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Expression of *AMIDASE1* (*AMI1*) is suppressed during the first two days after germination

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The regulation of cellular auxin levels is a critical factor in determining plant growth and architecture, as indole-3-acetic acid (IAA) gradients along the plant axis and local IAA maxima are known to initiate numerous plant growth responses. The regulation of auxin homeostasis is mediated in part by transport, conjugation and deconjugation, as well as by de novo biosynthesis. However, the pathways of IAA biosynthesis are yet not entirely characterized at the molecular and biochemical level. It is suggested that several biosynthetic routes for the formation of IAA have evolved. One such pathway proceeds via the intermediate indole-3-acetamide (IAM), which is converted into IAA by the activity of specific IAM hydrolases, such as Arabidopsis AMIDASE1 (AMI1). In this article we present evidence to support the argument that AMI1-dependent IAA synthesis is likely not to be used during the first two days of seedling development.

Key words: *Arabidopsis thaliana*, auxin biosynthesis, *AMIDASE1*, indole-3-acetic acid, indole-3-acetamide, *LEAFY COTYLEDON1*, seed development

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Auxins are versatile plant hormones that play diverse roles in regulating many aspects of plant growth and development.[1] To enable auxins to develop their activity, a tight spatiotemporal control of cellular indole-3-acetic acid (IAA) contents is absolutely necessary since it is well-documented that auxin action is dose dependent, and that high IAA levels can have inhibitory effects on plant growth.[2] To achieve this goal, plants have evolved a set of different mechanisms to control cellular hormone levels. On the one hand, plants possess several pathways that contribute to the de novo synthesis of IAA. This multiplicity of biosynthetic routes presumably facilitates fine-tuning of the IAA production. On the other hand, plants are equipped with a variety of enzymes that are used to conjugate free auxin to either sugars, amino acids or peptides and small proteins, respectively, or on the contrary, that act as IAA-conjugate hydrolases, releasing free IAA from corresponding conjugates. IAA-conjugates serve as a physiologically inactive storage form of IAA from which the active hormone can be quickly released on demand. Alternatively, conjugation of IAA can mark the first step of IAA catabolism. In general, conjugation and deconjugation of free IAA are ways to positively or negatively affect active hormone levels, which adds another level of complexity to the system. Additionally, IAA can be transported from cell to cell in a polar manner, which is dependent on the action of several transport proteins. All together, these means are used to form auxin gradients and local maxima that are essential to initiate plant growth processes, such as root or leaf primordia formation.[3]

Indole-3-Acetamide-Dependent Auxin Biosynthesis

The elucidation of auxin biosynthetic pathways in plants has proven to be very difficult. Until today, it has not been possible to confirm a complete single pathway on the molecular and biochemical level beyond doubt. However, several recent studies led to the identification of a couple of pathways that presumably operate in

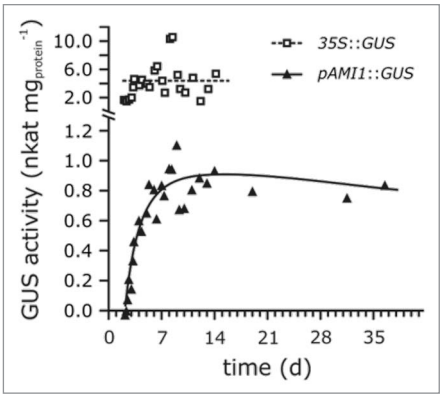

Figure 1. Fluorometric quantification of the GUS activity in *pAMI1*::*GUS* seedlings and in a constitutive *GUS* overexpression line (*35S*::*GUS*). *35S*::*GUS* and *pAMI1*::*GUS* seedlings were grown on half-strength MS medium (1% sucrose (w/v)) at short day conditions (8 h of light at 24°C, 16 h darkness at 20°C, photosynthetically active radiation 105 μmol photonsm^{-2}s^{-1} from standard white fluorescent tubes). Over a time period of 36 days (900 h) samples were taken. Quantification of the GUS activity was carried out according to Jefferson et al.[19]

planta.[4,5] One of this biosynthetic routes proceeds via indole-3-acetamide (IAM), which is hydrolyzed to IAA by specific IAM amidohydrolases, e.g., AMIDASE1 (AMI1) from Arabidopsis.[6] However, it is yet not entirely clear how IAM is produced in higher plants. In order for plant pathogens to re-program the cellular metabolism of their hosts for nutrient production, they synthesize IAA by an IAM-dependent two-step pathway. For this purpose, they use a tryptophan-2-monooxygenase (iaaM) to convert L-tryptophan (L-Trp) to IAM, and an IAM hydrolase (iaaH) to generate IAA in a subsequent reaction step. While bacterial iaaH sequences have successfully been used to search for homolog counterparts in plants, which led to the identification of *AMI1* and a homolog from tobacco, *NtAMI1*,[6,7] usage of the iaaM sequences did not return any valuable indication for the abundance of iaaM-like sequences in plant genomes. This finding, however, does not exclude the possibility that a functionally related enzyme catalyzes the conversion of L-Trp to IAM in planta. At least for Arabidopsis, it seems as if most of the IAM is derived from indole-3-acetaldoxime.[8]

Expression of Arabidopsis AMI1 during Seedling Development

Our previous work focused on the in vitro characterization of AMI1. After we found IAM to be endogenous to Arabidopsis[9] we identified *AMI1* from thale cress, which was the first IAM hydrolase known from plants.[6] Since that time, several studies were conducted to examine the properties of the enzyme, including intracellular localization studies, tissue specific expression analyses, and the analysis of the molecular mode of action of AMI1.[10,11] In order to enable in-depth studies on the regulation of the *AMI1* gene expression, we generated an *AMI1* promoter reporter gene construct (*pAMI1*::*GUS*) by fusing the complete intergenic region between the upstream located gene (At1g08970) and the predicted third exon of *AMI1* to the *uidA* (GUS) gene and re-entered it into Arabidopsis. Quantification of the GUS reporter activity in young seedlings has shown that *AMI1* expression is lacking during the first two days after seed imbibition. Thereafter, *AMI1* promoter activity strongly increases until a maximal expression level is reached (between days 7 and 14). Then, the GUS activity in the seedlings slowly declines (**Fig. 1**). This expression pattern nicely reflects the kinetic of IAA during the first two weeks of seedling development,[9] and the rapid growth of seedlings during that time. Seeds usually contain high levels of stored IAA, which facilitates initial seedling growth. After approximately two to three days this IAA storage pool is exhausted, or at least drastically reduced, and the seedling has to initiate its autonomous hormone production. Given that AMI1 functions as an IAM hydrolase not only in vitro but also in vivo, this would underline a role of AMI1 in auxin formation. Likewise, it would imply that IAM-dependent auxin synthesis does not play a role during the first two days of seedling development; as yet there is no indication for the abundance of an alternative enzyme with considerable IAM hydrolase activity from Arabidopsis or any other plant species.

AMI1 Expression is Presumably Suppressed by LEC1

With respect to the observations that *AMI1* expression is strongly upregulated in the *lec1-1* knockout mutant[12] and considerably suppressed in an inducible *LEC1* gain-of-function line,[13] it might be suggested that LEC1 is a suppressor of *AMI1* expression during seed and embryo development. *LEAFY COTYLEDON1* (*LEC1*) is a transcription factor that functions as one of the master regulators of seed development.[14-16] In its structure the LEC1 protein shows homology to the B-domain of the HAP3 (HEME ACTIVATED PROTEINS) subunit of the CCAAT-box binding factor. Recent studies have shown that LEC1 does not necessarily operate as a monomer, but rather tends to bind to other DNA-binding proteins to form functional complexes that regulate gene expression. From carrots it is known that the corresponding C-LEC1 protein interacts with several HAP2- and HAP5-homologs.[17] This might be of particular importance in so far as such a HAP factor (HAP5c, At1g08970) is located directly upstream of the *AMI1* gene.[6] Among other things, HAP factors are known to be involved in the regulation of flowering and to bind to CCAAT box motifs in the promoter region of their target genes.[18] Two such CCAAT boxes can be found in the *AMI1* promoter, 266 and 462 bp upstream of the start codon, respectively. Our hypothesis is further supported by the expression pattern of the corresponding genes as can be taken from publicly available databases (www.genevestigator.com/gv/index.jsp). *LEC1* and *HAP5c* show a development-dependent co-expression pattern. But perhaps more importantly, *AMI1* expression is suppressed at developmental stages or in tissues where *LEC1* and *HAP5c* expression

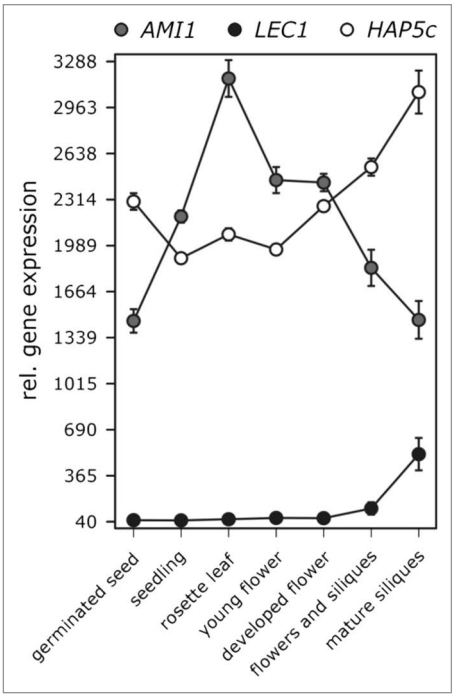

Figure 2. Expression pattern of *LEC1*, *AMI1* and *HAP5c* at various developmental stages. The relative gene expression of *LEC1* (At1g21970), *AMI1* (At1g08980) and *HAP5c* (At1g08970) at different developmental stages were compared by using Arabidopsis microarray-derived expression data as deposited in the genevestigator V3 database (www.genevestigator.com/gv/index.jsp).

becomes more pronounced, in particular in mature siliques (**Fig. 2**). It will be interesting to study these exciting correlations by yeast one-hybrid analyses and appropriate genetic approaches.

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