RESEARCH COMMUNICATION

Canopy shade causes a rapid and transient arrest in leaf development through auxin-induced cytokinin oxidase activity

Monica Carabelli,^{1,3} Marco Possenti,^{2,3}
Giovanna Sessa,¹ Andrea Ciolfi,^{1,2}
Massimiliano Sassi,¹ Giorgio Morelli,² and
Ida Ruberti^{1,4}

¹Institute of Molecular Biology and Pathology, National Research Council, 00185 Rome, Italy; ²National Research Institute for Food and Nutrition, 00178 Rome, Italy

A plant grown under canopies perceives the reduction in the ratio of red (R) to far-red (FR) light as a warning of competition, and enhances elongation growth in an attempt to overgrow its neighbors. Here, we report that the same low R/FR signal that induces hypocotyl elongation also triggers a rapid arrest of leaf primordium growth, ensuring that plant resources are redirected into extension growth. The growth arrest induced by low R/FR depends on auxin-induced cytokinin breakdown in incipient vein cells of developing primordia, thus demonstrating the existence of a previously unrecognized regulatory circuit underlying plant response to canopy shade.

Supplemental material is available at <http://www.genesdev.org>.

Received March 13, 2007; revised version accepted June 22, 2007.

Plants have evolved sophisticated mechanisms to perceive and respond to the presence of neighbors. Within a vegetation, the ratio of red (R) to far-red (FR) light is lowered by the absorption of R light by photosynthetic pigments. The low R/FR signal provides an early warning of shading and induces developmental responses that, when successful, result in the overgrowth of neighbors (Casal and Smith 1989). Upon sensing a low R/FR ratio, a shade-avoiding plant reacts very rapidly and increases the elongation of stem-like organs (including hypocotyl and petioles) at the expense of leaf development even before it is directly shaded (Ballaré 1999; Smith 2000). In the long term, low R/FR exposure leads to early flowering (Franklin and Whitelam 2005) with a reduced seed set, which is considered an escape mechanism because it shortens generation time.

Consistent with the rapidity of the elongation response to low R/FR and its reversibility upon perception of high R/FR, changes in gene expression are very rapid

and reversible (Carabelli et al. 1996; Salter et al. 2003; Roig-Villanova et al. 2006). The transcript level of the *Arabidopsis* HD-Zip *ATHB2* and basic helix-loop-helix (bHLH) *PIL1* transcription factor genes functionally implicated in the elongation response provoked by neighbor detection (Steindler et al. 1999; Salter et al. 2003) increases within a few minutes of low R/FR exposure. Significantly, *ATHB2* and *PIL1* transcript levels fall very rapidly after transfer from low to high R/FR (Carabelli et al. 1996; Salter et al. 2003). Interestingly, low R/FR also provokes a rapid induction of the *HFR1/SICS1* gene, a negative controller of the shade avoidance response, ensuring that an exaggerated reaction does not occur when the plant is unsuccessful in escaping canopy shade (Sessa et al. 2005).

Analogous to the phenomena of phototropism and gravitropism, several findings indicated that auxin and auxin transport systems are also important components of the elongation process induced by low R/FR. Consistent with the observation that phytochrome regulation of stem elongation is partly the result of changes in IAA levels, it was found that *axr1*, severely impaired in auxin response, does not elongate significantly in low R/FR. Furthermore, it has been shown that naphthylphthalamic acid (NPA), an auxin transport inhibitor, significantly reduces hypocotyl elongation of wild-type seedlings in response to low R/FR (Morelli and Ruberti 2000). Consistently, mutations in *BIG*, which is required in normal auxin efflux, result in attenuated shade avoidance responses (Kanyuka et al. 2003).

Despite considerable work in dissecting the shade avoidance response, the effects of low R/FR on leaf development have remained largely unknown. Here, we report that canopy shade causes a rapid and transient arrest in leaf primordium growth, promoting auxin-induced cytokinin breakdown in incipient vein cells of developing primordia.

Results and Discussion

A morphological analysis of fully expanded first leaves revealed that cell number, not cell size, contributes to the reduced leaf size of plants grown in low R/FR relative to high R/FR (Supplementary Fig. S1; Supplementary Table S1). Therefore, the effect of a brief low R/FR treatment on cell proliferation of developing first leaves was investigated using the *cyc1At::GUS* reporter. The GUS reporter is expressed in cells in the G2-M transition, and can be used as a marker for cells about to enter, or in the process of, division (Donnelly et al. 1999).

Seedlings were grown for 7 d in high R/FR, and then maintained in high R/FR or transferred to low R/FR for 8 h. Based on whole-mount analysis, *cyc1At::GUS*-expressing cells are not uniformly distributed throughout the leaf at this developmental stage (Fig. 1A). It is known that cessation of cell cycling occurs from leaf apex to base. In fact, while dividing cells are initially distributed uniformly throughout the primordia, cells near the apex cease dividing first, and the region of frequent divisions gradually becomes restricted to the leaf base, forming a strong longitudinal gradient (Donnelly et al. 1999). In seedlings grown in high R/FR, the number of GUS-stained cells is greatly reduced in distal sectors of the leaf blade compared with the median and proximal regions

[**Keywords:** *Arabidopsis*; shade avoidance response; light quality changes; leaf development; auxin signaling; cytokinin breakdown]

³These authors contributed equally to this work.

⁴Corresponding author.

E-MAIL ida.ruberti@uniroma1.it; FAX 39-06-4991-2500.

Article is online at <http://www.genesdev.org/cgi/doi/10.1101/gad.432607>.

Figure 1. The *cyc1At::GUS* pattern of developing leaves is rapidly and strongly affected by low R/FR. (A) Histochemical localization of GUS activity in axial subepidermal cells of the first leaf of *cyc1At::GUS* seedlings grown for 7 d in high R/FR (0 h), and then maintained in high R/FR or transferred to low R/FR for 8 h. Fifty *cyc1At::GUS* leaves were analyzed in each condition. Leaf area (average $\pm$ SEM): 0 h, 0.12 ± 0.007 mm²; 8 h high R/FR, 0.23 ± 0.010 mm²; 8 h low R/FR, 0.17 ± 0.008 mm². Bars, 100 μ m. (B) Cyclin indices for the axial subepidermal layer in the proximal (P), median (M), and distal (D) regions of the first leaf in high and low R/FR, presented with Box-and-Whiskers plot. The box delimits the first and third quartiles; the solid line within the box represents the second quartile; bars indicate upper and lower fence; dots represent outliers. The cyclin index was determined measuring 50 samples in each condition.

(Fig. 1A). Exposure to low R/FR results in a dramatic decrease in GUS expression, with all three sectors showing significantly fewer GUS-stained cells. Moreover, the labeling of these cells is weak relative to that in high R/FR (Fig. 1A).

To highlight the differences in cell cycling frequency in high and low R/FR, the cyclin index in the subepidermal cell layer was calculated (Donnelly et al. 1999). As expected from GUS analysis, there is a strong decrease in the cyclin index values in the leaves of seedlings exposed to low R/FR relative to those maintained in high R/FR (Fig. 1B). Interestingly, the effect of low R/FR is not homogenous throughout the leaf. In fact, the highest reduction in the cyclin index is observed in the distal region (4.3-fold), the lowest is observed in the proximal region (2.6-fold), and an intermediate effect is observed in the median region (3.7-fold).

To further investigate the effect of low R/FR on leaf cell proliferation, the early stages of leaf primordium development were analyzed. To this end, seedlings were grown for 4 d in high R/FR and then maintained in high R/FR or transferred to low R/FR for 1 and 2 d. From 4 d after germination, the leaf primordium enlarged rapidly in high R/FR, with a 2.3-fold and 6.6-fold increase in 1 and 2 d, respectively (Supplementary Table S2). In contrast, the growth of the primordium was almost arrested in seedlings transferred to low R/FR for 1 and 2 d, and the increase in size was only 1.2-fold and twofold, respectively (Supplementary Table S2). No significant difference in the axial subepidermal cell area of leaf primordia in high and low R/FR was observed (Supplementary Table S2).

Together, the data indicate that low R/FR rapidly reduces the frequency of leaf cell division. The data also suggest that low R/FR does not affect cell proliferation in a cell-autonomous manner. Conversely, the data support the hypothesis that low R/FR affects an apical-basal gradient of a diffusible signal involved in the regulation of cell division, likely auxin.

Auxin has been implicated in various aspects of plant development—including embryogenesis, root development, vascular patterning—as well as in growth responses to environmental signals. Auxin has also been shown to induce leaf and flower formation at the shoot apical meristem, and evidence for an auxin maximum as an essential element for leaf primordium development has recently accumulated (Benjamin et al. 2005; Fleming 2006; Golz 2006).

To investigate whether low R/FR affects auxin signaling in leaf primordia, we used the synthetic *DR5::GUS* early-auxin-responsive gene (Ulmasov et al. 1997), whose activity correlates with direct auxin measurements (Casimiro et al. 2001). Previous work demonstrated that *DR5::GUS* patterns in the developing leaf primordium presage the domains of procambial differentiation. At very early stages of development, a subset of the most distal subepidermal cells expresses GUS. At later stages, local *DR5* expression precedes the earliest stages of anatomically detectable procambial differentiation (Mattsson et al. 2003).

DR5::GUS seedlings were grown for 4 d in high R/FR, then maintained in high R/FR or transferred to low R/FR for 4 h. Figure 2A shows that the GUS staining is significantly higher in hypocotyl and cotyledons of seedlings exposed to low R/FR than in those grown in high R/FR. Interestingly, low R/FR also increases the expression of *DR5::GUS* in leaf primordia (Fig. 2A, insets). The

Figure 2. A low-R/FR pulse induces *DR5::GUS* expression during leaf procambium specification. (A) Histochemical localization of GUS activity in *DR5::GUS* seedlings grown for 4 d in high R/FR, and then maintained in high R/FR or transferred to low R/FR for 4 h. The insets show a magnification under DIC optics of the first leaf primordium in each panel. Fifty *DR5::GUS* leaf primordia were analyzed in each condition. Bars, 0.5 mm; insets, 25 μ m. (B) Histochemical localization of GUS activity in *DR5::GUS* leaves of seedlings grown for 6 d in high R/FR and then maintained in high R/FR or transferred to low R/FR for 4 h. Fifty *DR5::GUS* leaves were analyzed in each condition. Bars, 50 μ m.

auxin maximum at the tip of the primordium is visible exclusively in seedlings exposed to a brief low-R/FR treatment (Fig. 2A, insets).

To assess the effects of low R/FR on auxin signaling in leaf primordia at a later developmental stage, *DR5::GUS* seedlings were grown for 6 d in high R/FR, and then maintained in high R/FR or transferred to low R/FR for 4 h. At this stage, the initiation of the leaf lamina and the differentiation of the midvein occurred; *DR5::GUS* expression diminished in the midvein, while new expression zones appeared at the sites of future secondary vein loops, followed by others that coincided with the formation of the tertiary veins (Mattsson et al. 2003). No *DR5::GUS* pattern change was observed in 6-d-old leaf primordia exposed to a brief low-R/FR treatment relative to those maintained in high R/FR, but a conspicuous increase in GUS expression was noted (Fig. 2B).

To determine whether the transient arrest in leaf primordium growth provoked by low R/FR is indeed an auxin response, we analyzed seedlings lacking a functional TIR1 auxin receptor (Dharmasiri et al. 2005; Kepinski and Leyser 2005). Wild type and *tir1-1* (Ruegger et al. 1997) were grown for 4 d in high R/FR, and then maintained in high R/FR or transferred to low R/FR (Supplementary Fig. S2A; see Supplementary Fig. S3A and Supplementary Table S3 for adult phenotypes). Leaf primordia were measured after 8 and 24 h in these treatments. No significant alteration in leaf primordium size was observed in *tir1-1* relative to wild type in high R/FR (Fig. 3A,B). In wild type, as previously shown, low R/FR rapidly and strongly affects the growth of leaf primordia (Fig. 3A,B). In contrast, low R/FR has no effect on primordia development in *tir1-1* (Fig. 3A,B). No significant difference in the adaxial subepidermal cell area of wild-type and *tir1-1* leaf primordia was observed (see Fig. 3 legend). Interestingly, no significant difference in hypocotyl length was observed in *tir1-1* relative to wild type in low R/FR, implying that a distinct auxin receptor is

involved in the hypocotyl response or TIR1 acts redundantly with one or more TIR1 homolog (Supplementary Fig. S2C; Quint and Gray 2006).

Together, the data demonstrate that the rapid and strong reduction in leaf cell proliferation provoked by low R/FR is indeed an auxin response, occurring primarily in incipient vein cells of developing leaf primordia.

To identify negative regulators of leaf cell proliferation in low R/FR, we devised a two-step screen consisting of (1) the identification of genes rapidly and transiently induced by low R/FR, whose expression is also regulated by auxin, and (2) the selection of the corresponding mutants with an altered growth response of leaf primordia to low R/FR.

Affymetrix *Arabidopsis* Genome GeneChip array (ATH1) analyses on seedlings exposed to low R/FR for 1 h and 4 d (Supplementary Table S4; ArrayExpress database, accession no. E-MEXP-443; Sessa et al. 2005) identified 38 genes previously described as auxin inducible (fold change, ≥ 1.75) (Supplementary Table S5; Supplementary Fig. S4; Hagen and Guilfoyle 2002; Philippart et al. 2004; Redman et al. 2004; Overvoorde et al. 2005; Cheng et al. 2006). Among them is *AtCKX6*, a gene encoding a cytokinin oxidase involved in cytokinin breakdown (Werner et al. 2003), which is rapidly and transiently induced by auxin (Redman et al. 2004). Interestingly, the *AtCKX6* gene is also down-regulated by NPA, indicating that *AtCKX6* gene expression may depend on translocated auxin (Werner et al. 2006).

Cytokinin plays a crucial role in regulating the proliferation and differentiation of plant cells. Considerable efforts in the last few years have been directed toward the discovery of proteins and enzymes that function in cytokinin signaling and metabolism (Ferreira and Kieber 2005; Sakakibara 2006). However, the analysis of the *AtCKX* gene family demonstrated that cytokinin degradation is also tightly regulated. *AtCKX* genes have distinct, almost nonoverlapping expression domains, suggesting that the tissue-specific regulation of the cytokinin content has an important role in ensuring the proper regulation of cytokinin functions in plants. Interestingly, *AtCKX6::GUS* expression was localized primarily to the vascular system of developing cotyledons, leaves, and roots. GUS activity was strongest in the vasculature of young leaves; it gradually decreased basipetally in older leaves, and was undetectable in fully expanded leaves. Moreover, plants overexpressing *CKX* genes had increased cytokinin breakdown. Cytokinin deficiency resulted in diminished activity of the vegetative and floral shoot apical meristem and leaf primordia, indicating an absolute requirement for the hormone (Werner et al. 2003).

To determine whether *AtCKX6* is up-regulated by low R/FR in leaf primordia, *AtCKX6::GUS* seedlings (Werner et al. 2003) were grown for 5 d in high R/FR, then maintained in high R/FR or transferred to low R/FR for 4 h. Figure 4A shows that low R/FR significantly increases the expression of *AtCKX6::GUS* in preprovascular cells of young leaf primordia.

To investigate whether *AtCKX6* is functionally involved in the rapid arrest of leaf primordium growth provoked by low R/FR, we isolated a homozygous *ckx6* T-DNA insertional line (Salk_070071, *ckx6-1*). RT-PCR analysis indi-

Figure 3. Low R/FR causes a rapid arrest in leaf primordium growth through the action of TIR1. (A) Col-0 and *tir1-1* were grown for 4 d in high R/FR, and then maintained in high R/FR or transferred to low R/FR for the indicated times. The mean area of the first leaf primordium was determined measuring 50 samples in each condition. (B) Leaf primordia of Col-0 and *tir1-1* grown for 4 d in high R/FR, and then maintained in high R/FR or transferred to low R/FR for 24 h, observed under DIC optics. Adaxial subepidermal cell area (average $\pm$ SEM): Col-0 High R/FR, $30.1 \pm 0.9 \mu\text{m}^2$; Col-0 Low R/FR, $30.0 \pm 0.8 \mu\text{m}^2$; *tir1-1* High R/FR, $29.5 \pm 0.8 \mu\text{m}^2$; *tir1-1* Low R/FR, $29.3 \pm 0.5 \mu\text{m}^2$. Bars, 20 μm .

Figure 4. Low R/FR promotes cytokinin oxidase activity in incipient vein cells of leaf primordia. (A) Histochemical localization of GUS activity in *AtCKX6::GUS* seedlings grown for 5 d in high R/FR, and then maintained in high R/FR or transferred to low R/FR for 4 h. The insets show a magnification under DIC optics of the first leaf primordium in each panel. Fifty *AtCKX6::GUS* leaf primordia were analyzed in each condition. Bars, 0.5 mm; insets, 25 μ m. (B) Col-0 and *cks6-1* were grown for 4 d in high R/FR, and then maintained in high R/FR or transferred to low R/FR for the indicated times. The mean area of the first leaf primordium was determined measuring 50 samples in each condition. (C) Leaf primordia of Col-0 and *cks6-1* grown for 4 d in high R/FR, and then maintained in high R/FR or transferred to low R/FR for 24 h, observed under DIC optics. Adaxial subepidermal cell area (average $\pm$ SEM): Col-0 High R/FR, $32.2 \pm 1.3 \mu\text{m}^2$; Col-0 Low R/FR, $31.6 \pm 1.2 \mu\text{m}^2$; *cks6-1* High R/FR, $33.5 \pm 0.8 \mu\text{m}^2$; *cks6-1* Low R/FR, $32.1 \pm 1.1 \mu\text{m}^2$. Bars, 20 μ m.

cated the presence of a truncated transcript in *cks6-1* (Supplementary Fig. S5A,B). This may result in expression of a truncated protein lacking most of the substrate-binding domain as well as part of the FAD-binding domain (Supplementary Fig. S5C), and thus inactive.

To analyze the leaf primordium phenotype of the *cks6-1* line, wild type and *cks6-1* were grown for 4 d in high R/FR, and then maintained in high R/FR or transferred to low R/FR (Supplementary Fig. S2B; see Supplementary Fig. S3B and Supplementary Table S3 for adult phenotypes). Leaf primordia were measured after 8 and 24 h in these treatments. In wild type, as previously shown, low R/FR rapidly and strongly affects primordia growth (Fig. 4B,C). In contrast, low R/FR has essentially no effect on leaf primordia development in *cks6-1* (Fig. 4B,C). No significant difference in the adaxial subepidermal cell area of wild-type and *cks6-1* leaf primordia was observed (see Fig. 4 legend). As in *tir1-1*, no significant difference in the hypocotyl length of wild type and *cks6-1* in low R/FR was observed (Supplementary Fig. S2C).

Molecular and phenotypic analysis of a F2 segregating progeny produced from a cross between *cks6-1* and

Col-0 confirmed that the leaf primordium phenotype in low R/FR segregates with the T-DNA insertion in the *AtCKX6* gene. The same analysis also indicated that the *cks6-1* mutation is indeed recessive (Supplementary Table S6).

Together, the data indicate that the induction of the auxin-regulated *AtCKX6* gene by low R/FR promoting cytokinin breakdown diminishes cell proliferation in developing leaf primordia. This conclusion is further supported by the recent finding that the mature leaves of multiple cytokinin receptor mutants contained significantly fewer cells compared with those of the wild type. Despite the reduced cell number, the final size of the cells was the same, demonstrating that the cell division activity in leaves is diminished (Nishimura et al. 2004; Riefler et al. 2006). Similarly, the reduced leaf size of plants overexpressing *AtCKX1* was primarily the result of a decreased cell number (Werner et al. 2003).

Interestingly, the data also suggest that *AtCKX6* promotes cytokinin breakdown specifically in preprocambial cells. Hence, it is tempting to speculate that induction of cytokinin degradation in the developing vasculature is by itself sufficient to arrest leaf primordium growth.

The results described here demonstrate the existence of a previously unrecognized regulatory circuit underlying plant response to canopy shade that involves both auxin and cytokinin. The rapid changes in auxin signaling induced by low R/FR that are crucial for the plant to enhance elongation growth and compete with its neighbors are also essential to trigger a transient arrest of leaf development, ensuring that all plant resources are indeed redirected into extension growth. Strikingly, the role of auxin in leaf primordia is to induce cytokinin degradation through the action of *AtCKX6*, and therefore to inhibit leaf growth.

A major challenge in the future is to understand how HFR1/SICS1, a master negative regulator attenuating virtually all plant responses to canopy shade, relates to the TIR1 pathway.

Materials and methods

Plant growth and phenotypic analyses

Wild type used was *Arabidopsis thaliana* (L.) Heynh. var. Columbia (Col-0). Other lines used were *cyc1At::GUS* (Kang and Dengler 2002), *DR5::GUS* (Ulmasov et al. 1997), *AtCKX6::GUS* (Werner et al. 2003), *tir1-1* (NASC ID N3798; Ruegger et al. 1997), and *cks6-1* (Salk_070071, NASC ID N570071). Plants were grown as previously described (Sessa et al. 2005). Light outputs in high and low R/FR were as described in Sessa et al. (2005). Images of green leaves were taken under an MZ8 binocular microscope (Leica), using a Spot Jr. digital camera (Diagnostic Instrument, Inc.). Leaf primordia and leaves were cleared as described previously (Weigel and Glazebrook 2002). Cleared samples were excised under an MZ8 binocular microscope, and then analyzed under dark-field optics or with differential interference contrast (DIC) optics, with an Axioskop 2 plus binocular microscope (Zeiss). Images were taken with the Coolpix 990 digital camera (Nikon Corp.). To determine the mean leaf primordium and leaf area, at least 50 samples were measured with the NIH Image Analysis software (<http://rsb.info.nih.gov/ij/>). T-test statistical analysis was performed using QuickCalcs Online Calculators for Scientists (<http://graphpad.com/quickcalcs>).

Analysis of adaxial subepidermal and mesophyll cells

Adaxial subepidermal and mesophyll cells were analyzed in cleared leaf primordia and mature leaves, respectively. Cells were viewed with an

Axioskop 2 plus binocular microscope equipped with DIC optics, and photographed with the Coolpix 990. In leaf primordia, the mean cell area was determined on the whole organ; in mature leaves, the mean cell area was determined in a distal location from the base of the leaf, recognized in the upper part of the leaf blade, inside the first loop of secondary vein. To determine the mean cell area, 100 cells were measured in the entire organ or in the distal region of 10 leaf primordia and leaves, respectively (Horiguchi et al. 2005).

Histochemical detection of β -glucuronidase activity

For GUS detection, whole seedlings were treated as described by Scarpella et al. (2004). Seedlings were incubated in cold (-20°C) 90% acetone for 1 h at -20°C , and washed twice for 5 min with 100 mM phosphate buffer (pH 7.7). To facilitate the uptake of the X-gluc substrate, seedlings were pretreated with vacuum at room temperature for 15 min prior to incubation at 37°C . Incubation was 3 h for *cyc1At::GUS*, 6 h for *DR5::GUS*, and 2 h for *AtCKX6::GUS*. The reaction was terminated in 70% (v/v) ethanol and samples were stored at 4°C . For microscopy, samples were cleared as described (Weigel and Glazebrook 2002) and viewed with an Axioskop 2 plus binocular microscope equipped with DIC optics; images were taken with the Coolpix 990.

Cyclin index determination

In cleared leaves, the total cell number and the number of cells with GUS activity in the adaxial subepidermal layer were counted at three regions along the proximodistal axis of the blade (proximal, median, and distal, from base to apex) by photographing leaves under DIC optics. A 0.01-mm^2 area was recognized in each of the three locations. The 0.01-mm^2 area of the distal region was recognized in the upper part of the leaf blade closed inside the first secondary looped veins in the procambial stage, the median one was recognized at the boundary between the first and the second loop of secondary veins in the procambial stage, and the proximal was recognized close to the margin of the basal part of the leaf blade. The total cell number and the number of cells with GUS activity were counted and expressed as the cyclin index (number of cells with GUS activity/number of total cells $\times$ 100) (Donnelly et al. 1999). The data are presented in the form of a Box-and-Whiskers plot, generated by means of R software (<http://www.r-project.org>). A "box plot" function with default parameters was used. The box extends from the first quartile (Q1) of the distribution to the third quartile (Q3), and median value is indicated by the horizontal line to its inside. The interquartile range is calculated as follows: $\text{IQR} = \text{Q3} - \text{Q1}$. Bars indicate upper and lower fence, calculated as follows: $\text{Fence}_{\text{upper}} = \text{Q3} + (1.5 \times \text{IQR})$, $\text{Fence}_{\text{lower}} = \text{Q1} - (1.5 \times \text{IQR})$. Any value over the fences is an outside value and was plotted as a separate point on the graph.

Gene expression analysis

Affymetrix *Arabidopsis* Genome Gene Chip array (ATH1) data (Array-Express database, accession no. E-MEXP-443) were analyzed as described by Sessa et al. (2005) to find auxin-regulated genes varying significantly in low R/FR.

Acknowledgments

We thank ABRC for the 170G16T7 and E7A12T7 EST clones, NASC for the *tir1-1* (NASC ID N3798) and *ckx6-1* seeds (Salk_070071, NASC ID N570071), Nancy Dengler for the *cyc1At::GUS* seeds, Jiri Friml for the *PIN3*-specific probe, Ben Scheres for the *DR5::GUS* seeds, and Thomas Schmullig for the *AtCKX6::GUS* seeds. We are grateful to Sabrina Bruni for the isolation of the *ckx6-1* homozygous. This work was supported, in part, by grants from MUR, Strategic Program FIRB 2001 and 2003, and MiPAF, Agronanotech program.

References

Ballaré, C.L. 1999. Keeping up with neighbours: Phytochrome sensing and other signaling mechanisms. *Trends Plant Sci.* **4**: 97–102.
 Benjamin, R., Malenica, N., and Luschnig, C. 2005. Regulating the regulator: The control of auxin transport. *Bioessays* **27**: 1246–1255.
 Carabelli, M., Morelli, G., Whitelam, G., and Ruberti, I. 1996. Twilight-zone and canopy shade induction of the *Athb-2* homeobox gene in green plants. *Proc. Natl. Acad. Sci.* **93**: 3530–3535.
 Casal, J.J. and Smith, H. 1989. The end of day phytochrome control of

internode elongation in mustard: Kinetics, interaction with the previous fluence rate and ecological implications. *Plant Cell Environ.* **12**: 511–520.
 Casimiro, I., Marchant, A., Bhalerao, R.P., Beeckman, T., Dhooge, S., Swarup, R., Graham, N., Inze, D., Sandberg, G., Casero, P.J., et al. 2001. Auxin transport promotes *Arabidopsis* lateral root initiation. *Plant Cell* **13**: 843–852.
 Cheng, Y., Dai, X., and Zhao, Y. 2006. Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in *Arabidopsis*. *Genes & Dev.* **20**: 1790–1799.
 Dharmasiri, N., Dharmasiri, S., and Estelle, M. 2005. The F-box protein TIR1 is an auxin receptor. *Nature* **435**: 441–445.
 Donnelly, P.M., Bonetta, D., Tsukaya, H., Dengler, R.E., and Dengler, N.G. 1999. Cell cycling and cell enlargement in developing leaves of *Arabidopsis*. *Dev. Biol.* **215**: 407–419.
 Ferreira, F.J. and Kieber, J.J. 2005. Cytokinin signaling. *Curr. Opin. Plant Biol.* **8**: 518–525.
 Fleming, A.J. 2006. Plants signaling: The inexorable rise of auxin. *Trends Cell Biol.* **16**: 397–401.
 Franklin, K.A. and Whitelam, G.C. 2005. Phytochromes and shade avoidance responses in plants. *Ann. Bot. (Lond.)* **96**: 169–175.
 Golz, J.F. 2006. Signaling between the shoot apical meristem and developing lateral organs. *Plant Mol. Biol.* **60**: 889–903.
 Hagen, G. and Guilfoyle, T. 2002. Auxin responsive gene expression: Genes, promoters and regulatory factors. *Plant Mol. Biol.* **49**: 373–385.
 Horiguchi, G., Kim, G.T., and Tsukaya, H. 2005. The transcription factor AtGRF5 and the transcription co-activator AN3 regulate cell proliferation in leaf primordia of *Arabidopsis thaliana*. *Plant J.* **43**: 68–78.
 Kang, J. and Dengler, N. 2002. Cell cycling frequency and expression of the homeobox gene *ATHB-8* during leaf vein development in *Arabidopsis*. *Planta* **216**: 212–219.
 Kanyuka, K., Praekelt, U., Franklin, K.A., Billingham, O.E., Hooley, R., Whitelam, G.C., and Halliday, K.J. 2003. Mutations in the huge *Arabidopsis* gene *BIG* affect a range of hormone and light responses. *Plant J.* **35**: 57–70.
 Kepinski, S. and Leyser, O. 2005. The *Arabidopsis* F-box protein TIR1 is an auxin receptor. *Nature* **435**: 446–451.
 Mattsson, J., Ckurshumova, W., and Berleth, T. 2003. Auxin signaling in *Arabidopsis* vascular development. *Plant Physiol.* **131**: 1–13.
 Morelli, G. and Ruberti, I. 2000. Shade avoidance responses. Driving auxin along lateral routes. *Plant Physiol.* **122**: 621–626.
 Nishimura, C., Ohashi, Y., Sato, S., Kato, T., Tabata, S., and Ueguchi, C. 2004. Histidine kinase homologs that act as cytokinin receptors possess overlapping functions in the regulation of the shoot and root growth in *Arabidopsis*. *Plant Cell* **16**: 1365–1377.
 Overvoorde, P.J., Okushima, Y., Alonso, J.M., Chan, A., Chang, C., Ecker, J.R., Hughes, B., Liu, A., Onodera, C., Quach, H., et al. 2005. Functional genomic analysis of the auxin/indole-3-acetic acid gene family members in *Arabidopsis thaliana*. *Plant Cell* **17**: 3282–3300.
 Philipp, K., Ivashikina, N., Ache, P., Christian, M., Luthen, H., Palme, K., and Hedrich, R. 2004. Auxin activates *KAT1* and *KAT2*, two K^{+} -channel genes expressed in seedlings of *Arabidopsis thaliana*. *Plant J.* **37**: 815–827.
 Quint, M. and Gray, W.M. 2006. Auxin signaling. *Curr. Opin. Plant Biol.* **9**: 448–453.
 Redman, J.C., Haas, B.J., Tanimoto, G., and Town, C.D. 2004. Development and evaluation of an *Arabidopsis* whole genome Affymetrix probe array. *Plant J.* **38**: 545–561.
 Riefler, M., Novak, O., Strnad, M., and Schmullig, T. 2006. *Arabidopsis* cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. *Plant Cell* **18**: 40–64.
 Roig-Villanova, I., Bou, J., Sorin, C., Devlin, P.F., and Martinez-Garcia, J.F. 2006. Identification of primary target genes of phytochrome signaling. Early transcriptional control during shade avoidance responses in *Arabidopsis*. *Plant Physiol.* **141**: 85–96.
 Ruegger, M., Dewey, E., Hobbie, L., Brown, D., Bernasconi, P., Turner, J., Muday, G., and Estelle, M. 1997. Reduced naphthylphthalamic acid binding in the *tir3* mutant of *Arabidopsis* is associated with a reduction in polar auxin transport and diverse morphological defects. *Plant Cell* **9**: 745–757.
 Sakakibara, H. 2006. Cytokinins: Activity, biosynthesis, and transloc-

Carabelli et al.

- tion. *Annu. Rev. Plant Biol.* **57**: 431–449.
- Salter, M.G., Franklin, K.A., and Whitelam, G.C. 2003. Gating of the rapid shade avoidance response by the circadian clock in plants. *Nature* **426**: 680–683.
- Scarpella, E., Francis, P., and Berleth, T. 2004. Stage-specific markers define early steps of procambium development in *Arabidopsis* leaves and correlate termination of vein formation with mesophyll differentiation. *Development* **131**: 3445–3455.
- Sessa, G., Carabelli, M., Sassi, M., Ciolfi, A., Possenti, M., Mittempergher, F., Becker, J., Morelli, G., and Ruberti, I. 2005. A dynamic balance between gene activation and repression regulates the shade avoidance response in *Arabidopsis*. *Genes & Dev.* **19**: 2811–2815.
- Smith, H. 2000. Phytochromes and light signal perception by plants—An emerging synthesis. *Nature* **407**: 585–591.
- Steindler, C., Matteucci, A., Sessa, G., Weimar, T., Ohgishi, M., Aoyama, T., Morelli, G., and Ruberti, I. 1999. Shade avoidance responses are mediated by the ATHB-2 HD-Zip protein, a negative regulator of gene expression. *Development* **125**: 4235–4245.
- Ulmasov, T., Murfett, J., Hagen, G., and Guilfoyle, T.J. 1997. Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *Plant Cell* **9**: 1963–1971.
- Weigel, D. and Glazebrook, J. 2002. *Arabidopsis: A laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Werner, T., Motyka, V., Laucou, V., Smets, R., Van Onckelen, H., and Schmulling, T. 2003. Cytokinin-deficient transgenic *Arabidopsis* plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *Plant Cell* **11**: 2532–2550.
- Werner, T., Kollmer, I., Bartrina, I., Holst, K., and Schmulling, T. 2006. New insights into the biology of cytokinin degradation. *Plant Biol.* **8**: 371–381.

Canopy shade causes a rapid and transient arrest in leaf development through auxin-induced cytokinin oxidase activity

Monica Carabelli, Marco Possenti, Giovanna Sessa, et al.

Genes Dev. 2007, **21**:

Access the most recent version at doi:[10.1101/gad.432607](https://doi.org/10.1101/gad.432607)

Supplemental Material

<http://genesdev.cshlp.org/content/suppl/2007/07/25/21.15.1863.DC1>

References

This article cites 36 articles, 13 of which can be accessed free at:
<http://genesdev.cshlp.org/content/21/15/1863.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).