Hormonal Interactions in the Control of Arabidopsis Hypocotyl Elongation[1]

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The Arabidopsis hypocotyl, together with hormone mutants and chemical inhibitors, was used to study the role of auxin in cell elongation and its possible interactions with ethylene and gibberellin. When wild-type Arabidopsis seedlings were grown on media containing a range of auxin concentrations, hypocotyl growth was inhibited. However, when *axr1-12* and 35S-*iaaL* (which have reduced auxin response and levels, respectively) were grown in the same conditions, auxin was able to promote hypocotyl growth. In contrast, auxin does not promote hypocotyl growth of *axr3-1,* which has phenotypes that suggest an enhanced auxin response. These results are consistent with the hypothesis that auxin levels in the wild-type hypocotyl are optimal for elongation and that additional auxin is inhibitory. When ethylene responses were reduced using either the ethylene-resistant mutant *etr1* or aminoethoxyvinylglycine, an inhibitor of ethylene synthesis, auxin responses were unchanged, indicating that auxin does not inhibit hypocotyl elongation through ethylene. To test for interactions between auxin and gibberellin, auxin mutants were grown on media containing gibberellin and gibberellin mutants were grown on media containing auxin. The responses were found to be the same as wild-type Arabidopsis seedlings in all cases. In addition, 1 μM of the auxin transport inhibitor 1-naphthylphthalmic acid does not alter the response of wild-type seedlings to gibberellin. Double mutants were made between gibberellin and auxin mutants and the phenotypes of these appear additive. These results indicate that auxin and gibberellin are acting independently in hypocotyl elongation. Thus auxin, ethylene, and gibberellin each regulate hypocotyl elongation independently.

Plant morphogenesis is governed by coordinated cell division and cell expansion. A large body of evidence suggests that plant hormones are involved in regulating these events but little is known about how they interact to bring about environmentally and developmentally regulated growth. The Arabidopsis hypocotyl is a convenient system in which to study the interaction of plant hormones in cell expansion. The Arabidopsis hypocotyl has a simple structure: From apex to base there are only approximately 20 cells and during its development, although the hypocotyl may increase more than 10-fold in length, there are no significant cortical or epidermal cell divisions (Gendreau et al., 1997). Hypocotyl elongation is very plastic and is influenced strongly by factors that regulate cell elongation in the adult plant such as light, plant hormones, temperature, and touch. There are mutants in Arabidopsis with altered hormone biosynthesis or signaling and many of these mutations affect hypocotyl elongation. With the combination of this simple structure and the availability of hormone mutants, it is possible to use this system to discover more about the interactions between hormones in the regulation of cell elongation.

The plant hormone auxin is involved in diverse developmental processes including cell enlargement, vascular tissue differentiation, root initiation, gravitropic and phototropic responses, and apical dominance. Auxin has long been thought of as important in cell elongation. When added to isolated stem segments and coleoptiles, auxin is able to induce elongation. The response begins within 10 min and can result in a 5- to 10-fold increase in growth rate that can persist for days (Evans, 1985). Genetic approaches have identified many genes that are needed for a wild-type auxin response. The phenotypes of some of these mutants are consistent with a role for auxin in hypocotyl elongation. For example, mutations in *axr1* result in reduced responses to auxin (Lincoln et al., 1990; Leyser et al., 1993) and mutations in *axr3* have phenotypes consistent with an enhanced auxin response (Leyser et al., 1996; Rouse et al., 1998). Both of these mutants have shorter hypocotyls than wild-type seedlings. In transgenic 35S-*iaaL* plants, in which a bacterial enzyme is expressed that conjugates auxin to Lys, free auxin levels are reduced. Plants expressing this gene show reduced plant size, leaf size, apical dominance, fertility, and hypocotyl length in the light (Romano et al., 1991; Jensen et al., 1998).

In the hypocotyl, auxin appears to promote growth in some circumstances and to inhibit it in others. When intact seedlings are grown on media containing auxin, the usual response is an inhibition of hypocotyl elongation. This response suggests that the levels of auxin in the seedling are already optimal for elongation and addition of any more auxin makes the

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levels supra-optimal. There is one example of auxin promoting growth of intact seedlings. When seedlings are grown on very low-nutrient medium, exogenous auxin is able to promote hypocotyl elongation (Smalle et al., 1997). This elongation could happen because in such low-nutrient conditions, the seedling is not able to synthesize auxin at optimal levels. The role of auxin in hypocotyl elongation appears to be different in the light compared with the dark, with polar auxin transport being more important for hypocotyl elongation in light-grown seedlings (Jensen et al., 1998). It has been suggested that auxin could be inhibiting elongation through ethylene (Smalle et al., 1997) since auxin is known to be able to promote ethylene synthesis and ethylene is known to inhibit hypocotyl elongation (McKeon and Yang, 1995).

Like auxin, gibberellins are involved in the regulation of diverse developmental processes. Gibberellins can stimulate both cell division and stem elongation as well as induce germination and fruit setting. Many mutants in gibberellin biosynthesis and signaling have been identified. *ga1* and *ga4* are both mutants in gibberellin biosynthesis (Koorneef and van der Veen, 1980; Sun et al., 1992; Chiang et al., 1995) and have reduced levels of gibberellins. Both mutants have shorter hypocotyls than in the wild-type seedlings. *gai* and *spy* are both mutants in gibberellin signaling (Koorneef et al., 1985; Jacobsen and Olszewski, 1993; Jacobsen et al., 1996; Peng et al., 1997). Both GAI and SPY are thought to be negative regulators of gibberellin signal transduction that act early in the gibberellin signal transduction pathway. The phenotypes of the mutants are opposite because *gai* is a gain-of-function mutation, resulting in constitutive inhibition of the gibberellin response, and *spy* is a loss-of-function mutation, resulting in a constitutive gibberellin response. *gai* plants have short hypocotyls, are dwarfed, flower late, and are dark green, whereas *spy* plants show more stem elongation than wild-type plants, flower early, and are pale green. In the hypocotyl, it can be shown by using gibberellin-deficient and altered gibberellin response mutants that gibberellin regulates elongation in both the light and the dark. Exogenous gibberellin, however, promotes hypocotyl elongation only in light-grown hypocotyls; in the dark, the gibberellin response is close to saturation and gibberellin has little effect (Cowling and Harberd, 1999).

Both auxin and gibberellin are clearly important in hypocotyl elongation but it is unclear whether they act independently. Previous studies in pea have suggested that auxin and gibberellin may not be acting independently in the control of stem elongation. One study suggested that gibberellin may act, in part, by enhancing auxin action: The response to applied gibberellin was small in plants with low-auxin content (Yang et al., 1996). Another study, also in peas, obtained the opposite result: When auxin transport inhibitors were applied to elongating internodes of intact wild-type plants, the level of endogenous gibberellins was decreased below the application site (Ross, 1998). This response suggests that auxin could act by modulating gibberellin levels. In the pea stem, elongation is a result of both cell elongation and cell division, whereas in the hypocotyl there is only cell elongation.

We have used the Arabidopsis hypocotyl as a model system, together with hormone mutants, to investigate the role of auxin in hypocotyl elongation and its possible interactions with ethylene and gibberellin.

RESULTS

The Role of Auxin

The response of wild-type and mutant Arabidopsis hypocotyls to exogenous auxin was tested by germinating seedlings on a range of indole-3-acetic acid (IAA) concentrations. In our conditions, IAA does not promote the growth of wild-type hypocotyls at any concentration (Fig. 1). In contrast, IAA promotes the growth of both *axr1-12* hypocotyls and the hypocotyls of the transgenic plant 35S-*iaaL* (Fig. 1). This promotion in growth is likely to be cell elongation rather than cell division. *axr1-12* seedlings have the same number of cells in an epidermal cell file as wild type (17.06 ± 0.19 for wild type; 17.12 ± 0.20 for *axr1-12*; counted at 3 d old); therefore, the short hypocotyl is not a result of a reduced number of cells, and previous workers have demonstrated that there are no significant cortical or epidermal cell divisions during the postembryonic growth of wild-type hypocotyls (Gendreau et al., 1997). These data are con-

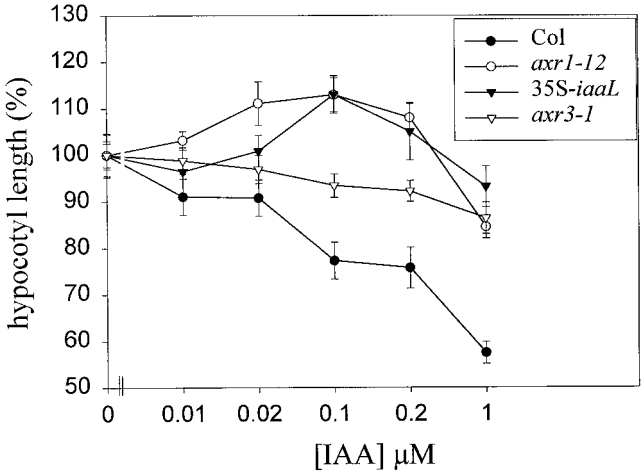

Figure 1. Dose responses of wild type, *axr1-12, axr3-1*, and 35S-*iaaL* to IAA. Seeds were germinated on media containing various concentrations of auxins, in the light (40 μM m^{-2} s^{-1}) at 26°C. Hypocotyl length was measured at 5 d. Data represent the mean hypocotyl length of at least 20 seedlings expressed as a percentage of hypocotyl length on no auxin. The mean hypocotyl lengths (in mm) on no auxin were as follows: 3.7 (Col), 2.4 (*axr1*), 3.3 (*axr3-1*), and 3.1 (35S-*iaaL*). Error bars represent the SE.

sistent with the hypothesis that in the wild-type hypocotyl, auxin levels are optimal and additional auxin is inhibitory, but in plants with reduced auxin levels or auxin responses, exogenous auxin can promote growth. This hypothesis is further supported by the auxin response of the *axr3-1* hypocotyl. Exogenous IAA is unable to promote the growth of *axr3-1* hypocotyls, consistent with their increased response to auxin (Fig. 1). As previously reported, the hypocotyls of *axr1* and *axr3* are resistant to inhibition by high levels of auxin (Lincoln et al., 1990; Leyser et al., 1996). The short final length of the *axr3-1* hypocotyl suggests that a supra-optimal auxin response inhibits elongation. In this context it is interesting that the growth pattern of the *axr3-1* hypocotyl is complex, with elongation rates above those of the wild type in the first 3 d followed by very slow rates below those of the wild type thereafter (Fig. 2). It is possible that the increased auxin response in this mutant leads to a greater rate of growth initially but that the auxin growth response then becomes saturated.

Auxin Does Not Act through Ethylene

Hypocotyl elongation is inhibited by the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) (Fig. 3). To investigate whether auxin acts through ethylene to inhibit hypocotyl elongation, the response of hypocotyls to exogenous auxin was tested by germinating seedlings on a range of IAA concentrations in conditions where ethylene responses were blocked or reduced. Ethylene responses were blocked by using the *etr1* ethylene-resistant mutant (Chang et al., 1993), and it was found that the elongation of the *etr1* mutant hypocotyl was inhibited by auxin to the same extent as the wild type (Fig. 4). Ethylene synthesis was blocked using AVG, a known inhibitor of ethylene synthesis (Yang and Hoffman, 1984). When AVG was added to the growth media, the response to auxin was also unchanged (Fig. 4).

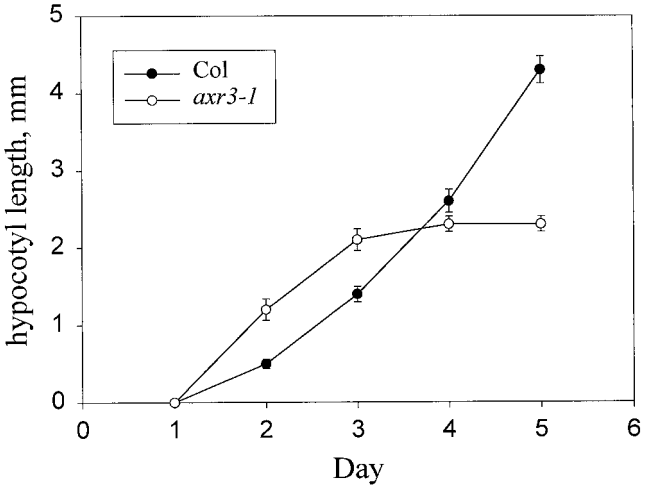

Figure 2. Growth curve of wild type (Col) and *axr3-1*. Seedlings were grown in the light (40 μM m^{-2} s^{-1}) at 26°C. Data represent the mean hypocotyl length of at least 20 seedlings. Error bars represent the SE.

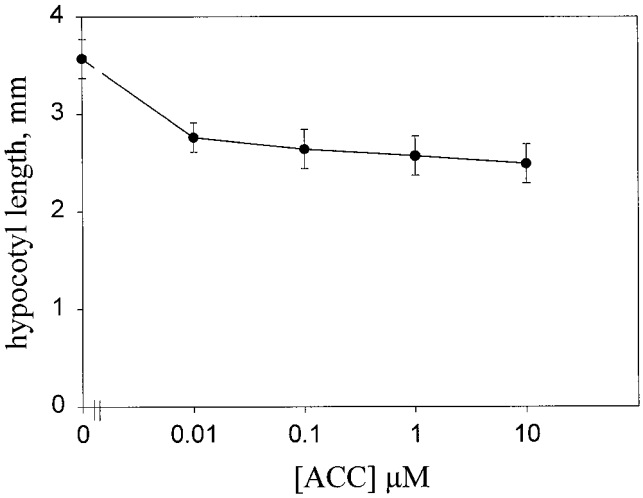

Figure 3. Dose response of wild type to ACC. Seedlings were grown in the light (40 μM m^{-2} s^{-1}) at 26°C. Hypocotyl length was measured at 5 d. Data represent the mean hypocotyl length of at least 20 seedlings. Error bars represent the SE.

Auxin and Gibberellin Act Independently

Next we investigated the interaction of auxin and gibberellin. When wild-type Arabidopsis seedlings are germinated on a range of gibberellic acid (GA_3) concentrations, concentrations above 0.1 μM stimulate hypocotyl elongation (Fig. 5). If gibberellin were

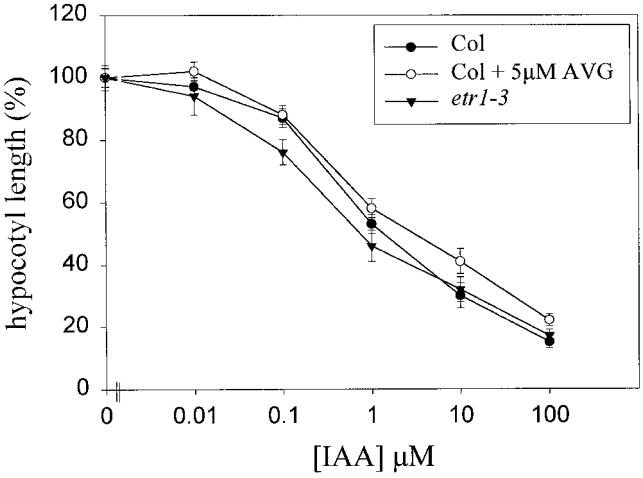

Figure 4. Dose responses of wild type (Col), *etr1*, and wild type with aminoethoxyvinylglycine (AVG) to IAA. Seeds were germinated on media containing various concentrations of auxin with or without 5 μM AVG. Seedlings were grown in the light (40 μM m^{-2} s^{-1}) at 26°C. Hypocotyl length was measured at 5 d. Data represent the mean hypocotyl length of at least 15 seedlings expressed as a percentage of hypocotyl length on no auxin. The mean hypocotyl lengths (mm) on no auxin were as follows: 2.3 (Col), 2.4 (*etr1*), and 1.7 (Col with AVG). Error bars represent the SE.

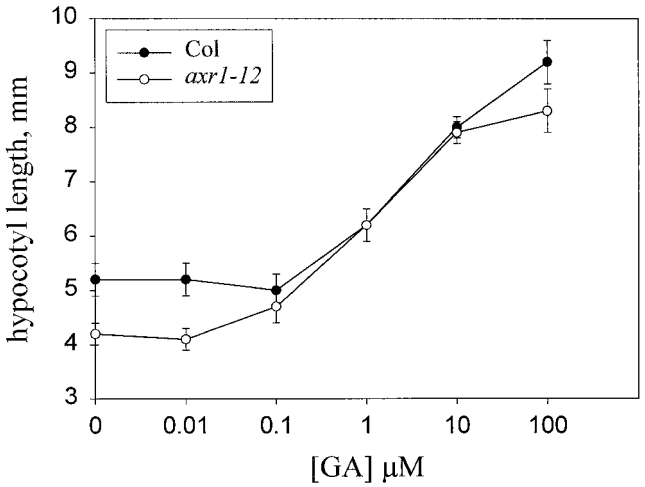

Figure 5. Dose responses of wild type (Col) and *axr1-12* to GA_3. Seeds were germinated on media containing various concentrations of gibberellin. Seedlings were grown in the light (40 μM m^{-2} s^{-1}) at 26°C. Hypocotyl length was measured at 5 d. Data represent the mean hypocotyl length of at least 20 seedlings. Error bars represent the SE.

acting by modulating auxin action, then a mutant with a reduced auxin response should show reduced responses to exogenous gibberellin. The *AXR1* gene is required for an early stage of auxin signal transduction and *axr1* mutants have reduced auxin sensitivity in all tissues tested and all assays used (Lincoln et al., 1990). When *axr1* seedlings were grown on a range of GA_3 concentrations, their response was not obviously different from wild-type seedlings (Fig. 5). If gibberellin acted by altering auxin transport, one would expect that the addition of an auxin transport inhibitor would reduce the hypocotyl response to gibberellin. However, the addition of 1 μM 1-naphthylphthalmic acid (NPA) does not alter the response of the hypocotyl to GA_3 (Fig. 6). These data suggest that exogenous gibberellin can act even if auxin response and auxin transport are reduced.

If auxin were acting by changing the gibberellin response, then gibberellin mutants would respond differently to exogenously applied auxin compared with wild type. To test this hypothesis, the gibberellin response mutants *spy* and *gai* and the gibberellin biosynthesis mutant *ga4* were germinated on a range of IAA concentrations. All showed wild-type IAA responses (Fig. 7). These mutants are in the Landsberg *erecta* background, so Landsberg *erecta* seedlings were used as the control.

To test genetically for gibberellin-auxin interactions, crosses were carried out between the auxin mutants *axr3-1* and *axr1-3* and the gibberellin mutants *spy-5* and *ga1-3* to obtain double mutants. The auxin mutants were in the Columbia background and the gibberellin mutants were in the Landsberg *erecta* background. All of the resulting double mutants had poor fertility and phenotypes characteristic of both parents (Fig. 8). Although the segregation of the *erecta* gene had a significant effect in adult plants, the seedling phenotypes appeared less affected. All single mutants were shorter than both Columbia and Landsberg *erecta* wild-type controls at d 7 (Fig. 9). *spy-5* has little effect on the hypocotyl length and, as expected, the hypocotyls of double mutants with *axr1-3* and *axr3-1* are similar in length to *axr1-3* and *axr3-1* hypocotyls, respectively (Fig. 9A). The hypocotyls of the *ga1-3 axr1-3* double mutant were shorter than either parent, suggesting that the phenotypes are additive. However, the hypocotyl of the *ga1-3 axr3-1* double mutant is longer than *ga1-3* but shorter than *axr3-1* at d 7 (Fig. 9b). To investigate this result further, earlier time points in hypocotyl elongation were examined. In the first few days of growth, *axr3-1* hypocotyls are longer than wild-type hypocotyls. The presence of the *axr3-1* mutation also promotes growth in a *ga1-3* background at this time, resulting in hypocotyls that are longer than the *ga1-3* single mutant (Fig. 10).

DISCUSSION

We have investigated the interactions between auxin and ethylene and auxin and gibberellin during the control of cell elongation in the Arabidopsis hypocotyl. This method is a convenient system in which to study the regulation of cell elongation. Hypocotyl growth occurs largely in the absence of cell division. The effects of exogenous hormones can be tested by adding them to the growth medium. Many hormone signaling and biosynthetic mutants are available to test possible interactions between the hormones.

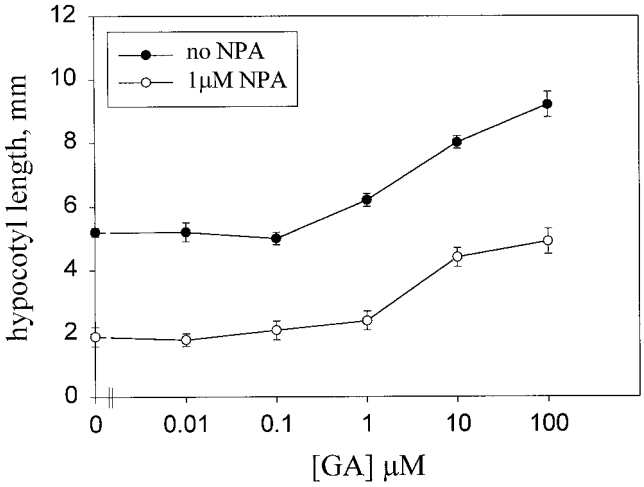

Figure 6. Dose responses of wild type, in the presence or absence of 1 μM NPA, to GA_3. Seeds were germinated on media containing various concentrations of gibberellin with or without NPA. Seedlings were grown in the light (40 μM m^{-2} s^{-1}) at 26°C. Hypocotyl length was measured at 5 d. Data represent the mean hypocotyl length of at least 20 seedlings. Error bars represent the SE.

The Level of Auxin in Wild-Type Seedlings Is Optimal for Hypocotyl Elongation

Under our conditions, when wild-type Arabidopsis seedlings are grown on media containing auxin, elongation of the hypocotyl is inhibited even though auxin is known to promote cell enlargement in stem and coleoptile segments (Evans, 1985). However, auxin is able to promote elongation in plants in which auxin levels or auxin sensitivity are reduced. *axr1* (Lincoln et al., 1990) has lowered auxin responses (Leyser et al., 1993), and the transgenic line 35S-*iaaL* conjugates IAA to Lys, giving reduced levels of free auxin (Jensen et al., 1998). Both have shorter hypocotyls than wild-type seedlings and in both cases adding auxin to the growth medium stimulates hypocotyl elongation, suggesting that auxin levels in the seedling are optimal for hypocotyl elongation and that a supra-optimal level of auxin will inhibit elongation.

Other groups have shown that under certain conditions auxin can stimulate elongation of hypocotyls of intact seedlings. For example, on nutrient-poor media, auxin can stimulate hypocotyl elongation (Smalle et al., 1997). Transgenic seedlings overexpressing the bacterial enzyme Trp monooxygenase (*iaaM*) have up to 4-fold higher IAA levels (Romano et al., 1995). These seedlings have longer hypocotyls than wild-type seedlings in white light. However, seedlings are indistinguishable from wild-type seedlings in red, far-red, and blue light, and shorter than wild-type seedlings in the dark. The superroot mutant, *sur1*, which also has increased levels of IAA, has a longer hypocotyl than wild type in the light and a shorter hypocotyl than wild type in the dark (Boerjan et al., 1995). These results contradict our conclusion that the levels of auxin in the wild-type hypocotyl are optimal for elongation. However, it is likely that the auxin levels are influenced by factors such as environmental conditions and the different results obtained by these workers could reflect this variation. For example, a reduction in auxin synthesis could explain why the hypocotyls of plants grown on nutrient-poor media can be stimulated to elongate by the addition of auxin (Smalle et al., 1997). The 35S-*iaaM* transgenic line produced by Romano et al. (1995) is in the RLD background whereas our work is in the Columbia background. There may be a difference in endogenous auxin levels or levels of response between ecotypes.

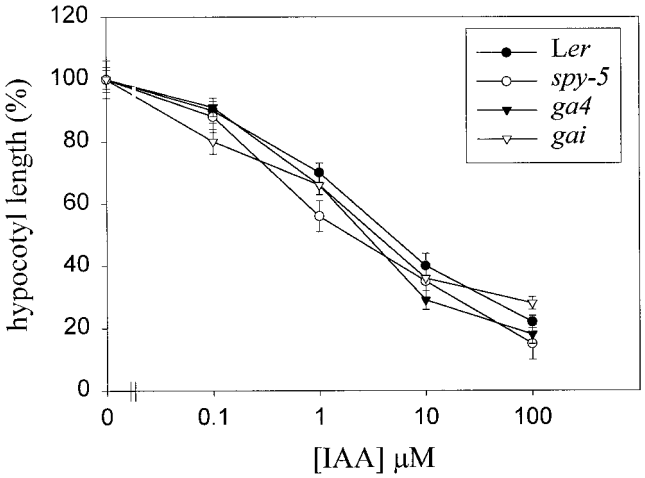

Figure 7. Dose responses of wild type (Landsberg *erecta*), *ga4*, *spy-5*, and *gai* to IAA. Seeds were germinated on media containing various concentrations of auxin. Seedlings were grown in the light (40 μM m^{-2} s^{-1}) at 26°C. Hypocotyl length was measured at 5 d. Data represent the mean hypocotyl length of at least 20 seedlings expressed as a percentage of hypocotyl length on no auxin. The mean hypocotyl lengths (in mm) on no auxin were as follows: 2.9 (L*er*), 3.0 (*ga4*), 3.1 (*spy-5*), and 1.4 (*gai*). Error bars represent the SE.

Figure 8. Top (from left to right): Columbia, *axr1–3*, *axr3–1*, Landsberg *erecta*, *spy-5*, and *ga1-3*. Bottom (from left to right): *axr3-1 ga1-3*, *axr3-1 spy-5*, *axr1-3 ga1-3*, and *axr1-3 spy-5*. Plants were grown in 16 h of light and 8 h of dark and were 5 weeks old. Scale bars = 3 cm.

Auxin-induced growth is also observed at higher temperatures (Gray et al., 1998). The observation that growth at 29°C stimulates auxin biosynthesis and auxin-dependent elongation in Arabidopsis hypocotyls suggests that additional auxin-induced elongation is possible over and above the elongation observed at 20°C. It is possible that the hypothetical optimal auxin level is higher at higher temperatures. These results illustrate how environmentally and genetically sensitive the effect of auxin on elongation is.

In some cases, increased auxin can promote hypocotyl elongation and in others it can inhibit it, consistent with a dual role for auxin in hypocotyl elongation. A similar situation exists in roots with auxin promoting elongation in some conditions and inhibiting it in others. At very low-auxin concentrations root elongation rates are increased and at higher concentrations they are reduced, suggesting that levels of auxin in roots are suboptimal for elongation (Evans et al., 1994).

The response of the dominant gain of function *axr3-1* mutant is more complex. Overall the phenotype of the mutant suggests an enhanced auxin response (Leyser et al., 1996). This enhanced response is reflected in the observation that *axr3-1* seedlings have longer hypocotyls than wild-type seedlings during the first 3 d of growth. However, after this time elongation in the mutant stops, suggesting that the response has reached a supra-optimal level and further growth is inhibited. Consistent with this idea, the data presented here demonstrate that exogenous auxin is unable to promote the growth of *axr3-1* hypocotyls. The *AXR3* gene is a member of the *Aux/IAA* gene family (Rouse et al., 1998). *Aux/IAA* genes are auxin-inducible and encode transcriptional regulators with characteristically short half-lives (Abel et al., 1994). The *axr3-1* mutation results in a stabilization of the AXR3 protein (Worley et al., 2000) and it is this increased longevity that most likely gives rise to the enhanced response to auxin found in *axr3-1* mutant plants. During seedling development, *AXR3* expression declines after d 4 (D. Rouse and O. Leyser, unpublished data). The persistence of axr3–1 protein later into seedling development may block further elongation.

The results obtained here can be compared with those obtained by Barratt and Davies (1996) who examined the response to exogenous auxin of pea stem segments at two different developmental stages. They showed that the growth of early-expansion stages, which have higher levels of endogenous auxin, was inhibited by exogenous auxin, whereas the growth of mid-expansion segments, which have lower levels of endogenous auxin, was promoted by exogenous auxin. Here again, when the levels of auxin in the plant are optimal for elongation, the addition of more auxin results in an inhibition of expansion. Moreover, the growth of early expansion segments of dwarf and severe dwarf pea plants can

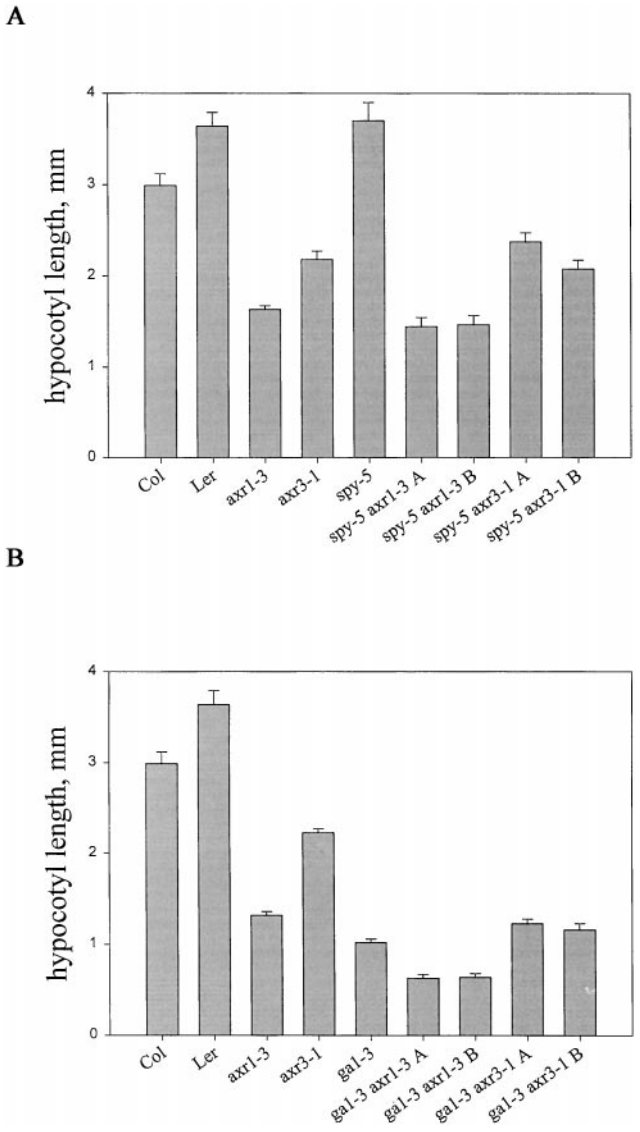

Figure 9. Hypocotyl length of Columbia (Col), Landsberg *erecta* (Ler), *axr1-3, axr3-1, spy-5, spy-5axr1-3, spy-5axr3-1* (A), and Columbia, Landsberg *erecta, axr1-3, axr3-1, ga1-3, ga1-3axr1-3, ga1-3axr3-1* (B). Seedlings were grown in the light (130 μM m^{-2} s^{-1}) at 23°C. All hypocotyls were measured at 7 d old. Seedlings in B were vernalized in 100 μM GA_3 to germinate. Two sets of data for each double mutant have been included to show that there is little variation in double mutant hypocotyl length, even though Landsberg *erecta* and Columbia genes are segregating differently.

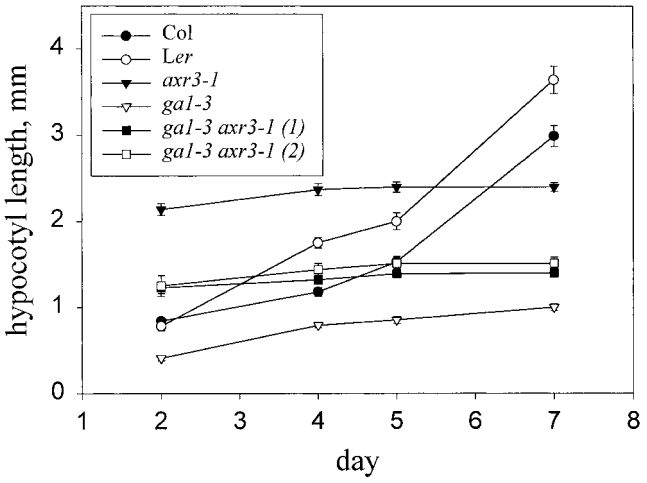

Figure 10. Growth curve of Columbia (Col), Landsberg *erecta* (L*er*), *axr3-1, ga1-3*, and double mutants. Seedlings were grown in the light (130 μM m^{-2} s^{-1}) at 23°C. Data represent the mean hypocotyl length of at least 20 seedlings. Error bars represent the SE.

be promoted by auxin, just as the growth of *axr1-12* and 35S-*iaaL* hypocotyls can be promoted by auxin.

All of the experiments described above were carried out in the light. It is important to note the role of auxin in hypocotyl elongation appears to be very different in the light versus the dark, with polar auxin transport being more important for hypocotyl elongation in light-grown seedlings (Jensen et al., 1998).

Auxin and Ethylene Act Independently

Our results show that auxin and ethylene act independently in the control of hypocotyl elongation. When ethylene responses were blocked, either using an ethylene-resistant mutant or using AVG to inhibit ethylene biosynthesis, the effect of auxin on hypocotyl elongation was unaltered. These data concur with those of Smalle et al. (1997) who also found that AVG did not alter the effect of auxin on hypocotyl elongation. In their system, auxin promoted hypocotyl elongation whereas in our system, auxin inhibits hypocotyl elongation. Furthermore, they found that silver ions, which interfere with ethylene perception, reduced the response to auxin. Results with inhibitors need to be interpreted with caution, since it is difficult to distinguish primary from secondary effects. However, here the result with AVG is consistent with the result using the *etr1* mutant and therefore cannot be dismissed as non-specific. In any case, it would be unlikely that the auxin-induced inhibition of hypocotyl elongation was mediated by ethylene since auxin can inhibit hypocotyl elongation by over 80% yet ACC inhibits hypocotyl elongation by only 30% (Figs. 3 and 4). We conclude that auxin does not inhibit hypocotyl elongation by acting through ethylene but that it acts by a separate mechanism. In dark-grown seedlings, Thomine et al. (1997) looked at the effect of anion-channel blockers on auxin and other hormone responses. These blockers were able to counteract the inhibition of hypocotyl elongation induced by auxin but not by ethylene or cytokinins, supporting our results that auxin and ethylene act independently.

Gibberellin and Auxin Act Independently

Our results show that auxin and gibberellin act independently in the control of hypocotyl elongation. The response of the auxin-resistant mutant *axr1-12* to gibberellin is very similar to the wild-type response; if anything, it responds slightly more to gibberellin than wild type, which is the opposite of what one would predict if a normal auxin response was necessary for a normal gibberellin response. Also, adding NPA, an inhibitor of auxin transport, makes no difference to the gibberellin response, which again suggests that auxin and gibberellin act independently. The auxin responses of the gibberellin mutants *spy-5, ga4*, and *gai* are no different from the wild type. Therefore, a normal gibberellin response is not necessary for a normal response to auxin in the hypocotyl.

Double-mutant analysis is consistent with the independence of auxin and gibberellin. The hypocotyl of the *ga1-3* (reduced gibberellin biosynthesis), *axr1-3* (reduced auxin response) double mutant is shorter than that of either parent, suggesting that the mutations are acting independently in the control of hypocotyl elongation. The caveat here is that *axr1-3* is not a complete loss-of-function allele. The hypocotyl of the double mutant between *ga1-3* and *axr3-1* (a gain-of-function mutant with increased auxin responses) is shorter than the hypocotyl of *axr3-1* but longer than the hypocotyl of *ga1-3.* Although the *axr3-1* hypocotyl is shorter than the wild-type mutant at d 7, in the first 3 d of growth, the *axr-1* mutant is longer than the wild type. In Figure 9, we see that at 2 d, the double-mutant hypocotyl is intermediate in length between *axr3–1* and *ga1–3*, as would be expected if the phenotypes were additive. By d 4, the *axr3-1* hypocotyl has stopped elongating and the *ga1-3* hypocotyl is elongating slowly. The rate of elongation of the double mutants is slower than that of *ga1-3* but faster than the zero rate of *axr3-1,* again indicating that the phenotypes are additive. *spy-5* hypocotyls are similar in length to wild-type hypocotyls and, as expected, the hypocotyls of double mutants with *axr1-3* and *axr3-1* are similar in length to *axr1-3* and *axr3-1* hypocotyls, respectively. In conclusion, the phenotypes of all double mutants between auxin and gibberellin mutants seem to be additive, suggesting that gibberellin and auxin are acting independently in hypocotyl elongation.

In pea internodes, it has been concluded that the effect of gibberellin might be mediated, in part, by auxin (Yang et al., 1996): The response of internodes to applied gibberellin was small in plants with low-IAA content. However, in contrast to the Arabidopsis hypocotyl, in pea internodes, elongation depends on both cell expansion and cell division. It is possible that the interaction of gibberellin and auxin is in cell division and not cell elongation.

Another study using pea stem segments found evidence that the mode of action of gibberellin is different at different stages of growth (Barratt and Davies, 1997). In early-expansion stages, the action of gibberellin is independent of endogenous IAA concentration whereas in mid-expansion segments, the magnitude of the response to gibberellin depends on endogenous IAA content. Therefore, although in the hypocotyl it appears that auxin and gibberellin act independently to regulate elongation, further studies are needed to discover whether they interact at other stages of Arabidopsis development. However, the adult phenotypes of the double mutants between the auxin and gibberellin mutants seem to be additive, suggesting that auxin and gibberellin are acting in-

dependently at this stage. A more detailed study of these plants would be needed to confirm this hypothesis. It is worth noting that the auxin in the experiments described here is taken up from the agar, whereas in nature, polar auxin transport is important. We are currently developing a system to allow auxin to be applied apically.

MATERIALS AND METHODS

The following mutants and transgenic lines are in the Columbia ecotype of Arabidopsis and have been described elsewhere: *axr1-3* and *axr1-12* (Lincoln et al., 1990), *axr3-1* (Leyser et al., 1996), *etr1*-3 (Chang et al., 1993), and 35S-*iaaL* (Jensen et al., 1998). The following mutants are in the Landsberg *erecta* ecotype of Arabidopsis and have been described elsewhere: *ga1-3* (Koorneef and van der Veen, 1980), *ga4* (Koorneef and van der Veen, 1980), *gai* (Koorneef et al., 1985), and *spy-5* (Wilson and Somerville, 1995).

Arabidopsis seeds were surface sterilized for 15 min in 10% (v/v) bleach and 0.1% (v/v) Triton, then placed in 70% (v/v) ethanol and rinsed five times with sterile, distilled water. Sterile seeds were placed in round 9-cm Petri dishes containing 20 mL of *Arabidopsis thaliana* salts growth medium (Wilson et al., 1990). All hormone and inhibitor stocks were dissolved in 70% (v/v) ethanol at a concentration 1,000 times greater than needed. Twenty microliters of hormone was added to the Petri dishes prior to the addition of media. After seed sowing, Petri dishes were chilled for 3 d at 4°C before being placed vertically in a 16-h-light, 8-h-dark growth room. Experiments for Figures 1 through 7 were carried out in a growth chamber at 26°C with a light intensity of 40 μM m^{-2} s^{-1}, and the experiments for Figures 9 and 10 were carried out in a growth chamber at 23°C with a light intensity of 130 μM m^{-2} s^{-1}. These conditions account for the variability in hypocotyl length observed.

etr1-3 seed was provided by the Nottingham Arabidopsis Stock Centre (Nottinghamshire, UK). 35S-*iaaL* was kindly provided by C. Romano (Monsanto, St. Louis). IAA, GA_3 (gibberellin), ACC, NPA, and AVG were obtained from Sigma (St. Louis, MO). All gibberellin used in these experiments was GA_3.

Measurements were made using LUCIA G software (version 3.52a, 1991, Laboratory Imaging, Nikon UK Limited, Kingston, UK).

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