

The DELLA protein RGL3 positively contributes to jasmonate/ethylene defense responses

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Keywords: gibberellin, jasmonate, ethylene, *RGL3*, plant defense

Abbreviations: GAI, GA INSENSITIVE; RGA, REPRESSOR OF GA1-3; RGL1, RGL2 and RGL3, RGA-LIKEs; JIN1/MYC2, JASMONATE INSENSITIVE1; EIN3, ETHYLENE INSENSITIVE3; EIL, EIN3-Like; JAZ, JA ZIM-domain; COI, CORONATINE INSENSITIVE1; ERF1, ETHYLENE RESPONSE FACTOR1; PDF1.2, PLANT DEFENSIN1.2

Gibberellins (GA) are phytohormones controlling major aspects of plant lifecycle including seed germination, growth and flower development. GA signaling is also involved in resistance to adverse conditions, thus providing a mechanism for environmentally responsive growth regulation. We recently characterized the function of a core component of the GA signal transduction pathway: RGL3. RGL3 belongs to the DELLA family of negative GA response regulators. Jasmonate (JA) rapidly induces *RGL3* expression, which in turn enhances the expression of JA-responsive genes by inhibiting the activity of key repressors of JA signaling, the JAZ proteins. JA and ethylene (ET) are well known to play synergistic roles in plant disease resistance. Accordingly, we showed that RGL3 regulates plant defense responses by modulating JA/ET-mediated defense signaling pathway.

GA plays critical roles in modulating plant growth by integrating both developmental and environmental stimuli. Key regulators of the GA signaling pathway are the nuclear-localized DELLA proteins (DELLAs), a subset of the GRAS family of transcriptional regulators that repress all aspect of GA response. GA promotes growth by stimulating the proteasome-dependent destruction of DELLAs.¹ Recently, DELLAs have been reported to be implicated in plant disease resistance. Upon pathogen attack, an interplay between the phytohormones salicylic acid (SA), JA and ET activates distinct defense response signaling cascades, depending on the lifestyle of the invading pathogen.² Whereas SA is generally associated with resistance against biotrophs, JA/ET are associated with resistance to necrotrophs. Moreover, the interaction between these two types of resistance is antagonistic and the activation of one attenuates the other.² Recent studies showed that DELLAs enhance resistance against necrotrophic pathogens while attenuating resistance against biotrophs by modulating the SA/JA balance.^{3,4} This is consistent with findings of DELLAs being able to interact with and inhibit the activity of JAZs, repressors of JA response, thus providing a mechanism for appropriate defense responses.⁵ Indeed, JAZs bind and inhibit the activity of a wide range of transcription factors including the basic helix-loop-helix JIN1/MYC2, MYC3 and MYC4 involved in JA-mediated expression of wound response genes,⁶⁻¹⁰ and EIN3 / EIL proteins involved in JA/ET-mediated expression

of pathogenesis-related genes.¹¹⁻¹⁴ In this addendum we discuss the role of the Arabidopsis DELLA protein RGL3 in regulating JA/ET-dependent defense responses through direct competitive binding to JAZs.

JA Induces *RGL3* Expression via Direct Binding of MYC2 to its Promoter Region

The *Arabidopsis thaliana* genome encodes five DELLAs, GAI, RGA, RGL1, RGL2 and RGL3, that play distinct but also overlapping functions in repressing GA response.^{11,15,16} Studies of loss-of-function alleles of DELLAs have revealed a specific role for each DELLA, except *RGL3*. This functional specificity is conferred by their expression pattern rather than by their molecular activity.¹⁷ We recently showed that JA positively regulates the expression of *RGL3* in contrast to the other DELLA genes.¹⁸ The induction is dependent on a functional JA-signaling cascade as it is abolished in the JA-receptor *coi1-1* mutant and in *myc2 myc3 myc4* triple mutant plants. Furthermore, chromatin immunoprecipitation (ChIP) analysis and electrophoretic mobility shift assays (EMSA) demonstrated that MYC2 regulates the transcription of *RGL3* through direct association with its promoter region (to a CACATG G-box-like motif located near the transcription start site).¹⁸ Thus *RGL3* expression is directly induced by JA through its signaling pathway.

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Submitted: 01/16/13; Accepted: 02/05/13

<http://dx.doi.org/10.4161/psb.23891>

Citation: Wild M and Achard P. The DELLA protein RGL3 positively contributes to jasmonate/ethylene defense responses. Plant Signal Behav 2013; 8: e23891.

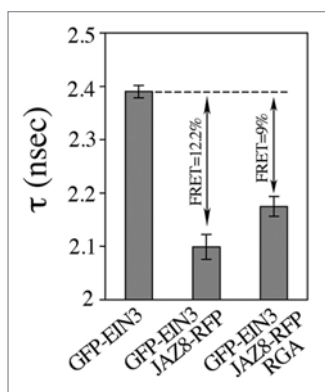


Figure 1. DELLA competes with EIN3 for binding to JAZ. Fluorescence lifetime analyses (in nsec) of GFP-EIN3, GFP-EIN3/JAZ8-RFP alone or together with RGA, and mean (\pm se) FRET value (%) in *Nicotiana benthamiana* agro-infiltrated leaves.

RGL3 Enhances MYC2 and EIN3 Activity via Competitive Binding to JAZs

Given the ability of DELLAs to interact and inhibit the activity of JAZs, repressors of MYC2, JA-mediated accumulation of RGL3 protein should enhance the expression of MYC2-regulated genes. Indeed, our results showed that induction of MYC2-regulated genes including *VSP2*, *TAT1* and *LOX2*, is reduced in *rgl3-5* mutant and enhanced in transgenic lines overexpressing *RGL3*. Thus RGL3 competes with MYC2 for binding to JAZs.^{5,18}

The JAZ proteins also interact with and inhibit EIN3 transcriptional activity to mediate JA/ET crosstalk.¹³ Whereas ET enhances EIN3 protein stability, JA releases EIN3 from the JAZs, thereby activating ET/JA-regulated processes.¹³ We next investigated whether DELLA function also modulates EIN3 activity via competitive binding to JAZs, in a similar manner than with MYC2. To this end, we monitored the interaction between JAZ8 and EIN3 by fluorescence lifetime imaging microscopy (FLIM) analysis of nuclei in transiently transformed *Nicotiana benthamiana* cells, in the presence and in the absence of a DELLA protein (RGA). Whereas without RGA, the fluorescence resonance energy transfer (FRET) between GFP-EIN3 and JAZ8-RFP was of 12.2%, expression of RGA significantly decreased the FRET to 9.0% (26.2% loss) (Fig. 1). This result demonstrates that the interaction between EIN3 and JAZ8 is diminished in the presence of DELLA. Consistent with this, overexpression of *RGL3* enhances EIN3-regulated genes, including *ERF1* and *PDF1.2*, in six week old Arabidopsis plants (Fig. 2A).^{12,14} In addition, JA-mediated induction of *PDF1.2* is reduced in *rgl3-5* mutant in comparison to wild-type plants (Fig. 2B). Taken together, our results indicate

that *RGL3* plays a critical role in modulating JA action by enhancing both MYC2 and EIN3 transcriptional activity.

RGL3 Contributes Positively to JA/ET-Mediated Plant Defense Responses

JA and ET play a crucial role in plant defense and are generally involved in the activation of defense responses against necrotrophic pathogens.² Our results showed that *rgl3-5* mutants are significantly more susceptible to the necrotrophic fungus *Botrytis cinerea* as represented by larger lesions correlated with increased pathogen growth as compared with wild-type.¹⁸ Conversely, *rgl3-5* mutants are more resistant to the hemibiotroph *Pseudomonas syringae* pv *tomato* DC3000 (*Pst*) compared with wild-type infected plants.¹⁸ This increased resistance is based on the capacity of *Pst* to activate the JA pathway via the production of a JA-mimicking phytotoxin, coronatine, therefore attenuating the defense response by exploiting the SA/JA antagonism.^{2,19} Consistent with this, induction of JA/ET-mediated expression of pathogenesis-related genes is compromised in *rgl3-5* infected leaves in comparison with wild-type infected leaves.¹⁸ Collectively, these results showed that RGL3 contributes positively to the JA/ET-defense pathway.

Previous studies have indicated that DELLAs can also interact with EIN3 and MYC2 to regulate apical hook development and the synthesis of sesquiterpenes in inflorescences, respectively.^{20,21} Further analyses will be required to assess the significance of these interactions in regulating plant defense responses.

Material and Methods

Fluorescence-lifetime imaging microscopy (FLIM). FLIM analysis was performed using a Nikon TE2000 microscope connected to a LiFA FLIM system. Fluorescence-lifetime was measured using the LiFLIM software version 1.2.8. on *N. benthamiana* agro-infiltrated leaves expressing GFP-EIN3 (control) and co-expressing various combinations of GFP-EIN3, JAZ8-RFP and RGA proteins. At least 60 nuclei per condition were analyzed.

Gene expression analyses. qRT-PCR were performed on at least two biological repeats as previously described.¹⁸

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Thomas Potuschak for EIN3 pENTRY vector. The authors' research group is currently supported by the Centre National de la Recherche Scientifique and Bayer.

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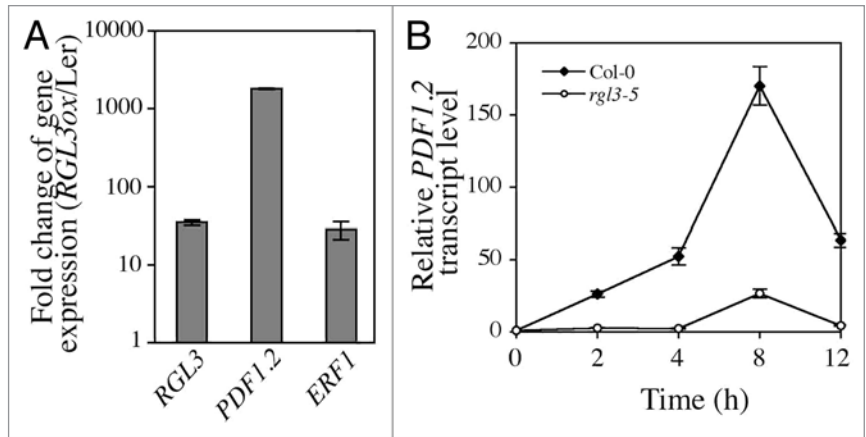


Figure 2. RGL3 enhances EIN3-dependent JA-responsive gene expression. **(A)** Relative transcript levels of *RGL3*, *PDF1.2* and *ERF1* in 6-week-old wild-type (Ler) and a line overexpressing *RGL3*. Data (mean \pm SD) are represented as fold change in gene expression (*RGL3ox/Ler*). **(B)** Time-course induction (mean \pm SD) of *PDF1.2* transcripts in 3-week-old wild-type (Col-0) and *rg13-5* mutant plants treated with 50 μ M of MeJA for the time indicated.