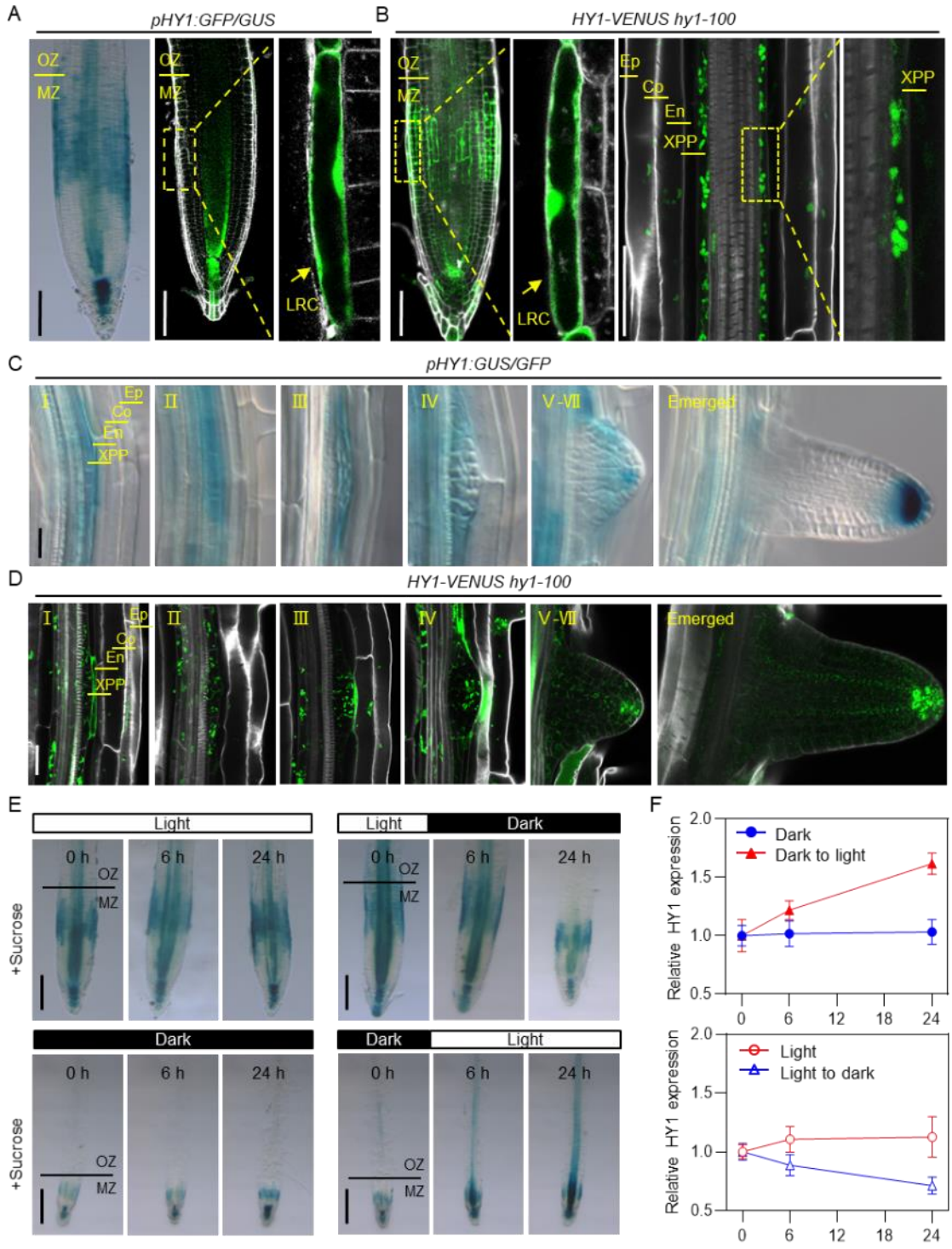

Figure 4. *HY1* exhibits a specific cell-type expression at root and its expression is regulated by light. (A and C) The expression of *pHY1:GUS/GFP* in the root and LRP at different stages. OZ, Oscillation zone; MZ, Meristem zone; LRC, Lateral root cap; Ep, Epidermis; Co, Cortex; En, Endodermis; XPP, Xylem pole pericycle. (B and D)

Subcellular localization of HY1-VENUS protein in the root and LRP. (E) Expression of *HY1* is regulated by light. GUS staining of 5-day-old transcriptional reporter lines *pHY1:GUS/GFP* grown on 1/2 MS with sucrose under light/dark treatment in the root tip. For light-to-dark treatment, the 5-day-old seedlings were grown under continuous light and transferred to the dark or kept in light for 24 h (100 μmol m^{-2} s^{-1}). For dark-to-light treatment, the 5-day-old seedlings were grown in darkness and switched to the light or kept in darkness for 24 h. (F) Comparison of *HY1* gene expression patterns. For light to dark, the 5-day-old seedlings were grown under continuous light at 22 ℃ and then transfer to dark for 24 h before samples collection at the indicated time points. For dark to light, the 5-day-old seedlings were grown under darkness at 22 ℃ and then transfer to light for 24 h before samples collection at the indicated time points. White bars represent the illuminated time and black represent the dark period. Scale bar, 50 μm.

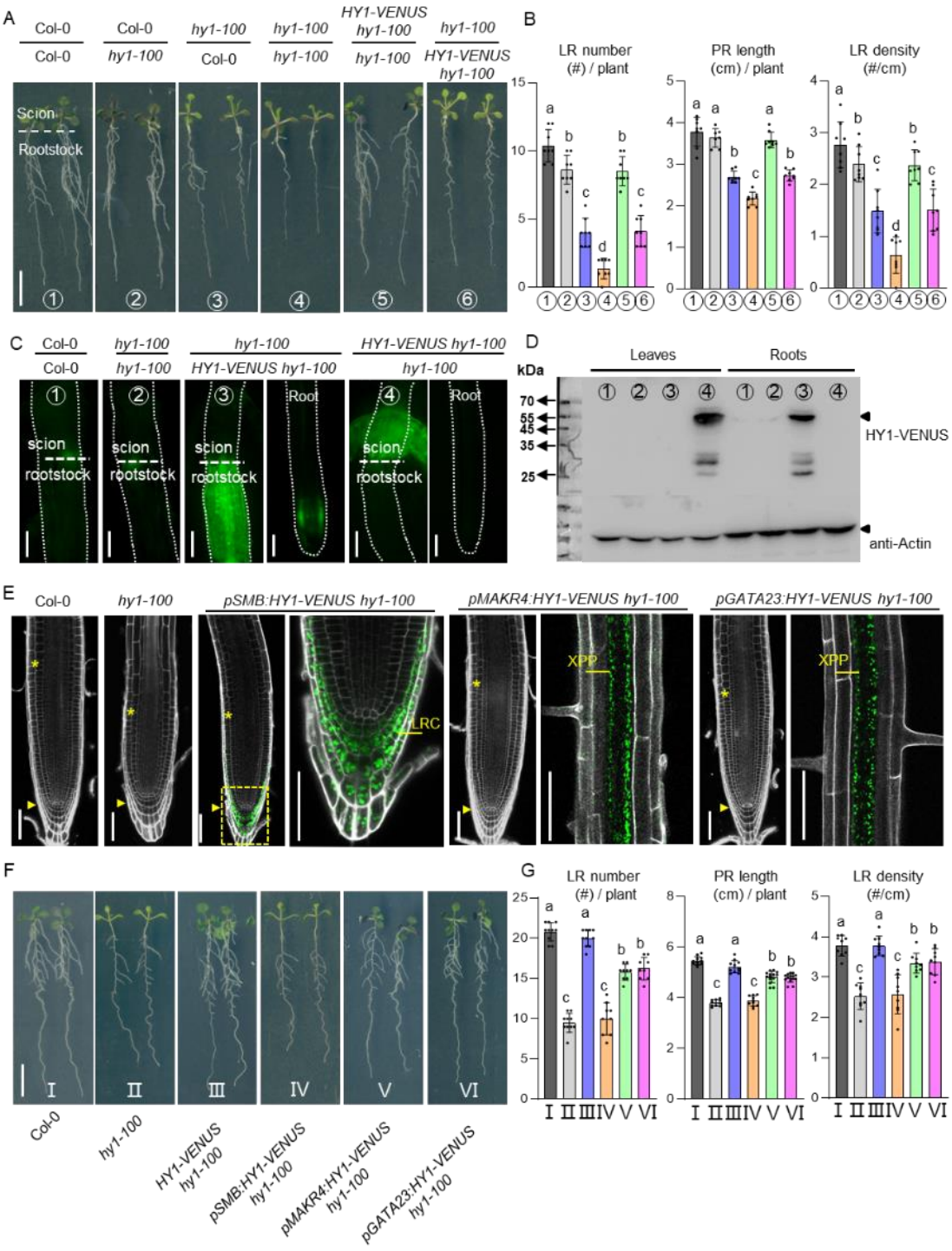

Figure 5. HY1 is not a shoot-to-root mobile signal and shoot- and root-derived HY1 both promote LR branching and PR elongation. (A and B) Root phenotype of Col-0, *hy1-100* and *HY1-VENUS hy1-100* reciprocal grafted seedlings. 5-day-old seedlings were kept in LD for another 10 days after grafting. The LR number, PR length

and LR density of indicated genotypes were measured. The dash line indicate the graft junction as scion/rootstock (n > 8). Scale bar, 1 cm. (**C**) HY1-VENUS protein is undetectable either in the scion or the rootstock of grafted seedlings. The dotted line indicates the root outline. The dash line indicate the graft junction as scion/rootstock. Scale bar, 250 μm. (D) Western blot analysis of HY1-VENUS either in the scion or the rootstock of grafted seedlings extracts using anti-GFP, with anti-Actin as a loading control. (E) Confocal images of root meristem zone and localization of seedlings expressing HY1-VENUS under the control of different tissue-specific promoters as shown in A. LRC, Lateral root cap; XPP, Xylem pole pericycle; asterisks indicate the approximate position where cells begin to elongate noticeably; White arrowheads indicate the quiescent center. Scale bar, 50 μm. (F and G) Root phenotype and quantification of LR number, PR length and LR density in 10-day-old light grown Col-0, *hy1-100*, *HY1-VENUS hy1-100, pSMB:HY1-VENUS hy1-100, pMAKR4:HY1-VENUS hy1-100,* and *pGATA23:HY1-VENUS hy1-100* seedlings (n>10). Scale bar, 1 cm. Error bars represent SD ($P < 0.05$) by one-way ANOVA and Tukey's test as post hoc analysis. Letter code labels the significant differences between the genotypes.

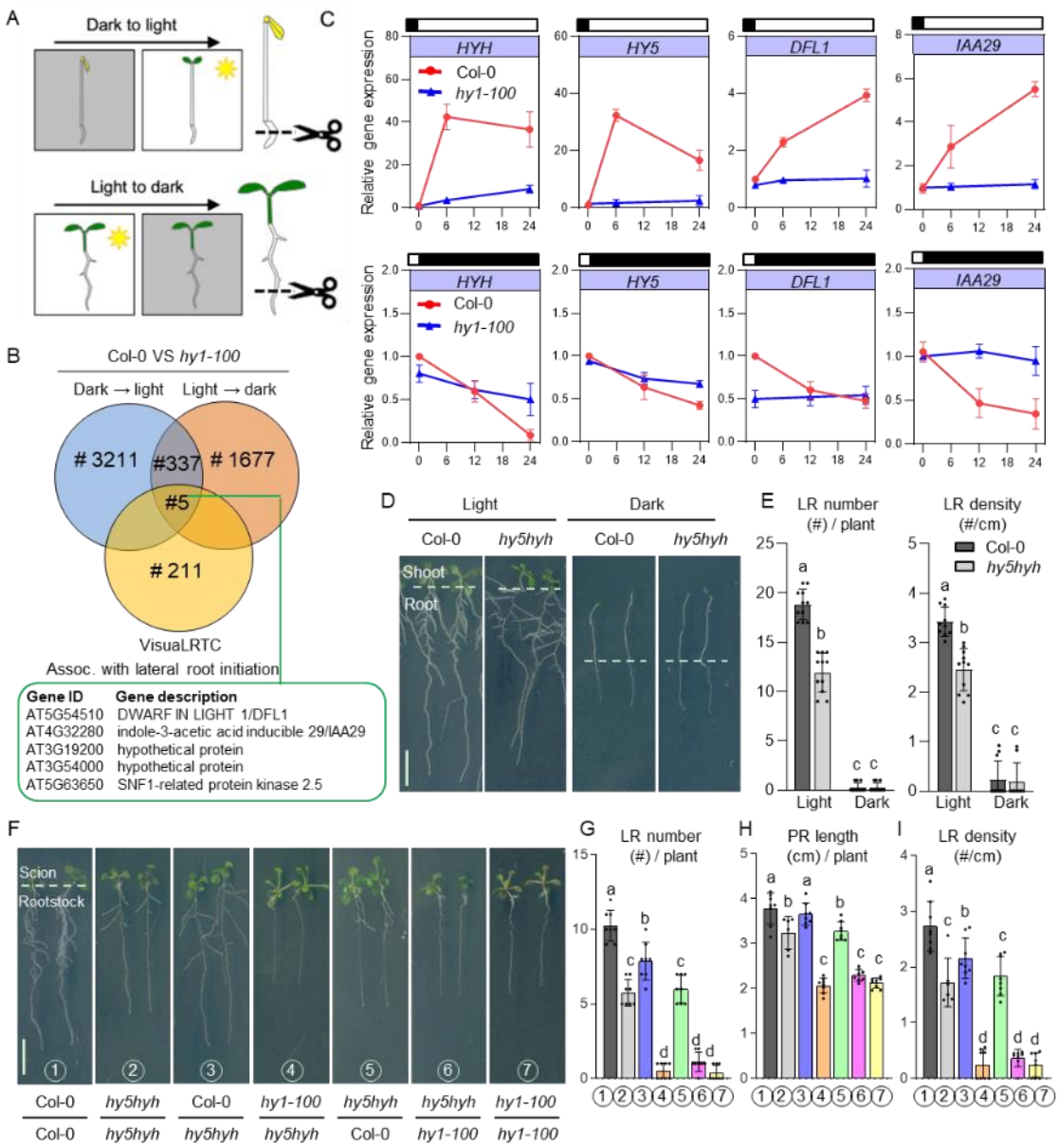

Figure 6. HY5 and its homologue HYH act downstream of the HY1 to regulate LR development. (A) Schematic representation of the seedlings with an indication of the root tip used for transcript profiling of Col-0 and *hy1-100* mutant seedlings after dark to light or light to dark treatment. RNA-seq analysis: RNA was extracted from the primary root tips of 7-day-old light- and dark-grown seedlings exposed to light or dark for 24 hours. (B) A Venn diagram displaying the clustering of *hy1*-dependent DEGs among the three datasets: DEGs of dark to light, DEGs of light-to-dark-, and

VisuaLRTC-based LRI-associated transcriptome datasets[34] (See Supplemental Methods). (C) Comparison of *HYH*, *HY5*, *DFL1* and *IAA29* gene expression patterns in the root tip of Col-0 and *hy1-100* mutant under dark to light or light to dark transition. White bars represent the illuminated time and black represent the dark period. Error bars represent SD of biological triplicates. (D and E) Root phenotype and LR number and density of Col-0 and *hyhhy5* mutant under light or darkness after 10 days germination (n>10). The dash lines indicate the shoot-root junction. (F-I) Col-0, *hy1-100* and *hy5hyh* seedlings grown in LD for 5 days were used for reciprocal grafting. Seedlings were kept in LD for another 10 days after grafting. The LR number, PR length, and LR density of indicated genotypes were measured (n > 8). The dash line indicate the graft junction as scion/rootstock. Scale bar, 1 cm. Error bars represent SD. Different letters represent a significant difference ($P < 0.05$) determined by one-way ANOVA with post hoc Tukey test. Letter code labels the significant differences between the genotypes.

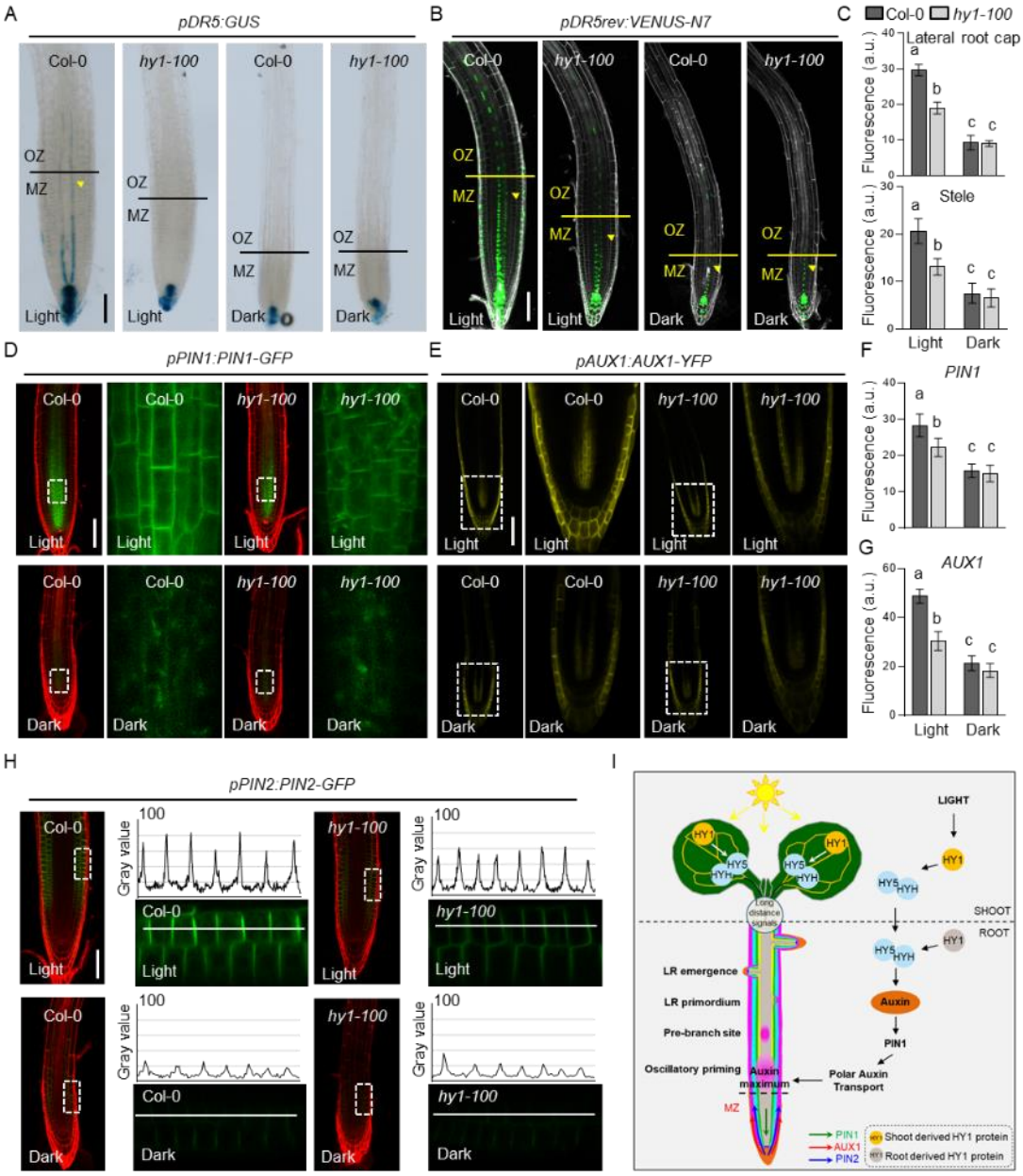

Figure 7. *HY1* enhances auxin accumulation by maintaining expression and intracellular localization of auxin transporters. (A) Expression of of *DR5:GUS* in root meristem zone of Col-0 and *hy1-100* mutant with or without light treatment (n >10). (B and C) Confocal images and quantification of *DR5rev:VENUS-N7* signal in the LRC and stele cells of 5-day-old seedlings under light or darkness treatment (n >10). Yellow arrows indicate the DR5 signal in the stele of the root tips. MZ, meristem zone. OZ, oscillation zone. EZ, elongation zone. DZ, differentiation zone. (D and E) Localization

of PIN1-GFP (D) and AUX1-YFP (E) in the root meristem zone of 5-day-old light- and dark-grown Col-0 and *hy1-100* seedlings (n >10). (F and G) Quantification of PM PIN1-GFP (F) and AUX1-YFP (G) levels in Col-0 and *hy1-100* seedlings grown as shown in D, E. (H) PIN2-GFP expression and quantification of PM PIN2-GFP levels in 5-day-old light- and dark-grown Col-0 and *hy1-100* seedlings (n >10). Graphs represent PM PIN2-GFP fluorescence intensity calculated along the white lines shown below. (I) A proposed working model depicting the HY1 regulatory module mediates light signaling. Error bars represent SD. Different letters represent a significant difference ($P < 0.05$) determined by one-way ANOVA with post hoc Tukey test. Letter code labels the significant differences between the genotypes. Scale bar, 50 μm.