

# Auxin metabolism and homeostasis during plant development

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## Summary

Auxin plays important roles during the entire life span of a plant. This small organic acid influences cell division, cell elongation and cell differentiation, and has great impact on the final shape and function of cells and tissues in all higher plants. Auxin metabolism is not well understood but recent discoveries, reviewed here, have started to shed light on the processes that regulate the synthesis and degradation of this important plant hormone.

**Key words:** Auxin metabolism, Biosynthesis, Conjugation and degradation, Plant hormone, *Arabidopsis thaliana*, Root and shoot development

## Introduction

The plant hormone auxin or indole-3-acetic acid (IAA) (Fig. 1) was discovered ~70 years ago, although the concept of plant hormones and speculation over their roles in plant development were conceived much earlier (reviewed by Abel and Theologis, 2010). Auxin research took off in the 1980s following the discovery of a battery of genes involved in auxin responses, and more recently with the discovery of the TIR1 auxin receptor family (reviewed by Calderón-Villalobos et al., 2010) and various auxin transporters (reviewed by Zažímalová et al., 2010). These discoveries boosted a wave of research into auxin signalling and the roles of these newly discovered genes during plant development, mostly using the model plant species *Arabidopsis thaliana* as an experimental system.

Although our understanding of auxin signalling has flourished during the last few decades, progress in understanding auxin metabolism (which includes auxin biosynthesis, conjugation and degradation) has been hampered by the lack of suitable tools for the quantification and visualisation of auxin metabolites on a tissue and cellular level. Until recently, key components in these metabolic pathways were missing and the regulation of auxin metabolism was poorly understood, but genetic and biochemical evidence in combination with sensitive methods for auxin metabolite identification and quantification have greatly improved our knowledge in this respect.

This Primer describes the recent progress that has been made in our understanding of auxin metabolism, with the aim of putting these discoveries into the context of plant growth and development. I will focus on auxin metabolism in *A. thaliana*, as these metabolic processes are much better understood in *Arabidopsis* than in other plant species. Still, it is important to remember that there are differences between plant species, and also that IAA metabolism in some species (including *Arabidopsis*) is intertwined with, and

affected by, the secondary metabolism of plant defence compounds (Normanly, 2010).

## An overview of auxin transport and signalling pathways

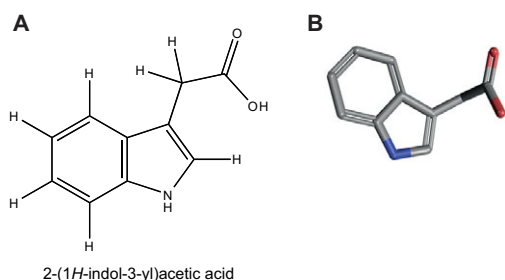
Auxin is a weak organic acid consisting of a planar indole ring structure coupled to a side chain harbouring a terminal carboxyl group (Fig. 1A,B). The carboxyl group is protonated at low pH, making the molecule less polar ( $\text{IAA}^- + \text{H}^+ \rightleftharpoons \text{IAA-H}$ ). In this form it can diffuse across cell membranes, whereas the molecule in its unprotonated, negatively charged form ( $\text{IAA}^-$ ) is too polar to diffuse. The pH in different cellular compartments varies, being ~5.0-5.5 in the apoplastic fluid of the cell wall and in vacuoles and ~7.0 in the cytosol. IAA-H in the apoplast and in vacuoles can thus diffuse over the surrounding membranes, whereas  $\text{IAA}^-$  is trapped within the cell and cannot escape from the cytosol without the aid of specific transporters (efflux carriers) (Zažímalová et al., 2010; Rosquete et al., 2012).

Two families of IAA efflux carrier proteins have so far been identified in *Arabidopsis*: the PIN family and the ABCB family (reviewed by Zažímalová et al., 2010). There are also specific IAA influx carrier proteins, such as the AUX1/LAX family, which are important for increasing IAA transport into specific cell types (reviewed by Swarup and Péret, 2012). Recently, a new group of transport proteins – the PIN-LIKES (or PILS proteins) – were identified, and these are postulated to have a function in IAA transport between the cytosol and the endoplasmic reticulum (ER) (Barbez et al., 2012). The localisation of influx and efflux carriers at the plasma membrane directs the transport of IAA in and out of the cell (also called ‘polar auxin transport’), providing the plant with a unique way of transporting this important hormone between different cells and tissues. There is plenty of evidence for the importance of polar transport in setting up auxin gradients in specific cell types, and such gradients provide developmental cues during, for example, embryogenesis and root development (Friml et al., 2003; Blilou et al., 2005; Benková et al., 2009).

Auxin signalling regulates cell responses to the different auxin levels that are formed by a combination of auxin metabolism and transport. One very important step forward for understanding auxin signalling was the discovery of the TIR1/AFB family of auxin receptors (Dharmasiri et al., 2005; Kepinski and Leyser, 2005). TIR1/AFBs are F-box proteins that, together with other proteins (ASK1, CUL1, RBX), form the ubiquitin protein ligase complex, SCF<sup>TIR1</sup>. TIR1/AFBs bind IAA, forming a co-receptor complex with Aux/IAA repressor proteins, which are negative regulators of auxin signalling (Fig. 2). At low IAA levels, the Aux/IAA proteins, together with the co-repressor TPL, can bind to and repress auxin response factors (ARFs), a group of transcription factors that regulate auxin responsive genes. At high IAA levels, however, the formation of the (TIR1/AFB)-IAA-(Aux/IAA) co-receptor complex targets the Aux/IAA proteins for degradation via the 26S proteasome (Calderón-Villalobos et al., 2010; Hayashi, 2012), and the ARFs are

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**Fig. 1. Structure of the plant hormone IAA.** (A) The chemical structure and IUPAC name for IAA, showing the planar conformation of the indole ring and the side chain harbouring the terminal carboxyl group. (B) The IAA carbon backbone (grey), showing the nitrogen molecule in blue and the oxygens in red (the hydrogens have been removed for clarity).

hence free to bind to genes containing auxin response elements (TGTCTC) in their promoters to activate or repress transcription (Fig. 2). The large number of Aux/IAA response genes (29 in *Arabidopsis*) and ARFs (23 in *Arabidopsis*) indicate that the auxin response is very complex, depending both on auxin levels and the specificity and strength of the TIR1-Aux/IAA and Aux/IAA-ARF interactions (Vernoux et al., 2011; Calderón-Villalobos et al., 2012).

An alternative, proteasome-independent auxin signalling pathway acting through the putative auxin receptor ABP1, which is located at both the ER and the cell wall, has been suggested to play important roles during cell division and cell elongation (reviewed by Sauer and Kleine-Vehn, 2011; Scherer, 2011) (Fig. 2). This signalling pathway has been shown to be important for cell wall loosening and the regulation of endocytosis and cytoskeleton rearrangement during cell expansion (Robert et al., 2010; Xu et al., 2010).

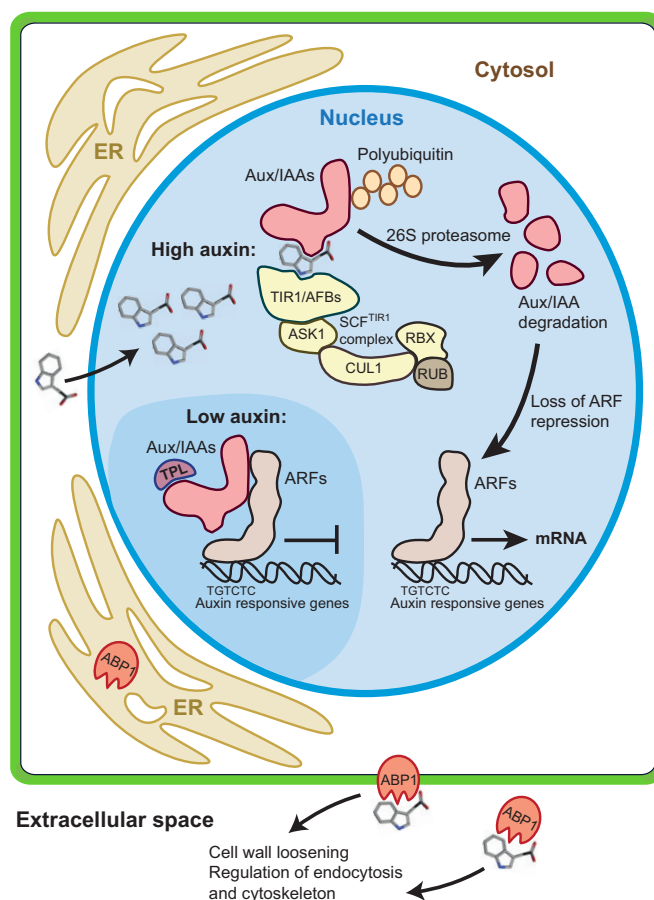
It is likely that both proteasome-dependent and proteasome-independent auxin signalling pathways play important roles during transcriptional regulation and function together with the rapid regulation of protein activity (e.g. enzyme and ion channel activity) that is independent of gene transcription. Data also indicate that these signalling pathways can interact to regulate specific cellular responses (Sauer and Kleine-Vehn, 2011).

### Auxin metabolism in higher plants

Most of our knowledge of the pathways for IAA biosynthesis and degradation comes from work on *Arabidopsis* mutants, and several genes involved in these metabolic pathways have been discovered in genetic screens for different developmental processes. For example, the *TAA1* gene, which encodes a tryptophan aminotransferase that converts the IAA precursor L-tryptophan (L-Trp) to indole-3-pyruvic acid (IPyA), was discovered simultaneously in a shade avoidance screen (Tao et al., 2008) and in a screen for ethylene effects on root development (Stepanova et al., 2008), and the *YUCCA1* gene was identified after screening activation-tagged mutant lines for developmental defects (Zhao et al., 2001). As summarised below, these screens, together with the detailed analysis of the mutants that they identify, are slowly beginning to unravel the various pathways used for IAA biosynthesis, degradation and conjugation. It must be noted that, although IAA is considered to be the main auxin in all higher plant species (Normanly, 2010), there are other indolic compounds (both endogenous and synthetic) that also have auxin-like activities (see Box 1).

### Pathways for auxin biosynthesis

IAA is believed to be synthesized mainly from precursors generated via the shikimate pathway (Fig. 3). This pathway



**Fig. 2. Key components in auxin perception and signalling.** Auxin can modulate both transcriptional regulation and transcription-independent responses. In the nucleus, IAA binds to its receptors, the TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALLING F-BOX proteins (TIR1/AFBs) and to the auxin/indole-3-acetic acid (Aux/IAA) proteins. TIR1/AFBs are F-box proteins that form part of the SCF<sup>TIR1</sup> complex, which consists of four subunits (TIR1/AFB, ASK1, CUL1 and RBX). An additional protein, RUB, binds to the SCF<sup>TIR1</sup> complex to regulate its function. The TIR1/AFB and Aux/IAA proteins function as co-receptors for IAA, binding IAA with high affinity. When IAA levels are low (darker blue background), the Aux/IAA proteins form heterodimers with auxin response factors (ARFs) to repress gene transcription. The TOPLESS (TPL) protein functions as a transcriptional co-repressor for Aux/IAAs. However, when IAA levels are high, the binding of IAA to its co-receptors targets the Aux/IAA proteins for degradation by the 26S proteasome, which leads to derepression of ARF transcriptional regulation and the expression of auxin responsive genes. AUXIN BINDING PROTEIN 1 (ABP1), which is located in the endoplasmic reticulum (ER) or at the plasma membrane and/or in the apoplast (extracellular space), is also believed to function as an IAA receptor, mediating rapid auxin responses such as cell wall loosening, cytoskeleton rearrangement and regulation of endocytosis, leading to cell expansion.

produces precursors for the biosynthesis of different indole compounds, aromatic amino acids (L-Trp, phenylalanine and tyrosine), alkaloids and other aromatic metabolites, lignins and flavonoids, and is therefore an essential pathway for primary and secondary metabolism in plants. The IAA precursor L-Trp is synthesized from chorismate, the final product of the shikimate pathway (Fig. 3A). The biosynthesis of L-Trp is well characterised in *Arabidopsis* and other plants, and genes in this pathway have been shown to be both transcriptionally and post-transcriptionally

### Box 1. Endogenous and synthetic compounds with auxin-like activity.

After the discovery of IAA, many compounds were synthesized and tested for their auxin-like activities in different growth assays. Some of these compounds have been used extensively in agriculture and horticulture as herbicides [e.g. 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-amino-3,5,6-trichloro-2-picolinic acid (picloram), 3,6-dichloro-2-methoxybenzoic acid (dicamba)] or as rooting agents for cuttings and in plant tissue culture [e.g. indole-3-butyric acid (IBA),  $\alpha$ -naphthaleneacetic acid ( $\alpha$ -NAA)]. The high auxin activities of these compounds are partly due to the fact that they are usually more stable than IAA in different growth media (e.g. 2,4-D and  $\alpha$ -NAA) and are therefore well suited for use in, for example, plant tissue culture (Dunlap et al., 1986; Nissen and Sutter, 1990). Some are also more easily taken up into cells by diffusion (e.g.  $\alpha$ -NAA), and some are more slowly metabolised (e.g. 2,4-D) (Hosek et al., 2012).

Besides IAA, other endogenous auxins have been identified in plants (reviewed by Simon and Petrášek, 2011). Phenylacetic acid (PAA) has been found in different plant species as well as in bacteria, although PAA has a much weaker auxin effect than IAA and its role during plant development is unclear. In pea seeds, the chlorinated form of IAA [4-chloroindole-3-acetic acid (4-Cl-IAA)] is very abundant (Tivendale et al., 2012), suggesting a role for 4-Cl-IAA during fruit development. IBA has also been suggested to function as an endogenous IAA precursor, forming IAA via peroxisomal  $\beta$ -oxidation and playing a role during seedling development (Strader et al., 2011). Whether IBA itself also has an auxin effect is still not fully understood, although genetic evidence suggests that this is not the case (Strader and Bartel, 2011).

regulated (Tzin and Galili, 2010). For example, abiotic and biotic stresses upregulate genes in both the shikimate and L-Trp biosynthesis pathways, as well as downstream genes involved in the synthesis of different defence compounds, and data indicate that co-regulation of these pathways by specific MYB transcription factors is important for different stress responses (Tzin and Galili, 2010).

Although, L-Trp-dependent biosynthesis of IAA is believed to be the main route of IAA biosynthesis in plants, evidence for a tryptophan-independent pathway of IAA synthesis branching from indole-3-glycerol phosphate (IGP) also exists (Ouyang et al., 2000) (Fig. 3A). The genes and enzymes involved in tryptophan-independent IAA biosynthesis are still largely unknown, and the existence of this alternative pathway is based mainly on feeding studies using stable labelled IAA precursors and different tryptophan biosynthesis mutants (Wright et al., 1991; Normanly et al., 1993). Further studies will hopefully shed light on the molecular mechanisms and role of this putative IAA biosynthesis pathway, which is suggested to exist in maize and *Arabidopsis* as well as in other plant species (Normanly, 2010; Mano and Nemoto, 2012).

In addition, the four-carbon side chain indole-3-butyric acid (IBA) has also been suggested to function as an endogenous IAA precursor, being converted to IAA in peroxisomes by  $\beta$ -oxidation (reviewed by Strader and Bartel, 2011).

Tryptophan-dependent IAA biosynthesis is also known to occur in microbes, and the metabolic pathways involved in bacterial IAA biosynthesis have been characterised (Spaepen et al., 2007). However, it has been surprisingly difficult to elucidate IAA biosynthetic pathways in higher plants. Feeding studies have shown that L-Trp is an important IAA precursor in maize (Kriechbaumer et al., 2006), *Arabidopsis* (Mashiguchi et al., 2011) and pea

(Quittenden et al., 2009), and genes involved in tryptophan-dependent IAA biosynthesis have been identified in many plant species (reviewed by Mano and Nemoto, 2012). Which of these pathways are most important during plant development is still under investigation, and key components of the pathways might still be missing. There is also considerable redundancy in many of the gene families involved in IAA biosynthesis, and we are far from understanding the developmental roles of all genes. Nonetheless, in recent years, important discoveries have increased our understanding of IAA biosynthesis in *Arabidopsis*, and the new data indicate that IAA biosynthesis could actually be less complex than previously believed.

Below is a summary of known tryptophan-dependent IAA biosynthesis pathways operating in higher plants, based on our present understanding. The pathways have been named after the first metabolite formed after L-Trp (Fig. 3B).

#### The indole-3-pyruvic acid (IPyA) pathway

In this pathway, the tryptophan aminotransferase TAA1 and its close homologues TAR1 and TAR2 convert L-Trp to IPyA (Stepanova et al., 2008; Tao et al., 2008; Yamada et al., 2009; Zhou et al., 2011), and the YUCCA (YUC) enzymes subsequently synthesize IAA from IPyA (Mashiguchi et al., 2011; Stepanova et al., 2011; Won et al., 2011). Although originally suggested to be operating in separate pathways, the *TAA1/TAR* and *YUCCA* gene families are now believed to work in the same IAA biosynthetic pathway, and genetic and biochemical evidence strongly suggest that this is one of the major pathways for IAA biosynthesis in diverse plants (Zhao, 2012). The putative role of indole-3-acetaldehyde (IAAld) as an intermediate in this pathway (reviewed by Normanly, 2010; Mano and Nemoto, 2012) has been questioned by recent data (Mashiguchi et al., 2011).

#### The indole-3-acetamide (IAM) pathway

The IAM pathway for IAA synthesis is well studied in bacteria, and *iaaM/iaaH* genes from *Agrobacterium tumefaciens* have been used for transgenic overproduction of IAA in different plant species. IAM is present in many plant species, including *Arabidopsis*, maize, rice and tobacco (Sugawara et al., 2009; Novák et al., 2012), and IAM hydrolases (AtAMI1, NtAMI1), which can convert IAM to IAA, have been isolated in *Arabidopsis* and tobacco (Pollmann et al., 2006; Nemoto et al., 2009).

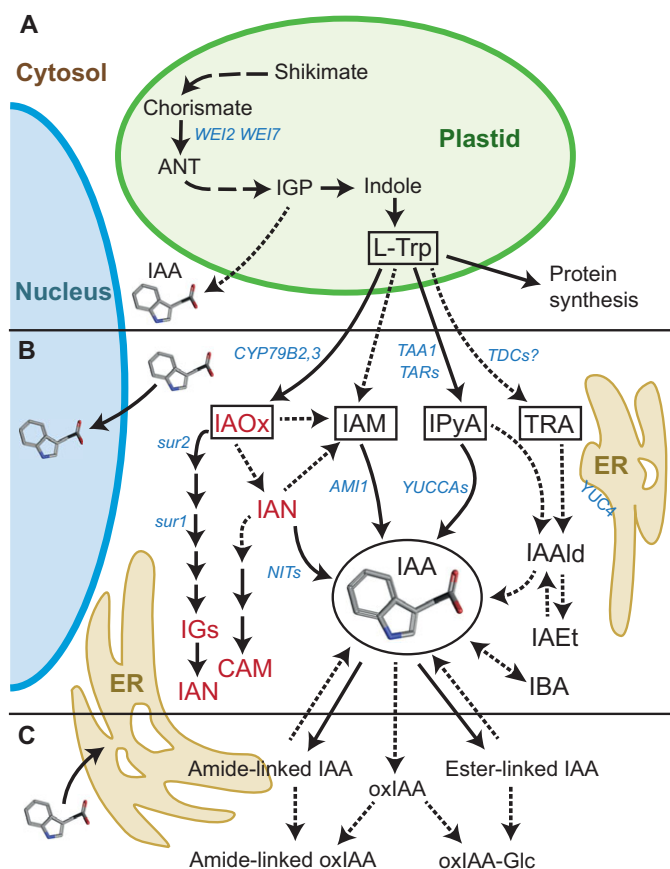
#### The tryptamine (TRA) pathway

TRA was originally believed to be an intermediate in the YUCCA pathway, but this has recently been questioned (Tivendale et al., 2010; Mano and Nemoto, 2012). TRA is found in very low levels compared with IAA and L-Trp in pea and *Arabidopsis* (Quittenden et al., 2009; Novák et al., 2012), and is believed to be the product of tryptophan decarboxylases (TDCs) (Mano and Nemoto, 2012). It is possible that TRA could function both as a precursor for IAA and in indole alkaloid and serotonin biosynthesis in different plant species (Quittenden et al., 2009; Mano and Nemoto, 2012).

#### The indole-3-acetaldoxime (IAOx) pathway

Using *Arabidopsis* as a model organism has greatly improved our knowledge of IAA metabolism in general, but there are also complications associated with studies using this plant species. In *Arabidopsis*, L-Trp also acts as a precursor for defence compounds such as camalexin (CAM) and indole glucosinolates (IGs) (Hansen and Halkier, 2005; Normanly, 2010), and perturbations in these pathways have been shown to affect IAA biosynthesis. The mutants





**Fig. 3. IAA metabolism in higher plants.** (A) The biosynthesis of IAA precursors, such as IGP and L-Trp, takes place in plastids. L-Trp, the major IAA precursor, is generated via the shikimate pathway. (B) The subsequent L-Trp-dependent IAA biosynthesis pathways are believed to be located in the cytosol. Four putative pathways for L-Trp-dependent IAA biosynthesis in higher plants are shown: the IAOx, IAM, IPyA and TRA pathways. The enzymes known to operate in each pathway are shown in blue. Intermediates that also act as precursors and degradation products of defence compounds (such as IGs and CAM) are in red. (C) Pathways for IAA degradation and conjugation. IAA can be conjugated to amino acids and sugars, or catabolised to form oxIAA. Some IAA conjugates can be regarded as storage forms that can be hydrolysed to form free IAA. Solid arrows indicate pathways in which the enzymes, genes or intermediates are known, and dashed arrows indicate pathways that are not well defined. ANT, anthranilate; CAM, camalexin; Glu, glucose; IAAld, indole-3-acetaldehyde; IAEt, indole-3-ethanol; IAM, indole-3-acetamide; IAN, indole-3-acetonitrile; IAOx, indole-3-acetaldoxime; IBA, indole-3-butyric acid; IGP, indole-3-glycerol phosphate; IGs, indole glucosinolates; IPyA, indole-3-pyruvic acid; L-Trp, L-tryptophan; NITs, nitrilases; oxIAA, 2-oxoindole-3-acetic acid; TDCs, tryptophan decarboxylases; TRA, tryptamine.

*sur1* and *sur2* are deficient in IG biosynthesis and seem to accumulate IAA and different IAA metabolites as a consequence of these mutations (Barlier et al., 2000; Mikkelsen et al., 2004; Sugawara et al., 2009; Novák et al., 2012), thus highlighting the link between auxin biosynthesis and the biosynthesis of IGs.

The cytochrome P450 monooxygenases CYP79B2 and CYP79B3 catalyze the conversion of L-Trp to IAOx, the common precursor for CAM, IGs and IAA, and CYP79B genes have so far only been identified in *Brassicaceae* species (Zhao et al., 2002; Sugawara et al., 2009), the family that contains *Arabidopsis* and other cruciferous plants.

Indole-3-acetonitrile (IAN) is a putative IAA precursor that is synthesized from IAOx (Sugawara et al., 2009) and is found in very high concentration in *Arabidopsis* tissues (Sugawara et al., 2009; Novák et al., 2012). IAN is also formed after hydrolysis of IGs by myrosinases, and IAN and other nitriles are believed to function in plant defence responses against biotic stresses (Hansen and Halkier, 2005). Nitrilases (NITs) have been suggested to convert IAN to IAA in both maize and *Arabidopsis* (Park et al., 2003; Normanly et al., 1997), but conclusive evidence for the role of these genes in IAA biosynthesis is still missing.

### Pathways for auxin conjugation and degradation

The regulation of auxin levels by *de novo* synthesis is one important homeostatic mechanism operating in plant cells, but the levels of IAA can also be attenuated by conjugation (mainly to amino acids and sugars) and by degradation (Normanly, 2010; Rosquete et al., 2012) (Fig. 3C). Genes involved in IAA conjugation and IAA conjugate hydrolysis have been identified, mainly in *Arabidopsis*, and include members of the auxin-inducible GH3 family of amido synthases and different amido hydrolases (reviewed by Woodward and Bartel, 2005; Ludwig-Müller, 2011). IAA conjugates are regarded as either reversible or irreversible storage compounds, although the function of IAA conjugates, and the genes that regulate their formation, during plant growth and development is still under investigation. IAA can be conjugated and stored in seeds and used for early seedling growth, and seeds have thus been a rich source for identifying different IAA conjugates in plants (Woodward and Bartel, 2005; Ludwig-Müller, 2011).

The metabolites 2-oxoindole-3-acetic acid (oxIAA) and oxIAA-glucose (oxIAA-Glc) are the major degradation products of IAA (Östin et al., 1998; Kai et al., 2007; Novák et al., 2012), but the genes involved in IAA catabolism have so far not been identified. OxIAA and oxIAA-Glc are found in high concentrations after IAA treatment (Östin et al., 1998), in stable IAA-overproducing lines (Stepanova et al., 2011; Novák et al., 2012) and after induction of IAA biosynthesis (Sairanen et al., 2012).

### The subcellular location of auxin biosynthesis, conjugation and degradation

The localisation of enzymes involved in IAA biosynthesis, conjugation and deconjugation suggests that different subcellular compartments are involved in IAA metabolism as well as in the storage of these compounds. Both the shikimate and L-Trp biosynthesis pathways are believed to be localised to plastids, based on protein localisation studies and the presence of specific plastid transit peptides in the enzymes involved in these pathways (Woodward and Bartel, 2005; Tzin and Gallili, 2010; Mano and Nemoto, 2012) (Fig. 3A). By contrast, the pathways downstream of L-Trp are believed to be localised to the cytosol (Woodward and Bartel, 2005; Mano and Nemoto, 2012) (Fig. 3B). However, as mentioned above, L-Trp is a precursor not only for IAA, but also for the synthesis of many defence compounds and for protein synthesis. As such, the pool of L-Trp is very large compared with that of IAA (Novák et al., 2012), suggesting a need for the compartmentalisation of the L-Trp pool or the strict regulation of the downstream steps in IAA biosynthesis to avoid IAA overproduction (Sairanen et al., 2012). The channelling of IAA precursors into an 'IAA synthase' complex has also been suggested (Pollmann et al., 2009).

It has recently been shown that the YUC4 enzyme can be localised both to the cytosol and to the cytosolic face of the ER

membrane, and that this localisation is regulated by alternative splicing (Kriechbaumer et al., 2012). Recent data suggest a role for the transport of IAA into the ER via specific PIN and PILS proteins, and subsequent IAA conjugation/deconjugation within the ER, as one mechanism for regulation of auxin homeostasis (Mravec et al., 2009; Barbez et al., 2012; Rosquete et al., 2012). Interestingly, several IAA-amino acid conjugate hydrolases have been shown to be located at the ER (Woodward and Bartel, 2005).

Very little is known about the localisation and concentration of IAA and its metabolites, as well as enzymes involved in IAA metabolism, in other subcellular compartments, such as vacuoles and the apoplast. These compartments could be important for the metabolism, storage and transport of IAA and different IAA metabolites. The concentration of IAA in the apoplast will also affect signalling via the auxin receptor ABP1.

### Regulation of auxin metabolism during plant growth and development

It is obvious that IAA biosynthesis, degradation and transport mechanisms need to be under strict control in order to regulate intracellular IAA concentrations, but we still know very little about how these regulatory mechanisms operate in plants. We know that most tissues, both in the root and in the shoot, have the capacity to synthesize IAA, although at different rates, and that they therefore can function both as source and sink tissues for this compound (Ljung et al., 2001). Long-range transport of IAA from source to sink tissues is important for developmental processes such as lateral root development and shoot branching, whereas short-range transport is believed to be the mechanism that is most important for setting up IAA gradients and maxima/minima in different tissues (Zařimalová et al., 2010).

#### Auxin metabolism in the root

Our understanding of how auxin biosynthesis and degradation are regulated in the root system under normal and different stress conditions is still very limited, but it is clear that auxin homeostasis has a major impact on root architecture (Jones and Ljung, 2012). Many genes believed to be involved in auxin biosynthesis are strongly expressed in the root apical meristem, where there is a high rate of IAA biosynthesis (Ljung et al., 2005). Furthermore, local auxin biosynthesis combined with polar auxin transport is involved in forming the auxin gradient within the root apex (Ljung et al., 2005; Petersson et al., 2009). An important role for local auxin biosynthesis in modulating the auxin gradient, planar polarity and hair positioning in the root was demonstrated by Ikeda et al. (Ikeda et al., 2009), but local IAA biosynthesis also has an impact on many other processes within the root, such as cell division, differentiation and elongation in the root apex and lateral root initiation and development (reviewed by Overvoorde et al., 2010).

Lateral root development is influenced both by local auxin production and by auxin transport (reviewed by Overvoorde et al., 2010; Jones and Ljung, 2012). Polar and phloem-based transport of auxin from the shoot stimulates lateral root primordia (LRP) development and outgrowth, and LRP initiation is also strongly influenced by local auxin biosynthesis in the root apex (Bhalerao et al., 2002). Accordingly, mutations in the *TAA1/TAR* gene family result in root-specific defects as well as ethylene insensitivity (Stepanova et al., 2008; Yamada et al., 2009), and *wei8 tar2* double mutants, mutated in both *TAA1* and *TAR2*, show a strong reduction in IAA levels, defects in gravitropism and vasculature development, reduced apical dominance and other auxin-related effects (Stepanova et al., 2008). Interactions between ethylene and

auxin biosynthesis have also been observed in other studies (Růzicka et al., 2007; Swarup et al., 2007). Furthermore, a reduction in the levels of other IAA precursors, such as anthranilate (ANT), has been shown to influence root development, as plants harbouring mutations in the *WEI2/ASA1* and *WEI7/ASB1* genes, which encode the anthranilate synthase  $\alpha$  and  $\beta$  subunits, respectively, are ethylene insensitive, have reduced auxin biosynthesis and show defects in root hair positioning (Ljung et al., 2005; Stepanova et al., 2005; Ikeda et al., 2009). Changes in the expression of the *YUCCA* genes also have an impact on auxin biosynthesis and root development (Stepanova et al., 2011; Won et al., 2011; Mashiguchi et al., 2011). Using a chemical genetic approach, He et al. (He et al., 2011) identified a compound [L-kynurenine (L-Kyn)] that inhibits TAA1/TAR activity, and treatment with L-Kyn caused effects that phenocopied those observed in plants harbouring loss-of-function mutations in *YUCCA* genes.

The influence of other plant hormones such as cytokinins (CKs) and abscisic acid (ABA) on auxin biosynthesis, and the roles of these interactions for normal plant growth and development as well as for different stress responses, have recently been demonstrated (Jones et al., 2010; Zhou et al., 2011; Lee et al., 2012). Very recently, the aminotransferase *VAS1* (*vas1* was identified as a *sav3/taal* suppressor) was identified as a key enzyme in the regulation of auxin and ethylene biosynthesis, converting IPyA and L-methionine to L-Trp and 2-oxo-4-methylthiobutyric acid, respectively (Zheng et al., 2013). This study shows that plants are able to coordinate the biosynthesis of these two important plant hormones in order to regulate growth responses such as shade avoidance.

#### Auxin metabolism in other organs and developmental pathways

IAA biosynthesis is also important for developmental processes in organs other than the root, and the *YUCCA* genes have been shown to play pivotal roles during, for example, *Arabidopsis* embryogenesis and leaf (Cheng et al., 2007; Wang et al., 2011), flower (Cheng et al., 2006), vascular (Cheng et al., 2006) and fruit (Eklund et al., 2010) development. In other species, such as rice, maize and potato, *YUCCA* genes are important for normal plant development and resistance to different stress conditions (Yamamoto et al., 2007; Gallavotti et al., 2008; Bernardi et al., 2012; Kim et al., 2012). In maize, the *YUCCA*-like gene *sparse inflorescence 1* (*spi1*) and the co-orthologue of *TAA1*, *vanishing tassel 2* (*vt2*), were shown to be important for both vegetative and reproductive development (Gallavotti et al., 2008; Phillips et al., 2011).

The PLETHORA (PLT) family of transcription factors has recently been suggested to play a role in the regulation of local IAA biosynthesis in the shoot apex, and PLT regulation of *YUC1* and *YUC4* expression was shown to influence phyllotactic patterns in *Arabidopsis* (Pinon et al., 2013).

It has recently been shown that light and sugar signalling interacts with IAA metabolism in *Arabidopsis*. IAA biosynthesis is strongly upregulated by soluble sugars (Sairanen et al., 2012), and this upregulation is mediated via the PHYTOCHROME INTERACTING FACTOR (PIF) transcriptional regulators and has an impact on hypocotyl elongation during early seedling growth and development (Lilley et al., 2012). Sugars have also been shown to regulate auxin biosynthesis in developing maize kernels (LeClere et al., 2010). The shade avoidance syndrome (SAS), which is a growth response to changes in the ratio of red

to far-red light, triggers a rapid increase in IAA biosynthesis via the *TAA1* pathway (Tao et al., 2008). This response also involves the *PIF4* and *PIF5* genes (Hornitschek et al., 2012). Other environmental factors can also influence IAA biosynthesis, and it was recently shown that *PIF*-mediated regulation of different IAA biosynthesis genes, such as *TAA1*, *CYP79B* and *YUC8*, is involved in temperature regulation of IAA biosynthesis (Franklin et al., 2011; Sun et al., 2012). The involvement of *PIF* genes in all these responses is intriguing, and suggests a common mechanism for the activation of IAA biosynthetic pathways under different conditions.

The regulation of IAA degradation has also been shown to impact various aspects of plant growth and development. Overexpression of the IAA amido synthetase genes *GH3.8* and *GH3.13* increases pathogen resistance and drought tolerance in rice, respectively (Ding et al., 2008; Zhang et al., 2009). In addition, an increase in the levels of IAA-aspartic acid and a subsequent decrease in IAA levels were observed during grape berry and tomato fruit ripening, correlating with elevated *GH3.1* expression levels (Böttcher et al., 2010). In *Arabidopsis*, a stress-responsive microRNA (miR167a) was shown to target the IAA amido hydrolase gene *IAA-ALANINE RESISTANT 3 (IAR3)*, and *IAR3* was also shown to be required for drought tolerance (Kinoshita et al., 2012).

## Conclusions

During the last ten years, our understanding of auxin metabolism and its role during plant growth and development has greatly improved. Nonetheless, there are still many gaps in our knowledge and we lack a deep understanding of these metabolic processes. There are probably many genes (and perhaps even entire metabolic pathways) that are either unknown or of poorly understood function, and our knowledge of auxin metabolism in species beyond *Arabidopsis* is very scarce. Besides thorough genetic and biochemical studies of the metabolic pathways involved, systems biology and modelling approaches will probably be crucial for deepening our understanding of auxin metabolism. The great challenge will be to integrate knowledge about auxin metabolism into the regulatory networks that act on different developmental processes operating in plants, and to understand how these processes work in different plant species under normal and stress conditions.

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## Competing interests statement

The author declares no competing financial interests.

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