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

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**Key words:** apical hook, hormone signaling, auxin/ethylene cross-talk, tomato, transcription factors

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.<sup>1-3</sup>

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.<sup>4</sup> 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.<sup>5</sup> 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.<sup>6</sup> 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.<sup>7-9</sup> An Arabidopsis mutant lacking differential growth in the apical region of the hypocotyl (*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.<sup>7</sup> Subsequently, a partial suppressor of *hls1* phenotype resulting from a loss-of-function mutation in the *ARF2* gene (Auxin Response Factor) was isolated.<sup>9</sup> Interestingly, our recent data indicate that antisense-mediated inhibition of *Sl-IAA3*, 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, *ProHLS::GUS*

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staining was restricted to the outer side of the hook curvature, whereas the *SI-IAA3* promoter drove GUS staining exclusively on the inner side (Fig. 1). These data suggest that *SI-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. *SI-IAA3* and *SI-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.

We postulate that *SI-IAA3* and *SI-HLS* may act in parallel pathways both of them involving ARF2 as a downstream component, or *SI-HLS* may act upstream of *SI-IAA3* to downregulate its expression which might explain why *SI-IAA3* is not expressed in the upper side of the hook where the expression of *SI-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 *SI-HLS* gene promoter region. The *SI-HLS* promoter was then fused to the  $\beta$ -glucuronidase (*GUS*) reporter gene in the plp100 binary vector<sup>10</sup> and used for stable tomato transformation [cv. MicroTom]. Growth conditions were performed as described previously.<sup>6</sup> For histochemical GUS analysis, *ProHLS::GUS* and *ProIAA3::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.

## References

- Alonso JM, Stepanova AN, Solano R, Wisman E, Ferrari S, Ausubel FM and Ecker JR. Five components of the ethylene-response pathway identified in a screen for weak ethylene insensitive mutants in Arabidopsis. *Proc Natl Acad Sci USA* 1993; 100:2992-7.
- Chilley PM, Casson SA, Tarkowski P, Wang KL-C, Hawkins N, Hussey PJ, et al. The POLARIS peptide of Arabidopsis regulates auxin transport and root growth via effects on ethylene signaling. *Plant Cell* 2006; 18:3058-72.
- Swarup R, Perry P, Hagenbeek D, van der Straeten D, Beemster GTS, Sandberg G, et al. Ethylene upregulates auxin biosynthesis in Arabidopsis seedlings to enhance inhibition of root cell elongation. *Plant Cell* 2007; 19:2186-96.
- Stepanova AN, Hoyt JM, Hamilton AA, Alonso JM. A link between ethylene and auxin uncovered by the characterization of two root-specific ethylene-insensitive mutants in Arabidopsis. *Plant Cell* 2005; 17:2230-42.
- Stepanova AN, Yun J, Likhacheva AV, Alonso JM. Multilevel interactions between ethylene and auxin in Arabidopsis roots. *Plant Cell* 2007; 19:2169-85.

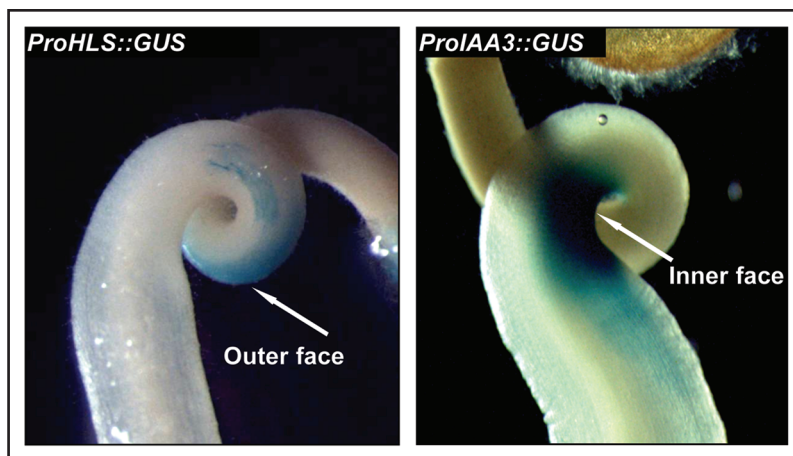


Figure 1. The expression of *SI-IAA3* and *SI-HLS* genes takes place on opposite sides of the hook. Tomato *ProHLS::GUS* and *ProIAA3::GUS* seedlings were dark-grown for 5 days and then treated for 48 h with  $10 \mu\text{l l}^{-1}$  of ethylene. The images are representative of at least three independent experiments with  $n > 30$  seedlings per experiment.

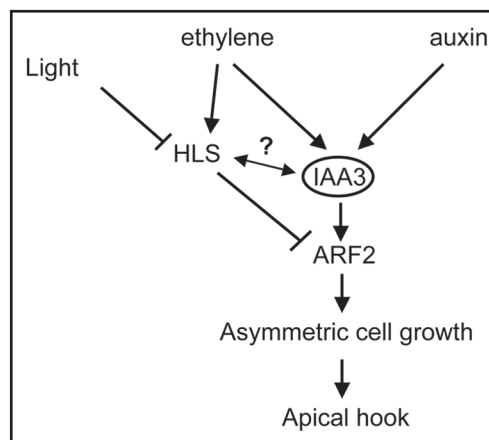


Figure 2. A model mechanism describing the different players of the auxin/ethylene interplay leading to differential growth in etiolated seedlings.

- Chaabouni S, Jones B, Delalande C, Wang H, Li Z, Mila I, et al. *SI-IAA3*, a tomato Aux/IAA at the crossroads of auxin and ethylene signalling involved in differential growth. *J Exp Bot* 2009; 60:1349-62.
- Lehman A, Black R, Ecker JR. *HOOKLESS1*, an ethylene response gene, is required for differential cell elongation in the Arabidopsis hypocotyl. *Cell* 1996; 85:183-94.
- Raz V and Ecker JR. Regulation of differential growth in the apical hook of Arabidopsis. *Development* 1999; 126:3661-8.
- Li H, Johnson P, Stepanova A, Alonso JM, Ecker JR. Convergence of signaling pathways in the control of differential cell growth in Arabidopsis. *Dev Cell* 2004; 7:193-204.
- Szabados L, Charrier B, Kondorosi A, de Bruijn FJ, Ratet P. New plant promoter and enhancer testing vectors. *Molec Breed* 1995; 1:419-23.