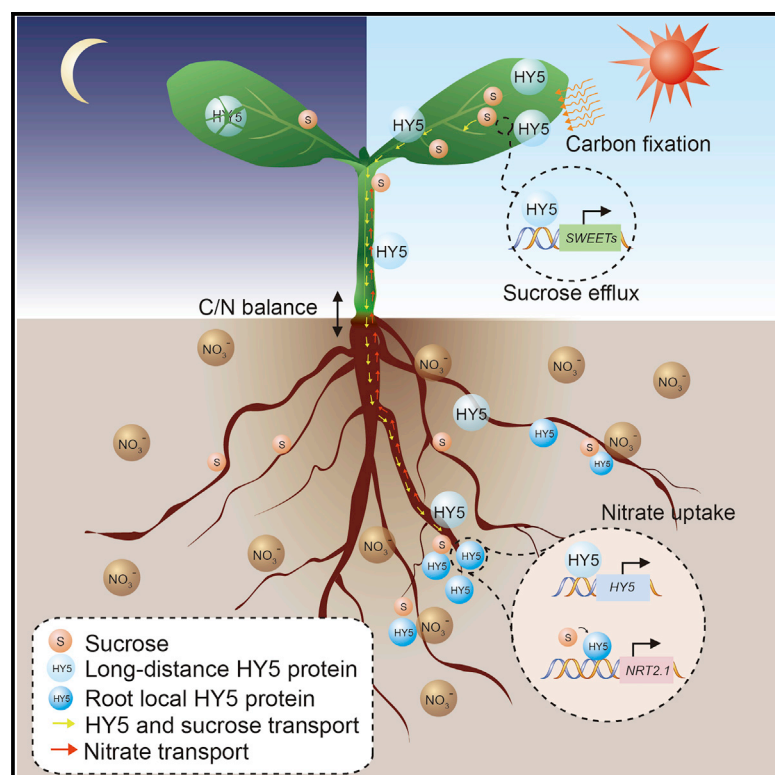


Current Biology

Shoot-to-Root Mobile Transcription Factor HY5 Coordinates Plant Carbon and Nitrogen Acquisition

Graphical Abstract



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In Brief

Chen et al. show that transcription factor HY5 is a shoot-to-root mobile signal that mediates light-responsive coupling of shoot growth and C assimilation with root growth and N uptake in *Arabidopsis*. HY5 mobility thus contributes to maintain homeostatic balance between whole-plant C and N metabolism in response to a fluctuating environment.

Highlights

- HY5 is essential for light-responsive coordination of the growth of shoots and roots
- Shoot-to-root translocated HY5 mediate light-activated root growth and N uptake
- Carbohydrate photosynthate-induced *NRT2.1* expression and N uptake depend upon HY5
- HY5 contributes to maintain balance of C and N metabolism at varying light fluence



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Shoot-to-Root Mobile Transcription Factor HY5 Coordinates Plant Carbon and Nitrogen Acquisition

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SUMMARY

Coordination of shoot photosynthetic carbon fixation with root inorganic nitrogen uptake optimizes plant performance in a fluctuating environment [1]. However, the molecular basis of this long-distance shoot-root coordination is little understood. Here we show that *Arabidopsis* ELONGATED HYPOCOTYL5 (HY5), a bZIP transcription factor that regulates growth in response to light [2, 3], is a shoot-to-root mobile signal that mediates light promotion of root growth and nitrate uptake. Shoot-derived HY5 auto-activates root HY5 and also promotes root nitrate uptake by activating *NRT2.1*, a gene encoding a high-affinity nitrate transporter [4]. In the shoot, HY5 promotes carbon assimilation and translocation, whereas in the root, HY5 activation of *NRT2.1* expression and nitrate uptake is potentiated by increased carbon photo-assimilate (sucrose) levels. We further show that HY5 function is fluence-rate modulated and enables homeostatic maintenance of carbon-nitrogen balance in different light environments. Thus, mobile HY5 coordinates light-responsive carbon and nitrogen metabolism, and hence shoot and root growth, in a whole-organismal response to ambient light fluctuations.

RESULTS AND DISCUSSION

Although plant shoots and roots follow distinct developmental trajectories, their biology is tightly coordinated to optimize whole-plant performance in a fluctuating environment. The coordination of metabolic assimilation is of key importance: shoots fix atmospheric carbon (C; CO₂), roots acquire soil ionic nitrogen (N; predominantly nitrate, NO₃[−]) [1]. Because N is essential, soil NO₃[−] levels frequently limit productivity in natural and agricultural environments [5, 6]. The regulation of rates of N and C acquisition is known to be tightly coupled [7, 8], with light regulating both processes [9]. However, the molecular mechanisms of the underlying regulatory long-distance shoot-root communication remain unknown.

HY5 Is Essential for Shoot-Illumination Promotion of Root Growth and NO₃[−] Uptake

As a visible reporter of regulatory shoot-root communication, and because roots are not usually exposed to light, we determined the effects of separate illumination of shoots or roots on root growth of 3-day-old seedlings, which were then exposed to 3 days of differential light treatment (Figures 1A and S1A). We found that the primary roots of wild-type (WT) *Arabidopsis thaliana* (Col-0 laboratory strain) seedlings whose shoots (only) were exposed to light [S(L)/R(D)] were of a similar length to those of WT seedlings fully exposed to light [S(L)/R(L); Figures 1B and 1C]. In contrast, the roots of WT S(D)/R(L) seedlings were shorter than those of S(L)/R(D) seedlings and of a similar length to S(D)/R(D) seedlings (Figures 1B and 1C). In addition, lateral root proliferation of S(L)/R(D) seedlings was similar to that of S(L)/R(L), whereas lateral root proliferation of S(D)/R(L) seedlings was much less and similar to that of S(D)/R(D) (Figure S1B). Thus, shoot illumination promotes root growth, most likely via shoot-to-root signaling.

We next screened for *A. thaliana* mutants specifically disrupted in shoot illumination promoted root growth, and we found that one of them contained a new HY5 loss-of-function allele (*hy5-526*; Figures 1B, S2A, and S2B). Further analysis showed that a *hy5* null allele also abolishes shoot-illumination-promoted root growth [10] (Figure 1C). HY5 encodes the photomorphogenic bZIP transcription factor HY5 [2, 3]. HY5 is regulated by the COP1 ubiquitin ligase, which targets HY5 for proteolytic degradation in the dark [11]. Accordingly, we found that the loss-of-function *cop1-4* mutant mimics shoot-illumination promotion of root growth in dark-grown seedlings (Figure 1C). These results suggest that HY5 mediates shoot-illumination promotion of root growth.

We next found that WT root NO₃[−] uptake is promoted by illumination of the shoot, but not of the root (Figure 1D). NO₃[−] is taken up by plants via dual low-affinity/high-affinity CHL1/NRT1.1 and high-affinity NRT2.1 transporters [4, 12–15]. Shoot-illumination-promoted NO₃[−] uptake was greatly diminished in the *nrt2.1-2* mutant lacking NRT2.1 [4] (Figure 1D) but relatively unaffected in a *chl1-5* mutant lacking CHL1/NRT1.1 [13] (Figure 1D), indicating that shoot-illumination promotion of root NO₃[−] uptake is predominantly NRT2.1 dependent. Furthermore, shoot-illumination-promoted root NO₃[−] uptake was largely abolished in both *hy5* and *hy5-526* (Figure 1D), suggesting that HY5 regulates NRT2.1-dependent shoot-illumination promotion of root NO₃[−] uptake.

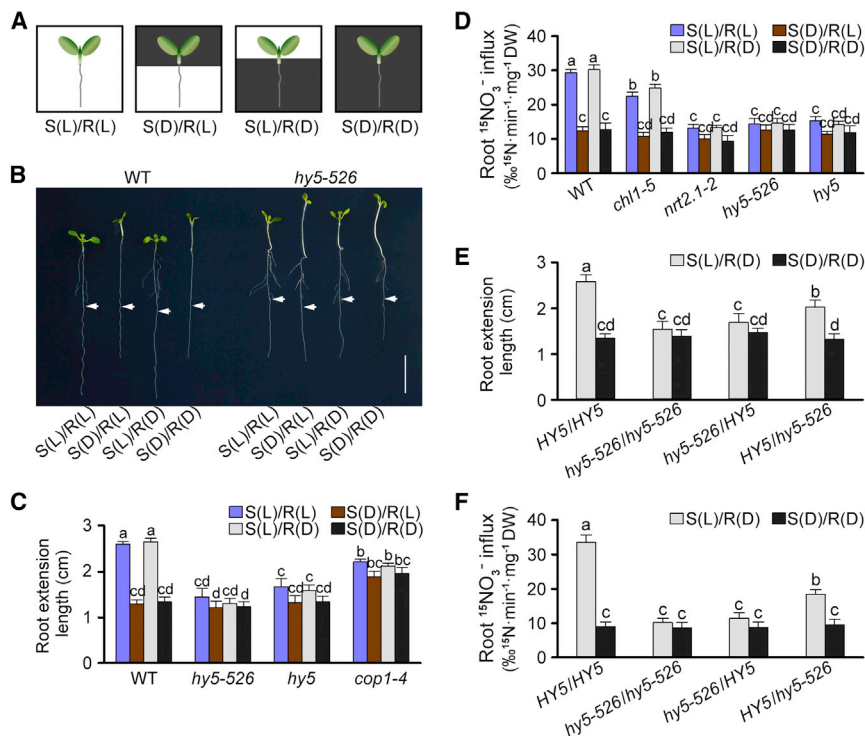


Figure 1. HY5 Mediates Shoot-Illumination Promotion of Root Growth and NRT2.1-Dependent NO_3^- Uptake

(A) Representation of differential shoot/root-illumination conditions. 3-day-old seedlings were exposed to 3 days of differential light treatment ($100 \mu\text{mol} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$): shoot illuminated [S(L)] or dark grown [S(D)] and root illuminated [R(L)] or dark grown [R(D)] (see also Figure S1A).

(B) Differential shoot/root-illumination effects on WT and *hy5-526* primary root growth (see Figure S1B for lateral root growth). Arrowheads indicate the root-tip position at the beginning of the experiment. Scale bar, 1 cm.

(C) Differential shoot/root-illumination effects on WT, *hy5-526*, *hy5*, and *cop1-4* seedling primary root extension length.

(D) Differential shoot/root-illumination effects on $^{15}\text{NO}_3^-$ uptake of seedling roots.

(E) Differential shoot/root-illumination effects on primary root extension length of 10-day-old grafted plants. Grafts are indicated as scion/stock (e.g., *HY5/hy5-526* has a *HY5* scion and a *hy5-526* stock).

(F) Differential shoot/root-illumination effects on $^{15}\text{NO}_3^-$ uptake of roots of plants grafted as in (E). (C–F) Data are shown as mean \pm SEM ($n = 30$). The same lowercase letter denotes a non-significant difference between means ($p < 0.05$). See also Figure S1.

HY5 Moves from Shoot to Root to Mediate Light-Activated Promotion of Root Growth and NO_3^- Uptake

Subsequent experiments used hypocotyl graft chimeras and found that a *HY5* scion permitted shoot-illumination promotion of root growth and NO_3^- uptake (versus a *hy5-526* scion; Figures 1E and 1F), suggesting that a *HY5*-dependent shoot-derived signal regulates root growth and NO_3^- uptake. To determine whether *HY5* is (part of) this signal, we next expressed transgenes encoding *HY5*-GFP or myc-*HY5* fusion proteins in *hy5* using non-tissue-specific *pHY5* [2], photosynthetic-tissue-specific *pCAB3* [16, 17], or phloem companion-cell-specific *pSUC2* promoters [18], finding that both *HY5*-GFP and myc-*HY5* complemented the *hy5* phenotype (Figures S2C and S2D) and thus retain *HY5* activity. We also found that *pHY5:HY5-GFP*, *pCAB3:HY5-GFP*, and *pSUC2:HY5-GFP* all restored shoot-illumination regulation of primary root growth to *hy5* (Figure 2A). Because *pCAB3*-driven expression is photosynthetic tissue specific, these observations suggest that *HY5* transcripts, *HY5*, or a *HY5*-dependent signal moves from shoot to root.

We next found that *HY5*-GFP was detectable throughout the roots of S(L)/R(D) *pHY5:HY5-GFP hy5* seedlings, but not in S(D)/R(D) (Figure 2B), suggesting that root *HY5* abundance is regulated by shoot illumination and that *HY5* is relatively stable in dark-grown roots. We additionally detected *HY5*-GFP in the roots of S(L)/R(D) *pCAB3:HY5-GFP hy5* seedlings, but not in S(D)/R(D) controls (Figure 2C). Because *HY5*-GFP transcripts are not detected in roots of *pCAB3:HY5-GFP hy5* seedlings (Figure S2D), this observation suggests that *HY5* moves from shoot to root. Accordingly, whereas myc-*HY5* transcripts were detectable only in S(L)/R(D) shoots of *pCAB3:myc-HY5 hy5* plants (Figures 2D and S2D), myc-*HY5* was detected in both shoots and

roots of S(L)/R(D) plants (Figure 2E). This result was confirmed by detection of *HY5*-GFP in roots of S(L)/R(D)-grown *pCAB3:HY5-GFP hy5/hy5* graft chimeras (Figures 2F and S2E). Additional experiments with *pHY5:HY5-GFP hy5/hy5* graft chimeras detected *HY5*-GFP in S(L)/R(D)-grown roots (Figures S2F–S2H), further suggesting that *HY5* moves from shoot to root.

We next expressed a fused *HY5*-GFP, TEV^{rs} (TEV protease recognition site), and double β -glucuronidase ($2 \times \text{GUS}$) ($2 \times \text{GUS-TEV}^{\text{rs}}$ -*HY5*-GFP) protein from *pCAB3*. This protein was detected in shoots, but not in roots, of S(L)/R(D) plants, most likely because its relatively large size prevents shoot-root mobility (Figures 2G and S2I). However, co-expression of *35S:TEVP* (expressing the TEV protease) enabled cleavage at the TEV^{rs} [19], resulting in detection of *HY5*-GFP in shoots and roots (Figures 2G and S2I). These changes in *HY5*-GFP mobility were reflected in parallel effects on shoot-illumination-promoted primary root growth (Figure 2H) and NO_3^- uptake (Figure 2I). Furthermore, *HY5*-GFP is detected in roots of S(L)/R(D)-grown *pSUC2:HY5-GFP hy5* seedlings and *pSUC2:HY5-GFP hy5/hy5* graft chimeras (Figure S2J), indicating that *HY5* is a shoot-root phloem-mobile signal that mediates light regulation of root growth and NO_3^- uptake.

Shoot-to-Root Translocated *HY5* Activates *NRT2.1* Expression and NO_3^- Uptake

The *HY5*-GFP distribution conferred on *hy5* roots by *pHY5:HY5-GFP* (Figure 2B) differs from that due to *pCAB3:HY5-GFP* (Figure 2C) or *pSUC2:HY5-GFP* (Figure S2J) and that seen in roots of *pHY5:HY5-GFP hy5/hy5* (Figure S2F) or *pSUC2:HY5-GFP hy5/hy5* graft chimeras (Figure S2J). A *pHY5:HY5-GFP* root

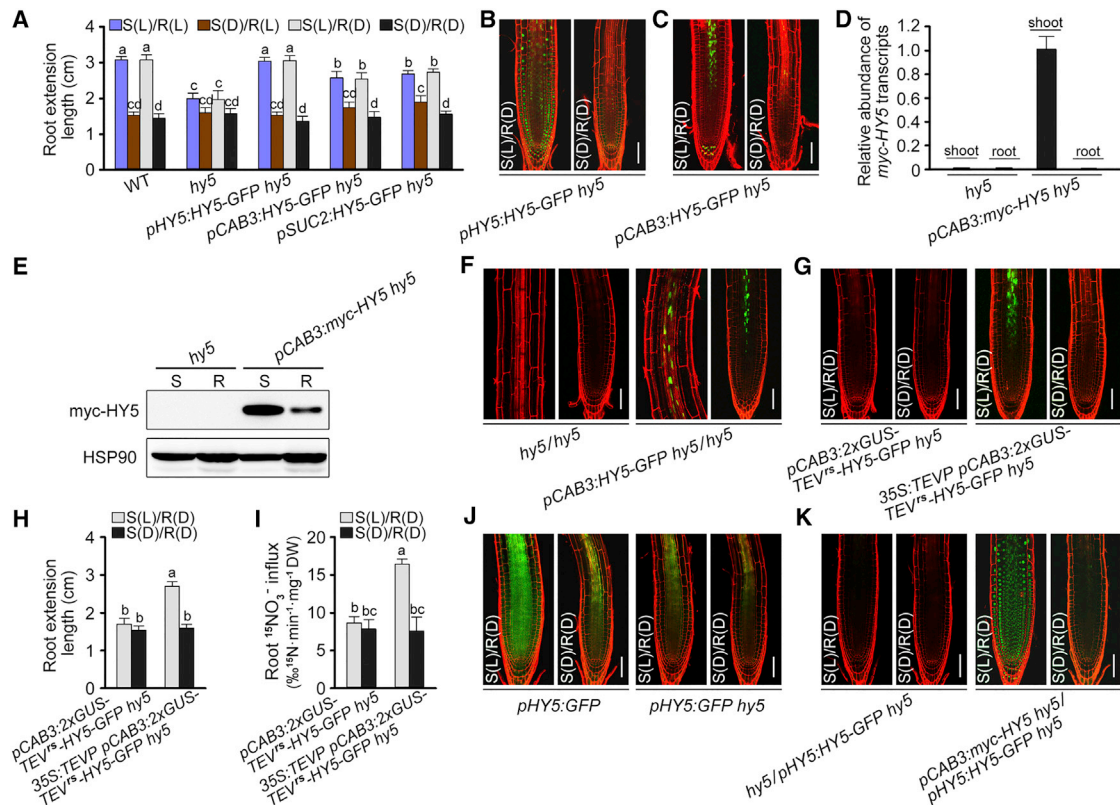


Figure 2. Shoot-to-Root Translocation of HY5 Regulates Root Growth and NO_3^- Uptake

(A) Differential shoot/root-illumination effects on primary root extension growth (seedling genotypes are as indicated) (see also Figures S2A–S2D).

(B) Distribution of HY5-GFP in *pHY5:HY5-GFP hy5* roots.

(C) Distribution of HY5-GFP in *pCAB3:HY5-GFP hy5* roots (see also Figure S2E).

(D) *myc-HY5* transcript abundance in the shoots and roots of 14-day-old plants (relative to that in *pCAB3:myc-HY5 hy5* shoots). Data are shown as mean \pm SEM ($n = 3$).

(E) Immunological detection of *myc-HY5* in shoots (S) and roots (R) of S(L)/R(D) plants, with HSP90 as a loading control (see Figures S2F–S2H for *pHY5:HY5-GFP hy5/hy5* graft chimera).

(F) Distribution of HY5-GFP in the roots of shoot-illuminated *pCAB3:HY5-GFP hy5/hy5* graft chimera.

(G) Detection of HY5-GFP in roots of shoot-illuminated *pCAB3:2 \times GUS-TEV^{rs}-HY5-GFP hy5* plants expressing TEV protease (see also Figure S2I).

(H) Differential shoot/root-illumination effects on primary root extension growth of plants expressing TEV protease.

(I) Differential shoot/root-illumination effects on root $^{15}\text{NO}_3^-$ uptake of plants expressing TEV protease.

(J) Differential shoot/root-illumination effects on expression of a *pHY5:GFP* transgene in roots.

(K) Distribution of HY5-GFP in seedling roots (grafted as indicated) (see also Figure S2J).

(B, C, F, G, J, K) Scale bar, 50 μm .

(A, H, I) Data are shown as mean \pm SEM ($n = 30$).

The same lowercase letter denotes a non-significant difference between means ($p < 0.05$). See also Figure S2 and Table S1.

genotype causes HY5-GFP to be detected throughout the root, whereas roots of *pCAB3:HY5-GFP hy5* and *pSUC2:HY5-GFP hy5* or *pHY5:HY5-GFP hy5/hy5* and *pSUC2:HY5-GFP hy5/hy5* chimeras exhibit a HY5-GFP location more closely associated with the root vasculature. This difference is most likely because HY5 activates root *HY5* expression [20]. First, shoot-illumination induction of root expression of a *pHY5:GFP* transgene (reporting *pHY5* activity by driving the expression of the *GFP* marker gene) is reduced in *hy5* (Figure 2J), suggesting that shoot-derived HY5 normally activates root *pHY5*. Second, HY5-GFP is detectable in roots of shoot-illuminated *pCAB3:myc-HY5 hy5/pHY5:HY5-GFP hy5* grafts, but not in roots of shoot-illuminated *hy5/pHY5:HY5-GFP hy5* grafts (Figure 2K). Third, electrophoretic mobility shift assays (EMSAs) and chromatin immunoprecipitation (ChIP) anal-

ysis using the roots of *pHY5:myc-HY5 hy5* seedlings confirmed binding of HY5 to *pHY5* [20] (Figures S3A and S3B). These observations indicate that shoot-root translocated HY5 activates root *HY5* via an auto-regulatory feedback loop and also explain the different patterns of HY5-GFP distribution conferred on *hy5* roots by *pHY5:HY5-GFP* (Figure 2B) versus *pCAB3:HY5-GFP* (Figure 2C) or *pSUC2:HY5-GFP* (Figure S2J).

We next found that shoot-illumination promotion of root *NRT2.1* transcript levels is largely abolished in *hy5* (Figure 3A). In addition, whereas shoot illumination partially promoted root *NRT2.1* transcript levels in *HY5/hy5* grafts, this effect was still further reduced in *hy5/HY5* grafts (Figure 3A). Further experiments showed that whereas either a *HY5* scion/*pHY5:HY5-GFP hy5* stock or a *pCAB3:HY5-GFP hy5* scion/*HY5* stock

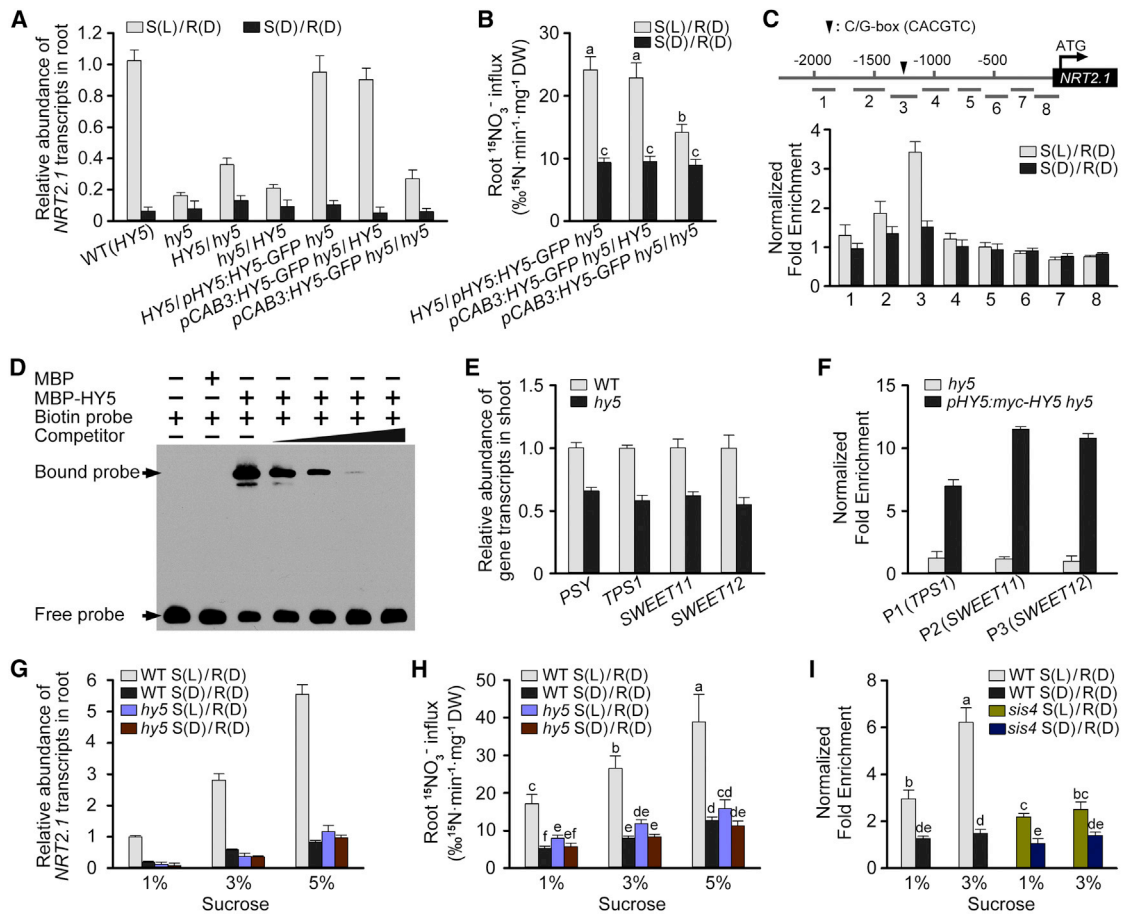


Figure 3. HY5 Coordinates N and C Metabolism

(A) *NRT2.1* transcript levels in primary seedling roots (genotypes/graft chimeras are as shown), with abundance relative to the level in WT roots. Data are shown as mean \pm SEM ($n = 3$).

(B) Root NO_3^- uptake of graft chimeras. Data are shown as mean \pm SEM ($n = 30$).

(C) *NRT2.1* promoter fragments used for ChIP analysis of extracts from 14-day-old *pHY5:myc-HY5 hy5* plants. Data are shown as mean \pm SEM ($n = 3$). An arrow indicates C/G box sequence motif.

(D) Fragment 3 from (C) was incubated with MBP-HY5. Competition was performed with 10-, 20-, 50-, or 100-fold unlabeled probe excess.

(E) Relative abundance of *PSY*, *TPS1*, *SWEET11*, and *SWEET12* transcripts in 7-day-old seedlings. Values are expressed relative to WT level. Data are shown as mean \pm SEM ($n = 3$).

(F) ChIP assays (see also Figures S3A and S3B). Fragments containing G box motifs in the *TPS1*, *SWEET11*, and *SWEET12* promoters were used for ChIP analysis of extracts from 14-day-old *pHY5:myc-HY5 hy5* (or control *hy5*) plants. Data are shown as mean \pm SEM ($n = 3$).

(G) Sucrose affects shoot-illumination promotion of root *NRT2.1* transcript abundance [relative to abundance in WT S(L)/R(D) seedlings in 1% sucrose] (see also Figures S3C and S3H). Data are shown as mean \pm SEM ($n = 3$).

(H) Sucrose affects shoot-illumination promotion of root $^{15}\text{NO}_3^-$ uptake (see also Figures S3D and S3I). Data are shown as mean \pm SEM ($n = 30$).

(I) In vivo binding of HY5 to fragment 3 (see C) of the *NRT2.1* promoter. ChIP-PCR analysis was performed using 10-day-old *pHY5:myc-HY5 hy5* plants (see also Figures S3E–S3G). Data are shown as mean \pm SEM ($n = 3$).

The same lowercase letter denotes a non-significant difference between means ($p < 0.05$). See also Figure S3 and Table S2.

permitted shoot-illumination-dependent elevation of root *NRT2.1* transcript levels to an extent similar to that seen in *HY5* plants, that of a *pCAB3:HY5-GFP hy5* scion/*hy5* stock was reduced versus that seen in *HY5* plants (Figure 3A). These differential effects were reflected in parallel effects on shoot illumination and *NRT2.1*-dependent NO_3^- uptake (Figure 3B) and relate to the need for a functional *HY5* in the root. ChIP and EMSA demonstrated both in vivo and in vitro binding of *HY5* to the *NRT2.1* promoter (Figures 3C and 3D), indicating that *HY5* directly regulates *NRT2.1* expression. Taken together, these re-

sults suggest that shoot-root mobile *HY5* activates root *NRT2.1* via a mechanism that is amplified by auto-activation of root *HY5*, thereby increasing NO_3^- uptake.

Mobile HY5 Contributes to Maintain Homeostatic Balance of C and N Metabolism in Response to Ambient Light Conditions

Recent studies showed that *HY5* modulates photosynthetic capacity in part via control of expression of chlorophyll biosynthesis and photosynthesis-related genes [21, 22]. For example,

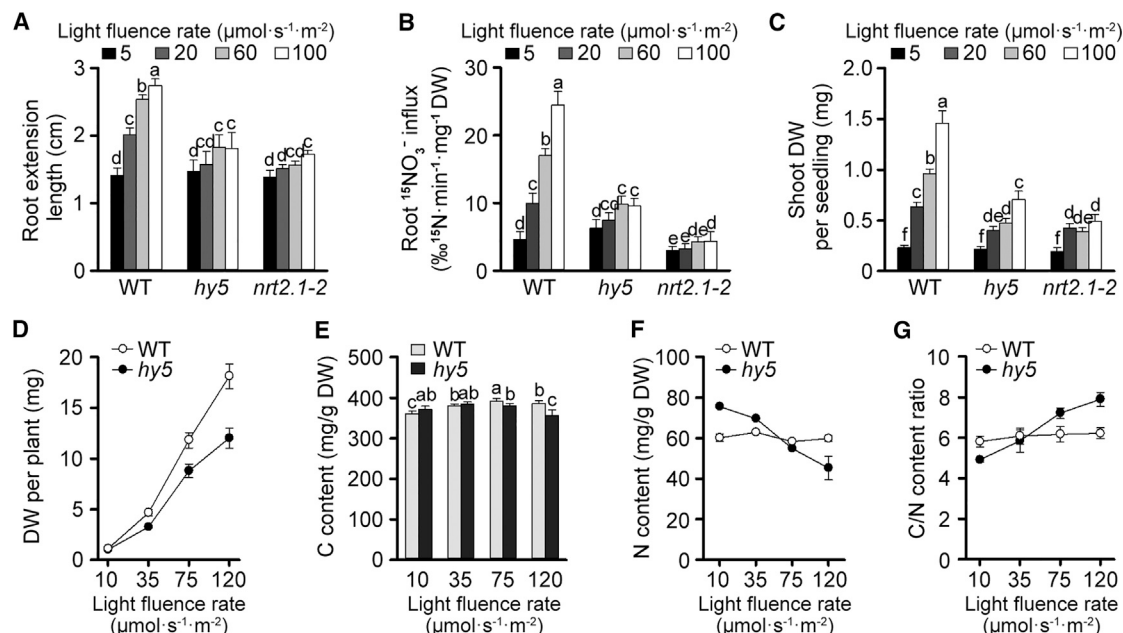


Figure 4. HY5 Coordinates Plant Growth and Nutrition in Responses to Fluctuating Light Environments

(A) Primary root extension lengths of seedlings grown in fluence rates as indicated. Data are shown as mean \pm SEM ($n = 30$).

(B) $^{15}\text{NO}_3^-$ uptake of seedlings grown in fluence rates as indicated. Data are shown as mean \pm SEM ($n = 30$).

(C) Seedling shoot biomass at different fluence rates. Data are shown as mean \pm SEM ($n = 30$).

(D) Whole-plant biomass of plants grown in soil for 21 days (16 hr photoperiod) at different light fluence rates (see also Figure S4).

(E) C contents of plants shown in (D).

(F) N contents of plants shown in (D).

(G) C/N content ratio of 21-day-old plants grown in fluence rates as indicated.

(D–G) Data are shown as mean \pm SEM ($n = 16$).

The same lowercase letter denotes a non-significant difference between means ($p < 0.05$). See also Figure S4.

HY5 promotes *PHYTOENE SYNTHASE* (*PSY*) expression by directly binding to the *PSY* promoter [22] (Figure 3E). Fixed C is predominantly transported to sink tissues (e.g., roots) through the phloem in the form of sucrose [23], and the trehalose precursor trehalose-6-phosphate (T6P) acts as a proxy for photosynthetic carbohydrate status [24]. We also found that HY5 affects both sucrose metabolism and shoot-root transport by promoting the expression levels of *TPS1*, a gene encoding trehalose-6-phosphate synthase [24], and *SWEET11* and *SWEET12* [23], genes encoding sucrose efflux transporters (Figure 3E). Further ChIP experiments confirmed in vivo binding of HY5 to the *TPS1*, *SWEET11*, and *SWEET12* promoters (Figure 3F). Thus, HY5 regulates both initial C fixation and subsequent movement of fixed C into phloem cells for shoot-root translocation.

Because C status regulates N status [7, 8], we next determined whether sugar level affects light-activated *NRT2.1*-dependent NO_3^- uptake. We found that both sucrose and glucose enhance the shoot-illumination promotion of root *NRT2.1* expression and NO_3^- uptake, effects that are largely abolished in *hy5* (Figures 3G, 3H, S3C, and S3D). Moreover, ChIP assays showed that sucrose promotes light-activated in vivo binding of myc-HY5 to the *NRT2.1* promoter and that this effect was abolished in the sugar-insensitive mutant *sis4* [25] (Figure 3I). Because neither *HY5-GFP* abundance nor *HY5-GFP* mobility is increased by sucrose (Figures S3E–S3G), increased HY5 levels are unlikely to contribute to sucrose-induced increases in the binding affinity of myc-

HY5 to the *NRT2.1* promoter. Whatever the mechanism, sucrose induction of *NRT2.1* expression and NO_3^- uptake is dependent upon HY5 (Figures S3H and S3I). Taken together, these results indicate that HY5 responds to sugar signals in the regulation of *NRT2.1*-dependent NO_3^- uptake, thus contributing to the coordinated homeostatic balancing of C and N metabolism.

Because natural fluctuation in incident light fluence affects both C fixation and HY5 abundance [26, 27], we next determined the effect of increasing fluence (5–100 $\mu\text{mol} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$) on seedling root growth and NO_3^- uptake. We found that increasing fluence promotes WT primary root extension growth (Figure 4A) and NO_3^- uptake (Figure 4B), that these effects are associated with a fluence-dependent increase in biomass accumulation (Figure 4C), and that they are largely abolished in *hy5* and *nrt2.1-2* mutant seedlings (Figures 4A and 4B and 4C). We next determined whether HY5 continues to coordinate light-responsive growth and C and N metabolism in more mature plants. First, as expected, increasing fluence increases the biomass of 21-day-old soil-grown WT plants (Figures 4D and S4), an effect that is reduced in *hy5* (Figure 4D). Next, we found that the whole-plant C content of 21-day-old WT and *hy5* plants remains relatively constant with increasing fluence (Figure 4E). In contrast, whereas WT whole-plant N content remains relatively constant, that of *hy5* falls markedly as fluence increases (Figure 4F). In consequence, lack of HY5 causes a fluence-dependent increase in the C/N content ratio, which remains relatively

constant in WT (Figure 4G). These findings suggest that mobile HY5 regulates the coordination of shoot and root growth and C and N acquisition throughout the plant life cycle. In particular, HY5 maintains homeostatic balance of C and N metabolism at varying light fluence.

Conclusions

Although a previous study implicates phloem-mobile sucrose as a cotyledon-derived signal to control primary root elongation during early seedling development in *Arabidopsis* [28], the molecular mechanism of the shoot-root long-distance signaling regulating lateral root growth and N uptake remains unclear [29]. Here, we show that HY5 is a shoot-root mobile signal that mediates light-regulated coupling of shoot growth and C assimilation with root growth and N uptake. This coupling is achieved via HY5 regulation of C fixation in the shoot and via sucrose-enhanced promotion of HY5-dependent N uptake in the root. In consequence, HY5 mediates homeostatic regulation of whole-plant C versus whole-plant N status. HY5 is already known to integrate multiple phytohormonal (e.g., abscisic acid) and environmental (e.g., low temperature) signaling inputs in the control of plant growth and development [30, 31]. Our discovery that HY5 is a mobile signal adds further dimension to this knowledge. Our finding that HY5 mobility mediates homeostatic coordination of C and N metabolism enhances understanding of how plant C and N nutrient balance is maintained in fluctuating environments and suggests novel strategies for the improvement of nutrient-use efficiency in crops.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.12.066>.

AUTHOR CONTRIBUTIONS

X.C. performed most of the experiments; Q.Y. and C.J. generated transgenic plants and analyzed the results; X.G. performed genetic screening; X.F. designed the experiments; and X.F. and N.P.H. wrote the manuscript. All authors have discussed the results and contributed to the manuscript.

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