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Mini review

Indole-3-acetamide-dependent auxin biosynthesis: A widely distributed way of indole-3-acetic acid production?

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ABSTRACT

During the course of evolution plants have evolved a complex phytohormone-based network to regulate their growth and development. Herein auxins have a pivotal function, as they are involved in controlling virtually every aspect related to plant growth. Indole-3-acetic acid (IAA) is the major endogenous auxin of higher plants that is already known for more than 80 years. In spite of the long-standing interest in this topic, IAA biosynthesis is still only partially uncovered. Several pathways for the formation of IAA have been proposed over the past years, but none of these pathways are yet completely defined. The aim of this review is to summarize the current knowledge on the indole-3-acetamide (IAM)-dependent pathway of IAA production in plants and to discuss the properties of the involved proteins and genes, respectively. Their evolutionary relationship to known bacterial IAM hydrolases and other amidases from bacteria, algae, moss, and higher plants is discussed on the basis of phylogenetic analyses. Moreover, we report on the transcriptional regulation of the *Arabidopsis AMI1* gene.

Introduction

Plants make use of small signaling molecules, collectively referred to as phytohormones, to adjust their growth and development to variable environmental and developmental triggers. In the framework of this phytohormone-based network, auxins are involved in a wealth of developmental processes as diverse as cell elongation growth, apical dominance, tropisms, initiation of adventitious and lateral roots, and both cell and vascular differentiation (Davies, 2004). The field of auxinology has a long-standing tradition in plant physiology, but despite nearly 80 years of intensive research since the detection of IAA in plants, the elucidation of auxin biosynthesis is still a remaining challenge. Over the last decades, several pathways of IAA-biogenesis have been proposed based on the identification of either putative intermediates, corresponding mutants with altered hormone levels, or enzymes capable of catalyzing conceivable reaction steps, but none of these pathways are yet completely defined. In all of the suggested pathways at least one reaction step has yet to be confirmed through the isolation of an appropriate enzyme and the corresponding gene. Currently, it is assumed that plants possess multiple pathways for the production of their major growth factor, which are assumed to operate either in parallel to each other or in a redundant manner (Woodward and Bartel, 2005; Pollmann et al., 2006a; Delker et al., 2008; Zhao, 2010). Such a multi-route system may facilitate effective compensation of a loss of a particular pathway and, besides the crucial function of auxin throughout plant development, could be an explanation for the lack of any auxin auxotroph mutant.

In this review, the proposed pathways of L-tryptophan (Trp)-dependent IAA formation are summarized. Herein, special attention will be paid to the IAM pathway, as there is mounting evidence that this pathway is operational not only in bacteria, but also in plants.

Proposed auxin biosynthetic pathways

Based on genetic, enzymatic, and metabolic data, four major Trp-dependent pathways for the biosynthesis of IAA as well as a Trp-independent pathway, which is not yet defined in detail, are currently matters of debate. Though a recent publication again emphasized the existence of Trp-independent IAA biosynthesis (Ehlert et al., 2008), all yet identified biosynthetic gene products belong to Trp-dependent pathways. For this reason, only the following four main Trp-dependent pathways will be discussed more closely: (i) the indole-3-acetaldoxime (IAOx) pathway; (ii) the tryptamine (TAM) pathway; (iii) the indole-3-pyruvic acid (IPyA) pathway, and (iv) the IAM pathway (Fig. 1).

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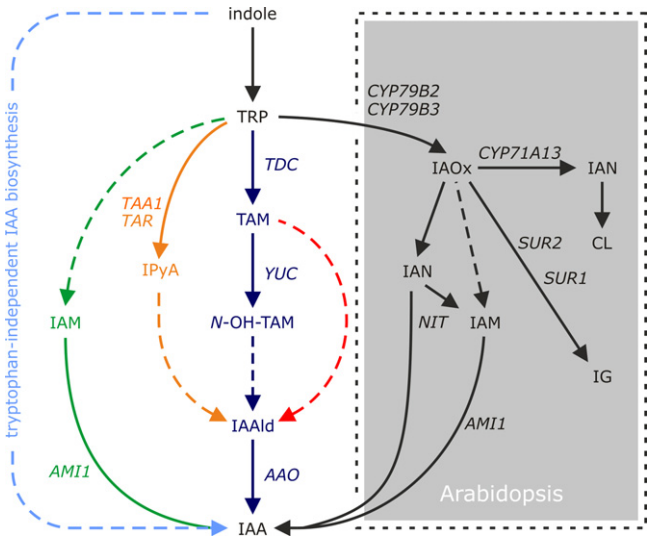

Fig. 1. Putative pathways of IAA-biogenesis in plants. The IAOx pathway that is seemingly restricted to IG-producing plant species is given in the grey box. In the middle, the TAM pathway is shown (dark blue), followed by the IPyA (orange) and the IAM pathway (green), respectively, further left. A tryptophan-independent pathway (light blue) of IAA formation is added on the left border of the scheme. Dashed lines indicate assumed reaction steps for which the corresponding enzymes have yet to be identified.

The indole-3-acetaldoxime pathway

In glucosinolate-producing plants such as *Arabidopsis*, IAOx is supposed to play an important role as a biosynthetic branching point, because it serves as a precursor that can be shunted to several secondary metabolites, e.g. IAA, indole glucosinolate (IG), or camalexin (CL). The IAOx pathway is apparently a special trait of *Arabidopsis* and perhaps of some other closely related glucosinolate-producing plants, as Trp-converting cytochrome P450 monooxygenases (CYP79B2, CYP79B3) have so far only been identified from thale cress (Hull et al., 2000; Mikkelsen et al., 2000). *CYP79B2* gain-of-function mutants have increased indole-3-acetonitrile (IAN), IAA (Zhao et al., 2002), and IG levels (Mikkelsen et al., 2000). In contrast, the *cyp79b2 cyp79b3* double null mutant exhibits phenotypes consistent with low free-IAA contents. Unexpectedly, a significant reduction of physiological active IAA is only detectable in *cyp79b2 cyp79b3* double knockout mutant seedlings when grown at increased temperatures (26 °C) (Zhao et al., 2002; Sugawara et al., 2009), though. Since previous work uncovered elevated IAA biosynthesis rates in excised hypocotyls in response to an increase in temperature (29 °C) (Gray et al., 1998), this may suggest that the IAOx pathway in *Arabidopsis* is, at least, involved in temperature-inducible IAA formation. However, recent findings that have been made by thorough mass spectrometric analyses in species like pea, rice, maize, and tobacco revealed that IAOx does not occur in these plants in considerable amounts (Quittenden et al., 2009; Sugawara et al., 2009). In conclusion, this most likely separates the IAOx pathway from the other more widely used pathways for the synthesis of IAA and emphasize its special role in IG-producing species.

The tryptamine pathway

Based on early reports of its auxin activity in *Avena* curvature tests and its growth promoting effect on excised *Avena* coleoptiles (Kuraishi and Yamaki, 1964; Winter, 1966), TAM has already been considered as a possible precursor for IAA biosynthesis for a long time. The first indication that tobacco plants use the TAM pathway was given by Phelps and Sequeira (1967). The first step in this pathway is the TRP DECARBOXYLASE (TDC)-catalyzed production of TAM from Trp. Corresponding genes and enzymes have already been reported from several plant species, such as *Catharanthus roseus* (De Luca et al., 1989), *Camptotheca acuminata* (Lopez-Meyer and Nessler, 1997), *Ophiorrhiza pumila* (Yamazaki et al., 2003), and *Oryza sativa* (Kang et al., 2007), while an appropriate TDC from *Arabidopsis* is yet to be discovered. By *in silico* analyses it was possible to identify two putative aromatic L-amino acid decarboxylases from the *Arabidopsis* proteome that show substantial similarity (62–73%) to the above-listed plant TDCs, but none of the *Arabidopsis* proteins accepted Trp as substrate. Rather, one of the proteins has recently been described as a specific L-TYROSINE DECARBOXYLASE (Lehmann and Pollmann, 2009). However, TAM is supposed to be converted to *N*-hydroxytryptamine in a rate-limiting step that is catalyzed by members of the YUCCA (YUC) family (Zhao et al., 2001; Kim et al., 2007). Recent work on garden pea provided evidence that *N*-hydroxytryptamine is possibly by-passed and TAM directly converted to indole-3-acetaldehyde (IAAld) (Quittenden et al., 2009). In *Arabidopsis*, the *YUC* gene family consists of 11 members that encode flavin-containing monooxygenase-like proteins, which are differentially expressed during plant development (Zhao, 2008). In contrast to the CYP79B2 and CYP79B3 monooxygenases, YUC-orthologous enzymes have been found outside the Brassicales, e.g. in petunia, tomato, and rice (Tobeña-Santamaria et al., 2002; Expósito-Rodríguez et al., 2007; Yamamoto et al., 2007). The YUC monooxygenases have firstly been identified in a gain-of-function screen, where they attracted attention by elongated hypocotyls (Zhao et al., 2001). Besides that, they show elongated petioles, epinastic cotyledons, and narrow leaf blades. Therein, the *YUC* overexpression lines resemble the phenotypes of several mutants known to have elevated auxin contents, such as *superroot 1* (*sur1*) (Boerjan et al., 1995; King et al., 1995), *superroot 2* (*sur2*) (Delarue et al., 1998), and lines expressing the *TRYPTOPHAN-2-MONOOXYGENASE* (*iaaM*) gene from *Agrobacterium tumefaciens* (Romano et al., 1995). In fact, mass spectrometric analyses corroborated the phenotypic observations and revealed increased IAA levels in the *YUC* gain-of-function lines (Zhao et al., 2001). However, the functional analysis of the YUC enzymes is hampered by the above-mentioned genetic redundancy. *YUCCA* single and double knockout mutations confer no auxin related phenotype. Only higher-order triple and quadruple null mutants begin to show severe alterations of the phenotype (Cheng et al., 2006, 2007). Interestingly, those mutants cannot be rescued by the exogenous application of auxin, but, in some cases, by the *YUC* promoter driven expression of the bacterial *iaaM* gene, which may indicate that not only auxin transport, but also the tight spatiotemporal control of auxin formation is a prerequisite for successful plant development. It is assumed that the TAM pathway further proceeds via IAAld that is, in a final reaction step, oxidized to IAA. This reaction could be catalyzed by suitable ACETALDEHYDE OXIDASES (AAOs). Four *AAO* isogenes have been identified in the *Arabidopsis* genome (Sekimoto et al., 1998; Akaba et al., 1999). It remains to be examined whether these enzymes are involved in IAA biosynthesis *in vivo*, because the *AAO* expression patterns do not correspond with a participation in auxin production (Seo et al., 2000). Further on, a null mutant of the most probable candidate, *aao1*, does neither have auxin related phenotypes nor reduced endogenous IAA levels (Seo et al., 2004). Overall, from these pieces of evidence, we may infer that the TAM pathway is widely distributed in the plant kingdom and that, in particular, the YUC enzymes are key players in IAA biosynthesis.

The indole-3-pyruvic acid pathway

The third major pathway of IAA biosynthesis proceeds via IPyA and IAAld to yield IAA. The early identification of IPyA to be endogenous to tomato and *Arabidopsis* already suggested an