MYC transcription factors: masters in the regulation of jasmonate biosynthesis in Arabidopsis thaliana

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Summary

Jasmonates (JAs), consisting of jasmonic acid (JA) and several of its cyclic precursors and derivatives, are signaling molecules involved in the regulation of a number of processes in plants, including certain developmental processes, senescence and responses to biotic and abiotic attack (Wasternack, 2007). JAs are oxylipins which are synthesized via the octadecanoid pathway. External stresses trigger the biosynthesis of JAs, which then switch on gene expression leading to the adaptation of plants to the changed environmental conditions. The major bioactive JAs is the amino acid conjugate jasmonoyl-isoleucine (JA-Ile) (Fonseca et al., 2009). Perception of JA-Ile by the F-box protein CORONATINE-INSENSITIVE1 (COI1), which is part of a Skp-Cullin-F-box protein (SCF) complex with putative E3 ubiquitin ligase activity leads to the degradation of JAZ repressor proteins, the release of JAZ-targeted transcription factors from repression and the subsequent activation of JAs-responsive genes (Gfeller et al., 2010).

Most of the enzymes leading to JAs biosynthesis and metabolism have been identified and the corresponding genes are known (Yan et al., 2013). The expression of most biosynthesis genes including *LOX*, *AOS*, *AOC*, *OPR3*, *JMT*, and *JAR1* is induced by JAs treatment and wounding (Wasternack, 2007), implying that JAs biosynthesis is regulated by a positive feedback loop. The transcriptional regulation of JAs biosynthesis genes by this feedback loop is widely accepted but its regulatory mechanisms remain to be elucidated. Our previous studies showed that overexpression of the AP2/ERF-domain transcription factor ORA47 resulted in elevated expression of JAs biosynthesis genes and increased levels of JAs. However, the expression of JAs biosynthesis genes was not altered by downregulation of the expression level of *ORA47*, suggesting that ORA47 regulates the positive feedback loop together with (an) unidentified transcription factor(s) (Pré, 2006; Khurshid, 2012). The current knowledge about the JAs biosynthesis pathway, the JAs signal transduction pathway and about JAs-related transcription factors is reviewed in **Chapter 1**.

It has been reported that the transcription factors MYC2 and ORA47 were able to activate the *LOX3* promoter in a transient assay in tobacco protoplasts (Pauwels et al., 2008). The bHLH-domain transcription factor MYC2 and the closely related proteins MYC3 and MYC4 are key regulators of JAs responses in Arabidopsis (Lorenzo et al., 2004; Fernández-Calvo et al., 2011). However, little is known about the roles of MYC2, MYC3 and MYC4 in the regulation of JAs biosynthesis. Therefore, the aim of the studies described in this thesis was to study the roles of MYC proteins in the auto-regulatory loop in JAs biosynthesis in *Arabidopsis thaliana*.

Chapter 2 describes the roles of MYC2, MYC3 and MYC4 in the expression of JAs biosynthesis genes. In *myc234* triple mutants the expression of a large number of genes encoding enzymes involved in JAs biosynthesis was dramatically reduced after treatment with MeJA or after wounding compared to wild-type plants. EMSAs showed that MYC proteins were able to bind *in vitro* to only one of the two G-boxes that are present in the *AOC2* promoter. This G-box was essential and sufficient for MYC-mediated activation of the *AOC2* promoter in Arabidopsis protoplasts. There was a perfect correlation between the *in vitro* binding of MYCs to the G-box sequences and the ability of MYCs to trans-activate *AOC2* promoter derivatives with mutated G-boxes *in vivo*. Furthermore, transient trans-activation assays showed that MYCs and ORA47 additively activated the promoters of the JAs biosynthesis genes *LOX2*, *AOS*, *AOC2* and *OPR3*. These results indicate that MYC2, MYC3 and MYC4 act together with ORA47 as key positive regulators of the auto-stimulatory loop in JAs biosynthesis.

Chapter 3 describes the roles of MYC2, MYC3 and MYC4 in the JAs-responsive expression of *ORA47*. As a positive regulator of JAs biosynthesis, ORA47 is itself encoded by a JAs-responsive gene. Based on literature data, the hypothesis that *ORA47* is regulated by the functionally redundant transcription factors MYC2, MYC3 and MYC4 was explored. The *ORA47* promoter contains 3 G-box and G-box-like motifs. The results showed that the MYC proteins could bind to only one of those G-box motifs *in vitro*. Transient trans-activation assays revealed that this G-box sequence was essential for MYC-mediated activation of the *ORA47* promoter and that the other G-box and a G-box-like sequence contributed to a higher expression level of the *ORA47* promoter *in vivo*. Triple knockout of the *MYC* genes or overexpression of a stable JAZ1 derivative abolished JAs-responsive *ORA47* expression, indicating the crucial role of the MYC-JAZ module in the regulation of *ORA47* expression.

JAs exert their function in crosstalk with gibberellins (GAs) signaling to regulate plant development and defense. **Chapter 4** aimed to study the effect of DELLA proteins, repressor proteins acting in the GAs signaling pathway, on the regulation of JAs biosynthesis in Arabidopsis. All five DELLAs have been reported to interact with MYC2 directly and to interfere with the MYC2-mediated JAs signaling output. Y2H and BiFC assays show that two members of the Arabidopsis DELLA protein family, RGA and GAI, interact with ORA47. In transient activation assays, RGA and GAI slightly promoted the activity of ORA47 in the activation of the *AOC2* promoter, which was partially attenuated by addition of JAZ1. The same two DELLA proteins,

GAI and RGA, significantly enhanced MYC2-mediated activation of the *ORA47* promoter. The expression of the JAs biosynthesis genes *LOX2*, *AOC2* and *OPR3* in response to JA, GA3 or both combined was not changed in the quintuple *della* mutant or in transgenic plants constitutively overexpressing RGA or GAI compared to wild-type. Thus, although members of the DELLA repressor family interact with the regulators MYC2, MYC3, MYC4 and ORA47, we did not find evidence that DELLAs modulate the expression of JAs biosynthesis genes in Arabidopsis.

In short, the studies described in this thesis show that MYC2, MYC3 and MYC4 positively regulate JAs biosynthesis genes directly and by controlling the expression of the *ORA47* gene. GAs may be involved in the regulation of JAs biosynthesis genes via the interaction of DELLA proteins with MYCs and ORA47. A model summarizing the main results presented in this thesis is depicted in Figure 1.

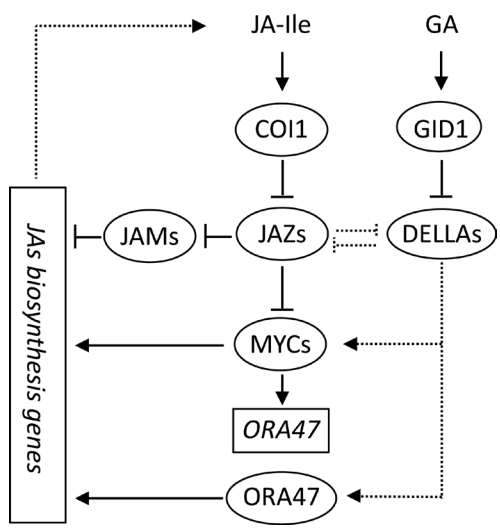

Figure 1. Model for the transcriptional regulation of the positive feedback loop in JAs biosynthesis in Arabidopsis. See text for details.

The rapid production of JA-Ile in response to changing environmental conditions triggers the degradation of JAZ repressors, resulting in the release of MYC transcription factors to activate their target genes including *ORA47*. MYCs and ORA47 respectively bind to G-box and GCC-box sequences in target gene promoters and additively activate the expression of JAs biosynthesis genes, which in turn promote JAs accumulation. Combining two distinct *cis*-elements, which are targeted by two distinct transcription factors, in single promoters may enhance the specificity and responsiveness of this positive feedback loop. Members of the DELLA repressor

family, which were identified as crucial components of the GAs signaling pathway, can interact with MYCs and ORA47. They may thus promote the expression of JAs biosynthesis genes. It was reported by others that JAZ proteins interact not only with the crucial MYC activator proteins but also with the JAM repressors, which are hypothesized to compete for binding to G-box sequences with MYCs. JAMs are therefore indicated in the model as negative regulators of JAs biosynthesis genes. This model highlights that plants have in place a complex regulatory system that should be able to fine-tune the auto-regulatory loop of JAs biosynthesis to ensure adequate JAs responses.

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