Plant Signaling & Behavior 6:3, 406-408; March 2011; © 2011 Landes Bioscience

Auxin acts independently of DELLA proteins in regulating gibberellin levels

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Shoot elongation is a vital process for plant development and productivity, in both ecological and economic contexts. Auxin and bioactive gibberellins (GAs), such as GA_1, play critical roles in the control of elongation,[1-3] along with environmental and endogenous factors, including other hormones such as the brassinosteroids.[4,5] The effect of auxins, such as indole-3-acetic acid (IAA), is at least in part mediated by its effect on GA metabolism,[6] since auxin upregulates biosynthesis genes such as GA 3-oxidase and GA 20-oxidase and downregulates GA catabolism genes such as GA 2-oxidases, leading to elevated levels of bioactive GA_1.[7] In our recent paper,[1] we have provided evidence that this action of IAA is largely independent of DELLA proteins, the negative regulators of GA action,[8,9] since the auxin effects are still present in the DELLA-deficient *la cry-s* genotype of pea. This was a crucial issue to resolve, since like auxin, the DELLAs also promote GA_1 synthesis and inhibit its deactivation. DELLAs are deactivated by GA, and thereby mediate a feedback system by which bioactive GA regulates its own level.[10] However, our recent results,[1] in themselves, do not show the generality of the auxin-GA relationship across species and phylogenetic groups or across different tissue types and responses. Further, they do not touch on the ecological benefits of the auxin-GA interaction. These issues are discussed below as well as the need for the development of suitable experimental systems to allow this process to be examined.

Key words: auxin, gibberellins, DELLA proteins, interactions, elongation

Submitted: 11/30/10

Accepted: 12/02/10

DOI: 10.4161/psb.6.3.14352

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Addendum to: O'Neill DP, Davidson SE, Clarke VC, Yamauchi Y, Yamaguchi S, Kamiya Y, et al. Regulation of the gibberellin pathway by auxin and DELLA proteins. Planta 2010; 232:1141–9; PMID: 20706734; DOI: 10.1007/s00425-010-1248-0.

Generality of the Auxin-GA Interaction

The strong promotion of bioactive GA levels by auxin appears to occur widely across angiosperms. It is present in both monocots (barley[11]) and broadly across the dicots (pea,[12] Arabidopsis[1,13] and tobacco[14]). However, the molecular basis of the interaction may differ from species to species. For example, with respect to GA_1 synthesis, the shoot-expressed *GA20ox1* gene seems to be only marginally upregulated in pea shoots, with the main effect attributable to the upregulation of Mendel's GA 3-oxidase gene, *LE*.[12,15] However, in tobacco GA 20-oxidation is strongly upregulated.[14] The reason for this difference is not clear, although at least two hypotheses can be suggested. Firstly, since the two gene families are closely related members of the 2-oxoglutarate-dependent dioxygenase group of enzymes, the auxin-GA interaction might pre-date the evolution of the split into these two enzyme specificities. Secondly, this may be a case of convergent evolution to yield the required effect on GA_1 levels because of the evolutionary importance to regulate elongation.

Developmental Responses

While the work by O'Neill et al.[1] focused on shoot elongation in pea and Arabidopsis, the effect of auxin on stimulating GA_1 levels is also seen in pea roots,[15] pods and seeds.[16,17] It therefore appears to be a common regulatory junction between the auxin and GA pathways. Detailed examination of these interactions in

Table 1. Elongation of the upper internode of phenotypically wild-type and the DELLA-deficient *la cry-s* mutant of pea seedlings either left intact or decapitated directly below the apical bud

	Intact		Decapitated	
	(cm)	n	(cm)	n
Wild-type	1.82 ± 0.13	6	1.29 ± 0.07	6
la cry-s	3.25 ± 0.46	4	3.63 ± 0.54	3

Elongation was measured over the 48 h period after decapitation. The plants were 12 days old at the time of decapitation.

DELLA-deficient *la cry-s* plants has not been carried out for all these developmental processes, so it is not clear if the action of auxin is independent of DELLA action. However, in roots the auxin action inhibitor PCIB caused significantly less inhibition of elongation in *la cry-s* plants than in phenotypically wild type plants, although this does not distinguish between the effect of auxin on GA synthesis or an effect on the GA response pathway.[15] Likewise, decapitation did not reduce the elongation of young expanding internodes in the DELLA-deficient pea mutant *la cry-s* (at this stage of development) as it does in plants with a wild-type phenotype (**Table 1**, $p < 0.01$). This presumably occurs since GA_1 levels and hence auxin levels are not important for elongation in the absence of the growth-inhibiting DELLA proteins. In pea pods (pericarps) it appears that it is only the chlorinated auxin, 4-Cl-IAA, and not IAA, that is involved in the regulation of GA metabolism genes.[17] This is suggested to be due to the ability of 4-Cl-IAA, but not IAA, to inhibit ethylene action.[18] The 4-Cl-IAA is thought to be exported from the developing seed to ensure pod growth is sufficient to allow seed development.[17] In *la cry-s* plants parthenocarpic pods of normal size develop from emasculated flowers showing that DELLA proteins are involved in pod development.[19]

A problem with studying plant hormones is how to perturb the system in a biologically relevant way. This is particularly the case where a hormone shows a clear optimum level for the control of a process, as is evident for auxin in stem and root elongation.[1,20] It is well known that auxin at high concentrations can inhibit elongation, but such concentrations, and the resulting internal levels of the applied compound, are probably unrealistically high and never encountered naturally. We suggest it is preferable to deplete auxin using auxin transport inhibitors,[21] decapitation[12,22] or excised segments.[1] This allows strong responses to be observed, both to the depletion and also to the addition of auxin to the auxin-depleted system. This may be much more important for work on auxin than for other hormones (e.g., GA, brassinosteroids) where distinct optima do not appear to exist and only saturation of the response is observed at high physiological concentrations.[4,23] The cause of this difference between auxin and other hormones is beyond the scope of this article but may reflect the direct effects that auxin has on other hormone levels, especially ethylene.[24] A further difficulty in working with auxin is that unlike other hormones, clear IAA-deficient mutants, specifically impaired in IAA biosynthesis, are unavailable.

Ecological Implications

Removal of the shoot apex has a major impact on the competitive capability of plants. This is particularly the case in herbaceous, mesophyllic, caulescent dicots where the apical bud is exposed (terminal) and the plants are competing for space and light. However, after removal of the apex, apical dominance is released, allowing the shoot to regenerate. As part of

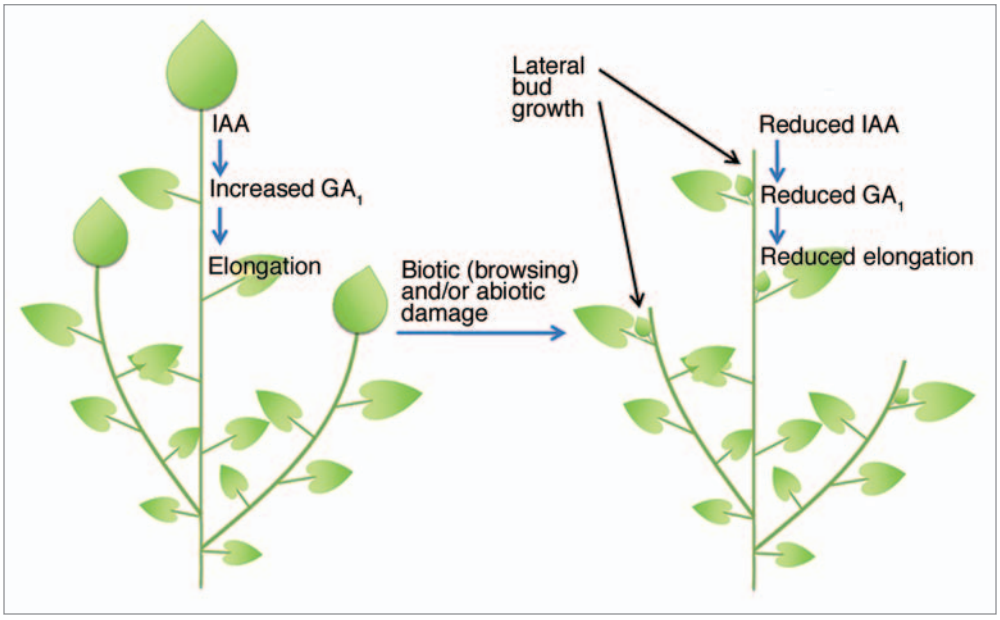

Figure 1. Biotic and abiotic removal of shoot apices results in reduced stem growth and conservation of resources for lateral bud outgrowth. This occurs since apex removal reduces auxin levels in young internodes which results in reduced bioactive GA levels and hence elongation.

this response the plant must redirect its resources from the elongating shoot that has been decapitated to the new laterals that have been released from dormancy or growth suppression. In pea, for example, the decapitated internode remaining can be less than 10% of the fresh weight of the fully expanded internode (data not shown). Traditionally, the drop in basipetal auxin transport from the shoot apex has been implicated in the outgrowth of lateral buds, although this involvement has been questioned.[25]

Interactions between auxin and other signals, including the recently identified branching hormone, strigolactone, are still being clarified.[26] The drop in GA_1 levels in the decapitated stem, attributable to reduced auxin content, is a vital component in this redistribution of resources, reducing elongation of the "stump" and internodes below this that are still elongating (Wolbang and Ross, unpub. data), thereby freeing up nutrition that presumably can be diverted into the new lateral shoot(s) (**Fig. 1**).

Experimental Approaches

With the increasing ease of monitoring gene expression, it has become common to base the suggested involvement of a hormone (i.e., the level of the hormone) in a particular process on changes in the expression of hormone synthesis/metabolism genes, rather than measuring the level of the hormone directly.[13,27,28] The validity of this approach has been discussed in the literature.[29] A major problem with using the expression of metabolism genes is that most are members of multi-gene families. Further, the pathways involved have many steps. Hence, it is hard to infer, even from comprehensive studies, what the level of the biologically active member of the pathway may be. Direct physiochemical measurements are frequently the only way to be certain about the hormone level, although such methods suffer from the need for relatively large tissue samples (at least mg quantities) and therefore may not provide a measure in a certain cell type or even tissue type. Molecular techniques may in future provide this specificity although the risk of other factors impinging on the reporter system needs to be considered, possibly on a case-by-case basis.

Even where the level of the active hormone is accurately measured it may still be important to use metabolic studies with labelled intermediates to understand why this level has changed. For example, in the original work on the effect of auxin on GA_1 levels[12] it was not clear if GA_1 levels were changed solely because of enhanced synthesis or by changes to both synthesis and catabolism. With multi-gene families involved with both processes and auxin sometimes regulating members of these families in opposite directions, metabolism studies are the only easy method for summing the actions of the gene family at the metabolite level, as was done in O'Neill et al.[1]

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