Regulation of ORA59, a key modulator of disease resistance in Arabidopsis

Körbes, A.P.

SUMMARY

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Fitness and survival of plants depend on efficient mechanisms to cope with adverse conditions present in natural environments. The initiation of defense responses against attacking organisms depends on the action of several endogenously produced phytohormones, including jasmonic acid (JA) and related jasmonates (JAs) and ethylene (ET). JAs play a major role in defense against wounding, insects and necrotrophic pathogens. The current knowledge of the octadecanoid pathway for biosynthesis of JAs and of the different components of the JA signaling pathway are reviewed in **Chapter 1.**

In defense against necrotrophic pathogens, the JA and ET signaling pathways synergize to activate a specific set of defense genes, including the *PDF1.*2 gene encoding the small antimicrobial protein PLANT DEFENSIN 1.2. The APETALA2 (AP2)-domain transcription factor OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2-domain protein 59 (*ORA59)* acts as the integrator of the JA and ET signaling pathways in *Arabidopsis thaliana* and is the key regulator of JA- and ET-responsive *PDF1.2* expression. The mechanisms by which ORA59 activates its target genes were not previously investigated. The studies described in this thesis focused on the functional analysis of the JA/ET-responsive transcription factor ORA59 in Arabidopsis.

Studies described in **Chapter 2** aimed at dissecting the interaction of ORA59 and the related transcription factor ETHYLENE-RESPONSIVE FACTOR 1 (ERF1) with the *PDF1.2* promoter. ERF1 had been previously suggested by others to also integrate JA and ET signaling pathways, but it plays a minor role if any since plants which lack *ORA59* expression do not express JA- and ET-responsive genes. We showed that two GCC boxes in the *PDF1.2* promoter are important for trans-activation by ORA59 and ERF1 in transient assays in protoplasts and for *in vitro* binding of these proteins. We did not observe a synergistic effect between ORA59 and ERF1 in trans-activating the *PDF1.2* promoter, indicating that each transcription factor acts independently on the *PDF1.2* promoter. Using the chromatin immunoprecipitation technique we were able to show that ORA59 binds to the *PDF1.2* promoter *in vivo*. In stably transformed plants single mutation of either GCC box completely abolished the expression of the *PDF1.2* promoter in response to JA alone or to the combination of JA with the ET-releasing agent ethephon. A tetramer of a single GCC box conferred JA/ethephon-responsive gene expression, demonstrating that the JA and ET signaling pathways converge to a single GCC box. Therefore ORA59 and two functionally equivalent GCC box binding sites form the module that enables the *PDF1.2* gene to respond synergistically to simultaneous activation of the JA and ET signaling pathways.

Defense responses need to be suppressed under normal growth conditions but when required should be quickly activated, a process which involves tight regulation of the activity of key transcription factors. Studies on the effects of JA on the activity of ORA59 protein are described in **Chapter 3.** The results show that JA caused stabilization as well as nuclear localization of ORA59. The re-localization of ORA59 depended on nuclear localization (NLS) and export (NES) signals in the protein. Besides the NLS and NES at least two other protein domains also affected ORA59 localization as well as stabilization. Interestingly, JA-responsive nuclear localization of ORA59 did not require the JAs receptor COI1. Based on the results in **Chapter 3** we postulate that Arabidopsis cells have a JAs receptor distinct from COI1, an F-box protein that targets ORA59 for degradation, and a repressor protein that sequesters ORA59 in the cytoplasm.

Therefore we set out to identify and functionally characterize ORA59-interacting proteins.

Chapter 4 describes the characterization of the ORA59-interacting CCCH zinc finger protein ZFAR1 identified as an interacting protein in yeast two-hybrid screening. A closely related protein called ZFAR2 also interacted with ORA59 in yeast. Bimolecular Fluorescent Complementation (BiFC) assays showed that ORA59 and ZFAR1 interacted in the cytoplasm of Arabidopsis cell suspension protoplasts. Re-localization studies of ORA59 showed that ZFAR1 interfered with JA-induced nuclear localization of ORA59. Moreover, ZFAR1 repressed ORA59 activity in trans-activation assays. Plant infection assays with the necrotrophic fungus *Botrytis cinerea* showed that transgenic plants overexpressing ZFAR1 showed accelerated disease progression, while a *zfar1zfar2* double knockout mutant was less severely affected than wild-type plants. The significant differences in resistance levels were not associated with major changes in the expression levels of JA/ET-dependent defense marker genes such as *PDF1.2*. In conclusion, our results indicate that ZFAR1 acts as a repressor protein that sequesters ORA59 in the cytoplasm to fine-tune basal resistance against pathogens.

The identification of the mechanisms whereby the transcription factor ORA59 is activated by JA at the protein level, the interaction of ORA59 with other transcription factors, and identification of the binding sites in the promoters of ORA59 target genes is of major importance to understand how JAs mediate defense responses. A model summarizing the main results presented in this thesis is depicted in Figure 1. ORA59 is present at very low levels in unelicited cells due to degradation via the 26S proteasome. Further control of the background expression level of target genes is achieved by retention of ORA59 in the cytoplasm via interaction with ZFAR1, which masks the NLS in ORA59 (Figure 1a). Upon infection with a necrotrophic fungus, JAs and ET are produced, which initiate signaling pathways leading to the release of ORA59 from the repressor ZFAR1 and causing ORA59 stabilization. ORA59 then moves to the nucleus, where it activates defense genes including *PDF1.2* leading to resistance (Figure 1b).

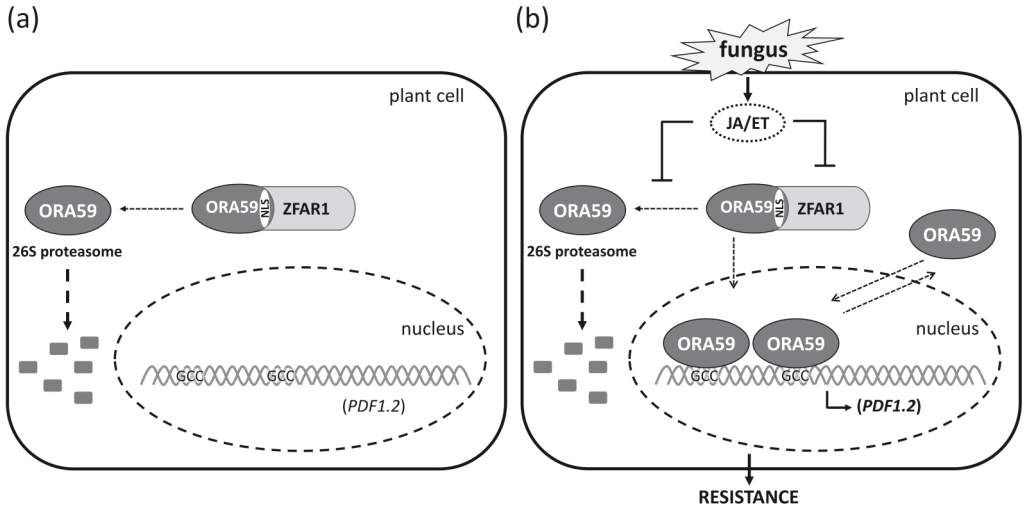

Figure 1. Model of ORA59 regulation. See text for details.