Jasmonate-responsive transcriptional regulation in Catharanthus roseus

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Chapter 5

General discussion

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Introduction

The biosynthesis of many different types of secondary metabolites that serve defensive functions in different plant species is regulated by hormones belonging to the group of jasmonate compounds. Regulation acts at the level of transcription of genes encoding biosynthetic enzymes. Chapters 3 and 4 describe mechanisms of signal transduction initiated by jasmonates leading to the activation of transcription factors. Here unifying models for jasmonate signal transduction regulating tobacco alkaloid biosynthesis and terpenoid indole alkaloid biosynthesis in *Catharanthus roseus* are presented.

How do JAZ proteins, MYC2 and COI1 interact?

One of the new findings in Chapter 4 was that the N-terminal domain of CrJAZ1 was able to interact with CrMYC2 in yeast. The C-terminal domain also interacted with CrMYC2 in yeast, in line with the report of Chini et al. (2007) of interaction between the C-terminal domain of AtJAZ3 and AtMYC2. These authors did not find an interaction between the N-terminal domain and AtMYC2. Chapter 4 also reports that the N-terminal and C-terminal domains of AtJAZ1 interacted with AtMYC2. This dual interaction therefore is a conserved property of JAZ1 proteins, which apparently differ from AtJAZ3.

The C-terminal domains of tomato JAZ1 (Katsir et al., 2008) and Arabidopsis JAZ1, JAZ3 and JAZ9 (Melotto et al, 2008) were shown to be necessary and sufficient for binding to COI1 in a JA-Ile or coronatine dependent manner. Importantly, no interaction between COI1 and JAZ proteins was detected in the absence of JAs (Thines et al., 2007; Katsir et al., 2008; Melotto et al., 2008).

This contrasts with the reported interaction between AtCOI1 and AtJAZ1 or AtJAZ3 in in vitro pull-down assays in the absence of JAs (Chini et al., 2007). In addition, Chini et al. (2007) showed that in the absence of JAs the N-terminal but not the C-terminal domain of AtJAZ3 interacts with AtCOI1 (Figure 1A).

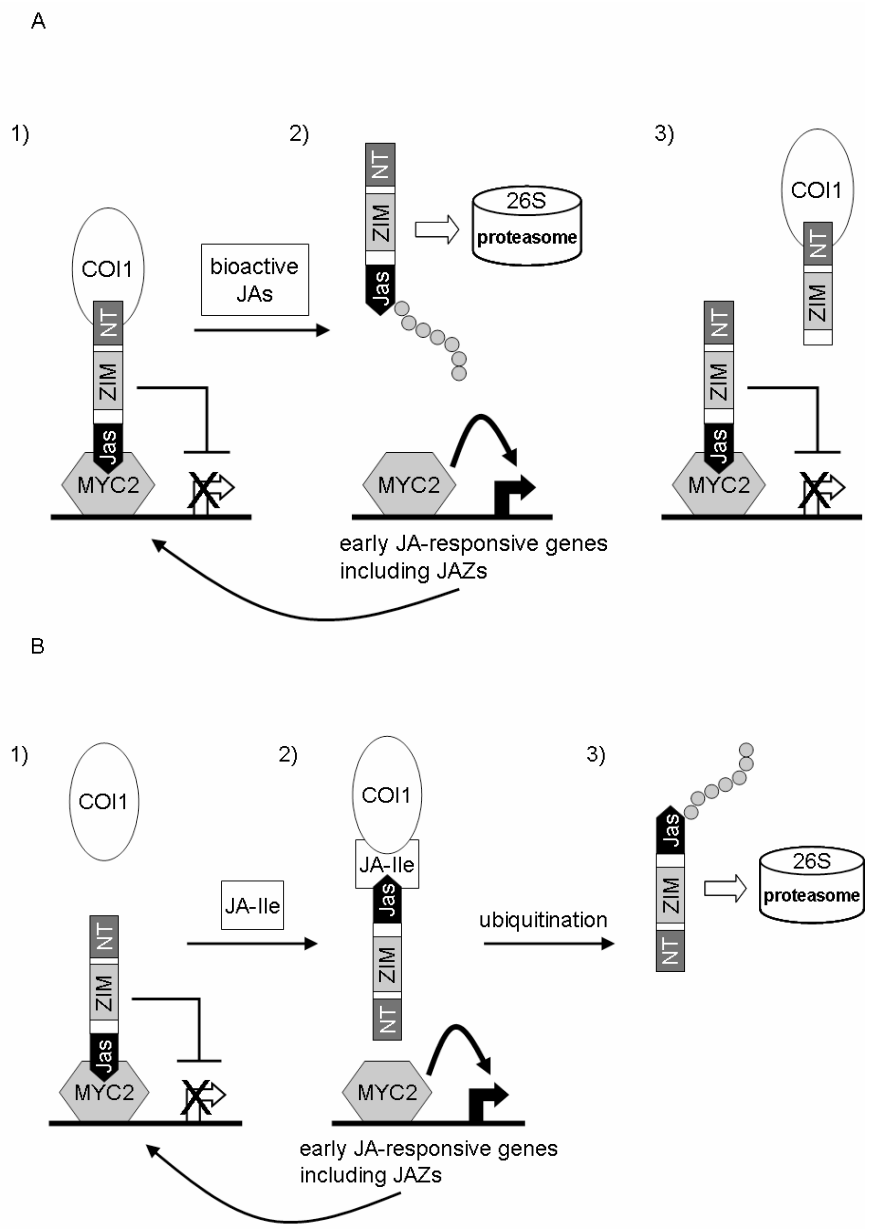
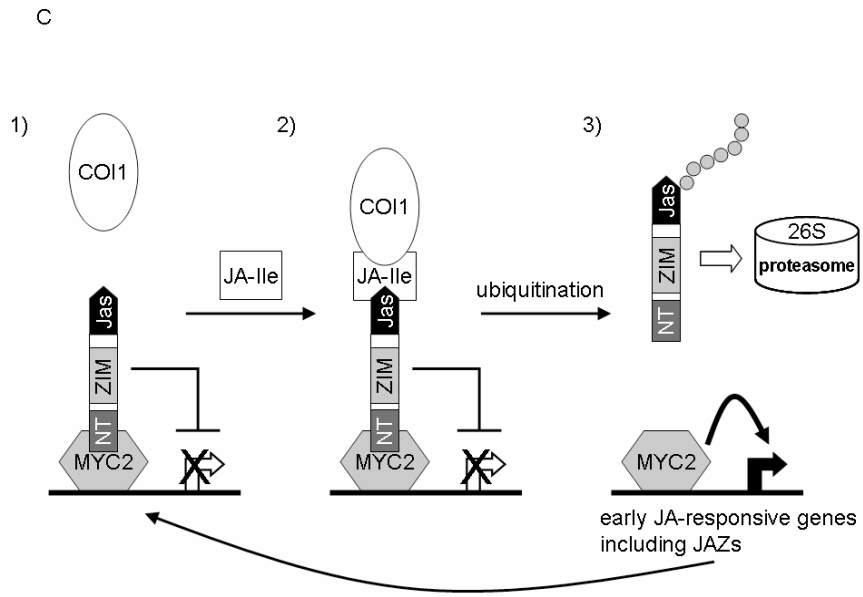


Figure 1. Models for jasmonate signal transduction leading to expression of AtMYC2-regulated genes. Although depicted as a single protein, COI1 forms part of the E3 ubiquitin ligase SCF^{COI1}. A) Model proposed by Chini et al. (2007). 1) In the absence of bioactive JAs, JAZ3 interacts through its Jas motif with AtMYC2 maintaining this transcription factor inactive. With its N-terminal domain JAZ3 interacts with COI1 in the absence of bioactive JAs. (To be continued on the next page)



(Continued from the previous page) 2) In the presence of bioactive JAs, JAZ3 is ubiquitinated by the SCF^{COI1} complex and degraded via the 26S proteasome. AtMYC2 is liberated and activates transcription of target genes, including genes encoding JAZ proteins, resulting in a negative feedback loop.3) Situation in the *jai3-1* mutant. The C-terminal deletion derivative of JAZ3 can still bind to COI1 and blocks its activity. As a result, other members of the JAZ family binding to AMYC2 cannot be ubiquitinated in the presence of bioactive JAs.

B) Model proposed by Melotto et al. (2008). 1) In the absence of JA-Ile, certain members of the JAZ family interact through a subdomain of the Jas motif with AtMYC2 maintaining this transcription factor inactive. 2) JA-Ile forms the molecular glue between another subdomain of the Jas motif and COI1. 3) SCF^{COI1} promotes the ubiquitination of bound JAZ proteins resulting in their subsequent degradation by the 26S proteasome. AtMYC2 is liberated and activates transcription of target genes.

C) Model proposed here. 1) In the absence of JA-Ile, certain members of the JAZ family interact through their N-terminal domain with AtMYC2 maintaining this transcription factor inactive, probably through the repressive action of the ZIM domain via interaction with a co-repressor. 2) JA-Ile forms the molecular glue between the Jas domain of certain members of the JAZ family and COI1. 3) SCF^{COI1} promotes the ubiquitination of bound JAZ proteins resulting in their subsequent degradation by the 26S proteasome. AtMYC2 is liberated and activates transcription of target genes.

The original model proposed by Chini et al. (2007) (Figure 1A), although consistent with all their reported data, has several elements that are difficult to understand. For example, what is the function of biologically active JAs in inducing ubiquitination of JAZ proteins if it is not by forming the molecular glue between COI1 and JAZ proteins? More importantly, why is a C-terminal deletion derivative of AtJAZ3 stable whereas according to the model it can still interact with AtCOI1? And why is this same deletion derivative, which still according to the model cannot interact anymore with AtMYC2, a strong repressor? The explanation presented by these authors is that the C-terminal deletion derivative of AtJAZ3 still binds to AtCOI1 and blocks its activity, thereby inhibiting degradation of other members of the AtJAZ family that can still bind and inactivate AtMYC2.

However, this “poison complex” model seems no longer tenable based on the convincing evidence that in the presence of JA-Ile the C-terminal domain of AtJAZ proteins including AtJAZ3 interacts with AtCOI1 (Melotto et al., 2008). Melotto et al. (2008) presented an alternative model (Figure 1B) which proposes that distinct subdomains of the Jas motif interact with AtMYC2 in the absence of JA-Ile and with AtCOI1 in the presence of JA-Ile. One major problem with that model is that it does not explain why a C-terminal deletion derivative of AtJAZ3 lacking the entire Jas motif which according to the model cannot interact with either AtMYC2 or AtCOI1 acts as a strong repressor.

Another problem with the model in Figure 1B is that it does not explain the observation of Chini et al. (2007) that the C-terminal deletion derivative of AtJAZ3 stabilizes other AtJAZ proteins in trans in the presence of JA-Ile. The model in Figure 1A explains this observation by proposing that the deletion derivative binds to AtCOI1 and blocks its activity. Apart from the fact that this interaction is being questioned (Melotto et al., 2008), it also does not appear to be fully consistent with other observations made by Chini et al. (2007). They report that in a genome-wide micro-array analysis only 31 genes showed a lower expression in the *jai3-1* mutant after JA treatment compared to JA-treated wild-type plants. If the C-terminal deletion derivative of AtJAZ3 expressed in the *jai3-1* mutant blocks AtCOI1, this should affect a much larger number of JA-

responsive COI1-dependent genes, which are estimated to comprise over 500 genes (Devoto et al., 2005).

A third model proposed here based on the data from Chapter 4 that the N-terminal domain of a JAZ protein from *C. roseus* interacts with the Catharanthus orthologue of MYC2 is shown in Figure 1C. It assumes that in the presence of JA-Ile the C-terminal JAZ domain interacts with COI1 in accordance with the data presented for Arabidopsis by Melotto et al. (2008). The model implies that a C-terminal deletion derivative of a JAZ protein that cannot interact anymore with COI1 is stable, and since it can still interact with MYC2 it is an active repressor. This model is in agreement with the results from our studies of CrJAZ1 and CrMYC2, but is not supported by the reported interaction between the C-terminal domain of AtJAZ3 and AtMYC2. The latter interaction was confirmed in a yeast two-hybrid assay (unpublished results) in accordance with the data reported by Chini et al. (2007). One possible explanation for these apparently contradictory results is that in planta the highest affinity interaction domains in AtJAZ3 are different from those in yeast, for example due to plant-specific protein modifications. A major problem with the model in Figure 1C is that it provides no explanation for the observation that a C-terminal deletion derivative of a JAZ protein stabilizes other JAZ proteins in trans. Maybe JAs-independent interaction between the N-terminal domain of a JAZ lacking the C-terminus and COI1 blocks the activity of the latter and thereby causes the dominant-negative effect as proposed by Chini et al. (2007).

It is clear that identification of the mechanisms of interaction between JAZ proteins, MYC2 and COI1 in planta requires more detailed studies, including crystal structure analyses of COI1-JAZ complexes in the presence and absence of JA-Ile or coronatine and of MYC2-JAZ complexes.

Jasmonate signalling in terpenoid indole alkaloid biosynthesis in *Catharanthus roseus*

The results in Chapter 3 demonstrate that MeJA-responsive *ORCA* expression is controlled by CrMYC2, and that at least for the *ORCA3* gene members of the

AT-hook family of transcription factors contribute to the level of expression (Figure 2).

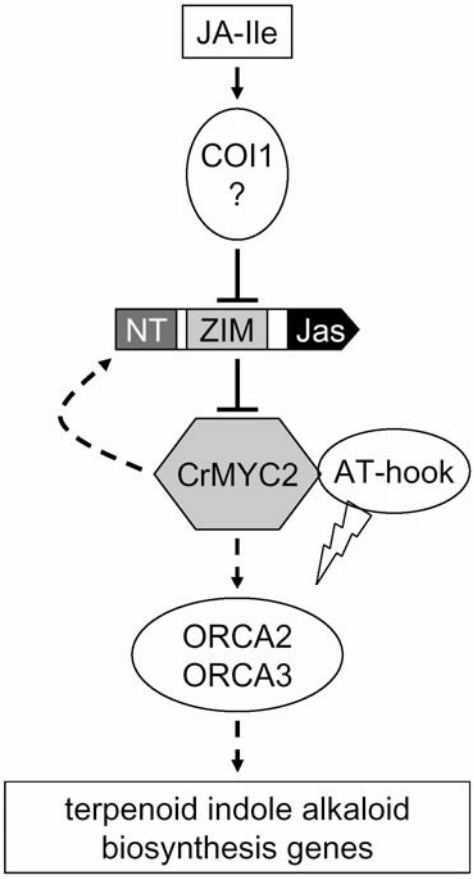


Figure 2. Model for jasmonate signal transduction leading to the expression of terpenoid indole alkaloid biosynthesis genes in *Catharanthus roseus*.

As depicted in Figure 1B, JA-Ile forms the molecular glue between CrCOI1 and CrJAZs, leading to degradation of the latter proteins. CrMYC2 then activates transcription of the genes encoding the ERF transcription factors ORCA2 and ORCA3, which in turn activate the expression of terpenoid indole alkaloid biosynthesis genes. CrMYC2 also activates transcription of *CrJAZ* genes as part of a negative feedback loop. Certain members of the AT-hook transcription factor family co-stimulate the expression of the *ORCA* genes. The position of CrCOI1 in this signal transduction pathway is hypothetical as indicated by the question mark. Solid lines indicate interactions between proteins and broken lines indicate interactions between proteins and genes.

Chapter 4 shows that the full-length CrJAZ proteins are repressors that negatively regulate CrMYC2 activity. For CrJAZ1 evidence was obtained that it is degraded in response to jasmonate via the 26S proteasome.

Based on the results in Chapters 3 and 4 the following model is proposed for signalling by JAs in *C. roseus* leading to alkaloid biosynthesis (Figure 2). Although MeJA was used in the studies, this compound is probably de-methylated in *C. roseus* cells, and then converted to the bioactive jasmonate JA-Ile. But this is clearly an issue that needs to be resolved. Perception of JA-Ile by CrCOI1 results in the degradation of CrJAZ proteins. CrMYC2 then activates the expression of the *ORCA* genes, which in turn activate the expression of a subset of TIA biosynthesis genes including *TDC* and *STR*. Simultaneous activation of *JAZ* genes by CrMYC2 restores the un-induced situation by inhibition of CrMYC2 activity. The involvement of CrCOI1 in this sequence of events still needs to be experimentally confirmed.

Jasmonate signalling in tobacco alkaloid biosynthesis

For jasmonate signalling in tobacco leading to the biosynthesis of nicotine and related alkaloids, a similar model is proposed. In transient assays in tobacco protoplasts two members of the tobacco ERF transcription factor family called NtORC1 and NtJAP1 were shown to upregulate the activity of the promoter of the tobacco gene encoding putrescine N-methyltransferase (PMT), which catalyzes the first committed step in nicotine biosynthesis (De Sutter et al., 2005). Together the transcription factors caused a strong synergistic activation of the *PMT* promoter. NtORC1 is a close homologue of the Catharanthus ERF transcription factor ORCA3. Both *NtORC1* and *NtJAP1* gene expression is induced by MeJA (Goossens et al., 2003).

Genetic studies using low-nicotine tobacco varieties demonstrated that the low-nicotine phenotype is caused by synergistic effects of two non-linked loci, called *nic1* and *nic2* (Katoh et al., 2005). Tobacco plants with the *nic1nic2* genotype have highly reduced nicotine contents (about 5% of wild type) and strongly decreased expression levels of nicotine biosynthesis genes. The genes corresponding to the *nic* loci have not been cloned yet.

MeJA-responsive nicotine biosynthesis is controlled by the jasmonate receptor COI1 and depends on degradation of members of the JAZ repressor family (Shoji et al., 2008). There are no published data yet about the nature of the transcription factor(s) repressed by the JAZ proteins in tobacco, but in the model in Figure 3 it is speculated that it is the tobacco homologue of AtMYC2 and CrMYC2. It is also hypothesized that this NtMYC2 transcription factor controls the MeJA-responsive expression of the *NtORC1* and *NtJAP1* genes, which in turn are hypothesized to control the MeJA-responsive expression of the nicotine biosynthesis genes.

Conclusion

Unifying models for jasmonate signalling regulating alkaloid biosynthesis in tobacco and in *Catharanthus roseus* are presented here. The models propose that perception of the jasmonate hormone JA-Ile by the receptor COI1 results in the degradation of JAZ proteins. Since these JAZ proteins repress the activity of the bHLH transcription factor MYC2, MYC2 then activates the expression of genes encoding certain members of the ERF family of transcription factors, which in turn activate the expression of alkaloid biosynthesis genes.

For both species, certain elements in the model have not yet been experimentally confirmed. For the Catharanthus model, the involvement of the jasmonate receptor COI1 has not been experimentally confirmed. Given the conservation of COI1 as a jasmonate receptor in Arabidopsis (Xie et al., 1998), tomato (Li et al., 2004; Katsir et al., 2008), tobacco (Shoji et al., 2008) and *Nicotiana attenuata* (Paschold et al., 2007), the position of COI1 in the jasmonate signal transduction pathway in Catharanthus seems highly probable. In tobacco, especially the identities and roles of transcription factors need more solid experimental confirmation. With the speed at which this research field currently progresses, it is anticipated that it will not take long to confirm or disprove the model.

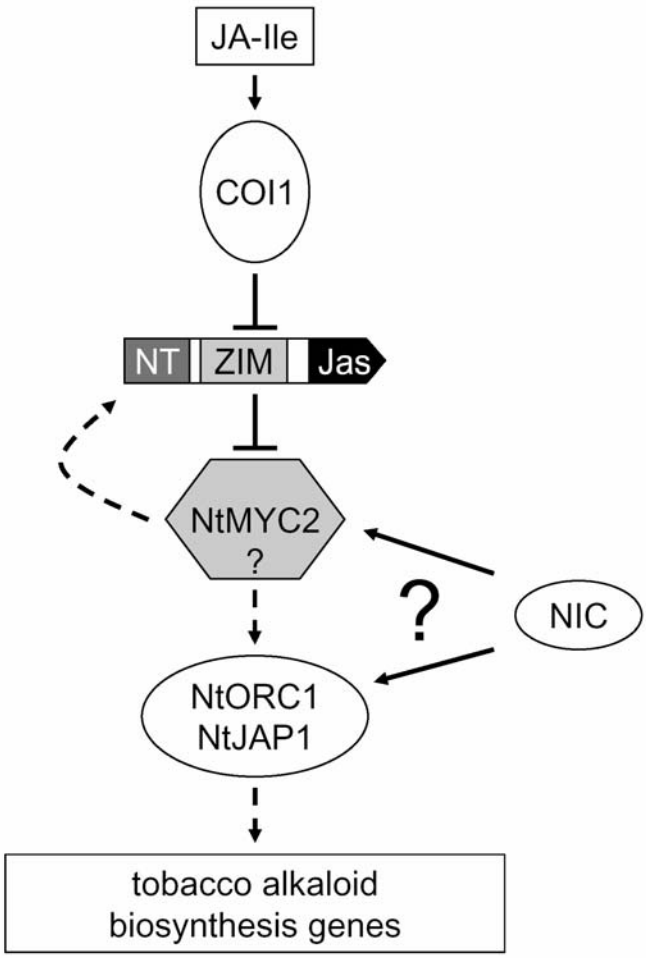


Figure 3. Model for jasmonate signal transduction leading to the expression of tobacco alkaloid biosynthesis genes.

As depicted in Figure 1B, JA-Ile forms the molecular glue between NtCOI1 and NtJAZs, leading to degradation of the latter proteins. NtMYC2 then activates transcription of the genes encoding the ERF transcription factors NtORC1 and NtJAP1, which in turn activate the expression of tobacco alkaloid biosynthesis genes. NtMYC2 also activates transcription of *NtJAZ* genes as part of a negative feedback loop. The elusive *NIC1* and *NIC2* genes may encode NtMYC2 and/or NtORC1 and NtJAP1 as indicated by the question mark. The position of NtMYC2 in this signal transduction pathway is hypothetical as indicated by the question mark. Solid lines indicate interactions between proteins and broken lines indicate interactions between proteins and genes.

It will be interesting to see to which degree the model will turn out to accurately reflect the mechanisms of jasmonate signal transduction in alkaloid biosynthesis, and to which degree this model applies to other secondary pathways regulated by JAs in different plant species.

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