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The *massugu1* Mutation of Arabidopsis Identified with Failure of Auxin-Induced Growth Curvature of Hypocotyl Confers Auxin Insensitivity to Hypocotyl and Leaf[1]

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Unilateral application of indole-3-acetic acid (IAA) in a lanolin base to hypocotyls of partially etiolated seedlings of wild-type *Arabidopsis thaliana* induced growth curvature in a dose-dependent manner. The effects of IAA in concentrations from 1 to 1000 μM were studied, with maximum IAA-induced curvature at 100 μM. Three IAA-insensitive mutants were isolated and are all in the same locus, *massugu1* (*msg1*). They did not undergo hypocotyl growth curvature at any of the IAA concentrations tested. *msg1* is recessive and is located on chromosome 5. *msg1* hypocotyl growth is resistant to 2,4-dichlorophenoxyacetic acid (2,4-D), but the roots are as sensitive to 2,4-D as the wild type. Growth of the hypocotyl was inhibited to essentially the same extent as the wild type by 6-benzylaminopurine, abscisic acid, and 1-aminocyclopropane-1-carboxylate, an ethylene precursor. The *msg1* leaves were also resistant to 2,4-D-induced chlorosis. The gravitropic response of the *msg1* hypocotyl takes much more time to initiate and achieve the wild-type degree of curvature, whereas the *msg1* roots responded normally to gravity. The mature plants and the etiolated seedlings of *msg1* were generally wild type in appearance, except that their rosette leaves were either epinastic or hyponastic. *msg1* is the first auxin-insensitive mutant in which its effects are mostly restricted to the hypocotyl and leaf, and *msg1* also appears to be auxin specific.

In the last 10 years molecular genetic studies of the model plant Arabidopsis have proven to be very powerful for dissecting the molecular mechanisms of various aspects of plant growth and development (Meyerowitz and Somerville, 1994). Auxin, a plant hormone, is a key controlling factor of growth and development. Thus, the actions of auxin have been a principal target of molecular genetic investigations of Arabidopsis (Hobbie and Estelle, 1994).

A number of mutants have been characterized that exhibit an abnormal phenotype with respect to auxin-related physiological phenomena, such as inhibition of growth at supraoptimal concentration, promotion of lateral root formation, and floral bud formation. Auxin-resistant mutants that can grow in the presence of inhibiting concentrations of auxin include *aux1* (Maher and Martindale, 1980), *axr1* (Estelle and Somerville, 1987), *axr2* (Wilson et al., 1990), *axr3* (Leyser et al., 1996), *axr4/rgr1* (Hobbie and Estelle, 1995; Simmons et al., 1995), and *dwf* (Mirza et al., 1984). The *AXR1* gene has been cloned by positional cloning and revealed to encode a polypeptide with homology to the ubiquitin-activating enzyme E1 (Leyser et al., 1993). The *AUX1* gene has also been cloned by T-DNA tagging, and the predicted protein sequence has similarity to an amino acid permease (Bennett et al., 1996). Study of the defects in regulation of lateral root formation has led to isolation of mutants with elevated levels of intracellular auxin, such as *rooty/superroot/alf1* (Boerjan et al., 1995; Celenza et al., 1995; King et al., 1995) and an IAA auxotroph mutant, *alf3* (Celenza et al., 1995). Analysis of the *pin1* mutant and study with auxin-transport inhibitors revealed that transport activity of auxin was required in an early stage of floral bud formation (Okada et al., 1991).

Auxin promotes cell elongation, and unilateral application of auxin to coleoptiles and stems produces growth curvature away from the application. The oat (*Avena sativa* L.) coleoptile curvature test, which was widely used for biological determination of auxin activity in the early history of auxin physiology (Stark, 1921), is based on this growth response. Usually, agar blocks containing test samples are applied unilaterally on decapitated coleoptiles for the curvature test. However, as an alternative choice lanolin paste is sometimes used as a carrier (Laibach, 1933). With lanolin, the test can be extended over a longer period of time because of the slower release of auxin from lanolin, and lanolin-treated intact plants will respond to high auxin concentrations by a much greater curvature than agar-treated decapitated plants (Larsen, 1961).

We found that the curvature test can be carried out with hypocotyls of partially etiolated seedlings of wild-type Arabidopsis. When lanolin paste containing IAA is smeared along one side of the hypocotyl, the hypocotyl exhibits a growth curvature, with the applied side becoming convex. The lanolin application is easiest when the plants are grown in a row. To our knowledge, IAA-induced differential growth of hypocotyl tissue has never been exploited to screen a mutagenized population of Arabidopsis. Use of a new screening method should reveal new genetic loci that are involved in the actions of auxin. By screening

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with this method, we have isolated three mutants that do not undergo auxin-induced growth curvature. Genetic analysis showed that all of the mutants fell into one recessive locus, which we designated *msg1* (*massugu1*, Japanese for "straight"). This nonresponsiveness to auxin in *msg1* is almost totally restricted to hypocotyl and leaves. To date, auxin-response mutants have had defects either in the root (*aux1*) or in the entire plant (*axr1*, *axr2*, *axr3*, *axr4*, and *dwf*). These results suggest the presence of organ-specific signal transduction pathways of auxin. In this article we describe the phenotype of a new auxin-insensitive mutant of Arabidopsis.

MATERIALS AND METHODS

M_2 seeds of *Arabidopsis thaliana* Columbia ecotype, mutagenized with ethyl methanesulfonate, were obtained from Lehle Seeds (Round Rock, TX). They were surface-sterilized as described by Watahiki et al. (1995), and were plated on nutrient agar that contained one-half-strength Murashige-Skoog salts (Murashige and Skoog, 1962), 1% (w/v) Suc, one-half-strength vitamin B5 (Gamborg et al., 1968), 1% (w/v) agar (Wako, Osaka, Japan), and 2.3 mM Mes, pH 5.8. In some experiments plants were grown at 23 to 26°C on a 1:1 (v/v) mixture of vermiculite:Metromix 350 (Scotts-Sierra Horticultural Products, Marysville, OH) with continuous illumination at a fluence rate of 16 W m^{-2} obtained from three 40-W white fluorescent tubes (FL40SW, Mitsubishi-Osram, Yokohama, Japan) and two 40-W pink fluorescent tubes (Homolux, Matsushita, Osaka, Japan).

Hypocotyl-Curvature Test

Arabidopsis seeds were plated in rows on the nutrient agar for the easiest application of auxin. Seeds that were surface-sterilized and allowed to imbibe overnight at 4°C were mixed with a 0.2% solution of agarose (LO3, Takara, Kyoto, Japan). They were sown by syringe application into a precut groove in about 2-mm-thick agar medium. Germination was induced by placing the dish under white light at 20 W m^{-2} for 48 h. The seedlings were then grown under dim white light at 0.8 W m^{-2} for 24 h to promote hook opening and elongation of the hypocotyl. The lanolin containing IAA was applied to one side of the hypocotyls of a few seedlings using a thin, plastic trowel. The seedlings were grown under dim red light (3.6 W m^{-2}) for 24 h at 23 to 26°C.

For measurements of the angle of growth curvature, an image of a treated seedling gently laid on an agar plate was taken by a charge-coupled device camera (XC-77, Sony, Tokyo, Japan) attached to a dissecting microscope (Stemi 2000, Zeiss) and processed by a computer (XL5100, Compaq, Houston, TX). The angle was determined on the image using image-analyzing software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD).

Lanolin paste containing IAA was prepared by adding anhydrous lanolin (Wako) to 50 μL of IAA solution dissolved in ethanol and 20 μL of 10% (v/v) Tween 20 up to a final volume of 1 mL and mixing vigorously. Dim white light was provided by filtering the light from five 40-W fluorescent tubes (FL40SW, Mitsubishi-Osram) through two sheets of gray plastic, each 2 mm thick (plate no. S909, Takiron, Osaka, Japan). Red light was provided by filtering the light described above through one sheet of 3-mm-thick red acrylic (Shinkolite 102, Mitsubishi Rayon, Tokyo, Japan).

Determination of Phytohormone Sensitivity

To measure growth of roots, plants were grown on the agar medium described above at 22°C under continuous white light at a fluence rate of 76 W m^{-2}. The agar plate was placed vertically so that the root would grow along the agar surface. Four days after sowing, seedlings were transferred onto a medium supplemented with various concentrations of 2,4-D so that their root tips were placed on a line drawn on the bottom of the plate. Three days later, the lengths of the roots below the line were determined.

Plants used for hypocotyl-growth measurements were grown hydroponically in one-half-strength Murashige-Skoog salts, 1% (w/v) Suc, one-half-strength vitamin B5, and 2.3 mM Mes, pH 5.8, at 22°C. They were first incubated under continuous white light at a fluence rate of 2.6 W m^{-2} for 36 h, with gentle shaking to induce germination. The medium was then exchanged with a medium supplemented with various concentrations of plant hormones and grown for 5 d in the dark. They were fixed with 5% formaldehyde and 10% acetic acid before measurement of hypocotyl length. Root and hypocotyl lengths were measured from a television image of a seedling as described previously (Kurata and Yamamoto, 1997).

For measurements of leaf chlorosis, seedlings germinated on 2,4-D-free agar medium for 4 d were grown on agar medium containing 2,4-D for 26 d at 22°C under continuous white light. Amounts of chlorophyll *a* and *b* in rosette leaves were measured as described by Moran and Porath (1980).

Genetic Characterization

Recombination mapping of *msg1* relative to simple sequence length polymorphism molecular markers (Bell and Ecker, 1994) between the Columbia and Landsberg ecotypes of Arabidopsis was done using PCR primers obtained from Research Genetics (Huntsville, AL). A homozygous *msg1–3* plant (ecotype Columbia) was crossed with a wild-type plant (ecotype Landsberg), and the resulting F_1 plants were allowed to self to generate an F_2 population. Auxin insensitivity in the F_2 population was scored by the hypocotyl-curvature test using lanolin containing 100 μM IAA.

Gravitropism

Seedlings were grown on vertically held plates in darkness for 4 d and then turned 90° to a horizontal position. Orientation of the hypocotyl was recorded continually in the dark with a time-lapsed video image-recording system, using IR radiation with a peak emission wavelength of 950

nm as a monitoring light. The system consisted of a charge-coupled device camera (XC-77, Sony) equipped with an extension tube and a television zoom lens (J6x11-II, Canon, Tokyo, Japan), an IR light-emitting diode (TLN115A, Toshiba, Tokyo, Japan), and a time-lapsed video recorder (VF90, Kowa, Tokyo, Japan). An angle of curvature of the recorded image was measured as described above.

RESULTS

Isolation of the *msg1* Mutants

When grown under dim white light, hypocotyls of Arabidopsis seedlings 24 h after germination grew to 6 to 10 mm long. When lanolin containing IAA was applied to one side of the hypocotyl and the seedling was left for 24 h under dim red light, the hypocotyl bent away from the side on which the lanolin had been applied (Fig. 1, top). The unilateral application of IAA induced hypocotyl growth curvature in a dose-dependent manner (Figs. 1 and 2). Wild-type hypocotyls undergo a curvature starting at 1 μM IAA, with the response reaching a maximum at 100 μM.

Using the hypocotyl-curvature test we screened 44,000 M_2 seeds (progeny of 14,000 M_1 seeds) of *A. thaliana* ecotype Columbia to find IAA-insensitive mutants. Seedlings that had not displayed growth curvature 8 to 12 h after unilateral IAA (10 μM) application were subjected to a second hypocotyl-curvature test for confirmation. Seedlings that did not respond to the two consecutive tests were selected. Three plants were recovered, none of which produced a hypocotyl curvature at any concentration of IAA tested (Figs. 1, middle, and 2). This indicates that hypocotyls of the mutants are unable to bend when responding to unilateral application of IAA. We designated the mutation *msg1* (see below).

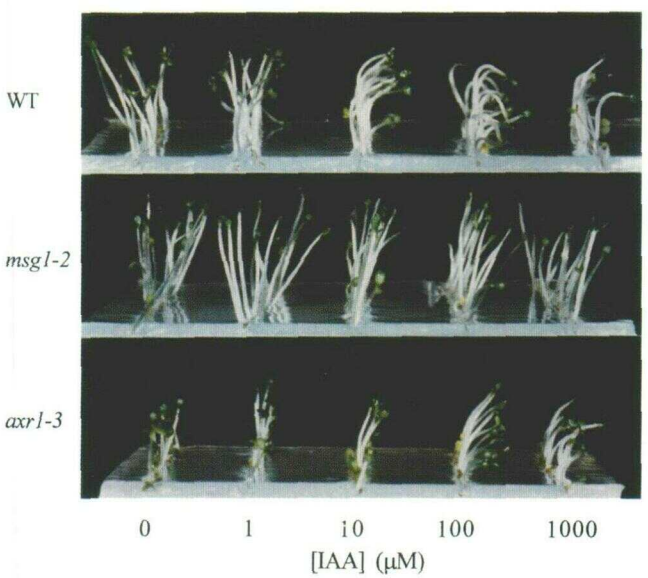

Figure 1. IAA-induced growth curvature of hypocotyls of wild type (WT; top), *msg1–2* (middle), and *axr1–3* (bottom). Lanolin containing IAA was applied to the left side of hypocotyls of the seedlings grown under dim white light for 24 h after germination. They were then incubated under dim red light for 24 h.

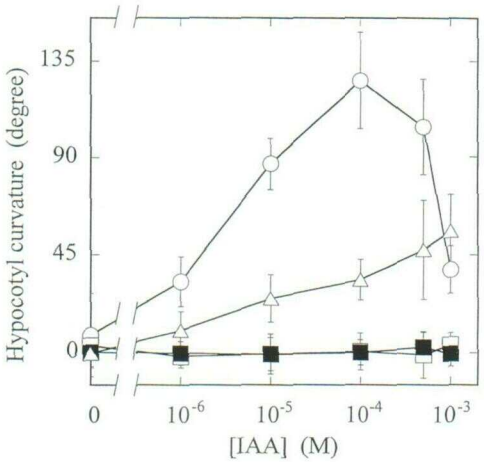

Figure 2. Dose-response curves of hypocotyl growth-curvature test using lanolin containing IAA in wild type (○), *msg1–2* (□), *msg1–3* (■), and *axr1–12* (△). The curvature test was carried out after seedlings were grown under dim white light for 36 h after germination. Values shown represent the means ± SD of eight seedlings.

Although the *msg1* mutants did not respond to IAA, they showed hypocotyl-growth curvature when lanolin-containing fusicoccin was applied unilaterally. Maximal fusicoccin-induced curvature occurs at 0.5 μM. *msg1* and the wild type responded essentially the same to fusicoccin (data not shown).

Genetic Characterization

To determine the genetic basis for these mutants' unresponsiveness to auxin, one of the three mutant plants was crossed to the wild type and the progeny were analyzed with respect to IAA-induced hypocotyl curvature. All of the F_1 plants resulting from this cross responded to auxin, and in the F_2-unresponsive seedlings segregated at a ratio of 3:1 (Table I). Thus, this mutation segregates in a Mendelian manner, which is most consistent with a single recessive gene. F_1 progeny from the cross between each of the three mutants were tested and found to be unrespon-

Table I. *Genetic analysis of msg1 mutants*

Cross	Generation	No. of Plants		χ^{2a}
		Sensitive[b]	Insensitive[c]	
(female × male)				
msg1-3 × wild type	F_1	25	0	
msg1-3 × wild type	F_2	505	164	0.085[d]
msg1-1 × *msg1-2*	F_1	0	34	
msg1-2 × *msg1-3*	F_1	0	34	
msg1-3 × *msg1-1*	F_1	0	23	
msg1-2 × *axr1-3*	F_1	16	0	
msg1-2 × *aux1-7*	F_1	27	0	

[a] χ^2 was calculated based on an expected ratio of 3 sensitive to 1 insensitive. [b] Growth curvature was observed with a hypocotyl-curvature test using lanolin containing IAA. [c] No growth curvature was observed with a hypocotyl-curvature test. [d] $P > 0.7$.

sive to IAA, demonstrating that all three plants are allelic in the *msg1* locus (Table I), which we designated *msg1–1* to *msg1–3*.

To determine whether the *msg1* mutants were alleles of previously isolated auxin-resistant mutants, they were crossed to the *axr1–3* (Lincoln et al., 1990) and the *aux1–7* plants (Maher and Martindale, 1980; Pickett et al., 1990). Hypocotyls of the *axr1–3* mutant showed a reduced sensitivity to the unilateral treatment of IAA-lanolin compared with the wild-type plants (Figs. 1, bottom, and 2), and *aux1–7* was as sensitive as the wild type (data not shown). All F_1 progeny from these crosses displayed a hypocotyl-growth curvature similar to the wild type, indicating that the *msg1* mutation is neither *axr1* nor *aux1*. The *msg1* mutation was mapped to chromosome 5 (Fig. 3) using simple sequence length polymorphism molecular markers (Bell and Ecker, 1994).

Morphology

The mature *msg1* plants were largely wild type in appearance except for their rosette leaves, but the morphology of the rosette leaves was allele dependent (Fig. 4). All of the leaves of *msg1–2* were strongly epinastic; about one-half of the *msg1–3* leaves were hyponastic, whereas the remaining leaves were epinastic. Leaves of *msg1–1* were somewhat epinastic but with less severity than those of *msg1–2*.

The etiolated seedlings of the mutants looked essentially the same as those of the wild type. The growth rate of the etiolated hypocotyls was not affected by the *msg1* mutation

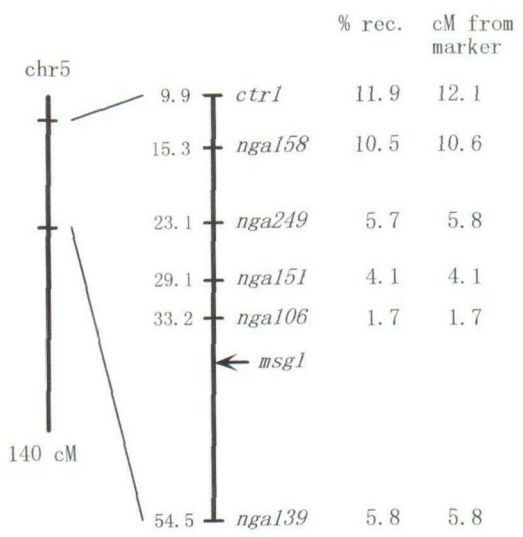

Figure 3. Genetic map location of *msg1* on Arabidopsis chromosome 5. Simple sequence length polymorphism markers on chromosome 5 (Bell and Ecker, 1994) were evaluated for linkage. The percentage of recombination (% rec.) was scored for 42 to 63 mutant plants from the F_2 generation. Map distance in centiMorgans (cM) was calculated according to the Kosambi function, as described by Koornneef and Stam (1992). Marker position is based on the method of Lister and Dean (1993; latest RI map of chromosome 5: December 1996, http://genome-www.stanford.edu/Arabidopsis/ww/Vol3iii/mapping/Chromosome5.html).

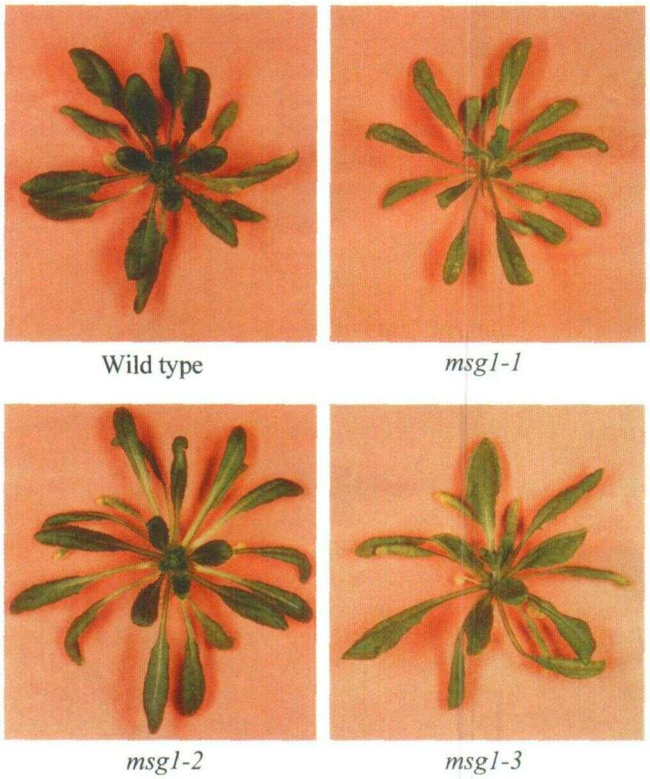

Figure 4. Rosettes of wild-type, *msg1–1*, *msg1–2*, and *msg1–3* plants grown for 36 d under continuous white light at 23 to 26°C.

(data not shown), indicating that *msg1* was not involved in hypocotyl growth in the dark. However, root growth of the *msg1* seedlings was significantly reduced (about two-thirds of the wild type) under continuous white light (see legend to Fig. 6).

Phytohormone Sensitivity

Auxin inhibited the growth of both hypocotyls and roots of wild-type Arabidopsis (Figs. 5 and 6), whereas *msg1* hypocotyl growth was more resistant to 2,4-D than was the wild type (Fig. 5). However, root growth of *msg1* was as sensitive to 2,4-D as was the wild type (Fig. 6), and the *axr1* hypocotyl was more resistant to 2,4-D than was *msg1* (Fig. 5). Growth of both the hypocotyl (Fig. 7) and the root (data not shown) of *msg1* was inhibited by ACC to a similar extent as the wild type, but the hypocotyl of *axr1–12* was resistant to ACC (Fig. 7). ABA and BA inhibited hypocotyl and root growth of *msg1* to a similar extent as the wild type (data not shown).

When wild-type seedlings were grown on 2,4-D-supplemented medium for 26 d, chlorosis was observed in the leaves. Determination of chlorophyll content of the *msg1* plants showed that they displayed less chlorosis than the wild type (Figs. 8 and 9). The *axr1–12* plants showed stronger resistance to 2,4-D than did *msg1*; chlorosis of the *aux1–7* leaves occurred to a similar extent as the wild type (Fig. 8). Rosette leaves of *msg1–3* showed hyponasty (Figs. 4, bottom right, and 9, bottom left). It is interesting that the

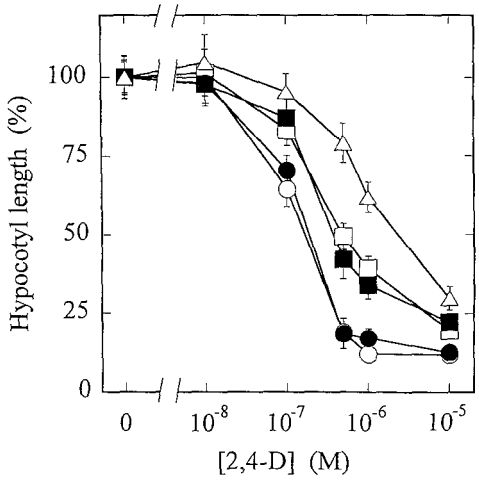

Figure 5. Effects of 2,4-D on hypocotyl elongation of wild type (○), *msg1–2* (□), *msg1–3* (■), *aux1–7*(●), and *axr1–12* (△). The plants were grown for 5 d in an aqueous medium containing 2,4-D at 22°C in the dark after germination was induced in the absence of 2,4-D. Elongation is expressed relative to the mean hypocotyl elongation of the same genotype on medium without 2,4-D. Each value represents the mean ± SD of at least 15 plants. Mean values for 100% hypocotyl length were 12.5 ± 0.8 mm for wild type, 15.6 ± 1.0 mm for *msg1–2*, 14.6 ± 0.8 mm for *msg1–3*, 11.8 ± 0.6 mm for *aux1–7*, and 14.6 ± 0.8 mm for *axr1–12*.

hyponastic leaves became flat when 2,4-D was added to the medium (Fig. 9).

Tropism

We examined gravitropism and phototropism of the *msg1* mutants. Vertically grown seedlings were reoriented by 90°, and the time course of the hypocotyl curvature was measured. Significant upward bending was observed in horizontally placed hypocotyls of wild type 2 h after the start of gravistimulation (Fig. 10). In the *msg1* mutants, however, hypocotyls showed a much slower response, although wild-type curvature values were eventually approached. Roots and the inflorescence stems of *msg1* showed normal gravitropism. No defects were observed in phototropism of the *msg1* hypocotyls by white light (data not shown).

DISCUSSION

A New Auxin-Insensitive Mutant, *msg1*

We have identified a new genetic locus of Arabidopsis, *msg1*, using a hypocotyl growth-curvature screen with auxin. In addition to the defective auxin-induced growth curvature in hypocotyls (Figs. 1 and 2), the *msg1* mutants are resistant to toxic levels of auxin, determined both by hypocotyl growth (Fig. 5) and leaf chlorosis (Fig. 8), and have reduced gravitropism in the hypocotyl (Fig. 10). Although root growth is reduced in the light condition by the mutation, the most striking feature of *msg1* is that most of the defects are restricted to the hypocotyl and the leaf. Since all of the auxin-resistant mutants of Arabidopsis reported so far display defects either in the root (*aux1* [Maher and Martindale, 1980]) or in the entire plant (*axr1* [Lincoln et al., 1990], *axr2* [Wilson et al., 1990], *axr3* [Leyser et al., 1996], *axr4/rgr1* [Hobbie and Estelle, 1995; Simmons et al., 1995], and *dwf* [Mirza et al., 1984]), the *msg1* mutant belongs to a new class of auxin-insensitive mutants. This result suggests that plants have multiple, organ-specific auxin signal transduction pathways.

Root growth of Arabidopsis seedlings, especially of the Columbia ecotype, is promoted by continuous irradiation with white light (Kurata and Yamamoto, 1997). *msg1* does not show this light-induced growth promotion of roots. Since the promotion of growth is caused primarily by photosynthesis in aerial parts of the plant (Kurata and Yamamoto, 1997), the growth defects observed in *msg1* roots might be a secondary effect of a disorder occurring in the cotyledon and the hypocotyl.

Hormone Specificity

Although hypocotyl growth of *msg1* is resistant to 2,4-D (Fig. 5), it is as sensitive to ACC (Fig. 7), BA, and ABA as the wild type. All of the auxin-resistant mutants so far reported show cross-resistance to plant hormones other than auxin: Roots of *aux1* (Maher and Martindale, 1980; Pickett et al., 1990; Hobbie and Estelle, 1994) and *axr1* (Hobbie and Estelle, 1994) are resistant to ethylene and cytokinin, those of *axr2* are resistant to ethylene and ABA (Wilson et al., 1990); those of *axr3* are resistant to ACC (Leyser et al., 1996), and those of *axr4* may show slight resistance to ABA (Hobbie and Estelle, 1995). We also showed that the *axr1* hypocotyl is resistant to ACC (Fig. 7). Thus, *msg1* seems to be the first Arabidopsis mutant that is

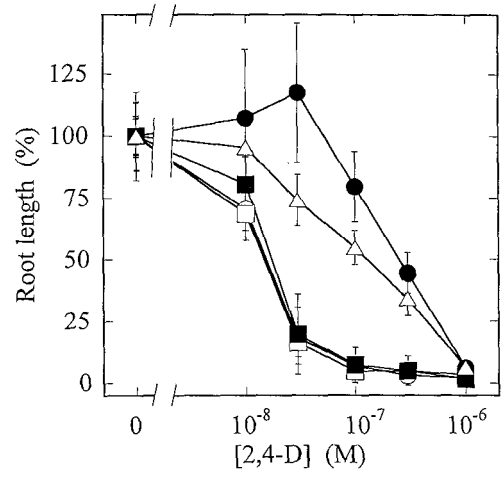

Figure 6. Effects of 2,4-D on root elongation of the wild type (○), *msg1–2* (□), *msg1–3* (■), *aux1–7* (●), and *axr1–12* (△). The plants were grown for 3 d in an agar medium in the presence of 2,4-D at 22°C under continuous light after germination was induced in the absence of 2,4-D. Elongation is expressed relative to the mean root elongation of the same genotype on medium without 2,4-D. Each value represents the mean ± SD of at least 11 plants. Mean values for 100% root length were 30.2 ± 2.5 mm for wild type, 23.6 ± 1.8 mm for *msg1–2*, 21.4 ± 2.9 mm for *msg1–3*, 17.6 ± 3.1 mm for *aux1–7*, and 28.2 ± 4.0 mm for *axr1–12*.

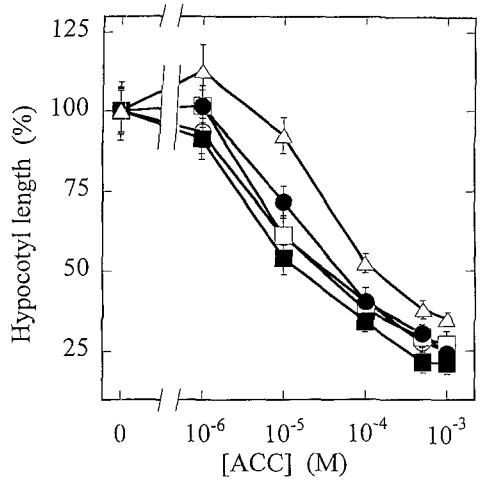

Figure 7. Effects of ACC on hypocotyl elongation of wild type (○), *msg1–2* (□), *msg1–3* (■), *aux1–7* (●), and *axr1–12* (△). For details, see the legend to Figure 5. Each value represents the mean ± SD of at least 13 plants. Mean values for 100% hypocotyl length were 12.0 ± 0.8 mm for the wild type, 14.3 ± 1.1 mm for *msg1–2*, 12.9 ± 1.2 mm for *msg1–3*, 11.1 ± 0.8 mm for *aux1–7*, and 11.6 ± 0.9 mm for *axr1–12*.

resistant to auxin in a specific manner. Blonstein et al. (1991) reported nine auxin-resistant mutants of *Nicotiana plumbaginifolia*, which were selected with respect to auxin resistance of hypocotyl growth and cotyledon expansion. All nine were specifically resistant to auxin; however, they are not likely to be tobacco counterparts of *msg1*, since their phenotypes are different from *msg1* in terms of morphology, root gravitropism, and dominance relationship. Isolation of the *msg1* mutant suggests the presence of a component involved only in auxin signal transduction, although multiple plant hormones have been known to have signal transduction components in common.

Gravitropic Response

All of the Arabidopsis mutants that are resistant to auxin in roots (*aux1* [Mirza et al., 1984], *axr1* [Estelle and Somerville, 1987], *axr2* [Wilson et al., 1990], *axr3* [Leyser et al., 1996], *axr4* [Hobbie and Estelle, 1995], and *dwf* [Mirza et al., 1984]) also show defects in root gravitropism. It can be concluded that auxin resistance and defects of gravitropism are closely coupled in the root. In the case of hypocotyl, however, auxin-resistant *axr1* (Knee and Hangarter, 1996; Fig. 5) and *axr4/rgr1* (Simmons et al., 1995) do exhibit normal gravitropism (Fukaki et al., 1996a). *msg1*, which is less resistant to 2,4-D than is *axr1* (Fig. 5), shows an aberrant hypocotyl gravitropism (Fig. 10). Thus, auxin resistance and gravitropism are not linked in the hypocotyl. IAA-induced growth curvature of the hypocotyl is probably produced by promotive effects of auxin on cell elongation (Larsen, 1961). The hypocotyl of *axr1* may perform gravitropism, since it can respond to auxin by promoting cell elongation, although the promotive response may be smaller than the wild type, as presumed from a smaller growth curvature induced by IAA (Fig. 2). The smaller response may be sufficient to express normal gravitropism. The hypocotyl of *msg1* does not respond to IAA at any concentrations tested in our hypocotyl-curvature test and shows slower gravitropism (Fig. 10). Thus, it appears likely that the auxin-induced growth curvature is correlated with gravitropism in the hypocotyl. *MSG1* gene may play an essential role in differential growth of the hypocotyl under gravistimulation through an auxin signal transduction cascade.

Bullen et al. (1990) reported that in approximately 40% of the Arabidopsis strains with alterations in gravitropism, only hypocotyl or root gravitropism was affected, and they concluded that hypocotyl and root gravitropisms were genetically separable. Our findings with *msg1* support this conclusion. Fukaki et al. (1996a, 1996b) recently identified genetic loci of Arabidopsis that are involved specifically in the gravitropic response of inflorescence stems. Thus, it appears that independent gravitropic pathways exist in the roots, hypocotyls, and inflorescence stems.

Differential Growth Mutants

Differential growth of the hypocotyl occurs according to a developmental program of plants, forming a hook structure in the dark, as well as in response to environmental stimuli such as light and gravity. The *axr1* (Lehman et al., 1996) and *hookless1* (*hls1*; Guzman and Ecker, 1990) mutants do not form the apical hook. The *HLS1* gene is thought to control differential cell growth by regulating auxin activity (Lehman et al., 1996). We found that the *hls1* mutant showed normal hypocotyl growth curvature upon unilat-

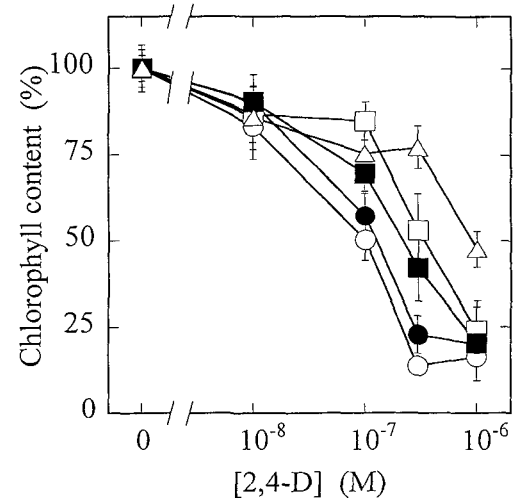

Figure 8. Effects of 2,4-D in agar medium on chlorophyll content in rosette leaves of wild type (○), *msg1–2* (□), *msg1–3* (■), *aux1–7* (●), and *axr1–12* (△). Seedlings were grown on agar medium containing 2,4-D for 26 d at 22°C under continuous white light after germination in the absence of 2,4-D. Values represent the mean percentages of chlorophyll retained in rosette leaves relative to the chlorophyll content of the same genotype on medium without 2,4-D. Each value represents the mean ± SD of five plants. Mean values for 100% chlorophyll were 2.61 ± 0.10 mg/g for wild type, 2.50 ± 0.13 mg/g for *msg1–2*, 2.24 ± 0.28 mg/g for *msg1–3*, 2.32 ± 0.09 mg/g for *aux1–7*, and 2.48 ± 0.17 mg/g for *axr1–12*.

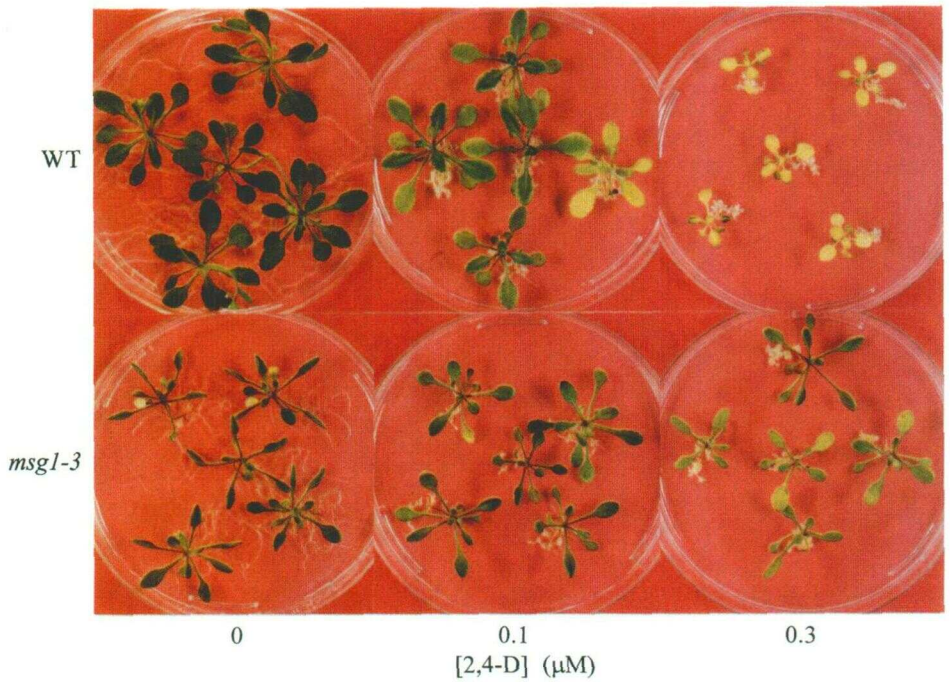

Figure 9. Effects of 2,4-D in agar medium on growth of wild type (WT) and *msg1–3* under continuous light at 22°C. Seedlings germinated on 2,4-D-free medium for 4 d were grown on 2,4-D-supplemented medium for 21 d.

eral application of auxin (data not shown). In contrast, *axr1* is partially defective in the auxin-induced hypocotyl curvature (Figs. 1 and 2). *msg1*, which exhibits no response to unilateral auxin application, has a wild-type hook structure in the dark (data not shown). These results indicate that there is no correlation between the differential cell growth observed in the apical hook formation and that induced by exogenous auxin.

The *MSG1* locus affects differential cell growth induced by exogenously applied auxin and gravitropism and is not involved in the two other cases of asymmetrical growth, because *msg1* mutants display growth curvature upon unilateral application of fusicoccin and the normal hypocotyl phototropic response (data not shown). These observations, as well as the apical hook formation data described above, indicate that various differential growths of hypocotyl occur through multiple, independent reaction pathways.

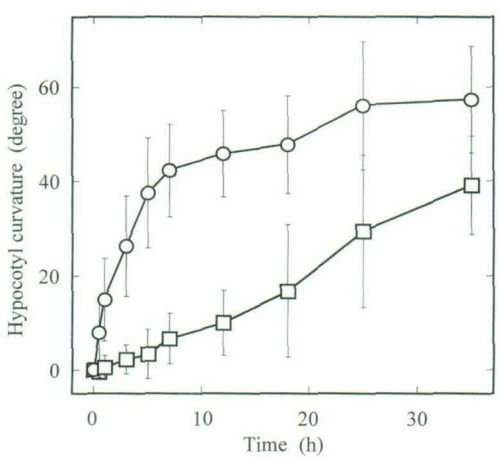

Figure 10. Hypocotyl reorientation in response to gravity. Seedlings of wild type (○) and *msg1–2* (□) grown on vertically held plates for 4 d in the dark were turned 90° to a horizontal position and the orientation of the hypocotyl was measured at the indicated times thereafter; 90° represents complete reorientation upward. Values shown represent the means ± SD of eight seedlings.

Leaf Epinasty

Leaves of the *msg1* plants show epinasty or hyponasty, depending on their alleles (Fig. 4). *msg1–3* shows strongly hyponastic leaves on agar medium (Fig. 9), but when it is grown in soil, its leaves are either hyponastic or epinastic (Fig. 4). On the other hand, *axr1* (Lincoln et al., 1990) and *axr2* (Wilson et al., 1990) have twisted and wrinkled leaves, respectively, and *axr4* shows epinastic leaves (Hobbie and Estelle, 1995). These altered morphologies of leaves strongly suggest that defects in auxin sensitivity affect the concerted growth of leaf cells, resulting in aberrant curvature of leaves (Hobbie and Estelle, 1995). Currently, it cannot be explained why the leaf curvature, epinasty, or hyponasty is allele dependent in *msg1*. Recently, the role of auxin and ethylene in epinasty was assessed using transgenic Arabidopsis plants (Romano et al., 1993). This investigation revealed that leaf epinasty is primarily controlled by auxin rather than auxin-induced ethylene. Our results with *msg1* are consistent with this conclusion, since sensitivity to ethylene does not change in *msg1*, at least in its hypocotyl (Fig. 7).

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