Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 7197–7202, June 1998
Plant Biology

High temperature promotes auxin-mediated hypocotyl elongation in *Arabidopsis*

WILLIAM M. GRAY*, ANDERS ÖSTIN†, GÖRAN SANDBERG†, CHARLES P. ROMANO‡, AND MARK ESTELLE*§

*Department of Biology, Indiana University Bloomington, IN 47405; †Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Science, S-901 83 Umeå, Sweden; and ‡Cereon Genomics LLC, Cambridge, MA 02139

Edited by Bernard O. Phinney, University of California, Los Angeles, CA, and approved April 9, 1998 (received for review September 24, 1997)

ABSTRACT **Physiological studies with excised stem segments have implicated the plant hormone indole-3-acetic acid (IAA or auxin) in the regulation of cell elongation. Supporting evidence from intact plants has been somewhat more difficult to obtain, however. Here, we report the identification and characterization of an auxin-mediated cell elongation growth response in *Arabidopsis thaliana*. When grown in the light at high temperature (29°C), *Arabidopsis* seedlings exhibit dramatic hypocotyl elongation compared with seedlings grown at 20°C. This temperature-dependent growth response is sharply reduced by mutations in the auxin response or transport pathways and in seedlings containing reduced levels of free IAA. In contrast, mutants deficient in gibberellin and abscisic acid biosynthesis or in ethylene response are unaffected. Furthermore, we detect a corresponding increase in the level of free IAA in seedlings grown at high temperature, suggesting that temperature regulates auxin synthesis or catabolism to mediate this growth response. Consistent with this possibility, high temperature also stimulates other auxin-mediated processes including auxin-inducible gene expression. Based on these results, we propose that growth at high temperature promotes an increase in auxin levels resulting in increased hypocotyl elongation. These results strongly support the contention that endogenous auxin promotes cell elongation in intact plants.**

Plant growth and development are regulated by both external environmental factors, such as light quantity and quality, and by a set of endogenous regulators collectively known as the phytohormones. In many instances, these two sets of determinants interact with one another. For example, phytohormones mediate many of the stress responses that facilitate adaptation to environmental changes. One of the most studied examples of this occurs during periods of drought stress, when the phytohormone abscisic acid mediates stomatal pore closure, resulting in reduced transpirational water loss (1).

The *Arabidopsis* hypocotyl is a useful model for investigating the regulation of plant growth. Hypocotyl elongation in *Arabidopsis* is the result of regulated cell expansion that is under both environmental and hormonal controls (2). In the absence of light, seedlings undergo skotomorphogenic development. The cotyledons remain closed, an apical hook is formed, and the hypocotyl becomes greatly elongated. Light induces the photomorphogenic developmental program resulting in cotyledon expansion, leaf development, the initiation of photosynthesis, and limited hypocotyl growth. In addition to light, all of the known plant hormones have been implicated in the control of hypocotyl elongation. Brassinosteroids, auxin, and gibberellins (GAs) promote hypocotyl growth, whereas cytokinins and abscisic acid (ABA) have growth inhibitory effects (3–7). Ethylene both positively and negatively regulates hypocotyl elongation. In darkness, ethylene inhibits elongation, but at least under some conditions in the light, ethylene promotes elongation (8). Precisely how this complex array of hormonal controls is integrated with regulation by light and other environmental factors is poorly understood. We have identified an additional environmental control of hypocotyl growth. We find that high temperature promotes dramatic hypocotyl elongation in light-grown *Arabidopsis* seedlings. This temperature-induced growth response depends on indole-3-acetic acid (IAA or auxin).

The ability of exogenous auxin to promote cell elongation in excised stem and hypocotyl segments has been studied extensively (9). This strong growth-promoting property of auxin provided the basis for its identification as the first known plant hormone by Went nearly 70 years ago. The role of auxin in regulating cell expansion in intact plants is somewhat less clear, however, because application of exogenous auxin often does not stimulate elongation. The inability of exogenous auxin to stimulate prolonged cell expansion led to the suggestion that auxin plays little if any role in regulating plant growth by cell elongation (10). More recent studies, however, have found a correlation between endogenous auxin levels and stem and/or hypocotyl length (4, 11, 12). Furthermore, by using a continuous auxin infusion system, Yang *et al.* (13) were able to demonstrate that exogenous IAA can promote prolonged stem growth in pea seedlings. Our findings that temperature-induced hypocotyl elongation (*i*) correlates with an increase in IAA levels, (*ii*) depends on the auxin transport and response systems, and (*iii*) is diminished in transgenic seedlings containing reduced levels of free IAA strongly support the contention that endogenous auxin is capable of promoting cell elongation in intact plants.

MATERIALS AND METHODS

Plant Material and Growth Conditions. The *Arabidopsis thaliana etr1–1*, *ein2–1*, *aba-1*, and *ga4–1* mutant lines were obtained from the Arabidopsis Biological Resource Center at Ohio State University and have been described previously (14–17). *det2–1* seed was the generous gift of J. Chory (18). The auxin response and transport mutants used in this study also have been described elsewhere (19–21). The *aba-1* and *ga4–1* mutations are in the Landsberg erecta background. Wild-type Landsberg erecta seedlings were used in experiments involving these two mutants. All other wild-type, mutant, and transgenic lines used in this study are in the Columbia (Col-0) ecotype. Transgenic plants expressing the bacterial IAA–lysine synthase (*iaaLys*) gene originally were generated in the RLD background with pMON690 as described (4, 22, 23). The *35s-iaaLys* transgene then was backcrossed exten-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

This paper was submitted directly (Track II) to the *Proceedings* office.
Abbreviations: GAs, gibberellins; ABA, abscisic acid; IAA, indole-3-acetic acid; GUS, β-glucuronidase; NPA, naphthylphthalamic acid.
§To whom reprint requests should be addressed. e-mail: mestelle@bio.indiana.edu.

sively into Col-0. Expression of the iaaLys protein was confirmed by immunoblot analysis. As previously reported for other plant species expressing this IAA-conjugating enzyme (22), we found that the *35s-iaaLys Arabidopsis* line used in this study contains a lower level of free (unconjugated) IAA than corresponding wild-type seedlings (see Table 2 below). Plants harboring the *SAUR-GUS* and *P. sativum pIAA4-GUS* reporter genes were the gifts of P. Green (Michigan State Univ.) and A. Theologis (Plant Gene Expression Center), respectively. The 35S-GUS reporter line CS6151 was obtained from the Arabidopsis Biological Resource Center at Ohio State University. Plants were grown under sterile conditions in vertical Petri plates containing ATS nutrient media (19). Hormones and auxin transport inhibitors were added after autoclaving. Plants were grown in incubators at 20°C or 29°C under constant light at an intensity of 85 μE m^{-2} sec^{-1}. Data were collected from 9-day-old seedlings unless noted otherwise.

Measurement of Hypocotyl and Cell Length. Hypocotyl lengths of 9-day-old seedlings were measured from shadow projections with a Simmon Omega variable condenser. For *axr1–12* and naphthylphthalamic acid (NPA)-treated seedlings, the cotyledons were excised to facilitate the measurement of extremely short hypocotyls. Epidermal cell length was measured by using a Zeiss phase contrast microscope equipped with an optical micrometer. All measurements of hypocotyl and cell length are presented as the mean ± SEM.

Measurement of Endogenous IAA Levels. Seedlings were grown as described above. The hypocotyls from pools of 25 seedlings were excised, weighed, and frozen under liquid nitrogen. Quantitative measurements of endogenous IAA were performed on 1–10 mg of tissue by selected reaction monitoring MS as described (24). Calculation of isotopic dilution was based on the addition of 5 pg of [13C6]IAA/mg of tissue. Special care was used in sample preparation in an ultra clean environment, and random analyses of blank samples were performed to avoid contamination problems associated with analysis of extremely low amounts of IAA. Measurements are presented as the mean of five to six individual samples each consisting of material from 25 seedlings.

β-Glucuronidase (GUS) Staining and Quantitation. *SAUR-GUS* and *pIAA4-GUS* expression was visualized by incubating seedlings in an X-Gluc (Sigma) solution overnight at 37°C and destaining with 70% ethanol as described (25). To quantitate GUS activity, crude extracts were prepared from the shoots of 9-day-old seedlings grown at 20°C or 29°C, and GUS activity was assayed by using a spectrophotometric assay (26). Reported values are the average of at least four independent assays.

RESULTS

High Temperature Promotes Hypocotyl Elongation in Light-Grown Seedlings. The effect of sustained high temperature on plant growth and development was examined by growing wild-type *Arabidopsis* seedlings at 29°C. Germination, growth, and development proceeded relatively normally at 29°C with the plants progressing through the entire life cycle and producing viable seed. Development was slightly accelerated at high temperature, with lateral roots and primary leaves initiating 1–2 days earlier than plants grown at 20°C. Plants grown at 29°C also bolt, flower, and senesce significantly earlier than control plants (data not shown). In young seedlings, the most noticeable affect of high temperature was a dramatic increase in hypocotyl and petiole length in light-grown plants. The hypocotyls of wild-type seedlings grown at 29°C were 4- to 5-fold longer than the hypocotyls of 20°C grown seedlings (Fig. 1 *a* and *f*).

Temperature-Induced Hypocotyl Elongation Is Auxin-Dependent. Several studies have implicated auxin in hypocotyl elongation (4, 9, 13). To investigate whether auxin was involved in mediating hypocotyl elongation in response to growth at high temperature, auxin response mutants, auxin transport mutants, and lines with altered auxin levels were examined. The effects of mutations in the auxin response pathway on temperature-induced hypocotyl elongation were examined by using the *axr1–12* and *tir1–1* mutations. *AXR1* encodes a protein related to the N terminus of the ubiquitin-activating enzyme E1 and has been proposed to mediate auxin response by the modification of a key regulatory protein(s) by ubiquitin or a related protein (27, 28 21). The *axr1–12* mutation confers resistance to exogenous auxin, and mutants exhibit severe defects in auxin response (19). The *TIR1* gene encodes a member of a protein family that also has been implicated in ubiquitin-mediated processes, and genetic analysis suggests

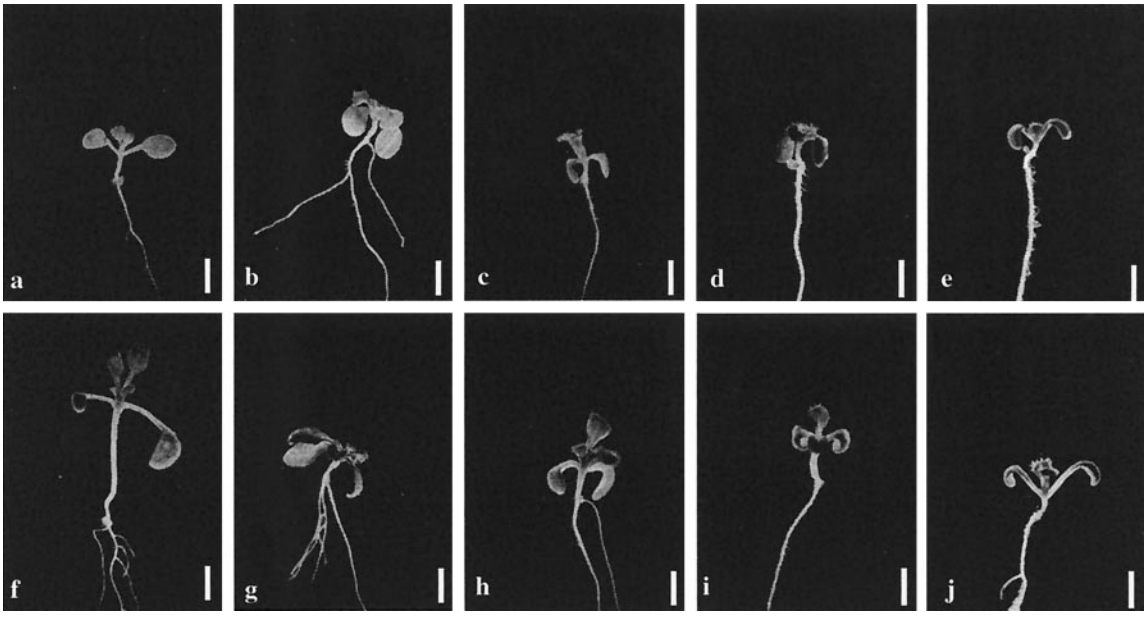

FIG. 1. Temperature-induced hypocotyl elongation in *Arabidopsis*. Nine-day-old *Arabidopsis* seedlings were grown under continuous light at either 20°C (*a–e*) or 29°C (*f–j*). Col (*a* and *f*); *axr1–12* (*b* and *g*); 35S-iaaLys (*c* and *h*); *tir3–1* (*d and i*); Col grown on medium supplemented with 1 μM NPA (*e* and *j*). (Bars = 2 mm.)

that TIR1 functions with AXR1 in the auxin response pathway (21). Mutations in *TIR1*, however, confer much less severe defects in auxin response than mutations in *AXR1*. When grown at 29°C, no temperature-induced hypocotyl elongation was observed for *axr1–12* mutant seedlings (Figs. 1 *b* and *g* and 2) whereas the hypocotyls of the *tir1–1* mutant seedlings elongated slightly but significantly less than those of wild type (Fig. 2). Similarly, transgenic plants expressing the bacterial *iaaLys* IAA-conjugating enzyme also were impaired severely in hypocotyl elongation in response to growth at high temperature (Figs. 1 *c* and *h* and 2). Expression of the *iaaLys* gene results in a modest reduction in the level of free IAA (ref.22 and see below). To investigate the effects of impaired auxin transport on temperature-induced hypocotyl elongation, the *tir3–1* mutant was examined. *tir3–1* mutants are defective in polar auxin transport (20). Like the auxin response mutants and 35S-*iaaLys* seedlings, the *tir3–1* mutant exhibited reduced temperature-induced hypocotyl elongation (Figs. 1 *d* and *i* and 2). Consistent with this result, the auxin transport inhibitor NPA was able to abolish hypocotyl elongation in response to high temperature (Figs. 1 *e* and *j* and 2). Together, these data indicate that temperature-induced hypocotyl elongation depends on both the auxin response and the auxin transport systems.

To investigate the possibility that the temperature-induced hypocotyl elongation response simply was delayed rather than impaired in the auxin-deficient lines, the hypocotyls of wild-type, *axr1–12*, and 35S-*iaaLys* seedlings grown at 20°C and 29°C were measured over a 2-week period. As shown in Fig. 3 for the wild-type and 35S-*iaaLys* seedlings, hypocotyl length at both temperatures plateaued before day 7. Similar results were obtained with the *axr1–12* mutant (data not shown).

Auxin has been implicated in cell division as well as cell elongation. Hypocotyl growth during normal *Arabidopsis* development occurs primarily, if not entirely, by cell elongation (2). To confirm that temperature-induced hypocotyl elongation also was caused by increased cell expansion rather than increased cell division, we measured hypocotyl epidermal cells from wild-type, *axr1–12*, and 35S-*iaaLys* seedlings grown at

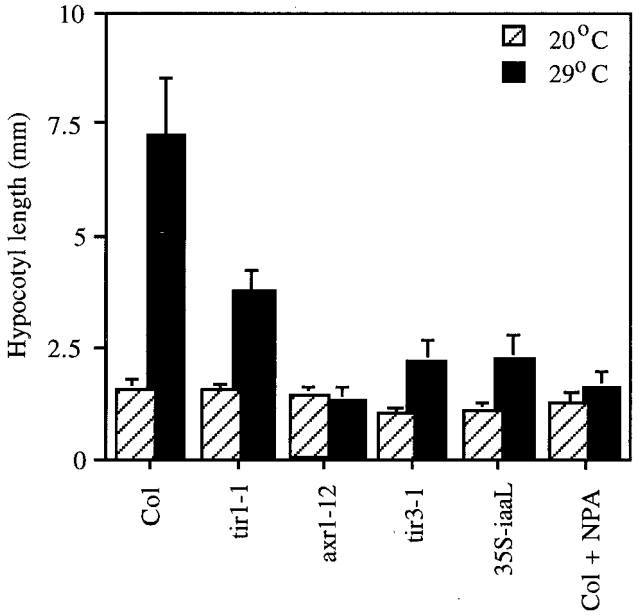

FIG. 2. Temperature-induced hypocotyl elongation is auxin-dependent. Wild-type (Col), transgenic 35S-iaaLys and mutant lines were grown at 20°C or 29°C under constant light. Hypocotyls were measured from 9-day-old seedlings. Growth medium was supplemented with 1 μM NPA where noted. Data represent mean ± SEM (n = 10).

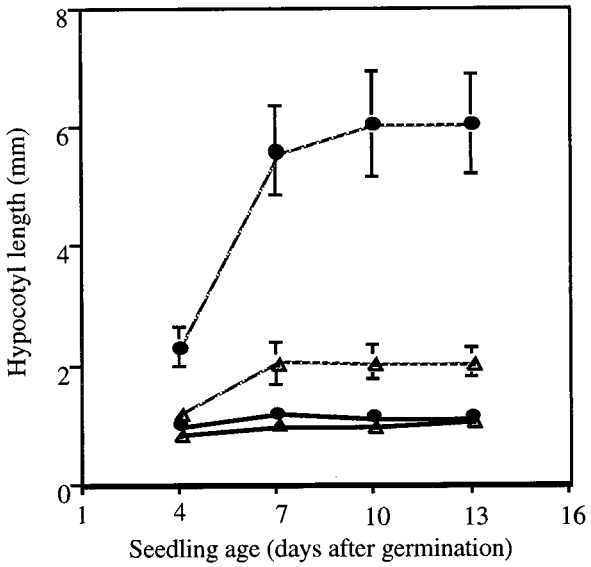

FIG. 3. Hypocotyl elongation over time. Wild-type (Col) and 35S-*iaaLys* lines were grown at 20°C or 29°C under constant light. Hypocotyls were measured 4, 7, 10, and 13 days after germination. Data represent mean ± SEM (n = 10). Col 20°C, solid line with circles; Col 29°C, broken line with circles; *iaaLys* 20°C, solid line with triangles; and *iaaLys* 29°C, broken line with triangles.

20°C and 29°C. Hypocotyl epidermal cells of wild-type seedlings grown at high temperature were ≈3.5-fold longer than the hypocotyl cells of 20°C grown control seedlings (Table 1). In contrast, high temperature growth resulted in less than a 2-fold increase in hypocotyl epidermal cell length for 35S-*iaaLys* seedlings and had no effect on the hypocotyl epidermal cell length of *axr1–12* mutants (Table 1). The temperature-induced changes in cell length for the wild-type, 35S-*iaaLys*, and *axr1–12* lines correlate well with the observed increases in hypocotyl length, suggesting that auxin-mediated cell expansion is primarily responsible for temperature-induced hypocotyl elongation.

Temperature-Induced Hypocotyl Elongation Is Not Affected by Mutations in the ABA, GA, or Ethylene Pathways. Ethylene, GA, and brassinosteroids also have been shown to positively regulate hypocotyl growth. The effects on temperature-induced hypocotyl elongation of mutations in the bisynthetic and/or response pathways of these hormones, as well as of ABA (which mediates several physiological stress responses in vegetative tissues), were examined. Mutations in the GA and ABA biosynthetic pathways and in the ethylene response pathway had essentially no effect on temperature-induced hypocotyl elongation (Fig. 4). Mutations in the *DET2* gene, however, which encodes an enzyme required for brassinosteroid biosynthesis, resulted in a moderate reduction in temperature-induced hypocotyl elongation (Fig. 4).

High Temperature Does Not Promote Hypocotyl Elongation in Etiolated Seedlings. To investigate whether the reduced temperature-induced hypocotyl elongation of auxin pathway mutants is caused by a general defect in cell expansion or a

Table 1. Effect of temperature on hypocotyl epidermal cell length

Background	Growth temperature, °C	Cell length, μm*
Col	20	69.7 ± 11.9
	29	245.4 ± 36.8
35S-iaaLys	20	65.6 ± 8.1
	29	104.9 ± 12.9
axr1-12	20	56.8 ± 10.8
	29	53.8 ± 11.4

*Data represent mean epidermal cell length ± SEM (n = 80).

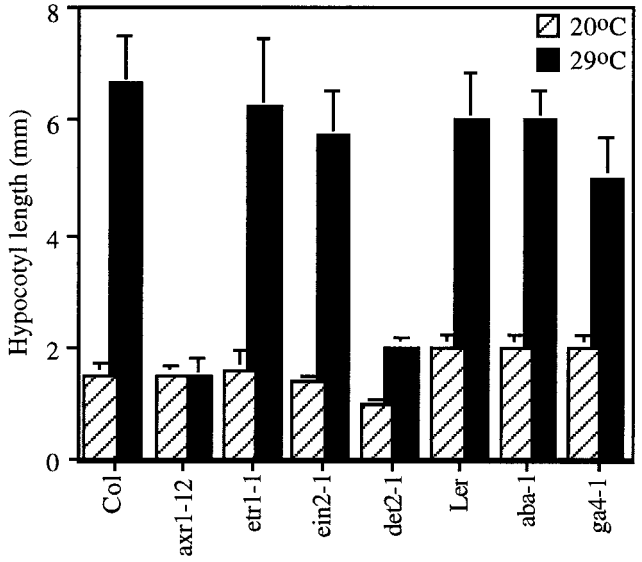

FIG. 4. Temperature-induced hypocotyl elongation in hormone mutants. Wild-type and mutant lines were grown at 20°C or 29°C under constant light. Hypocotyls were measured from 9-day-old seedlings. The *axr1–12*, *etr1–1, ein2–1,* and *det2–1* mutations are in the Col background. *aba-1* and *ga4–1* are in the Ler background. Data represent mean ± SEM (n = 10).

specific defect in response to high temperature, hypocotyl elongation in etiolated seedlings was examined. As reported (19), the hypocotyls of etiolated *axr1–12* seedlings were slightly shorter than those of wild type. We also observed that mutations in *TIR1*, as well as expression of the *iaaLys* transgene, also caused modest reductions in hypocotyl length in dark-grown seedlings (Fig. 5). Nonetheless, when grown in darkness, the auxin response mutants and *iaaLys* plants still exhibited considerable hypocotyl elongation, suggesting that these plants are not defective in cell elongation per se but rather in cell elongation in response to high temperature.

In contrast to light-grown plants, high temperature did not stimulate hypocotyl elongation in dark-grown, wild-type seedlings. Instead, the hypocotyls of plants grown at 29°C were slightly shorter than those of plants grown at 20°C (Fig. 5). Similar to wild-type seedlings, the hypocotyls of auxin response mutants and *iaaLys* plants grown at high temperature

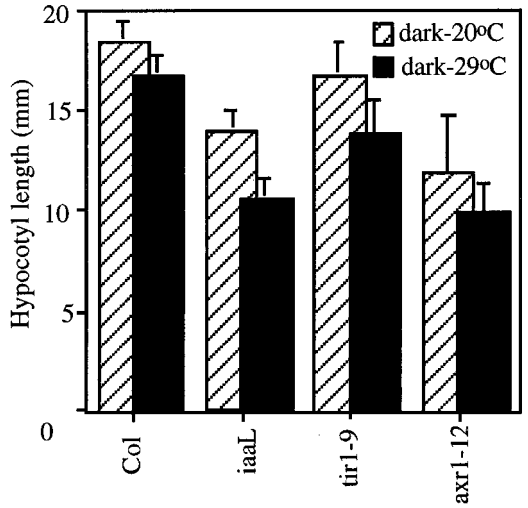

FIG. 5. Temperature-induced hypocotyl elongation is a light-specific growth response. Wild-type and mutant lines were grown at 20°C or 29°C in darkness. Hypocotyls were measured from 9-day-old seedlings. Data represent mean ± SEM (n = 10).

were slightly shorter than those of plants grown at 20°C (Fig. 5).

Seedlings Grown at High Temperature Contain Elevated IAA Levels. The physiological analysis described above clearly implicates auxin in playing an important role in the temperature-induced hypocotyl elongation growth response. One possible explanation is that high temperature promotes cell elongation by increasing IAA levels. Alternatively, temperature may modulate the activity of a component of the auxin response pathway or regulate a distinct pathway that merges with, or is regulated by, the auxin response pathway. To begin to explore these possibilities, the level of endogeonous IAA was measured in the hypocotyls of 9-day-old wild-type seedlings grown at 20°C and 29°C. Compared with seedlings grown at 20°C, a 1.75-fold increase in the concentration of free IAA was detected in the hypocotyls of seedlings grown at high temperature (Table 2).

The concentration of free IAA also increased in response to high temperature in *axr1* and *35s-iaaLys* seedlings (Table 2). In *axr1–3* hypocotyls, a 2.2-fold increase in free IAA was detected in response to temperature. Like *axr1–12* mutants, plants carrying the *axr1–3* mutant allele exhibit no temperature-induced hypocotyl elongation (data not shown). The absence of temperature-induced hypocotyl elongation despite a significant increase in IAA concentration is consistent with AXR1 functioning in auxin response.

The level of free IAA in *35s-iaaLys* hypocotyls was modestly but significantly less than that of wild-type seedlings grown at 20°C (Table 2). High temperature promotes a relative increase in free IAA concentration in *35s-iaaLys* hypocotyls (1.64-fold) that is nearly equivilent to that seen with wild type (1.75-fold). However, the absolute level of free IAA in *35s-iaaLys* hypocotyls at 29°C is significantly less than wild type. Thus, this finding is consistent with the suggestion that *35s-iaaLys* seedlings are impaired in the temperature-induced hypocotyl elongation growth response because they contain less free IAA.

High Temperature Stimulates Additional Auxin-Dependent Responses. Because seedlings grown at high temperature contain higher IAA levels than seedlings grown at 20°C, we examined the effect of high temperature on additional auxin-inducible processes to investigate whether elevated IAA levels conferred a corresponding increase in the activity of the auxin response pathway. The auxin-inducible *pIAA4-GUS* and *SAUR-AC1-GUS* reporter genes were used to investigate the effect of high temperature on auxin-dependent gene expression. Histochemical staining for β-GUS activity indicated an increase in both *pIAA4-GUS* (Fig. 6) and *SAUR-AC1-GUS* (data not shown) expression in plants grown at 29°C relative to plants grown at 20°C. Quantitation of GUS activity in seedling shoots revealed 3- to 4-fold and ≈2-fold increases in *pIAA4-* and *SAUR-AC1*-driven *GUS* expression, respectively (Table 3). In contrast, the activity of a 35*s*-GUS fusion was not affected by high temperature. In addition to increased expression levels, the 29°C-grown plants exhibited an expanded range of expression for both reporters. Although *GUS* expression from the reporters was confined largely to the stems and leaves of plants grown at 20°C, expression also was seen throughout the root

Table 2. Effect of temperature on free IAA levels in *Arabidopsis* hypocotyls

Line	Free IAA*		Fold increase
	20°C	29°C	
Col	78.1 (5.9)	137 (11.1)	1.75
35s-iaaLys	61.6 (7.9)	101.2 (14.3)	1.64
axr1-3	70.9 (10.7)	156.2 (17.8)	2.2

*IAA levels are expressed as pg of IAA/mg fresh weight of tissue. Values represent the average of at least five independent assays. SDs are indicated in parenthesis.

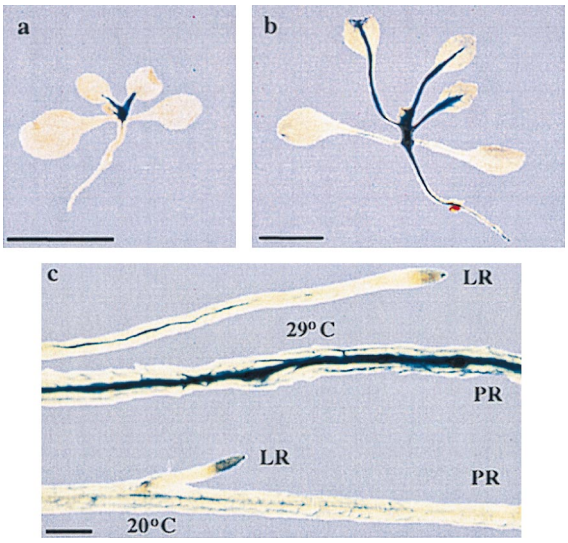

FIG. 6. High temperature stimulates auxin-inducible gene expression. Wild-type (Col) seedlings carrying a *pIAA4-GUS* reporter gene were grown for 10 days at 20°C or 29°C on ATS nutrient media and stained for *GUS* expression. (*a*) 20°C; (*b*) 29°C; (*c*) *pIAA4-GUS* expression in the roots of 10-day-old wild-type seedlings grown at 29°C (*Upper*) or 20°C (*Lower*). LR, lateral root; PR, primary root. (Bars: *a* and *b* = 5 mm, *c* = 0.5 mm.)

system when the plants were grown at high temperature (Fig. 6). The increase in auxin-inducible gene expression observed in plants grown at 29°C is consistent with the hypothesis that a temperature-mediated increase in IAA levels promotes increased auxin signaling at high temperature.

DISCUSSION

When grown in the light at high temperature, *Arabidopsis* seedlings display dramatic hypocotyl elongation. This elongation is caused by increased cell expansion and correlates with an increase in the level of IAA. The role of auxin in cell expansion has been studied extensively (9). Application of auxin to excised stem and coleoptile segments induces rapid cell elongation. Although similar treatment of intact seedlings does not cause dramatic increases in cell size, several studies provide supporting evidence for a role of auxin in cell elongation. Notably, plants that overproduce IAA, including the *Arabidopsis rooty* mutant (*rty/sur1*) and transgenic lines expressing bacterial auxin biosynthesis genes, display increased hypocotyl elongation resulting from increased cell expansion (4, 11). Also, the auxin transport inhibitor NPA recently was shown to inhibit hypocotyl elongation in light-grown *Arabidopsis* seedlings (29). Our finding that the temperature-induced hypocotyl elongation growth response is an auxin-dependent process supports these earlier studies and provides a simple assay for investigating auxin-mediated cell elongation.

Table 3. Effect of high temperature on auxin-inducible gene expression

	GUS activity*		
	35s-GUS	pIAA4-GUS	SAUR-AC1-GUS
Temperature			
Col - 20°C	4.57×10^4	1.67×10^2	3.24×10^4
Col - 29°C	5.13×10^4	6.76×10^2	5.91×10^4
Fold increse	1.12×	4.05×	1.83×

*Extracts were prepared from the stems and leaves of 9-day-old seedlings grown at the appropriate temperature. Units of β-GUS activity are expressed as picomoles pPNG cleaved/mg extract/min. Values represent the average of at least four independent assays. SDs ≤ 30% of the mean.

Temperature-induced hypocotyl elongation is only observed with light-grown seedlings. Curiously, the aforementioned effects of auxin overproduction and NPA on hypocotyl elongation also were detected only with light-grown plants (4, 11, 29). Jensen *et al.* (29) proposed that auxin plays a much greater role in *Arabidopsis* hypocotyl elongation in light-grown plants than it does in etiolated seedlings. Our results (Fig. 5) are consistent with this hypothesis. However, we cannot rule out the possibility that the cellular elongation rate is at its maximum during etiolated growth such that high temperature cannot promote additional cellular expansion. Nonetheless, the isolation of the *prc1* mutant, which is specifically impaired in hypocotyl elongation during etiolated growth, clearly demonstrates that distinct genetic pathways regulate hypocotyl cell elongation in light- and dark-grown seedlings (30). Thus, it is possible that auxin regulates only one of these pathways, or alternatively, regulates the two pathways differentially.

A recent report demonstrated that exogenous auxin was capable of promoting mild increases in hypocotyl length in light-grown seedlings grown on low nutrient medium (8). These increases were found to be caused by auxin-mediated stimulation of ethylene biosynthesis and were not a direct effect of auxin treatment. In contrast to these findings, temperature-induced hypocotyl elongation is highly auxin-dependent and is unaffected by mutations in the ethylene response pathway, indicating that temperature-induced hypocotyl elongation involves a distinct regulatory pathway. Mutations in the GA and ABA biosynthetic pathways also did not confer defects in hypocotyl elongation in response to high temperature. The *det2–1* brassinosteroid mutant displayed a moderate defect in temperature-induced hypocotyl elongation similar to some of the less severe auxin pathway mutants. Brassinosteroids are known to have a role in hypocotyl elongation on both light- and dark-grown seedlings (31). Thus, it is possible that some functional interactions occur between auxins and brassinosteroids that are required for temperature-induced hypocotyl elongation. Alternatively, because the *det2–1* mutation confers a much more severe dwarf phenotype than any of the auxin pathway mutants examined, the reduced temperature-induced elongation of *det2* mutants may simply be a reflection of a general cell expansion defect.

The increase in free IAA levels observed when plants are grown at 29°C suggests that temperature may directly regulate auxin levels to achieve this growth response in *Arabidopsis* seedlings. Because the level of IAA conjugates also is elevated in these plants (data not shown), regulation presumably occurs at the level of IAA synthesis. An alternative possibility is that temperature negatively regulates IAA catabolism. Although it is formally possible that the increase in IAA levels is a secondary effect of growth at high temperature rather than the actual mediator of the response, several lines of evidence suggest that temperature-induced hypocotyl elongation is due to elevated endogenous auxin levels. First, the growth response is abolished in auxin response and transport mutants. Second, the response can be inhibited pharmacologically with auxin transport inhibitors. Third, transgenic *iaaLys* seedlings containing lower than normal IAA levels exhibit a diminished response. Fourth, previous studies have reported hypocotyl elongation in mutant and transgenic lines that contain higher than normal IAA levels (4, 11). And fifth, the elevated expression levels and expanded expression patterns of the auxin-inducible reporters *pIAA4-GUS* and *SAUR-AC1-GUS* suggest that a corresponding increase in auxin signaling does indeed occur at high temperature. We also have observed changes in the number and distribution of lateral roots in seedlings grown at high temperature (data not shown). Lateral root formation is also an auxin-dependent process.

We and others have shown that addition of exogenous auxin does not promote hypocotyl elongation at 20°C (ref. 10 and data not shown). One possible explanation for the failure of

exogenous auxin at low temperature to phenocopy the hypocotyl elongation seen at high temperature may be that increased auxin levels are necessary but not sufficient for increased cell elongation. Alternatively, the tissue specificity and timing of auxin synthesis and transport may be important factors for temperature-induced hypocotyl elongation.

The physiological relevance of temperature-induced hypocotyl elongation is unclear. One possibility is that increased elongation is an adaptive response to high temperature. Hypocotyl elongation elevates the photosynthetic and meristematic tissues away from the heat-adsorbing soil and may allow the plant to take better advantage of the cooling effect of moving air. This response could potentially provide the plant with an adaptive advantage under unfavorable conditions.

It seems unlikely that this temperature-dependent growth response is unique to *Arabidopsis*. There have been previous reports of temperature promoting hypocotyl elongation in various plants. In cotton seedlings, for example, hypocotyl length was found to increase as a function of soil temperature (32). Whether this growth response is auxin-mediated as it is in *Arabidopsis* remains to be seen. The identification of temperature-induced hypocotyl elongation as an auxin-mediated growth response provides a strong, easily scorable phenotype that may serve as a useful assay for future physiological and genetic investigations. This simple and rapid assay should greatly facilitate the genetic and molecular analysis of auxin-mediated cell elongation as well as provide insight into the environmental regulation of auxin metabolism.

We thank the staff of the Ohio State Stock Center and Drs. J. Chory, P. Green, and A. Theologis for seed stocks; Dr. Phil Jensen and other members of the Estelle lab for advice and support; and Dr. Roger Hangarter for advice and critical review of this manuscript. This work was supported by the National Institutes of Health (PHS-GM 43644 to M.E. and GM 18680 to W.M.G.) and by NFR/SJFR and The European Commission DG XII Biotechnology Program (to A.O. and G.S.).

1. Blatt, M. R. & Thiel, G. (1993) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **44,** 543–567.
2. Gendreau, E., Traas, J., Desnos, T., Grandjean, O., Caboche, M. & Hofte, H. (1997) *Plant. Physiol.* **114**, 295–305.
3. Clouse, S. D. (1996) *Plant J.* **10,** 1–8.
4. Romano, C. P., Robson, P. R. H., Smith, H., Estelle, M. & Klee, H. (1995) *Plant Mol. Biol.* **27**, 1071–1083.
5. Jacobsen, S. E. & Olszewski, N. E. (1993) *Plant Cell* **5,** 887–896.
6. Chaudhury, A. M., Letham, S., Craig, S. & Dennis, E. S. (1993) *Plant J.* **4,** 907–916.
7. Koornneef, M. & Karssen, C. M. (1994) in *Arabidopsis*, eds. Meyerowitz, E. M. & Somerville, C. R. (Cold Spring Harbor Lab. Press, Cold Spring Harbor, NY), pp. 313–334.
8. Smalle, J., Haegman, M., Kurepa, J., Van Montagu, M. & Van Der Straeten, D. (1997). *Proc. Natl. Acad. Sci. USA* **94,** 2756–2761.
9. Cleland, R. E. (1995) in *Plant Hormones: Physiology, Biochemistry, and Molecular Biology*, ed. Davies, P. J. (Kluwer, Dordrecht, The Netherlands), pp. 214–227.
10. Hanson, J. B. & Trewavas, A. J. (1982) *New Phytol.* **90,** 1–18.
11. Boerjan, W., Cervera, M.-T., Delarue, M., Beeckman, T., Dewitte, W., Bellini, C., Caboche, M., Van Onckelen, H., Van Montagu, M. & Inze, D. (1995) *Plant Cell* **7,** 1405–1419.
12. Law, D. M. & Davies, P. J. (1990) *Plant Physiol.* **93,** 1539–1543.
13. Yang, T., Law, D. M. & Davies, P. J. (1993) *Plant Physiol.* **102,** 717–724.
14. Bleecker, A. B., Estelle, M. A., Somerville, C. & Kende, H. (1988) *Science* **241,** 1086–1089.
15. Guzman, P. & Ecker, J. (1990) *Plant Cell* **2,** 513–524.
16. Koornneef, M., Jorna, M. L., Brinkhorst-van der Swan D. L. V. C. & Karssen, C. M. (1982) *Theor. Appl. Genet.* **61,** 385–393.
17. Talon, M., Koornneef, M. & Zeevaart, J. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7983–7987.
18. Chory, J. Nagpal, P. & Peto, C. (1991) *Plant Cell* **3,** 445–460.
19. Lincoln, C., Britton, J. H. & Estelle, M. (1990) *Plant Cell* **2,** 1071–1080.
20. Ruegger, M., Dewey, E., Hobbie, L., Brown, D., Bernasconi, P., Turner, J., Muday, G. & Estelle, M. (1997) *Plant Cell* **9,** 745–757.
21. Ruegger, M., Dewey, E., Gray. W. M., Hobbie, L., Turner, J. & Estelle, M. (1998) *Genes Dev.* **12,** 198–207.
22. Romano, C. P., Hein, M. B. & Klee, H. J. (1991) *Genes Dev.* **5,** 438–446.
23. Valvekens, D., van Montagu, M. & Van Lijsebettens, M. (1992) *Proc. Natl. Acad. Sci. USA* **85,** 5536–5540.
24. Edlund, A., Eklof, S., Sundberg, B., Moritz, T. & Sandberg, G. (1995) *Plant Physiol.* **108,** 1043–1047.
25. Stomp, A.-M. (1991) in *GUS Protocols*, ed. Gallagher, S. R. (Academic, London), pp. 103–113.
26. Herrera-Estrella, L. & Simpson, J. (1988) *in Plant Molecular Biology: A Practical Approach*, ed. Shaw, C. H. (IRL Press, Oxford), pp. 301–303.
27. Leyser, H. M. O., Lincoln, C., Timpte, C., Lammer, D., Turner, J. & Estelle, M. (1993) *Nature (London)* **364,** 161–164.
28. Lammer, D., Mathias, N., Laplaza, J. M., Jiang, W., Liu, Y., Callis, J., Goebl, M. & Estelle, M. (1998) *Genes Dev.*, **12,** 914–926.
29. Jensen, P. J., Hangarter, R. P. & Estelle, M. (1998) *Plant Physiol.* **116,** 455–462.
30. Desnos, T., Orbovic', V., Bellini, C., Kronenberger, J., Caboche, M., Traas, J. & Hofte, H. (1996) *Development (Cambridge, U.K.)* **122,** 683–693.
31. Kauschmann, A., Jessop, A., Koncz, C., Szerkes, M., Willmitzer, L. & Altmann, T. (1996) *Plant J.* **9,** 710–713.
32. Chu, Y. N., Coble, C. G. & Jordan, W. R. (1991) *Crop Sci.* **31,** 410–415.