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Auxin biosynthesis within the network of tryptophan metabolism

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ABSTRACT

A number of pathways starting from tryptophan have been proposed for the biosynthesis of the auxin indole-3-acetic acid (IAA), a key regulator of plant development. Many aspects of auxin metabolism have been investigated in the model species *Arabidopsis thaliana* that, in addition to IAA, synthesizes tryptophan-derived indole glucosinolates and camalexin as defence compounds. This results in a complex metabolic network, which makes it difficult to assign specific enzymatic functions and biosynthetic genes to IAA biosynthesis. In this review, the Arabidopsis system is compared to the maize system, where the branch point between IAA and secondary metabolite biosynthesis occurs prior to tryptophan.

Key words : tryptophan, auxin, IAA, Arabidopsis, maize

Tryptophan metabolism and IAA biosynthesis

Tryptophan is an essential amino acid for human nutrition. In addition to its role in protein synthesis tryptophan is the precursor of a large variety of secondary metabolites like terpenoid indole alkaloids, indole glucosinolates, and indolic phytoalexins (reviewed in ref. (1-3)). Terpenoid indole alkaloids have been found in a number of plant families, including the *Apocynaceae*, which comprise many medicinal plants. The vinblastine producer *Catharanthus* roseus is of particular economic importance. Indole glucosinolates and indolic phytoalexins are mainly found in the *Brassicaceae* and related families. The biologically active degradation products of glucosinolates, which are formed when glucosinolates are hydrolyzed by myrosinases under tissue disruption, are well known as the characteristic flavour compounds in mustard or in Brassica vegetables like cabbage. An essential tryptophan-derived metabolite throughout the plant kingdom is the auxin indole-3-acetic acid (IAA), which is involved in numerous plant processes including embryo development, apical dominance, lateral root formation, vascular development, and tropisms. Auxin homeostasis is controlled by biosynthesis, degradation, conjugation, hydrolysis of conjugates, and transport (4). While recently significant progress was made in understanding other factors controlling IAA homeostasis (4), the IAA biosynthetic pathways still remain to be resolved. Approaches and drawbacks in the investigation of IAA biosynthesis in the model plant systems Arabidopsis and maize will be discussed in this review.

Tryptophan-independent IAA biosynthetic pathways have been proposed based on the analysis of mutants in tryptophan synthase and labelling experiments with indole and anthranilate in relation to tryptophan (5,6). The corresponding enzymatic steps involved and the origin of the acetic acid side chain of IAA remain to be identified.

On the other hand several pathways leading from tryptophan to IAA have been suggested after incorporation experiments with specific precursors and the identification of enzymatic steps *in vitro* (see **Fig 1**).

A pathway from tryptophan via indole pyruvate and in-

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dole-3-acetaldehyde (IAAld) was characterized in bacteria and indole pyruvate was identified as endogenous compound of tomato shoots (7). Also tryptamine, a potential IAA precursor has been identified in tomato shoots (8). Tryptamine is formed by the decarboxylation of tryptophan and the corresponding enzymes (tryptophan decarboxylases) have been well characterized in plants that produce indole alkaloids from tryptamine, e.g. in *Catharanthus roseus* cell cultures that accumulate indole alkaloids after treatment with a fungal elicitor. Thereby alkaloid formation was preceded by rapid transient increases in tryptophan decarboxylase activity (9) and induction of the corresponding transcript, which can be repressed by addition of auxin (10). An overexpression of the *Catharanthus* tryptophan decarboxylase in *Brassica napus*

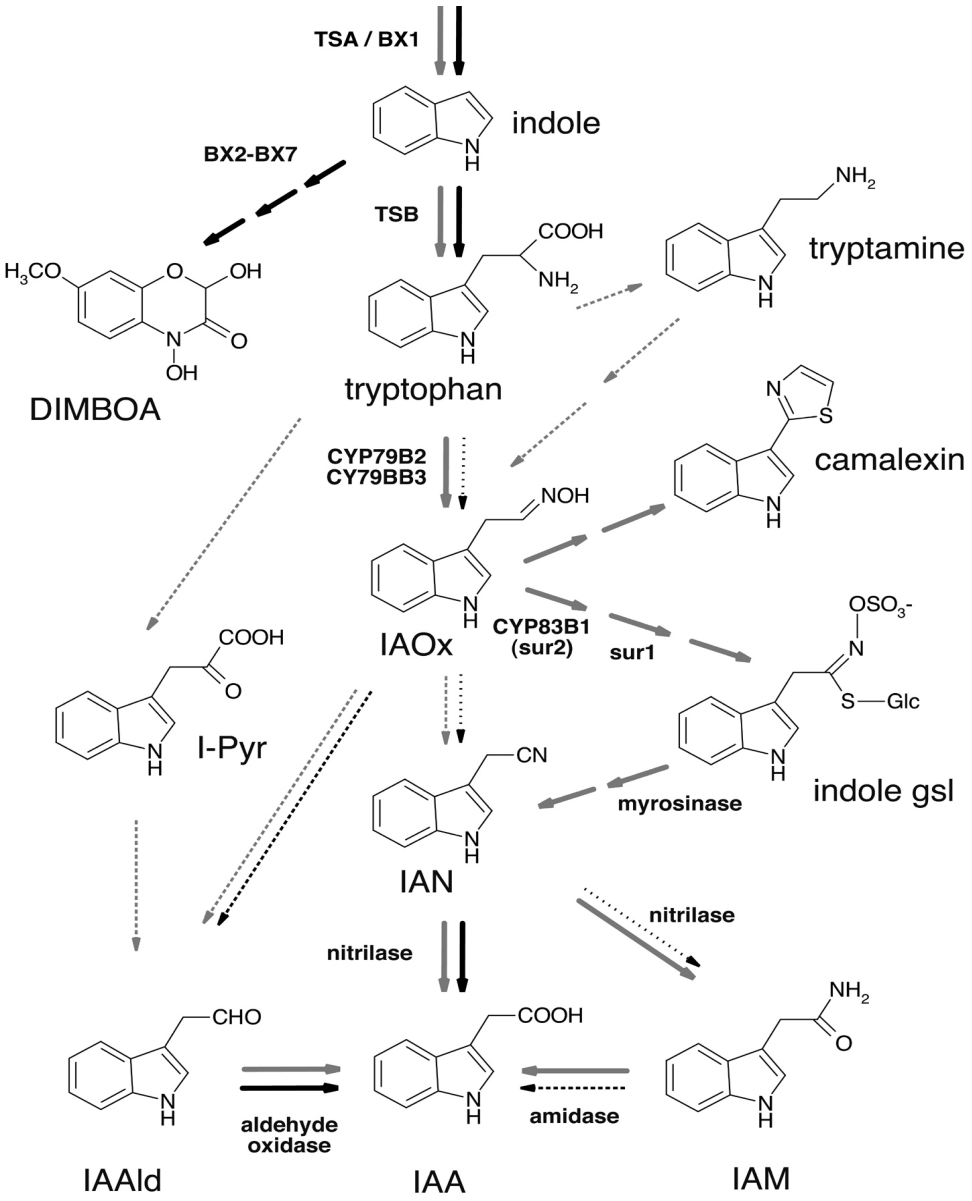

Fig 1. Tryptophan metabolism and IAA biosynthesis in maize (black arrows) and Arabidopsis (grey arrows). IAOx: indole-3-acetaldoxime; IAN: indole-3-acetonitrile; IAA: indole-3-acetic acid; I-Pyr: indole-3-pyruvic acid; IAAld: indole-3-acetaldehyde; IAM: indole-3-acetamide; DIMBOA: 2,4-dihydroxy-7-methoxybenzoxazin-3-one; gsl: glucosinolate.

resulted in a dramatic reduction in indole glucosinolate levels, probably due to a competition for tryptophan. Nonetheless the morphological appearance of the plant remained unaffected (11), from which it can be assumed that there is no flow from tryptamine to IAA in this system.

Alternative pathways via the intermediates indole-3-acetaldoxime (IAOx), indole-3-acetonitrile (IAN), indole-3-acetamide (IAM) and indole-3-acetaldehyde (IAAld) have been mainly investigated in the model system Arabidopsis thaliana, a cruciferous plant that synthesizes indole glucosinolates and the indolic phytoalexin camalexin from tryptophan (**Fig 1**).

The Arabidopsis system

In addition to IAA *Arabidopsis thaliana* produces indole glucosinolates from tryptophan, which act as phytoanticipins against pathogens and herbivores. Indole glucosinolates accumulate in Arabidopsis leaves to levels of app. 6 μmol/g dry weight (12) and therefore are a major sink for tryptophan. Particularly, in relation to glucosinolates total IAA content is at least one order of magnitude lower (13).

Recently major progress has been made in the identification of the indole glucosinolate biosynthetic pathway (14). The first step in indole glucosinolate biosynthesis is catalysed by two cytochrome P450 enzymes (CYP79B2 and CYP79B3), which convert tryptophan to IAOx (15,16). *CYP79B2* and *CYP79B3* are expressed throughout the plant but can be additionally induced by methyl jasmonate, which is in accordance to an enhanced indole glucosinolate formation (17). In addition, *CYP79B2* is induced under pathogen infection where the IAOx-derived phytoalexin camalexin is synthesized (18). *Cyp79B2/B3* double knock-out plants are devoid of indole glucosinolates and are not able to synthesize camalexin (18,19). While under normal growth conditions leaves of these plants accumulate wild type auxin concentrations, only 65% of free IAA compared to wild type levels are formed when these plants are grown under heat stress (26℃) (19). In addition, root-localized IAA synthesis was diminished in a *cyp79B2/B3* double knock-out, suggesting an important role for Trp-dependent IAA synthesis pathways in the root (20).

In an attempt to understand mechanisms that control auxin homeostasis and physiological processes controlled by auxin action, mutants were isolated that showed severe auxin overproducing phenotypes like enhanced adventitious root formation, and were therefore termed *superroot1* (*sur1*) (21), allelic to *alf1* (22), *rooty* (23), and *hookless3* (24) and *superroot2* (*sur2*) (25), allelic to *rnt1* (26). It turned out that the corresponding genes encode for CYP83B1 (*sur2*) (27,28) and a C-S lyase (*sur1*) (29), catalyzing the subsequent steps of indole glucosinolate biosynthesis from IAOx to indole-3-thiohydroxymate. Therefore the block of the indole glucosinolate biosynthetic pathway in these mutants, the major sink for tryptophan in Arabidopsis, may result in accumulation of the intermediate IAOx, which is then converted to IAA. Enzyme activities converting IAOx to the IAA precursors IAN or IAAld have been found in the crucifers *Brassica campestris* and *Isatis tinctorea* (30). Whether an IAA biosynthetic pathway via IAOx significantly contributes to the IAA pool also under wild type conditions remains to be investigated.

An alternative IAA-biosynthetic pathway has been proposed based on the phenotype of the activation tagged *YUCCA* gene, encoding a flavin monooxygenase, which accepts tryptamine as a substrate (31). It was suggested that the N-hydroxylated product should be subsequently converted to IAOx. Due to the lack of indole glucosinolates and camalexin in the *cyp79B2/B3* double knock-out plants, an independent IAOx pool, which does not feed into indole glucosinolates and camalexin, has to be assumed in this model.

Cyp79B2/B3 double knock-out plants have strongly reduced but clearly detectable levels of indole-3-acetonitrile (IAN) (19). This suggests a dual role for IAN as a breakdown product of indole glucosinolates as well as an intermediate of IAA biosynthesis. Arabidopsis contains four nitrilase encoding genes (*NIT1-4*). NIT1-3 have been shown to convert IAN to IAA *in vitro*. In all cases the enzymes had a broad substrate spectrum and an apparent K_M value towards IAN in the millimolar range (32), implying an efficient substrate channelling to permit involvement in IAA biosynthesis. A possible mode of channelling could be that a nitrilase is part of an IAA-synthase complex (33). Alternatively, nitrilases could be modified *in vivo* to allow higher efficiency, which is supported by the fact that IAN has auxin activity in the micromolar range (34).

The major isoform NIT1 plays a role during infection with *Plasmodiophora brassicae* that causes the clubroot disease. The formation of root galls coincides with higher IAA levels and higher nitrilase levels in infected, but not in healthy cells. Knock-out mutants of *nit1* developed smaller clubs, which correlated with a lower content of free IAA in comparison to wild type (35). At the protein level, recently an additional specific mode of NIT1 regulation has been observed. Mechanical wounding induces rapid aggregation of NIT1 protein directly abutting wound sites, which is one of the earliest events associated with wound and herbicide-induced cell death (36). NIT2 might have a specific IAA biosynthetic function in breaking seed dormancy (32) and is induced by pathogen infection (37) and leaf senescence (38) NIT3 is specifically expressed in sulphur-deprived roots. It was suggested that IAA synthesized by NIT3 induces lateral roots to access new areas of the soil and therefore new potential sulphur sources (39). AtNIT4 does not accept IAN as a substrate, but shows high activity towards ß-cyanoalanine and is therefore probably involved in cyanide detoxification (40).

An alternative source of IAA involves indole-3-acetamide as an intermediate, which has been identified in sterile grown rosette leaves (41). An amidase (AtAMI1) that is expressed in leaves and preferentially hydrolyzes IAM to IAA has been identified (42). As the Arabidopsis nitrilases 1, 2, and 3 also show some nitrile hydratase activity (41) IAM could be a product of IAN.

A hypothetical pathway not interconnected with glucosinolate metabolism involves indole-3-pyruvic acid, which has been detected in seedlings (43). In several bacteria indole-3-pyruvic acid is converted to indole-3-acetaldehyde (IAAld) that is further oxidized to IAA by members of the aldehyde oxidase family. Aldehyde oxidase activity is 5 fold induced in *superroot1* seedlings compared to wild type (44). As IAOx is probably a second source for IAAld, which is suggested by the conversion of ^{14}C-IAOx to ^{14}C-IAAld in a soluble enzyme extract from *Brassica campestris* (45), this induction could be due to a detoxification process in the IAOx accumulating *sur1* mutant.

The maize system

Driven by its economic importance maize has been a well-studied classical genetic model. In contrast to Arabidopsis the branch point between IAA biosynthesis and secondary metabolism is earlier, as maize does not form tryptophan- but indole-derived phytoanticipins (DIBOA and DIMBOA). For phytoanticipin biosynthesis indole, synthesized by the tryptophan synthase alpha homolog BX1, is oxidized to 2,4-dihydroxy-benzoxazin-3-one (DIBOA) by a series of four cytochrome P450-reactions extending the indole to a benzoxazine ring (46,47). As benzoxazinoid biosynthesis and degradation do not share common intermediates with proposed IAA biosynthetic pathways, the maize system makes it possible to exclude a function of candidate IAA biosynthetic genes in secondary metabolism.

The maize mutant *orange pericarp* is deficient in detectable tryptophan synthase beta activity and therefore tryptophan auxotroph (5). As tryptophan biosynthesis is feedback controlled the *orange pericarp* kernels accumulate large amounts of the tryptophan precursors, anthranilate and indole. Because *orange pericarp* seedlings are able to produce elevated IAA concentrations tryptophan-independent IAA biosynthesis was inferred. Whether this pathway is important in wildtype maize has been debated. Developing kernels are the main IAA biosynthetic tissue in maize and large quantities of IAA esters are stored in the endosperm (48). In kernels the tryptophan-dependency of IAA biosynthesis was addressed by application of the general precursors [U-$^{13}C_6$]glucose und [1,2-$^{13}C_2$]acetate and determination of the metabolic history of IAA by retrobiosynthetic NMR analysis. In these experiments no evidence for tryptophan-independent IAA biosynthesis was obtained (49).

For the turnover of tryptophan to IAA a pathway involving IAAld as intermediate has been suggested for maize. An aldehyde oxidase that converts IAAld to IAA was purified from maize coleoptiles (50) and an apparent K_M value of 3.2 μM for IAAld was determined. The enzyme shows a relatively broad substrate spectrum with respect to aldehydes, so other metabolic processes in addition to IAA biosynthesis, as e.g. abscisic acid biosynthesis have to be considered as natural function for the aldehyde oxidases as well. Based on the amino acid sequence two homologous genes (*ZmAO-1 and ZmAO-2*) were isolated (51).

IAN has been proven to be an endogenous compound in

maize kernels and seedlings (52) and shows auxin effects in the micromolar range (Kriechbaumer, Park, and Glawischnig, unpublished results). As maize is not synthesizing glucosinolates, this highlights the potential role of IAN as an IAA precursor. Maize expresses two nitrilases ZmNIT1 and ZmNIT2, both in the kernel tissue, where also IAA is synthesized. Heterologously expressed ZmNIT2 was shown to convert IAN to IAA at least 7 to 20 times more efficiently than the nitrilases from Arabidopsis (52). As like for Arabidopsis nitrilases the apparent K_M value is in the millimolar range, protein modification and/or efficient substrate channelling has to be proposed. We currently investigate this hypothesis by identifying protein interaction partners for the maize nitrilases. The pathway of IAN formation in maize is not known, particularly no CYP79B homolog has been identified and IAOx has not been reported in grasses. *In vitro* plasma membrane fractions from maize hypocotyls convert tryptophan to IAOx (53) and in a maize mesocotyl preparation IAN and IAAld were formed from IAOx (30). However, it has been questioned whether *de novo* biosynthesis is relevant in seedlings or IAA is rather imported from the kernel (54).

Whether the nitrilases significantly contribute to the IAA pool will be clarified by the analysis of knock-out mutants. The overlapping expression pattern of the nitrilases and the aldehyde oxidases could entail that multiple mutants will have to be created in order to obtain auxin deficient mutants.

Concluding remarks

Despite considerable effort analyzing the function of candidate genes of IAA biosynthesis no IAA biosynthetic pathway has been conclusively identified at the level of the biosynthetic genes. Possible reasons include redundancy of biosynthetic pathways and lethality of auxin deficient mutants. In Arabidopsis, the linkage of secondary metabolism with IAA biosynthesis has added an additional level of complexity. This resulted in initial annotation of genes involved in secondary metabolism as related to IAA, which is exemplified by the *superroot* genes. In this respect the investigation of IAA biosynthesis in other model plants apart from maize that do not synthesize tryptophan-derived defence compounds is meaningful. In addition to the sequencing of the genomes currently large mutant collections are established in rice, *Medicago, Lotus, Physcomitrella*, and other model plants, which will be powerful tools to determine general mechanisms of IAA metabolism throughout the plant kingdom.

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