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Article Addendum

Arabidopsis thaliana GH3.9 in Auxin and Jasmonate Cross Talk

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ABSTRACT

Plant growth and development are governed by an intricate web of signaling networks controlled by phytohormones, such as auxin and jasmonic acid. Auxin influences all aspects of plant growth and development, ranging from embryogenesis to root and shoot morphogenesis and organ patterning. Three major groups of auxin-responsive genes have been classified as *IAA/AUX*, *GH3* and *SAUR* families. Some Group I and II GH3 proteins biochemically function in conjugating amino acids to methyl jasmonate and auxin, respectively. We recently demonstrated that *GH3.9*, a previously uncharacterized Group II *GH3* gene family member, influences primary root growth. Whereas several *GH3* family members are transcriptionally induced by auxin, *GH3.9* was repressed by exogenous indole-3-acetic acid (IAA) in whole seedlings. *GH3.9* promoter::*GUS* reporter transgenic seedlings showed expression in several tissues, and application of exogenous IAA led to a shift in promoter activity from primary roots to lateral root tips, supporting the hypothesis that *GH3.9* maintains auxin homeostasis by redistribution of active auxin pools in roots. *GH3.9* mutations influenced both IAA- and methyl jasmonate (MeJA)-mediated root growth inhibition. In this addendum, we expand on a possible role for *GH3.9* in crosstalk between auxin and jasmonate signal transduction pathways controlling plant development.

Hormones play a critical role in growth and development of multicellular organisms. Plants are no exception to this rule with the cues to organ initiation and development, reproduction, and resistance to biotic and abiotic stresses governed by a fine interplay between phytohormones. Among the well-studied plant hormones, auxin is a major player due to its pleiotropic effects throughout the plant life cycle. Indole-3-acetic acid (IAA) is the prevalent form of naturally occurring auxin and has been implicated in cell division, cell elongation and cell differentiation.[1,2] Jasmonic acid (JA) and its derivatives, collectively referred to as jasmonates, comprise another group of plant hormones that are essential for seed germination, root growth, fertility, and defense.[3] Isolation of mutants resistant to exogenous JA (e.g., *jar1*, *coi1* and *jin* mutants) or auxin (e.g., *axr* mutants) has enhanced our understanding of the mechanisms by which these hormones regulate plant development and biotic and abiotic stress responses.[2,4] The physiological effects of these phytohormones are, in part, manifested by altered expression of JA- and auxin-responsive genes.[5]

Auxin-responsive genes fall into three major families, the so-called *AUX/IAA*, *GH3* and small auxin up RNA (*SAUR*).[6] We recently reported a function for *GH3.9* in primary root growth.[7] *GH3.9* is a Group II *GH3* gene postulated to act as an IAA-amido synthetase to conjugate free auxin to amino acids.[8,9] Other Group II *GH3* gene family members influence primary root growth, hypocotyl elongation, apical dominance, leaf formation and stress responses.[10-13] No obvious morphological alterations in the aerial tissues were observed in *gh3.9* mutants, possibly due to functional redundancy. However, the *gh3.9-1* mutant, *GH3.9* RNAi transgenics, and additional T-DNA insertion mutants displayed a long-root phenotype, moderate sensitivity to IAA-mediated root growth inhibition, and moderate resistance to MeJA-mediated root growth inhibition.[7] Unlike most other Group II family members, exogenous IAA repressed *GH3.9* expression in seedlings. *GH3.9* promoter::*GUS* transgenic seedlings provided greater detail on where *GH3.9* might function. The *GH3.9* promoter drove expression in root vascular tissues, siliques, seedling root-hypocotyl junctions and mature embryos. Interestingly, exogenous IAA caused increased expression in lateral root tips with a concomitant decrease in primary roots, both sites of auxin biosynthesis.[7,14] Polar transport of auxin from root tip to outer cells in root meristem by PIN and PAT proteins is essential for maintaining physiological levels of auxin to ensure proper establishment of root architecture.[15] Therefore, *GH3.9* may complement

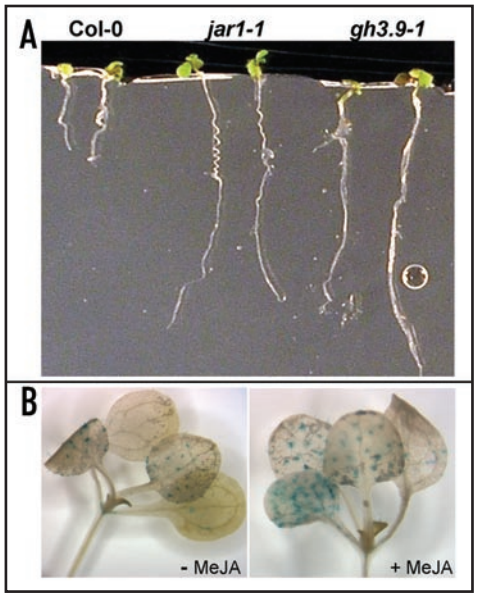

Figure 1. (A) Photographs of representative seedlings (genotypes; wild-type Col-0, mutants *jar1-1*, and *gh3.9-1*) after ten days of growth on MS-agar media supplemented with 10 μM MeJA. (B) Photographs of representative transgenic plants harboring a *GH3.9* promoter::*GUS* construct. *GH3.9* is expressed in trichomes during leaf development, and exogenous MeJA (10 μM for 60 minutes) enhanced GUS activity in trichomes.

auxin transport proteins to regulate active auxin levels in specific root tissues/cell types. In this report, we would like to further comment on *GH3.9* possibly affecting plant growth and development regulated by JA-mediated signaling pathways.

GH3.9 IN AUXIN AND JASMONIC ACID CROSS TALK?

Biosynthesis, metabolism and transport of plant hormones finely tune gene expression in response to various stimuli. Cross talk between intersecting hormone signaling pathways is the paradigm. For example, expression of JA-responsive genes (JRGs) have been reported to be either repressed or induced by exogenous auxin, supporting both antagonism and synergism between JA- and auxin-mediated signaling pathways.[16,17] The extent of this cross talk is exemplified by identification of *auxin-resistant1* (*axr1*) mutants with altered JRG expression in separate genetic screens for mutants resistant to MeJA- and auxin-mediated growth inhibition.[16,18] *AXR1* encodes an E1 Nedd8/RUB1-activating enzyme, and *COI1* encodes an F-box protein that is a subunit of SCF^{COI1} E3 ubiquitin ligase.[19,20] Therefore, both jasmonate and auxin signal transduction depends on small modifier protein-dependent proteasome-mediated degradation.[4]

The *jar1* mutant, identified in MeJA-mediated root growth inhibition screen, is defective in a Group I *GH3* gene family member, *JAR1* (*GH3.11*).[9] JAR1 and GH3.9 likely regulate hormone activity by conjugating amino acids to MeJA and auxin, respectively.[8,9] JA-isoleucine conjugate levels were significantly reduced in *jar1-1*, while levels of other conjugates, including JA-phenylalanine, were increased, implying that additional JA-conjugating enzymes exist.[21] The *jar1-1* mutant displayed a short-root phenotype opposite of the *gh3.9-1* long-root phenotype on unsupplemented media, prompting us to investigate the response of the *gh3.9-1* mutant to MeJA-mediated root growth inhibition. Unexpectedly, we noted similar roles for *JAR1* and *GH3.9* in MeJA-mediated root growth inhibition, that is both mutants were insensitive to MeJA-mediated root growth inhibition (Fig. 1A).[7] Also like *jar1-1*, fertility was unaffected in *gh3.9-1* (data not shown). However, exogenous IAA caused significant changes in GUS activity patterns in roots of *GH3.9* promoter::*GUS* transgenic seedlings, and MeJA had no apparent effect.[7] Our observed MeJA-dependent increase in GUS activity in young leaf trichomes may provide another example of integration of auxin and JA-mediated signaling pathways, similar to that performed by AXR1.[19,20]

GH3.9 IN JA-MEDIATED TRICHOME DEVELOPMENT?

Jasmonate signaling has been implicated in trichome development by increased JA-dependent trichome density and *JRG* promoter activity.[22,23] To investigate whether *GH3.9* expression in trichomes is regulated by JA, *GH3.9* promoter::*GUS* transgenics were treated with exogenous MeJA (10 μM), resulting in a moderate increase in the GUS activity in the trichomes of young leaves (75% GUS positive) compared to untreated seedlings (54% GUS positive; Fig. 1B). Trichome density in the *gh3.9-1* mutant was similar to wild-type plants (data not shown) and was also unaffected in the *jar1-1* mutant.[23] However, given the similar phenotypes related to MeJA-mediated root growth inhibition, it would be interesting to determine whether trichome development is affected in a *jar1*/*gh3.9* double mutant. This double mutant could also be assessed for both IAA- and JA-amino acid conjugate levels and auxin-, JA- or other hormone-responsive gene expression.[21,24] These experiments are likely to enhance our understanding of the physiological functions of *GH3.9* and *JAR1*, the importance of hormone conjugation to amino acids and the intricacy of hormone cross talk in plant development.

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