Local auxin production underlies a spatially restricted neighbor-detection response in *Arabidopsis*

Olivier Michaud[a], Anne-Sophie Fiorucci[a], Ioannis Xenarios[b], and Christian Fankhauser[a,1]

[a]Center for Integrative Genomics, Faculty of Biology and Medicine, University of Lausanne, CH-1015 Lausanne, Switzerland; and [b]Swiss Institute of Bioinformatics, University of Lausanne, CH-1015 Lausanne, Switzerland

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Competition for light triggers numerous developmental adaptations known as the "shade-avoidance syndrome" (SAS). Important molecular events underlying specific SAS responses have been identified. However, in natural environments light is often heterogeneous, and it is currently unknown how shading affecting part of a plant leads to local responses. To study this question, we analyzed upwards leaf movement (hyponasty), a rapid adaptation to neighbor proximity, in *Arabidopsis*. We show that manipulation of the light environment at the leaf tip triggers a hyponastic response that is restricted to the treated leaf. This response is mediated by auxin synthesized in the blade and transported to the petiole. Our results suggest that a strong auxin response in the vasculature of the treated leaf and auxin signaling in the epidermis mediate leaf elevation. Moreover, the analysis of an auxin-signaling mutant reveals signaling bifurcation in the control of petiole elongation versus hyponasty. Our work identifies a mechanism for a local shade response that may pertain to other plant adaptations to heterogeneous environments.

neighbor detection | organ-specific response | hyponasty | auxin | PIF

The availability of essential resources, including micro- and macronutrients, water, CO_2, and sunlight, is an important regulator of plant phenotypic plasticity (1, 2). A well-known example is the response of plants to foliar shade known as the "shade-avoidance syndrome" (SAS) (3–5). In shade-avoiding plants the SAS comprises a suite of growth and developmental responses including elongation of hypocotyls, stems, and petioles and repositioning of the leaves to higher positions in the canopy (known as "leaf hyponasty") (3). These responses confer an adaptive advantage, with shade-avoiding plants having improved relative fitness in environments with high plant density (6, 7). Interestingly, many of the physiological responses elicited by neighboring plants are triggered before shading, a response known as "neighbor detection" that enables plants to anticipate potentially unfavorable light conditions (3, 8, 9).

A primary signal informing plants about the presence of neighbors is the red (R) to far-red (FR) ratio (3). In sunlight the R/FR is slightly above a value of 1, but, because of the strong absorbance of R and blue by photosynthetic pigments and the substantial reflection of FR by leaves, this ratio drops before actual shading and decreases further in true shade (3, 10, 11). Phytochromes (phy) sense the R/FR ratio, with phyB playing a predominant function in shade and neighbor detection (3). In sunlight a substantial fraction of phyB is active in preventing the SAS, while a reduction of the R/FR ratio gradually enhances elongation of hypocotyls, petioles, and stems (11). These growth responses are controlled by extensive transcriptional reprograming mediated primarily by three members of the phytochrome-interacting factor (PIF) family of basic helix–loop–helix (bHLH) transcription factors acting immediately downstream of phyB (12–14). In sunlight, phyB inhibits these PIFs through complex mechanisms, but in the shade this inhibition is released, resulting in PIF-mediated promotion of elongation (3, 5). Shade cues are sensed mostly in leaf blades (or cotyledons), leading to auxin production in green tissues (15, 16). Auxin then is transported to the elongating parts of the plant (e.g., petioles and hypocotyls) to elicit the growth response (17, 18). A key step in this process is PIF-dependent expression of several members of the YUCCA family of auxin biosynthetic enzymes (12, 13, 19). PIFs also control the expression of additional players contributing to growth regulation, including several hormonal pathways and cell-wall components (20–25).

In natural environments, shading is often heterogeneous, leading to situations in which plants are only partly shaded by competitors. This heterogeneous shading led to the concept of foraging for light that is mediated by local tuning of the SAS specifically in the shaded part of the plant, thus promoting canopy gap filling (26). Examples of such local responses have been identified in several species (27, 28). We decided to investigate the molecular basis of such local shade responses in *Arabidopsis* by studying leaf hyponasty, an early response to increasing plant density that is induced rapidly by lowering the R/FR (29). Our experiments show that auxin production in the leaf blade is necessary and sufficient to trigger a leaf hyponastic response. Interestingly, the response depends on the site of auxin production/application and selectively affects the treated leaf, thereby providing a molecular basis for local shade responses in *Arabidopsis* leaves.

Results

The PIF-YUC Regulon Controls Low R/FR-Induced Leaf Hyponasty. Leaf hyponasty is a complex, dynamic response, and the position of leaves is controlled by both internal (e.g., circadian) and external cues (30). To study this process dynamically, we tracked

Significance

Being photoautotrophic, plant growth is exquisitely sensitive to the light environment. In response to light cues from potential competitors, plants initiate a neighbor-proximity response favoring direct access to sunlight. This response includes elevation of the leaf (hyponasty) that is rapidly triggered following perception of neighbors. Light signals emanating from surrounding vegetation are heterogeneous; however, it is unknown how plants trigger a localized response in such conditions. We show that auxin synthesis in the leaf blade coupled with transport into the petiole induces a hyponastic response restricted to the leaf perceiving the signal. Moreover, we identify a branch of auxin signaling controlling petiole elevation while not affecting elongation. Our work uncovers a mechanism underlying plant responses to a heterogeneous environment.

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[1]To whom correspondence should be addressed. Email: christian.fankhauser@unil.ch.

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leaf position (tip elevation angle) with high spatial and temporal resolution in plants growing in control (high R/FR) and low R/FR (simulated neighbors) conditions using previously described methodology (31). We typically monitored leaves 1 and 2, which are at the same developmental stage, but similar response patterns were observed in younger leaves (Fig. 1 and Fig. S1 *A* and *B*) (31). A photogrammetric approach showed that tip and petiole elevation angles are highly correlated, justifying the choice of tip position as a proxy for leaf movement (Fig. S1 *C* and *D*). In wild-type (Col-0 accession) plants a reduction in R/FR led to an increase in the leaf elevation angle starting 3–4 h after transfer into simulated shade, and leaves reached maximal elevation in the late afternoon (Fig. 1*A* and Fig. S1*A*). In leaves 1 and 2 the effect of low R/FR was more pronounced during the first day of treatment but was less apparent in younger leaves with more growth potential (Fig. 1*A* and Fig. S1*A*). In addition, shade led to a higher baseline for the diurnal movements, resulting in an approximate 20° increase in the lowest elevation angle in low R/FR as compared with high R/FR (Fig. 1*A* and Fig. S1*A*). *phyB* mutants show a constitutive shade-avoidance phenotype including leaf hyponasty (32), a phenotype that we confirmed in our growth conditions (Fig. S2 *A* and *B*). PIF4, PIF5, and PIF7 act immediately downstream of phyB to promote shade-induced hypocotyl and petiole elongation (14, 33, 34). We therefore analyzed shade-regulated leaf movements in *pif7*, *pif4pif5*, and *pif4pif5pif7* mutants. The amplitude of leaf movement in *pif4pif5* and *pif4pif5pif7* mutants was reduced in control conditions (Fig. 1*A* and Fig. S2 *B–D*) (31). Moreover, a reduction of the R/FR led to a strongly reduced leaf hyponastic response in *pif4pif5pif7* and *pif7* mutants, indicating that the low R/FR-controlled leaf position is predominantly regulated by PIF7 (Fig. 1*A* and Fig. S2 *B–D*).

PIF-controlled auxin biosynthesis is an essential step in shade-regulated hypocotyl and petiole elongation (11). Moreover, a role for auxin biosynthesis in shade-regulated leaf hyponasty was previously identified by analyzing the *taa1/sav3* mutant (35). The amplitude of leaf movement in *taa1/sav3* plants was reduced in high R/FR (Fig. S3*A*), in contrast with other aspects of the *taa1/sav3* phenotype that are normal in control conditions (17) but correlating with the reduced indole-3 acetic acid (IAA) levels in the mutant (17). In addition, we confirmed the strongly diminished shade-mediated leaf hyponastic response in *taa1/sav3* mutants (Fig. S3*A*). PIFs control auxin production downstream of TAA1, at the level of *YUC* expression, and a *yuc2yuc5yuc8yuc9* mutant lacking four shade-induced *YUC* genes lacks several shade responses (19, 25). Therefore, we analyzed the *yuc2yuc5yuc8yuc9* mutant and found that it maintained normal leaf movements in control conditions but was unresponsive to a reduction of the R/FR (Fig. 1*B*). Consistent with these genetic data, pharmacological inhibition of YUC enzymes also inhibited low R/FR-induced hyponasty (Fig. S3*B*). To control petiole and hypocotyl elongation, auxin needs to be transported from the site of synthesis to the site of action, a process requiring several members of the PIN-FORMED family of auxin efflux carriers (16, 18, 25). Therefore, we examined the *pin3pin4pin7* mutant and found that these plants displayed severely reduced movements in control conditions as well as in response to shade (Fig. S3*C*). In contrast, a mutant lacking *PIN1* retained normal diurnal and shade-induced hyponasties (Fig. S3*D*). Considering these findings together, we conclude that both auxin synthesis and PIN3,4,7-mediated auxin transport are required to trigger low R/FR-induced leaf hyponasty.

Auxin Synthesized in the Blade and Transported to the Petiole Induces Hyponasty. To test whether auxin is sufficient to trigger leaf hyponasty, we applied auxin to the tip of wild-type leaves. We focused on the leaf tip because previous studies identified the leaf margin as the major source of newly synthesized auxin during shade responses (16, 17). Auxin (IAA) application led to

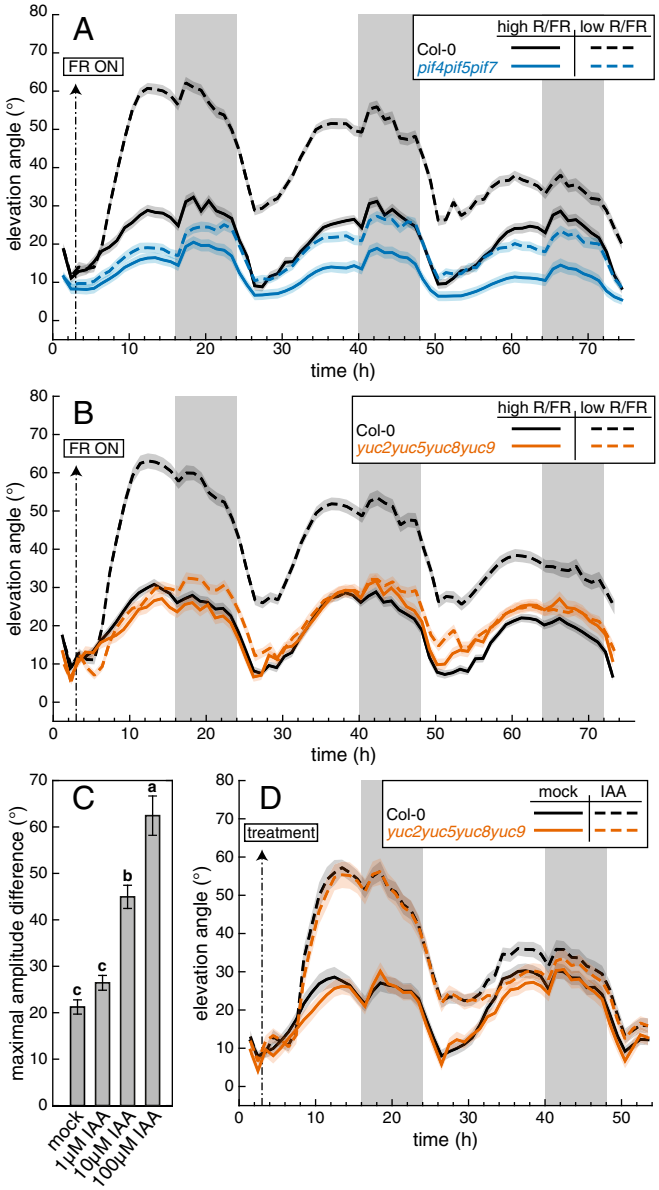

Fig. 1. Low R/FR-induced hyponasty is controlled by PIFs and requires YUC-mediated auxin biosynthesis. (*A*) Leaf elevation angle of leaves 1 and 2 in Col-0 plants (black lines) and *pif4pif5pif7* mutants (blue lines) in high (solid lines) versus low (dashed lines) R/FR conditions. Leaf elevation angles are mean values (n = 55–58). (*B*) Leaf elevation angle of leaves 1 and 2 in Col-0 plants (black lines) and *yuc2yuc5yuc8yuc9* mutants (orange lines) in high (solid lines) versus low (dashed lines) R/FR conditions. Leaf elevation angles are mean values (n = 51–60). In *A* and *B*, shade treatment started on day 15 at ZT3 (t = 3) by adding FR light (FR ON) to decrease the R/FR. (*C*) Bar plot representing the amplitude of leaf movement between maximum and minimum leaf elevation angles over the time period from ZT3 (t = 3) to ZT16 (t = 16) on day 15 and computed for each individual leaf analyzed in Fig. S4*A*. Error bars represent the twofold SE of mean estimates. One-way ANOVAs followed by Tukey's Honestly Significant Difference (HSD) test were performed, and different letters were assigned to significantly different groups ($P < 0.05$). (*D*) Leaf elevation angle of leaves 1 and 2 treated with mock solution (solid lines) or 10 μM IAA (dashed lines) in Col-0 plants (black lines) and *yuc2yuc5yuc8yuc9* mutants (orange lines). Leaf elevation angles are mean values (n = 35–40). Shaded bands around mean lines in *A*, *B*, and *D* represent the 95% CIs of mean estimates. Vertical gray bars represent night periods. In *C* and *D* a 1-μL drop of solution was applied to the leaf tip (adaxial side) at ZT3 on day 15. Plants were grown for 14 d in standard long-day [LD, 16-h light, 8-h dark (16/8)] conditions. Imaging started on day 15 at ZT0 (t = 0), and plants were maintained in LD conditions.

a leaf hyponasty that increased with the concentration of the phytohormone (Fig. 1*C* and Fig. S4*A*). Moreover, the kinetics of the response was comparable to shade treatments (Fig. 1 and Fig. S4*A*). We also noticed that the initial response to auxin application was a decrease in the leaf elevation angle that was followed by a rapid increase (Fig. S4*A*). A similar but less pronounced pattern was also observed in some low R/FR treatments. By comparing the effect of IAA application on the leaf tip with an application on the margin in the middle of the longitudinal blade axis, we found that tip application was most efficient in triggering leaf hyponasty (Fig. S4*B*). However, IAA application on one side of the leaf also led to a lateral repositioning of the leaf (Fig. S4*C*). Given that shade-induced leaf hyponasty depends on YUC-mediated auxin biosynthesis (Fig. 1*B* and Fig. S3*B*), we tested whether induction of *YUC* expression at the leaf tip also triggers an upward movement of leaves. Application of estradiol on the leaf tip of a YUC3-inducible line (iYUC3) resulted in strong leaf hyponasty, confirming that application or production of auxin at the leaf tip was sufficient to elicit the response (Fig. S4*D*). Importantly, auxin application restored leaf hyponasty in the *yuc2yuc5yuc8yuc9* auxin biosynthetic mutant but not in the *pin3pin4pin7* auxin transport mutant, confirming that auxin biosynthesis at the leaf tip followed by PIN-mediated transport is required for the hyponastic response (Fig. 1*D* and Fig. S4*E*). In line with this conclusion, we found that simultaneous application of IAA on the leaf tip and 1-naphthylphthalamic acid (NPA) on the blade–petiole boundary inhibited auxin-induced leaf hyponasty (Fig. S4*F*).

Low R/FR-Induced Leaf Hyponasty Is Restricted to the Treated Leaf. Because shade treatments on parts of a plant result in local effects for some responses but trigger systemic effects for others (16, 27, 36), we aimed at determining whether application of auxin on one leaf selectively affected the movement of the treated leaf or led to systemic effects. We found that only the auxin-treated leaf responded to hormone application (Fig. 2*A* and Fig. S5 *A*–*C*). Next we treated individual leaves with a low R/FR to determine whether a localized shade treatment also leads to a local response. We found that the hyponastic response was restricted to the leaf that was treated with low R/FR on its tip, indicating that a local reduction of the R/FR does not lead to a systemic signal affecting other leaves (Fig. 2 *B* and *C* and Fig. S5 *D* and *E*). Because leaf hyponasty is produced primarily by a change in the petiole angle (31), we tested whether reducing the R/FR on the petiole rather than on the leaf blade also triggered the response. Interestingly, an increase in leaf elevation angle was specifically induced upon reduction of the R/FR on the leaf tip but not on the petiole (Fig. 2 *B*–*D*). Therefore, we also compared leaf movement following auxin production/application at the tip versus the petiole. As observed following a reduction of the R/FR, applying auxin or inducing *YUC3* expression at the petiole did not trigger leaf hyponasty (Fig. S6). In contrast, we noticed that such treatments instead led to a reduction of the leaf angle (Fig. S6). We observed similar results when those treatments were performed at the petiole–blade junction (Fig. S6). Collectively these experiments indicate that auxin production in the leaf blade, but not in the petiole, induces a local leaf hyponastic response.

To study the auxin response triggered by shade or auxin application, we used the *DR5:GUS* auxin response marker line. As reported previously, a reduction of the R/FR led to an increase in GUS signal at the leaf margins (Fig. 3 *A* and *B*) (17). In addition, in the petiole we observed that the GUS signal was concentrated in the vasculature (Fig. 3 *A* and *B*, *Insets*). When auxin was applied to the leaf tip, we observed broad staining in the blade, whereas in the petiole the signal again was strongest in the vasculature (Fig. 3*C*, *Right Inset*). Moreover, the restriction of auxin-induced *DR5:GUS* expression to the blade and petiole

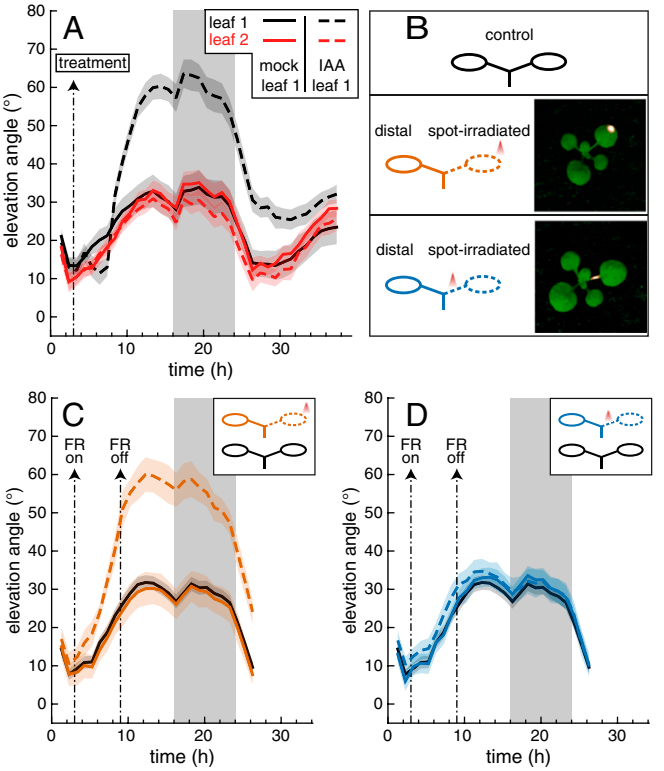

Fig. 2. Shade-induced hyponasty is restricted to the treated leaf and requires sensing at the tip rather than at the petiole. (*A*) Leaf elevation angle of Col-0 leaf 1 (black lines) and leaf 2 (red lines) with mock solution (solid lines) or 10 μM IAA (dashed lines) applied to the tip of leaf 1. Plants were grown as in Fig. 1 except that imaging started at ZT0 on day 16 ($t = 0$), and at ZT3 a 1-μL drop of solution was applied to the tip of leaf 1 (adaxial side). Data are mean of n = 14–15. (*B*) Illustration of the different treatments applied in *C* and *D*. (*Upper*) Leaves 1 and 2 growing in standard high R/FR conditions (control solid black outlines). (*Middle*) One single leaf (leaf 1 or 2) was spot-irradiated with FR on the tip (indicated by an orange triangle; the irradiated leaf is drawn with a dashed orange outline), while the opposite leaf was growing in high R/FR conditions (distal leaf drawn with a solid orange outline). Note the illumination on the representative plant shown on the right. (*Lower*) One single leaf (leaf 1 or 2) was spot-irradiated with FR on the petiole (indicated by an orange triangle; the leaf is drawn with a dashed blue outline) while the opposite leaf was growing in high R/FR conditions (distal leaf drawn with a solid blue outline). Note the illumination on the representative plant on the right. (*C*) Leaf elevation angles of Col-0 leaves grown in control conditions (solid black line, n = 48) or with FR spot illumination on the leaf tip (dashed orange line, n = 12), and of the distal untreated leaves of the same plants (solid orange line, n = 12). (*D*) Leaf elevation angle of Col-0 leaves grown in control conditions (solid black line, n = 48) or with FR spot illumination on the petiole (dashed blue line, n = 12) and of the distal untreated leaves of the same plants (solid blue line, n = 12). Plants in *C* and *D* were grown as in Fig. 1 except that FR illumination started on day 15 at ZT3 ($t = 3$) and stopped at ZT9 ($t = 9$). Leaf elevation angles are mean values. Opaque bands around mean lines in *A*, *C*, and *D* represent the 95% CIs of mean estimates. Vertical gray bars represent night periods.

of the treated leaf further confirmed the local nature of the auxin response (Fig. 3*C*) (37). The GUS signal in the vasculature of the petiole was prevented by the simultaneous application of IAA on the tip and NPA on the blade–petiole boundary, a treatment that also inhibited leaf hyponasty (Fig. 3*D* and Fig. S4*F*). When IAA was applied on one side at the margin in the middle of the longitudinal blade axis, we observed that the GUS signal was restricted to half of the leaf blade, with the midvein acting as a boundary (Fig. S7*A*). Following such a treatment, staining in the petiole vasculature was also prominent, but in addition we

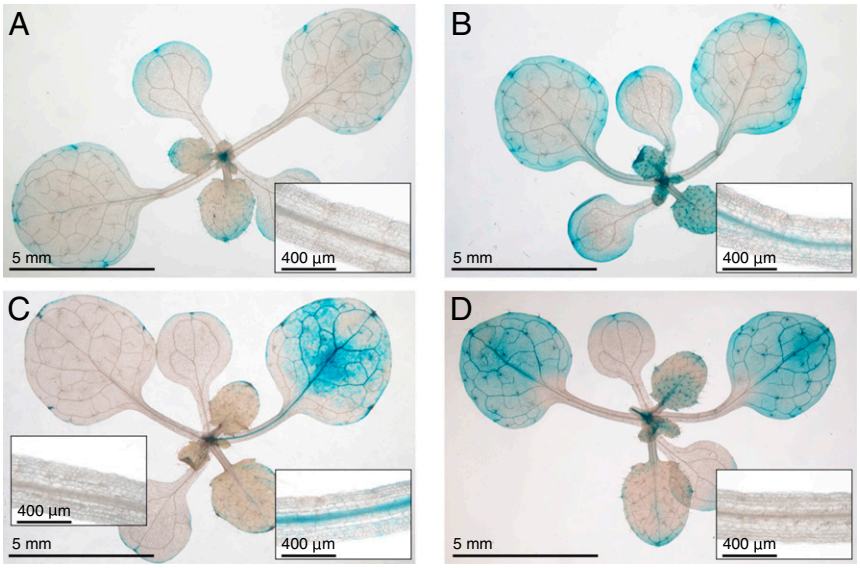

Fig. 3. Shade and auxin application both lead to an increased auxin response within the vasculature of the petiole. The auxin response was visualized in leaves of *DR5:GUS* reporter plants after 7 h in high R/FR (*A*), in low R/FR (*B*), after exogenous auxin application on the tip of leaf 1 in high R/FR (*C*, leaf on the right), or after simultaneous exogenous auxin and NPA applications on the tip and petiole–blade junction, respectively, of leaves 1 and 2 in high R/FR (*D*). Plants were grown as in Fig. 1. (*B*) Shade treatment started on day 15 at ZT3. (*C*) At ZT3 on day 15, a 1-µL drop of 10 µM IAA was applied to the tip of leaf 1 (adaxial side). The petioles of both the treated (*Right Inset*) and untreated (*Left Inset*) leaves are shown. (*D*) At ZT3 on day 15, 1-µL drops of 10 µM IAA and 20 µM NPA were administered simultaneously to the tip and the petiole–blade junction, respectively, of leaves 1 and 2 (adaxial side). Plants were harvested on day 15 at ZT10. *Insets* in all panels show close-ups of petioles.

observed a lateral GUS gradient across the petiole (Fig. S7*A*). We also applied auxin to the petiole–blade junction and to the petiole of *DR5:GUS* plants. Such IAA applications resulted in a broader GUS signal in the petiole (Fig. S7 *B* and *C*). When IAA was applied to the petiole–blade junction, the signal was very broad close to the application site but was concentrated inside the vasculature at the petiole base (Fig. S7*B*), suggesting that polar auxin transport, which is required for shade-induced hyponasty (Figs. S3 and S4), leads to a focused auxin response in the vasculature.

Auxin Signaling in the Vasculature and the Epidermis Controls Low R/FR-Induced Hyponasty. To determine whether a similar trend was also observed for other auxin-regulated genes, we analyzed an *IAA19:GUS* reporter line, because *IAA19* expression is induced by shade and auxin. Low R/FR enhanced the GUS signal, particularly in the petiole vasculature of expanding leaves (Fig. S8 *A* and *B*) (38). To determine the functional importance of IAA19 in this process, we used the *massugu2* (*msg2*) allele expressing a stabilized IAA19 auxin-signaling inhibitor (39). Interestingly, this mutant displayed a diurnal leaf hyponastic pattern similar to that of the wild type in control conditions but a reduced low R/FR-induced response (Fig. 4*A* and Fig. S8 *C* and *D*). This observation is noteworthy, because the *msg2* mutant exhibited normal shade-induced petiole elongation (Fig. 4*B*) (40), thereby demonstrating that IAA19 function is restricted to specific shade-induced responses. Taken together, our data show that a neighbor-proximity signal leads to an auxin response that is particularly strong in the petiole vasculature and suggests that this response is important for the leaf hyponastic response (Figs. 3 and 4 and Fig. S8).

It was previously proposed that elevation of the petiole angle is caused by differential growth between the adaxial and abaxial sides of the petiole (41, 42). Moreover, auxin signaling in the epidermis is important to control low R/FR-induced hypocotyl elongation (43), suggesting that auxin signaling in the epidermis is important to mediate shade-induced leaf hyponasty. To test this notion we used epidermal-specific expression of *axr3-1* (*CER6:axr3-1*), coding for a stable version of the IAA17 auxin-signaling inhibitor (43). In this mutant leaf movements were altered in control conditions, and low R/FR-induced leaf hyponasty was largely suppressed (Fig. 5 *A* and *B* and Fig. S9 *A*–*C*). Moreover, we found that application of auxin on the tip of *CER6:axr3-1* leaves did not trigger leaf hyponasty (Fig. 5*C* and Fig. S9*D*). Because epidermal expression of *axr3-1* leads to obvious morphological alterations (Fig. S9*A*) (43), rendering leaf tip tracking more difficult, we validated our observation by photogrammetric experiments (Fig. S9). This method allowed us to show that neither the leaf nor the petiole angle increased when *CER6:axr3-1* leaves were treated with low R/FR or auxin (Fig. S9). Collectively our data suggest that shade-induced leaf hyponasty requires auxin signaling in both the vasculature and the epidermis.

Discussion

Neighbor proximity triggers a reduction in the R/FR elicited by FR light reflected from surrounding plants (3, 8). In *Arabidopsis*, this light cue is perceived primarily by phyB, which controls a suite of low R/FR- or shade-induced responses (3). Key players controlling hypocotyl and petiole elongation have been identified.

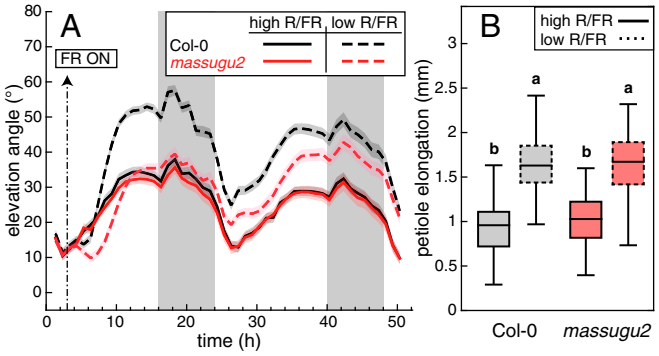

Fig. 4. A gain-of-function mutation in *IAA19* confers reduced shade-induced leaf hyponasty while maintaining a wild-type elongation response. (*A*) Leaf elevation angle of leaves 1 and 2 in Col-0 plants (black lines) and *massugu2* mutants (red lines) in high (solid lines) versus low (dashed lines) R/FR conditions. Plants were grown as in Fig. 1. Leaf elevation angles are mean values ($n = 57$–59). Opaque bands around mean lines represent the 95% CIs of mean estimates. Vertical gray bars represent night periods. (*B*) Boxplots representing petiole elongation over the time period from ZT2 on day 15 ($t = 2$) to ZT2 on day 16 ($t = 26$) computed for each individual leaf analyzed in *A*. Solid and dashed outlines represent data from high R/FR and low R/FR conditions, respectively. Two-way ANOVAs followed by Tukey's HSD test were performed, and different letters were assigned to significantly different groups ($P < 0.05$).

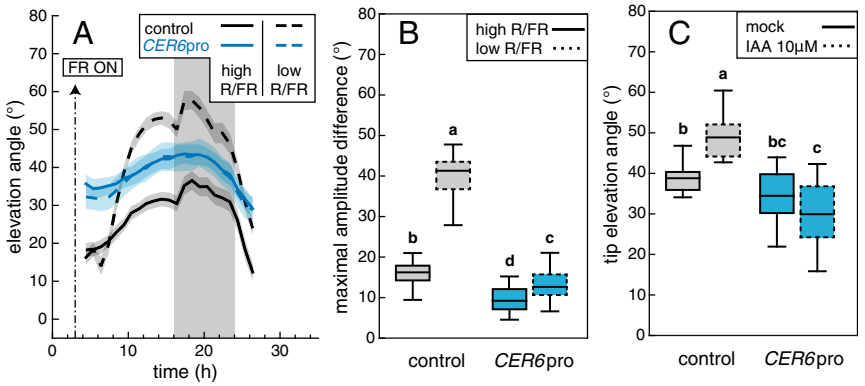

Fig. 5. Auxin signaling in the epidermis is required for shade- and auxin-induced leaf hyponastic responses. (*A*) Leaf elevation angle of leaves 1 and 2 in control plants (black lines) and *CER6:axr3-1* mutants (blue lines) in high (solid lines) versus low (dashed lines) R/FR conditions. Plants were grown as in Fig. 1. Leaf elevation angles are mean values (n = 24–30). Opaque bands around mean lines represent the 95% CIs of mean estimates. Vertical gray bars represent night periods. (*B*) Boxplots representing the amplitude of leaf movement between maximum and minimum leaf elevation angles over the time period from ZT3 (t = 3) to ZT16 (t = 16) on day 15 and computed for each individual leaf analyzed in *A*. Plots with solid and dashed outlines represent data from high and low R/FR conditions, respectively. (*C*) Boxplots representing tip elevation angle for leaf 1 at ZT11 on day 15 (n = 12–16) in control plants (gray plots) and *CER6:axr3-1* mutants (blue plots) treated with mock solution (solid outlines) or 10 μM IAA (dashed outlines). Plants were grown as in Fig. 1, with the exception of the application of solution to the tip of leaf 1 (adaxial side). For *B* and *C*, two-way ANOVAs followed by Tukey's HSD test were performed, and different letters were assigned to significantly different groups (P < 0.05). Groups with multiple assigned letters are not significantly different from groups sharing one of these letters, at least.

They include PIFs orchestrating transcriptional reprograming induced by shade cues (12, 13, 21, 24). Among the numerous PIF targets, several *YUC* genes were directly linked to auxin production and elongation responses (12, 19, 25). In this study we show that the PIF-YUC regulon is also essential for low R/FR-induced leaf hyponasty (Fig. 1 and Fig. S2). Moreover, PIN3, 4, and 7 are required for both shade-regulated growth responses and leaf hyponasty (Fig. S3) (18, 24, 25). Despite the involvement of common elements controlling petiole growth and position, our study reveals a bifurcation in the signaling pathways underlying these responses. Indeed, in low R/FR the *msg2* mutant elongates its petiole normally, but leaf hyponasty is impaired (Fig. 4 and Fig. S8) (40).

We establish the importance of auxin for low R/FR-induced hyponasty based on both gain- and loss-of-function studies (Fig. 1 and Figs. S3 and S4). The phenotype of the *yuc* quadruple mutant lacking the *YUC* genes that are rapidly induced upon shade treatment is particularly noteworthy (12, 19, 25). This mutant displays a normal diel-regulated leaf position in high R/FR, but it is essentially unresponsive to the reduction in the R/FR (Fig. 1). Given that the application of auxin or induction of *YUC* expression at the leaf tip triggers a hyponastic response with kinetics comparable to a low R/FR treatment (Fig. 1 and Fig. S4), we conclude that localized shade-induced auxin synthesis is both necessary and sufficient to trigger leaf hyponasty.

The site of auxin production strongly influences the hyponastic response. This conclusion is based on localized reduction of the R/FR (Fig. 2), *YUC* expression (Fig. S6), or auxin application (Fig. 1 and Figs. S4 and S6). In all cases, treatment of the petiole did not lead to leaf hyponasty, whereas the same treatment on the leaf margin was effective. IAA application to the leaf tip led to stronger hyponasty than application on the middle of the margin (Fig. 1 and Fig. S4). However, the latter also resulted in lateral displacement of the leaf (Fig. S4*C*), suggesting that local modulation of IAA levels triggers highly plastic leaf repositioning, as previously observed in densely grown *Arabidopsis* plants (28). Local auxin application to *DR5:GUS* plants gave a strong GUS response irrespective of the application site, indicating that auxin is perceived throughout the leaf (Fig. 3 and Fig. S7). Moreover, it was previously shown that low R/FR is sensed either at the petiole or the blade to trigger petiole elongation (15). Finally, low R/FR induces *YUC* expression in both the blade and the petiole (40). Therefore, we conclude that the absence of hyponastic response following treatments on the petiole is not caused by its inability to produce or sense auxin. In contrast, this situation is analogous to shade-induced elongation, in which low R/FR sensing and auxin production occur in the cotyledon/leaf blade, but the response is observed distally in the hypocotyl/petiole (Fig. 2 and Figs. S4 and S6) (15, 16, 25). An interesting question is why *Arabidopsis* uses information from the blade rather than from the petiole to control leaf hyponasty. Our data based on local shade/auxin application suggest that this mechanism allows highly plastic repositioning of the leaf (Fig. S4 *B* and *C*). Moreover, the advantage of using the leaf tip to perceive the presence of competitors was predicted using a modeling approach in the accompanying paper by Pantazopoulou et al. (44) in this issue of *PNAS*.

That the regulation of auxin levels directly in the petiole does not induce a hyponastic response suggests that auxin needs to be distributed properly within the petiole to trigger the response. Indeed, polar auxin transport and PIN proteins have been implicated in the control of leaf hyponasty (Fig. S3) (45). We show that low R/FR-induced hyponasty requires the activities of PIN3, 4, and 7 but not PIN1 (Fig. S3). To identify sites with a strong auxin response correlating with the hyponastic response, we used *DR5:GUS* lines, which suggest the importance of the petiole vasculature (Fig. 3 and Fig. S7) (46). In addition, the petiole vasculature also corresponds to a site of strong shade-induced *IAA19/MSG2* expression (Fig. S8 *A* and *B*) (38). Finally, the *msg2* mutant expressing a stable form of the IAA19 protein has a reduced hyponastic response in low R/FR (Fig. 4, S8). We therefore propose that auxin signaling in the vasculature is important for shade-mediated leaf elevation. In addition, we show that auxin signaling is also required in the epidermis (Fig. 5 and Fig. S9), as previously observed for shade-induced hypocotyl elongation (43). We thus propose that auxin, which is synthesized in the blade, must be canalized toward the midvein, from which point it forms a gradient in the petiole leading to directional leaf movement through asymmetric auxin-controlled epidermal cell expansion (Fig. 3 and Figs. S4 and S7) (41, 42, 44). Of note, communication between events modulating auxin content in the blade with an effect on the petiole was observed more than 60 years ago (47).

When considering the entire plant, leaf elevation represents a good example of a localized response (Fig. 2 and Fig. S5). In this respect, leaf hyponasty differs from systemic shade responses such as the control of stomatal density (36) or hypocotyl elongation (16). Shade control of stomatal density occurs through developmental regulation of newly emerging leaves that have not experienced the shade treatment (36). In this case a shade signal influences young leaf primordia, a response that may involve distal shade signals leading to reprograming of gene expression at the shoot apex (48). Local shade responses, which have been observed in several species (9, 26, 28), are

considered to be particularly important in the heterogeneous light conditions that are typical in natural environments (26, 27). However, the molecular mechanism underlying local shade responses was unknown. Our study provides a mechanism underlying one such response which combines localized low R/FR-controlled IAA production coupled with PIN3, 4, 7-mediated transport. Auxin produced in the shaded leaf is transported toward the lower parts of the plant where it influences the growth of hypocotyls, stems, or roots as well as branching (3). However, in agreement with models explaining the major routes of auxin transport (49), production of auxin in one leaf does not appear to trigger an auxin response in other rosette leaves (Fig. 3).

Materials and Methods

Detailed experimental procedures are provided in *SI Materials and Methods*.

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