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Auxin Metabolism in Plants

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The major natural auxin in plants, indole-3-acetic acid (IAA), orchestrates a plethora of developmental responses that largely depend on the formation of auxin concentration gradients within plant tissues. Together with inter- and intracellular transport, IAA metabolism—which comprises biosynthesis, conjugation, and degradation—modulates auxin gradients and is therefore critical for plant growth. It is now very well established that IAA is mainly produced from Trp and that the IPyA pathway is a major and universally conserved biosynthetic route in plants, while other redundant pathways operate in parallel. Recent findings have shown that metabolic inactivation of IAA is also redundantly performed by oxidation and conjugation processes. An exquisite spatiotemporal expression of the genes for auxin synthesis and inactivation have been shown to drive several plant developmental processes. Moreover, a group of transcription factors and epigenetic regulators controlling the expression of auxin metabolic genes have been identified in past years, which are illuminating the road to understanding the molecular mechanisms behind the coordinated responses of local auxin metabolism to specific cues. Besides transcriptional regulation, subcellular compartmentalization of the IAA metabolism and posttranslational modifications of the metabolic enzymes are emerging as important contributors to IAA homeostasis. In this review, we summarize the current knowledge on (1) the pathways for IAA biosynthesis and inactivation in plants, (2) the influence of spatiotemporally regulated IAA metabolism on auxin-mediated responses, and (3) the regulatory mechanisms that modulate IAA levels in response to external and internal cues during plant development.

The extensive search for the plant molecule responsible for tropic responses to light and gravity led to the identification of the first auxin molecule, indole-3-acetic acid (IAA), more than 80 years ago (Abel and Theologis 2010). Since then, it has been determined that auxin is not only involved in the control of tropisms, but also regulates numerous plant developmental responses that mainly rely on the spatiotemporal control of cell division, growth, and differentiation (Zhao 2018; Gallei et al. 2020). In addition to IAA, phenylacetic acid (PAA) and 4-chloro-indole-3-acetic acid (4-Cl-IAA) are naturally occurring auxins in plants. Although both are perceived by the auxin signaling machinery (Shimizu-Mitao and Kakimoto 2014; Jayasinghege et al. 2019), 4-Cl-IAA is not widespread (Lam et al. 2015) and PAA has been studied far less than IAA due to generally less potent effects (Cook 2019).

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Because plant responses to auxin concentrations are threshold-dependent, IAA levels must be finely regulated during plant growth in response to external and internal cues. Tuning of the IAA concentration within cells and tissues is largely performed by directional transport and localized biosynthesis (Brumos et al. 2018; Robert et al. 2018) as well as inactivation of IAA (Zheng et al. 2016; Di Mambro et al. 2019). A classical model for auxin gradient formation in plants is based on a primary synthesis of IAA in the shoot—mainly in the young leaves and cotyledons—which is then distributed throughout the plant by polar transport with a major rootward component. However, early reports already suggested a role for the root tip in auxin biosynthesis (van Raalte 1936; van Overbeek 1939; Davies and Mitchell 1972). Improvements in the sensitivity of analytical technologies that allowed quantification of IAA from minute amounts of plant tissue, along with the identification of newly synthesized auxin in these tissues using stable isotope labeling (Novák et al. 2012, 2017), have helped researchers establish that auxin biosynthesis occurs locally in different plant organs and, albeit at different rates, in every root cell type (Ljung et al. 2001a, 2005; Petersson et al. 2009). Together with advances in analytical methodologies, the availability of the *Arabidopsis* genome sequence boosted the identification of genes associated with auxin biosynthesis, conjugation, and degradation. The detailed genetic and biochemical evaluation of these genes and their regulatory networks has revealed the importance of an initially disregarded role of auxin metabolism in plant development.

AUXIN BIOSYNTHESIS IN PLANTS

Several decades of research on auxin metabolism have firmly established the aromatic amino acid L-tryptophan (Trp) as a central precursor for IAA biosynthesis in plants. Trp is produced in chloroplasts via the shikimate pathway, a route through which most living organisms—excluding animals—produce aromatic amino acids (Maeda and Dudareva 2012). Far from being linear, Trp-dependent auxin biosynthesis involves various parallel pathways converging at the production of IAA, being IAOx (indole-3-acetaldoxime), IAM (indole-3-acetamide) and IPyA (indole-3-pyruvic acid) the most common intermediates (Fig. 1). A Trp-independent pathway for auxin synthesis was proposed after finding that maize and *Arabidopsis* mutants defective in Trp biosynthesis were still producing IAA (Wright et al. 1991; Normanly et al. 1993). It was later suggested that a cytosolic indole synthase (INS) mediates Trp-independent IAA production via the conversion of indole-3-glycerol-phosphate to indole (Zhang et al. 2008; Wang et al. 2015). However, the biochemical pathway for the Trp-independent conversion of indole to IAA remains unclear (Nonhebel 2015). The following section will describe the Trp-dependent pathways for auxin synthesis.

The Main Pathway for IAA Biosynthesis

Whereas auxin production from IAOx and IAM is not yet fully understood, the IPyA pathway has been established as the prevailing route for IAA synthesis in plants. It consists of a two-step reaction in which Trp is first deaminated to IPyA by TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1 (TAA1) and TAA1-RELATED proteins (TARs) (Stepanova et al. 2008; Tao et al. 2008; Yamada et al. 2009). IPyA is then decarboxylated to IAA in a rate-limiting and irreversible reaction catalyzed by flavin-containing monooxygenases from the YUCCA (YUC) family (Mashiguchi et al. 2011; Stepanova et al. 2011; Won et al. 2011). *TAA1* and *YUC* homologs are found across the genomes of vascular and nonvascular plants (Yue et al. 2014; Eklund et al. 2015; Poulet and Kriechbaumer 2017; Matthes et al. 2019), and functional conservation of *TAA1* and *YUC* homologs has been shown in maize (Gallavotti et al. 2008; Phillips et al. 2011; Bernardi et al. 2012), rice (Yamamoto et al. 2007; Yoshikawa et al. 2014), and *Marchantia polymorpha* (Eklund et al. 2015). Taken together, this suggests that the IPyA pathway is a universal route for IAA synthesis in land plants. Compared to single mutants in *TAA1*/*TARs* and *YUCCA* genes, which show only subtle developmental

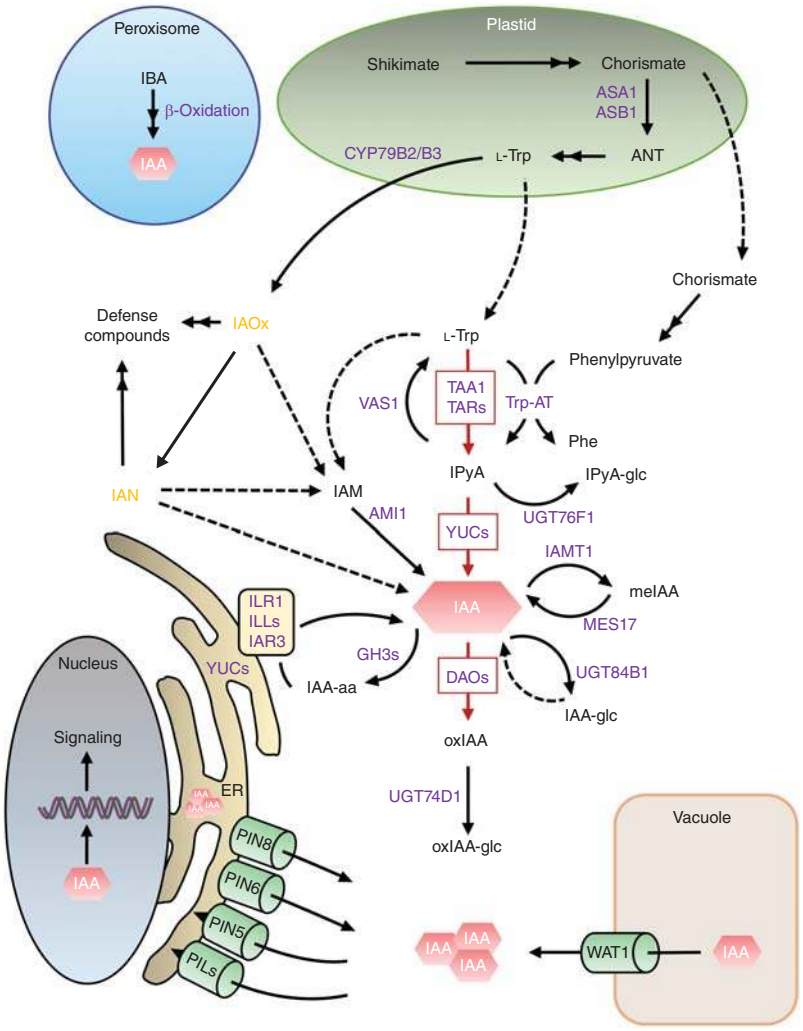

Figure 1. Main pathways for indole-3-acetic acid (IAA) metabolism in plants. Biosynthesis of the IAA precursor L-tryptophan (Trp) takes place in the plastid (green oval). Subsequent IAA biosynthesis, catabolism, and conjugation reactions commonly operate in the cytoplasm. IAA molecules are indicated as red hexagons and IAA-related metabolites are shown in black. Enzymes catalyzing the metabolic reactions are indicated in purple. Major components for IAA biosynthesis and inactivation are indicated with red arrows. Solid arrows indicate pathways in which the enzymes, genes, or intermediates are known, while dashed arrows indicate pathways that are not yet well defined. *Brassica*-specific metabolites are indicated in orange. Green cylinders indicate intracellular IAA transporters and the attached arrows indicate the IAA movement direction. The blue circle, the gray oval, and the pale orange square indicate peroxisome, nucleus, and vacuole, respectively. Endoplasmic reticulum (ER) is indicated by the dark yellow structure attached to the nucleus. Organelles are not drawn to scale. (AMI1) AMIDASE-LIKE PROTEIN 1, (ANT) anthranilate, (ASA1) ANTHRANILATE SYNTHASE α SUBUNIT 1, (ASB1) ANTHRANILATE SYNTHASE β SUBUNIT 1, (CYP79B2/B3) CYTOCHROME P450, family 79, subfamily B, polypeptides 2 and 3, (DAO) DIOXYGENASE FOR AUXIN OXIDATION, (GH3) GRETCHEN HAGEN3, (IAA-glc) IAA-glucose, (IAM) indole-3-acetamide, (IAMT1) indole-3-acetate *O*-methyltransferase 1, (IAN) indole-3-acetonitrile, (IAOx) indole-3-acetaldoxime, (IBA) indole-3-butyric acid, (IAR3) IAA-ALANINE RESISTANT3, (ILLs) ILR1-LIKE, (ILR1) IAA-LEUCINE RESISTANT1, (IPyA) indole-3-pyruvic acid, (meIAA) methylindole-3-acetic acid, (MES17) METHYLESTERASE 17, (oxIAA) 2-oxindole-3-acetic acid, (Phe) phenylalanine, (PILS) PIN-likes, (PIN) PIN-FORMED, (TAA1) TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1, (TAR) TRYPTOPHAN AMINOTRANSFERASE-RELATED PROTEIN, (Trp-AT) TRYPTOPHAN AMINOTRANSFERASE, (UGTs) URIDINE-DIPHOSPHATE GLYCOSYLTRANSFERASE, (VAS1) REVERSAL OF SAV3 PHENOTYPE 1, (WAT1) WALLS ARE THIN1, (YUC) YUCCA flavin-containing monooxygenases.

phenotypes, higher-order *taa1*/*tar* and *yuc* mutants that bypass functional redundancy result in marked reductions in IAA levels and severe developmental defects in *Arabidopsis*, including abnormal embryo patterning, reduced stature, aberrant vasculature, defective root growth and gravitropic response, abnormal apical hook formation, and altered leaf and floral patterning (Cheng et al. 2006, 2007; Stepanova et al. 2008; Chen et al. 2014). Loss-of-function of the maize *vt2* gene, a TAA1 ortholog, resulted in reduced plant growth and dramatic effects in inflorescence development, which entails a severe sterility of the mutant plants (Phillips et al. 2011). Similar reproductive defects were observed in the maize *spi1* mutants, impaired in the function of a monocot-specific YUC-like protein (Gallavotti et al. 2008). In the liverwort *Marchantia*, the knock-out of its single *TAA* gene resulted in severe growth and developmental defects caused by a loss of cell and tissue differentiation (Eklund et al. 2015). The severity of the above-mentioned phenotypes, along with the seedling lethality observed in the *Arabidopsis taa1 tar1 tar2* triple mutant (Stepanova et al. 2008), points to the IPyA pathway as a major and essential route for IAA biosynthesis in plants.

Parallel Pathways for IAA Biosynthesis

The Trp derivative IAOx is an intermediate in an IAA biosynthetic route that is yet to be fully understood. The conversion of Trp to IAOx is mediated by two isozymes from the cytochrome P450 (CYP) monooxygenase family, CYP79B2 and CYP79B3 (Hull et al. 2000; Mikkelsen et al. 2000; Zhao et al. 2002). Both IAOx and *CYP79B2/3* genes have so far only been found in *Brassica* species (Sugawara et al. 2009), which suggests that this pathway is restricted to the Brassicaceae family (Fig. 1). Moreover, the *cyp79b2 cyp79b3* double mutant shows conditional auxin phenotypes when grown at high temperatures (Zhao et al. 2002; Sugawara et al. 2009), further suggesting a role for the IAOx-dependent IAA synthesis specifically during adverse conditions. IAOx is a well-known precursor of indole glucosynolates (IGs) and camalexin, which serve as defense metabolites in plants (Hull et al. 2000; Glawischnig et al. 2004; Nafisi et al. 2007). Nevertheless, an increase in IAOx levels—through either genetic disruption of the IG pathway or overexpression of genes associated with IAOx biosynthesis—results in elevated IAA and high-auxin phenotypes (Boerjan et al. 1995; King et al. 1995; Delarue et al. 1998; Barlier et al. 2000; Zhao et al. 2002; Grubb et al. 2004; Sugawara et al. 2009; Novák et al. 2012; Kong et al. 2015). A study in which isotope-labeled IAOx was fed to *Arabidopsis* seedlings revealed that IAOx, IAM, and IAN (indole-3-acetonitrile) are intermediates of IAA biosynthesis (Sugawara et al. 2009). IAN-to-IAA conversion by a family of plant nitrilases (NITs) is thought to account for the IAOx-dependent auxin biosynthesis (Lehmann et al. 2017). However, the lack of direct genetic and biochemical evidence for NIT-mediated auxin synthesis *in planta*, together with the knowledge that nitrilases participate in cyanide and glutathione detoxification (Piotrowski 2008; Niehaus et al. 2019), means that the biochemical route from IAOx to IAA remains unresolved.

IAM is a well-known auxin biosynthesis intermediate in certain plant-associated bacteria (Patten et al. 2013) in which Trp is converted to IAA through the formation of IAM. While it was demonstrated that IAM can be produced from IAOx in *Arabidopsis* (Sugawara et al. 2009), IAM has also been detected in non-*Brassica* species that lack IAOx (Pollmann et al. 2002; Sugawara et al. 2009; Novák et al. 2012). IAM application results in classical high-auxin phenotypes (Sugawara et al. 2009; Gao et al. 2020), indicating that IAM-to-IAA conversion operates *in planta*. However, the disruption of the main IAM hydrolases in *Arabidopsis*, IAMH1, and IAMH2, did not lead to substantial developmental defects or variations in IAA contents, suggesting that the IAM pathway only plays a minor role in auxin homeostasis under standard growth conditions (Gao et al. 2020).

Indole-3-butyric acid (IBA) is a compound that has been shown to stimulate an auxin response when applied to plants. However, it is very unlikely (1) that IBA itself is perceived by

the plant (Strader and Bartel 2011; Uzunova et al. 2016), and (2) that it is transported via polar transport (Liu et al. 2012). Instead, the effects of IBA can be attributed to its conversion to IAA by a group of peroxisomal enzymes (Zolman et al. 2008). IBA-to-IAA conversion has been found to be relevant to plant development (Frick and Strader 2018). How plants synthesize IBA is still unknown and, as such, it is not clear whether IBA is an IAA precursor or storage molecule. Also, whether endogenous IBA is present at physiologically relevant concentrations in plants has been questioned (Novák et al. 2012) and is still under debate (Frick and Strader 2018).

AUXIN METABOLIC INACTIVATION

Together with directional transport and local biosynthesis, metabolic inactivation of IAA also modulates auxin concentrations across plant cells and tissues. Indeed, research has shown that the majority of plant IAA exists as (1) inactive conjugates and methyl ester forms that can be reversibly converted to IAA (auxin storage forms), to rapidly fine-tune auxin levels without the need for de novo synthesis; and (2) as irreversible inactive IAA (auxin catabolites), which is the result of the removal of excess auxin or a regulated response to create auxin minima. The most extensively studied inactive forms of auxin will be summarized in the following section.

IAA Storage Forms

Many different auxin storage forms have been identified in plants (Korasick et al. 2013). These forms fall into three main groups: ester-linked IAA conjugates, amide-linked IAA conjugates, and methyl IAA (meIAA). The most prevalent and abundant ester-linked auxin in plants is IAA-glucose (IAA-glc), which is present at higher levels than any other directly measured conjugate (Pěnčík et al. 2009, 2018; Brunoni et al. 2020). IAA-glc has been detected in seedling extracts from different plants (Kai et al. 2007), and is the predominant IAA metabolite throughout *Arabidopsis* tissues (Porco et al. 2016). IAA-glc and its metabolic derivative IAA-myo-inositol (IAA-Ins) are particularly abundant in plant seeds (Hall 1980; Cohen and Bandurski 1982), and are thought to be the main auxin source during early seedling establishment in vascular plants (Bartel et al. 2001; Ljung et al. 2001b). High-molecular-weight IAA-glycan and -glycoprotein conjugates have also been found in plants (Korasick et al. 2013), although their specific roles in auxin homeostasis are not yet understood. Hydrolases that release free IAA from IAA-glc and IAA-Ins were identified in maize kernels (Jakubowska and Kowalczyk 2005), and rice (TGW6; Ishimaru et al. 2013). Specific UDP-glycosyltransferases (UGTs) that produce IAA-glc have been identified in plants (Szerszen et al. 1994; Jackson et al. 2002; Ludwig-Müller et al. 2005; Liu et al. 2019). Overexpression of these *UGT*s results in increased levels of IAA-glc and reduced levels of amide-linked auxins (Jackson et al. 2002; Ludwig-Müller et al. 2005), indicating that IAA homeostasis was disturbed. The availability of knockout mutants for the known auxin UGTs, together with the discovery of additional IAA glycosylases and hydrolases, will help clarify the roles of ester-linked auxins throughout plant development.

Amide-linked auxins encompass a group of compounds in which IAA is conjugated to amino acids, small peptides and proteins, among which IAA-amino acid (IAA-aa) conjugates are the best characterized. The formation of the IAA-amide bond is catalyzed by a group of IAA acyl acid amido synthetases from the GRETCHEN HAGEN3 (GH3) family (Staswick et al. 2005; Ludwig-Müller et al. 2009). *GH3* genes (Terol et al. 2006; Okrent and Wildermuth 2011), along with different IAA-aa conjugates (Korasick et al. 2013; Záveská Drábková et al. 2015), are found all across land plants. *GH3* co-orthologs have been functionally characterized in vascular and nonvascular plants (Staswick et al. 2005; Ludwig-Müller et al. 2009; Brunoni et al. 2020). The application of various IAA-aa conjugates results in plant phenotypes that are similar to what can be observed upon the addition of exogenous IAA, which provides strong evidence that IAA-aa conjugates can serve as IAA storage forms (LeClere et al. 2002; Rampey et al. 2004). Several IAA-aa ami-

dohydrolases, including IAA-LEUCINE RESISTANT1 (ILR1), ILR1-LIKE proteins (ILLs), and IAA-ALANINE RESISTANT3 (IAR3), were identified in screens for mutants that are insensitive to IAA-aa conjugates (Bartel and Fink 1995; Davies et al. 1999; LeClere et al. 2002). These amidohydrolases appear to be functionally conserved across plant species. IAR3 was found to be required for root architectural changes under osmotic stress in *Arabidopsis* (Kinoshita et al. 2012), and to mediate defense responses of tomato and potato plants upon infection (D'Ippolito et al. 2016). GH3s are known to mediate different responses to biotic and abiotic stress (Park et al. 2007; Zhang et al. 2007, 2009; Ding et al. 2008; Du et al. 2012; Kirungu et al. 2019). A note of caution is due here since certain GH3 enzymes that function as IAA amido synthetases, as GH3.3, GH3.5, and GH3.6, present a promiscuous conjugating activity toward different substrates like salicylic acid, jasmonic acid, and benzoic acid (Zhang et al. 2007; Gutierrez et al. 2012; Westfall et al. 2016), at least in vitro. Hence, GH3-related phenotypes might be the result of the perturbation of the homeostasis of not just IAA, but also of other phytohormones.

The methyl ester of IAA, meIAA, also serves as an auxin storage form in plants. IAA methylation at the carboxyl group is mediated by IAA CARBOXYL METHYLTRANSFERASE1 (IAMT1) (Zubieta et al. 2003; Qin et al. 2005). As an inactive form of auxin, melAA does not interfere with the auxin signaling machinery (Li et al. 2008; Abbas et al. 2018). Nevertheless, the application of meIAA results in auxin-related phenotypes in plants (Qin et al. 2005), a dynamic that can be attributed to the hydrolysis of meIAA to IAA by METHYLESTERASE 17 (MES17) and related enzymes (Yang et al. 2008). Research using knockout *iamt1* mutants revealed that auxin methylation has little impact on auxin levels, both locally or in whole seedlings (Abbas et al. 2018; Takubo et al. 2020). meIAA is a nonpolar compound that is transported in plants by both passive influx and PIN-mediated efflux and, thus, affects IAA gradients rather than IAA levels (Li et al. 2008; Abbas et al. 2018). Specific expression of *IAMT1* in the hypocotyl endodermis was shown to be important for gravitropic growth (Abbas et al. 2018). The role of auxin methylation in regulating plant development is, however, still under debate (Takubo et al. 2020).

Irreversible IAA Catabolites

The amide-linked IAA-Asp and IAA-Glu conjugates, unlike other IAA-amino acid conjugates, are not hydrolyzed back to IAA *in planta* (Östin et al. 1998; Rampey et al. 2004) and are thus considered catabolites. These two irreversible conjugates are found in plants at much higher levels than the reversible IAA-aa conjugates (Kowalczyk and Sandberg 2001; Pěnčík et al. 2009). GH3 IAA-amido synthetases show different substrate preferences for Asp and Glu (Staswick et al. 2005; Brunoni et al. 2020). GH3.17, and to a lesser extent GH3.5, is known to preferentially use Glu as a cosubstrate (Staswick et al. 2005). Accordingly, *gh3.17* plants show remarkably reduced levels of IAA-Glu in their hypocotyls (Zheng et al. 2016) and roots (Di Mambro et al. 2017), while the *gh3.1,2,3,4,5,6* sextuple mutant does not produce any IAA-Asp, although IAA-Glu production is up-regulated, and is likely supported by the still functional GH3.17 (Porco et al. 2016).

The major catabolic pathway that regulates IAA levels in plants is the irreversible oxidation of IAA to oxIAA (2-oxindole-3-acetic acid), with further glycosylation to oxIAA-glc (Östin et al. 1998; Kai et al. 2007; Kubeš et al. 2012; Novák et al. 2012; Pěnčík et al. 2013). There is extensive evidence that the levels of these oxidative catabolites rapidly increase after IAA application (Östin et al. 1998; Kubeš et al. 2012). Moreover, they are prevalent at higher levels than amide-linked catabolites at physiological conditions in algae, as well as in vascular and nonvascular land plants, which suggests that oxidation is a major pathway for IAA catabolism across the plant kingdom (Novák et al. 2012; Pěnčík et al. 2013; Záveská Drábková et al. 2015; Porco et al. 2016; Žižková et al. 2017). Conifers were found to be an exception, as conjugation and not oxidation dominates IAA homeostasis (Brunoni et al. 2020). The conversion

of IAA to oxIAA is catalyzed by DIOXYGENASE FOR AUXIN OXIDATION (DAO) proteins (Zhao et al. 2013), which belong to the 2-oxoglutarate-dependent Fe(II) dioxygenase superfamily (Kawai et al. 2014; Nadi et al. 2018), while the UGT74D1 enzyme participates in the glycosylation of oxIAA to oxIAA-glc (Tanaka et al. 2014). *Arabidopsis DAO1* accounts for most de novo IAA oxidation and is widely expressed in plant tissues. However, *DAO1* loss-of-function results in only mild developmental defects. Because amide-linked catabolites are greatly increased in *dao1* mutants, while decreased in *DAO1* overexpressors, DAO and GH3 enzymes are proposed to function redundantly in regulating IAA levels (Mellor et al. 2016; Porco et al. 2016; Zhang et al. 2016). Despite this redundant IAA catabolism, multicellular modeling of auxin gradients has shown that impaired oxidation perturbs IAA levels in specific root tissues (Mellor et al. 2016), which is supported by the localized, albeit subtle, phenotypes of *dao1-1* mutant roots (Porco et al. 2016; Zhang et al. 2016).

LOCALIZED IAA METABOLISM COORDINATES PLANT DEVELOPMENT

The identification and characterization of several genes related to auxin metabolism has revealed an intricate spatiotemporal orchestration of their localized expression (exemplified in Fig. 2) that turned out to contribute to the regulation of local auxin concentrations. *YUCCA* genes represent an excellent example of specialization, as several rounds of gene duplication have resulted in multiple *YUCs* that have unique expression domains (Zhao 2018). For example, a group of shoot- and root-specific *YUCCAs* has been defined (Won et al. 2011; Chen et al. 2014). *YUC1* and *YUC4* are specifically expressed in the shoot apical meristem, along with flower and leaf primordia (Cheng et al. 2006, 2007). In the embryo, *YUC1*, *YUC4*, *YUC10*, and *YUC11* were found to be expressed in the apical cells, with each showing certain temporal changes in expression domain as embryo development progressed (Cheng et al. 2007). The quadruple *yuc1 yuc4 yuc10 yuc11* mutant was found to lack the hypophysis, a root meristem precursor cell, and thus germinated without a primary root (Cheng et al. 2007). Localized expression of *TAA1* in the developing embryo was also found to be critical for root and apical embryonic meristem specification (Stepanova et al. 2008; Robert et al. 2013). In roots, *TAA1* is specifically expressed at the quiescent center (QC), while *TAR2* expression was reported in the root provasculature (Stepanova et al. 2008). Disruption of both of these genes resulted in the complete loss of the stem cell niche and root growth abortion early after germination (Stepanova et al. 2008). This dynamic and localized expression, together with the developmental abnormalities noted for plants with loss-of-function mutations in the *TAA1*/*TAR* and *YUC* genes, supports a role for IPyA-dependent local auxin biosynthesis in embryo patterning, root meristem maintenance, gynoecium formation, and leaf and floral development (Cheng et al. 2006, 2007; Stepanova et al. 2008). Localized expression of *CYP79B2*/*B3* genes at the root meristem and lateral root primordia initiation sites additionally suggests that the IAOx pathway participates in local IAA synthesis during root development (Ljung et al. 2005).

Cooperation between local IAA biosynthesis and polar transport generates auxin concentration gradients that drive plant growth (Ikeda et al. 2009; Brumos et al. 2018). For example, the spatiotemporally coordinated expression of *TAA1* and *YUCs* in the basal and apical embryo, together with the resulting auxin-triggered PIN polarization, was shown to define the apicobasal embryo axis (Robert et al. 2013; Wabnik et al. 2013). However, auxin transport cannot always compensate for deficiencies in local synthesis. Proper root development largely depends on auxin production in the root (Bhalerao et al. 2002; Chen et al. 2014), and localized auxin biosynthesis at the root QC was found to be sufficient for root meristem maintenance in the absence of functional polar transport (Brumos et al. 2018). Recently, the capacity of the roots to regenerate their tips after wounding was shown to be highly dependent on local auxin biosynthesis mediated by TAA1 and YUCs near the cut site and in the protoxylem and

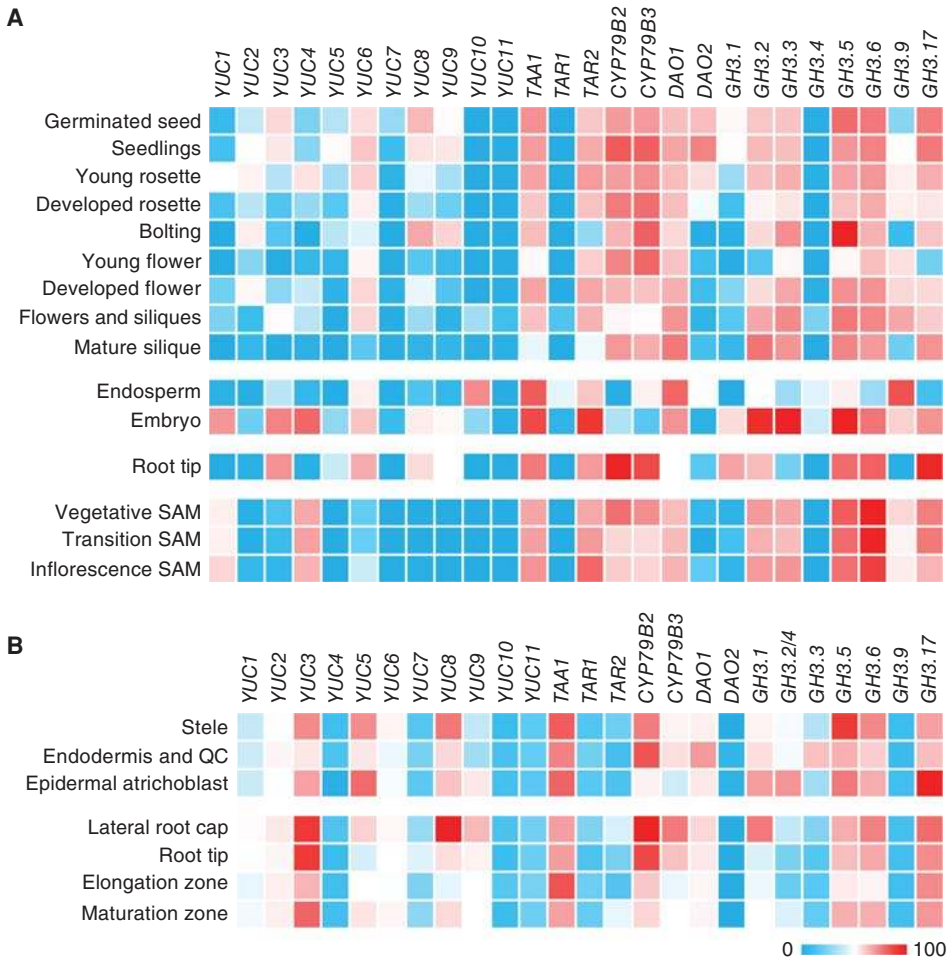

Figure 2. Relative expression level of the main genes for auxin metabolism in different developmental stages, organs, and tissues from *Arabidopsis*. Data was retrieved from Genevestigator (genevestigator.com; Hruz et al. 2008) using datasets from (*A*) RNA-seq experiments, and (*B*) Affymetrix *Arabidopsis* ATH1 Genome Array. Results are expressed in percentage of expression potential (the maximum expression a gene reaches across all experiments). *GH3.2* and *GH3.4* share array probes in the results shown in *B*. AGI codes: *YUC1* (At4G32540), *YUC2* (At4G13260), *YUC3* (At1G04610), *YUC4* (At5G11320), *YUC5* (At5G43890), *YUC6* (At5G25620), *YUC7* (At2G33230), *YUC8* (At4G28720), *YUC9* (At1G04180), *YUC10* (At1G48910), *YUC11* (At1G21430), *TAA1* (At1G70560), *TAR1* (At1G23320), *TAR2* (At4G24670), *CYP79B2* (At4G39950), *CYP79B3* (At2G22330), *DAO1* (At1G14130), *DAO2* (At1G14120), *GH3.1* (At2G14960), *GH3.2* (At4G37390), *GH3.3* (At2G23170), *GH3.4* (At1G59500), *GH3.5* (At4G27260), *GH3.6* (At5G54510), *GH3.9* (At2G47750), and *GH3.17* (At1G28130).

xylem-pole-pericycle, while PIN-mediated transport was found dispensable (Matosevich et al. 2020).

Localized auxin degradation was also found to play various roles in plant growth and development. The IAA-amido synthetase GH3.17 was shown to modulate hypocotyl elongation in response to shade and temperature independently of auxin transport and de novo biosynthesis (Zheng et al. 2016). *GH3.17*, *GH3.5*, and *GH3.6* are specifically expressed in the lateral root cap (Di Mambro et al. 2019; Pierdonati et al. 2019), with GH3.17—at the very least—participating in the IAA degradation required

to create an auxin minimum at the transition zone, which influences root meristem size (Di Mambro et al. 2019).

REGULATION OF AUXIN METABOLISM

Decades of intensive research have resulted in a deep understanding of the network of interconnected and redundant pathways involved in IAA synthesis and inactivation. To ensure plastic and coordinated plant development, these pathways need to be fine-tuned by an array of regulating mechanisms that control the activation/deactivation of various synthetic and catabolic routes. However, our understanding of the regulation of this complex homeostatic machinery is mainly based on research that has been conducted during the past decade. The regulatory mechanisms underlying IAA synthesis and inactivation include (1) metabolic regulation, (2) hormonal cross talk, (3) transcriptional regulation, (4) posttranslational modifications, and (5) subcellular compartmentalization.

Metabolic Regulation of IAA Metabolism

It has been well established that IAA biosynthesis is controlled by feedback inhibitory mechanisms dependent on IAA levels (Ljung et al. 2001a). Auxin perception and signaling was shown to cause the down-regulation of genes related to the IPyA biosynthetic pathway and, consequently, to decrease endogenous IAA levels in a feedback loop (Suzuki et al. 2015; Takato et al. 2017). Likewise, the impaired conversion of IAA-aa conjugates, IBA, and meIAA to free IAA has been shown to up-regulate the expression of genes associated with IPyA-dependent auxin synthesis (Spiess et al. 2014). Moreover, the disrupted oxidative degradation of auxin in *Arabidopsis dao1* mutant plants up-regulates not only redundant GH3-mediated degradation, but also de novo auxin biosynthesis, which is a rather counterintuitive way of returning to homeostasis after an increase in auxin (Mellor et al. 2016; Porco et al. 2016).

Chorismate, the terminal product of the shikimate pathway, is a common precursor in the synthesis of the aromatic amino acids Trp, tyrosine (Tyr), and phenylalanine (Phe) (Maeda and Dudareva 2012). While the Tyr and Phe biosynthetic routes share common intermediates, Trp synthesis proceeds independently. However, a recent investigation revealed metabolic interplay between the Phe and IAA biosynthesis pathways. Phenylpyruvate, an intermediate in cytosolic Phe synthesis, can also serve as an amino acceptor in the Trp-to-IPyA conversion by Trp amino transferases and, thus, modulates IAA biosynthesis in response to Phe fluctuations (Lynch et al. 2020). Such metabolic interplay might also play a role under specific stresses, such as wounding (Lynch et al. 2020).

A screening for suppressors of the impaired shade-avoidance response observed in the *TAA1* mutant *sav3-1* identified the aminotransferase VAS1, which converts IPyA back to Trp and, thus, limits IAA synthesis (Zheng et al. 2013). VAS1 uses the ethylene intermediate methionine as a preferred amino donor, which revealed a link in the metabolic control of auxin and ethylene biosynthesis (Zheng et al. 2013). VAS1-mediated regulation is specifically, but most likely not exclusively, required for shade-induced elongation of hypocotyl and petioles (Zheng et al. 2013) and for the control of parthenocarpy in Solanaceae species (Matsuo et al. 2020).

Additional metabolic control of the IPyA pathway relies on the glycosylation of IPyA to IPyA-glc by UGT76F1, which was recently identified as a fine-tuning mechanism in the modulation of IPyA availability for IAA biosynthesis during light- and temperature-induced hypocotyl elongation (Chen et al. 2020).

Hormonal Cross Talk Modulates IAA Metabolism

The conversion of chorismate to anthranilate, which represents the first, rate-limiting step in the Trp biosynthetic pathway, is catalyzed by the anthranilate synthase complex (Niyogi and Fink 1992). Previous research has identified anthranilate production as a central hub in the modulation of IAA biosynthesis by other hormonal pathways. A screen for mutants with altered responses to ethylene identified two mutants, *wei2* and *wei7*, which harbored mutations in genes

encoding anthranilate synthase α1 (ASA1) and β1 (ASB1) subunits, respectively (Stepanova et al. 2005). The *ASA1* promoter was later found to be a direct target of ETHYLENE RESPONSE FACTOR 1 (ERF1), which up-regulates *ASA1* expression in response to ethylene, resulting in increased auxin biosynthesis and root growth inhibition (Mao et al. 2016). ASA1 was also found to be at the core of jasmonate-auxin metabolic cross talk (Sun et al. 2009), in a mechanism that relies on the direct up-regulation of *ASA1* expression by the jasmonate-responsive ETHYLENE RESPONSE FACTOR 109 (ERF109) (Cai et al. 2014). ERF109 additionally binds to *YUC2* promoter to stimulate auxin biosynthesis in response to an increase in jasmonate levels (Cai et al. 2014). *YUC8* and *YUC9* might also be involved in jasmonate-regulated auxin biosynthesis, as their expression is promoted by methyl jasmonate (Hentrich et al. 2013).

The interplay between auxin and cytokinin has long been recognized as crucial to plant growth and development (Moubayidin et al. 2009). In *Arabidopsis*, cytokinin signal transduction was shown to modulate the rate of IAA biosynthesis (Jones et al. 2010). This metabolic cross talk involves the cytokinin-mediated up-regulation of *YUC1* and *YUC4* at the gynoecium primordia (Müller et al. 2017) and of *YUC8* in roots (Di et al. 2016). The cytokinin-response transcriptional effector ARABIDOPSIS RESPONSE REGULATOR 1 (ARR1) promotes auxin biosynthesis in the stem cell niche via the up-regulation of ASB1 (Moubayidin et al. 2013). While a direct molecular link for cytokinin-regulated auxin biosynthesis remains unknown, cytokinin was found to directly modulate IAA degradation. More specifically, ARR1 directly binds to *GH3.17* and activates its transcription in response to cytokinin, thus promoting auxin degradation (Di Mambro et al. 2017). This regulatory module was found to be a prerequisite for root meristem size determination (Di Mambro et al. 2017, 2019).

Transcriptional Regulation of IAA Metabolism

The coordinated response of auxin biosynthesis and inactivation to specific physiological and environmental cues is mediated by the direct modulation that specific transcription factors and components of the epigenetic machinery exert on the expression of auxin metabolic genes. Although little is known about the transcriptional control of auxin metabolism, several transcription factors and epigenetic regulators have been identified and associated to the control of auxin metabolism during specific plant developmental responses (see Tables 1 and 2).

Specific posttranslational histone modifications, also termed histone marks, are associated with an active or repressed transcriptional state. Whole-genome occupancy studies of the histone repressive mark H3K27me3 found that epigenetic mechanisms control auxin-related genes. In comparisons of dividing and differentiated cells, differential H3K27me3 modifications were observed at genes involved in auxin biosynthesis (*YUCs*, *CYPs*, *TAA1/TARs*, *SUR1*, *NITs*), inactivation (*GH3s*, *IAMT*), transport (*PINs*, *AUX/LAXs*), and signaling (*TIR1/AFBs*, *IAAs*, *ARFs*), thus revealing that this histone mark exerts a profound effect on auxin action (Lafos et al. 2011; He et al. 2012). *YUC1* and *YUC4*, for example, were found to be specifically involved in early auxin-mediated de novo root regeneration, in direct correlation with an H3K27me3 drop along their promoter regions (Chen et al. 2016). The Polycomb Repressive Complex 2 (PRC2) accessory protein LIKE HETEROCHROMATIN 1 (LHP1) (Derkacheva et al. 2013) are recruited to *YUC1*, *YUC2*, *YUC4*, *YUC5*, *YUC6*, *YUC8*, *YUC9*, and *YUC10* promoters to control their expression (Rizzardi et al. 2011). Beyond general correlations and whole-genome comparisons, specific mechanisms related not only to histone modifications —but also DNA methylation and small RNAs— have been shown to alter auxin homeostasis by regulating the transcription of *YUCs* (for review, see Mateo-Bonmatí et al. 2019).

During the first steps of flower determination—a process in which floral meristem cells stop proliferating and initiate a floral organ primordium—flower primordia formation requires auxin-driven rapid cell expansion and elongation. The required increase in auxin levels is, at least in part, achieved by the activation of

Table 1. Transcription factors that have been shown to directly modulate the expression of genes related to IAA metabolism

Target	Transcriptional regulator(s)	Structural type	Biological process(es)	References
YUC1	ARR1, ARR10, ARR12	B-ARR	Shoot stem cell niche maintenance	Meng et al. 2017
	SUP	C2H2-type zinc finger	Floral patterning	Xu et al. 2018
YUC4	LEC2	B3 domain	Embryogenesis	Stone et al. 2008
	SHI/STY1	RING-like zinc finger	Leaf and flower development	Eklund et al. 2010
	ARR1, ARR10, ARR12	B-ARR	Shoot stem cell niche maintenance	Meng et al. 2017
	LEC2, FUS3	B3 domain	Lateral root formation	Tang et al. 2017
	SUP	C2H2-type zinc finger	Floral patterning	Xu et al. 2018
	CRC	C2C2 type zinc finger	Floral determinacy	Yamaguchi et al. 2018
	AG	MADS	Floral determinacy	Yamaguchi et al. 2018
YUC5	IDD14, IDD15, IDD16	IDD	Organ morphogenesis, gravitropism	Cui et al. 2013
YUC8	PIF4	bHLH	Hypocotyl elongation at high temperature	Franklin et al. 2011
YUC9	EIN3	EIL	Aluminum-induced root growth inhibition	Liu et al. 2016b
TAA1	PIF4	bHLH	Hypocotyl elongation at high temperature	Franklin et al. 2011
	IDD14, IDD15, IDD16	IDD	Organ morphogenesis, gravitropism	Cui et al. 2013
	SPT	bHLH	Gynoecium development	Reyes-Olalde et al. 2017
	ARR1s	B-ARR	Light-induced tissue-specific IAA synthesis	Yan et al. 2017
CYP79B2	PIF4	bHLH	Hypocotyl elongation at high temperature	Franklin et al. 2011
UGT76F1	PIF4	bHLH	Hypocotyl elongation at high temperature	Chen et al. 2020
OsYUC8	OsEIL1	EIL	Ethylene-mediated primary root elongation	Qin et al. 2017
ZmGH3.2	ZmDREB2A	AP2/ERF	Longevity of maize seed	Han et al. 2020
GH3.2 *GH3.6*	MYB30	R2R3-MYB	Root growth	Zhao and Xue 2020

(Os) *Oryza sativa*, (Zm) *Zea mays*; otherwise *Arabidopsis thaliana*.

YUC4 by the chromatin-remodeling factors CHROMATIN REMODELLING 11 (CHR11) and CHR17, both of which are specifically recruited to the *YUC4* promoter during floral primordium formation (Yamaguchi et al. 2018). Later in floral organ development, the C2H2-type zinc-finger transcription factor *SUPERMAN* (*SUP*) actively represses *YUC1* and *YUC4* expression and, thereby, auxin biosynthesis at the boundaries between carpels and stamen primordia (Xu et al. 2018). *SUP*-mediated transcriptional silencing of *YUC1* and *YUC4* further involves the recruitment of members of the PRC2 machinery, such as CURLY LEAF (CLF) or LHP1, for the trimethylation of H3K27 (Xu et al. 2018).

Table 2. Epigenetic regulators that have been shown to directly modulate the expression of genes involved in IAA biosynthesis in a specific biological process

Target	Epigenetic regulator(s)	Biological process(es)	References
YUC1	LHP1	Floral patterning (−)	Xu et al. 2018
	CLF-PRC2		
YUC2	CMT3, DRM1, DRM2	Leaf development/growth (−)	Forgione et al. 2019
	LOCUS_77297	Ambient temperature (−)	Gyula et al. 2018
YUC4	LHP1	Floral patterning (−)	Xu et al. 2018
	CLF-PRC2		
	CHR11	Floral determinacy (+)	Yamaguchi et al. 2018
	CHR17		
YUC8	MRG2	Hypocotyl elongation (shade) (+)	Peng et al. 2018
	HDA9	Hypocotyl elongation (temperature) (+)	van der Woude et al. 2019
YUC9	ARP4	Hypocotyl elongation (shade) (−)	Lee and Seo 2017
YUC10	FIS2-PRC2	Endosperm development (−)	Figueiredo et al. 2015

(ARP4) ACTIN-RELATED PROTEIN 4, (CHR11) CHROMATIN REMODELLING 11, (CLF) CURLY LEAF, (CMT3) CHROMOMETHYLASE 3, (DRM1) DOMAINS REARRANGED 1, (FIS2) FERTILIZATION INDEPENDENT SEED 2, (HDA9) HISTONE DEACETYLASE 9, (LHP1) LIKE HETEROCHROMATIN 1, (MRG2) MORF-RELATED GENE 2, (PRC2) POLYCOMB REPRESSIVE COMPLEX 2. The (-) and (+) reflect transcriptional repression and activation of the target, respectively.

During angiosperm fertilization, auxin biosynthesis is constitutively repressed in maternal-derived tissues by the action of the FERTILIZATION-INDEPENDENT SEED-PRC2 (FIS-PRC2) complex (Figueiredo et al. 2015). Mutations in genes encoding subunits of this complex lead to premature expression of *YUC10* in the nonfertilized diploid central cell, which will result in the development of empty seeds. Recent reports indicate that additional mechanisms, driven by EMSY-like Tudor/Agenet H3K36me3 histone readers EMSY-Like protein 1 (EML1) and EML3, actively repress auxin biosynthesis, transport, and signaling during seed coat and endosperm development (Milutinovic et al. 2019).

Auxin integrates internal and external signals as sugar levels, shade, and temperature into regulated plant growth responses (Sairanen et al. 2012; for review, see Zhao 2018). A good model to exemplify such interaction is the auxin biosynthesis-driven hypocotyl elongation in response to shade or high temperature (Gray et al. 1998; Tao et al. 2008). In the hypocotyls of plants grown under normal light conditions, *YUC9* is actively repressed by the action of two AT-HOOK-CONTAINING NUCLEAR-LOCALIZED (AHL) proteins, AHL27 and AHL29 (Lee and Seo 2017). AHL29 recruits ACTIN-RELATED PROTEIN 4 (ARP4), a member of the SWI2/SNF2-RELATED1 (SWR1) chromatin-remodeling complex, to the *YUC9* regulatory region to promote the deposition of histone variant H2A.Z and, hence, block the access of RNApol II to the DNA (Lee and Seo 2017). Similar mechanisms for transcriptional regulation are driven by ARP6 under normal temperatures (Kumar and Wigge 2010). However, independent mechanisms trigger *YUC8* expression in plant hypocotyls to facilitate their elongation under shade or high temperature. The transcription factor PHYTOCHROME-INTERACTING FACTOR 7 (PIF7) and the H3K4me3/H3K36me3-binding protein Morf-Related Gene 2 (MRG2) bind to the *YUC8* promoter in response to shade, and facilitate its transcription by allowing the acetylation of H3 and H4 (Peng et al. 2018). At high temperature, HISTONE DEACETYLASE 9 (HDA9) accumulates, facilitating the H2A.Z removal from the *YUC8* locus and providing a looser chromatin environment that allows PIF4-mediated activation of *YUC8* transcription (van der Woude et al. 2019). High temperatures additionally promote auxin biosynthesis through the temperature-specific recruitment of PIF4 to the promoters of the IPyA glycosylase *UGT76F1* and the IAOx-pathway-related *CYP79B2* gene to repress

and promote their transcription, respectively, by unknown epigenetic mechanisms (Chen et al. 2020).

DNA methylation of cytosines is another important epigenetic mark mostly associated with gene repression that has also been shown to participate in auxin homeostasis. In animals, DNA methylation is almost entirely restricted to CG dinucleotides while plant DNA can additionally be methylated at CHG and CHH sequence contexts (with H representing A, T, or C) (Zhang et al. 2018; Gallego-Bartolomé 2020). The cytosine methylation is mediated by RNA-directed DNA methylation (RdDM), in which 24-nt small interfering RNA (siRNA) guides the DNA methyltransferases DOMAINS REARREANGED METHYLTRANSFERASE 1 (DRM1) and DRM2 to the target region (Zhang et al. 2018). Following DNA replication, cell-specific methylation patterns are maintained by another subset of DNA methyltransferases (METHYLTRANSFERASE 1 [MET1], CHROMOMETHYLASE 2 [CMT2], and CMT3) which, in contrast to DRMs, function in a context-specific manner, targeting CG, CHH, and CHG sequences, respectively (Zhang et al. 2018). Interestingly, several phenotypes that have been linked with auxin deficiency, such as root agravitropism, aberrant embryogenesis, and vascular disorders, were also observed in a triple *drm1 drm2 cmt3* mutant (Forgione et al. 2019). Tissue-specific expression analyses in *drm1 drm2 cmt3* seedlings revealed that the auxin biosynthetic genes *YUC2* and *TAA1* were specifically up-regulated in leaves. Me-DIP (methylated DNA immunoprecipitation)-PCR experiments further confirmed a reduction of non-GC DNA methylation at the *YUC2* promoter, thus linking RdDM with the regulation of auxin biosynthesis (Forgione et al. 2019). A search for thermoresponsive regulatory RNAs identified a novel temperature-regulated 24-nt siRNA, coined Locus_77297, in the vicinity of the *YUC2* promoter (Gyula et al. 2018). The expression of this siRNA in leaves was positively correlated with CHH methylation at the *YUC2* promoter. Plants grown at high temperatures showed severe reductions in both Locus_77297 expression and CHH methylation, which triggered the up-regulation of *YUC2* (Gyula et al. 2018).

Posttranslational Regulation of IAA Metabolism

After transcriptional control, additional regulatory mechanisms also govern protein function posttranslationally. Posttranslational modifications (PTMs) refer to covalent modifications that generally modulate protein folding or activity. There is recent evidence that auxin homeostasis is also modulated by PTMs. *Arabidopsis* TAA1 phosphorylation at the Thr101 was shown to be triggered by auxin perception itself, and to serve as an on/off switch controlling the activity of the enzyme, and therefore IAA biosynthesis (Wang et al. 2020). DAO1 auxin oxidase is known to be barely induced by IAA (Porco et al. 2016). Instead, experiments in rice demonstrated that DAO activity is regulated posttranslationally by substrate-mediated multimerization, which involves the IAA-triggered formation of DAO dimers that show increased affinity for IAA (Takehara et al. 2020). Interestingly, IAA-triggered TAA1 phosphorylation also enables this enzyme to dimerize with homologous TAR enzymes, a dynamic that likely regulates IAA biosynthesis through the control of several isoenzymes (Wang et al. 2020). Whether DAO dimerization also depends on IAA-triggered phosphorylation remains unexplored, and future research might identify a common mechanism for the posttranslational control of auxin metabolism.

Subcellular Compartmentalization of the IAA Metabolism

The cellular compartmentalization of bioactive IAA, IAA metabolites, and IAA metabolic enzymes represents yet another mechanism through which intracellular levels of IAA are regulated (Skalický et al. 2018). The first compartmentalization of IAA regards its biosynthesis, as the central precursor Trp is produced in chloroplasts, and the IAOx-pathway-related CYP79B2 and CYP79B3 contain a chloroplast transit peptide (Hull et al. 2000). Nevertheless,

the major routes for IAA biosynthesis and degradation are believed to take place in the cytosol, as the enzymes TAA1, YUC1, YUC2, YUC3, YUC6, YUC11, and DAO1 have been shown to share a cytoplasmic localization (Stepanova et al. 2008; Tao et al. 2008; Zhao et al. 2013; Kriechbaumer et al. 2016; Porco et al. 2016; Zhang et al. 2016). The IAA amido synthetase GH3.17 is also localized to the cytosol (Di Mambro et al. 2019). However, TAR2, YUC5, YUC7, YUC8, and YUC9 colocalize with ER-membrane markers (Kriechbaumer et al. 2016). *YUC4* is expressed as two tissue-specific splice variants, with one localized to the cytosol and the other localized to the cytosolic face of the endoplasmic reticulum (ER) membrane (Kriechbaumer et al. 2012). This suggests that part of IAA biosynthesis is compartmentalized to the ER. In line with this hypothesis, isolated ER microsomes were shown to significantly contribute to IPyA-dependent IAA biosynthesis (Kriechbaumer et al. 2015, 2016). Additionally, the IAA amidohydrolases ILR1, IAR3, and ILL2 have been shown to localize to the ER (Sanchez Carranza et al. 2016).

Together with the apparent subcellular localization of the IAA metabolism, active transport between organelles and the cytosol is a major factor determining cellular IAA homeostasis. The atypical members of the PIN family of auxin transporters PIN5, PIN6, and PIN8, together with PIN-LIKES proteins (PILS), have been detected at the ER (Mravec et al. 2009; Barbez et al. 2012; Dal Bosco et al. 2012; Ding et al. 2012; Simon et al. 2016). Genetic analyses on these PINs and PILS mutants and overexpressors suggest that ER-compartmentalization of IAA regulates auxin signaling by limiting the available cytosolic IAA that can enter to the nucleus (Mravec et al. 2009; Béziat et al. 2017; Feraru et al. 2019). Genetic manipulation of the ER transporters leads to altered levels of IAA as well as the IAA-Asp and IAA-Glu conjugates (Mravec et al. 2009; Dal Bosco et al. 2012; Ding et al. 2012; Simon et al. 2016), which further suggests that the compartmentalization of certain IAA metabolites is important to maintaining auxin homeostasis. Vacuole-associated auxin transporters that move IAA out of the vacuole have also been found (Ranocha et al. 2013; Liu et al. 2016a), pointing to a role of the vacuole in intracellular IAA regulation (Fig. 1). The presence of IAA precursors, as well as ox-IAA and IAA-glc, in *Arabidopsis* vacuoles suggests that this organelle is involved in at least part of IAA metabolism (Ranocha et al. 2013). Additionally, the IBA vacuolar transporter TOB1 was shown to move IBA from the cytoplasm to the vacuole, which provides further evidence that the vacuole participates in subcellular IAA metabolism (Michniewicz et al. 2019).

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Auxin is mainly produced in plants from Trp through the formation of IPyA by the action of plant-conserved members of the TAA and YUC enzyme families. The IPyA pathway is a major and essential pathway for auxin biosynthesis, while other parallels routes for auxin production turned out to have minor or environmentally restricted roles during plant development. Irreversible IAA degradation by the redundant action of DAO oxidases and GH3 amido synthetases additionally control auxin concentrations across plant tissues. Research on when and where these genes are expressed has revealed a strict requirement of localized auxin metabolism for regulated plant growth and development. Major breakthroughs during the past decade have shed light on the transcriptional control of auxin biosynthesis, as several transcription factors and epigenetic regulators have been described to control local auxin production during specific developmental processes in response to environmental and physiological cues (Tables 1 and 2). However, we are only starting to understand the molecular basis for such regulation. As such, the transcriptional control of genes associated with auxin inactivation remains largely unexplored. Whether the so-far-known regulatory mechanisms operate in a similar way across different tissues and/or developmental times needs further exploration. Moreover, when considering the number of transcription factors that have been reported to directly control auxin biosynthesis (Table 1), it

seems probable that many additional epigenetic mechanisms for the regulation of auxin metabolism will be elucidated in the coming years. The recently described posttranslational modulation of the activity of the auxin biosynthetic enzyme TAA1 (Wang et al. 2020) and the auxin oxidase DAO (Takehara et al. 2020) represents a thrilling starting point to explore similar mechanisms controlling the activity of other enzymes from the pathway.

Despite extensive efforts to unravel intracellular IAA dynamics to understand the role of organelles such as the ER and the vacuole on cellular auxin homeostasis, many critical questions remain unanswered. For example, the subcellular localization of several enzymes involved in IAA metabolism, notably, most of the GH3s, remains unexplored. Moreover, our understanding of how various IAA metabolites are distributed among different organelles, and how this compartmentalization influences intracellular auxin levels, transport, and signaling, is still at a rudimentary level. Fluorescent activated cell sorting has been employed to characterize the hormone distribution in different *Arabidopsis* root cell types (Petersson et al. 2009; Antoniadi et al. 2015). Sorting pure fractions of organelles by fluorescent activated organelle sorting (FAOS), a technique successfully used in mammalian cells (Gauthier et al. 2008), followed by high-resolution IAA metabolite profiling is expected to advance our knowledge regarding how the intracellular compartmentalization of different enzymes and metabolites influences auxin homeostasis (Novák et al. 2017; Skalický et al. 2018). Novel techniques, however, will be required to study the dynamic changes of subcellular IAA distribution and metabolism in response to different stimuli *in planta*. As most of the IAA is sensed in the nucleus, determination of IAA levels in nuclei during different responses to auxin in living plants, and the specific role of other cell compartments in modulating the available IAA to be sensed, remains an exciting challenge.

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