Cell, Vol. 85, 183–194, April 19, 1996, Copyright ©1996 by Cell Press

HOOKLESS1, an Ethylene Response Gene, Is Required for Differential Cell Elongation in the Arabidopsis Hypocotyl

Anne Lehman,* Robert Black,† and Joseph R. Ecker*
*Department of Biology
Plant Science Institute
University of Pennsylvania
Philadelphia, Pennsylvania 19104-6018
†Department of Biology
Pennsylvania State University
Media, Pennsylvania 19603

Summary

Bending in plant tissues results from differential cell elongation. We have characterized Arabidopsis "hookless" mutants that are defective in differential growth in the hypocotyl. *HOOKLESS1* was cloned and its predicted protein shows similarity to a diverse group of N-acetyltransferases. *HOOKLESS1* mRNA is increased by treatment with ethylene and decreased in the ethylene-insensitive mutant *ein2*. High level expression of *HOOKLESS1* mRNA results in constitutive hook curvature. The morphology of the *hookless* hypocotyl is phenocopied by inhibitors of auxin transport or by high levels of endogenous or exogenous auxin. Spatial patterns of expression of two immediate early auxin-responsive genes are altered in *hookless1* mutants, suggesting that the ethylene response gene *HOOKLESS1* controls differential cell growth by regulating auxin activity.

Introduction

The process of tissue bending in response to environmental stimuli is important for plant development and survival and represents a fundamental difference in how plants and animals react to environmental change. Unlike animals, which actively move to meet their needs for nutrients and protection, higher plants can only alter their growth patterns in response to a variety of signals. Plants respond to light (phototropism), gravity (gravitropism), and certain stresses by initiating differential growth (Firn and Digby, 1980; reviewed in Smith, 1995). The stress-induced hormone ethylene promotes differential growth in the seedling (Abeles et al. 1992; Ecker, 1995).

The apical hook of the hypocotyl, found in dark grown seedlings, is one example of differential cell growth in plants. During germination, the hook structure acts to protect the cotyledons and delicate meristematic primordia of the apex as they emerge through the soil (Darwin and Darwin, 1896). Kinematic analysis of the hypocotyl revealed that the rate of elongation of apex cells on the outer edge of the hook exceeded that of the inner edge (Silk and Erickson, 1978). Once cells were displaced past the midpoint of the hook, the rate of expansion of cells on the inner surface of the hypocotyl exceeded the outer and caused the hypocotyl to straighten. Thus, during seedling growth, the hypocotyl hook is maintained as a morphological structure while the cells that comprise it are continually replaced. These analyses suggest that coordinated regulation of the rate of cell elongation is required to achieve differential growth and formation of the apical hook.

The plant hormones ethylene and auxin have been implicated as possible regulators of differential growth in the apical hook (Kang et al., 1967; Schwark and Schierle, 1992). Auxin acts to the stimulate cell expansion and hypocotyl elongation while ethylene, whose production can be induced by physical stress during seedling germination, has an antagonistic effect on these processes (Cleland, 1987; Abeles et al., 1992). Moreover, the auxin, indole-3-acetic acid (IAA), and the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), are found unequally distributed in apical hook cells of bean (Schwark and Bopp, 1993). Arabidopsis mutants that are blocked in auxin (auxin resistant) or ethylene (ethylene insensitive) signaling show much reduced differential growth in the apical hook (Roman et al., 1995). Conversely, application of exogenous ethylene, mutations that result in ethylene overproduction (*eto1)*, or constitutive activation of the ethylene response pathway (*ctr1*) cause exaggeration in the curvature of the apical hook (Guzman and Ecker, 1990; Kieber et al., 1993). A mutant, called *hookless1 (hls1)*, was isolated that showed no differential growth in the apical hook (Guzman and Ecker, 1990). Genetic epistasis analysis revealed that the *hls1* mutation was able to suppress the constitutive exaggerated hook phenotype observed in *eto1* and *ctr1* mutants (Roman et al., 1995).

Herein we describe the identification and characterization of *HOOKLESS1*, an ethylene response gene, that is essential for differential cell elongation in the hypocotyl. Molecular cloning of this gene revealed that its predicted protein shows significant amino acid sequence similarity to a class of N-acetyltransferases not identified previously in plants. These studies reveal a link between the ethylene and auxin response pathways, and provide new insight into how these two growth regulators may act to coordinate elongation and differential growth in plant cells.

Results

Mutations That Disrupt Differential Growth in the Hypocotyl

Three mutants were identified in Arabidopsis, in screens of three-day-old dark-grown seedlings, that showed no differential growth in the apical region of the hypocotyl: *hookless1* (*hls1-1*; Guzman and Ecker, 1990), *constitutive photomorphogenic2* (*cop2-1*; Hou et al., 1993), and *auxin resistant1* (*axr1-12*; Lincoln et al., 1990) (Figure 1). These "hookless" mutants lacked differential growth in the hypocotyl and showed different amounts of bending upon treatment with exogenous ethylene (Figure 1A).

Twenty-five new alleles of *hls1* were identified from independent lots of mutagenized seeds (Table 1). The alleles could be placed into two distinct phenotypic classes (Table 1 and Figure 1). "Strong" alleles (i.e. *hls1-1*) exhibited no differential growth in the hypocotyl when grown in the presence or absence of ethylene. "Weak" *hls1* alleles (i.e. *hls1-10*) lacked differential

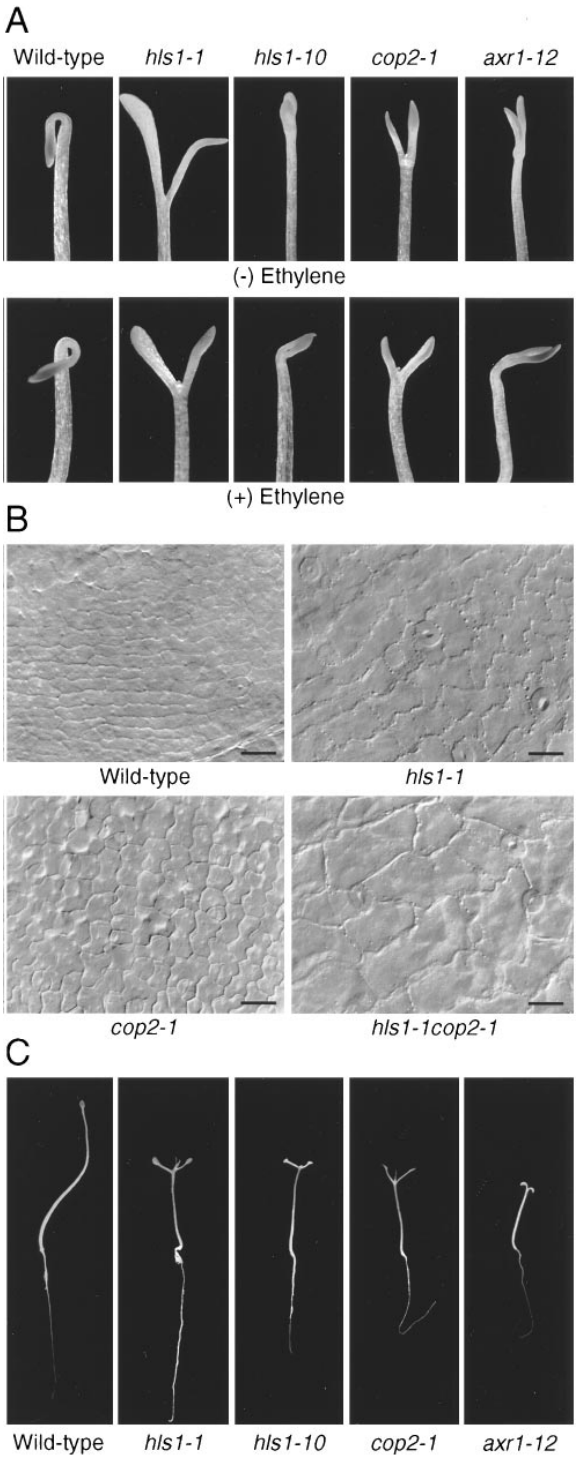

Figure 1. Morphology of Wild-Type and Mutant Seedlings

(A) Wild type and mutants were grown either in air or 10 μl ethylene per liter of air in the dark as described in Experimental Procedures.

(B) Effect of *hookless1* and *constitutive photomorphogenic2* on cell size. Cotyledons from three-day-old etiolated seedlings were cleared and mounted as described in Experimental Procedures. Photographs of epidermal cells were taken with Nomarski optics. The stomata are visible as doughnut shaped cells. Scale bars, ~22 μm.

(C) Seeds of wild type and the mutants grown in hydrocarbon-free air for ten days in the dark.

growth in the hypocotyl when grown in the absence of exogenous ethylene but showed significant curvature in the apical region of the hypocotyl when treated with ethylene. Interestingly, the putative photomorphogenic mutant *cop3-1* (Hou et al., 1993) was found to be an allele of *hls1 (hls1-26)* (Table 1).

The effects of *hls1* mutations on development were not restricted to the apical hook. The cotyledons and petioles of etiolated seedlings were more elongated than wild type, (Figures 1B and 2C). The surface areas of *hls1-1* (856.7 $\mu m^2 \pm 24.6$) epidermal cells were significantly larger than wild type (346.8 $\mu m^2 \pm 7.13$). Thus, the increased length of *hls1-1* cotyledons can be attributed to an increase in cell expansion. *hls1* seedlings also showed reduced elongation of the hypocotyl and root (Figures 1C, 2A, and 2D). Adult *hls1-1* plants showed a decrease in apical dominance (wild type = 2.65 inflorescences, *hls1-1* = 3.25 inflorescences, $t = 2.118$, $p < 0.05$) and a reduction in the length of the primary inflorescence in strong alleles when compared with wild type (wild type = 29.3 cm, *hls1* = 27.4 cm, $t = -2.281$, $p < 0.05$). *hls1* plants flowered 2–4 days earlier than wild type, but with the same number of leaves (Figure 2B). The early flowering phenotype may be explained by the increased rate of leaf initiation that occurred during the first 2–3 days of development (Figure 2B).

cop2-1 and *axr1-12* mutant seedlings were also defective in apical hook formation (Figure 1A). *cop2-1* was very similar in phenotype to strong alleles of *hls1*: no differential growth was observed in the apical region of the hypocotyl and the hypocotyl showed less elongation than wild type (Figures 1C and 2A). The surface areas of *cop2-1* (503.1 $\mu m^2 \pm 17.1$) epidermal cells were also significantly larger than wild type (346.8 $\mu m^2 \pm 7.13$). *axr1-12* mutant seedlings lacked the apical hook and, compared with wild type, showed less elongation of the hypocotyl (Figures 1A and 1C). Upon treatment with exogenous ethylene, bending of the hypocotyl was observed in *axr1-12* but not to the same extent as wild type (Figure 1A).

Interestingly, a synergistic effect on the size of the cotyledons was observed in the *hls1-1cop2-1* double mutant. The surface area of epidermal cells in *hls1-1cop2-1* (1856.1 $\mu m^2 \pm 66.9$) was significantly greater than either of the single mutants (*hls1-1*, 856.7 $\mu m^2 \pm 24.6$; *cop2-1*, 503.1 $\mu m^2 \pm 17.1$). The hypocotyl of the *hls1-1cop2-1* double mutant showed significantly less elongation than either single mutant and was only one-third the length of wild type (Figure 2A). Thus, in the hypocotyl, an additive interaction of these two mutations was found. The *hls1-1* mutant also showed reduced root elongation when compared with wild type and *cop2-1* (Figure 2D). *hls1-1cop2-1* adult plants also formed leaves at a rate faster than either single mutant during the first three days of development, resulting in an early flowering phenotype (Figure 2B). Similar to *hls1-1*, leaf initiation in *hls1-1cop2-1* ceased after eleven days and flowering was initiated.

Cloning and Characterization of the *HLS1* Gene and Mutant Alleles

hls1-2, an insertion allele, was isolated from a population of 4000 mutagenized families containing T-DNA insertions (Feldmann, 1991). Consistent with the presence of

Table 1. Quantitation and Molecular Analysis of *hookless 1* Alleles

hookless 1 Allele	Mutagen[a]	Hook Angle[b]	Sequence Change
Strong alleles[b]			
hls1-1	EMS	2.2° ± 0.9°	amino acid 346, E to K
hls1-4	DEB	+	amino acid 346, E to K
hls1-5	DEB	1.3° ± 0.5°	nucleotide 164, t to a
hls1-6	EMS	2.1° ± 1.0∞	amino acid 327, L to W
hls1-7	DEB	3.0° ± 1.3°	nucleotide 164, t to a
hls1-8	EMS	2.1° ± 1.2°	amino acid 181, R to UGA stop
hls1-9	EMS	6.3° ± 1.5°	amino acid 11, R to UGA stop
hls1-11	T-DNA	3.0° ± 1.2°	ND[c]
hls1-14	T-DNA	+	ND
hls1-15	FN	+	ND
hls1-16	FN	+	ND
hls1-19	EMS	+	ND
hls1-20	EMS	+	ND
hls1-21	EMS	+	ND
hls1-23	EMS	+	ND
hls1-24	DEB	+	ND
hls1-25	EMS	+	ND
hls1-26	*cop3*[d]	+	ND
Weak alleles[b]			
hls1-2	T-DNA	26.2° ± 3.2°	T-DNA insertion, nucleotide −1030
hls1-3	X-ray	8.1° ± 1.8°	~4.8 kb deletion of the promoter
hls1-10	EMS	23.2° ± 3.0°	amino acid 1, M (start) to I
hls1-12	EMS	+++	no change in coding region or introns
hls1-13	EMS	+++	no change in coding region or introns
hls1-17	FN	+++	ND
hls1-18	EMS	+++	ND
hls1-22	DEB	+++	ND

[a]Mutagen indicates the type of mutagen used to generate the allele. EMS, ethylmethanesulfonate; DEB, diepoxybutane; FN, fast neutrons.
[b]Hook angle indicates severity of the allele, with wild type in the presence of ethylene having a designation of ++++ = 298° ± 6.6°, weak alleles having a designation of +++ = 6.5° to 27°, and strong alleles a designation of + = 0° to 6.5°.
[c]ND, Not Determined.
[c]*cop3*, obtained from X. W. Deng (Hou et al., 1993).

a closely linked T-DNA insertion, each of 336 T5 kanamycin-resistant plants segregated the *hls1-2* mutation. An 800 bp fragment of plant DNA that flanked the T-DNA insertion site was isolated and used to identify lambda cDNA (λ76-1c) and genomic (λ12) clones (Figure 3A). Mapping of the cDNA clone to the bottom of chromosome 4 indicated close proximity of this gene to *hls1-1* (6.0 ± 1.5 cM distal to pCITd99). Southern analysis using λ76-1c revealed a polymorphism between wild type and *hls1-2* (data not shown). Additionally, Northern blot analysis of total RNA revealed the presence of a transcript in wild type that was undetected in *hls1-2* (data not shown).

To confirm that λ76-1c indeed corresponded to the *HLS1* cDNA, genomic DNA from both wild-type and *hls1* mutant alleles was isolated and the DNA sequences were determined (Figure 3B). Eight *hls1* alleles were found to contain alterations in the gene sequence when compared with wild type (Table 1 and Figure 3A). Sequence analysis of the promoter region of *hls1-2* revealed that the proximal insertion site of the Agrobacterium T-DNA was 710 bp upstream of the first nucleotide in the longest *HLS1* cDNA clone (Figure 3A). Analysis of genomic DNA from *hls1-3*, an X-ray-induced weak allele, revealed the presence of a 4.8 kb deletion within the *HLS1* promoter (Table 1 and data not shown). In another weak allele, *hls1-13*, no sequence changes were found in the regions of the gene that correspond to the protein coding and 5′ or 3′ untranslated leader sequence. However, Northern blot analysis demonstrated that *HLS1* mRNA was significantly reduced in this mutant (Figure 4A, lanes 1 and 2). The mutations in *hls1-3* and *hls1-13* may define important control regions in the promoter of the *HLS1* gene. Interestingly, sequence analysis of the *HLS1* promoter revealed the presence of an ethylene response element, a GCC box (Figure 3A). This DNA sequence element has been demonstrated previously to confer ethylene responsiveness to a minimal promoter in transgenic plants (Ohme-Takagi and Shinshi, 1995).

HLS1 Shows Amino Acid Sequences Similarity to a Diverse Family of N-Acetyltransferases

The protein product derived from conceptual translation of the *HLS1* cDNA sequence was compared with all proteins in the current public databases (January 1996). Significant sequence similarity was found in the first 158 amino acids of HLS1 to a recently defined class of N-acetyltransferases found in bacteria, yeast, and mammals (Figure 3C). A consensus motif of amino acids that define this class of enzymes has been identified (Tercero et al., 1992; Coon et al., 1995) and the predicted translation product of *HLS1* matches the consensus at each of the critical residues (Figure 3C). While the overall amount of amino acid similarity of HLS1 to the N-acetyltrasferases is relatively low (25%–30% identity), HLS1

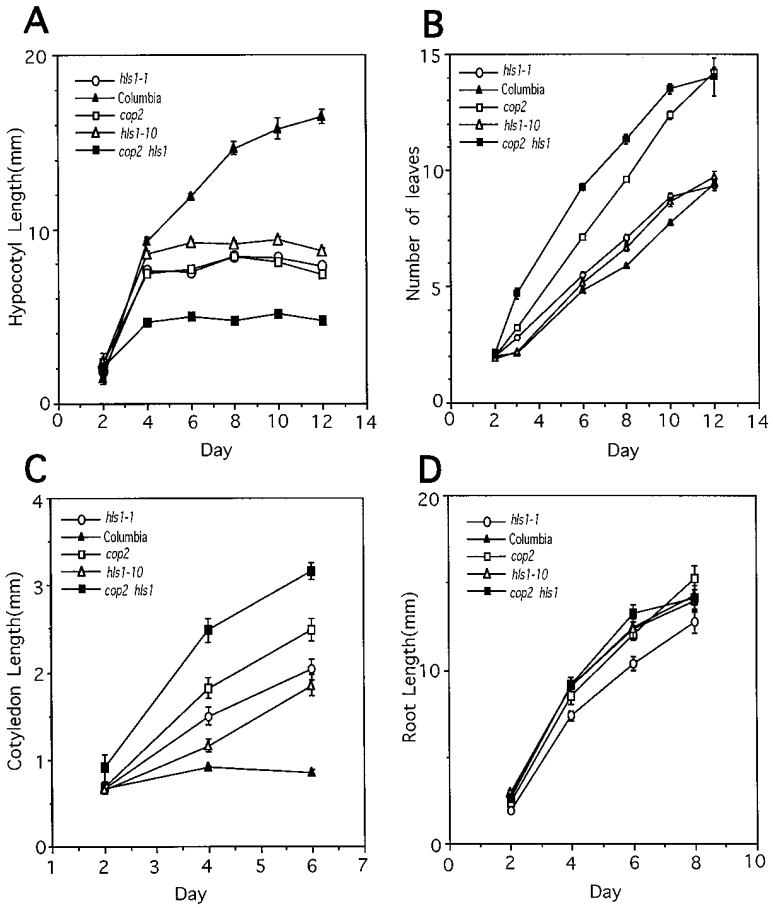

Figure 2. Quantitative Analysis of *hookless1* Development

(A) Hypocotyl lengths. Seedling hypocotyls were measured at the specified days after germination as described in Experimental Procedures.

(B) Leaf initiation rates. Leaf numbers were examined in light-grown seedlings by counting the number of true leaves visible on designated days after germination. The same seedlings were followed for 14 days.

(C) Cotyledon lengths. Seedlings cotyledons were measured at indicated days after germination and growth in darkness. The measurement was taken from the base of the petiole of the cotyledon to the tip of the cotyledon as described in Experimental Procedures.

(D) Root lengths. Seedlings roots grown on vertically oriented plates were measured at specified days after germination and growth in darkness as described in Experimental Procedures.

is as similar to any of these known acetyltransferases as they are to each another. The COOH-terminal 226 amino acids of HLS1 showed no significant similarity to any protein in the database. However, *hls1-1, hls1-4*, *hls1-6*, and *hls1-8* all contain mutations in this part of the protein, indicating that it is also essential for HLS1 activity (Table 1).

HLS1 mRNA Expression Is Regulated by Ethylene and Is Requisite for Differential Elongation of Cells in the Hypocotyl

To study the expression of *HLS1* mRNA in a variety of plant tissues and to examine the effect of ethylene gas treatment, Northern blots and in situ RNA localization studies were performed. *HLS1* mRNA was detected in etiolated seedlings and was also present, but at much reduced levels (20-fold less) in adult flowers, stems, and leaves (Figure 4A). The level of *HLS1* mRNA expression was significantly reduced (6.7-fold less) in the ethylene-insensitive mutant *ein2-1*, while its accumulation increased 5-fold in wild-type seedlings exposed to exogenous ethylene (Figure 4A).

The possibility that *HLS1* RNA was differentially localnized in cells of the hypocotyl hook was investigated. In situ hybridization of *HLS1* RNA in tissue sections of wild-type seedlings showed no differential localization of this RNA; the level of *HLS1* RNA in cells of the inner and outer regions of the hook appeared to be equivalent (Figure 4B). In keeping with these results, length measurements (data not shown) revealed that cells throughout the "hook" region of *hls1* mutants were longer than those of cells in the wild type. More specifically, while epidermal cells on both adaxial (outside) and abaxial (inside) surfaces of the *hls1* hook were equal in size, they were approximately twice the length of cells on the adaxial surface and ten times the length of abaxial cells in the wild-type hypocotyl hook. These results indicate that HLS1 is normally required to limit the expansion of cells throughout the hypocotyl hook. *HLS1* RNA was also found to be present in both differentially and uniformly elongating cells of the hypocotyl, cotyledons, and root (Figure 4B).

To examine the effects of aberrant *HLS1* expression on differential growth, plants were transformed with a wild-type *HLS1* gene whose expression was under the control of the 35S cauliflower mosaic virus promoter. Two independent transformed lines (H13 and H15) were identified that contained high levels of *HLS1* RNA (>200-fold; Figure 5A). Etiolated seedlings from these lines showed a substantial increase in differential cell elongation in the hypocotyl, and constitutive exaggerated hook formation was observed (Figure 5B). H13 and H15 also had longer hypocotyls when compared with wild type (data not shown). Interestingly, etiolated seedlings of another transgenic plant (line H1) showed a hookless phenotype (Figure 5B). Northern blot analysis revealed

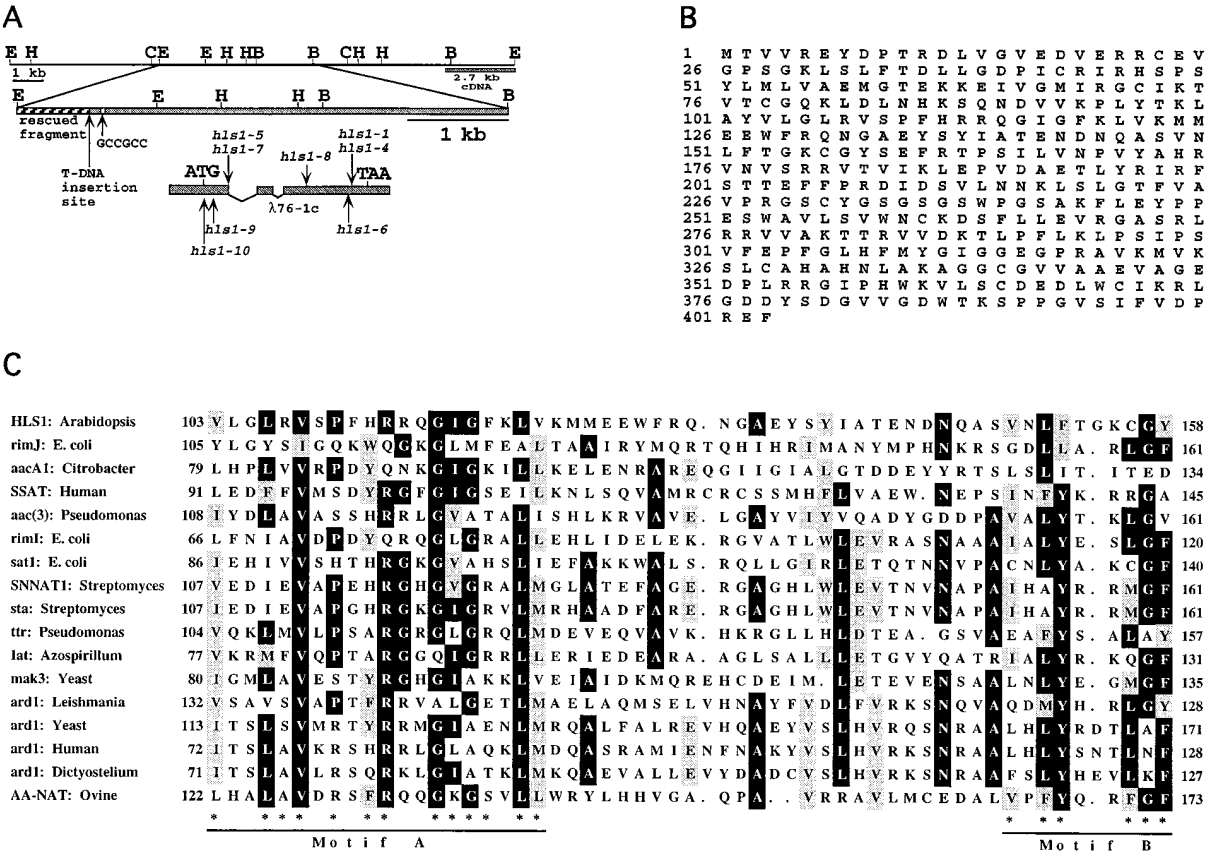

Figure 3. Structure of *HLS1* Genomic and cDNA Clones, Amino Acid Sequence, and Alignment with Similar N-Acetyltransferases

(A) *HLS1* genomic clone. A restriction map showing the location of the mRNA (cDNA). The magnified region displayed below the genomic clone (EcoRI to BamHI) specifies the location of the T-DNA insertion and a genomic plant DNA fragment isolated by left border rescue. Also indicated is the ethylene response element box (GCCGCC), a putative ethylene response element. λ76-1c, the 1.7 kb *HLS1* cDNA clone, is shown below corresponding genomic regions. The boxed regions represent exons and the lines represent introns. The arrows show the locations of mutations in the indicated alleles.

(B) Predicted amino acid sequence of the *HLS1* gene (GenBank accession #U50400). The intron–exon boundaries were determined by comparison with the cDNA sequence (GenBank accession #U50399) . The numbers for the amino acids are shown at the left with the M from the predicted ATG start assigned as number +1.

(C) Amino acid sequence similarity with N-acetyltransferases. An alignment of the amino acids 103–158 of HLS1 with a class of N-acetyltransferases was generated using the PILEUP program within the GCG package. Light gray highlighted amino acids indicate similar residues of family members. Solid highlighting with white characters indicates a residue in common between at least half of the family members. Asterisks indicate consensus residues, and the underscored motifs A and B define regions previously identified to be critical for N-acetyltransferase activity (Tercero et al., 1992; Coon et al., 1995). The family members are: AA-NAT from ovine (Coon et al., 1995), ARD1, MAK3 from yeast (Mullen et al., 1989; Tercero et al., 1992), ARD1 from Dictyostelium and Leishmania, TE2 from human (Ryan et al., 1993; Tribioli et al., 1994), RIMI and RIMJ from E. coli (Yoshikawa et al., 1987), STAT and NAT1 from Streptomyces, SAT1 from E. coli, aacA1 from Citrobacter, aac(3)-Ib from Pseudomonas (Horinouchi et al., 1987; Tenover et al., 1988; Heim et al., 1989; Krugel et al., 1993), TTR gene from Pseudomonas (Anzai et al., 1990), IAAT from Azospirillum (Zimmer et al., 1991), and SSAT from human (Casero et al., 1991).

that these plants contained an undetectable level of *HLS1* RNA (Figure 5A). Thus, introduction of the transgene in this line likely caused transinactivation (Jorgensen, 1995) of the endogenous *HLS1* gene, resulting in a hookless phenotype. Together, these results demonstrate that the presence of *HLS1* mRNA is essential for differential growth in the hypocotyl.

Bending in the Hypocotyl Is Dependent upon the Level and Distribution of Auxin

Auxin may be produced in the cotyledons and transported in a polar manner from the apex to the base of the seedling (reviewed in Lomax et al., 1995). Precise regulation of both the level and distribution of auxin may be required for the establishment of a hormone gradient and for differential elongation of cells in the hypocotyl. To examine the role of auxin in hypocotyl hook curvature, etiolated seedlings were grown in the presence of the polar auxin transport inhibitors, naphthylphthalamic acid (NPA) and triiodobuteric acid (TIBA) (Morgan, 1964; Rubery, 1990). Etiolated seedlings treated with NPA were hookless (Figure 6A); a similar effect was also observed in TIBA-treated seedlings (data not shown). The *eto1* and *ctr1* mutants, which normally show constitutive

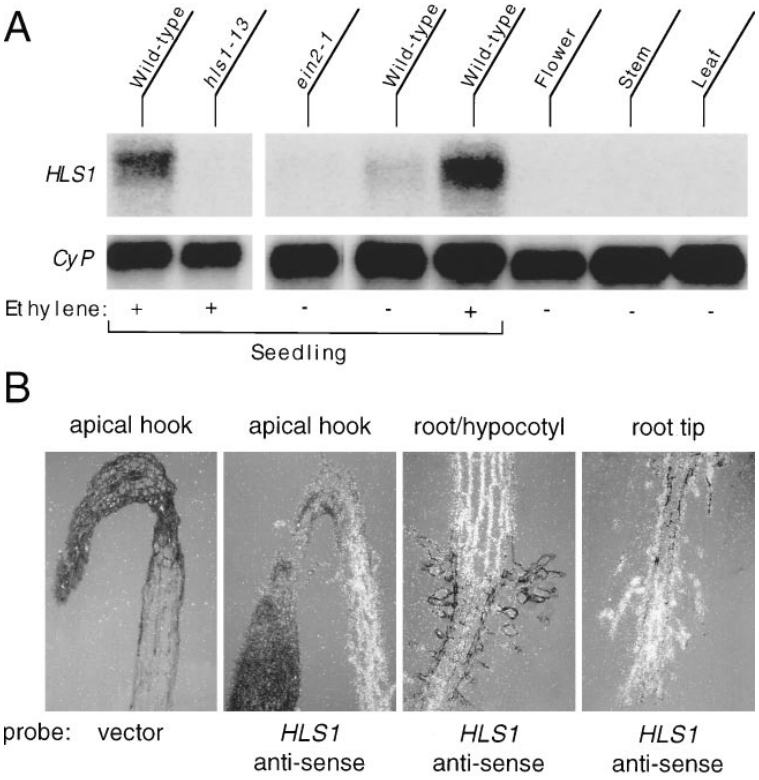

Figure 4. Analysis of *HLS1* Expression

(A) Northern blot of RNA isolated from three-day-old etiolated seedlings grown in hydrocarbon-free air or 10 μl ethylene per liter in air and adult tissues. Poly(A)+ RNA (5–7 μg) was loaded in each lane and Northern blots were performed as described in Experimental Procedures. The blot was probed with the *HLS1* cDNA and with a cyclophilin (*CYP*) probe as a loading control.

(B) Three-day-old etiolated seedlings grown in air or 10 μl ethylene per liter of air were fixed, embedded, and sectioned as described in Experimental Procedures. The apical hook sections shown here are from air-grown seedlings, and the root sections are from an ethylene grown seedling. Micrographs were taken under dark-field microscopy with conditions in which the developed grains of the emulsion appear white.

curvature of the apical hook (Guzman and Ecker, 1990; Kieber et al., 1993), also failed to form an apical hook upon treatment with NPA (Figure 6A) or TIBA (data not shown). Similarly, the constitutive hook phenotype observed in the HLS1 overexpression lines (H13 and H15) was prevented by treatment of seedlings with NPA (data not shown).

Hypocotyl hook development was also abolished in etiolated wild-type seedlings treated with the auxin mimic 2-4 dichlorophenoxyacetic acid (2,4-D). Similarly, we identified a mutant called *hls3* in which the apical hook does not form (Figure 6B) (also called *alf1*, *rty*, and *superroot*; Boerjan et al., 1995; Celenza et al., 1995; King et al., 1995). Examination of the level of free IAA in *hls3* revealed that this mutant produced 6-fold higher levels of endogenous free indole-3-acetic acid (IAA) (*hls3-1*:

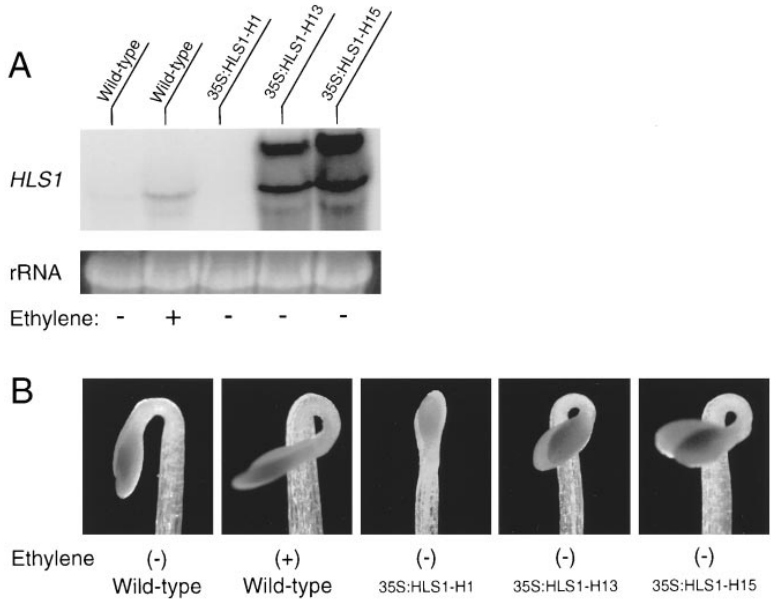

Figure 5. Effects of Overexpression of *HLS1*

(A) Northern-blot analysis of *HLS1* RNA in three-day-old etiolated seedlings of wild type and transgenic lines H1, H13, and H15. Total RNA (20 μg) from each line was used to prepare a Northern blot, and the blot was probed with radiolabeled *HLS1* cDNA. The *HLS1* RNA and rRNA are approximately the same size, and the single *HLS1* RNA appears as two bands.

(B) Three-day-old etiolated seedlings of wild type and lines H1, H13, and H15 grown in the absence of exogenous ethylene. Exaggerated apical hook curvature (angle >200°) observed in HLS1 overexpression lines H13 and H15 was found in 29% and 8% of the transformed seedlings, respectively. There were no apical hooks with angles >200° found upon examination of over 50 wild-type nontransformed seedlings.

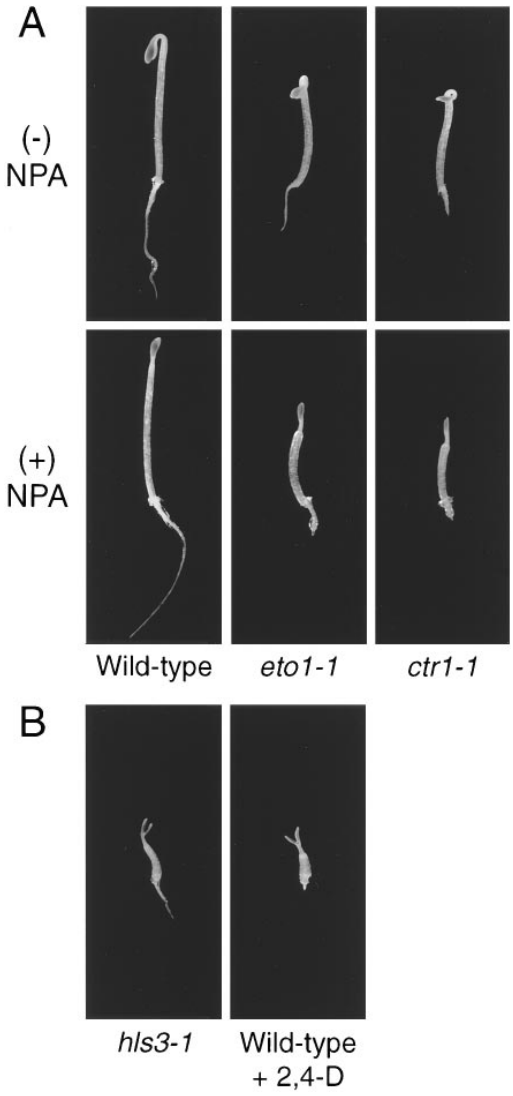

Figure 6. Absence of the Apical Hook in Wild-Type NPA and 2,4-D Treated Seedlings and the *hls3* Mutant

(A) Three-day-old etiolated seedlings of wild type, *eto1-1*, and *ctr1-1* treated with 1 μM NPA.

(B) Wild-type seedling treated with 1 μM 2,4-D (a synthetic auxin) for 3 days in the dark and a three-day-old dark-grown *hls3* seedling.

362 ± 22 pmol/g fresh weight, wild type: 58 ± 16 pmol/g). Together, results from both pharmacological and genetic studies revealed an essential role for auxin in the establishment of differential elongation of cells required for hypocotyl curvature in Arabidopsis.

hookless1 Mutations Alter the Spatial Expression Patterns of Primary Auxin-Responsive Genes

To further explore the relationship between HLS1 and auxin, the effect of the *hls1* mutation on the pattern of expression of several auxin-regulated genes was examined. The *AtAux2-11* gene (also referred to as the *IAA4* gene) has been shown to be a primary auxin response gene and a member of a large multigene family of early auxin-inducible mRNAs (Wyatt et al., 1993; Abel et al., 1995). A narrow range of auxin concentrations, with an optimum of 5×10^{-5}M IAA, was reported for *AtAux2-11* induction by exogenous auxin. Levels of hormone above or below this concentration inhibited *AtAux2-11* expression (Wyatt et al., 1993; Abel et al., 1995). *AtAux2-11* is expressed specifically on the elongating side of seedlings undergoing differential growth in response to gravity (Wyatt et al., 1993). Transgenic plants that contained the *AtAux2-11*/*LacZ* reporter gene were crossed to *hls1* plants (*hls1-1* and *hls1-10*) and F2 seedlings containing the transgene were stained for LacZ activity (Figure 7A). Wild-type (*HLS1*/*HLS1)* transgenic seedlings showed intense staining throughout the seedling including the hypocotyl, root, and apical hook. In the *hls1-1* and *hls1-10* mutants, expression of *AtAux2-11*/*LacZ* was greatly decreased in the part of the hypocotyl where the apical hook is normally found. No staining was observed in the apical hook region, and only a low level of expression was observed in the more apical portion of the cotyledons. These results suggest that normal expression of *AtAux2-11*/*LacZ* in the hypocotyl and apical hook requires HLS1 activity.

Like *AtAux2-11,* the small auxin up-regulated RNA (*SAUR*) genes have been demonstrated to be primary auxin-responsive genes (Li et al., 1994). Similar studies have also been carried out using the auxin-responsive *SAUR-AC1*/*GUS* reporter gene (Gil et al., 1994). These immediate early genes show cyclohexamide-independent induction of transcription (Gil et al., 1994; Li et al., 1994). *SAUR* mRNAs are rapidly redistributed when plants undergo differential growth in response to gravity (Li et al., 1991). The expression of this reporter gene was examined in wild-type and *hls1* seedlings. *SAUR-AC1*/*GUS* plants were crossed with *hls1-1* and *hls1-2*, and the F2 plants were stained for GUS activity (Figure 7B). Wild-type (*HLS1*/*HLS1*) transgenic seedlings showed no staining in the apical hook portion of the hypocotyl, whereas in *hls1-1*, strong staining was present in the part of the hypocotyl where the hook is normally found (opposite to the staining pattern of *AtAux2-11*/*LacZ*). Expression of *SAUR-AC1*/*GUS* in the weak allele, *hls1-2*, was intermediate between wild type and *hls1-1*. These results indicate that *HLS1* normally acts to suppress auxin-regulated *SAUR* gene expression in the cotyledons and apical hook.

Since inhibitors of auxin transport, such as NPA, can phenocopy the hookless morphology, the effect of this drug on primary auxin responsive gene expression in the hypocotyl hook was also examined. The *SAUR-AC1*/*GUS* and *AtAux2-11*/*LacZ* plants were treated with NPA and then examined for GUS or LacZ activity, respectively (Figure 7). Like the *hls1* mutations, NPA blocked differential elongation and apical hook formation in the seedling hypocotyl. Similarly, in the presence of NPA, the patterns of expression of both transgenes precisely mimicked those observed in the *hls1-1* mutant. In the *AtAux2-11*/*LacZ* seedlings, no staining was detected in the hook region, whereas in the *SAUR-AC1*/*GUS* seedlings, strong staining was visible. These results suggest that, like NPA, *hls1* mutations may cause a change in the level of active auxin in cells or in the sensitivity of cells to this hormone.

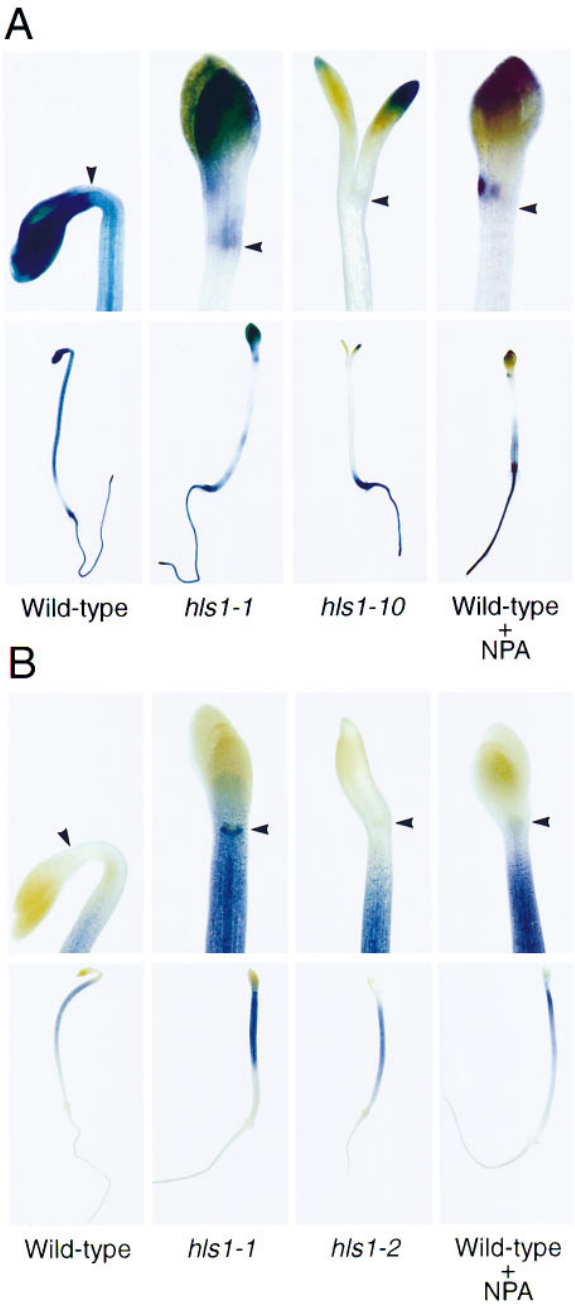

Figure 7. *AtAux2-11*/*LacZ* and *SAUR-AC1*/*GUS* Expression in Wild-Type and *hls1* Seedlings

(A) Wild-type and mutant plants transformed with the *AtAux2-11*/*LacZ* construct were grown in the presence and absence of 1 μM NPA in the dark for three days fixed and stained for LacZ activity as described.

(B) Wild-type and mutant plants transformed with the *SAUR-AC1*/*GUS* were grown in the presence and absence of 1 μM NPA in the dark for three days, fixed, and stained for GUS activity as described. Arrowheads indicate the junction of the hypocotyl and cotyledons in each seedling.

Discussion

The *HLS1* Gene Encodes a Putative N-Acetyltransferase

The HLS1 protein shows significant similarity to a diverse class of N-acetyltransferases and represents the first member of this family to be identified in plants. N-acetyltransferases are amino group acetylating proteins and, as such, have a wide range of substrates. Members of this family act to acetylate relatively small molecules, such as aminoglycosides and polyamines (Heim et al., 1989; Casero et al., 1991). Interestingly, molecules with structural similarity to IAA, such as serotonin and tryptamine, are acetylated by a member of this family (Coon et al., 1995). In addition, the *Iat* gene from Azospirillum was identified as part of a trpGDC cluster, which is involved in IAA production, and *Iat* is proposed to encode an IAA acetyltransferase (Zimmer et al., 1991). Thus, it is possible that HLS1 could utilize an IAA-related metabolite as substrate.

An important role of acetyltransferases has been observed in plants, yeast, and other systems where reversible acetylation occurs on core histones at the ϵ-amino groups of lysyl residues. Acetylation of histones is believed to play a significant role in transcriptional control (reviewed in Davie and Hendzel, 1994). A further role of these enzymes may be to modulate protein turnover (reviewed in Driessen et al., 1985). Approximately 50%–90% of all eucaryotic proteins are N-terminally acetylated, and this modification has been shown to block protein degradation. Through studies of auxin-regulated gene expression, it has been observed that changes in protein stability may play a significant role in the auxin response pathway (Oeller et al., 1993; discussed in Hobbie and Estelle, 1994). The rate of protein turnover by the ubiquitin cytoplasmic proteolysis system is dependent on a free (unacetylated) amino group at the amino terminus of proteins (reviewed in Driessen et al., 1985). In this regard, it is intriguing that the *AXR1* gene has been shown to encode a protein related to the ubiquitin-activating enzyme E1, and it has been suggested that AXR1 may function in the ubiquitin cytoplasmic proteolysis system (Leyser et al., 1993). HLS1 may acetylate the N-terminal amino group of a protein(s) that are required for auxin transport, or may contribute to the stability or activity of such a protein(s).

Hookless Genes Act to Control Differential Growth in the Hypocotyl

Arabidopsis mutants that are defective in differential cell elongation have been identified and characterized. The *hls1*, *cop2*, and *axr1* mutants are unable to form a hypocotyl hook and are also inhibited in hypocotyl elongation. The similarity of each of the single mutant phenotypes indicates that these genes may affect the same developmental process, although our genetic studies suggest that they act in different pathways. The recessive nature of these mutations indicates that *HLS1*, *COP2*, and *AXR1* normally act to promote cell elongation and differential growth in the hypocotyl. The enlarged cotyledons of *hls1* and *cop2* mutants are due to an increase in cell size, indicating that these two genes

normally act to inhibit expansion of cotyledon cells. Interestingly, the *hls1-1* mutant also shows inhibition of root elongation. Thus, HLS1 appears to promote cell elongation in the hypocotyl and root but inhibit cell expansion in the cotyledons.

The observation that genes such as *COP2* and *AXR1* are also required for differential growth in the hypocotyl implies that *HLS1* is not entirely sufficient for apical hook development. The *AXR1* gene is believed to be important for normal auxin response throughout the plant (Lincoln et al., 1990), and mutations in this gene cause a complete loss of auxin-inducible *SAUR* gene expression (Timpte et al., 1995). The absence of an apical hook in *axr1-12* mutants also suggests a role for auxin responsiveness in the establishment or maintenance of differential growth in the hypocotyl. Interestingly, *cop2-1* has been shown recently to be allelic to the mutations, *primordia timing-1* (*pt-1*; data not shown) and *altered meristem program1* (*amp1*; Chaudhury et al., 1993; S. Poethig, personal communication). The *amp1* mutant contains increased levels of the cytokinins (Chaudhury et al., 1993). Since many auxin-affected processes are regulated by the ratio of auxin and cytokinin (Tamas, 1987), increasing the level of cytokinin in plant tissues by mutations such as *cop2*/*amp1*/*pt-1* may be functionally equivalent to mutations such as *hls1* and *axr1* that are proposed to cause a localized decrease in levels of active auxin or response to auxin, respectively. The synergistic interaction observed in size of *hls1cop2* cotyledon epidermal cells may reflect an alteration in this ratio.

Auxin-Mediated Gene Expression in the Hypocotyl Requires HLS1

Auxins are believed to be transported by a unique polar transport mechanism from their source at the apex of seedlings toward the base of the hypocotyl (reviewed in Lomax et al., 1995). NPA specifically blocks auxin transport (Rubery, 1990), resulting in an accumulation of auxin in the apical portions (cotyledons and hypocotyl hook) of the seedling. The role of auxin in apical hook development was further established by determining the effects of inhibitors of auxin transport on plant morphology and gene expression patterns. Seedlings were unable to establish an apical hook when the normal auxin balance in cells was disrupted. Similarly, elevated levels of auxin or a mutation (*hls3*) that causes high levels of endogenous free auxin also blocks apical hook formation.

In *hls1* mutants, the observed pattern of expression of the auxin primary response genes *SAUR* and *AtAux2-11* differs significantly from wild type. *SAUR-AC1*/*GUS* expression is absent in the hypocotyl hook of wild-type seedlings, but in *hls1* mutants it is highly expressed in this region of the hypocotyl. In contrast, strong *AtAux2-11*/*LacZ* expression was found in the hypocotyl hook in wild-type seedlings but was absent in this region in the *hls1* mutant. Seedlings grown in the presence of NPA display a staining pattern that is identical to the pattern found in *hls1* plants. Blocking the transport of auxin and blocking the *hls1* mutation both cause similar affects on auxin primary response genes. These results are consistent with a role for HLS1 affecting auxin localization in seedlings.

The Role of HLS1 in Apical Hook Development

Several lines of evidence reveal a strong correlation between the degree of differential growth (bending) observed in the hypocotyl and the level of *HLS1* gene expression. Treatment of seedlings with ethylene promotes both exaggeration in the curvature of the apical hook and higher levels of *HLS1* mRNA in seedling tissues. In contrast, ethylene-insensitive mutant seedlings, such as *ein2*, contain very low levels of *HLS1* mRNA and show much reduced apical hook curvature. Weak alleles of *hls1*, which contain reduced amounts of *HLS1* mRNA, show significant bending only upon exposure to exogenous ethylene. In contrast, transgenic seedlings that produce high levels of *HLS1* mRNA exhibit exaggeration in hook curvature even in the absence of exogenous ethylene.

HLS1 may also promote elongation in the hypocotyl and root and limit expansion of cells in the cotyledons by controlling the distribution of auxin in the seedling. This suggestion is supported by studies of the expression of auxin-inducible reporter genes which indicate that *hls1* mutations cause significant alterations in the level of auxin-regulated gene expression in the hypocotyl. If HLS1 is required for normal transport of auxin, then a mutation in this gene may cause auxin accumulation (increased auxin) at the source of auxin production, the apex of the seedling (cotyledons and apical hook), with a corresponding reduction in the level found in hypocotyl of *hls1* plants. The enlarged cotyledons and the inhibition of hypocotyl and root elongation observed in *hls1*, may result from levels of auxin that are not optimal for normal cell expansion. These observations suggest that *HLS1* may regulate the distribution of auxin or response to auxin. Local auxin concentrations may be modulated by a variety of mechanisms, including changes in auxin biosynthesis, catabolism, transport, or conjugation (Reinecke and Bandurski, 1987). It is currently not possible to directly measure subtle differences in auxin concentration in hypocotyl tissues. However, recent studies suggest that indirect measurements of IAA using antibodies might be feasible (Kerk and Feldman, 1995).

The Arabidopsis *HLS1* gene encodes a putative member of a family of N-acetyltransferases not previously known in plants and is the first plant gene known to be essential for differential elongation of cells. This ethylene response gene regulates differential growth in the hypocotyl, possibly via regulation of the transport or chemical modification of the critical cell growth regulator auxin. These studies reveal a link between the ethylene and auxin response pathways and provide new insight into how these two hormones act to coordinate elongation and differential growth in plant cells.

Experimental Procedures

Arabidopsis Strains, Growth, and Mutagenesis Conditions

Plants were grown as described (Guzman and Ecker, 1990). Etiolated seedlings were grown in the dark in the presence and absence of ethylene, and M2 mutagenized seedlings were examined for the presence or absence of the apical hook as previously described (Guzman and Ecker, 1990). The ethyl methanesulfonate–induced *hls1-1* mutant was isolated from Arabidopsis thaliana ecotype Columbia. Additional alleles of *hls1* were isolated in M2 populations

following X-ray, diepoxybutane, and fast neutron mutagenesis of seeds of the Columbia ecotype. X-ray and diexpoxybutane mutagenesis were performed according to Kieber et al., 1993. Fast neutron populations were provided by M. Ahmad (Univ. of Pennsylvania). T-DNA alleles in the Wassilewskija ecotype were found in populations generated by T-DNA mutagenesis (Feldmann, 1991).

Genetic Analysis

Crosses were performed following Guzman and Ecker, 1990. Progeny from complementation crosses were examined in the F1 and F2 generations. Double mutants were constructed by crossing the two parents and collecting seed from the F1 plant. F2 seedlings were allowed to germinate in the dark in hydrocarbon-free air and 10 μl ethylene per liter hydrocarbon-free air, then screened for seedlings expressing both parental phenotypes. Putative double mutants were verified by their failure to complement each parental mutation.

Quantification of Phenotypes

Seedlings were measured using a WILD (Wild Heerbrugg, Switzerland) dissecting microscope with an ocular micrometer. Roots were measured after growing seedlings on vertical Murashige and Skoog (MS) agar plates (Guzman and Ecker, 1990). Cotyledons were measured from the base of the petiole to the tip of the cotyledon. Leaf initiation rates were calculated by counting the number of leaves visible on designated days postgermination. For dark-grown seedlings, different representative groups of seedlings were measured at each time point to eliminate any effect that light exposure might have on seedling development.

Cell size measurements were conducted on seedlings grown on MS agar for three days in darkness. The cotyledons were cleared by incubating seedlings for 18–24 hr at room temperature, then for 1 hr at 55°C–60°C in chloral hydrate (8 parts chloral hydrate:3 parts water). The cotyledons were mounted on slides, examined under Nomarski optics, and photographed. Epidermal cells were traced from photographs (10 cotyledons per treatment, 30 cells per cotyledon) and quantified using NIH Image (Wayne Rasband, National Institutes of Health, USA). Statistical analyses were done with Statview 512+ (BrainPower, Inc., Ventura, CA).

NPA and 2,4-D

NPA containing 23.7% 2[(1-Naphthalenylamino)carbonyl]benzoic acid, sodium salt was provided as ALANAP-L (Uniroyal Chemical Company, Inc.). Seedlings were surface sterilized and plated on MS agar media containing 1 μM NPA. 2,4-D was added to MS agar media at a concentration of 1 μM.

Staining for LacZ and GUS Expression

AtAux2-11/*LacZ* line 3E-16 (Wyatt et al., 1993) transgenic plants were crossed to the *hls1-1* and *hls1-10* mutants. Fixation and staining were performed according to Wyatt et al., 1993, on three-day-old etiolated F2 seedlings that contained the *hls1-1* or *hls1-10* mutation and the *AtAux2-11*/*LacZ* transgene. Transgenic plants of *SAUR-AC1*/*GUS* (Gil and Green, 1996) were crossed to the *hls1-1* and *hls1-2* mutants. Three-day-old etiolated F2 seedlings containing the *hls1-1* or *hls1-2* mutations and *SAUR-AC1*/*GUS* were fixed in 1% formaldehyde, 100 mM sodium phosphate (pH 7.0) for 20 min under vacuum. Fixed seedlings were washed 5 times in excess 100 mM sodium phosphate (pH 7.0) and stained 12–16 hr at 37°C after vacuum infiltration for 2 min in staining buffer (100 mM $NaPO_4$, 10 mM EDTA, 0.5 mM $K_4Fe(CN)_6$, 0.5 mM $K_3Fe(CN)_6$, 1% Triton X-100, 1 mM 5-bromo-4-chloro-3-indolyl β-D glucuronic acid [X-Gluc, New Jersey Lab Supply]).

Gene Isolation and Characterization

Plant DNA was isolated from *hls1-2* by left border plasmid rescue according to the procedures described in Kieber et al. (1993). The fragment was used to screen an Arabidopsis Columbia ecotype genomic library in λDASH (provided by N. Crawford, UCSD). A genomic clone (λ12) was then used to identify cDNA clones from a three-day-old etiolated seedling Columbia ecotype λZAP II cDNA library (Kieber et al., 1993). The longest cDNA was sequenced completely as described below.

Sequencing and Analysis of Wild-type and Mutant DNAs

The λ12 genomic clone and the cDNA clones were subcloned into pKS (Stratagene) and exonuclease III deletions were generated following the protocols of the manufacturers (New England Biolabs and Stratagene). DNA sequencing of the genomic (λ12) and cDNA (λ76-1c) clones was done manually by [^{35}S]ATP dideoxy sequencing using Sequenase according to the instructions of the manufacturer (United States Biochemical), or by use of the Applied Biosystems automated sequencer (model 373A) using dye terminators as recommended by the manufacturer.

Genomic DNA was isolated from wild type and mutant plants by cesium chloride purification (Pruitt and Meyerowitz, 1986) and using a plant DNA miniprep procedure (Edwards et al., 1991). Two sets of gene-specific oligonucleotide primers (19 bp, ~50% G+C) were used to amplify the *HLS1* gene as two overlapping fragments using polymerase chain reaction (PCR). Amplified fragments were sequenced directly using the Applied Biosystems automated DNA sequencer. Sequence differences were verified by sequencing both strands from at least 2 independent PCR reactions.

The *HLS1* predicted amino acid sequence was used to search the DNA and protein sequence databases using BLAST (Altschul et al., 1990). The alignment of N-acetyltransferases was generated using the Pileup program within the GCG program package, version 7 (Genetics Computer Group). Similarity consensus was identified using the program Pretty with the default symbol comparison table based on the Dayhoff PAM-250 matrix; default program settings were used.

Northern Analysis

Three-day-old wild-type and etiolated seedlings were grown and harvested according to Kieber et al. (1993). Adult tissues were harvested at 4–5 weeks of age. Flowers, stems and leaves were removed separately and frozen in liquid nitrogen. All tissues were stored at −80°C until used. Total RNA was isolated as described by Kieber et al. (1993). RNA was denatured and electrophoresed then transferred to Hybond N+ (Amersham). The blot was cross-linked with ultraviolet light (0.3 J/cm^2), hybridized with ^{32}P-radiolabeled probes, washed, and exposed 4 days using a Phosphorimager (Molecular Dynamics, Sunnyvale, CA).

Measurement of Auxin Levels

Three-day-old etiolated wild-type and *hls3* seedlings were collected from a segregating population and transferred to 50 ml of liquid culture (Gamborg's B-5 medium without hormones, GIBCO) in a 125 ml flask shaken at 50 rpm for 25 days in the light. Tissue was then harvested and frozen in liquid nitrogen. IAA concentrations were determined using the isotope dilution high pressure liquid chromatography method for isolation and quantitation of IAA, utilizing electrochemical detection (Black et al., 1986).

Plant Transformation and Overexpression of *HLS1*

The *HLS1* cDNA clone insert was subcloned into pKYLX7 (Schardl et al., 1987), which contains the 35S promoter from cauliflower mosaic virus to generate the 35S-*HLS1* plasmid. Agrobacterium strain LBA4404, transformed with 35S-*HLS1*, was used to transform Arabidopsis ecotype Columbia plants according to Bechtold et al., 1993.

In Situ RNA Hybridization

Three-day-old etiolated seedlings grown in the presence of 10 μl of ethylene per liter of air or in hydrocarbon-free air were used for in situ hybridization. In situ hybridization of sectioned seedling tissues was performed essentially according to Cox and Goldberg, 1988. Antisense *HLS1* and pKS plasmid RNA probes were synthesized by in vitro transcription using a transcription kit (Promega).

Acknowledgments

Correspondence should be addressed to J. R. E. T-DNA and fast neutron mutagenized populations were generously provided by K. Feldmann (Arizona State University) and M. Ahmad (University of Pennsylvania), respectively. Transgenic seeds containing the *SAUR-AC1*/*GUS* and *AtAux2-11*/*LacZ* were generously provided by P. Green (Michigan State University) and R. T. Nagao (University of

Georgia), respectively. The *cop2-1*, *axr1-12*, and *pt-1* mutants were obtained from X. Deng (Yale University), M. Estelle (Indiana University), and the Arabidopsis Biological Resource Center (Ohio State University), respectively. We would like to thank S. Poethig, A. Binns, M. Estelle, S. Theologis and members of the Ecker, Poethig, and Theologis labs for a critical reading of this manuscript. This work was supported by the Department of Energy and the National Science Foundation.

Received January 17, 1996; revised March 6, 1996.

References

Abel, S., Nguyen, M.D., and Theologis, A. (1995). The PS-IAA4/5-like family of early auxin inducible messenger RNAs in *Arabidopsis thaliana*. J. Mol. Biol. *251*, 533–549.

Abeles, F.B., Morgan, P.W., and Saltveit, M.E., Jr. (1992). Ethylene in Plant Biology, Second Edition (San Diego, California: Academic Press).

Altschul, S.F., Gish, W., Miller, W., Meyers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. J. Mol. Biol. *215*, 403–410.

Anzai, H., Yoneyama, K., and Yamaguchi, I. (1990). The nucleotide sequence of *tabtoxin resistance gene (ttr)* of *Pseudomonas syringae* pv. tabaci. Nucl. Acids Res. *18*, 1890.

Bechtold, N., Ellis, J., and Pelletier, G. (1993). *In planta* Agrobacterium-mediated transfer by infiltration of adult *Arabidopsis thaliana* plants. Comptes rendus de Academic des sciences *316*, 1194–1199.

Black, R.C., Kuleck, G.A., and Binns, A.N. (1986). The initiation of auxin autonomy in tissue from tobacco plants carrying the auxin biosynthesizing genes from the T-DNA of *Agrobacterium tumefaciens*. Plant Physiol. *80*, 145–151.

Boerjan, W., Cervera, M., Delarue, M., Beeckman, T., Desitte, W., Bellini, C., Caboche, M., Van Onckelen, H., Van Montagu, M., and Inze, D. (1995). *superroot*, a recessive mutation in *Arabidopsis* confers auxin overproduction. Plant Cell *7*, 1405–1419.

Casero, R.A., Celano, P., Ervin, S.J., Applegren, N.B., Wiest, L., and Pegg, A.E. (1991). Isolation and characterization of a cDNA clone that codes for human spermidine/spermine *N'*1-acetyltransferase. J. Biol. Chem. *266*, 810–814.

Celenza, J.L., Grisafi, P.L., and Fink, G.G.R. (1995). A pathway for lateral root formation in *Arabidopsis thaliana*. Genes Dev. *9*, 2131–2142.

Chaudhury, A.M., Letham, S., Craig, S., and Dennis, E.S. (1993). *amp1*—a mutant with high cytokinin levels and altered embryonic pattern, faster vegetative growth, constitutive photomorphogenesis and precocious flowering. Plant J. *4*, 907–916.

Cleland, R.E. (1987). Auxin and cell elongation. In Plant Hormones and Their Role in Plant Growth and Development, P.J. Davies, ed. (Hingham, Massachusetts: Martin Nijhoff Publishers), pp. 132–148.

Coon, S.L., Roseboom, P.H., Baler, R., Weller, J.L., Namboodiri, M.A.A., Koonin, E.V., and Klein, D.C. (1995). Pineal serotonin *N*-acetyltransferase expression cloning and molecular analysis. Science *270*, 1681–1683.

Cox, K.H., and Goldberg, R.B. (1988). Analysis of plant gene expression. In Plant Molecular Biology: A Practical Approach (Oxford: IRL Press), pp. 1–34.

Darwin, C., and Darwin, F. (1896). The Power of Movement in Plants (New York: D. Appleton and Co.), pp. 87–94.

Davie, J.R., and Hendzel, M.J. (1994). Multiple functions of dynamic histone acetylation. J. Cell. Biol. *55*, 98–105.

Driessen, H.P.C., de Jong, W.W., Tesser, G.I., and Bloemendal, H. (1985). The mechanism of *N*-terminal acetylation of proteins. CRC Crit. Rev. Biochem. *18*, 281–306.

Ecker, J.R. (1995). The ethylene signal transduction pathway in plants. Science *268*, 667–675.

Edwards, K., Johnstone, C., and Thompson, C. (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucl. Acids Res. *19*, 1349.

Feldmann, K.A. (1991). T-DNA insertion mutagenesis in *Arabidopsis*: mutational spectrum. Plant J. *1*, 71–82.

Firn, R.D., and Digby, J. (1980). The establishment of tropic curvatures in plants. Annu. Rev. Plant Physiol. *31*, 131–148.

Gil, P., and Green P.J. (1996). Multiple regions of the *Arabidopsis SAUR-AC1* gene control transcript abundance: the 3′ untranslated region functions as an mRNA instability determinant. EMBO J., in press.

Gil, P., Yang, L., Orbovic, V., Verkamp, E., Poff, K.L., and Green, P.J. (1994). Characterization of the auxin inducible SAUR-AC1 gene for use as a molecular genetic tool in *Arabidopsis*. Plant Physiol. *104*, 777–784.

Guzman, P., and Ecker, J.R. (1990). Exploiting the triple response to identify ethylene-related mutants. Plant Cell *2*, 513–523.

Heim, U., Tietze, E., Weschke, W., Tschape, H., and Wobus, U. (1989). Nucleotide sequence of a plasmid born streptothricin-acetyltransferase gene (*sat-1*). Nucl. Acids Res. *17*, 7103.

Hobbie, L., and Estelle, M. (1994). Genetic approaches to auxin action. Plant Cell Environ. *17*, 525–540.

Horinouchi, S., Furuya, K., Nishiyama, M., Suzuki, H., and Beppu, T. (1987). Nucleotide sequence of the streptothricin acetyltransferase gene from *Streptomyces lavendulae* and its expression in heterologous hosts. J. Bact. *169*, 1929–1937.

Hou, Y., von Arnim, A.G., and Deng, X.W. (1993). A new class of *Arabidopsis* constitutive photomorphogenic genes involved in regulating cotyledon development. Plant Cell *5*, 329–339.

Jorgensen, R.A. (1995). Cosuppression, flower color patterns and metastable gene expression states. Science *268*, 686–691.

Kang, B.G., Yocum, C.S., Burg, S.P., and Ray, P.M. (1967). Ethylene and carbon dioxide: mediation of hook opening response. Science *156*, 958–959.

Kerk, N.M., and Feldman, L.J. (1995). A biochemical-model for the initiation and maintenance of the quiescent center—implications for organization of root-meristems. Development *121*, 2825–2833.

Kieber, J.J., Rothenberg, M., Roman, G., Feldmann, K.A., and Ecker, J.R. (1993). *CTR1*, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the Raf family of protein kinases. Cell *72*, 427–441.

King, J.J., Stimart D.P., Fischer, R.H., and Bleecker, A.B. (1995). A mutation altering auxin homeostasis and plant morphology in *Arabidopsis*. Plant Cell *7*, 2023–2037.

Krugel, H., Fiedler, G., Smith, C., and Baumberg, S. (1993). Sequence and transcriptional analysis of the nourseothricin acetyltransferase-encoding gene *nat1* from *Streptomyces noursei*. Gene *127*, 127–131.

Leyser, H.M.O., Lincoln, C.A., Timpte, C., Lammer, D., Turner, J., and Estelle, M. (1993). *Arabidopsis* auxin-resistant gene *AXR1* encodes a protein related to ubiquitin-activating enzyme E1. Nature *364*, 161–164.

Li, Y., Hagen, G., and Guilfoyle, T.J. (1991). An auxin-responsive promoter is differentially induced by auxin gradients during tropisms. Plant Cell *3*, 1167–1175.

Li, Y., Strabala, T.J., Haben, G., and Guilfoyle, T.J. (1994). The soybean *SAUR* open reading frame contains a cis element responsible for cyclohexamide-induced mRNA accumulation. Plant Mol. Biol. *24*, 715–723.

Lincoln, C., Britton, J.H., and Estelle, M. (1990). Growth and development of the *axr1* mutants of *Arabidopsis*. Plant Cell *2*, 1071–1080.

Lomax, T.L., Muday, G.K., and Rubery, P.H. (1995). Auxin transport. In Plant Hormones: Physiology, Biochemistry, and Molecular Biology. P.J. Davies, ed. (Norwell: Kluwer Academic Press), pp. 509–530.

Morgan, D.G. (1964). Influence of α-naphthylphthalamic acid on the movement of indolyl-3-acetic-acid in plants. Nature *201*, 476–477.

Mullen, J.R., Kanye P.S., Moerschell, R.P., Tsunasawa, S., Gribskov, M., Colavito-Shepanski, M., Grunstein, M., Sherman, F., and Sternglanz, R. (1989). Identification and characterization of genes and mutants for an *N*-terminal acetyltransferase from yeast. EMBO J. *8*, 2067–2075.

Oeller, P.W., Keller, J.A., Parks, J.E., Silbert, J.E., and Theologis, A.

(1993). Structural characterization of the early indole-acetic acid-inducible genes, *PS-IAA4/5* and *PS IAA6* of Pea (*Pisum sativum* L). J. Mol. Biol. *233*, 789–798.

Ohme-Takagi, M., and Shinshi, H. (1995). Ethylene inducible DNA binding proteins that interact with an ethylene-responsive element. Plant Cell *7*, 173–182.

Pruitt, R.E., and Meyerowitz, E.M. (1986). Characterization of the genome of *Arabidopsis thaliana*. J. Mol. Biol. *187*, 169–183.

Reinecke, D.M., and Bandurski, R.S. (1987). Auxin biosynthesis and metabolism. In Plant Hormones and Their Role in Plant Growth and Development, P.J. Davies, ed. (Hingham, Massachusetts: Martin Nijhoff Publishers), pp. 24–42.

Roman G., Lubarsky, B., Kieber, J.J., Rothenberg, M., and Ecker, J.R. (1995). Genetic analysis of ethylene signal transduction in *Arabidopsis thaliana*: five novel mutant loci integrated into a stress response pathway. Genetics *139*, 1393–1409.

Rubery, P.H. (1990). Phytotropins: receptors and endogenous ligands. Soc. Exp. Bot. *44*, 119–146.

Ryan, K.A., Garraway, L.A., Pescoteaux, A., Turco, S.J., and Beverly, S.M. (1993). Isolation of virulence genes directing surface glycosylphosphatidylinositol synthesis by functional complementation of *Leishmania*. Proc. Natl. Acad. Sci. USA *90*, 8609–8613.

Schardl, C.L., Byrd, A.D., Benzion, G., Altschuler, M.A., Hildebrand D.F., and Hunt, A.G. (1987). Design and construction of a versatile system for the expression of foreign genes in plants. Gene *61*, 1–11.

Schwark, A., and Bopp, M. (1993). Interaction of ethylene and auxin in the regulation of hook growth. II. The role for ethylene in different growing regions of the hypocotyl hook of *Phaseolus vulgaris*. J. Plant Physiol. *142*, 585–592.

Schwark, A., and Schierle, J. (1992). Interaction of ethylene and auxin in the regulation of hook growth. I. The role of auxin in different growing regions of the hypocotyl hook of *Phaseolus vulgaris*. J. Plant Physiol. *140*, 562–570.

Silk, W.H., and Erickson, R.O. (1978). Kinematics of hypocotyl curvature. Am. J. Bot. *65*, 310–319.

Smith, H. (1995). Physiological and ecological function within the phytochrome family. Annu. Rev. Plant Physiol. *46*, 289–315.

Tamas, I.A. (1987). Hormonal regulation of apical dominance. In Plant Hormones and Their Role in Plant Growth and Development, P.J. Davies, ed. (Hingham, Massachusetts: Martin Nijhoff Publishers), pp. 393–410.

Tenover, F.C., Filpula, C., Phillips, K.L., and Plorde, J.J. (1988). Cloning and sequencing of a gene encoding an aminoglycoside 6'-*N*-acetyltransferase from an R factor of *Citrobacter diversus*. J. Bact. *170*, 471–473.

Tercero, J.C., Riles L.E., and Wickner, R.B. (1992). Localized mutagenesis and evidence for post-transcriptional regulation of MAK3. J. Biol. Chem. *267*, 20270–20276.

Timpte, C., Lincoln, C., Pickett, F.B., Turner, J., and Estelle, M. (1995). The *AXR1* and *AUX1* genes of *Arabidopsis* function in separate auxin-response pathways. Plant J. *8*, 561–569.

Tribioli, C., Mancini, M., Plassart, E., Bione, S., Rivella, S., Sala, C., Torri, G., and Toniolo, D. (1994). Isolation of new genes in distal Xq28: transcriptional map and identification of a human homologue of the ARD1 *N*-acetyl transferase of *Saccharomyces cerevisiae*. Hum. Mol. Genet. *3*, 1061–1067.

Wyatt, R.E., Ainley, W.M., Nagao, R.T., Conner T.W., and Key, J.L. (1993). Expression of the *Arabidopsis AtAux 2–11* auxin responsive gene in transgenic plants. Plant Mol. Biol. *22*, 731–749.

Yoshikawa, A., Isono, S., Sheback, A., and Isono, K. (1987). Cloning and nucleotide sequencing of the genes *rimI* and *rimJ* which encode enzymes acetylating ribosomal proteins S18 and S5 of *Escherichia coli* K12. Mol. Gen. Genet. *209*, 481–488.

Zimmer, W., Aparicio, C., and Elmerich, C. (1991). Relationships between tryptophan biosynthesis and indole-3-acetic acid production in *Azospirillum*: identification and sequencing of a trpGDC cluster. Mol. Gen. Genet. *299*, 41–51.

GenBank Accession Numbers

Accession numbers for the sequences reported in this paper are as follows: *HLS1* gene amino acid sequence, U50400; cDNA sequence, U50399.