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Auxin-Gibberellin Interactions in Pea: Integrating the Old with the New

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ABSTRACT

Recent findings on auxin-gibberellin interactions in pea are reviewed, and related to those from studies conducted in the 1950s and 1960s. It is now clear that in elongating internodes, auxin maintains the level of the bioactive gibberellin, GA_1, by promoting GA_1 biosynthesis and by inhibiting GA_1 deactivation. These effects are mediated by changes in expression of key GA biosynthesis and deactivation genes. In particular, auxin promotes the step GA_{20} to GA_1, catalyzed by a GA 3-oxidase encoded by Mendel's *LE* gene. We have used the traditional system of excised stem segments, in which auxin strongly promotes elongation, to investigate the importance for growth of auxin-induced GA_1. After excision, the level of GA_1 in wild-type (*LE*) stem segments rapidly drops, but the auxin indole-3-acetic acid (IAA) prevents this decrease. The growth response to IAA was greater in internode segments from *LE* plants than in segments from the *le-1* mutant, in which the step GA_{20} to GA_1 is impaired. These results indicate that, at least in excised segments, auxin partly promotes elongation by increasing the content of GA_1. We also confirm that excised (light-grown) segments require exogenous auxin in order to respond to GA. On the other hand, decapitated internodes typically respond strongly to GA_1 application, despite being auxin-deficient. Finally, unlike the maintenance of GA_1 content by auxin, other known relationships among the growth-promoting hormones auxin, brassinosteroids, and GA do not appear to involve large changes in hormone level.

Key words: Auxin; Elongation; Gene expression; Gibberellin; Hormone interactions; Hormone transport; *Pisum sativum*

INTRODUCTION

Between 1950 and 1970 many studies were conducted on the possible interactions between the ''traditional'' plant growth hormones, auxin and gibberellin (GA). Auxin strongly promotes elongation in excised segments from elongating internodes, and in many of the early experiments, excised pea stem segments were incubated with auxin, GA, or a combination of both hormones. It was often suggested, on the basis of the growth responses, that GA might affect auxin content, but the reverse relationship was rarely, if ever, mentioned. Therefore, from an historical perspective it was surprising to discover recently that in pea, auxin strongly promotes the biosynthesis of the bioactive GA, GA_1 (Ross and others 2000; O'Neill and Ross 2002).

It now appears that auxin from the apical bud maintains GA_1 levels in elongating pea internodes, thus ensuring a normal rate of internode elongation. When auxin levels are reduced, as in decapitated stems or isolated stem segments, so too is GA_1 biosynthesis (Ross and others 2000; O'Neill and

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Ross 2002). When auxin is added to these systems, GA_1 biosynthesis is restored. The interaction involves substantial changes in GA_1 level: decapitation reduces both IAA and GA_1 content by about 10-fold (Ross and others 2000). A dose-response curve further indicates the dependence of GA_1 accumulation on auxin in pea internodes (O'Neill and Ross 2002).

Auxin and GA_1 Biosynthesis

In pea, the main GA biosynthesis step affected by IAA is the final, activation step, GA_{20} to GA_1 (Figure 1). There is no evidence as yet for a marked effect on the biosynthetic steps prior to GA_{20}. The step GA_{20} to GA_1 is catalyzed by a GA 3-oxidase, encoded by Mendel's *LE* gene (Lester and others 1997; Martin and others 1997). The gene *LE* is also referred to as *PsGA3ox1*. We showed previously (Ross and others 2000) that *LE* transcript levels are dramatically reduced in auxin-deficient decapitated stems, and are restored by application of IAA. Recently we showed that the auxin up-regulation of *LE* transcript level can occur quite rapidly after auxin application (within 2 h) (O'Neill and Ross 2002). However, this up-regulation is inhibited by the protein synthesis inhibitor cycloheximide, indicating that *de novo* protein synthesis is required for the effect of auxin on *LE*. Thus it appears that auxin first affects a ''primary'' auxin response gene, which in turn mediates an effect on *LE* transcript level. The *LE* gene is, therefore, a ''late'' auxin response gene (Abel and Theologis 1996).

Auxin and GA_1 Deactivation

As well as promoting GA_1 biosynthesis, auxin also inhibits GA_1 deactivation. We showed this by feeding $[^{14}C]GA_1$ to decapitated plants with and without exogenous IAA (O'Neill and Ross 2002). Internodes without auxin appeared to convert more of the substrate to $[^{14}C]GA_8$ than did auxin-treated internodes. Importantly, we have now obtained the same result with excised stem segments, adding the $[^{14}C]GA_1$ substrate to the incubation medium (Figure 2).

These findings are consistent with the effect of auxin on the expression of *PsGA2ox1*, which encodes a GA 2-oxidase. GA 2-oxidases catalyze GA deactivation steps such as GA_1 to GA_8 (Figure 1). We showed originally that auxin down-regulates *PsGA2ox1* expression (Ross and others 2000), and recently that this effect occurs quite rapidly (O'Neill and Ross 2002). However, the most convincing evidence that *PsGA2ox1* mediates the effects of auxin on GA_1 deactivation comes from studies on *sln*, a null mutation in *PsGA2ox1* (Lester and others 1999).

In wild-type plants, decapitation reduces the IAA content in stems, and as a result speeds up the

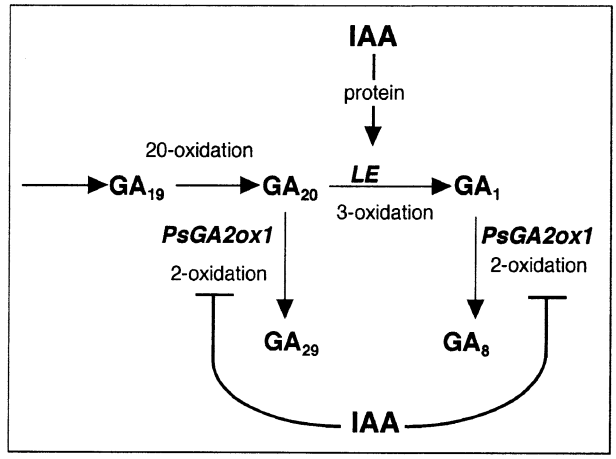

Figure 1. Later stages of the GA pathway in pea shoots, showing steps affected by auxin.

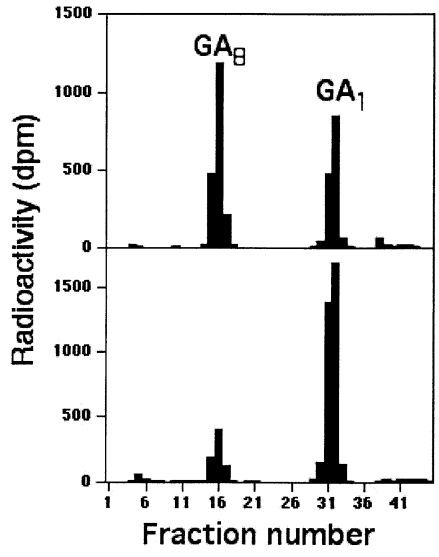

Figure 2. Effect of IAA on the conversion of $[^{14}C]GA_1$ to $[^{14}C]GA_8$ in excised stem segments (line 205+, *LE*). Top, control; bottom, IAA (5 $\mu g.ml^{-1}$). For each replicate 8 segments from internode 10 to 11 were incubated for 6 hours in Murashige and Skoog medium as described previously (O'Neill and Ross 2002). The medium contained $[^{14}C]GA_1$ at a concentration of 40,000 $dpm.ml^{-1}$. GAs were analyzed by HPLC-radiocounting as before (Ross and others 2000). Similar results were observed in a second replicate (not shown).

deactivation of GA_1 to GA_8. However, decapitation does not speed up GA_1 deactivation in the *sln* mutant. As a result, in decapitated internodes there is a large difference in GA_1 deactivation rate between *sln* and the wild type (O'Neill and Ross 2002). This indicates that *PsGA2ox1* is the predominant gene for GA_1 deactivation in decapitated wild-type plants. Decapitation cannot up-regulate GA_1 deactivation in *sln* plants because they possess a nonfunctional *PsGA2ox1* protein.

The important implication of these observations is that in intact plants IAA from the apical bud inhibits the expression of *PsGA2ox1*, and therefore GA_1 deactivation, in elongating internodes (O'Neill and Ross 2002). Because *PsGA2ox1* expression is relatively low in intact stems, the effect of other 2-oxidase genes becomes more important. In intact stems these other genes can compensate for the loss of functional *PsGA2ox1* protein, and this explains why *sln* only weakly affects GA_1 deactivation in intact plants (Ross and others 1995).

Interestingly, the expression of another GA 2-oxidase gene, *PsGA2ox2*, was actually up-regulated by IAA (O'Neill and Ross 2002). The reason for this, given that auxin down-regulates GA_1 deactivation, is not clear, but it might result from the GA_1 which would have accumulated in response to auxin treatment. There is evidence that GA_1 up-regulates GA deactivation genes as part of a feed-forward mechanism (Thomas and others 1999; Elliott and others 2001).

Our research on the pea 2-oxidase genes demonstrates the value of metabolism experiments when studying the regulation of biochemical pathways. Although gene expression studies are essential, they could be misleading. For example, on its own, the auxin up-regulation of *PsGA2ox2* expression would indicate that auxin *promotes* GA_1 deactivation, whereas the opposite is the case. Clearly, when a given factor regulates members of a gene family in opposite ways, metabolism experiments are essential for understanding the overall regulation.

Integrating the Old with the New

We would expect that in rapidly elongating internodes, auxin-induced GA_1 is important for growth, because GA_1-deficient mutants are dramatically shorter than the wild type (Reid and Ross 1993). However, that expectation does not constitute proof that auxin-induced GA_1 is significant for growth, rather than a mere secondary consequence of auxin action. To what extent does GA_1 mediate the growth-promoting effect of auxin? We have addressed that question using excised stem segments, the system in which the promotion of growth by auxin has been most studied. This system was widely used in early auxin research, and has since been valuable for studies on the mechanism of auxin action (Cleland 1995), including auxin-regulated genes (Theologis and others 1985; Koshiba and others 1995).

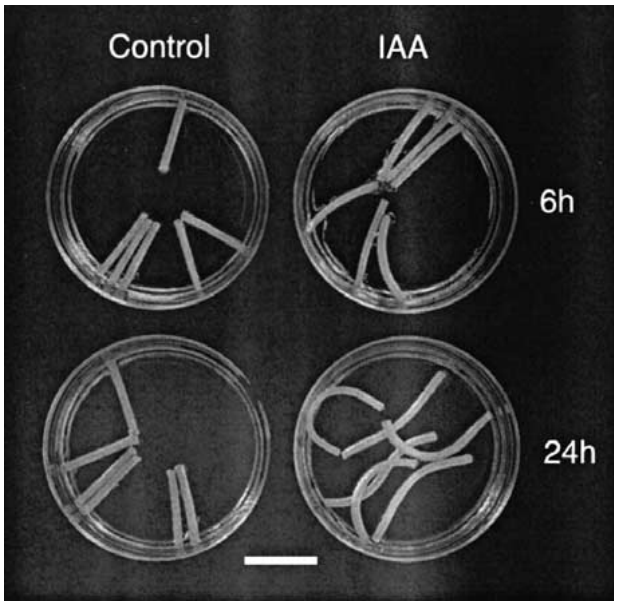

Figure 3. Effect of IAA on the elongation of excised pea stem segments. Shown are segments at 6 hours (top) and 24 hours (bottom) after excision. Left, controls; right, IAA (5 $\mu g.ml^{-1}$). Segments (20 mm) were cut from internode 7–8 of line 205+ (*LE*) plants, and incubated in Murashige and Skoog medium. Scale bar 20 mm.

In excised segments from light-grown wild-type pea plants the growth response to auxin is typically quite striking (Figure 3). Under our conditions there is a visible difference between auxin-treated and control segments after 6 hours of incubation, and by 24 hours, the auxin-treated segments are not only much longer than the controls, they are lighter in color as well.

We now know that in excised segments auxin promotes the formation of $[^{14}C]GA_1$ from $[^{14}C]GA_{20}$ (O'Neill and Ross 2002) and inhibits the deactivation of $[^{14}C]GA_1$ (Figure 2). However, these results do not show that auxin affects *endogenous* GA_1 levels in excised segments, since they do not exclude the possibility that the segments quickly ''run out'' of GA_1 precursors. Therefore, we quantified endogenous GA_1 in segments incubated with and without IAA. After only 6 h, the level of GA_1 was very low in segments without IAA (about 0.3 $ng.g^{-1}$), but was

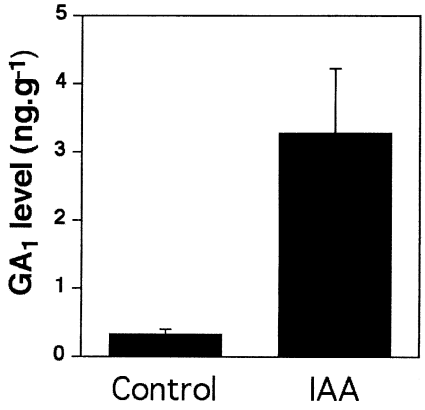

Figure 4. Effect of IAA (5 μg.ml^{-1}) on endogenous GA_1 content of excised stem segments, 6 hours after excision (line 205+, genotype *LE*). Segments (20 mm), from internode 7–8, were incubated in Murashige and Skoog medium. GA_1 content was determined by GC-MS with internal standards, as before (Ross and others 2000). Shown are the means and standard errors of 6 replicates, each consisting of 7 or 8 segments.

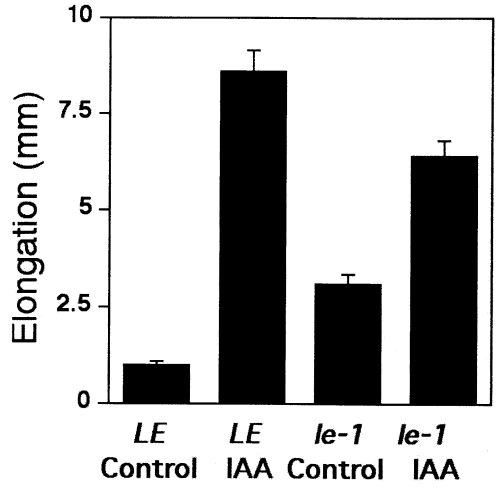

Figure 5. Effect of the *le-1* mutation on the elongation response to IAA (5 μg.ml^{-1} Segments (10 mm) were excised from the middle of internode 6–7 of 205+ (*LE*) and 205−(*le-1*) plants, when these internodes were 30–40% expanded (internode 6–7 was approximately 45 mm long in genotype *LE* and 12 mm in *le-1*). Segments were incubated in Murashige and Skoog medium with and without IAA. The length of segments was measured after 24 hours.

10-fold higher when IAA was present (Figure 4). At the time of excision the segments would typically have contained at least 3 ng.g^{-1} GA_1, as shown previously (Ross and others 2000). It appears that in control segments, the GA_1 level rapidly drops after excision, but is maintained at a high level if IAA is present.

Thus, it is entirely possible that in the early experiments investigating the relationship between auxin and GA (for example, Galston and Warburg 1959), auxin stimulated GA_1 biosynthesis. This means that growth effects attributed directly to auxin might actually have been mediated by GA_1. The early workers did not measure the effects of auxin on GA content. Indeed, at that time little was known about which GA was important for growth in pea shoots.

We reasoned that if the GA_1 induced by IAA in segments is important for growth, the elongation response to auxin should be less in genotypes where the GA pathway is impaired. The *le-1* mutation is an obvious choice for this approach because it blocks the step activated by IAA, GA_{20} to GA_1, resulting in a dwarf phenotype (Ingram and others 1984).

In fact, a comparison between *le-1* and *LE* segments already existed in the literature. Ockerse and Galston (1967) and Ockerse (1970) reported that the growth response to IAA was indeed less in *le-1* segments than in *LE* segments. These workers used the unrelated lines Alaska (*LE*) and Progress No. 9 (*le-1*), but we have obtained essentially similar results with isogenic lines (Figures 5 and 6). Barratt

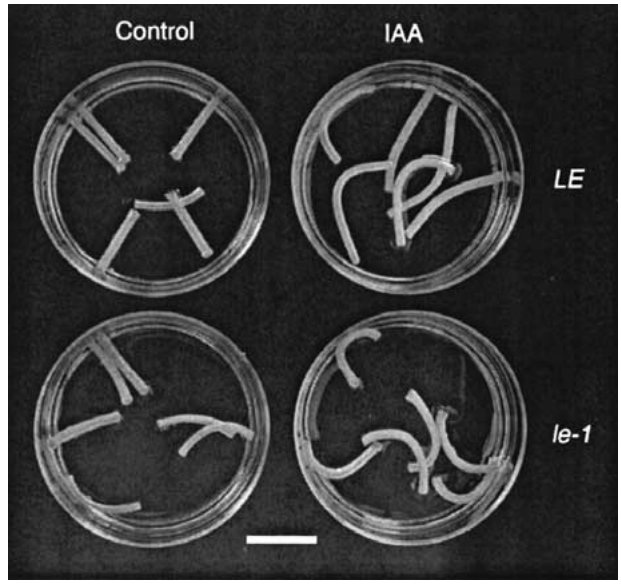

Figure 6. Effect of the *le-1* mutation on the elongation response to IAA. Segments (18 mm) were excised from the middle of internode 7–8 of 205+ (*LE*, top) and 205− (*le-1*, bottom) plants, when these internodes were approximately 50% expanded (internode 7–8 was approximately 60 mm long in genotype *LE* and 20 mm in *le-1*). Segments were incubated in Murashige and Skoog medium with (right) and without (left) IAA (5 μg.ml^{-1}).

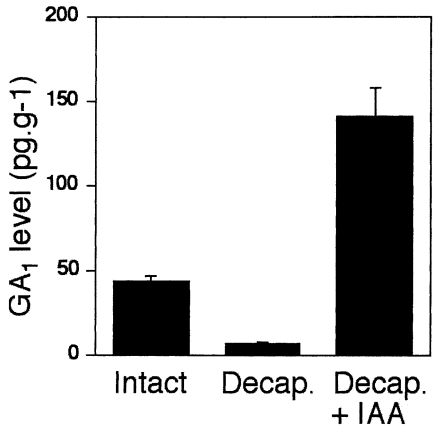

Figure 7. Effects of decapitation and IAA treatment on the endogenous GA_1 content of *le-3* (line NGB5839) internodes. Plants were decapitated immediately below node 10, and lanolin or lanolin containing 1 $mg.g^{-1}$ IAA was applied to the stump, and re-applied after a further 19 and 42 hours. Internode 9–10 was harvested 48 hours after excision. GA_1 was quantified by GC-MS with an internal standard.

and Davies (1997) also reported a stronger effect of IAA on segments from a tall, compared with a dwarf (*le-3*) line, in ''mid-expansion'' segments.

Ockerse and Galston (1967) and Ockerse (1970) suggested that as the phenotypic difference between their tall and dwarf lines was most likely due to a difference in GA content, the growth response to auxin is dependent on GA. That is, they suggested that GA present in the tissue enhances the auxin response. Our present results show that the level of bioactive GA in internode segments rapidly drops after excision, and that auxin prevents this from happening. Integrating our findings with the Ockerse theory, we now suggest that the growth response of segments to auxin depends on, or indeed is mediated by, the endogenous GA_1 *induced by auxin*. In other words, we suggest that auxin acts (partially) via changes in GA_1 content.

It should be noted that even in *le-1* segments there is a substantial growth response to auxin, and indeed some of the early studies were performed entirely with *le-1* lines such as Meteor (Brian and Hemming 1958). In the *le-1* mutant, as in the wild type, part of the auxin growth response is probably mediated by auxin-induced GA_1, since *le-1* is a leaky mutation (Lester and others 1997; Martin and others 1997). Data from another leaky *le* mutant, *le-3*, show the effect of IAA on GA_1 content (Figure 7; although in this case decapitated plants rather than segments were used).

Ockerse (1970) also reported that treating *LE* plants with 2-chlorethyl trimethylammonium chloride (CCC), an inhibitor of the early stages of GA biosynthesis, reduced the growth response to IAA. In retrospect, the CCC treatment probably acted by reducing the amount of GA_{20} substrate available for the auxin-induced GA 3-oxidase. The same reasoning might also explain why another inhibitor of GA biosynthesis, 2-isopropyl-4-(trimethylammonium chloride)-5-methylphenylpiperidine-1-carboxylate (AMO1618), inhibited the auxin growth response in cucumber (Katsumi and others 1965).

It is well known that auxin can stimulate elongation growth within approximately 15 min (Cleland 1995). This initial growth response appears too rapid to be due to changes in GA content. However, the auxin response is often biphasic (Cleland 1995; Barratt and Davies 1997) and we suggest that it is the long-term response that is mediated (at least in part) by GAs.

Auxin is also Required for the GA Response in Excised Sections (From Light-Grown Plants)

The early researchers typically found that in the absence of auxin, excised segments from light-grown plants respond poorly to GA_3, and we have recently confirmed that finding using GA_1 (data not shown). This poor GA response was probably one of the reasons why it was not suggested that auxin might promote elongation by increasing GA levels. The addition of auxin enhances the response to GA in excised sections, and Brian and Hemming (1958) and Ockerse (1970) reported a synergistic response to IAA and GA in *le-1* segments. However, in segments from the *LE* line Alaska, the synergism was much reduced (Ockerse 1970). In retrospect, we suggest that this was probably because the *LE* segments showed a strong growth response to auxin alone, in turn because they could synthesize endogenous GA_1.

In contrast to segments from light-grown plants, segments from etiolated (dark-grown) plants typically elongate in response to GA. In fact, segments cut from the uppermost stem section of dark-grown plants (and cultured in the dark) can show a greater growth response to GA than to IAA (Purves and Hillman 1958). Such observations are consistent with the greater general GA responsiveness of dark-grown plants compared with light-grown plants (Reid 1988; O'Neill and others 2000). The discussion in this article is confined mainly to light-grown plants.

In a different system, *Arabidopsis* roots, auxin again appears to be required for the GA response (Fu and Harberd 2003). In this system GA is thought to promote growth by opposing the effects of DELLA protein growth repressors, and this effect appears to be enhanced by auxin (Fu and Harberd 2003).

Decapitated Plants can Show a Strong Growth Response to GA, Despite Being Auxin Deficient

In contrast to excised segments, the internodes of decapitated light-grown pea plants usually respond strongly to GA application. Ockerse and Galston (1967) reported that GA_3 markedly stimulated elongation in decapitated Progress No. 9 plants (genotype *le-1*). More recently, Ross and others (2002) found that decapitated 205− (*le-1*) plants respond strongly to GA_1, showing 70% of the response observed in intact plants. These data show that a strong response to GA_1 *in planta* does not depend on the presence of auxin.

However, there are some exceptions to the rule that decapitated plants respond to GA, and in some cases decapitated plants behave like excised segments in this respect. For example, Brian and Hemming (1958) and Kuraishi and Muir (1964) found virtually no effect of GA application to decapitated *le-1* plants, and Brian and Hemming reported a synergistic effect of IAA and GA_3 in that system.

It is interesting that in our hands decapitated plants generally respond to GA whereas isolated segments do not. Auxin is deficient in both situations, and it can be speculated that internodes *in planta* (even without an apical bud) are provided with some other compound(s) that enhances the GA response.

The Auxin-GA Interaction and the Concept of Hormone Transport

The concepts of hormone transport and ''action at a distance'' continue to be relevant to plant hormone biology (Weyers and Paterson 2001; Davies 1995). The question of GA_1 mobility has been addressed in the past using the dwarf *le-1* mutant, which contains 10 to 20-fold less GA_1 than the wild-type (Ross and others 1992; Smith and others 1992). Peas are relatively easy to graft, and if GA_1 is a mobile hormone, it should be possible to graft together the *le-1* and *LE* genotypes in such a way as to affect shoot elongation. However, elongation is not affected by grafting (McComb and McComb 1970; Reid and others 1983), and it is now accepted that endogenous GA_1 is not transported over long distances within the pea shoot. This is consistent with recent evidence that elongating internodes are themselves capable of converting GA_{20} to GA_1 (O'Neill and Ross 2002).

Exogenous GA_1, in contrast to endogenous GA_1, is readily transported right around the vegetative pea plant (Ross and others 1995), demonstrating that the movement of an applied substance does not necessarily reflect that of the endogenous compound.

Considering auxin and GA_1 together as a two-factor control system satisfies the criterion of ''action at a distance.'' Auxin can be viewed as the mobile factor, required for GA_1 biosynthesis in internodes, and GA_1 as an actual effector of elongation. Auxin is transported *via* a specialized cell-to-cell system with recently characterized influx and efflux carriers (Friml and Palme 2002). In addition to GA_1, auxin also affects the levels of other signalling compounds, and in general acts as a ''master hormone'' (see later). Therefore, by transporting one hormone, auxin, in a controlled manner, several developmental phenomena can be regulated. The advantages of a specialized hormone transport system, compared with the xylem and phloem, have been discussed by Weyers and Paterson (2001).

Unlike for GA_1 itself, there is evidence that GA_1 precursors are transported within the vegetative shoot system. In germinating *Arabidopsis* seedlings, there is a spatial separation between the sites of expression of genes encoding enzymes from early and late in the GA biosynthetic pathway. This implies that early GA precursors such as *ent*-kaurene might move short distances from one tissue to another (Yamaguchi and others 2001).

There is also evidence for the long-distance movement of GA_1 precursors. Unlike the *le-1* mutant, *na* scions elongate dramatically when grafted to wild-type stocks (Reid and others 1983). Because *na* blocks the oxidation of the early GA precursor, *ent*-kaurenoic acid (Davidson and others 2003), the transported intermediate must be past that step. The *na* grafting results remain the best evidence that in some circumstances at least, GA_1 precursors are mobile within the shoot system. However, reciprocal grafting (wild-type scions on *na* stocks) did not reduce elongation of the scion, indicating that the immature wild-type scions were able to synthesize adequate levels of GA_1 for normal growth even in the absence of intermediates imported from below.

Certain graft combinations used by Reid and others (1983) implicate the mature shoot tissue as a source of transported GAs. However, whether or not mature tissue is capable of synthesizing GAs is somewhat controversial, and it is often implied that GA biosynthesis is confined to elongating, immature tissue (Hedden 1999; Davies 2002). This belief is based largely on papers by Aach and others (1995, 1997), and is not consistent with the view that mature tissue can synthesize precursors of GA_1 and export them to the elongating zone.

In pea, GA_{20} and GA_1 levels are certainly very low in mature, fully expanded tissue (Proebsting and others 1992; Smith and others 1992; Ross and others 2003). However, Ross and others (2003) suggest that this is not primarily due to reduced GA biosynthesis in mature tissue, but rather to rapid deactivation of GA_{20} and GA_1. Mature tissue is not markedly deficient in GA_{19}, which might therefore be the mobile GA_1 precursor that moves across graft unions (Reid and others 1983). The rapid deactivation of GA_{20} and GA_1 in mature tissue does not appear to be due to a low level of auxin (Ross and others 2003).

Although IAA is the main auxin in pea shoots, fruits contain substantial amounts of another auxin, 4-chloro-IAA, which appears to move from young seeds into the pods, where it stimulates the conversion of GA_{19} to GA_{20} (van Huizen and others 1997; Ozga and Reinicke, this issue). Previously we noted that the significance of GA_1 for pod elongation is still unclear (MacKenzie-Hose and others 1998; Ross and others 2002), mainly because GA-deficient pods, such as those on *le-1* plants, are not shorter than wild-type pods (Santes and others 1993). However, a reexamination has revealed some differences between the isolines 205+ (*LE*) and 205− (*le-1*), which bear ''sugar'' or non-parchmented pods (controlled by genes *P* and *V*), and which were the lines used by Santes and others (1993). Figure 8 shows that *le-1* pods are narrower (suture to suture) and more tapered than *LE* pods. Thus GA_1 deficiency in the *le-1* mutant might, after all, affect some aspects of pod development, although not pod length itself.

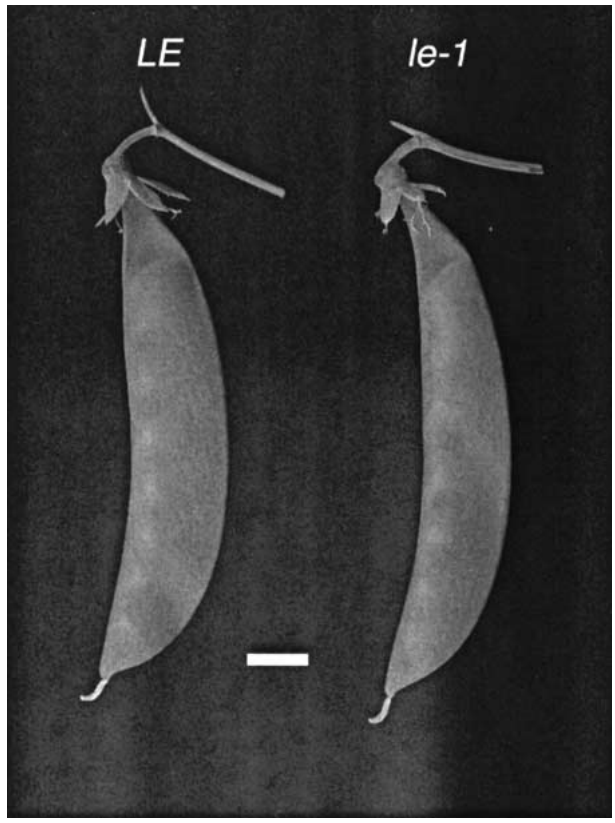

Figure 8. Effects of the *le-1* mutation on the development of ''sugar'' (non-parchmented) pods. Shown are 9-day-old pods from *LE* (line 205+) and *le-1* (205−) plants. The mean maximum suture-to-suture width of 10 to 12 day-old *LE* pods was 17.5 ± 0.3 mm (n = 12), and that of *le-1* pods was 15.4 ± 0.12 mm (n = 12). In addition, note the greater degree of tapering of the *le-1* pod.

Other Potential Interactions Between Growth Hormones in Pea Stem Elongation

Discovering the promotion of GA biosynthesis by IAA is significant in historical terms, because to a large extent it resolves an issue that has been under investigation for decades. However, is the auxin-GA interaction merely one of many in pea where one hormone markedly affects the level of another? Or does it ''stand out'' from other relationships between pairs of plant hormones?

It is important to note at the outset that large changes in the level of a hormone do not necessarily cause large changes in other hormone levels. The large deficiency of GA_1 in the *le-1* mutant, for example, is accompanied by only a small (25%) change in IAA level (Law and Davies 1990), and GA_1 application to the mutant, which increased GA_1 content by 5000-fold, increased IAA content by less than 2-fold (Ross and others 2002). Thus, the GA_1-auxin interaction is much weaker than the auxin-GA interaction. Indeed, the strong growth response to applied GA_1 by decapitated (that is, auxin-deficient) plants allowed us to obtain evidence that GA_1 does not promote growth primarily by increasing auxin content (Ross and others 2002).

It is instructive also to consider a third growth hormone group, the brassinosteroids (BRs). It is

now clear from the short stature of BR-deficient mutants that BRs are important for elongation (Altmann 1999). Including the BRs with IAA and GA_1 increases from two to six the number of potential interactions whereby one hormone might affect the level of another. As discussed above, auxin markedly affects GA levels but the reverse does not hold, leaving four potential interactions. Two of these can be examined by measuring IAA and GA_1 levels in the *lkb* mutant, which is BR-deficient (Nomura and others 1997, 1999).

The level of IAA is not reduced in apical portions of *lkb* plants, but is reduced in the elongating internodes (by approximately 2-fold in internodes 15–30% of their final length and 3-fold at the 50–100% stage; McKay and others 1994). The significance of this reduction for elongation is not yet clear. It is true that auxins can stimulate the elongation of *lkb* internodes (McKay and others 1994), but it has not been excluded that auxin sensitivity is enhanced in the mutant. The BR-related *sax* mutant of *Arabidopsis* has been reported to affect auxin responsiveness (Ephritikhine and others 1999).

Turning to the GAs, Lawrence and others (1992) showed that *lkb* apical portions (apical buds and expanding internodes, analyzed together) are not deficient in GA_1. GAs have also been quantified in the d^x mutant of tomato, which is BR-deficient (Bishop and others 1999). Hedden and Lenton (1988) reported small (approximately 2-fold) increases in the levels of GA_{19}, GA_{20} and GA_1 in the d^x mutant, compared with the wild-type, whereas Nadhzimov and others (1988) reported quite large accumulations of GA_{20} in the mutant. On the other hand, Bouquin and others (2001) reported that the expression of a key GA biosynthesis gene was reduced in mutants affected in BR levels or response, implying that BR deficiency might lead to deficiencies of GA_{20} and therefore GA_1. Clearly, it will be important to quantify GAs in BR-deficient *Arabidopsis* mutants.

Thus it appears that of the potential interactions among auxin, GA and BRs in pea, the auxin-GA interaction is the only one where changes in hormone content are sufficiently large to substantially affect growth. Other interactions, such as the relatively small effect of BR deficiency on auxin content of internodes, are of interest from the point of view of hormone homeostasis, but there is no evidence as yet that auxin deficiency in *lkb* mediates a substantial portion of the mutation's dwarfing effect.

Nevertheless, the effect of auxin on GA_1 content may be part of a more general phenomenon whereby auxin acts as a ''master hormone,'' regulating the levels of a range of signalling molecules. Auxin suppresses the content of cytokinins in root sap (Bangerth 1994), and this might be important in the inhibition of lateral branching. There is also evidence for an auxin-polyamine interaction in *Arabidopsis* (Hanzawa and others 2000). Furthermore, auxin stimulates ethylene biosynthesis in a range of species, including pea (Burg and Burg 1966). However, it is doubtful whether ethylene plays a role in regulating the internode length of light-grown, wild-type pea shoots during most of the vegetative growth phase, because such shoots show no response to application of the ethylene synthesis inhibitor aminoethoxyvinylglycine (AVG; data not shown).

As the focus on plant hormone interactions intensifies, many small effects of one hormone on another will no doubt be discovered, but at least some of these will be of limited significance. When constructing models relating signals to plant development, it will be important to concentrate on the more significant interactions to ensure that the resulting models are not unduly complex.

Conclusions

It now appears that a key function of auxin in the pea plant is to maintain the GA_1 content of the elongating internodes. Auxin performs this role by promoting GA_1 biosynthesis and inhibiting GA_1 deactivation, by regulating the genes *LE* (*PsGA3ox1*) and *SLN* (*PsGA2ox1*), respectively. Auxin rapidly affects the transcript levels of these genes, although we have shown that *LE* is a late, rather than an early, auxin response gene. Auxin can now be viewed as the mobile component of a two-factor system, with GA_1 itself synthesized in the elongating internodes.

In excised stem segments, a traditional system for studying the auxin-GA interaction, the GA_1 content of *LE* segments drops rapidly after excision, and auxin prevents this decrease. The auxin-induced GA_1 is important for internode elongation, as shown by the reduced auxin growth response of *le-1* stem segments. Indeed, GA_1 can be viewed as a component of the auxin signal transduction pathway, and *LE* can be classified as a late auxin response gene with a well-defined function that links auxin with the regulation of stem elongation. As discovered in early experiments, auxin is also important for the GA *response* in excised stem segments. Decapitated plants, on the other hand, can respond strongly to applied GA.

Other possible interactions among auxin, BRs and GAs in pea that have been investigated so far do

not involve large effects of one growth hormone on another. At this stage, the auxin-GA interaction remains the only case where there is strong evidence that one hormone, auxin, significantly promotes elongation by altering the level of a second hormone, GA.

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