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Article Addendum

Tomato Aux/IAA3 and HOOKLESS are important actors of the interplay between auxin and ethylene during apical hook formation

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Plants implement differential cell growth as an adaptation process in order to direct their development in a way that allow them to better cope with the environmental conditions. This process requires the complex integration of multiple hormone signalings, though, a lot remain to be known about the mechanisms and the molecular actors that take part in this hormonal dialogue. We have previously shown that Sl-*IAA3*, an *Aux/IAA* gene, is a molecular link between auxin and ethylene responses in tomato plants. We show here that the expression of Sl-*IAA3* in etiolated seedlings is restricted to the inner side of the apical hook, opposite to that of the *HOOKLESS* gene whose loss-of-function mutation results in the absence of hook formation. We propose a model on how auxin and ethylene modulate the expression of *Auxin Response Factor 2* (*ARF2*) via IAA3 and HLS protein to regulate hypocotyl bending.

The coordination of plant developmental processes and growth adaptation rely on complex interplay between individual hormone and non-hormone signalings. The phytohormones auxin and ethylene are essential regulators of plant development and the mechanisms governing the interactions between these two hormones are becoming better understood even though, so far, only few molecular players of this cross-talk have been identified.[1-3]

The ethylene-mediated regulation of auxin biosynthesis occurs through the activation of WEI2/ASA1 and WEI7/ASB1, anthranilate synthase subunits that catalyse the first step in tryptophane biosynthesis. The reciprocal effect of auxin on ethylene biosynthesis through the activation of several ACC synthases has also been described.[4] A comprehensive study combining physiological, genetic and genomic approaches uncovered a simple mechanistic model for the interaction between the two hormones in roots. Indeed, in addition of acting independently on the same target genes, ethylene and auxin can reciprocally regulate each other's biosynthesis and influence each other's response pathways.[5] This model provides a likely explanation for the strong ethylene response defects observed in auxin mutants. In accordance with these data we have recently shown that phenotypic responses to the downregulation of Sl-*IAA3* gene in tomato include alterations to the classical auxin-regulated processes of apical dominance and hypocotyl elongation as well as to typical ethylene responses such as apical hook formation in etiolated seedlings and leaf epinasty in light-grown plants.[6] These results suggest that Sl-*IAA3* is an integral regulator of auxin and ethylene responses in tomato plants.

The induction of apical hook formation in Arabidopsis represents one of the best described examples of auxin-ethylene cross-talk in plants and the hook is a result of differential cell elongation on opposite sides of the hypocotyls.[7-9] An Arabidpsis mutant lacking differential growth in the apical region of the hypocothyl (*hookless1)* has been identified and it was proposed that the mutated gene encodes a key regulator that integrates ethylene and auxin signaling pathways during apical hook formation.[7] Subsequently, a partial suppressor of *hls1* phenotype resulting from a loss-of-function mutation in the *ARF2* gene (Auxin Reponse Factor) was isolated.[9] Interestingly, our recent data indicate that antisense-mediated inhibition of Sl-*AA3*, a tomato *Aux/IAA* gene, results in exaggerated hook formation associated with a downregulation of ARF2 expression. By contrast, accumulation of Sl-*HLS* transcripts is not altered in the *AS-IAA3* plants suggesting that the exaggerated hook formation in the transgenic lines does not involve an alteration in Sl-*HLS* expression.

To further investigate potential interactions between Sl-*HLS* and Sl-*IAA3* in controlling hook formation we analyzed the spatial expression of the Sl-*HLS* and Sl-*IAA3* in the apical hook by native promoter-reporter constructs. We generated transgenic lines expressing a 1.3 kb fragment of the Sl-*HLS* promoter fused to the *GUS* reporter gene and assessed the GUS staining in etiolated tomato seedlings treated with ethylene. Remarkably, Pro*HLS*::GUS

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staining was restricted to the outer side of the hook curvature, whereas the Sl-*IAA3* promoter drove GUS staining exclusively on the inner side (Fig. 1). These data suggest that Sl-IAA3 acts as a repressor of auxin/ethylene-mediated cell elongation on the inner surface of the apical hook and/or conversely that SI-HLS1 is involved in promoting cell elongation on the outer surface. Sl-*IAA3* and Sl-*HLS* genes provide therefore tissue-specific markers for the inner and outer sides of the apical hook, respectively, and the corresponding promoters could be useful to target the ectopic expression of transgenes to a specific side of the hook.

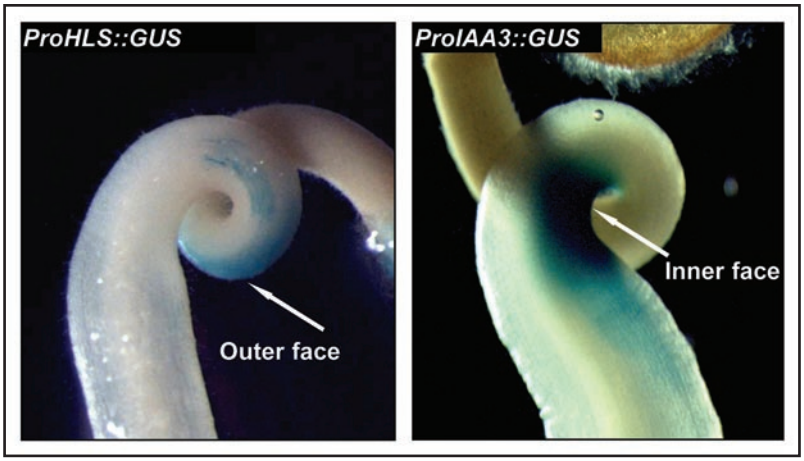

Figure 1. The expression of Sl-*IAA3* and Sl-*HLS* genes takes place on opposite sides of the hook. Tomato Pro*HLS*::GUS and Pro*IAA3*::GUSseedlings were dark-grown for 5 days and then treated for 48 h with 10 µl l^{-1} of ethylene. The images are representative of at least three independent experiments with n > 30 seedlings per experiment.

We postulate that Sl-IAA3 and Sl-HLS may act in parallel pathways both of them involving ARF2 as a downstream component, or Sl-*HLS* may act upstream of Sl-*IAA3* to downregulate its expression which might explain why Sl-*IAA3* is not expressed in the upper side of the hook where the expression of Sl-*HLS* is high. Figure 2 depicts a model mechanism on how the convergence of ethylene and auxin signaling impacts differential growth of etiolated seedlings in an IAA3-dependent manner. IAA3 serves as central integrator of ethylene and auxin. In this model, HLS-dependent and IAA3-dependent regulation of ARF2, a negative regulator of the differential auxin response, leads to enhanced differential growth and exaggerated hook curvature. In conclusion, we suggest that the process of hook formation requires an interplay between HLS, IAA3 and ARF2 proteins.

Continued efforts are now engaged to determine the post-translational regulation of IAA3 by auxin and ethylene and to test the potential interaction between IAA3 and ARF2 proteins.

The Universal Genome Walker Kit (Clontech Laboratories, Inc., Palo Alto, CA, USA) was used to isolate 1.3 kb of the Sl-*HLS* gene promoter region. The Sl-*HLS* promoter was then fused to the β-glucuronidase (*GUS*) reporter gene in the plp100 binary vector[10] and used for stable tomato transformation [cv. MicroTom]. Growth conditions were performed as described previously.[6] For histochemical GUS analysis, Pro*HLS*::GUS and Pro*IAA3*::GUS transgenic lines were incubated at 37°C for 5 h with GUS-staining solution (100 mM sodium phosphate buffer, pH 7.2, 10 mM EDTA, 0.1% Triton and 1 mM 5-bromo-4-chloro-3-indolyl-b-D-glucuronic acid). Following GUS staining, samples were washed several times to extract chlorophyll using a graded ethanol series.

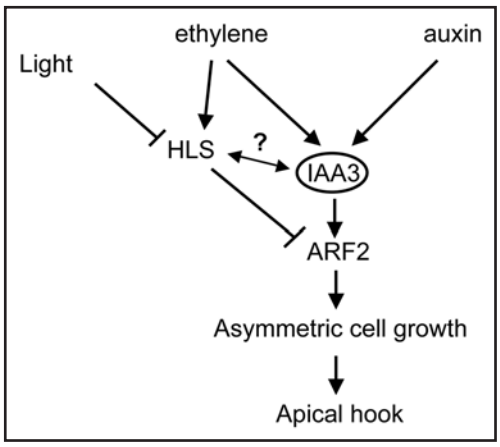

Figure 2. A model mechanism describing the different players of the auxin/ethylene interplay leading to differential growth in etiolated seedlings.

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