

Phytochrome regulates translation of mRNA in the cytosol

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An array of photoreceptors including cryptochromes, phototropin, and phytochromes regulates various light responses in plants. Among these photoreceptors, phytochromes perceive red and far-red light by switching between two interconvertible spectral forms (Pr and Pfr). The Pfr form promotes light responses partly by destabilizing negatively acting, phytochrome-interacting basic helix-loop-helix transcription factors (PIFs), thus modulating transcription in the nucleus. The Pfr form is also present in the cytosol. However, the role of phytochromes in the cytosol is not well understood. Here we show that the Pfr form interacts with the cytosolic protein PNT1 and inhibits the translation of protochlorophyllide reductase (*PORA*) mRNA. PNT1 possesses five C3H-type zinc finger domains and displays similarity to various RNA binding proteins including Tristetraprolin, which regulates stabilities of mRNAs such as TNF- α mRNA in humans. Consistent with its function as an RNA binding protein, PNT1 directly binds to mRNA of a key chlorophyll biosynthetic gene, protochlorophyllide reductase in vivo and inhibits the translation of *PORA* mRNA in the presence of phytochromes. The present results demonstrate that phytochromes transmit light signals to regulate not only transcription in the nucleus through PIFs, but also translation in the cytosol through PNT1.

light signaling | protein–protein interaction | post transcriptional regulation | translational regulation | nucleocytoplasmic shuttling

Phytochromes are dimeric proteins consisting of an apoprotein and a chromophore. They are synthesized in the cytosol in the Pr form, which can be converted into the Pfr form by red light and back into Pr by far-red light (1). Pfr translocates into the nucleus and inhibits a set of negatively acting phytochrome-interacting basic helix-loop-helix transcription factors (PIFs), partly by destabilizing them (2–7). Among PIFs, four PIFs (PIF1, PIF3, PIF4, and PIF5) play key negative roles to repress light responses in the dark. A *pif* quadruple mutant (*pif1/pif3/pif4/pif5*, collectively referred as quadruple mutants *pifq*) is therefore constitutively photomorphogenic and expresses many light responsive genes, even in the dark (8–10). However, dark-grown quadruple mutant seedlings still have longer hypocotyls than light-grown wild-type seedlings and the correlation coefficient of gene expression is only 0.7 between dark-grown *pifq* mutant seedlings and red light-grown wild-type seedlings (9, 10). This implies that phytochromes transmit light signals not only through these four PIFs, but also through other phytochrome-interacting proteins such as PIF7 and PHYTOCHROME KINASE SUBSTRATE 1 (PKS1) (8, 11, 12).

Ever since researchers discovered the nuclear translocation of Pfr and identified the nuclear-localized PIFs, the nucleus has been considered the focal site of phytochrome signaling (13). Mutant or transgenic phytochromes that are localized to the cytosol are unable to induce the majority of light responses, including hypocotyl elongation (14–16), supporting the notion that the phytochromes mainly function in the nucleus. The PIFs, which are transcription factors, also function in the nucleus. A genome-wide chromatin immunoprecipitation (ChIP-chip) study coupled with microarray analysis showed that one of the PIFs (PIF1) bound to 748 sites in vivo and regulated the expression of

166 target genes (105 positively and 61 negatively) in imbibed seeds (17). Among PIF1 binding sites, 59% were found to possess G-box elements (CACGTG). Other PIFs have also been shown to bind to individual G-box elements both in vitro and in vivo and to regulate the expression of target genes (18–25). Collectively, these previous results indicate that phytochromes regulate light responses through PIFs in the nucleus.

However, a few lines of evidence suggest that the cytosol should also be considered as a site of phytochrome action and processes occurring in the cytosol as a target of phytochrome signaling. First, cytoplasmic motility is accelerated by red light within a few seconds (26). Second, ion flux is also changed very rapidly by red light (27, 28). Third, hypocotyl negative gravitropism and red-enhanced phototropism are partly regulated by cytoplasmic phytochrome A (phyA) (29). Consistent with the evidence of cytoplasmic phytochrome signaling, phytochromes are present in the cytosol. The Pr form is abundantly present in the cytosol and the presence of the Pfr form of phytochrome is also present in the cytosol, as indicated by the sequestering of the Pfr of phyA in the cytosol and the slow accumulation of phytochrome B (phyB) nuclear speckles after red light irradiation (30–32). In addition, PKS1, which negatively regulates phytochrome signaling, was shown to interact with both phytochrome and phototropin 1 in the cytosol (11, 33). The molecular function of PKS1 has not been fully elucidated.

Translation is one of the cytosolic processes that could be regulated by phytochromes. To investigate this possibility, we sought to identify phytochrome-interacting proteins that might control translation in the cytosol. The translation of most eukaryotic mRNAs is initiated by attachment of the 43S pre-initiation complex (comprising the 40S subunit, the eIF2 complex, eIF3, eIF1, and eIF1A) to the capped 5' end region of the mRNA (34, 35). This attachment is facilitated by the eIF4F complex, which binds to the cap. Once the 43S complex is attached, it scans downward to find the initiation codon, where it is subsequently joined by the 60S subunit to form the elongation-competent 80S ribosome. In animals, translation is extensively controlled by different mechanisms, including protein modification of eIFs and the selection of specific mRNAs by RNA-binding proteins. Translational control also occurs in plants, where it plays important roles in various plant processes, including the responses to environmental stress, pathogens, and hormonal signaling. However, the role of phytochromes in translational control is not yet known (36, 37). Here, we report that the Pfr forms of phyB can interact with a cytosolic zinc finger protein that binds

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to the 5'-UTR of the protochlorophyllide reductase (*PORA*) mRNA to light-dependently inhibit translation of the *PORA* mRNA. Our results demonstrate that phytochromes can control gene expression not only through transcriptional regulation in the nucleus, but also through translational control in the cytosol.

Results

PENTA1 Is a Cytosolic Phytochrome-Binding Protein That Promotes Far-Red (FR) Block of Greening. In the present study, a putative cytosolic phytochrome-interacting protein was identified through yeast two-hybrid screening and named PENTA1 (PNT1) on the basis of its structure, which includes five C3H-type zinc finger motifs (Fig. 1A). The five CX₈CX₅CX₂H motifs are present in two clusters that consist of three N-terminal zinc finger motif clusters and two C-terminal zinc finger motif clusters. Proteins

with multiple C3H-type zinc finger motifs are found in various eukaryotic proteins ranging from yeast and rice to human proteins, and some of which have been shown to be RNA binding proteins (38, 39). A database search indicated that *Arabidopsis* contains 10 proteins with similar multiple zinc finger motifs and rice contains 7 proteins. (Fig. S1). One of them, known as ENHANCER OF AG-4 1 (HUA1), was shown to bind an intron of *AGAMOUS* mRNA to regulate the splicing (40).

The interaction between PNT1 and phytochrome was further probed by in vivo coimmunoprecipitation assay using transgenic plants expressing either GFP-tagged PNT1 for phyA or both GFP-tagged PNT1 and myc-tagged phyB for phyB. PNT1-GFP was functional as its phenotype is similar to transgenic lines expressing *PNT1* without any tag (*PNT1-OX1*). PhyB-myc was also functional (Fig. S2). PNT1-GFP was immunoprecipitated with anti-GFP antibody after either red or far-red light pulse and the precipitated phyA was detected by anti-phyA antibody, whereas the precipitated phyB-myc was detected by anti-myc antibody. The assay showed that PNT1 binds preferentially to the Pfr form of both phyA and phyB (Fig. 1B). A subcellular localization of PNT1 was determined by transgenic plants expressing PNT1-GFP. Similar to PKS1, PNT1 is localized in the cytosol rather than in the nucleus and red or far-red light did not change its subcellular localization (Fig. 1C). Taken together, these results indicate that PNT1 is a cytosolic C3H-type zinc finger protein that interacts preferentially with the Pfr form of both phyA and phyB.

Mutation and overexpression of *PNT1* alter a subset of phytochrome-mediated light responses. Among these light responses, FR block of greening was strongly enhanced in *PNT1* overexpressing lines, whereas it was mildly suppressed in two *pnt1* mutant alleles with different ecotype backgrounds (Col-0 for *pnt1-1* and WS-2 for *pnt1-2*) (Fig. 2A). The FR block of greening response is caused by photobleaching when FR-grown seedlings are transferred to white light (41). The FR block of greening was quantified by measuring the amount of produced chlorophyll after transferring to white light. *PNT1-OXs* (*PNT1-OX1*, *PNT1-GFP1*) accumulated chlorophyll at approximately half the rate of wild type when FR-grown seedlings older than 3 d were transferred to white light, whereas the *pnt1* mutants accumulated more chlorophyll than wild-type seedlings (Fig. 2B). A previous study showed that gibberellins (GA) promote photobleaching by repressing the expression of all *POR* mRNAs (42). GA promoted photobleaching both in wild type and the *pnt1* mutant (Fig. S3), suggesting that they function independently. Unlike the FR block of greening, light-dependent hypocotyl elongations were not affected by *PNT1* (Fig. S4).

Phytochrome and PENTA1 Inhibit the Translation of *PORA* mRNA Light Dependently. In previous studies, FR-grown seedlings were shown to photobleach when transferred to white light, partly because phyA represses the expression of *PORA* mRNA (41), which leads to an imbalance between chlorophyll intermediates and protochlorophyllide reductase (POR) under FR conditions. Irrespective of *PNT1* mutation, all plants expressed similar levels of two key chlorophyll biosynthetic genes, *HEMA* and *GUN5* (Fig. 3A). *PORA* mRNA levels were also either similar in the *pnt1* mutant or slightly higher in the *PNT1-OXs* under far-red light conditions (Fig. 3A). These results suggest that PNT1 does not promote the FR block of greening through transcriptional repression of *PORA* gene or activation of other chlorophyll biosynthetic genes.

Instead, PNT1 was found to inhibit the translation of *PORA* mRNA in the light. Western blot analysis indicated that *PORA* protein levels were similar or slightly higher in the *pnt1* mutant, but much lower in the *PNT1-OXs* than in the wild type, both under red and far-red light conditions (Fig. 3B). The decrease in *PORA* protein levels could be caused by the repression of *PORA* mRNA translation. The effect of PNT1 on the inhibition of

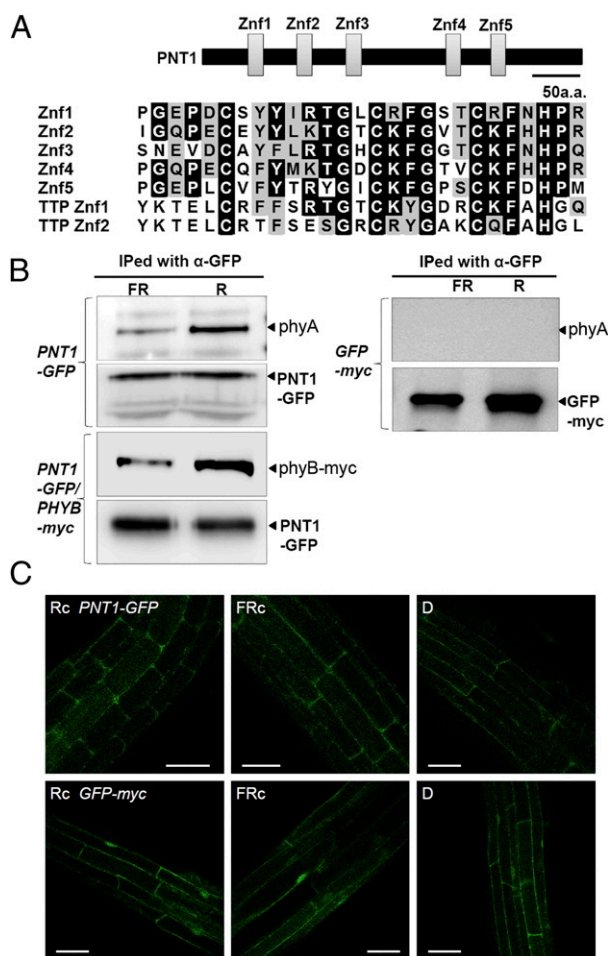


Fig. 1. Phytochromes interact with PENTA1 (PNT1), a C3H-type zinc finger protein. (A) Diagram showing the five zinc finger motifs in PNT1 and their amino acid sequence alignment. The five zinc finger motifs are indicated by boxes. The amino acid sequences of the five zinc finger motifs (Znf1 to Znf5) are aligned with two zinc finger motifs (TTP Znf1 and TTP Znf2) found in human Tristetraprolin (NP_003398). (B) In vivo coimmunoprecipitation assay showing the preferential interaction between PNT1 and the Pfr form of phyA and phyB. PNT1-GFP or GFP were immunoprecipitated with an anti-GFP antibody. PhyA was detected with an anti-phyA antibody and phyB-myc was detected with anti-myc antibody. The precipitated PNT1-GFP or GFP were detected with an anti-GFP antibody. FR and R indicate plant samples irradiated with far-red (2.4 $\mu\text{mol}/\text{m}^2/\text{s}$, 15 min) and red light (4.4 $\mu\text{mol}/\text{m}^2/\text{s}$, 15 min), respectively, before immunoprecipitation. (C) Confocal microscopic images showing the cytosolic localization of PNT1-GFP irrespective of light conditions. D, dark; Rc, continuous red light; FRC, continuous far-red light. GFP alone was used as a control.

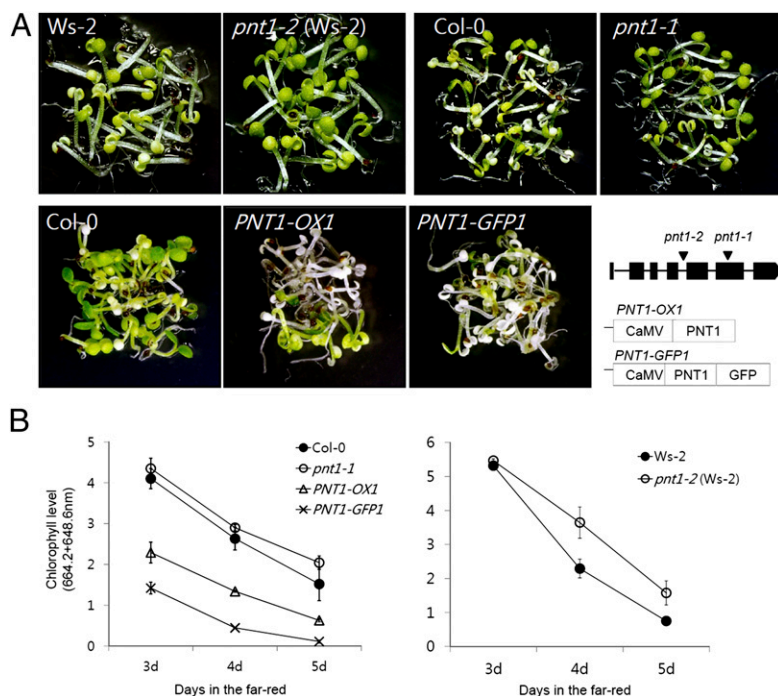


Fig. 2. PNT1 regulates a subset of phytochrome-mediated light responses. (A) Seedlings displaying more severe (*PNT1-OXs*) or less severe [*pnt1* mutants: *pnt1-1* (Col-0) and *pnt1-2* (*Ws-2*)] photobleaching than wild-type plants (Col-0 and *Ws-2*) are shown. FR (2.4 $\mu\text{mol}/\text{m}^2/\text{s}$)-grown seedlings were transferred to white light (100 $\mu\text{mol}/\text{m}^2/\text{s}$) for 4 d. Genomic structure of *PNT1* with two T-DNA insertion sites (inverted triangles) and transgenes for two *PNT1-OXs* are shown. (B) Quantification of remaining chlorophylls after transferring FR-grown seedlings of various ages to white light for 4 d (SD, $n = 3$).

the *PORA* mRNA translation was further investigated by constructing a luciferase reporter gene with or without the 5'- and 3'-UTRs of the *PORA* gene under the constitutive 35S promoter and generating transgenic *Arabidopsis* plants. Compared with transgenic plants harboring a luciferase reporter gene without UTRs, transgenic plants harboring the luciferase gene with *PORA* UTRs showed decreased luciferase activities both under red and far-red light conditions but not in the dark (Fig. 3C). This light-induced decrease of luciferase activity was not due to a decreased stability of luciferase mRNA with UTRs, as levels of luciferase mRNA were similar irrespective of the UTRs (Fig. 3D). The role of PNT1 was also investigated by generating transgenic *pnt1* mutants harboring the reporter gene with *PORA* UTRs. Luciferase activities were decreased less by red and far-red light in the *pnt1* mutant than in wild-type plants, indicating that PNT1 is partially responsible for the translational repression in red and far-red light (Fig. 3E). The partial release of the repression in the *pnt1* mutant may be caused by the presence of additively acting PNT1 homologs (Fig. S1).

To further determine which UTR is necessary for the translational repression of *PORA* mRNA, constructs containing the luciferase reporter gene with the 5'-UTR alone or the 3'-UTR alone were generated and a transient expression assay using protoplasts was performed (Fig. 3F). In constructs containing no UTRs or only the 3'-UTR fused to the reporter gene, luciferase activities were not decreased by light. However, when the 5'-UTR or both the 5'-UTR and 3'-UTR were fused, luciferase activities were strongly decreased by light. The correct sequence of the 5'-UTR was required to repress the translation of the reporter mRNA, as insertion of the 5'-UTR in the reverse direction had no effect. These results suggest that PNT1 represses the translation of *PORA* mRNA through its 5'-UTR.

The role of phytochromes in light signaling-mediated inhibition of the translation of the reporter gene with *PORA* UTRs was investigated using a transient expression assay. Red light reduced luciferase activity only partially in the *phyB* mutant (Fig.

3G), suggesting that phyB is not the only phytochrome-mediated red light signaling. A *phyA phyB* double mutant did not show any inhibition by red light, suggesting that both phyA and phyB additively mediate red light signaling to inhibit the translation of *PORA* mRNA. Continuous far-red light also reduced luciferase activity in wild type but not in *phyA* mutants, supporting the role of phyA in mediating far-red light signaling. Consistent with the inhibitory role of phytochromes on the translation of *PORA* mRNA, the overexpression of phyB caused more severe photobleaching in a PNT1-dependent manner (Fig. S5). Taken together, the present results indicate that phytochromes mediate red and far-red light signaling to inhibit the translation of *PORA* mRNA in the cytosol.

To demonstrate that cytosolic phytochromes are capable of inhibiting the translation, we introduced the same luciferase reporter gene with the *PORA* UTRs into *phyA* mutant and *fhy1/fhl* double mutant to generate stable transgenic lines. Due to the lack of carrier proteins, phyA protein is localized in the cytosol of the *fhy1/fhl* double mutant (29, 43). Compared with the *phyA* mutant, luciferase activities were decreased in the *fhy1/fhl* double mutant both under red and far-red light conditions but not in the dark (Fig. 3H). Because the inhibition of translation under far-red light condition is solely caused by phyA (Fig. 3G), the results further support that cytosolic phyA can inhibit the translation.

PNTA1 Binds to 5'-UTR of *PORA* mRNA and Recruits Phytochrome to Inhibit the Translation. PNT1 possesses a putative RNA binding domain, suggesting that PNT1 binds to *PORA* mRNA in vivo. RNA immunoprecipitation (RIP) analysis was performed using transgenic plants expressing PNT1-GFP and the luciferase reporter gene sandwiched between the 5'-UTR and 3'-UTR of the *PORA* gene. Immunoprecipitation of PNT1-GFP by an anti-GFP antibody precipitated high levels of endogenous *PORA* mRNA compared with levels of *PP2A* mRNA (Fig. 4A), indicating that PNT1 binds to *PORA* mRNA in vivo. To further investigate the light dependency of the association between PNT1 and *PORA*

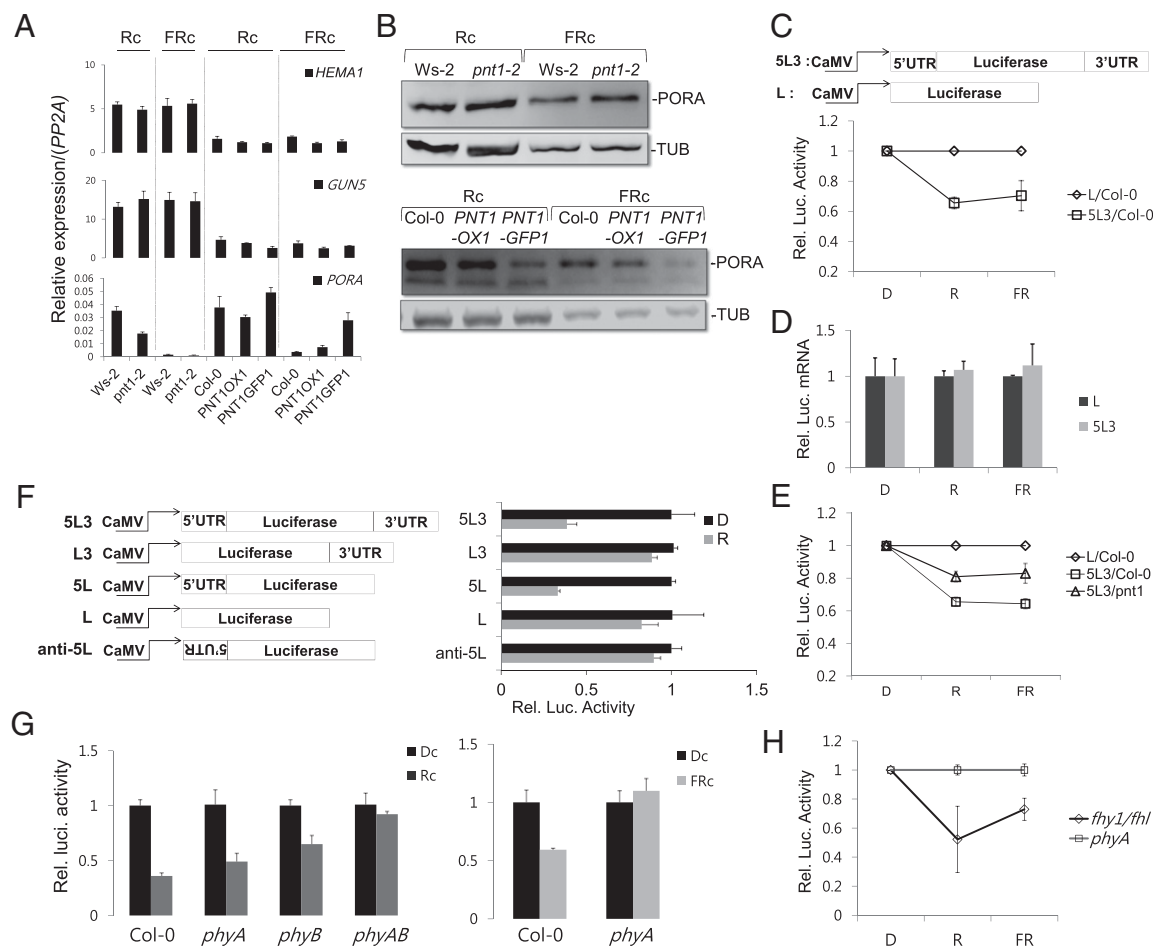


Fig. 3. Phytochromes inhibit the translation of *PORA* mRNA through *PNT1*. (A) Expression levels of chlorophyll biosynthetic gene mRNAs in *pnt1* and *PNT1-OX1*s. Results are expressed as relative levels of *PP2A* mRNA. Four-day-old red light-grown (Rc) or FR-grown seedlings were used for the expression analysis. (B) Altered POR protein levels in *pnt1* and *PNT1-OX1*. Tubulin was used as a loading control. (C) Light-dependent decrease of luciferase activity in transgenic *Arabidopsis* harboring a luciferase reporter gene with 5'- and 3'-UTRs of *PORA* (5L3) in comparison with a luciferase gene without any UTRs (L). Upper diagrams describe the 5L3 and L constructs. Relative luciferase activities of L samples were taken as one for each light condition. (D) Similar levels of luciferase reporter mRNAs irrespective of UTRs in transgenic *Arabidopsis*. (E) Reduced light-dependent decrease of luciferase activity in the *pnt1* mutant harboring a luciferase reporter gene with 5'- and 3'-UTRs of *PORA* (5L3). (F) Sufficiency of 5'-UTR of *PORA* for the translational inhibition of luciferase reporter gene in protoplasts. Various reporter constructs are shown. Relative luciferase activities of dark samples were taken as one for each construct. (G) Abolishment of light-dependent translational inhibition of the luciferase reporter with UTRs of *PORA* in protoplasts of phytochrome mutants. Relative luciferase activities of dark samples were taken as one for each phytochrome mutant. (H) Light-dependent decrease of luciferase activity in transgenic *fhy1/fhl* double mutant harboring a luciferase reporter gene with *PORA* UTRs. Relative luciferase activities of *phyA* mutant samples were taken as one for each light condition (SD, $n = 3$).

mRNA, transgenic plants harboring a luciferase reporter gene with the *PORA* UTRs were used. The RNA-IP assay showed that *PNT1* precipitated high levels of the luciferase reporter mRNA irrespective of the light conditions, whereas it did not enrich the mRNA of the *HPTII* transgene (present in the same T-DNA with the luciferase reporter gene) (Fig. 4B). These results indicate that *PNT1* is a phytochrome-interacting protein that binds to *PORA* mRNA through its UTR in vivo irrespective of light conditions.

Phytochromes do not regulate the binding of *PNT1* to its target RNA as evidenced by the light-independent binding of *PNT1* to the reporter gene containing the UTRs of *PORA*. Phytochromes also do not regulate the protein stability of *PNT1*, which is in contrast with the degradation of *PIF3* under light conditions (Fig. 4C). Instead, *phyB* was found to be recruited to *PORA* mRNA through *PNT1*. The RNA-IP of *phyB*-myc with an anti-myc antibody enriched the *PORA* mRNA fraction at much higher levels in *PNT1-OX1* than in the *pnt1* mutant (Fig. 4D). Consistent with the preferential binding of the Pfr form to *PNT1*,

the binding of *phyB*-myc to *PORA* mRNA was increased by red light (Fig. 4E). Taken together, these results indicate that the Pfr form of *phyB* inhibits the translation of *PORA* mRNA after recruited to the 5'-UTR of *PORA* mRNA through *PNT1*.

Discussion

The present results uniquely show that phytochrome directly inhibits translation of mRNA in the cytosol. We show that *PNT1*, possessing multiple C3H-type zinc finger motifs, binds to *PORA* mRNA in vivo and recruits the Pfr form of phytochrome to the 5'-UTR of *PORA* mRNA. The light-dependent recruitment of *phyB*, and presumably *phyA* too, leads to the translational inhibition of *PORA* mRNA in the light (Fig. 4F). Our discovery indicates that both nuclear and cytosolic phytochrome signaling events work toward the same goal of reducing *PORA* protein levels: phytochromes inhibit the transcription of *PORA* through their effect on nuclear signaling events (10, 41, 44, 45), and they inhibit the translation of *PORA* mRNA through cytosolic signaling events. This concerted phytochrome-mediated inhibition

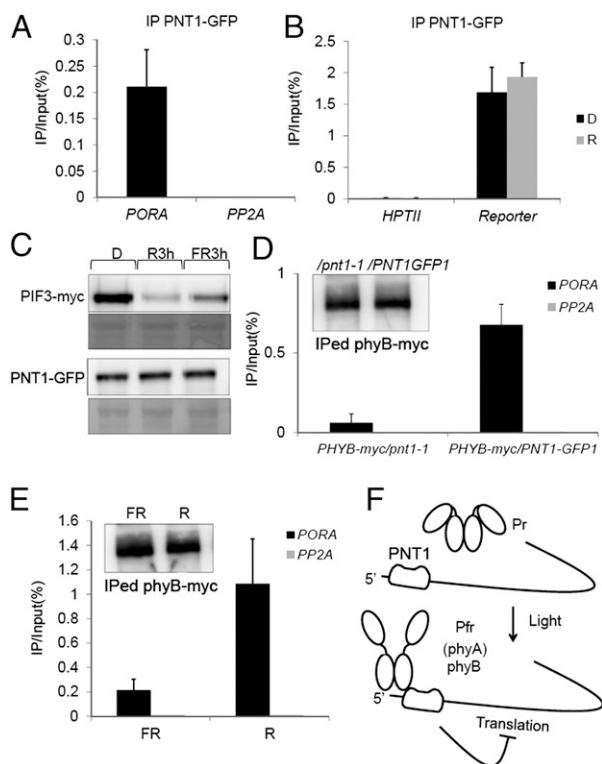


Fig. 4. PNT1 binds to the UTR of *PORA* mRNA and recruits phytochromes to inhibit the translation of *PORA* mRNA. (A) Immunoprecipitation of *PORA* mRNA by PNT1 using RIP. *PP2A* mRNA was used as a nonbinding control. (B) Light-independent immunoprecipitation of luciferase reporter mRNA with UTRs of *PORA* but not *HPTII* mRNA in transgenic plants. (C) PNT1 was not degraded in response to light. Unlike PNT1, PIF3 is rapidly degraded by light. R3h and FR3h indicate 3 h after the transfer of etiolated seedlings to red and far-red light, respectively. (D) PNT1-dependent immunoprecipitation of *PORA* mRNA by phyB-myc. phyB-myc/PNT1-GFP1 and phyB-myc/pnt1 indicate transgenic plants expressing phyB-myc either in the *PNT1-GFP1* line or in the *pnt1-1* mutant. (Inset) Amount of precipitated phyB-myc in different plants. (E) Light-dependent recruitment of phyB to *PORA* mRNA. R and FR indicate samples treated with either red light or far-red light before precipitation. (Inset) Amount of precipitated phyB-myc under different light conditions. (F) Model showing the translational repression of *PORA* mRNA by phytochromes and their interacting protein PNT1 (SD, $n = 3$).

of both transcription and translation is likely to rapidly reduce the level of *PORA* protein during the dark-light transition. Finally, it is noteworthy that Pfr is the functional form of phytochromes in both the nucleus and the cytosol.

Different explanations may account for how phytochromes and PNT1 inhibit the translation of the *PORA* mRNA. The recruitment of phytochromes to the 5'-UTR of the *PORA* mRNA may inhibit the activity of one or more of the eIFs required for loading of the 43S preinitiation complex onto the 5' cap and the subsequent formation of the 48S initiation complex (34, 35). In mammals, the phosphorylation of various eIFs (e.g., eIF1, eIF2, eIF3, eIF4E, and eIF4G) by mammalian kinases can affect the activities of these eIFs. Because phytochromes were shown to have protein kinase activity in vitro (46), the recruited phytochrome may phosphorylate some of the eIFs, thereby inhibiting their activities. Alternatively, the phy-PNT1 complex on the 5'-UTR may inhibit the loading of the 43S preinitiation complex or other proteins onto the 5'-UTR by steric hindrance. A well-known example of this process is the interaction between the iron response element (IRE) in the 5'-UTR of ferritin mRNA and the iron response protein (IRP) (47). When the concentration of iron is low, IRP binds to the IRE and blocks loading of

the 43S preinitiation complex onto the ferritin mRNA (48), thereby inhibiting translation of the ferritin mRNA. Analogous to IRP, the phy-PNT1 complex could inhibit translation by blocking the loading of the 43S preinitiation complex or other protein components. However, future work will be required to unravel the detailed molecular mechanism(s) of translational inhibition by PNT1 and phytochromes.

Our results further suggest that there may be additional cytosolic phytochrome-mediated signaling events. The translational regulation of the *PORA* mRNA does not seem to account for the classical cytoplasmic light responses (e.g., rapid changes in cytoplasmic motility and ion fluxes) (26–28), indicating that there is likely to be cytosolic phytochrome-mediated signaling other than the PNT1-mediated translational control described herein. In addition, although PNT1 was identified as a *PORA*-binding protein, PNT1 may also bind and regulate other mRNAs. PNT1 itself belongs to a small protein family that has 10 members in *Arabidopsis* and 7 members in rice (Fig. S1). It is somewhat analogous to the PIFs, which belong to a subgroup of the bHLH transcription factors having 15 members in *Arabidopsis* and at least 6 members in rice (49, 50). As many PIF family members play key roles in nuclear phytochrome signaling, it would be interesting to determine the potential roles of other PNT1 family members in cytosolic phytochrome signaling.

Methods

In Vivo Pull Down Assay. In vivo pull down assay was performed with 4-d-old dark-grown seedlings. Total proteins were solubilized in extraction buffer (50 mM Tris-Cl pH 7.5, 150 mM NaCl, 10% glycerol, 0.1% Nonidet P-40, 1× complete mini protease inhibitor, 100 μ M MG132) and filtered through a QIAshredder. The filter through was divided into two and anti-GFP antibody was added. Each sample was irradiated with either red (4.4 μ mol/m²/s) or far-red (2.4 μ mol/m²/s) light for 15 min and incubated in the dark for 1 h with gentle rotation at 4 °C. Antibody-bound protein complexes were precipitated by protein A agarose. Beads were recovered and washed three times with 500 μ L binding buffer in spin columns (Pierce). Proteins were eluted and analyzed by Western blot using anti-phyA (Agrisera) and anti-myc (Santa Cruz) antibody. All procedures were performed in the dark or under safety green light.

RIP. All procedures were basically performed as described previously, which was reported in the mammalian cell (51). To optimize RIP in *Arabidopsis* we introduced minor changes. In brief, 2 g of long-day grown *PNT1-GFP1* plants were dark adapted for 24 h before sampling and grinding in liquid nitrogen. Alternatively, 2 g of 4- to 6-d-old dark grown seedlings were used in the experiments. Total proteins were solubilized in the same volume of poly-some lysis buffer (100 mM KCl, 5 mM MgCl₂, 10 mM Hepes pH 7.0, 0.5% Nonidet P-40, 1 mM DTT, 100 units ml⁻¹ RNase inhibitor, 400 μ M VRC, 1× complete mini protease inhibitor). In the case of phyB-myc RIP, we added 100 μ M MG132 to prevent the degradation of phyB-myc during the procedure. The lysate was filtered through two layers of miracloth and diluted two-fold with NT2 buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM MgCl₂, 0.05% Nonidet P-40). A total of 15 μ g of GFP antibody (Ab frontier) and 100 μ L of protein A agarose/salmon sperm DNA slurry (Millipore) were added and incubated 4 h in 4 °C with gentle rotation. Beads were collected and washed thoroughly five to six times with NT2 buffer. RNAs were released by adding 10 μ L proteinase K. DNase1 was treated to remove genomic DNA contamination. The RNAs were purified by TRIzol. Reverse transcription was performed with total eluted RNA, random hexamer and oligo dT. Transcript levels were detected by real-time PCR with specific primers for each gene. Amplified products were reconfirmed by gel electrophoresis. For the light treatment experiments, all procedures were performed in the dark or under safety green light.

Plant materials and other methods including a primer list (Table S1) can be found in *SI Methods*.

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