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GENETIC AND PHYSIOLOGICAL STUDIES ON AUXIN-INSENSITIVE MUTANTS
OF Arabidopsis thaliana WITH A NEW ORGAN SPECIFICITY

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Environmental Earth Science of
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ABSTRACT

Auxin is a phytohormone which was originally isolated as a growth regulator of elongation growth of plant tissue in 1930's. In addition to cell elongation, auxin controls various kinds of reactions in growth and development of plant.

In an effort to dissect molecular mechanism of auxin-induced elongation, a new screening method was developed for isolation of auxin-specific mutants in *Arabidopsis*. Unilateral application of auxin-containing lanolin paste on coleoptiles or stems produces a growth curvature. It was found that a test utilizing this curvature response was able to be carried out with a large number of partially etiolated *Arabidopsis* seedlings. Three mutants which did not produce a growth curvature upon application of natural auxin, indole-3-acetic acid (IAA), were isolated from 40,000 M2 seeds mutagenized with ethyl methanesulfonate. They were recessive and fell into the same locus, *msg1*, mapped in chromosome 5. The mutation was thought new since mutants of similar phenotype were not reported near this locus. This locus was named *massugul* (*msg1*). *msg1* mutation caused lesion in sensitivity to IAA in hypocotyl since *msg1* plants did not perform a growth curvature at any concentrations of IAA from 1 μM to 1 mM. Visible phenotype of *msg1* was normal except for morphology of leaf.
leaves of *msgl*-1 and *msgl*-2 showed epinasty and those of *msgl*-3 showed hyponasty. Size of mature plants and etiolated seedlings was essentially the same as wild type.

Application of auxin or ethylene precursor, 1-aminocyclopropane-1-carboxylate (ACC), to medium inhibited growth of root and hypocotyl of wild type. In *msgl* plants, hypocotyl was auxin resistant but root was not. Leaves of *msgl* plants were also auxin resistant (see below). ACC inhibited growth of *msgl* in both root and hypocotyl in a similar manner to wild type. On the other hand, the auxin-resistant mutants so far identified showed growth resistance to auxin in either root only (aux1) or in a whole plant (axr1, axr2 and axr4). All of them were also resistant to ethylene. Thus, *msgl* is the only one mutation that confers auxin-specific resistance to hypocotyl and leaf.

It is well known that auxin induces production of ethylene, and that ethylene inhibits growth of plant. An addition of an inhibitor of ethylene biosynthesis, aminoethoxyvinylglycine (AVG), partially rescued a growth inhibition of *msgl* hypocotyl induced by high concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D). The growth inhibition in hypocotyl of wild type was not rescued by AVG at all. These results suggest that the auxin-induced growth inhibition in hypocotyl consists of two separable portions, growth inhibition by auxin-induced ethylene and that induced by auxin per se. Most of the growth
inhibition in wild type was found due to an inhibitory action of auxin alone. MSG1 gene was involved in the inhibitory pathway inherent in auxin.

When wild-type plants were grown on 2,4-D-containing medium, chlorosis was observed in the leaves. Leaves of msg1 showed resistance to 2,4-D with respect to the chlorosis. Hyponastic leaves of msg1-3 became flat probably due to epinastic response when 2,4-D was added to medium. This indicates that auxin acts in multiple pathways in leaves; one is involved in chlorosis of leaf and another in leaf epinasty. MSG1 gene was responsible only for chlorosis.

A tomato mutant known as diageotropica (dgt) is nearly insensitive to exogenously applied auxin with respect to hypocotyl and root elongation and ethylene production. Auxin sensitivity of dgt was examined with a hypocotyl curvature test and auxin resistance was also examined with respect to growth inhibition by application of IAA to medium. Hypocotyl of dgt did not perform a growth curvature and showed auxin resistance.

Hypocotyl growth of msg1 and axrl was resistant to 2,4-D. Hypocotyl of axrl showed a smaller curvature upon unilateral treatment of IAA-containing lanolin compared to wild-type plants. Hypocotyl of msg1 showed abnormal gravitropism, however, that of axrl was normal. All the so-far reported mutants that are resistant to auxin in root showed abnormal gravitropism in root. Therefore, auxin-resistance is coupled
to abnormal gravitropism in root, but not linked in hypocotyl. These results suggest that auxin action in gravitropism is different between hypocotyl and root.

Phenotype of double mutants between msgl and axrl or auxl was additive with respect to morphology of leaves and growth inhibition by auxin in hypocotyl. It is concluded that msgl acts independently from axrl and auxl.

Many auxin-induced processes are thought to include changes in gene expression, and a number of mRNAs have been identified that increase in abundance in response to auxin treatment. Gene expression of early auxin-inducible pea gene PS-IAA4/5 was examined in msgl background by staining msgl plants harboring PS-IAA4/5/GUS reporter gene fusion. GUS staining of etiolated hypocotyl of msgl was weaker than wild type; significant but much lower induction was observed than wild type upon application of auxin. Induction of the staining detected in root of msgl was similar to wild type. Thus, MSGl gene is located upstream of PS-IAA4/5 gene in the signal transduction chain of auxin, and functions as an enhancer.

In an effort to isolate other mutations that confer unresponsiveness to IAA with respect to a hypocotyl curvature response, screening for gravitropism mutants specific to hypocotyl was carried out. Three mutants were isolated and named jyuuryoku (jrk) (jrk48 and jrk197) and #59. Since hypocotyl of jrk197 showed a reduced response to IAA in a growth
curvature test, jrk197 could be classified into massugu class of mutation. Hypocotyl of jrk48 produced the growth curvature to a similar extent to wild type, but it was defective in gravitropism of inflorescence stem and hypocotyl. jrk48 is likely to be a mutant defective in perception of gravistimulation. #59 was allelic to msgl.

I have isolated an auxin-insensitive mutant, msgl, in which defects were restricted in hypocotyl and leaf. Abnormal expression of auxin-inducible PS-IAA4/5 gene was also observed only in hypocotyl. Therefore, msgl was found to be an auxin-insensitive mutant with a new organ specificity, because auxin-resistant mutants which had already been reported showed lesion in only root or in a whole plant. These results indicate that a number of signal transduction pathways with definite organ specificities exist for auxin, and that they were mostly independent each other.
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Abbreviations

$A_{\lambda}$  Absorbance at $\lambda$ nm
ACC  1-Aminocyclopropane-1-carboxylic acid
AVG  Aminoethoxyvinylglycine
2,4-D  2,4-Dichlorophenoxyacetic acid
EDTA  Ethylenediaminetetraacetic acid
EMS  Ethyl methanesulfonate
GUS  $\beta$-Glucuronidase
IAA  Indole-3-acetic acid
MES  2-Morpholinoethanesulfonic acid
MS  Murashige and Skoog
PCR  Polymerase chain reaction
SSLP  Simple sequence length polymorphisms
T-DNA  Transfer-deoxyribonucleic acid
INTRODUCTION

In the last 10 years, molecular genetic study of a model plant, *Arabidopsis*, has been shown to be very powerful for dissecting molecular mechanisms of various aspects of plant growth and development (Meyerowitz and Somerville, 1994). Auxin, a plant hormone, is one of key controlling factors of the growth and development (Went and Thimann, 1937; Davies, 1995). Thus, auxin action has been one of principal objectives of molecular genetic investigation of *Arabidopsis* (Hobbie and Estelle, 1994; Ecker and Theologis, 1994).

A number of mutants have been characterized that exhibit abnormal phenotypes with respect to auxin-related physiological phenomena, such as inhibition of growth at its supraoptimal concentrations, promotion of lateral root formation and floral bud formation. Auxin resistant mutants that can grow even in the presence of inhibiting concentrations of auxin, include aux1 (Maher and Martindale, 1980), axrl (Estelle and Somerville, 1987), axr2 (Wilson et al., 1990) and axr4 (Hobbie and Estelle, 1995). 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 sequence has similarity to an amino acid permease (Bennett et al., 1996). Study on defects in regulation of lateral root formation has led to isolation of mutants with elevated level of intracellular auxin concentrations, such as rooty/superroot/alf1 (King et al., 1995; Boerjan et al., 1995; Celenza et al., 1995), and an IAA auxotroph mutant, alf3 (Celenza et al., 1995). Analysis of pin1 mutant and experiments with auxin-transport inhibitors revealed that transport activity of auxin was required in the early stage of flower bud formation (Okada et al., 1991). Although we have learned much from these studies, current information on auxin signal transduction still remains very fragmentary in a molecular genetic term.

Molecular genetic study of ethylene signal transduction has been successful among the similar attempts to dissect molecular mechanism of growth and development in plants (Ecker, 1995). The success seems to result from the use of triple response as a screening method for mutants. Triple response is a set of ethylene-induced responses, that is, inhibition of hypocotyl and root elongation, radial swelling of the hypocotyl and root and exaggeration in the curvature of the apical hook, which is specific to ethylene. A number of mutants have been isolated that do not show the triple response after application of ethylene (ethylene resistant and ethylene insensitive; Bleecker et
al., 1988, Guzmán and Ecker, 1990), or do show the response constitutively in the absence of ethylene \textit{(constitutive triple response; Kieber et al., 1993)}. Nearly a dozen of loci in the ethylene transduction pathway was identified, and epistatic relationship among them was established. A few loci have been cloned; ETR1 was revealed to encode a receptor-kinase-like protein (Chang et al., 1993), and CTR1 product similar to the Raf family of protein kinases (Kieber et al., 1993). It seems that utilization of a specific response as a screening method makes it possible to identify many loci in a single transduction pathway.

The molecular genetic analysis has been carried out with the auxin-resistant mutants most extensively in auxin physiology. They have been isolated utilizing growth-inhibiting activity of synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D). As described above, they can grow in the presence of the auxin. Since growth of root is more susceptible to auxin than hypocotyl and epicotyl (Went and Thimann, 1937), and since root is on close contact with culture media containing auxin, all of them inevitably acquire auxin resistance in root. On the other hand, many substances inhibit growth of plants. As far as only plant hormones are considered, cytokinin, abscisic acid, ethylene and jasmonic acid are inhibitory for growth of plants as well as auxin at the inhibitory
concentration (Davies, 1995). Therefore, screening with respect to growth inhibition can not be specific for only one growth-inhibiting substance, for example, auxin. If a gene encodes a downstream component in a reaction pathway leading to growth inhibition, mutation of the gene should confer growth resistance to multiple substances. In fact, all the auxin-resistant mutants show resistance to at least one other hormone than auxin (see Discussion for detail). Obviously, development of new screening method that is more specific to auxin is needed to dissect auxin physiology in a molecular genetic term.

Until instrumental analyses of auxin became available using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), auxin had been quantitated with a few kinds of bioassays, among which a growth curvature test was widely used (Larsen, 1961). In this assay, unilateral application of auxin to coleoptiles or stems produces the growth curvature. The curvature occurs since auxin promotes cell elongation of the applied side more than the opposite side. This gradient of growth rate across a coleoptile or stem is established because of the polar transport of auxin which is characteristic to only auxin among the growth-promoting substances. Since induction of a growth curvature is very specific to auxin, the growth curvature test was utilized as a bioassay for auxin. In the Avena coleoptile curvature test, that had
been a standard assay for auxin activity, agar blocks which contained test samples were applied unilaterally on decapitated coleoptiles (Stärk, 1921). However, lanolin paste was sometimes an alternative choice of a career (Laibach, 1933), since the test was able to be extended over a longer period of time because of slower release of auxin from lanolin, and since lanolin-treated intact plants responded to high auxin concentrations by a much greater curvature than agar-treated decapitated plants (Larsen, 1961).

We found that the curvature test was able to be carried out with Arabidopsis hypocotyl if growth condition was controlled adequately. When indole-3-acetic acid (IAA)-containing lanolin paste was smeared along one side of the hypocotyl, it performed a growth curvature, the applied side becoming convex. It was also found that the curvature test was applicable to a large number of plants when they were grown in a row. The IAA-induced differential growth of hypocotyl tissue has never been exploited to screen a mutagenized population of Arabidopsis. Introduction of a new screening method would lead to a finding of new genetic loci that were involved in auxin action.

By screening with this method, I have isolated three mutants that do not perform the growth curvature. Genetic analysis showed that all the mutants fell into one
recessive locus, which I designated *msgul* (*msgl*), since their hypocotyls stood *massugu* (straight in Japanese) after the unilateral treatment with IAA-lanolin paste. Studying phenotype of the mutants, I learned that defects observed in them were restricted in hypocotyl and leaf. This type of organ specificity has never been observed in the auxin-insensitive or -resistant mutants. *msgl* is likely to belong to a new class of auxin-insensitive mutations. Double mutants between *msgl* and *axrl* or *auxl* were created to examine genetic relationship among the loci. It is well known that auxin rapidly activates gene expression of early auxin-inducible genes. The genes may function in auxin signal transduction (Hagen, 1995; Abel and Theologis, 1996). Thus, gene expression of one of them was measured in *msgl* background after β-glucuronidase (GUS) reporter gene fusion was introduced into *msgl* by crossing.

It was also found that auxin insensitivity was closely linked with defects in gravitropism. Because screening with a gravitropic response is more labor-saving and needs less skill than that with the hypocotyl curvature test, the former screening was carried out to obtain more mutants with the hypocotyl-specific defects. This part of study is being under way, and obtained mutants have not fully been characterized yet. However, one of the mutants obtained by this screening showed defects in formation of an auxin-
induced growth curvature and did not look like msg1, suggesting that it was a mutant of another locus of msg, possibly msg2. In an effort to discover molecular mechanism of auxin action, especially in cell elongation, genetic and physiological characterization of these mutants is described in this study.
MATERIALS AND METHODS

1. Plant materials

M₂ seeds of Arabidopsis thaliana Columbia ecotype, mutagenized with ethyl methanesulfonate (EMS) or fast neutron, were obtained from Lehle Seeds (Tucson, AZ, U.S.A.). Seeds of sgr3 and sgr4 were obtained from Dr. Masao Tasaka (Kyoto University, Kyoto, Japan). Seeds of T-DNA tagged lines and the other Arabidopsis mutants were obtained from the Arabidopsis Biological Resource Center (Ohio State University, Columbus, OH, U.S.A.) or the Nottingham Arabidopsis Stock Centre (University of Nottingham, Nottingham, U.K.). Seeds of transgenic Arabidopsis carrying PS-IAA4/5/GUS gene were obtained from Dr. Athanasios Thologis, Plant Gene Expression Center, Albany, CA, U.S.A.

Seeds of tomato (Lycopersicon esculentum) wild type (Rutger) and diageotropica were generous gifts from Dr. Masayasu Nagata (National Research Institute of Vegetables, Ornamental Plants and Tea, Ano, Japan).
2. Growth condition

Arabidopsis seeds were surface sterilized for 5 min in a 1.5% solution of sodium hypochlorite that contained 0.02% Triton X-100 and rinsed with sterile water five times (Watahiki et al., 1995). They were plated on nutrient agar that contained half-strength MS salts (Murashige and Skoog, 1962), 1%(w/v) sucrose, half-strength B5 vitamin (Gamborg et al., 1968), 1%(w/v) agar (Wako, Osaka, Japan), and 2.3 mM 2-morpholinoethanesulfonic acid (MES), pH 5.8. In some experiments Arabidopsis plants were grown at 23 to 26°C on a 1:1(v/v) mixture of vermiculite and Metromix 350 (Grace-Sierra, Milpitas, U.S.A.), with continuous white illumination at a fluence rate of 16 W m⁻² obtained from three 40-W fluorescent tubes for plant breeding (Homolux; Matushita, Osaka, Japan) and two 40-W florescent tubes (FL40SW; Mitsubishi-Osram, Yokohama, Japan).

Dim white light was provided by filtering the light from five 40-W fluorescent tubes (FL40SW) through two layers of gray plastic sheet, each 2-mm thick (Takiron plate S909; Takiron, Osaka, Japan). Red light was provided by filtering the light described above through one layer of red acrylic sheet, 3-mm thick (Shinkolite 102; Mitsubishi Rayon, Tokyo, Japan).

In an experiment for investigation of effects of auxin
on chlorosis of leaf, plants were grown at 22°C in a growth cabinet (MLR-350T; Sanyo, Moriguchi, Japan) with six 40-W fluorescent tubes (FL40SSW; Sanyo).

3. Measurement of length and growth curvature

Root and hypocotyl lengths were measured from images of a seedling taken by a CCD camera (XC-77; SONY, Tokyo, Japan) attached to a dissecting microscope (Stemi2000; Zeiss, Jena, Germany), and captured to a desktop computer (PC9801 BA2; NEC, Tokyo, Japan). The image was measured using image analyzing software (RIPP-2 ver. 1.05; Rise, Sendai, Japan).

For a measurement of growth curvature, seedlings were gently laid on an agar plate. Images of seedlings were recorded as described above. Angles of curvature were measured using image analyzing software (Image Pro; Media Cybernetics, Silver Spring, MD, U.S.A.). In this procedure, one straight line was made along the lower part of the image of the seedling frozen on the screen. A second line was then made as a tangent to the curved portion of the hypocotyl below the hook. The angle of curvature was the angle between these intersecting lines.
4. Hypocotyl curvature test

IAA-containing lanolin paste 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 final volume of 1 ml and mixing vigorously. Arabidopsis seeds were immersed in water overnight and were surface sterilized as described above. Several grooves were cut in 2-mm-thick agar medium in a petri plate. The seeds were mixed with 0.2% agarose (L03; Takara, Kyoto, Japan). The mixture was poured into the groove using a microdispenser (Drummond, Broomall, U.S.A.) and solidified. Germination was induced by placing the plate under white light at 20 W m⁻² for 48 h at 23 to 26°C. The seedlings were grown under dim white light at 0.8 W m⁻² for 24 h to promote hook opening and elongation of hypocotyl. About 60 nl of the IAA-containing lanolin was applied to one side of hypocotyl using a small piece of thin plastic sheet as a trowel. The seedlings were then incubated under red light at 3 W m⁻² for 24 h. Fusicoccin (Sigma, St. Louis, U.S.A.)-containing lanolin was prepared in a similar manner to IAA-containing one.
5. Determination of phytohormone sensitivity

To measure sensitivity of hypocotyl to phytohormone, seeds were germinated in an aqueous medium containing half-strength MS salts, 1% (w/v) sucrose, half-strength B5 vitamin, and 2.3 mM MES, pH 5.8, at 22°C under the continuous white light for 36 h with gentle shaking. The medium was then exchanged for a medium supplemented with various concentrations of a phytohormone. The plants were incubated in the medium for 5 days in darkness thereafter. Thus, all the organs of plants were always on contact with the hormone included in the medium. They were fixed with 5% formaldehyde and 10% acetic acid for measurement of hypocotyl length.

To measure the sensitivity of root, plants were grown on an agar medium described above at 22°C under continuous white light of a fluence rate of 76 W m\(^{-2}\) all through the experiment. 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 a phytohormone. Root tips of the transferred seedlings were placed just on a line drawn on the bottom of the plate. Three days later, the amount of root growth was determined.

Seeds of tomato were surface sterilized as described above and germinated on filter papers (3 MM; Whatman,
Maidstone, U.K.) under the continuous white light at 22°C for 2 days. Germinated seeds were brought on MS medium containing 1% agar and IAA, and grown there at 25°C for 12 days in darkness.

6. Genetic characterization

The genetic location of msgl was established by determining linkage between the mutant gene and SSLPs which were identified and mapped by Bell and Ecker (1994). Oligonucleotides for PCR were obtained from Research Genetics (Huntsville, AL, U.S.A.). A homozygous msgl-3 plant (ecotype Columbia) was crossed to a wild type plant (ecotype Landsberg), and the resulting F₁ plants were allowed to self in order to generate F₂ population of plants segregating both the msgl mutation and SSLPs between Columbia and Landsberg ecotype. Auxin insensitivity was scored by the hypocotyl curvature test using lanolin containing 100 μM IAA.

Double mutants were obtained by progeny testing F₂ plants with a hypocotyl curvature test using lanolin containing 10 μM IAA or growth inhibition of root in 1 μM 2,4-D. Testcrosses were also carried out using the same assays.
7. Gravitropism

To measure gravitropic curvature, seedlings were grown on vertically-held plates in darkness for 4 days and the plates were turned 90° to a horizontal position (time 0 h). The orientation of the hypocotyl was recorded continually with a time-lapsed video image recording system. Infrared radiation was used to monitor the seedlings in this system. The recording station, which was set in a dark room, consisted of an CCD camera (XC-77, SONY), an infrared light source (TLN115A, Toshiba, Tokyo, Japan) with a peak emission wavelength of 950 nm, and a time-lapsed video recorder (VF90; Kowa, Japan). The camera was equipped with an extension tube and a lens (J6x11-II, Canon, Tokyo, Japan) to magnify images of seedlings. Images of the seedlings were also monitored while being recorded throughout an experiment. Angles of curvature of the images were measured as described in Section 3 of this chapter.

8. β-Glucuronidase assay
Histochemical assays of β-glucuronidase (GUS) activity in transgenic lines were performed as described by Jefferson and Wilson (1991). Whole seedlings were fixed with 2% formaldehyde, 50 mM sodium phosphate, pH 7.0, and 0.05% Triton X-100 at 4°C for one h following vacuum infiltration. Seedlings were rinsed with 50 mM sodium phosphate, pH 7.0, and incubated at 37°C for 24 h in 50 mM sodium phosphate, pH 7.5, containing 1 mM Na₂ EDTA, 0.1% Triton X-100 and 1 mM 5-bromo-4-chloro-β-D-glucuronide (Wako). Incubation time was 24 h following vacuum infiltration. Samples were then transferred to 70% ethanol to clear the tissue of pigments. A photograph of histochemical staining was taken by a camera (OM-4, Olympus, Tokyo, Japan) or a light microscope (Axioplan, Zeiss).

Twenty seedlings were assayed for quantitative GUS assay. Proteins were extracted with 200 μl of extraction buffer which consisted of 50 mM sodium phosphate, pH 7.5, 5 mM dithiothreitol, 1 mM Na₂ EDTA, 0.1% sodium lauryl sarcosyl and 0.1% Triton X-100. Twenty μl of the protein solutions were incubated with 500 μl of the extraction buffer that also contained 50 μg bovine serum albumin, 0.02% NaN₃, 1 mM p-nitrophenyl β-D-glucuronide at 37°C. Ninety μl of the samples were taken at 0, 2, 4 and 6 h and the enzyme reaction was stopped by an addition of 810 μl of 0.2 M Na₂CO₃. GUS activities were quantified.
spectrophotometrically with a Beckman DU-65 spectrophotometer (Beckman, Fullerton, CA, U.S.A.) by following the appearance of the product, p-nitrophenol, at 405 nm.

9. Screening of gravitropism mutants

To examine gravitropic response of a large number of seedlings efficiently, they should be grown in a row with a sufficient interval between them. For this purpose, a small amount of lanolin was spotted in a row at interval of about a few mm on a piece of filter paper. When Arabidopsis seeds were spread evenly on the filter paper, they stuck to the spots of lanolin, resulting in a row of seeds with a certain interval on filter paper. A piece of filter paper with seeds was soaked in deionized water and incubated at 4°C for 24 h. Germination was induced by placing it under white light at 22°C for 2 days. After the germinated seedlings were grown in darkness at 22°C for three days, they were turned 90° and grown further in darkness for one day. The seedlings of which hypocotyl did not show negative gravitropism were picked up, and subjected to a second assay of gravitropism. Namely, they were placed horizontally on a vertically-held agar plate,
and grown in darkness for one day. The seedlings that did not respond to the two consecutive tests were selected as a gravitropic mutant.

10. Miscellaneous

Chlorophyll was extracted by N,N-dimethylformamide (Wako) with an extraction ratio of 5 - 10% (fresh weight/volume) at room temperature for 24 h. It was quantified with a spectrophotometric assay using a spectrophotometer (DU-65; Beckman) as described by Moran and Porath (1980). The amount of chlorophyll a and b was calculated by equation:

\[ Ct = 7.04 A_{664} + 20.27 A_{647}, \]

where Ct is total chlorophyll concentration, expressed in μg/ml.
RESULTS

1. Isolation and initial characterization of msssgul (msg1) mutants

1.1. Hypocotyl curvature test

Germination of seeds was induced by cold treatment for 2 days and subsequent irradiation with white light at 24°C for 36 h. Hypocotyl of the seedlings was about 6 - 10 mm long 24 h after the induction of germination when they were grown under continuous white light of a low fluence rate (0.8 W m\(^{-2}\)). When IAA-containing lanolin was applied to one side of the hypocotyl and the seedling was left for 24 h under continuous dim red light (3.6 W m\(^{-2}\)), the hypocotyl bent away from the applied side (Fig. 1 and Fig. 2, top). Unilateral application of IAA produced growth curvature of hypocotyl in a dose-dependent manner (Figs. 1, 2 and 3). Hypocotyls of wild type began to perform a curvature at 1 \(\mu M\) IAA and the response reached a maximum at 100 \(\mu M\). IAA was inhibitory for the curvature at 1,000 \(\mu M\). Hypocotyl length between the point of curvature and the base of cotyledon was longer in seedlings treated with 10 \(\mu M\) IAA (Fig. 1, middle left) than those treated with 100 and 500
μM IAA (Fig. 1, middle right and bottom left).

The hypocotyl curvature test was carried out with partially etiolated seedlings with a fully opened hook. In fact, this test was applicable to totally etiolated seedlings with a closed hook. However, the presence of a closed hook made it difficult to apply lanolin evenly on surface of hypocotyl. Therefore, partially etiolated seedlings grown under dim white light were preferred for the experimental material. The curvature test was carried out under dim red light. The growth curvature did not develop under dim white light; in total darkness, hypocotyl performed a curvature to a smaller extent than under continuous irradiation with red light. From these observations it was concluded that the most reliable curvature test was conducted with partially etiolated seedlings under dim red light.

1.2. Isolation of msg1 mutants

Using the hypocotyl curvature test, I screened 44,000 M₂ seeds (progeny of 14,000 M₁ seeds mutagenized with EMS) of A. thaliana ecotype Columbia and 84,000 T₄ T-DNA tagged lines (Feldmann, 1991; progeny of 6,500 T₁ seeds) of A. thaliana ecotype Wassilewskija (WS) to find IAA-insensitive
mutants. For screening, seedlings that had not performed a hypocotyl growth curvature 8 to 12 h after unilateral application of IAA-containing lanolin, were subjected to a second hypocotyl curvature test. Seedlings that did not respond to the two consecutive tests were selected. Three plants were recovered from the EMS-mutagenized population. Hypocotyl of these mutant plants showed no curvature upon unilateral application of lanolin containing 10 µM IAA, at which concentration the screening was carried out. Furthermore, hypocotyl of the mutant plants did not produce a curvature at any concentrations of IAA tested (Fig. 2, middle, and Fig. 3). This indicates that the mutants lack ability to bend responding to unilateral application of IAA. The mutation was designated massugul (msgl).

1.3. Genetic characterization

To determine the genetic basis for the unresponsiveness to auxin in these mutants, each of the three mutant plants were crossed to wild type and the progeny were analyzed with respect to the IAA-induced hypocotyl curvature. The F1 plants resulting from this cross all responded to auxin, and in the F2, unresponsive seedlings segregated at a ratio of 3:1 (Table 1). Thus,
this mutation is recessive and segregates in a manner most consistent with a single Mendelian gene. F₁ progeny between each of the three mutants were tested and found unresponsive to IAA, demonstrating that all the three plants were allelic in \textit{msgl} locus (Table 1).

To determine if \textit{msgl} mutants were alleles of previously isolated auxin-resistant mutants, they were crossed to \textit{axrl-3} (Lincoln et al., 1990) and \textit{auxl-7} plants (Maher and Martindale, 1980; Pickett et al., 1990). Hypocotyls of \textit{axrl-3} mutant showed a smaller curvature upon unilateral treatment of IAA-containing lanolin compared to wild-type plants. They also did not exhibit inhibitory effects of 1,000 μM IAA on the growth curvature (Fig. 2, bottom, and Fig. 3). \textit{auxl-7} was as sensitive as wild type with respect to the growth curvature test. All F₁ progeny from crosses between \textit{msgl} and \textit{axrl} or \textit{auxl} displayed a growth curvature of hypocotyl upon unilateral treatment of IAA to a similar extent to wild type (Table 1), indicating that \textit{msgl} mutation was neither \textit{axrl} nor \textit{auxl}. \textit{msgl} mutation was mapped on chromosome 5 (Fig. 4) using SSLP molecular markers (Bell and Ecker, 1994).

1.4. Morphology
1.4.1. Leaf

Morphology of rosette leaves of msgl plants was allele dependent (Fig. 5). Leaves of msgl-2 was epinastic, namely they curled downward along both their long and short axes. Leaves of msgl-3 were both epinastic and hyponastic, namely some of them curled upward and others curled downward. Leaves of msgl-1 were somewhat epinastic, but not so obvious as those of msgl-2. F₁ progeny between msgl-2 and msgl-3 showed both epinastic and hyponastic leaves in a plant (Fig. 6).

1.4.2. Root

Elongation of msgl root was essentially the same as that of wild type in darkness (Table 2). However, root elongation of light-grown msgl was shorter than that of wild type (Table 2). When culture plates are inclined to 45° to the direction of gravity, the path of root growth changes to a waving pattern (Okada and Shimura, 1990). Roots of msgl plants showed essentially the same waving pattern induced by obstacle-touching stimulus as was observed in wild type.

1.4.3. Hypocotyl and inflorescence stem

Hypocotyl of dark-grown seedlings was as long as wild type (Fig. 7, Table 2) and they looked essentially the same
as those of wild type in terms of development of cotyledon and hook formation (Fig. 8). There were no differences in the height of inflorescence stems among wild-type and msg1 plants (Table 2).

1.5. Phytohormone sensitivity

1.5.1. Hypocotyl

When a synthetic auxin, 2,4-D, was added to culture medium, growth of both hypocotyl and root of wild type was inhibited. Growth of root was blocked at lower concentrations of 2,4-D than that of hypocotyl (Figs. 9 and 10), as had been shown more than 60 years ago (Went and Thimann, 1937). In the case of msg1, hypocotyl growth was more resistant to 2,4-D than wild type (Fig. 9). Hypocotyl of axrl was more resistant to 2,4-D than msg1; hypocotyl of auxl-7 was as sensitive as that of wild type. Inhibitory effects of a natural auxin, IAA, were 50-fold weaker than 2,4-D (Fig. 11). Hypocotyls of msg1 and axrl showed similar resistance in this case. auxl hypocotyl was not resistant to IAA.

Auxin induces ethylene production by rapidly activating expression of genes encoding 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, a key
enzyme of ethylene biosynthesis (Yoshii and Imaseki, 1981; Nakagawa et al., 1991; McKeon, et al., 1995). Produced ethylene, in turn, inhibits growth of plants (Ecker and Theologis, 1994; Ecker, 1995; Reid, 1995). Consequently, auxin-induced growth inhibition could be separated into two portions: a growth inhibition mediated through ethylene and that induced auxin alone. The relative role of ethylene and auxin in the growth inhibition is able to be estimated by the use of ACC, the immediate precursor of ethylene biosynthesis, and aminoethoxyvinylglycine (AVG), an inhibitor of ACC synthase. AVG is an effective inhibitor of pyridoxal phosphate-mediated enzymes, thus inhibits ACC synthase activity both in vitro and in vivo (Yang and Hoffman, 1984). Growth inhibition caused by ACC is regarded as that induced by ethylene only, and that observed in the presence of AVG is considered due to effects of auxin only.

An addition of ACC to culture medium inhibited growth of wild-type hypocotyl. Growth of hypocotyl of msgl and auxl was also inhibited by ACC to a similar extent as in wild type. Growth of axr1-12 hypocotyl was significantly resistant to ACC (Fig. 12).

An addition of AVG had no effects on hypocotyl growth in wild type in the absence of 2,4-D and it did not rescue growth inhibition of hypocotyl observed in the presence of 2,4-D (Fig. 13). An addition of AVG had also no effects on
hypocotyl growth in msgl-2 and msgl-3 without 2,4-D. However, hypocotyls of msgl-2 and msgl-3 grown with AVG were longer than those without AVG when 2,4-D was present in the culture media. The recovery of growth inhibition by AVG was incomplete (Fig. 13). This result indicates that inhibition of growth of msgl hypocotyl in the presence of 2,4-D is partly mediated by ethylene.

1.5.2. Root

When 2,4-D was added to agar medium, growth of root of msgl was inhibited to a similar extent to wild type (Fig. 10). Root of axr1 (Maher and Martindale, 1980) and aux1 (Estelle and Sommerville, 1987) showed significant resistance to 2,4-D as reported previously.

1.5.3. Leaf

When wild type plants were grown on 2,4-D-containing medium for 26 days, chlorosis was observed in the leaves (Fig. 14, top). Determination of chlorophyll contents of msgl plants showed that they displayed less chlorosis than wild type (Figs. 14 and 15). axr1-12 plants showed stronger resistance to 2,4-D than msgl. Chlorosis of aux1-7 leaves occurred to a similar extent to that of wild type (Fig. 15).

All rosette leaves of msgl-3 showed hyponasty in an
in-vitro condition (Fig. 14, bottom left) though they showed both hyponasty and epinasty in soil (Fig. 5). Interestingly, the up-rolled hyponastic leaves became flat when 2,4-D was added to the medium (Fig. 14).

1.6. Fusicoccin-induced hypocotyl curvature

Fusicoccin, a fungal toxin, has been known to promote growth of plant tissue (Cleland, 1976; Marré, 1979). In wild type, unilateral application of fusicoccin produced growth curvature of hypocotyl in a dose-dependent manner (Fig. 16, top). Hypocotyls of wild type began to perform a curvature at 0.05 μM fusicoccin and the response reached a maximum at 0.5 μM. Fusicoccin was inhibitory for the curvature from 2.5 to 5 μM. Hypocotyls of all the three alleles of msgl showed similar growth curvature responses to the unilateral treatment of fusicoccin (Fig. 16).

1.7. Tropism

When msgl seedlings were held in a horizontal position in darkness, hypocotyls of msgl showed reduced negative gravitropism than wild type, but the roots bent downward
normally (Fig. 17). Hypocotyls of auxl-7 and axr1-3 showed normal negative gravitropism; root of auxl-7 showed agravitropism and that of axr1-3 showed weaker gravitropism (Fig. 17) as described previously (Maher and Martindale, 1980; Lincoln et al., 1990). Vertically grown seedlings were reoriented by 90° and the time course of hypocotyl curvature was measured (Fig. 18). Horizontally oriented hypocotyls of wild type began to bend upward significantly 2 h after the start of gravistimulation. In msgl-2 mutant, however, hypocotyls showed only reduced gravitropism even 30 h after gravistimulation. An inflorescence stem of msgl-2 showed normal gravitropism (Fig. 19).

When continuous white light was irradiated unidirectionally from one side of hypocotyls for 24 h, hypocotyl of msgl showed phototropism like that of wild type.

1.8. Growth habit

Since msgl hypocotyls did not respond to change in gravitational vector in a normal manner, growth pattern of hypocotyl was investigated in wild type and msgl under dim white light. Although both wild-type and msgl hypocotyls grew straight, their growth orientation was not the same.
Wild-type plants grew upward. But, *msgl* hypocotyls grew in more random direction, which was indicated by an increase in the standard deviation of the frequency distribution histogram of growth orientation (Fig. 20). This result indicates that defects of *msgl* in hypocotyl gravitropism are expressed even when *msgl* plants are cultured in a normal condition. Next, growth pattern of *msgl* hypocotyls was found dependent on whether they were grown on a horizontally- or vertically-held plates. *msgl* plants grew straight at a various angle to the vertical on a horizontally-held plate as described above. On a vertically-held plate, however, they grew straight upward initially after germination (Fig. 21), and gradually performed a growth curvature, resulting in somewhat lazy growth habit (Fig. 8, bottom). This spontaneous growth curvature observed in *msgl* hypocotyl did not occur in wild type on either horizontally- or vertically-placed plate (for seedlings on a vertically-placed plate, see Fig. 8, top).

1.9. IAA-induced hypocotyl curvature of related mutants

A tomato mutant known as *diageotropica (dgt)* is nearly insensitive to exogenously applied auxin with respect to
hypocotyl (Kelly and Bradford, 1986) and root elongation (Muday et al., 1995) and ethylene production (Zobel, 1973; Bradford and Yang, 1980). Auxin sensitivity of dgt was examined with a hypocotyl curvature test. Seedlings of tomato were grown under continuous white light for 12 days in soil and subjected to a hypocotyl curvature test using IAA-containing lanolin. Hypocotyl of wild type performed a curvature at 10 and 100 μM IAA, but that of dgt did not perform a growth curvature at either concentration (Fig. 22). When IAA was added to MS medium, growth of both hypocotyl and root of wild type was inhibited; root of dgt was resistant to IAA with respect to their growth (Figs. 23) as reported previously (Muday et al., 1995). Hypocotyl of dgt was resistant to IAA as well as root. Therefore, both organs were resistant to IAA in dgt plants.

Hypocotyl curvature test was also conducted with a few dozens of other Arabidopsis mutants defective in action of plant hormones or elongation growth. Except for axr1 as described above (Fig. 3), all of them exhibited a normal growth curvature (Table 3).
2. Double-mutant analysis of *msgl* and auxin-resistant mutants

2.1. Auxin sensitivity

To determine if *msgl*, *axrl* and *auxl* genes functioned in the same reaction pathway, I had constructed and characterized double mutant plants. *msgl*-2 *axrl*-3 and *msgl*-2 *auxl*-7 double mutants were obtained by progeny testing F2 plants of each parental mutant phenotype: for *msgl*-2, hypocotyl growth curvature test was carried out; for *axrl*-3 and *auxl*-7, root growth was tested in the presence of 2,4-D. The genotype of double mutant plants was confirmed by outcrossing to *msgl*-2, *auxl*-7 and *axrl*-3 plants and scoring progeny for *msgl*-2, *auxl*-7 and *axrl*-3 phenotype (Table 4). *msgl*-2 *axrl*-3 double mutants grew in the presence of 2,4-D. In this condition, hypocotyl length of the double mutants was significantly longer than that of *msgl*-2 or *axrl*-3 (Fig. 24). Thus, *msgl*-2 and *axrl*-3 interact in an additive manner. Growth of *auxl* hypocotyl was inhibited by application of 2,4-D to a similar extent to wild type. No difference was observed in hypocotyl length of *msgl*-2 *auxl*-7 double mutants and *msgl*-2 mutants, indicating that *msgl* and *auxl* were independent each other.
2.2. Morphology

Rosette leaves of axr1-3 plants were irregular in shape and tended to be wrinkled or twisted in this study (Fig. 25, middle left), although Lincoln et al. (1990) reported that they curled downward. Rosette leaves of msg1-2 curled downward along the main vein of the leaf (Fig. 25, top right). Rosette leaves of msg1-2 axr1-3 double mutants showed additive effects: they were both wrinkled and epinastic (Fig. 25, middle right). Rosette leaves of aux1-7 were similar to those of wild type (Fig. 25, top left and bottom left), and those of msg1-2 aux1-7 double mutants showed msg1-2 phenotype (Fig. 25, bottom right). These results indicate that phenotype observed in msg1-2 axr1-3 double mutants was additive with respect to leaf shape as is the case with 2,4-D resistance of hypocotyl as described above. aux1 mutation was independent from msg1-2 with respect to leaf shape, which was also consistent with 2,4-D resistance in its hypocotyl.
3. Expression of auxin-induced gene in msg1

Ballas et al. (1995) have identified a promoter region in the pea PS-IAA4/5 gene that confers IAA inducibility to the gene. Two functional modules (domain B and domain A) and PS-IAA4/5 core promoter were fused to GUS gene and introduced to A. thaliana ecotype Columbia (Oono and Theologis, unpublished). Transgenic plants that contained the PS-IAA4/5/reporter gene were crossed to msg1-2 plants and F2 seedlings containing the transgene and msg1-2 mutation were selected. Segregation of PS-IAA4/5/GUS reporter gene was checked at F3 progeny and a homozygous line was selected.

Neither wild-type (MSG1/MSG1) nor msg1 transgenic seedlings grown in darkness showed any staining in hypocotyl and root (Fig. 26). When wild-type seedlings were submerged in half-strength MS medium for 5 h, significant staining was observed in hypocotyl, but not in root (Figs. 26 and 27). An addition of 0.1 and 100 μM IAA to the MS medium enhanced the staining of hypocotyl, and staining of root tip was also evident in these concentrations (Fig. 28). Staining in msg1-2 hypocotyls was far fainter than that of wild type (Figs. 26, 28 and 29), although significant induction of the GUS gene was
observed (Fig. 29). This result indicates that MSG1 gene is involved in enhancement of the induced expression of the GUS gene in hypocotyl. Staining of root tip of the IAA-treated msgl-2 was observed to a similar extent to wild type (Fig. 27).

Quantitative GUS assay was carried out with whole seedlings. A dose response of PS-IAA4/5 promoter-driven GUS expression was shown as a function of IAA concentrations in Figure 30. In seedlings of wild type, the GUS expression was induced by application of IAA in a dose-dependent manner. No induction of the GUS gene was observed in msgl seedlings, which is inconsistent with the results of histochemical assay (Fig. 29). Weak GUS staining may become undetectable after extraction with buffer solution because the staining is diluted by it.
4. Isolation and initial characterization of gravitropism mutants, jyuuryoku (jrk)

4.1. Isolation of jrk mutants

Seeds of Arabidopsis were grown on filter paper placed in a vertical position for three days in darkness after induction of germination. Then, the filter paper with the seedlings was turned 90° and left for one day in darkness. Hypocotyls of wild type showed negative gravitropism and grew upward in this culture condition, whereas mutants showed no bending away from gravity (Fig. 31).

I screened 30,000 M2 seeds (progeny of 21,000 M1 seeds mutagenized with fast neutron) of A. thaliana ecotype Columbia to find curvature mutants to gravistimulation, using the gravistimulation test described above. For screening, seedlings that had not shown negative gravitropism one day after gravistimulation, were subjected to a second gravistimulation test. Seedlings that did not respond to the two consecutive gravistimulation tests were selected. Four plants were recovered from the mutagenized population. Hypocotyls of the four mutant plants showed no curvature on gravistimulation. Hypocotyls of #59 mutants did not perform an IAA-induced growth curvature when tested
with the hypocotyl curvature test. F1 progeny between #59 and msgl was tested and found unresponsive to IAA, demonstrating that #59 and msgl were allelic in msgl locus. We designated #59 msgl-4. The other mutants were collectively named as jyuuryoku: namely, jrk48, jrk110, and jrk197, however, they were not examined allelism test among them. Since jrk48 and jrk110 were isolated from the same batch of M1 plants, the two mutants were most likely to be progeny of the identical mutation. Thus, only jrk48 plants were used in further experiments.

4.2. IAA-induced hypocotyl curvature

Hypocotyls of jrk197 showed a reduced but significant response to the unilateral treatment of lanolin containing 1 μM IAA. However, curvature of jrk197 is not significant at 10 to 1000 μM IAA. jrk48 was as sensitive as wild type with respect to the growth curvature test (Fig. 32).

4.3. Morphology

4.3.1. Inflorescence stem

Growth pattern of inflorescence stems of jrk48 and
jrk197 was different from that of wild type. Lateral shoots of inflorescence stems of wild type usually grew at an angle to the vertical smaller than 90° ( plagiotropically) (Fig. 33, top left). However, those of jrk48 grew horizontally (diageotropically) at first and bent downward in a later stage (Fig. 33, top right). Inflorescence stems of jrk197 were shorter than wild type (Fig. 33, bottom). jrk197 plants did not bear fruits (Fig. 33, bottom), but they produced normal amounts of seeds when pollinated with wild-type pollen. Thus, jrk197 was male sterile.

4.3.2. Seedling

Hypocotyl of dark-grown seedlings of jrk48 was as long as wild type, but that of jrk197 was shorter than wild type (13.7 ± 1.1 mm for wild type, 12.4 ± 0.7 mm for jrk48, and 8.8 ± 1.3 mm for jrk197, when plants were grown for 4 days after induction of germination). Root of the mutants was as long as wild type (3.9 ± 0.8 mm for wild type, 4.2 ± 0.6 mm for jrk48, and 3.8 ± 0.4 mm for jrk197).

4.4. Gravitropism

The normal response of wild-type seedlings to gravity
is illustrated in Figure 31 (top). However, growth orientation of both \textit{jrk48} and \textit{jrk197} hypocotyls ranged in a random fashion from $-75^\circ$ to $90^\circ$ (Fig. 31, middle and bottom, and Fig. 34).

An inflorescence stem of \textit{jrk48} did not bend away from gravity (Fig. 19, bottom), but that of \textit{jrk197} showed normal gravitropism (Fig. 35). Root of \textit{jrk48} and \textit{jrk197} showed normal positive gravitropism in darkness within 24 h.
DISCUSSION

1. msgl and its organ specificity

In the present study, several mutant lines of Arabidopsis were isolated that were defective in auxin-induced growth curvature in hypocotyl. Three lines among them were found to be allelic of single locus, msgl (Table 1), and genetic and physiological characterization was carried out mainly on msgl mutation. Besides the defect in the growth curvature in hypocotyl (Figs. 2 and 3), msgl mutants show growth resistance of hypocotyl to auxin (Figs. 9 and 11), resistance to auxin with respect to chlorosis in leaves (Fig. 15), and reduced gravitropism in hypocotyl (Fig. 18). The most striking feature of msgl is that all the defects are restricted to hypocotyl and leaf. Since all the auxin-resistant mutants in Arabidopsis reported so far (aux1, Maher and Martindale, 1980; axr1, Lincoln et al., 1990; axr2, Wilson et al., 1990; and axr4, Hobbie and Estelle, 1995) display defects either in root or in an entire plant, msgl mutant is likely to belong to a new class of auxin-insensitive mutants. This result indicates that plants have multiple auxin signal transduction chains with determined organ specificity.
Root of light-grown *msgl*-2 and *msgl*-3 plants is shorter than that of wild type (Table 2, Fig. 10). Since all of *msgl* plants have been backcrossed two times, it is not likely that their short roots result from a second mutation. Recently, Kurata and Yamamoto (1997) reported that photosynthetic activity in cotyledons greatly promotes growth of roots in *Arabidopsis* seedlings, probably by affecting availability of sucrose. This suggests that growth of roots is affected by many physiological activities in the aerial part of seedlings. It is possible that *msgl* mutation secondarily alters physiology of hypocotyls and possibly cotyledons. Then, growth of root could be influenced indirectly by *msgl* mutation, even if the mutation is specific to hypocotyl and leaf. Root of *msgl*-1 plants elongates to a similar extent to wild type (Fig. 10). Epinasty in *msgl*-1 leaves is not so obvious as that of *msgl*-2 as described below (Fig. 5). *msgl*-1 may be a weak allele of *msgl*, and this could be a reason of normal growth of *msgl*-1 roots.

2. Hypocotyl growth curvature test

I have found three kinds of *Arabidopsis* mutants, *msgl*, *jrk197* and *axr1* (Lincoln et al., 1990), that are deficient
in IAA-induced growth curvature in hypocotyl (Figs. 3 and 32). Although *jrk197* mutant have not been fully characterized yet, are most likely to be mutant in a new locus distinct from the other two loci since morphology of *jrk197* mutant is clearly different from that of *msgl* and *axrl* (Fig. 33). Each of the three mutants shows a different dose-response curve of IAA-induced growth curvature: *msgl* does not respond to IAA at all the concentrations tested (Fig. 3). The growth curvature of *axrl* is smaller than that of wild type, and is proportional to IAA concentrations, namely *axrl* lacks inhibitory effects of higher concentrations of IAA on the growth curvature, which are observed in wild type (Fig. 3). Although a normal and small growth curvature is detected at 1 μM IAA in *jrk197*, application of higher concentrations of IAA does not promote the curvature (Fig. 32). Complete loss of the curvature response in *msgl* may indicate that the insensitivity is not due to any changes in uptake and metabolism of applied IAA. Estelle and Somerville (1987) investigated uptake and metabolism of 2,4-D in wild-type and *axrl* plants using labeled 2,4-D, and concluded that *axrl* mutation did not disturb these processes. It was likely to reduce sensitivity to IAA. Although *msgl* and *axrl* mutations seem to affect sensitivity of hypocotyl to IAA to a different extent, their growth of hypocotyl in
darkness is similar (Figs. 7 and 9), suggesting that IAA sensitivity observed in the growth curvature test is not correlated with hypocotyl growth. Hypocotyl of jrk197 is smaller than wild type by about 30% (Section 4.3.2 in Results). Mature jrk197 plants are also smaller than wild type (Fig. 33). These characteristics of jrk197 suggest an occurrence in jrk197 of profound changes in growth, which may result from changes in composition of cell-wall components or mechanical properties of cell wall. Such changes may produce a different dose-response relationship of the IAA-induced growth curvature. On the other hand, Shinkle and Briggs (1984) showed a biphasic dose-response curve for IAA-stimulated growth in oat coleoptile segments. This raises a possibility that jrk197 mutation only disrupts a growth curvature response to higher concentrations of IAA, leaving the response to lower IAA concentrations intact.

Fusicoccin is the major toxin produced by Fusicoccum amygdali Del. and is responsible for the most of the pathological symptoms induced by this fungus on peach and almond tree (in review of Marrè, 1979). Fusicoccin has been known to promote growth of plant tissue (Marrè, 1979). In wild type, unilateral application of fusicoccin produced growth curvature of hypocotyl in a dose-dependent manner (Fig. 16). Hypocotyls of all the three alleles of msg1
showed similar growth curvature responses to the unilateral treatment of fusicoccin (Fig. 16). Therefore, hypocotyl of msgl does not lack an ability to bend and msgl mutation does not cause changes composition of cell-wall components or mechanical properties of cell wall. This result indicate that lesion of hypocotyl bending in msgl is specific to the action of auxin.

The hypocotyl curvature test was carried out under continuous red light of low fluence rate, since more consistent results were obtained under the red light than in darkness. Liscum and Hangarter (1993) showed that irradiation with red light partially canceled gravitropism of Arabidopsis hypocotyls. Treatment of hypocotyls with IAA-containing lanolin under red light, therefore, could produce a curvature more readily due to a smaller interference with stimulus of gravity. Irradiation with red light has been also known to decrease IAA contents in coleoptiles of etiolated maize seedlings (Iino, 1982; Koshiba et al., 1995). Possible reduction of auxin level under red-light irradiation might make Arabidopsis hypocotyls more responsive to IAA applied exogenously.

3. Sensitivity to phytohormones
3.1. Cross-resistance

Growth of root of msg1 mutant is inhibited by 2,4-D to essentially the same extent as wild type (Fig. 10), while that of hypocotyl is resistant to 2,4-D (Fig. 9) and IAA (Fig. 11). All the auxin-resistant mutants so far reported with respect to root growth show resistance to other phytohormones. Root growth of auxl (Maher and Martindale, 1980; Pickett et al., 1990; Hobbie and Estelle, 1994) and axr1 (Hobbie and Estelle, 1994) is also resistant to ethylene and cytokinin; root of axr2 is also resistant to ethylene and ABA (Wilson et al., 1990). Hobbie and Estelle (1995) showed that axr4 mutant was specifically resistant to auxin. However, root of axr4 plants was actually more resistant than wild type to ACC, kinetin and ABA at certain concentrations of them (Hobbie and Estelle, 1995). These cross-resistances observed in the auxin-resistant mutants reflect interactions among the plant hormones: either direct effects on synthesis, metabolism and transport of plant hormones, or more complex interactions such as cross talk among signal transduction pathways can be considered as a molecular basis of the cross-resistance (Hobbie and Estelle, 1994). For example, it has been well known that auxin induces ethylene synthesis through rapid activation of ACC synthase genes which encode a key enzyme of ethylene
biosynthesis (Nakagawa et al., 1991). Therefore, if observed growth inhibition by auxin is completely mediated by the auxin-induced ethylene, plants resistant to ethylene should acquire auxin resistance as far as growth inhibition is concerned. Cytokinin inhibits root elongation in wild-type Arabidopsis seedlings. Since the inhibition is partially blocked by the action of ethylene inhibitors or ethylene-resistant mutations, and since ethylene production is stimulated by cytokinin, the inhibitory effect of cytokinin appears to be mediated largely by the production of ethylene (Cary et al., 1995). The growth resistance of axr1 root to cytokinin mentioned above could be attributed to its resistance to ethylene.

Growth of hypocotyl of msgl plants is inhibited by ACC to a similar extent to wild type (Fig. 12). All the other auxin-resistant mutants in Arabidopsis display resistance to auxin and ethylene as described above. Thus, msgl is the first auxin-resistant mutant in Arabidopsis in which ethylene sensitivity is not changed. 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 of the nine mutants were specifically resistant to auxin.
3.2. Auxin and ethylene actions in growth inhibition

AVG, an inhibitor of ACC synthase, significantly reduces growth inhibition of msg1 hypocotyl induced by high concentrations of 2,4-D (Fig. 13). This suggests that the growth inhibition of msg1 hypocotyl is partly caused by ethylene, and that ethylene is produced in response to auxin in msg1 hypocotyl. Thus, insensitivity to auxin conferred by msg1 mutation does not seem to affect ethylene production induced by auxin. On the other hand, 2,4-D-induced growth inhibition of wild-type hypocotyl is not restored by an addition of AVG (Fig. 13). This indicates that most of the growth inhibition in wild type by 2,4-D results from the inhibitory action inherent in auxin. Since the inhibition of hypocotyl growth is a saturable response, the inhibitory effects of the auxin-induced ethylene, if any, should be masked in wild type at saturating levels of 2,4-D. In other words, msg1 mutation makes it visible that ethylene-mediated growth inhibition constitutes a part of auxin-induced growth inhibition. Thus, MSG1 gene is probably a component of the auxin signal transduction cascade which is separated from the ethylene signal transduction cascade. It should be examined further whether AVG can restore growth inhibition induced by lower
concentrations of 2,4-D in wild type, in order to evaluate the role of ethylene more precisely in the auxin-triggered growth inhibition.

Rosette leaves of msgl plants are resistant to 2,4-D with respect to their chlorosis (Figs. 14 and 15). In contrast, hyponastic leaves of msgl-3 become flat by an addition of 2,4-D in medium as described below. This suggests that auxin controls multiple physiological responses in leaves via separate signaling cascades. MSG1 gene might be responsible only for chlorosis of leaf by 2,4-D, and might not be involved in the leaf-opening response.

4. Epinasty and hyponasty of leaf

Leaves of msgl plants show epinasty or hyponasty, depending on their alleles (Fig. 5). msgl-3 shows hyponastic leaves on agar medium (Fig 14B), but when it is grown in soil, its leaves are either hyponastic or epinastic (Fig. 5). On the other hand, the auxin-resistant mutants, axr1 (Lincoln et al., 1990) and axr2 (Wilson et al., 1990), have twisted and wrinkled leaves, respectively. These phenotypes of leaves probably reflect unconcerted growth of cells in the leaves. Epinasty and hyponasty are
observed when differential growth between the upper side and the lower side of leaves occurs uniformly all over the leaves (Palmer, 1985). Wrinkled or twisted leaves may be produced when unconcerted growth of cells occurs to a various degree in many, localized regions of leaves. These observations suggest that defects in auxin sensitivity affect the concerted growth of leaf cells, resulting in aberrant curvature of leaves. Currently it can not be explained why the leaf curvature, epinasty or hyponasty, is allele-dependent in msg1.

It has been well known for more than 60 years that epinasty is induced by application of auxin (Went and Thimann, 1937) or ethylene (for review, see Palmer, 1985; Kang, 1979). Since auxin-induced epinasty is confined to plants that become epinastic in the presence of ethylene, it has been postulated that applied auxin acts indirectly by stimulating ethylene formation, which is the primary cause of the observed curvature (Palmer, 1985). Recently, the role of auxin and ethylene in epinasty has been reassessed using transgenic Arabidopsis plants (Romano et al., 1993). Transgenic plants expressing the auxin-overproducing Agrobacterium iaaM gene (19S–iaaM, i.e., the cauliflower mosaic virus 19S RNA promoter fused to the iaaM gene) show strong leaf epinasty. They are crossed to plants expressing ACC deaminase (ACCase) transgene (FMV-
ACCase, i.e., the figwort mosaic virus 35S RNA promoter fused to the ACCase), or the ethylene-insensitive ein1 and ein2 mutants. Since ACCase breaks down ACC, transgenic plants harboring the ACCase gene produce a lower amount of ethylene than wild type. Leaf epinasty is as severe in 19S-iaaM/FMV-ACCase plants with low ethylene production as it is in the 19S-iaaM plants with high ethylene production. Furthermore, neither the ein1-l nor ein2-1 mutation relieves the leaf epinasty caused by the 19S-iaaM transgene. These results indicate that leaf epinasty is primarily controlled by auxin rather than auxin-induced ethylene (Romano et al., 1993), which is contrary to the conventional view described above. Obviously, further work is needed to resolve the relative roles of auxin and ethylene in leaf epinasty.

Leaves of msg1-3 are hyponastic and epinastic (Fig. 5). The hyponasty is exaggerated when the mutants are grown in Petri plates; the leaf blades are almost closed in some of the leaves (Fig. 14B). Interestingly, the closed leaf blades become open when they are cultured in the presence of 2,4-D (Fig. 14B). This response could occur if cells of the adaxial (upper) surface expand more relative to the abaxial (lower) surface of the leaves. If this is the case, the phenomenon could be regarded as an epinastic response induced by 2,4-D. Considering the above discussion, we do
not know whether it is caused by auxin alone or auxin-
induced ethylene, since ethylene is likely to be produced
in response to auxin in msgl plants (see Section 3.2 in
Discussion). If it is induced by auxin alone as suggested
by Romano et al. (1993), the epinastic response would
indicate that msgl plants keep a second auxin-dependent
reaction intact in the leaves although they are defective
in auxin sensitivity in hypocotyl and leaf (Section 1 in
Discussion).

Interestingly, leaves of tomato on a clinostat show
epinasty and ethylene plays a role in the epinastic leaf
bending (Salisbury and Wheeler, 1981). This suggests that
a loss of graviperception in plants promotes ethylene
production, which then induces leaf epinasty. It is
reasonable to assume that msgl leaves are defective in
gravitropism as well as its hypocotyl, since both organs
are affected similarly by msgl mutation with respect to
growth resistance to auxin. The loss of graviperception in
msgl leaves may induce ethylene production, which could be
a cause of epinasty of msgl leaves. If this is the case,
MSGl gene should be involved in a sensing process of
gravity.
5. Gravitropism

5.1. Organ specificity

Various gravitropism mutants have been characterized in *Arabidopsis* as well as pea and tomato. Organ specificity of gravitropic defects in *Arabidopsis* mutants is summarized in Table 5. In msg1 (Figs. 18 and 20) and jrk197 (Fig. 34) gravitropism of hypocotyl is altered, but that of root (Figs. 17, 31) and inflorescence stem (Figs. 19 and 34) is normal. Gravitropic response of msg1 hypocotyl is slower than wild type (Fig. 18). Poff (1992) reported the presence of a number of *Arabidopsis* strains which showed no response to gravity during a 10-h presentation time, but did show some gravitropism after much longer time. msg1 seems to belong to this type of mutants.

Fukaki et al. (1996a, b) have recently isolated six kinds of *Arabidopsis* mutants with no or reduced gravitropism in inflorescence stems (sgr1 to 6). They classified the sgr's and other gravitropism mutants into five groups according to their organ specificity (Table 5): Type I: mutants showing abnormal gravitropism in both inflorescence stems and hypocotyls; type II: mutants showing abnormal gravitropism in inflorescence stems only;
type III: mutants showing abnormal gravitropism in all three tropic organs (that is, inflorescence stems, hypocotyls, and roots); type IV: mutants of abnormal gravitropism in both hypocotyls and roots, which includes a mutant line which was isolated as a second mutation of phyB-1 (Fukaki et al., 1996a; Robson and Smith, 1996); and type V: mutants showing abnormal gravitropism only in roots. Classifying the gravitropic mutants in this way, Fukaki et al. (1996a) suggest that gravitropic reaction chains are genetically different between inflorescence stems and hypocotyls in Arabidopsis.

On the other hand, Bullen et al. (1990) reported that in approximately 40% of the Arabidopsis strains with alterations in gravitropism of the hypocotyl or root, the other organ appeared unaffected, and concluded that hypocotyl and root gravitropisms were genetically separable. Finding of msg1 and jrk197 in this study have made it clear that there exists the sixth type of gravitropic mutants with defects restricted only in hypocotyl. The results confirm the conclusions above, and further demonstrate that each of the three gravitropic organs contains a reaction pathway of gravitropism specific to it.

In jrk48 gravitropism of hypocotyl and inflorescence stem is altered (Fig. 19) but that of root is normal (Fig. 31), indicating that jrk48 is a type I gravitropism mutant.
Morphology of *jrk48* is normal except for diageotropic nature of lateral shoot of inflorescence stem (Fig. 33). The other type I mutants, *sgr1*, *sgr2* and *sgr4*, show different phenotypes: *sgr1* has smaller leaves and thin and short stems. *sgr2* and *sgr4* show twisting and zigzag stems, respectively. Therefore, *jrk48* is most likely to be a novel type I mutation of gravitropism.

Gravitropism mutation does not always confer changes in auxin sensitivity. For example, *jrk48* which is defective in hypocotyl gravitropism responds to auxin normally in a hypocotyl growth curvature test (Fig. 32). However, auxin-insensitive mutants show lesion of gravitropism without exception in the organ where changes in auxin sensitivity occur. This result is consistent with a widely accepted view that auxin acts as an effector system of gravitropism (Kaufman et al., 1995). *jrk48* mutation might occur in a step upstream of the effector system, such as a step of perception of gravistimulation.

Organ specificity of gravitropism mutants in Table 5 shows that only the mutants defective in both inflorescence stem and root have not been found. Inflorescence stem and root are not connected directly; they are separated by epicotyl and hypocotyl. It may be possible to speculate that two organs which are not connected directly could not share the identical reaction pathway of gravitropism.
Obviously, more efforts should be concentrated on isolation of a number of gravitropism mutants so that we can get a full spectrum of organ specificity in gravitropism.

5.2. Auxin sensitivity and gravitropism

Relationship between resistance to auxin-induced growth inhibition, auxin-induced growth curvature and gravitropism in root and hypocotyl is summarized in Table 6 for each of the auxin-related Arabidopsis mutants. All the mutants that are resistant to auxin in root (axr1, axr2, axr4, aux1 and dwf) show defects in root gravitropism. It can be concluded that auxin resistance and lesion of gravitropism are closely coupled in root. On the other hand, axr1 and axr4 which are resistant to auxin in hypocotyl do exhibit normal gravitropism in the same organ. Thus, auxin resistance and gravitropism are not linked in hypocotyl. This discrepancy between hypocotyl and root could be explained by physiological nature of the two organs.

Auxin produced in a shoot apex is transported to the base of shoot first. After reaching root, it moves to the root tip, and enters the central region of the root cap through the acropetal transport system. In a vertically
oriented root auxin is redistributed symmetrically in the cap and transported in equal amounts toward the elongating zone. After gravistimulation auxin entering the root cap is transported preferentially downward and then back toward the lower side of the elongation zone. The increased amount of auxin on the lower side leads to growth inhibition and downward curvature (Kaufman et al., 1995). Auxin-resistant mutations are less susceptible to the inhibition, thus causing no curvature. On the other hand, gravistimulation causes redistribution of auxin to the lower side of hypocotyl and leads to growth promotion and upward curvature. In other words, auxin action is different in hypocotyl and root in gravitropism.

Gravitropism of root and hypocotyl is caused by growth inhibition and promotion, respectively. IAA-induced growth curvature of hypocotyl is probably produced by promotive effects of auxin on cell elongation (Larsen, 1961). Hypocotyl of axr1 may perform gravitropism because it can respond to auxin by promoting cell elongation, although the promotive response may be smaller than wild type as presumed from a smaller growth curvature induced by IAA (Fig. 3). The smaller response may be enough to express gravitropism. Hypocotyl of msg1 does not respond to IAA at all concentrations tested in a hypocotyl curvature test, and shows slower gravitropism (Fig. 16). Thus, it could be assumed that growth curvature induced by application of
auxin is correlated with gravitropism in hypocotyl. MSG1 gene may play an essential role in differential growth of hypocotyl under gravistimulation through an auxin signal transduction cascade.

Recently, Knee and Hangarter (1996) reported that IAA elongates a hypocotyl of axrl in the range of concentrations from \(10^{-10}\) to \(10^{-5}\) M. This observation supports that hypocotyl of axrl does not lose an ability to elongate in response to IAA. In my study, however, axrl hypocotyl does not show growth promotion at IAA concentrations from \(10^{-7}\) to \(5\times10^{-6}\) M, and \(10^{-5}\) M IAA is already inhibitory (Fig. 11). Since experimental conditions in this study are different from those of Knee and Hangarter (1996), the results of the two studies are not comparable directly.

A phototropic mutant, JK345, isolated by Khurana and Poff (1989) shows no "first positive" phototropism, but shows reduced gravitropism and "second positive" phototropism in hypocotyl. They conclude that gravitropism and phototropism share at least one common element. Since msgl mutants show normal phototropism upon unilateral irradiation with white light, MSG1 gene is not related to the elements common to gravitropism and phototropism.
6. *msgl* and other related mutants

Blonstein et al. (1991) isolated nine tobacco mutants which were specifically resistant to auxin. In one of them, R15, leaf hyponasty was observed like *msgl*-3. But gravitropism of its root was also changed contrary to *msgl*-3. R15 and another mutant, R20, showed relatively normal phenotype like *msgl* but they were partially dominant. Thus, these mutations are not likely to be *msgl*. A hypocotyl curvature test should be done in the tobacco mutants to determine whether they are the tobacco counterparts of *msgl*.

The *diageotropica* (*dgt*) mutant of tomato is characterized by its diageotropic habit (a habit to grow horizontally) in shoots, plagiotropic habit (a habit to grow obliquely) dark green hyponastic leaves, and lack of lateral roots (Zobel, 1973). Hypocotyl sections of *dgt* do not elongate in response to IAA in the range of concentrations up to 100 μM (Kelly and Bradford, 1986). Hypocotyl of *dgt* shows auxin resistance (Fig. 23). *msgl* and *dgt* are similar in altered gravitropism of hypocotyl, hyponastic leaves (Zobel, 1973), and resistance to auxin in hypocotyl. But, *DGT* gene also affects root tissue: roots of *dgt* seedlings tend to grow plagiotropically when grown between vertical sheets of blotter paper, and primary and adventitious roots are without lateral branching (Zobel,
1973). Thus, functions of MSG1 and DGT resemble each other but their organ specificity is different.

*ageotropum* mutants of pea have defects in gravitropism in both epicotyl and root (Schöldéen and Burström, 1960; Eason et al., 1987). The root grows in the presence of auxin (Eason et al., 1987). This result is consistent with the above conclusion that auxin resistance and abnormal gravitropism are linked in root. Although gravitropism of epicotyl was changed in *ageotropum*, the epicotyl performed a growth curvature upon application of IAA-containing lanolin (Schöldéen and Burström, 1960), which is similar to jrk48 and sgr4. The root also performs a auxin-induced growth curvature (Schöldéen and Burström, 1960). Although change of auxin sensitivity detected with a hypocotyl growth curvature test is associated with auxin resistance in *msg1* and *axrl* (Table 6), this relationship does not exist in root of *ageotropum*. This result may indicate that correlation between auxin-induced growth curvature and auxin resistance is different between in hypocotyl/epicotyl and root.

7. Double mutants

Rosette leaves of *msg1-2 axrl-3* double mutant show
additive effects: they are both wrinkled and epinastic (Fig. 25). When grown in the presence of 2,4-D, hypocotyl length of msg1-2 axr1-3 double mutants is significantly longer than that of msg1-2 or axr1-3 (Fig. 24). Therefore, MSG1 and AXR1 genes are not likely to function in a single pathway. However, it should be admitted that there remains a possibility that the both genes function in a single pathway. AXR1 gene encodes a polypeptide of 540 amino acid residues and axr1-3 mutation is a missense mutation that replaces the cysteine at position 154 with a tyrosine (Leyser et al., 1993). Auxin resistance and morphological defects are weaker in axr1-3 than axr1-12, showing that axr1-3 is a weak allele (Lincoln et al., 1990). Although no response in a growth curvature test in msg1-2 hypocotyl (Fig. 3) suggests that msg1-2 is a null mutation, it is possible that msg1-2 is a leaky mutant since the MSG1 gene product and its function have not been identified yet. If msg1-2 is leaky, an interaction between MSG1 and AXR1 genes could not be determined.

Timpte et al. (1995) show that axr1 and aux1 genes function in separate auxin-response pathways using an axr1 aux1 double mutant. axr1 and aux1 mutations are additive in auxin resistance in root, suggesting that each mutation confers resistance by a different mechanism. axr1 mutants are characterized by a reduction in stature and wrinkled,
irregularly shaped leaves. The auxl mutant has no morphological abnormalities in the aerial part. axrl-12 auxl-7 double mutants are clearly less severe than axrl-12. These result indicate that the auxl mutation acts to partially suppress the morphological defects caused by the axrl mutation.

SAUR-AC1 is one of early auxin-inducible genes in Arabidopsis as described below. Although no morphological changes are seen in the aerial part of auxl, SAUR-AC1 mRNA is only induced about 70% of wild type in rosette leaves by application of auxin (Timpte et al., 1995). Therefore, auxl mutation affects rosette leaf as well as root. On the other hand, phenotype of msgl auxl double mutants shows that their interaction is additive, as far as auxin resistance of hypocotyl (Fig. 24) and shape of rosette leaves (Fig. 25) are concerned. No suppression or promotion of msgl characteristics is observed in the double mutants. This result indicate that msgl and auxl mutations are independent each other. In conclusion, msgl is independent from axrl and auxl in auxin signal pathways.

8. Gene expression in msgl

Many auxin-induced processes are thought to include
changes in gene expression, and a number of mRNA's have been identified that increase or decrease in abundance in response to auxin treatment (for review, Abel and Theologis, 1996; Hagen, 1995). Aux22 and Aux28 from soybean are the early auxin-inducible genes that have been identified first (Ainley et al., 1988). They are members of a large gene family, AUX/IAA gene family. This family includes GH1 from soybean (Guilfoyle et al., 1993), PS-IAA4/5 and PS-IAA6 from pea (Oeller et al., 1993), ARG3 and ARG4 from mung bean (Yamamoto et al., 1992), and at least 14 expressed genes from Arabidopsis (IAA1 to IAA14; Conner et al., 1990; Abel et al., 1995a). SAUR is another early auxin-inducible gene. It constitutes a small gene family in soybean (McClure et al., 1989). In a specific response to auxin, steady-state mRNA levels of most of these genes rapidly increase within 5 to 60 min. This response does not require de novo protein synthesis, which indicates direct gene activation. This also implies that components required for their transcriptional activation are preexisting, and that an extracellular stimulus such as auxin is transduced to a nucleus via post translational processes (Abel and Theologis, 1996). Although functions of the early auxin-induced genes remain unknown, they have been postulated to have some physiological significances since their gene expression corresponds well to cell
elongation observed in gravitropism (McClure and Guilfoyle, 1989; Wyatt et al., 1993).

Linker-scanning mutagenesis of pea PS-IAA4/5 promoter has identified two positive domains [domain A (48-bp long; -203 to -156 of the promoter) and domain B (44-bp long; -299 to -256)] responsible for transcriptional activation of PS-IAA4/5 by IAA (Ballas et al., 1995). Domain A contains a highly conserved sequence 5'-TGTCCCAT-3' found among various IAA inducible genes (PS-IAA6: Oeller et al., 1993; GmAux22 and GmAux28: Ainley et al., 1988; AtAux2-11 and AtAux2-28: Conner et al., 1990; rolB and rolC: Slightom et al., 1986; GH3: Hagen et al., 1991; Ach5: Körber et al., 1991; SAUR gene family: McClure et al., 1989; OS-ACS1: Zarembinski and Theologis, 1993) and behaves as the major auxin responsible element. Domain B functions as an enhancer element which may also contain a less efficient auxin-responsible element. The two domains act cooperatively to stimulate transcription. The two functional modules (domain B and domain A) and a PS-IAA4/5 core promoter were fused to GUS gene (PS-IAA4/5/GUS) and introduced to A. thaliana ecotype Columbia (Oono and Theologis, unpublished).

Expression of PS-IAA4/5/GUS reporter gene in msg1 hypocotyl is weaker than wild-type hypocotyl, however, small increase of the staining upon application of IAA is
observed in msgl hypocotyl (Figs. 28 and 29). This indicates that MSG1 gene is located upstream of PS-IAA4/5 gene in an auxin signal transduction pathway. Furthermore, MSG1 gene probably plays a role in enhancement of the expression of PS-IAA4/5. Thus, the MSG1 gene product could be a candidate for the preexisting components for the transcriptional activation. Expression of PS-IAA4/5 gene in msgl root is normal (Fig. 27). This is consistent with organ specificity of msgl, again.

Auxin-induced gene expression of Arabidopsis SAUR (SAUR-AC1; Gil et al., 1994) is reduced in seedlings of axr1 (Timpte et al., 1994), axr2 (Gil et al., 1994) and aux1 (Gil et al., 1994; Timpte et al., 1994). Steady-state mRNA levels of IAA's are severely reduced in auxin-treated axr1-12 seedlings; the reduction rate ranges from three folds for IAA12 and IAA14 to 15 folds for IAA7 (Abel et al., 1995b). However, auxin inducibility of the IAA mRNA accumulation in axr1-12 appears not to be affected. In axr2-1 plants, auxin inducibility of IAA mRNA is either unaffected (IAA2, IAA9, IAA11 and IAA13), reduced (IAA1, IAA4 and IAA14) or abolished (IAA3, IAA5, IAA6, IAA7, and IAA12). ACS4 encodes an ACC synthase of Arabidopsis and ACS4 is specifically induced by IAA in etiolated seedlings. Relative to the expression in mock-treated and auxin-treated wild-type tissue, steady-state ACS4 mRNA levels are
severely reduced (greater than 10-fold) in respectively treated axr1-12 and axr2 seedlings; however, significant induction of ACS4 mRNA is still observed in axr1 but abolished in axr2 (Abel et al., 1995a). Therefore, steady-state level of most of the auxin-inducible mRNAs were affected by axr1 mutation but their auxin inducibility does not change. In contrast, auxin inducibility of IAA3, 5, 6, 7 and 12 (Abel et al., 1995b) and SAUR-AC1 (Gil et al., 1994) is abolished in axr2. Although expression of PS-IAA4/5 is not examined in axr1, control of PS-IAA4/5 expression by auxin in msg1 is similar to that of axr1. These results suggest that these auxin-insensitive mutations function as a component of auxin signal transduction pathways, regulating gene expression of the early auxin-inducible genes.

9. Conclusion

The results obtained in this study are summarized in Figure 36. Reported data concerning auxin-resistant mutants in root are also included for comparison. Hypocotyl of msg1, jrk197 and axr1 performs an aberrant growth curvature upon unilateral application of auxin, but their gravitropic responses are not the same. Thus, the
three corresponding genes must locate somewhere in an auxin signal transduction cascade (Fig. 36B), but their relationship each other is not clear right now. Quantitative examination of gravitropic responses in double mutants of them will reveal it rather soon. Since hypocotyl of jrk48 performs a growth curvature by auxin, the corresponding gene may encode a sensor or transducer of gravitropism (Kaufman, 1995). The signalling pathway of auxin-induced growth inhibition (Fig. 36C) may be different from that of auxin-induced growth curvature since an analysis of msg1 axrl double mutants indicates that msg1 and axrl act independently each other.

Defects of msg1 are restricted in hypocotyl and leaf. Growth of msg1 hypocotyl is resistant to auxin but sensitive to ethylene. The present study shows the existence of an auxin-insensitive mutant specific to hypocotyl and leaf, and not cross-resistant to ethylene. These results indicate the presence of organ-specific and auxin-specific auxin signalling pathway(s). By characterizing more auxin-specific mutants in each organ, molecular mechanism of the auxin signal transduction will be disclosed near future.
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Table 1. Genetic analysis of *msgl* mutants

<table>
<thead>
<tr>
<th>Cross</th>
<th>Generation</th>
<th>Number of plants</th>
<th>$\chi^2$&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>$msgl$-1 x $msgl$-2</td>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>$msgl$-2 x $msgl$-3</td>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>$msgl$-3 x $msgl$-1</td>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>$msgl$-2 x <em>axr</em>1-3</td>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>$msgl$-2 x <em>aux</em>1-7</td>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>0</td>
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<td>$msgl$-3 x wild type</td>
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<td>25</td>
<td>0</td>
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<tr>
<td>$msgl$-3 x wild type</td>
<td>F&lt;sub&gt;2&lt;/sub&gt;</td>
<td>505</td>
<td>164</td>
</tr>
</tbody>
</table>

<sup>a</sup> $\chi^2$ was calculated based on an expected ratio of 3 sensitive to 1 insensitive.

<sup>b</sup> Growth curvature was observed with a hypocotyl curvature test using IAA-containing lanolin.

<sup>c</sup> No growth curvature was observed with a hypocotyl curvature test.

<sup>d</sup> $P>0.7$. 
Table 2. Size of *msgl* mutants

<table>
<thead>
<tr>
<th>Organ</th>
<th>Length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type</td>
</tr>
<tr>
<td><strong>Root</strong></td>
<td></td>
</tr>
<tr>
<td>in darkness&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.7 ± 1.7</td>
</tr>
<tr>
<td>under white light&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.2 ± 2.5</td>
</tr>
<tr>
<td><strong>Hypocotyl in the dark</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.7 ± 3.6</td>
</tr>
<tr>
<td><strong>Inflorescence stem</strong>&lt;sup&gt;d&lt;/sup&gt;</td>
<td>283 ± 28</td>
</tr>
</tbody>
</table>

<sup>a</sup> Grown on vertically-held plates for 4 days in the dark. Each value represents the mean ± SD of at least 35 plants.

<sup>b</sup> Grown on vertically-held plates for 7 days under continuous white light. Each value represents the mean ± SD of at least 11 plants.

<sup>c</sup> Grown on vertically-held plates for 7 days in the dark. Each value represents the mean ± SD of at least 14 plants.

<sup>d</sup> The lengths were measured after the plants had withered to death. Each value represents the mean ± SD of at least 33 plants.
Table 3. List of the *Arabidopsis* mutants that showed IAA-induced hypocotyl curvature

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phytohormone mutants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aux1</td>
<td>Auxin resistant</td>
<td>Maher and Martindale, 1980</td>
</tr>
<tr>
<td>etr1</td>
<td>Ethylene resistant</td>
<td>Bleecker et al., 1988</td>
</tr>
<tr>
<td>eto1</td>
<td>Ethylene overproducer</td>
<td>Guzman and Ecker, 1990</td>
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<tr>
<td>hls1</td>
<td>Hookless</td>
<td>Guzman and Ecker, 1990</td>
</tr>
<tr>
<td>ga2</td>
<td>Gibberellin sensitive</td>
<td>Koornneef and van der Veen, 1980</td>
</tr>
<tr>
<td>ga3</td>
<td>Gibberellin sensitive</td>
<td>Koornneef and van der Veen, 1980</td>
</tr>
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<td>Gibberellin sensitive</td>
<td>Koornneef and van der Veen, 1980</td>
</tr>
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<td>gai</td>
<td>Gibberellin insensitive</td>
<td>Koornneef et al., 1985</td>
</tr>
<tr>
<td><strong>Long hypocotyl mutants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hy1</td>
<td>Long hypocotyl under white light</td>
<td>Koornneef et al., 1980</td>
</tr>
<tr>
<td>hy3 (phyB)</td>
<td>Long hypocotyl under white light</td>
<td>Koornneef et al., 1980</td>
</tr>
<tr>
<td>hy5</td>
<td>Long hypocotyl under white light</td>
<td>Koornneef et al., 1980</td>
</tr>
<tr>
<td>hy6</td>
<td>Long hypocotyl under white light</td>
<td>Chory et al., 1989</td>
</tr>
<tr>
<td>hy8 (phyA)</td>
<td>Long hypocotyl under far red light</td>
<td>Kurata and Yamamoto, 1997</td>
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<tr>
<td><strong>Gravitropism mutants</strong></td>
<td></td>
<td></td>
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<tr>
<td>sgr3</td>
<td>Shoot gravitropism defective</td>
<td>Fukaki et al., 1996a</td>
</tr>
<tr>
<td>sgr4</td>
<td>Shoot gravitropism defective</td>
<td>Fukaki et al., 1996b</td>
</tr>
<tr>
<td><strong>Small mutants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vi</td>
<td>Compact plants</td>
<td>CS3196&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>dw</td>
<td>Dwarf</td>
<td>CS232&lt;sup&gt;b&lt;/sup&gt; and CS233&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Hyponastic leaf mutants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>foliomut</td>
<td>Leaf margin rolled up and dwarf + or not ++</td>
<td>N330&lt;sup&gt;c&lt;/sup&gt;, N443&lt;sup&gt;++c&lt;/sup&gt;</td>
</tr>
<tr>
<td>rosetomut</td>
<td>Leaf margin rolled up and dwarf +++ or extreme dwarf *</td>
<td>N345&lt;sup&gt;+++c&lt;/sup&gt;, N442&lt;sup&gt;***d&lt;/sup&gt;</td>
</tr>
<tr>
<td>rosflosmut</td>
<td>Leaf margin rolled up and semi-dwarf</td>
<td>N346&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>folflosmut</td>
<td>Leaf margin rolled up and dwarf *** or extreme dwarf ***</td>
<td>N350&lt;sup&gt;***c&lt;/sup&gt;, N419&lt;sup&gt;***d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>foliomut</td>
<td>Main flowering stem short with secondary stems much longer</td>
<td>N307&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Isolated by G.P. Redei and deposited to Arabidopsis Biological Resource Center, Ohio State University (Columbus, OH). Stock number is indicated.

<sup>b</sup>Isolated by I. Vizir and deposited to Arabidopsis Biological Resource Center, Ohio State University (Columbus, OH). Stock number is indicated in above.

<sup>c</sup>Isolated by G. Robbelen and deposited to Nottingham Arabidopsis Stock Center, University of Nottingham (Nottingham, UK). Stock number is indicated.

<sup>d</sup>Isolated by A.R. Kranz and deposited to Nottingham Arabidopsis Stock Center, University of Nottingham (Nottingham, UK). Stock number is indicated.
<table>
<thead>
<tr>
<th>Double mutant</th>
<th>Testcross (^a)</th>
<th>Hypocotyl curvature</th>
<th>Growth inhibition of root</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitive(^b)</td>
<td>Insensitive(^c)</td>
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<tr>
<td>WT</td>
<td></td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>msg1-2</td>
<td></td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>aux1-7</td>
<td></td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>axr1-3</td>
<td></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>msg1-2 axr1-3</td>
<td></td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>msg1-2 aux1-7</td>
<td>(\times) WT</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>msg1-2 aux1-7</td>
<td>(\times) aux1-7</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>msg1-2 aux1-7</td>
<td>(\times) msg1-2</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>msg1-2 aux1-7</td>
<td>(\times) aux1-7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>msg1-2 aux1-7</td>
<td>(\times) msg1-2</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>msg1-2 aux1-7</td>
<td>(\times) aux1-7</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>msg1-2 aux1-7</td>
<td>(\times) msg1-2</td>
<td>0</td>
<td>15</td>
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</tbody>
</table>

\(^a\) All testcrosses were performed in F\(_1\) progeny.

\(^b\) Growth curvature was observed with a hypocotyl curvature test.

\(^c\) No growth curvature was observed with a hypocotyl curvature test.
Table 5. Organ specificity of gravitropism mutants in *Arabidopsis*

<table>
<thead>
<tr>
<th>Locus</th>
<th>Inflorescence stem</th>
<th>Hypocotyl</th>
<th>Root</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>msgI</td>
<td>+</td>
<td>- b</td>
<td>+</td>
<td>This study</td>
</tr>
<tr>
<td>jrk197</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>This study</td>
</tr>
<tr>
<td>jrk48</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>This study</td>
</tr>
</tbody>
</table>

**Type I**

- sgrI  | +                  | +         | Fukaki et al., 1996a
- sgr2  | +                  | +         | Fukaki et al., 1996a
- sgr4  | +                  | +         | Fukaki et al., 1996b

**Type II**

- sgr3  | -                  | +         | Fukaki et al., 1996a
- sgr5  | -                  | +         | Fukaki et al., 1996b
- sgr6  | -                  | +         | Fukaki et al., 1996b

**Type III**

- pgm   | -                  | -         | -    | Casper and Pickard, 1989
- axr2  | -                  | -         | -    | Kiss et al., 1989

**Type IV**

- cop4  | +                  | -         | -    | Hou et al., 1993
- dwf4  | ?                  | -         | -    | Mirza et al., 1984

**Type V**

- agr   | +                  | +         | -    | Bell and Maher, 1990
- auxI  | +                  | +         | -    | Mirza et al., 1984
- axrI  | +                  | +         | -    | Lincoln et al., 1990
- axr4/rgrI | +      | +         | -    | Hobbie and Estelle, 1995
- eirI  | +                  | +         | -    | Roman et al., 1995

*a* +, normal gravitropism.

*b* -, reduced or no gravitropism.

*c* ?, not determined.

*d* The assignment is tentative due to the absence of gravitropic data of inflorescence stem.
Table 6. Organ specificity of auxin resistance and sensitivity and gravitropism of auxin-insensitive mutants in *Arabidopsis*.

<table>
<thead>
<tr>
<th></th>
<th>Root</th>
<th>Hypocotyl</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Auxin resistance</td>
<td>Gravitropism</td>
<td>Auxin resistance</td>
</tr>
<tr>
<td><strong>WT</strong></td>
<td>+*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>msg1</strong></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>aux1</strong></td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>aux1</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>axr2</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>axr4/rgr1</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>dwf</strong></td>
<td>-</td>
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</table>

* The wild-type phenotype is shown as "+"; phenotypes different from wild type are shown as "-".
Figure 1. IAA-induced growth curvature of wild-type hypocotyl. About 60 nl of lanolin containing 0 μM (top left), 1 μM (top right), 10 μM (middle left), 100 μM (middle right), 500 μM (bottom left), and 1 mM (bottom right) IAA, was applied to the left side of hypocotyls of wild-type plants grown under dim white light for 24 h after germination. The seedlings were then incubated under dim red light for 24 h.
Figure 2. IAA-induced growth curvature of hypocotyls of wild type (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 3. Dose-response curves of hypocotyl growth curvature test using IAA-containing lanolin in wild type (O), 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 mean ± SD of 8 seedlings.
Figure 4. Genetic map location of msg1 on Arabidopsis chromosome 5. Single sequence length polymorphisms (SSLPs) markers on chromosome 5 (Bell and Ecker, 1994) were evaluated for linkage. The order of 7 markers and msg1 on chromosome 5 was determined by MapMaker (Lander et al., 1987).
Figure 5. Rosettes of wild type, msgl-1, msgl-2, and msgl-3 plants, grown for 36 days under continuous white light at 23 to 26°C.
Figure 6. Morphology of F₁ progeny between msg1-2 and msg1-3 plants. Plants were grown at 23°C for 5 weeks under continuous light.
Figure 7. Time course of hypocotyl growth of wild type (O), msg1-2(□), and msg1-3(△). Hypocotyl length of seedlings grown in the dark on vertical plates at 22°C was measured. Each value represents the mean ± SD of at least 14 seedlings.
Figure 8. Seedlings of wild type and msg1-2 grown on vertically-held plates for 4 days in the dark.
Figure 9. Effects of 2,4-D on hypocotyl elongation of wild type (○), msgl-2 (□), msgl-3 (■), auxl-7 (●), and axrl-12 (△). The plants were grown for 5 days 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 msgl-2, 14.6 ± 0.8 mm for msgl-3, 11.8 ± 0.6 mm for auxl-7, and 14.6 ± 0.8 mm for axrl-12.
Figure 10. Effects of 2,4-D on root elongation of wild type (○), msg1-1 (▲), msg1-2 (□), msg1-3 (■), auxl-7 (●), and axrl-12 (△). The plants were grown for 3 days 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, 32.0 ± 2.2 mm for msg1-1, 23.6 ± 1.8 mm for msg1-2, 21.4 ± 2.9 mm for msg1-3, 17.6 ± 3.1 mm for auxl-7, and 28.2 ± 4.0 mm for axrl-12.
Figure 11. Effects of IAA on hypocotyl elongation of wild type (○), msg1-2 (□), msg1-3 (■), aux1-7(●) axr1-12 (△), and etr1-3 (▲). The plants were grown for 5 days in the presence of IAA at 22°C in the dark after germination was induced in the absence of IAA. Elongation is expressed relative to the mean hypocotyl elongation of the same genotype on medium without IAA. Each value represents the mean ± SD of at least 13 plants. Mean values for 100% hypocotyl length were 12.2 ± 1.0 mm for wild type, 14.4 ± 1.2 mm for msg1-2, 13.5 ± 0.9 mm for msg1-3, 11.0 ± 0.7 mm for aux1-7, 14.3 ± 2.0 mm for axr1-12, and 15.7 ± 1.1 mm for etr1-3.
Figure 12. Effects of ACC on hypocotyl elongation of wild type (O), msgl-2 (□), msgl-3 (■), auxl-7 (●) axrl-12 (△), and etrl-3 (▲). The plants were grown for 5 days in the presence of ACC at 22°C in the dark after germination was induced in the absence of ACC. Elongation is expressed relative to the mean hypocotyl elongation of the same genotype on medium without ACC. Each value represents the mean ± SD of at least 13 plants. Mean values for 100% hypocotyl length were 12.0 ± 0.8 mm for wild type, 14.3 ± 1.1 mm for msgl-2, 12.9 ± 1.2 mm for msgl-3, 11.1 ± 0.8 mm for auxl-7, 11.6 ± 0.9 mm for axrl-12, and 17.9 ± 1.9 mm for etrl-3.
Figure 13. Effects of 10 µM AVG on 2,4-D-induced inhibition of hypocotyl elongation of wild type (circle), msgl-2 without AVG (square), and msgl-3 (triangle). The plants were grown for 5 days in the presence of 2,4-D and AVG (closed symbol) or 2,4-D only (open symbol) at 22°C in the dark after germination was induced in the absence of AVG and 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 13 plants. Mean values for 100% hypocotyl length were 10.1 ± 0.8 mm for wild type without AVG, 12.3 ± 0.7 mm for msgl-2 without AVG, 10.0 ± 0.9 mm for msgl-3 without AVG, 10.4 ± 1.0 mm for wild type with AVG, 11.4 ± 0.7 mm for msgl-2 with AVG, and 10.7 ± 0.7 mm for msgl-3 with AVG.
Figure 14. Effects of 2,4-D in a growth medium on growth of wild type (A) and msg1-3 (B) under continuous light at 22°C. Seeds were germinated on 2,4-D-free medium for 4 days and then transferred to 2,4-D-containing agar medium. They were grown for 21 days thereafter.
Figure 15. Effects of 2,4-D in agar medium on chlorophyll contents in rosette leaves of wild type (○), msgl-2 (□), msgl-3 (■), auxl-7 (●), and axrl-12 (△). Seeds were germinated on 2,4-D-free agar medium for 4 days, and then transferred to the same media containing 2,4-D. They were grown at 22°C under continuous light for 26 days thereafter. Values represent the mean percent chlorophyll retained in rosette leaves relative to the chlorophyll contents of the same genotype on medium without 2,4-D. Each value represents the mean ± SD of 5 plants. Mean values for 100% chlorophyll were 2.61 ± 0.10 mg/g for wild type, 2.50 ± 0.13 mg/g for msgl-2, 2.24 ± 0.28 mg/g for msgl-3, 2.32 ± 0.09 mg/g for auxl-7, and 2.48 ± 0.17 mg/g for axrl-12.
Figure 16. Fusicoccin (FC)-induced growth curvature of hypocotyl. Lanolin containing FC was applied to the left side of hypocotyls of wild type (WT), msgl-1, msgl-2, and msgl-3 grown under dim white light for 24 h after germination. The seedlings were then incubated under dim red light for 24 h.
Figure 17. Reorientation of hypocotyl and root of wild type (WT), msgl-1, msgl-2, msgl-3, auxl-7, and axrl-3 in response to gravity. Seedlings grown on a vertically-held plate for 4 days in the dark were turned 90°. The photograph was taken after 24 h.
Figure 18. Hypocotyl reorientation in response to gravity. Seedlings of wild type (O) and msg1-2 (□) grown on vertically-held plates for 4 days in the dark were turned 90° to a horizontal position and the orientation of the hypocotyl measured at the indicated time thereafter; 90° represents complete reorientation upward. Values shown represent the mean ± SD of 8 seedlings.
Figure 19. Reorientation of an inflorescence stem of wild type (WT), msgl-2 and jrk48 in response to gravity. An inflorescence stem was cut out from a 5-week-old plant, and stuck into grass wool wetted with one thousand-fold diluted solution of Hyponex. The stem was placed horizontally for 24 h at 23°C in the dark.
Figure 20. Frequency distribution histogram for hypocotyl growth orientation of wild type and msg1-2 grown on horizontally-placed plates. Seedlings were grown in a row for 36 h under dim white light after germination. A photograph of the seedlings was taken from the direction perpendicular to the row of the seedlings. The orientation of the hypocotyl growth was measured on the photograph. 0° represents complete orientation upward. Mean curvature (X), standard deviation (SD), and total number of individuals measured (N) are given.
Figure 21. Frequency distribution histogram for hypocotyl growth orientation of wild type and msg1-2 grown on vertically-placed plates. Seedlings were grown for 36 h under dim white light (0.8 W m\(^{-2}\)) after germination. Orientation of the hypocotyl was measured at its base. 0° represents complete orientation upward. Mean curvature (X), standard deviation (SD), and total number of individuals measured (N) are given.
Figure 22. IAA-induced growth curvature of hypocotyl of tomato wild type (top) and dgt (middle) grown under continuous white light for 12 days after germination. Lanolin containing IAA was applied to the left side of the hypocotyls. The seedlings were then incubated under white light for 24 h.
Figure 23. Effects of IAA in culture medium on hypocotyl and root elongation of wild type tomato (○) and dgt (□). The plants were grown for 12 days in the presence of IAA at 25°C in the dark after germination was induced in the absence of IAA. Elongation is expressed relative to the mean hypocotyl elongation of the same genotype on medium without IAA. Each value represents the mean ± SD of at least 5 plants. Mean values for 100% hypocotyl length were 160 ± 16 mm for wild type and 92 ± 16 mm for dgt. Mean values for 100% root length were 37 ± 7 mm for wild type and 24 ± 4 mm for dgt.
Figure 24. Effects of 2,4-D on hypocotyl elongation of wild type (○), msgl-2 (□), auxl-7(■), axr1-3 (△), msgl-2 auxl-7 (●), and msgl-2 axr1-3 (▲). The plants were grown for 5 days in the presence of 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 14 plants. Mean values for 100% hypocotyl length were 12.5 ± 0.8 mm for wild type, 15.6 ± 1.0 mm for msgl-2, 11.8 ± 0.6 mm for auxl-7, 13.3 ± 1.1 mm for axr1-12, 12.3 ± 1.4 mm for msgl-2 auxl-7, and 13.9 ± 0.7 mm for msgl-2 axr1-3.
Figure 25. Morphology of wild type (WT), msgl-2, axrl-3, auxl-7, msgl-2 axrl-3, and msgl-2 auxl-7. Plants were grown at 23°C for 4 weeks under continuous light.
Figure 26. Promoter activity of PS-IAA4/5 in seedlings of wild type and msg1. The PS-IAA4/5-GUS seedlings were grown for 5 days in the dark and submerged in MS medium for 5 h (right). Control samples were not subjected to the submergence (left). The seedlings were stained with 5-bromo-4-chloro-β-D-glucuronide.
Figure 27. Promoter activity and its responsiveness to exogenous auxin of PS-IAA4/5 in a root tip of wild type and msg1. The PS-IAA4/5-GUS seedlings were grown for 5 days in the dark, and then submerged for 5 h in MS medium containing 0.1 μM IAA. They were subsequently stained for GUS activity.
Figure 28. Promoter activity and its responsiveness to exogenous auxin of PS-IAA4/5 in seedlings of wild type and msg1. The PS-IAA4/5-GUS seedlings were grown as described in Figure 27.
Figure 29. Promoter activity and its responsiveness to exogenous auxin of PS-IAA4/5 in middle parts of hypocotyl of wild type and msg1. The PS-IAA4/5-GUS seedlings was grown as described in Figure 27.
Figure 30. Quantitative GUS assays in seedlings containing the PS-IAA4/5 promoter-GUS fusion. The plants were grown in petri dishes for 5 days at 22°C in the dark after germination. Half-strength MS solution containing IAA was then poured into the dishes until the plants were soaked in the solution completely. They were left for 5 h in the dark thereafter.
Figure 31. Hypocotyl reorientation of wild type (WT), \textit{jrk48} and \textit{jrk197} in response to gravity. Seedlings grown on a vertically-held plate for 4 days in the dark were turned 90° (direction of gravity is indicated on the right). The photograph was taken after 24 h.
Figure 32. Dose-response curves of hypocotyl growth curvature test using IAA-containing lanolin in wild type (○), jrk197 (□), and jrk48 (△). The curvature test was carried out after seedlings were grown under dim white light for 36 h after germination. Values shown represent the mean ± SD of 6 seedlings.
Figure 33. Morphology of wild type, *jrk48*, and *jrk197* plants. Wild type and *jrk197* plants were grown at 23°C for 6 weeks under continuous light. *jrk48* plants were grown for 5 weeks. Note that all the plant pots used were the same in size (11 cm in diameter).
Figure 34. Frequency distribution histogram for negative gravitropism of hypocotyl grown on vertically-placed plates. Seedlings of wild type, *jrk48* and *jrk197* were grown for three days in the dark after germination. Orientation of the hypocotyl was measured at its base. 0° represents complete orientation upward. Mean curvature (X), standard deviation (SD), and total number of individuals measured (N) are given.
Figure 35. Reorientation of an inflorescence stem of *jrkl97* in response to gravity. Six-week-old plants in a pot were turned horizontally and left for 1 day at 23°C in the dark.
Figure 36. Putative auxin signal transduction cascades in different organs.  

**A. Leaf:** Auxin $\rightarrow$ [MSG1, AXR2, AXR1] $\rightarrow$ Chlorosis

- Auxin $\rightarrow$ AXR1 $\rightarrow$ Growth promotion
- Growth curvature

**B. Hypocotyl:** Gravi-stimulation $\rightarrow$ JRK48 $\rightarrow$ Auxin $\rightarrow$ [MSG1, JRK197] $\rightarrow$ Growth inhibition
- Growth curvature

**C. Hypocotyl:** Auxin $\rightarrow$ MSG1 $\rightarrow$ DWF, AXR4/RGR1, AXR2 $\rightarrow$ Growth inhibition
- Ethylene $\rightarrow$ AXR1 $\rightarrow$ AXR1

- Ethylene

**D. Root:** Gravi-stimulation $\rightarrow$ Auxin $\rightarrow$ [AUX1, AXR4/RGR1, AXR2, DWF] $\rightarrow$ Growth inhibition
- Growth curvature

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*Epistatic relationship of these genes is not known.*  
*This study (data not shown).*  
*AXR1 may be located in either position. Both positions could be influenced by AXR1 (Lincoln and Estelle, 1991).*  
*These genes are located in the same signal transduction pathway, but their relative positions have not been determined (Hobbie and Estelle, 1995; Timpte et al., 1995).*  
*Sensitivity to ethylene is not determined.*