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Differential expression of the auxin primary response gene *MASSUGU2/IAA19* during tropic responses of Arabidopsis hypocotyls

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We have examined the expression pattern of an auxin primary response gene, *MSG2/IAA19*, during photo- and gravitropic responses of hypocotyls by using a transgenic Arabidopsis harboring *MSG2/IAA19 promoter::GUS*. The upper portion of most etiolated hypocotyls showed uniform β-glucuronidase (GUS) staining with the strongest activity in the pericycle. When hypocotyls were irradiated with unilateral blue light, GUS activity on the concave side of hypocotyls was decreased, resulting in differential GUS staining with a stronger signal on the convex side. The number of differentially stained hypocotyls peaked at 24 h after the onset of the phototropic stimuli, while hypocotyl curvature continued to increase for the entire 36-h experimental period. This result suggests that the *MSG2/IAA19* expression precedes the phototropic responses. When seedlings were grown under dim white light, their hypocotyls displayed

almost no GUS activity. The light-grown hypocotyls also showed differential GUS staining after phototropic stimuli as result of the increase in GUS activity on the convex side of hypocotyls, especially in the epidermis, the outer cortex and pericycle, although GUS acitivity was much weaker than that observed in etiolated hypocotyls. Similar but less obvious differential staining was obtained for gravitropic response of hypocotyls. Considering the recent finding that Aux/IAA proteins are immediate targets of the auxin F-box receptors, *MSG2/IAA19* is likely to act as one of master genes for tropic responses.

Abbreviations -- ARF, auxin response factor; GUS, β-glucuronidase; RT-PCR, reverse transcription-polymerase chain reaction.

Introduction

Auxin appears to play a central role in tropic responses since malfunction of factors involved in either polar auxin transport or auxin signal transduction leads to severe defects in the tropic responses. Factors involved in polar auxin transport include auxin pin-formed efflux facilitators (PINs), a few ATP binding cassette transporters such as P-glycoprotein1 (PGP1) and auxin influx carriers such as auxin resistant1 (AUX1) (Geisler and Murphy 2006). The latter includes auxin F box receptors (AFBs and transport inhinitor response1 (TIR1); Dharmasiri et al. 2005a, Kepinski and Leyser 2005), the auxin primary response proteins, Aux/IAAs, and auxin response factors

(ARFs) (Hagen et al. 2004). These findings strongly suggest that photo- and gravistimuli modulate polar auxin flow, resulting in a lateral gradient of auxin concentration. This subsequently activates the auxin signal transduction pathway and leads to transcriptional regulation through ARFs in a differential manner. In fact, higher concentrations of auxin are found on the shaded side than on the irradiated side of maize (Fuchs et al. 2003) and rice (Haga et al. 2005) coleoptiles, rape hypocotyls (Esmon et al. 2006) and pea epicotyls (Haga and Iino 2006) in phototropism. Higher concentrations are also found on the lower side than on the upper side of maize coleoptiles in gravitropism (Philippar et al. 1999). These observations are consistent with the historical Cholodny-Went hypothesis (Went and Thimann 1937).

As for ARFs and Aux/IAAs, we have identified two tropism-defective Arabidopsis mutants, *nph4/msg1* of a recessive nature (Liscum and Briggs 1995, Watahiki and Yamamoto 1997) and *msg2* of a dominant nature (Tatematsu et al. 2004). *NPH4* encodes ARF7 (Harper et al. 2000) and *MSG2* encodes IAA19 (Tatematsu et al. 2004). Because the two mutants display similar phenotypes, MSG2/IAA19 is thought to repress NPH4/ARF7 function by binding through the protein-protein interaction domains shared by the two protein families (Hagen et al. 2004, Muto et al. 2006, Tatematsu et al. 2004). Aux/IAA proteins including MSG2/IAA19 are also thought to be primary targets of auxin F box receptors, and are thus degraded quickly after auxin perception by the receptors (Dharmasiri et al. 2005a, Kepinski and Leyser, 2005). This degradation activates downstream ARFs such as

NPH4/ARF7. On the other hand, *MSG2/IAA19* is an auxin primary response gene, and its transcription is also turned on through NPH4/ARF7. Therefore, NPH4/ARF7 function will be shut down again when MSG2/IAA19 accumulates to a sufficiently high level. This negative feedback loop is likely to be the molecular basis of the transient nature of tropic responses (Tatematsu et al. 2004).

The above mechanistic scheme of tropism is based on analyses of the phenotypes of the *nph4* and *msg2* mutants and mRNA and protein levels of involved factors in auxin-treated whole seedlings. We still do not have enough knowledge on when and where the above-mentioned factors function in plant organs during tropic responses. Considering the above model, *MSG2/IAA19* could be a master gene for tropism. However, it is unknown whether and how fast *MSG2/IAA19* responds to the lateral gradient of auxin concentration that is likely to be established during tropistic responses. Here, we attempted to answer these questions using a *MSG2/IAA19* promoter-β-glucuronidase (*GUS*) reporter gene (Tatematsu et al. 2004) in Arabidopsis hypocotyls.

Materials and methods

Plant materials and growth conditions

Seeds of the *MSG2/IAA19* promoter-*GUS* line (Tatematsu et al. 2004), which is *Arabidopsis thaliana* Columbia ecotype background, were first imbibed in

water in the dark at 4°C for 3 days. They were surface sterilized with 1% hypochlorite and sown on horizontally held agar plates that contained half-strength Murashige and Skoog (MS) salts (Murashige and Skoog 1962), 1% (w/v) sucrose and 0.9% (w/v) agar. After seeds were kept at 23°C under continuous white light illumination for 24 h to induce germination, seedlings were grown in the dark for 4 days. In some experiments, seedlings were grown under continuous dim white light (2.1 W m^{-2}) prepared by filtering output of three 40-W white fluorescent tubes (FL40SW; NEC, Tokyo, Japan) through grey acrylic sheet.

β-Glucuronidase staining and measurement of tropic responses

For the second-positive phototropism of hypocotyls, seedlings grown as above were irradiated with unilateral blue light at a fluence rate of 15 mW m^{-2} (or 0.059 μmol m^{-2} s^{-1}) obtained by blue light-emitting diodes (λ_{max} = 470 ± 30 nm; Stick-B16, Tokyo Rikakikai, Tokyo). Then, they were taken off agar plates and fixed with ice-cold 50% acetone for 45 min. After rinse with 50 mM sodium phosphate, pH 7.0, twice, they were stained for GUS activity by incubation in 50 mM sodium phosphate, pH 7.0, containing 1 mg ml^{-1} 5-bromo-4-chloro-3-indolyl-β-D-glucuronide, 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, 1 mM EDTA and 0.1% Triton X-100, at room temperature for 26 h. Then, the seedlings were rinsed with water twice and incubated in 70% ethanol for 24 h with one exchange of the same solution to get rid of pigments. After a brief rinse with water, stained seedlings were laid on the

bottom of a petri dish filled with water and were observed with a dissecting microscope (MZ12; Leica, Wetzlar, Germany) equipped with a digital camera (DXM1200; Nikon, Tokyo, Japan). Light-grown seedlings were stained with the 2 mg ml^{-1} GUS substrate for 50 h.

Phototropic curvature was determined between the basal and apical parts of hypocotyls on their images using IMAGE-PRO PLUS (Media Cybernetics, Silver Spring, MD). Seedlings were classified into four groups according to the pattern of GUS staining in the upper part of the hypocotyls: (1) uniform staining; (2) no staining; (3) stronger staining on the convex side; and (4) stronger staining on the concave side. At time 0, some of the straight hypocotyls, which amounted to 6.8%, were not stained uniformly but stained only on one flank of the hypocotyls. Half of these hypocotyls were placed in group 3 and the other half were placed in group 4. For gravitropic responses, seedlings grown as above were turned 135° and kept in this position in the dark for various time periods. Then, they were treated in the same manner as seedlings were treated in the phototropic measurements described above.

We examined about 100 etiolated seedlings for each time point except for the time 0 samples, where about 50 seedlings were measured. For light-grown seedlings we examined 30 - 40 hypocotyls. All the presented values are mean and the SE of the three independent experiments unless stated otherwise.

Microscopic examination

Stained seedlings were observed with a microscope (Zeiss Axioplan, Oberkochen, Germany) with the above-mentioned digital camera after immersion in clearing solution (chloral hydrate : glycerol : water = 100 g : 10 g : 25 ml). Transverse sections of stained hypocotyls were made with a microslicer (DTK-3000; Dohan EM, Kyoto, Japan) after they were embedded in 5% agarose.

Reverse transcription-polymerase chain reaction

About 20 mg of seeds of the *MSG2/IAA19* promoter-*GUS* plants were grown hydroponically in 1/2 MS liquid media under continuous white-light conditions of four different light fluence rates plus in the dark condition for 6 days. Seedlings were ground to powder in liquid nitrogen in a mortar with a pestle. Total RNA was prepared from the most part of the plant powder using RNeasy Plant Mini kit (QIAGEN, Hilden, Germany).

Reverse transcription-polymerase chain reaction (RT-PCR) analyses of the *MSG2/IAA19* mRNA were done essentially in the same way as described by Nakamoto et al. (2006). The PCR primers for the *MSG2/IAA19* cDNA were 5'-TCGGTTTCCGTGGCATCGGTGTGGCCTTGA -3' and 5'-TCACTCGTCTACTCCTCTAGGCTGCAGCCC-3'. *ACTIN8* gene was used as an internal control for the RT-PCR with two primers, 5'-TCTATCCTTGCTTCCCTCAGCACTTTCCAGCAG -3' and 5'-GCTACAAACAAACAAACAAATGGGGCTACAAACAAACAAACAAATGG

A-3'. After fractionated on 1.4% agarose gel and stained with ethidium bromide, fluorescence image of PCR products was taken with a digital camera (AE-6905H; Atto, Tokyo, Japan), and its intensity was quantified with IMAGEJ (NIH, Bethesda, MD). Quantitativeness of PCR was verified by always conducting PCR with two different concentrations of template cDNA.

Fluorometric assay of GUS activity

Protein extracts were made by mixing about 1/10 of the above-described plant powder and 0.2 ml of extraction buffer and centrifuging at 16000 *g* for 15 min at 4°C. Extraction buffer consisted of 50 mM sodium phosphate, pH 7.0, 10 mM 2-mercaptoethanol, 10 mM EDTA, 0.1% sodium lauryl sarcosine and 0.1% Triton X-100. GUS activity was determined fluorometrically as described by Gallagher (1992). Twenty-five microliters of protein extracts appropriately diluted with extraction buffer were mixed with 25 μl of extraction buffer containing 2 mM 4-methylumbelliferyl-β-D-glucuronide and incubated for 10 min at 37°C. Reaction was stopped by an addition of 39 volumes of 0.2 M sodium carbonate. Fluorescence at 455 nm was measured with excitation wavelength of 365 nm by the use of a spectrofluorometer (F-4500; Hitachi, Tokyo, Japan). Quantitativeness of the GUS assay was verified by conducting the GUS reaction with two different concentrations of protein extracts. Protein contents of the protein extracts were determined with the dye-binding method (Protein Assay Kit;

Bio-Rad, Hercules, CA; Bradford 1976) using bovine serum albumin as a standard.

Results

Tropic responses of dark-grown hypocotyls

We examined the staining pattern of *MSG2/IAA19* promoter-*GUS* during the second-positive phototropic responses of dark-grown seedlings. Etiolated seedlings were grown on horizontally-held agar plates for 4 days and were subjected to unilateral blue light irradiation for various time durations. Then, they were taken off agar plates, fixed and stained for GUS activity. Because phototropic curvature was determined between the basal and apical parts of hypocotyls for seedlings removed from the agar medium, we did not know the angles between the direction of irradiating blue light and the growth direction of hypocotyls in the present study. However, larger curvatures of hypocotyls were observed as seedlings were stimulated for a longer time (Fig. 1A, squares), indicating that phototropic response of hypocotyls did occur in this experimental condition.

Staining pattern of the *MSG2/IAA19* promoter-*GUS* activity was so variable in each seedling that we examined about 100 seedlings for each time point, and divided hypocotyls into 4 groups with different staining patterns as described in Materials and methods. At time 0, an upper part of most hypocotyls (86%) was uniformly stained blue (Fig. 1A, closed triangles; Fig.

2A, F). Hypocotyls bent slightly (7°) even without the blue light stimulus, and 9.6% of hypocotyls showed differential staining with a stronger signal on the convex side (Fig. 1A, open circles). When seedlings were subjected to unilateral blue light stimulus, the number of hypocotyls that showed differential staining with a stronger signal on the convex side (Fig. 1A, open circles; Fig. 2E, G) increased at the expense of the uniformly stained hypocotyls (Fig. 1A, closed triangles; Fig. 2C). The number of hypocotyls with no staining also increased to a lesser extent (Fig. 1A, open triangles; Fig. 2D). Although phototropic curvature increased monotonously during the entire 36-h-long experimental period, the number of hypocotyls with a stronger signal on the convex side reached a plateau of 58% at 24 h. Based on a comparison of stained seedlings before and after phototropic stimulation (Fig. 2A, F vs. 2E, G), it appears that the differential staining pattern of the GUS activity occurred as a result of its reduction on the concave side of hypocotyls.

As a control experiment, seedlings were irradiated uniformly with blue light from above, whose fluence rate was exactly the same as that of the unilateral irradiation. Then, the number of hypocotyls with stronger staining on the convex side did not increase significantly for 36 h ($P = 0.11$ in *t*-test) (Fig. 1B, open circles). The percentage of the uniformly stained hypocotyls decreased to a lesser extent (Fig. 1B, closed triangles) compared with the decrease observed in the unilateral blue light irradiation (Fig. 1A, closed triangles). The number of the unstained hypocotyls also increased to a lesser extent (Fig. 1B, open triangles). Even in the dark-kept seedlings

(Fig. 1C), the uniformly stained population (closed triangles) gradually decreased and the unstained population (open triangles) gradually increased.

The same experiments were conducted for the gravitropic response. Because the gravitropic response was weaker than the phototropic response under the present experimental conditions, seedlings were subjected to gravistimulation of 135° turning instead of common 90° turning. Hypocotyls kept bending under the gravistimuli for the entire experimental period (Fig. 1D, squares). The differential staining with a stronger signal on the convex side was seen in more hypocotyls as gravitropic curvature of hypocotyls developed (Fig. 1D, closed triangles). The number of differentially stained hypocotyls stopped increasing at 25 h in spite of continued increase in the gravitropic curvature. Although the observed time course of the change in the staining patterns was qualitatively similar to that of the above phototropic response, the magnitude of the gravitropic response was smaller than that of the phototropic response. Together, these results show that either unilateral blue light exposure or gravitropic stimulus induced differential expression of the *MSG2/IAA19* promoter-*GUS* activity in hypocotyls with a stronger expression on the convex side.

Tropic responses of light-grown hypocotyls

The above examination of staining pattern of *MSG2/IAA19* promoter-*GUS* did not reveal whether GUS activity was promoted on the convex side of

hypocotyls during tropic responses because most hypocotyls were already stained uniformly at the onset of tropic stimuli and the initial staining level was rather high (Fig. 2A). *MSG2/IAA19* promoter-*GUS* activity has been shown to be repressed by photomorphogenetic responses (Tatematsu et al. 2004). Thus, we attempted to examine the change in staining pattern during tropistic responses of seedlings grown under a dim white light condition (Fig. 3). In this case, hypocotyls were stained in a two-fold higher concentration of the GUS substrate for 50 h, twice as long as the staining period for dark-grown hypocotyls because overall GUS activity was much lower than that of dark-grown seedlings. Almost all of the hypocotyls (97%) showed no GUS signals in the light-grown seedlings (Fig. 2J, M and 3A). After 24-h-long unilateral blue light exposure, the hypocotyls showed bending of 46°, and 83% of the hypocotyls displayed differential GUS staining with a stronger signal on the convex side of hypocotyls (Fig. 2L, N). GUS activity appeared also on the concave side of hypocotyls, showing that the *MSG2/IAA19* promoter activity was increased on both flanks of hypocotyls but that it was induced more on the convex side than on the concave side of hypocotyls. The differential GUS activity was usually observed in the entire zone of hypocotyls above the bending part (Fig. 2L). The number of the uniformly stained hypocotyls also increased from 2.0 to 14% (Fig. 3A) after phototropic stimulation.

When seedlings were subjected to uniform blue light irradiation from above, the population of uniformly stained hypocotyls increased to 55%, but the population of differentially stained hypocotyls was only 9.3%. About a

third of hypocotyls remained unstained in this condition (Fig. 3B). Even when seedlings were kept under a dim white light condition for 24 h without tropic stimulation, the number of the uniformly stained hypocotyls increased from 2.0 to 12% at the expense of the number of unstained hypocotyls (Fig. 3C). When seedlings were kept in the dark for 24 h without tropic stimulus, most of the hypocotyls (80%) became stained uniformly, probably because of the release from repression of the *MSG2/IAA19* promoter activity by light (Figs. 2K and 3D). No significant curvatures developed in hypocotyls for 24 h in any light conditions except for that of the unilateral blue light irradiation.

The gravitropic response of hypocotyls of the light-grown seedlings was weaker than the phototropic response described above. When seedlings were turned 135° and kept in the dark for 24 h, hypocotyls bent only 20° (Fig. 3E). In this condition, 23% of the hypocotyls exhibited GUS staining with a stronger signal on the convex side, while 54% of the hypocotyls were stained in a uniform fashion. These results as well as the above phototropic results show that GUS activity was more promoted on the convex side than on the concave side of hypocotyls when tropic responses were induced in light-grown seedlings.

We also examined GUS activity of the auxin-responsive *DR5::GUS* transgenic line (Sabatini et al. 1999) during the tropic responses. However, GUS staining was detected in <1% of hypocotyls in either tropism, irrespective of whether seedlings were raised in the dark or under dim white light condition. Even when dark-grown seedlings were exposed to a 10-fold

stronger unilateral blue light (150 mW m^{-2} or 0.59 μmol m^{-2} s^{-1}) for 24 h, the hypocotyls showed no significant GUS staining although their curvature increased from 48° to 67°.

Histology of the *MSG2/IAA19* promoter-*GUS* activity

Microscopic examination of dark-grown hypocotyls showed GUS activity in the epidermis, cortex and the central stele of hypocotyls, the last of which displayed a stronger activity than the others (Fig. 2F - I). The pericycle stained most intensely because it was only one stained cell species when the GUS staining was carried out for a shorter time (Fig. 2O - Q). GUS activity was also differentially developed in the pericycle cell layer with a stronger signal on the convex side during the phototropic response (Fig. 2P, Q). GUS activity has been found in the pericycle in the root tip and in an early phase of lateral root formation (Tatematsu et al. 2004).

Relevance of *MSG2/IAA19* promoter-*GUS* as a reporter gene for *MSG2/IAA19*

Finally we investigated whether the *MSG2/IAA19* promoter-*GUS* activity reflected the *MSG2/IAA19* mRNA level by determining both of them in the same seedling sample. The *MSG2/IAA19* promoter-*GUS* plants were grown under light conditions with 4 different light fluence rates for 6 days. We also checked dark-grown seedlings. Total RNA and protein were extracted

from the same population of seedlings, and levels of the *MSG2/IAA19* mRNA and GUS activity were determined by RT-PCR and fluorometric method, respectively. Results in Fig. 4 show that the *MSG2/IAA19* mRNA level relative to that of *ACTIN8* decreased monotonously as fluence rates increased. In contrast, GUS activity in the dark condition was just as high as that at the lowest fluence rate (0.09 W m^{-2}), although it decreased almost monotonously in the range of fluence rates higher than 0.09 W m^{-2}. These results show that the *MSG2/IAA19* promoter-*GUS* activity may not correctly represent the mRNA level of *MSG2/IAA19* when it is highly expressed as is in the dark condition.

Discussion

msg1/nph4/arf7 and *msg2/iaa19* are twin Arabidopsis mutants that have been identified as defective in hypocotyl bending when auxin-containing lanolin paste is unilaterally applied to hypocotyls (Watahiki and Yamamoto 1997). The hypocotyls of these mutants show aberrant photo- and gravitropisms (Liscum and Briggs 1995, Tatematsu et al. 2004). Therefore, it is likely that an accumulation of *msg2/iaa19* protein brought about by an amino-acid substitution in the conserved domain II of Aux/IAA proteins represses the transcriptional activities of NPH4/ARF7, resulting in tropic defects in *msg2/iaa19*.

In the present study, we have shown that the *MSG2/IAA19* promoter-*GUS* was expressed differentially with a stronger expression on

the convex side of hypocotyls in photo- and gravitropic responses. The population of the hypocotyls with the differential staining reached a maximum 24 h after the start of phototropic blue light exposure, while phototropic bending of hypocotyls continued to develop for the entire experimental period of 36 h. This suggests that differential expression of *MSG2/IAA19* precedes phototropic bending. Because *MSG2/IAA19* is at least partially expressed through NPH4/ARF7 (Tatematsu et al., 2004), the present results suggest that differential transcriptional activation of NPH4/ARF7 occurs before hypocotyl bending. Similar, but less pronounced results were obtained in gravitropism of hypocotyls (Fig. 1D).

Before etiolated seedlings were subjected to the tropic stimuli, GUS activity was observed uniformly in the upper portion of hypocotyls in most seedlings. After the start of the tropic stimuli, the population of the seedlings that exhibited differential GUS staining increased, where the concave side of hypocotyls was less intensely stained than before. Therefore, the differential staining appears to occur as a result of a decrease in the GUS signal on the concave side of hypocotyls. The uniformly observed GUS activity of 4-day-old etiolated hypocotyls decreased slowly even in the prolonged incubation in the dark or under the uniform blue light, suggesting that the unilateral blue light stimuli accelerates the decrease in the GUS activity on the concave side of hypocotyls. However, we showed that the enzymatic activity of GUS driven by the 2-kb-long *MSG2/IAA19* promoter used in the present study was proportionate to the *MSG2/IAA19* mRNA level only when the *MSG2/IAA19* expression level was modest or low. It was not

reliable in a higher expression range. Therefore, we can not exclude the possibility that the *MSG2/IAA19* expression not only decreases on the concave side of hypocotyls, but also increases on the convex side of etiolated hypocotyls after tropic stimuli. Thus, our present results together with data available from the literature, suggests a model where hypocotyl bending occurs, at least partly because of decreases in activities of NPH4/ARF7 on the concave side of hypocotyls. Recently Haga et al. (2005) reported that unilateral blue light exposure has almost no effect on the growth of the convex side of rice coleoptiles, but that it significantly inhibits that of the concave side during phototropism. This differential growth results in coleoptile bending. In contrast, in pea epicotyls grown in red light, growth of the convex side is promoted by phototropic stimuli, while growth of the concave side remains almost unchanged (Haga and Iino 2006).

In contrast to the etiolated Arabidopsis seedlings, light-grown seedlings show differential GUS staining of hypocotyls because of the greater GUS activity on the convex side than on the concave side of hypocotyls. The GUS activity of the 4-day-old light-grown hypocotyls was increased when they were left in the uniformly irradiated blue light condition. This implies that the unilateral blue light exposure induces inhibition of the GUS expression in the concave side of hypocotyls, which leads to the differential GUS staining. In the phototropically stimulated light-grown hypocotyls, the epidermal and outer cortical cell layers as well as the pericycle cells are most intensively stained. The epidermis has been proposed to be the growth-limiting tissue and the target tissue for auxin in coleoptiles and

stems (Kutschera 1987). However, Fukaki et al. (2005) found that the pericycle is dispensable in root gravitropism of Arabidopsis.

Esmon et al. (2006) recently carried out transcriptional profiling of the concave and convex sides of etiolated *Brassica oleracea* hypocotyls using Arabidopsis microarrays. They found eight genes that were expressed more strongly on the convex flank than on the concave flank of hypocotyls during photo- and gravitropic responses. Their transcription occurs before development of hypocotyl curvature in gravitropism and is dependent on NPH4/ARF7. Therefore, expression of the genes is likely to be regulated by MSG2/IAA19. A soybean *SAUR* (McClure and Guilfoyle 1989), the Arabidopsis *AtAux2-11/IAA4* (Wyatt et al. 1993) and *IAA2* (Nadella et al., 2006), and the maize auxin-inducible K^+ channel gene (Philippar et al. 1999) have been shown to express differentially with stronger expression on the convex side. The synthetic *DR5* promoter, whose acitivity is widely used as an indicator of auxin concentrations, also has been shown to be differentially expressed in phototropically stimulated Arabidopsis hypocotyls (Friml et al. 2002). Our failure to detect the *DR5* activities in the present study may arise from any differences in experimental conditions and suggests that the *MSG2/IAA19* promoter may be more sensitive to a putative auxin gradient than the *DR5* promoter. At present, none of the four auxin F box receptors appears to function specifically in tropistic responses (Dharmasiri et al., 2005b). Therefore, in conclusion, *MSG2/IAA19* is likely to be one of the master genes of tropisms in hypocotyls, regulating transcriptional activity of NPH4/ARF7 directly downstream of the auxin receptors.

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Figure legends

Fig. 1. Time course of phototropic (A) and gravitropic (D) curvature and changes in staining pattern of *MSG2/IAA19 promoter-GUS* in hypocotyls of dark-grown seedlings. Development of tropic curvature (square) is shown as well as percent population of hypocotyls showing four different GUS staining patterns: uniform staining (closed triangle), no staining (open triangle), and differential staining with a stronger signal on the convex side (open circle) or the concave side (closed circle). For the blue light control in B, seedlings were irradiated uniformly from above with blue light, which was as strong as that of the unilateral blue light used for the tropic stimuli in A. For the dark control in C, seedlings were kept in the dark without any light irradiation. For gravitropism in D, seedlings were turned 135^{o} and kept in the dark thereafter. Symbols used for the staining pattern and the bending curvature in B to D are the same as those in A. Data represented show mean and the SE of the three independent experiments, in which 50 - 100 seedlings were counted for each time point.

Fig. 2. Stained *MSG2/IAA19 promoter-GUS* seedlings grown in the dark (A - I and O - Q) or continuous white light (J - N) for 4 days. (A) An etiolated seedling before application of tropic stimuli. (B - E) Etiolated seedlings kept in the dark for 24 h (B) or subjected to the unilateral blue light exposure for 24 h. The latter hypocotyls show uniform (C), no (D) or differential (E) GUS staining. (F - I) Enlarged pictures (F and G) and cross sections (H and I) of etiolated hypocotyls before application of the stimuli (F and H) or after

24-h-long blue light exposure (G and I). E, G and I exhibit a stronger staining on the convex side of hypocotyl. The left side of the picture corresponds to the convex side of hypocotyl in I. (J) A light-grown seedling before application of tropic stimuli. (K and L) Light-grown seedlings kept in the dark for 24 h (K) or subjected to the unilateral blue light exposure for 24 h showing differential GUS staining, with a stronger staining on the convex side of hypocotyl (L). (M and N) Enlarged pictures of light-grown hypocotyls before application of the stimuli (M) or after 24-h-long blue light exposure (N). (O - Q) Etiolated hypocotyls before (O) or after (P and Q) the unilateral blue light irradiation were stained for the GUS activity for 3.5 h. Q is an enlarged picture of P. Arrowheads in G, N and Q show amyloplasts in the endodermis. Scale bar in A is 2 mm; magnifications in A - E and J - L are the same. The other scale bars are all 0.2 mm; magnifications in F - I, M and N are the same, and magnifications in O and P are the same.

Fig. 3. Phototropic (A - D) and gravitropic (E) curvature and changes in staining pattern of *MSG2/IAA19 promoter-GUS* in hypocotyls of light-grown seedlings. Hypocotyl curvature and the GUS-staining pattern were examined before (open bars) and after (shaded bars) exposure to unilateral blue light (A) or gravitropic stimuli by turning 135° (E) for 24 h. (A - D) Four-day-old seedlings were exposed to unilateral blue light (A), uniform blue light from above (B), uniform white light under which they had been raised since germination (C), or kept under the dark condition (D). (E) Four-day-old seedlings were subjected to gravitropic stimuli in the dark.

Data represented show mean and the SE of three (A - D) or four (E) independent experiments, in which 30 - 40 seedlings were counted for each point.

Fig. 4. Effects of light fluence rates on the *MSG2/IAA19* mRNA level (closed circles) and the *MSG2/IAA19* promoter-GUS activity (open circles). The *MSG2/IAA19 promoter-GUS* seedlings were grown for 6 days in the dark or in white light conditions with various fluence rates. The mRNA levels and the GUS activities were measured in the same samples of seedlings with semiquantitative RT-PCR and a fluorometric method, respectively. In RT-PCR, levels of the *MSG2/IAA19* mRNA were normalized to *ACTIN8* mRNA levels, and were expressed relative to the values obtained for samples grown in the dark. The data indicate the mean and SD of three independently grown samples.

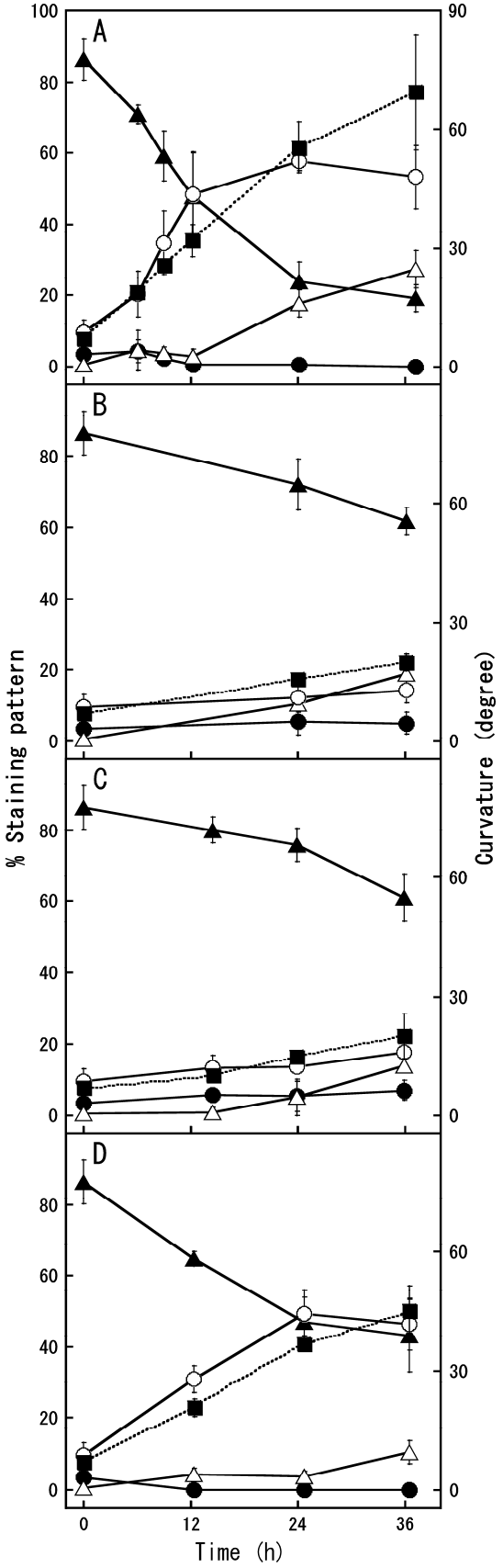
A
B
C
D
% Staining pattern
Curvature (degree)
Time (h)
0
12
24
36
100
80
60
40
20
0
90
60
30
0

Fig. 1

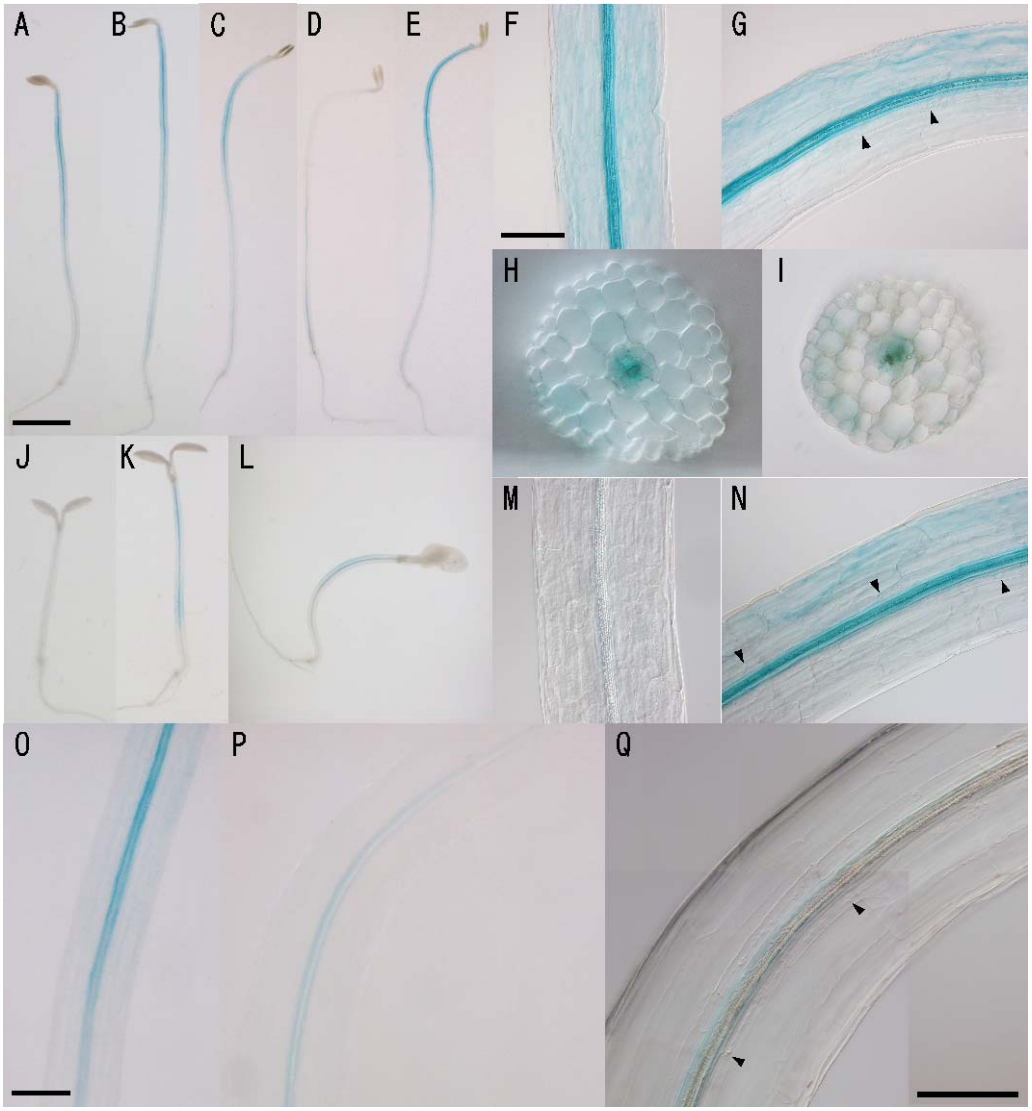

Fig. 2

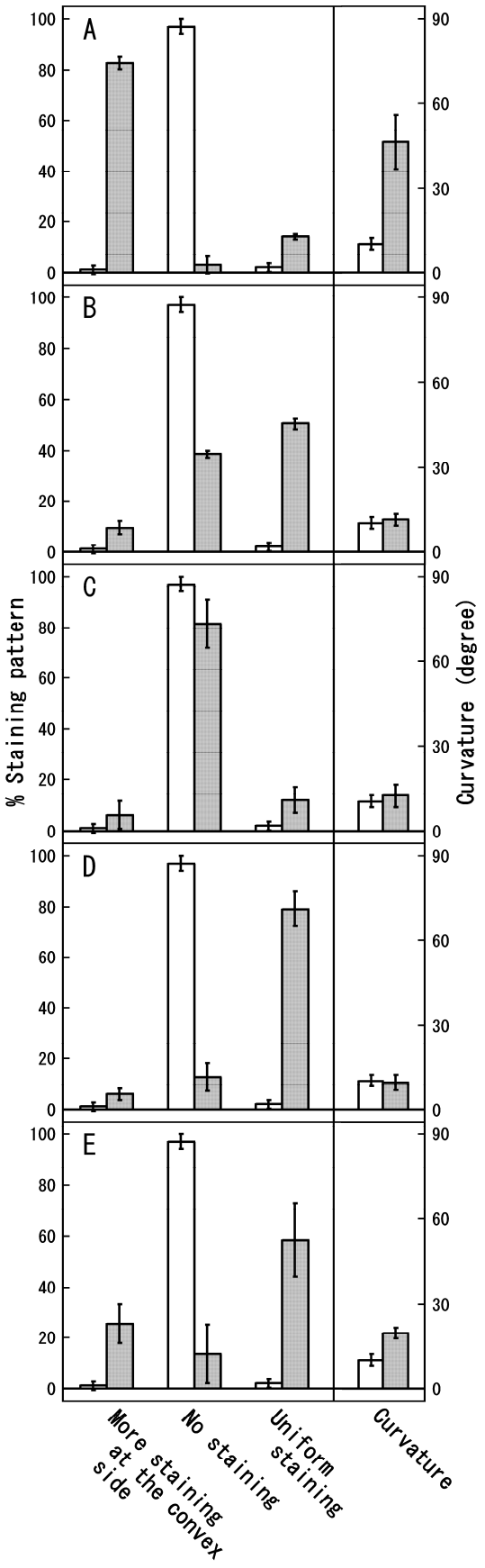
A
B
C
D
E
% Staining pattern
Curvature (degree)
0
20
40
60
80
100
30
90
More staining at the convex side
No staining
Uniform staining
Curvature

Fig. 3

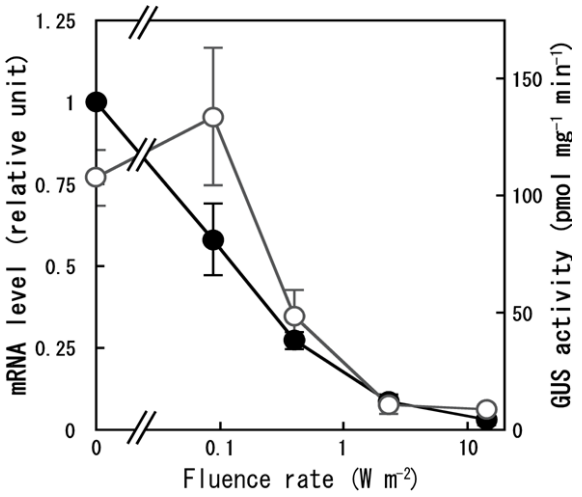
1.25
1
0.75
0.5
0.25
0
mRNA level (relative unit)
150
100
50
0
GUS activity (pmol mg-1 min-1)
0
0.1
1
10
Fluence rate (W m-2)

Fig. 4