The SAR1 gene of Arabidopsis acts downstream of the AXR1 gene in auxin response

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SUMMARY

A screen for suppressors of the auxin resistant mutant axr1 in Arabidopsis thaliana has identified at least three second site suppressor loci called Suppressor of Auxin Resistance (SAR). In this study we focus on the SAR1 gene. Previous studies have documented the effects of the axr1 mutations on auxin-inhibition of root growth, auxin-induced gene expression, seedling morphology and aerial morphology. In this study, we show that the axr1 mutations also affect root hair development and epidermal cell length. The sar1-1 mutation suppresses at least partially, every aspect of the axr1 phenotype. Genetic experiments indicate that this suppression is gene specific. When crossed with the auxin-resistant mutant aux1-7*, the suppressor has little affect on auxin response. However, the morphology of sar1-1 aux1-7 inflorescences is different from either of the single mutants indicating that both genes play a role in auxin mediated development of the inflorescence. The sar1-1 mutation also affects morphology in an AXR1 background. sar1-1 plants are shorter than wild-type, have altered leaf morphology, flower earlier than wild-type plants and appear to have reduced cell division in the primary root. In most respects sar1-1 axr1 and sar1 AXR1 plants are indistinguishable, indicating that sar1 both suppresses and is epistatic to axr1. Based on these results, we propose that SAR1 acts after AXR1 and that a major function of AXR1 is to relieve SAR1 mediated repression of auxin response.

Key words: Arabidopsis, auxin response, SAR1, plant growth

INTRODUCTION

Plant growth and development requires the integration of signals from diverse environmental factors such as water and nutrients, temperature, and light. This integration is accomplished, in part, through the action of a group of compounds called the plant hormones. Auxin, one of the most extensively studied hormones, has been shown to regulate cell division, cell elongation, and cell differentiation (Evans et al., 1984). There is also evidence supporting a role for auxin in the establishment and maintenance of apical dominance, the regulation of root hair formation and lateral root development (Wightman and Thimann, 1980; Klees and Estelle, 1991; Masucci and Schiefelbein, 1996; King et al., 1995; Celenza et al., 1995). Despite a long history of study, the mechanism(s) of auxin action has yet to be elucidated.

One approach that has been used to gain insight into the physiological action of auxin has been the isolation and characterization of mutant plants defective in auxin response (Hobbie and Estelle, 1994 for review). In Arabidopsis, five auxin response loci (AXR1, AXR2, AXR3, AXR4, AUX1) have been identified by screening for mutants that are resistant to the growth inhibiting effects of the endogenous auxin, indole acetic acid (IAA) or the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D; Maher and Martindale, 1980; Lincoln et al., 1990; Wilson et al., 1990; Hobbie and Estelle, 1994; Leyser et al., 1996). To date, two of these genes, AUX1 and AXR1 have been characterized at the molecular level. The AUX1 gene encodes a putative integral membrane protein with similarity to a family of amino acid permeases (Bennett et al., 1996). These authors suggest that AUX1 functions in auxin transport.

The axr1 mutants have been extensively characterized (Lincoln et al., 1990; Timpte et al., 1995) and are defective in a variety of auxin-regulated processes. Plants homozygous for a severe allele, such as axr1-12, display reduced hypocotyl elongation in the dark, lack of apical hook formation, a reduction in apical dominance, and a reduction in auxin-induced callus production (Lincoln and Estelle, 1991; Lincoln et al., 1990). The roots of axr1 plants elongate faster than wild-type roots but produce fewer lateral branches (Lincoln et al., 1990 and Hobbie and Estelle, 1995). At the molecular level, the axr1 mutants are defective in the regulation of several families of early auxin-response genes suggesting that AXR1 is required for rapid auxin responses (Timpte et al., 1995; Abel et al., 1995). The AXR1 gene was cloned and shown to encode a protein related to the ubiquitin-activating enzyme (E1), a highly conserved enzyme that functions in the ubiquitin conjugation pathway (Leyser et al., 1993). Although related to E1, AXR1 is approximately half the size of E1 and lacks a cysteine residue known to be essential for conventional E1 activity. Recently, genetic studies as well as genome sequencing projects have shown that AXR1 is the first member of a protein...
family with representatives in diverse eukaryotes including Saccharomyces cerevisiae (Lammer and Estelle, unpublished), Caenorhabditis elegans, (Wilson et al., 1994) and humans (Chow et al., 1996; S. Handeli, FHCRC, pers. comm.). We speculate that AXR1-like proteins are present in all eukaryotes.

The biochemical function of AXR1 and the role of the protein in auxin response remains uncertain. Recently, we have found that deletion of a yeast AXR1-like gene called ENR2 (E1 N-terminus Related) produces synthetic lethality in combination with the cdc34-2 mutation (Lammer and Estelle, unpublished). The CDC34 gene encodes a ubiquitin-conjugating enzyme with a key role in cell cycle regulation (Goebel et al., 1988). This genetic interaction strongly suggests that Enr2p and the other AXR1-like proteins function in ubiquitin-mediated processes. In animal systems, members of the AXR1 family have been implicated in diverse aspects of cellular regulation. Chow et al. have recently shown that a human AXR1-like protein (69% similar and 39% identical to AXR1), called APP-BP1, interacts with the cytoplasmic tail of the amyloid precursor protein (APP). APP is an integral membrane protein that is the precursor of α-amyloid, a short peptide that accumulates in patients with Alzheimer disease. The normal function of APP is unclear, but the protein has some of the qualities of an integrin and may function as a receptor. Chow et al. have proposed that APP-BP1 acts to mediate APP signaling (Chow et al., 1996).

In another study, Handeli and Weintrab (pers. comm.) have found that a protein nearly identical to APP-BP1 is mutated in a hamster mutant cell line called ts41. The ts41 cells have a complex cell cycle arrest phenotype which includes successive S phases without intervening mitoses (Handeli and Weintrab, 1992). This suggests that ts41 (APP-BP1) functions in cell cycle regulation. These findings hint at possible roles for AXR1 in cell growth regulation and signal transduction, but do not directly address how AXR1 functions in the cell to regulate auxin mediated growth and development.

In order to better understand how AXR1 functions in auxin regulation of growth, we have undertaken a screen for suppressors of the axr1 mutants. Suppressor analysis has been used in a number of systems as a means to further investigate gene function and to define additional interacting genes (For examples see Barbazuk et al., 1994; Maddock et al., 1994; Preat et al., 1993). In Arabidopsis, suppressor screens have been used to identify genes involved in ABA biosynthesis (Koornneef et al., 1982) and gibberellin signal transduction (Jacobsen and Olszewski, 1993; Wilson and Somerville, 1995; We report here the isolation and characterization of a suppressor of axr1.

**MATERIALS AND METHODS**

**Morphometric analysis**

Plants were grown according the methods of Lincoln et al. (1990), except the light level used was between 100 uE/m²/second and 120 uE/m²/second and plants were fertilized on weeks two and four. Seedlings were thinned to between eight and ten plants per pot when the first leaves became visible. Bolting time was defined as the time that the first sign of the inflorescence was observed. Rosette leaf numbers were counted between the opening of the first and fifth flower and it was at this time that plants were weighed. Epidermal peels were conducted for inflorescence epidermal cell length assays by peeling away the epidermis between the first and second lateral branches of the plant, while the plants were still healthy and vigorous (approximately 25 days old). The epidermal cells were measured under 100x magnification by making a wet mount of the peel. 100 cells from five plants were measured. Plant heights were determined after the plants had stopped initiating new flowers. Lateral branches and lateral shoots were counted at the time that plant heights were measured.

Plants used for lateral root assays were grown according to the methods of Hobbie and Estelle (1995), except seedlings were transferred on the fourth day to 15×150 mm Petri plates containing minimal medium and lateral roots counted on the eighth day after transfer.

Seedlings used for hypocotyl measurements were grown according to the methods of Timpte et al. (1992), except the seedlings were grown for 8 days in the dark for hypocotyl measurements and 3 days for apical hook documentation.

Seedlings used for root hair and epidermal cell measurements were sown onto 100×15 mm Petri plates containing minimal medium (Lincoln et al., 1990) at a density of 20-30 seeds per plate. Root hairs were counted or photographed on the fifth day after placement into the growth chamber. Root hairs were counted over a 2 mm section of root located in the approximate middle of the root and photographs taken of the approximate middle at 40x magnification. Root epidermal cells were measured by viewing unstained wet mounts of whole roots under 100x magnification. 100 or more cells, located in the approximate middle of the root, from five individual plants were measured.

Seedlings used for growth rate measurements were grown according to the methods of Lincoln et al. (1990), except root measurements were taken for five days after transfer.

**Physiology**

Hormone assays were done according to the methods of Lincoln et al. (1990) with the following modifications: sterile seeds were sown onto plates containing minimal medium, the seeds were then cold treated for 2 days. The seedlings were allowed to grow on these plates for 4 days before being transferred to plates made up with minimal medium containing the various concentrations of auxin used. After transfer, the position of the root tip was marked on the bottom of the plate. The plates were incubated under the conditions used by Lincoln et al. (1990) for 3 days, after which time the change in root length between day 0 and day 3 was measured.

**SSLP mapping**

Crosses between sar1-1 in a Colombia background and Niedersentz wild type were carried out and F2 plants were scored for the sar1-1 phenotype. F2 seeds were collected from these plants, sown, and F3 tissue collected after 15 days. Genomic DNA was isolated according to the methods of Delaporta et al. (1983) and amplified using the SSLP markers nga248 and nga280 (Bell and Ecker, 1994). PCR conditions and the amounts of DNA and primers used were the same as those used by Bell and Ecker (1994) except that the reaction buffer consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2 and 0.05% Nonidet P-40. 50 F3 families were used for the initial mapping.

**RNA isolation**

1 g of tissue for each concentration of auxin used was harvested from 15-day old rosettes and depleted of endogenous auxin according to the methods of Timpte et al. (1995). The tissue was then exposed to 0 M or 50 M 2,4-D (Timpte et al., 1995). RNA was then extracted, electrophoresed on a formaldehyde gel, blotted and probed according to Timpte et al. (1995). The probe used was the IAA2 gene, provided by A. Theologis (Abel et al., 1995).

**RESULTS**

**Screening for suppressors of axr1**

To better understand the function of the AXR1 gene, a screen for second site suppressors of the axr1-3 mutation was performed.
The basis for this initial screen was the suppression of auxin resistance in the elongating root of axr1-3 (a weak allele of axr1). Approximately 1,000-3,500 M2 seedlings from each of 23 M2 populations were germinated on medium containing 0.2 μM 2,4-D. Each M population was derived from 1,500 M1 EMS mutagenized axr1-3 gl-1 seeds. axr1-3 seedlings display significant root growth on this medium while the roots of wild-type seedlings are completely inhibited. Suppressor candidates with short roots were recovered from the plates and transferred to medium without 2,4-D to allow the seedlings to recover.

A total of eleven suppressor lines were recovered from this initial screen. To determine the genetic basis for suppression, each of the mutants was crossed to homozygous axr1-3 plants. For each cross, the F1 plants were all resistant to 2,4-D resistance in the elongating root of axr1. The basis for this initial screen was the suppression of auxin resistance in the elongating root of axr1-3 (a weak allele of axr1). Approximately 1,000-3,500 M2 seedlings from each of 23 M2 populations were germinated on medium containing 0.2 μM 2,4-D. Each M population was derived from 1,500 M1 EMS mutagenized axr1-3 gl-1 seeds. axr1-3 seedlings display significant root growth on this medium while the roots of wild-type seedlings are completely inhibited. Suppressor candidates with short roots were recovered from the plates and transferred to medium without 2,4-D to allow the seedlings to recover.

The effects of axr1-3 (a weak allele) and axr1-7 (a strong allele) on auxin responses were determined using a series of experiments. These included root elongation on media containing various concentrations of 2,4-D, and the RNA run out on a denaturing gel and blotted. The RNA was probed for auxin induced IAA2 RNA accumulation in axr1-12 plants. Two-week old rosette leaves were depleted of endogenous auxin and exposed to 0 or 50 μM 2,4-D and the RNA run out on a denaturing gel and blotted. The blot was probed with IAA2 DNA. Numbers below each blot represent RNA accumulation in axr1-12 plants. Two-week old rosette leaves were depleted of endogenous auxin and exposed to 0 or 50 μM 2,4-D and the RNA run out on a denaturing gel and blotted. The blot was probed with IAA2 DNA. Numbers below each blot represent RNA level as determined by densitometry, corrected for differences in loading. All values were normalized to wild type without 2,4-D. To compare loading levels, the blot was stripped and reprobed with the 18S rRNA gene. Four of the eleven lines recovered, CLS1, CLS2, CLS4, and CLS5, displayed a characteristic phenotype (see below). To determine if these lines were mutated at the same locus, crosses between the lines were performed. These crosses produced only auxin-sensitive seedlings and the mature plants all had the mutant morphology indicating that these four lines carry allelic mutations. CLS1 and CLS2 were recovered from a single M2 pool and are likely to represent the same mutation. The locus defined by these mutations was named SAR1 (suppressor of axr1). In F2 populations derived from sar1 and axr1-3 parents, the axr1 phenotype always cosegregated with auxin sensitivity indicating that suppression and the morphological effects are caused by the same mutation. Of the three sar1 alleles, we chose to examine sar1-1 (CLS4) in detail because it has the strongest phenotype. The second allele, sar1-2 is similar to sar1-1, while sar1-3 appears to be a weak allele.

The sar1-1 mutation was mapped using an F2 population generated by crossing the original mutant line (ecotype Col-0) to a wild-type Nd-0 line. Segregation of the sar1-1 visible phenotype (see below) as well as several PCR-based markers was scored in an F2 population of 50 plants. The mutation mapped to the centromere region of chromosome one between markers nga280 and nga248.

**Effects of sar1-1 on auxin responses**

(I) Root elongation

The basis for the initial screen for suppressors of axr1 was the suppression of auxin resistance in the elongating axr1-3 root. To further examine the effect of sar1-1 on this aspect of the axr1 phenotype, root elongation on media containing various concentrations of 2,4-D was measured. The sar1-1 mutation suppresses auxin resistance in both axr1-3, a weak allele (data not shown) and axr1-12, a strong allele (Fig. 1A) indicating that suppression is not allele-specific. The concentrations of 2,4-D that cause 50% inhibition of root elongation for wild

![Graph](image-url)

**Fig. 1.** sar1-1 suppresses auxin resistance of axr1 but not aux1-7 plants. (A) The effect of 2,4-D on root elongation in sar1-1 and axr1 plants. (□) wild-type; (●) sar1-1; (○) the double mutant axr1-12 sar1-1; and (△) axr1-12 mutants. Percentage inhibition is relative to plants of the same genotype grown on medium without hormones. (B) The effect of 2,4-D on root elongation in sar1-1 and axr1-7. (□) wild-type; (●) sar1-1; (△) the double mutant aux1-7 sar1-1; and (○) aux1-7. Error bars represent standard error. Each point represents 20-30 seedlings.

![Graph](image-url)

**Fig. 2.** sar1-1 increases the level of auxin induced IAA2 RNA accumulation in axr1-12 plants. Two-week old rosette leaves were depleted of endogenous auxin and exposed to 0 or 50 μM 2,4-D and the RNA run out on a denaturing gel and blotted. The blot was first probed with IAA2 DNA. Numbers below each blot represent RNA level as determined by densitometry, corrected for differences in loading. All values were normalized to wild type without 2,4-D. To compare loading levels, the blot was stripped and reprobed with the 18S rRNA gene.
type, sar1-1, axr1-12 sar1-1 and axr1-12 were 0.025 μM, 0.030 μM, 0.050μM and 0.6μM, respectively. These results indicate that sar1-1 and axr1-12 sar1-1 are similar to wild type in auxin response, while axr1-12 requires twenty-four times the amount of 2,4-D to achieve 50% inhibition of root elongation compared to wild type. Similar results were also obtained using IAA (data not shown).

To determine if the effects of sar1-1 on auxin response are specific to the axr1 mutants, we measured auxin response in sar1 aux1 seedlings. The aux1 mutation confers a level of auxin resistance similar to that of the axr1 mutants. However, genetic studies indicate that the aux1 and axr1 mutants function in distinct pathways (Timpte et al., 1995). The data shown in Fig. 1B indicates that sar1-1 does not increase the auxin response of aux1 seedlings. In fact, there appears to be a slight additive effect in the sar1-1 aux1-7 double mutant. 50% inhibition of root elongation was observed at 0.25 μM 2,4-D and 0.3 μM 2,4-D for aux1-7 and sar1-1 aux1-7 respectively. These findings support the conclusion that sar1-1 is acting as a gene-specific suppressor of auxin resistance. Thus, SAR1 and AXR1 appear to function in the same auxin response pathway, while, consistent with earlier studies (Timpte et al., 1995), AUX1 functions in a separate pathway.

(II) Auxin-regulated gene expression

Previous work has shown that the axr1 mutants are deficient in auxin-regulated expression of the SAUR-AC1 (Small Auxin Up regulated RNA) gene (Timpte et al., 1995) as well as several members of the IAA family of genes (Abel et al., 1995). We examined the effect of sar1-1 on auxin regulated expression of the IAA genes. RNA blot analysis was performed with RNA isolated from 2-week old rosettes. The data in Fig. 2 show that accumulation of IAA2 RNA is dramatically reduced in axr1-12 plants compared to wild type. In axr1-12 sar1-1 plants, the level of IAA2 RNA accumulation is restored. Quantification by densitometry and normalization to 18s rRNA levels indicate that induction of the IAA2 gene is similar in wild-type and axr1-12 sar1-1 plants. These data indicate that sar1-1 acts to suppress the gene expression defect in axr1-12 plants. Similar results were obtained with IAA5.

Effects of sar1-1 on seedling development

(I) Root elongation

The axr1 mutants have an increased rate of root elongation relative to wild-type plants (Lincoln et al., 1990). The effect of sar1-1 on this aspect of the axr1 phenotype was examined and is shown in Fig. 3A. Our results indicate that the sar1-1 mutant has an elongation rate approximately one half that of wild type and one third that of axr1-12 mutants. The double mutant, axr1-12 sar1-1 has an elongation rate similar to that of wild type, indicating that sar1-1 suppresses this aspect of the axr1-12 phenotype. Similar results were obtained for axr1-3 (data not shown). These data could be interpreted as evidence for an additive interaction. However, because axr1-12 and

Fig. 3. The effects of sar1-1 and axr1 on seedling morphology. (A) Root elongation. Root elongation of wild-type (□); sar1-1 (○); axr1-12 sar1-1, (×); axr1-12, (△). Each point represents 10-12 seedlings. Error bars indicate standard error. (B) Root epidermal cell length. Error bars indicate standard error. Cell lengths represent at least 100 epidermal cells from five individual plants. (C) Number of lateral roots per cm of primary root. Error bars indicate standard error. Lateral roots were counted from 10-12 plants. (D) Hypocotyl elongation. Error bars indicate standard error. At least 25 hypocotyls from each genotype were measured. (E-H) Apical hooks of 3-day old dark-grown wild-type (E), axr1-12 (F), axr1-12 sar1-1 (G), sar1-1 (H) plants.
sar1-1 do not interact in an additive fashion with respect to other aspects of their phenotypes, we do not favor this interpretation.

Changes in the rate of root elongation could be due to alterations in cell division and/or cell elongation. It is possible that axr1 is affecting one or both of these processes. To obtain information on the cause of the observed changes in root elongation, root epidermal cell size was determined in plants with both weak and strong alleles of axr1 as well as the corresponding sar1-1 double mutants. Fig. 3B shows that the length of root epidermal cells in sar1-1 and wild-type plants is similar. The epidermal cells of axr1-12 are approximately 25% longer than those of wild type. The cell length of axr1-12 sar1-1 double mutants is similar to that of wild type indicating that sar1-1 restores wild-type cell length to axr1-12 plants. Again, similar results were obtained with the axr1-3 mutant. These data suggest that the enhanced root elongation observed in axr1 plants (Fig. 3A) could be explained by an increase in cell elongation. In contrast to axr1, the decreased length of sar1-1 roots is probably due to a decrease in the rate of cell division. This is supported by the reduced elongation rate of sar1-1 roots and a root epidermal cell size similar to that of wild type.

(II) Lateral root formation

A variety of studies suggest that lateral root formation is an auxin-mediated process (Boerjan et al., 1995; Wightman and Thimann, 1980; Celenza et al., 1995). Consistent with this suggestion, the axr1 mutants are deficient in lateral root formation (Hobbie and Estelle, 1995). To further characterize the interactions between sar1 and axr1, the effects of these mutations on lateral root formation were determined (Fig. 3C). sar1-1 plants have approximately the same number of lateral roots/cm of primary root as wild-type plants while both axr1-12 and axr1-3 plants have fewer lateral roots per cm of primary root than wild type or sar1-1 plants. These data also show that sar1-1 is able to partially suppress the defect in lateral root formation of axr1-12 seedlings, the severe allele, but not of axr1-3, the weak allele.

(III) Hypocotyl elongation and apical hook formation

The axr1-12 mutation reduces the height of dark-grown hypocotyls (Lincoln et al., 1990). The effects of sar1 on hypocotyl height are shown in Fig. 3D. At 8 days sar1-1 hypocotyls are approximately two-thirds the height of wild type while axr1-12 hypocotyls are about one half that of wild-type height. These data also show that axr1-3 is capable of elongating its hypocotyl to wild-type levels. The height of both axr1-12 sar1-1 and axr1-3 sar1-1 hypocotyls are the same as those of sar1-1 indicating that sar1-1 is epistatic to axr1. Previous work (Lehman et al., 1996) has shown that dark-grown axr1-12 seedlings are unable to form an apical hook. Examination of 3-day old dark-grown seedlings show that sar1-1 mutants are able to form apical hooks, while axr1-12 mutants do not, and that sar1 is able to suppress this defect in axr1-12 (Fig. 3E-H).

(IV) Root hair formation in axr1 and sar1-1 seedlings

Root hair formation also appears to be auxin and ethylene regulated (Masucci and Schiefelbein, 1996). In Arabidopsis, root hairs form at the apical end of cells located in specific files of the epidermal cell layer. These files are typically located over the outer periclinal walls of the underlying cortical cells (Dolan et al., 1994). A number of Arabidopsis mutants with defects in root hair development have been isolated (Schiefelbein and Sommerville, 1990; Masucci and Schiefelbein, 1994). One of these, the rhd6 mutant can be rescued by the application of auxin or ACC (the biosynthetic precursor to ethylene) to the growth media. During the course of the studies on root morphology, it became apparent that axr1 plants have a reduced number of root hairs compared to wild-type plants (Fig. 4A-D). To characterize this defect in more detail, visible root hairs in a 2 mm section of wild-type and axr1-12 roots were counted. The results, displayed in Fig. 4F, show that axr1-12 plants have approximately one third the root hairs of wild-type plants. When we examined axr1-12 roots at higher magnification, we observed small bulges on epidermal cells where...
a root hair is expected to elongate (Fig. 4E). The presence of these bulges suggests that axr1-12 plants can initiate root hairs normally, but are deficient in hair elongation. Fig. 4A-D, and F also shows that sar1-1 plants have more root hairs than wild type and that the sar1 mutation acts to increase root hair number of axr1-12 plants to sar1-1 levels. Similar results were obtained for axr1-3 (data not shown).

Effects of sar1 on the mature plant
The original axr1-3 sar1-1 M2 plant had a phenotype distinct from both axr1-3 and wild type. To determine the contribution of sar1 to this phenotype we characterized mature sar1-1 plants in axr1-3, axr1-12, and AXR1 backgrounds. The results of this characterization are shown in Table 1, Figs 5 and 6. Several aspects of our findings are noteworthy. First, the sar1-1 mutation causes a number of changes in growth and development in an AXR1 background. Most striking, homozygous sar1-1 plants flower approximately 10 days earlier than wild type under our growth conditions (Table 1). Since the number of rosette leaves at the time of flowering is reduced in sar1-1 compared to wild type, mutant plants must flower at an earlier developmental stage. In addition, the height of the mutant stems is reduced compared to wild type. Second, in virtually every respect, the appearance of axr1-12 sar1-1 and axr1-3 sar1-1 plants is similar to that of sar1-1 AXR1 (Figs 5 and 6), indicating that sar1-1 is epistatic to axr1. At the same time, this interaction results in the partial or complete suppression of most aspects of the axr1 phenotype. For example, a comparison of axr1-12 and axr1-12 sar1-1 plants reveals that sar1-1 acts to increase the height and internode distance of axr1-12 plants. In addition, sar1-1 suppresses the striking defect in axr1-12 leaf morphology (Fig. 6).

DISCUSSION
The AXR1 gene is required for a variety of auxin responses including auxin-induced cell proliferation (Lincoln and Estelle, 1991), auxin inhibition of root growth (Evans et al., 1994) and auxin-induced gene expression (Timpte et al., 1995; Abel et al., 1995). As a means to gain insight into the function of the AXR1 gene and its role in auxin response, we have isolated 11 recessive suppressor lines of the axr1-3 mutation. Four of the suppressor lines are allelic and define a new locus on chromosome one that we have called suppressor of auxin resistance-1 (SAR1). Our studies show that the sar1 mutations suppress auxin resistance in several axr1 alleles. In an axr1 background, sar1 acts to increase both auxin inhibition of root growth and
Table 1. sar1-1 and axr1 morphometric analysis

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<th>Genotype</th>
<th>WT</th>
<th>axr1-12</th>
<th>axr1-12 sar1-1</th>
<th>sar1-1</th>
<th>axr1-3</th>
<th>axr1-3 sar1-1</th>
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<tr>
<td>Bolting time (days)</td>
<td>33.6±0.67*</td>
<td>32.9±0.61</td>
<td>21.3±0.33</td>
<td>21.8±0.48</td>
<td>32.8±0.39</td>
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<td>Leaf number at bolting</td>
<td>19.4±0.75</td>
<td>21.8±2.09</td>
<td>10.4±0.29</td>
<td>8.4±0.24</td>
<td>19.7±0.6</td>
<td>9.3±0.33</td>
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<td>Weight at bolting (g)</td>
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<td>Height at the end of flowering (cm)</td>
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<td>Siliques per cm</td>
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<td>4.3±0.23</td>
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<td>1.8±0.04</td>
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*± standard error.

auxin induction of gene expression. In contrast, sar1-1 does not suppress auxin resistance conferred by the aux1-7 mutation. These results indicate that sar1 acts in a gene-specific but not allele-specific manner.

Role of the AXR1 and SAR1 genes in cell growth

The phenotypes of both axr1 and sar1 plants indicate that the respective proteins are required for normal cell growth. In both mutants, root, hypocotyl and inflorescence elongation are altered. In addition, both mutations affect leaf morphology. Although the cellular basis for these changes has not been determined, measurement of epidermal cell sizes in the root and inflorescence suggest that changes in both cell division and cell elongation are involved. In axr1 plants, increased root elongation is associated with increased root epidermal cell length. Thus a change in cell elongation may be sufficient to explain the increase in root growth. In contrast, the length of epidermal cells in sar1-1 seedlings is unchanged, suggesting that the decreased rate of root elongation is caused by a reduction in the rate of cell division. The picture is complicated by the fact that in the inflorescence, the length of stem epidermal cells in both sar1-1 and axr1 mutants is reduced (Fig. 5F). Thus both mutations appear to have very different effects in the root and aerial parts of the plant. It is also important to note that cell division and cell elongation are coupled processes and that changes in the dynamics of cell division can have profound affects on cell elongation. We are presently examining cell division rates in axr1 and sar1 root meristems to learn more about the effects of these mutations on cell growth. Another example of a mutation that has disparate effects on related processes in different tissues is ttg (Galway et al., 1994). In this case, mutant plants lack trichomes but have an increased number of root hairs.

Both axr1 and sar1 plants also display defects in root hair morphology and numbers. Root hair development is an orderly and highly regulated process with hair forming epidermal cells being located over the anticlinal walls of two underlying cortical cells. Determination of hair bearing cells and/or growth of the hair, has been shown to be mediated by auxin and ethylene (Dolan et al., 1994; Masucci and Schiefelbein, 1996 and Tanimoto et al., 1995). The results described here provide additional support for a role for auxin in hair morphogenesis. When viewed at high magnification, small protuberances or bumps, which we interpret to be root hairs, are visible at the appropriate position on the trichoblasts (hair forming cells) of axr1 roots. These data indicate that axr1 trichoblasts can initiate root hairs, but the hairs do not elongate properly. In this respect, axr1 resembles the rhd2 mutant (Schiefelbein and Sommerville, 1990). The sar1-1 mutation appears to have the opposite effect on root hair development. Hairs on sar1-1 plants are thicker and tend to have a bulge on the end. Visual comparison of sar1-1 plants to wild type suggest that they have increased expansion with respect to wild type as well as a higher number of ectopically placed hairs. This observation is currently being investigated.

The SAR1 gene and floral evocation

The sar1-1 mutation decreases the time required for flowering by approximately 10 days under continuous light conditions. Early flowering is associated with a reduction in the number of rosette leaves indicating that the floral transition is occurring at an earlier developmental stage in sar1 plants. Because sar1 and axr1 sar1 plants are healthy and robust, early flowering is probably not caused by physiological stress. There have been several reports of auxin effects on flower evocation (Bernier, 1988; King et al., 1996). However, the specific role of auxin in this process is not known. It is important to note that the axr1 mutants flower at the same time as wild-type plants suggesting that auxin is not the primary signal for the floral transition. In contrast, studies by King et al. (1996) show that the rty mutant has high levels of auxin and rarely flowers suggesting that auxin can inhibit transition to flowering. Genetic studies in Arabidopsis have led to the development of a model in which floral transition occurs when the level of a negative regulator falls below a threshold level (Martinez-Zapater et al., 1994; Weigel, 1996; Araki and Komeda, 1993; Sung et al., 1992; Zagotta et al., 1992). It is possible that SAR1 plays a role in the negative regulation of flowering and in the absence of SAR1 protein the floral transition is accelerated. Further experiments, including double mutant analysis with other mutants that affect floral evocation, should provide more information on the role of SAR1 during flowering.

Interaction between sar1-1 and aux1-7

The sar1-1 and aux1-7 mutations have either an additive or synergistic interaction depending on the organ examined. The roots of sar1-1 aux1-7 seedlings are more auxin resistant than either single mutant, suggesting that the two genes function in
different pathways. Curiously, the two mutations interact in a synergistic fashion in the aerial portion of the plant (Fig. 5E). The aux1 mutation has no apparent effect on aerial morphology and neither mutation alone has a significant effect on apical dominance. In combination however, the two mutations result in a dramatic loss of apical dominance. Timpte et al. (1995) found that aux1-7 also modified the aerial phenotype of the axr1-12 mutant, but in that case, aux1-7 acted to partially ameliorate the effects of axr1-12. The axr1 sar1-1 and sar1-1 aux1-7 double mutants suggest that AUX1 also functions in the aerial part of the plant, despite the lack of a mutant phenotype for aux1 plants. Recently, Bennett and colleagues have reported that the AUX1 protein is related to a family of amino acid permeases and suggest that AUX1 may function in auxin uptake (Bennett et al., 1996). Thus it is possible that auxin transport is compromised in the inflorescences of aux1 plants, and in combination with either axr1 or sar1 this defect results in changes in morphology.

The AXR1-SAR1 pathway

A comparison of wild-type, axr1-12 SAR1 and axr1-12 sar1-1 plants reveals that the sar1-1 mutation suppresses (at least partially) every aspect of the axr1-12 phenotype. For example, sar1-1 restores near normal leaf morphology and root hair number to axr1-12 plants. Inflorescence height and internode distance are also increased in axr1-12 sar1-1 plants relative to axr1-12 SAR1. However, sar1-1 does not restore axr1 plants to a wild-type appearance. In an AXR1 background, the sar1-1 mutation affects diverse aspects of plant growth and development ranging from root morphology to flowering time and inflorescence structure. All of these defects are also present in axr1-12 sar1-1 plants. In fact, AXR1 sar1-1 and axr1-12 sar1-1 plants are nearly identical in appearance indicating that sar1 is epistatic to axr1. In a sar1-1 mutant background, the axr1 gene has little affect on phenotype. This finding suggests that a major role of the AXR1 protein is to act upon the SAR1 gene or its product.

As outlined above, our genetic evidence indicates that a major function of AXR1 is to act (directly or indirectly) on either the SAR1 gene or its product. Based on these data we propose that SAR1 is a regulator of cell growth and that AXR1 acts to modify the activity of SAR1. Since AXR1 is likely to have a role in protein degradation, AXR1 may promote the degradation of SAR1 in an auxin-dependent manner. Experiments on the auxin-induced genes suggest one possible model for SAR1 function. Several groups have shown that transcription of both the SAUR and IAA genes is induced by cycloheximide (McClure et al., 1989; Abel et al., 1995). These results have led Theologis and colleagues to propose that transcription of the auxin-regulated genes is normally repressed by a short-lived repressor protein (Ballas et al., 1995). SAR1 may function as such a repressor. In an axr1 background, SAR1 may be high, resulting in a decreased auxin response. Mutations in SAR1 relieve this repression. If this model were correct, one might expect to see constitutive expression of the auxin-regulated genes in a sar1 background. The fact that we do not observe this may be explained by partial gene redundancy. We note that we have identified two additional SAR genes that may encode functionally related proteins. Alternatively, AXR1 and SAR1 may function in auxin-mediated cell cycle regulation. Recent studies in CHO cells indicate that a mutation in an AXR1-related gene called ts41 (also called APP-BP1), results in cell cycle defects (Handeli and Weintraub, 1992; Chow et al., 1996; Handeli, pers. comm). The molecular characterization of the SAR1 gene is likely to provide additional information on the role of AXR1 in auxin action and plant development.

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