Review

The shifting paradigms of auxin biosynthesis

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Auxins are an important group of hormones found in all land plants and several soil-dwelling microbes. Although auxin was the first phytohormone identified, its biosynthesis remained unclear until recently. In the past few years, our understanding of auxin biosynthesis has improved dramatically, to the stage where many believe there is a single predominant pathway in *Arabidopsis* (*Arabidopsis thaliana* L.). However, there is still uncertainty over the applicability of these findings to other plant species. Indeed, it appears that in certain organs of some species, other pathways can operate. Here we review the key advances that have led to our current understanding of auxin biosynthesis and its many proposed pathways.

A panoply of pathways to auxin

Auxins are a structurally diverse group of phytohormones involved in a large number of plant developmental processes. Initial hints of auxin date back as far as 1872, when Theophil Ciesielski postulated the presence of a 'transmitted influence', which was present in the tip of plant roots and responsible for gravitropism [1]. This idea was further developed by Charles and Frances Darwin, who showed that this 'transmitted influence' also has a role in shoot phototropism [2]. In addition, auxins have been shown to play roles in lateral root formation, control of shoot architecture (reviewed in [3]), organ patterning ([4,5], reviewed in [6]), and vascular development (reviewed in [6]). The action of auxins is thought to be regulated at three points: biosynthesis, polar auxin transport, and perception/signal transduction (reviewed in [7]).

In 1937, the structure of the primary naturally occurring auxin, IAA (see Table 1 for a list of abbreviations related to auxin biosynthesis), was determined ([8], reviewed in [9]). For a range of plant and microbial species, many IAA biosynthetic intermediates were suggested, including TAM [10], IPyA [11], IAOx [12], IAAld [13], IEt [14,15], IAN [12], and IAM [16–19]. By the 1980s the amino acid Trp, synthesized from anthranilate via indole [20], had been well established as an IAA precursor in a wide variety of plant species (for reviews, see [3,21,22]) and many IAA biosynthetic pathways had been proposed (Figure 1). Yet, since the elucidation of the structure of IAA, over 80 years ago, there has been much confusion and debate over IAA biosynthesis; discovery of a primary biosynthetic route has been particularly problematic and IAA biosynthetic schemes that incorporate data from all species are intensely complicated (e.g., Figure 2 in [23] and Figure 1 in [24]). However, in a single species, the situation may be much simpler. Recently, there have been dramatic improvements in our understanding of auxin biosynthesis and simplifications of proposed pathways but, as we will show in this review, one must remain cautious about drawing conclusions based on as-yet incomplete data. Four interconnected Trp-dependent IAA biosynthetic pathways have been proposed (Figure 1), each named after the intermediate immediately downstream of Trp – the IAOx, IAM, TAM, and IPyA pathways (see reviews mentioned above). A Trp-independent pathway has also been proposed (Box 1).

The IAOx pathway

In *Arabidopsis*, the conversion of Trp to IAOx is catalyzed by the cytochrome p450 enzymes CYP79B2 and CYP79B3 [25–27]. At one stage it was suggested that IAOx can also be produced from the putative TAM pathway intermediate NHT [28]. However, it was later shown that the CYP79B2/3 enzymes were responsible for the vast majority of IAOx production in *Arabidopsis* [29] and ectopic expression of either the CYP79B2 or CYP79B3 genes from *Arabidopsis* in tobacco (*Nicotiana tobacum* L.) resulted in IAOx biosynthesis [30]. Various lines of evidence indicate the IAOx pathway is restricted to the Brassicaceae [29,31], where it might be more important for producing indole glucosinolates than IAA, because knocking out both *Arabidopsis CYP79B* genes had little effect on IAA content [27,29]. Nevertheless, the *sur1* and *sur2* mutants, which accumulate IAOx, contain substantially elevated levels of IAA, indicating that relatively large amounts of IAA can be produced from IAOx [32]. Furthermore, a *cyp79B2 cyp79B3* double mutant did show a significantly reduced rate of IAA biosynthesis in apical sections of excised roots [27].

The IAM pathway

In the bacteria *Agrobacterium tumerfaciens* and *Pseudomonas savastanoi* [33,34], Trp is converted to IAM (catalyzed by the tryptophan-2-monooxygenase, iaaM), which is

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Table 1. Common abbreviations for the names of auxin-related compounds, enzymes, and genes

Abbreviation	Definition
4-Cl-IAA	4-Chloroindole-3-acetic acid
4-Cl-Trp	4-Chlorotryptophan
AMI1	AMIDASE1
AO	Aldehyde oxidase
CYP79B2	Cytochrome P450 79B2
CYP79B3	Cytochrome P450 79B3
IAA	Indole-3-acetic acid
IAAld	Indole-3-acetaldehyde
IAM	Indole-3-acetamide
IAN	Indole-3-acetonitrile
IAOx	Indole-3-acetaldoxime
IEt	Indole-3-ethanol
IGP	Indole-3-glycerol phosphate
IPDC	Indole-3-pyruvate decarboxylase
IPyA	Indole-3-pyruvic acid
NHT	*N*-Hydroxytryptamine
ORP1	ORANGE PERICARP1 (Tryptophan synthase β1)
ORP2	ORANGE PERICARP2 (Tryptophan synthase β2)
SAV3	SHADE AVOIDANCE3
SPI1	SPARSE INFLORESCENCE1
TAA1	TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1
TAM	Tryptamine
TAR1–4	TRYPTOPHAN AMINOTRANSFERASE RELATED 1 to 4
TDC	Tryptophan decarboxylase
Trp	L-Tryptophan
VT2	VANISHING TASSEL2
WEI8	WEAK ETHYLENE INSENSITIVE8
YUC	YUCCA (Flavin monooxygenase)

subsequently hydrolyzed to IAA (catalyzed by the IAM hydrolase, iaaH; Figure 1). IAM has also been identified as an endogenous compound in *Arabidopsis* [29,35], rice (*Oryza sativa* L.), maize (*Zea mays* L.), and tobacco [29], and is thought by some to be widespread in the plant kingdom (reviewed in [36]). In *Arabidopsis*, there is evidence that IAM is mainly generated from IAOx, which is synthesized from Trp by CYP79B2/3 enzymes [29]. However, rice, maize, and tobacco lack the CYP79B2/3 homologs and are devoid of endogenous IAOx [29]. It is therefore proposed that in species other than *Arabidopsis* and its relatives, Trp is converted by iaaM-type enzymes to IAM (Figure 1), which is then converted to IAA by members of an enzyme family termed AMI1, reportedly widespread among plants [36]. Although it had been proposed that this pathway may be an important route of auxin biosynthesis in all plants [37], it was recently shown that in seeds of pea (*Pisum sativum* L.) IAM is not converted to IAA and that endogenous IAM was below the limit of detection [38]. Furthermore, there are as yet no reports of mutations that specifically affect the IAM pathway.

The TAM pathway

The TAM pathway was first proposed in 1966 [10] after auxin-like activity of TAM was observed in *Avena* coleoptile elongation assays. TAM has been reported as an endogenous compound in tomato (*Solanum lycopersicum* L.) [39,40], rice [41], *Arabidopsis* [29], barley (*Hordeum vulgare* L.) [40], and pea [31]. However, some early studies cast doubt over the importance of this pathway in IAA biosynthesis. A study in 1991 [39] reported the labeling of IAA and TAM after applying D_2O to tomato shoots [35]. Initially, IAA and TAM became labeled at a similar rate, but TAM continued to accumulate label up to 21 h, whereas IAA did not. It was inferred that TAM and IAA are synthesized from separate Trp pools, and that TAM is not a major IAA precursor. Similarly, tobacco plants overexpressing Trp decarboxylase accumulate high levels of TAM, whereas IAA levels were unaffected [42].

Nevertheless, in the early 2000s, interest in the TAM pathway increased, largely due to the discovery of the *YUC* gene family [28] in *Arabidopsis*. It was proposed that YUCs catalyze a rate-limiting step in the TAM pathway, the *N*-hydroxylation of TAM to produce NHT [28]. YUC homologs were then identified from several other species, including tomato [43], maize [44], pea [45], and possibly potato (*Solanum tuberosum* L.) [46], although the role of these enzymes in TAM-dependent IAA biosynthesis was called into question [45,47]. The reports of NHT as an *in vitro* product of YUC enzymes supplied with TAM were based on chromatographic and/or mass spectral analyses with no authentic NHT used for comparison [28,43,48]. Analysis of authentic NHT by mass spectrometry [45] showed that the previously published spectra do not correspond to that compound.

However, these findings do not exclude the TAM pathway as an important source of IAA in certain species. For example, in pea roots TAM is converted to IAA, although the majority of TAM appeared to be converted to *N*ω-acetyltryptamine [31]; it is conceivable that this occurs also in tobacco, which may explain why the high TAM level reported in [42] was not accompanied by high IAA content. In contrast to pea roots, it was shown that the TAM pathway does not operate in pea seeds [45]. These findings highlight one of the potential major sources of confusion in the study of IAA biosynthesis: even if one pathway is found to operate in a particular organ of a particular species, it is often not possible to extrapolate that finding directly to other species, or even to other organs of the same species.

The IPyA pathway

The IPyA pathway (Figure 1) is important in several IAA-synthesizing microorganisms ([49,50], reviewed in [51]). By the early 1990s, there was also some evidence for this pathway in certain plant species [52,53]. However, it is only in the past 5 years that there have been significant advances in our understanding of the IPyA pathway in plants and how it diverges from that occurring in microbes [38,54–61]. This pathway is now thought to be widespread among flowering plants, because IPyA has been isolated from a range of species [38,39,49,53,55]. However, study of this pathway is somewhat complicated because IPyA is easily oxidized and degrades to IAA at room temperature [50,53,62,63], making it difficult to demonstrate that the conversion of IPyA to IAA is enzymatically controlled [50]. Furthermore, it has been shown that IPyA exists as a mixture of two tautomers: keto and enol (Box 2) [38,64].

Trp aminotransferases, which convert Trp to IPyA (Figure 1), have been isolated from *Arabidopsis* [57,59],