The pathway of auxin biosynthesis in plants

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Received 24 October 2011; Revised 27 February 2012; Accepted 1 March 2012

Abstract

The plant hormone auxin, which is predominantly represented by indole-3-acetic acid (IAA), is involved in the regulation of plant growth and development. Although IAA was the first plant hormone identified, the biosynthetic pathway at the genetic level has remained unclear. Two major pathways for IAA biosynthesis have been proposed: the tryptophan (Trp)-independent and Trp-dependent pathways. In Trp-dependent IAA biosynthesis, four pathways have been postulated in plants: (i) the indole-3-acetamide (IAM) pathway; (ii) the indole-3-pyruvic acid (IPA) pathway; (iii) the tryptamine (TAM) pathway; and (iv) the indole-3-acetaldoxime (IAOX) pathway. Although different plant species may have unique strategies and modifications to optimize their metabolic pathways, plants would be expected to share evolutionarily conserved core mechanisms for auxin biosynthesis because IAA is a fundamental substance in the plant life cycle. In this review, the genes now known to be involved in auxin biosynthesis are summarized and the major IAA biosynthetic pathway distributed widely in the plant kingdom is discussed on the basis of biochemical and molecular biological findings and bioinformatics studies. Based on evolutionarily conserved core mechanisms, it is thought that the pathway via IAM or IPA is the major route(s) to IAA in plants.

Key words: Auxin, auxin biosynthesis, IAA, indole-3-acetaldehyde, indole-3-acetaldoxime, indole-3-acetamide, indole-3-acetic acid, indole-3-pyruvic acid, plant hormone.

1. Introduction

In 1880, Charles Darwin proposed that some plant growth responses are regulated by ‘a matter which transmits its effects from one part of the plant to another’ (Darwin and Darwin, 1880). Then, half a century later, this substance termed auxin was identified as indole-3-acetic acid (IAA) (Kögl and Kostermans, 1934; Went and Thimann, 1937). This phytohormone auxin is a key regulator of many aspects of plant growth and development, including cell division and elongation, differentiation, tropisms, apical dominance, senescence, abscission, and flowering (Woodward and Bartel, 2005; Teale et al., 2006).

Although much progress has been made in defining IAA signalling pathways, the biosynthesis of IAA and its regulation by environmental and developmental signals remain poorly understood. De novo auxin biosynthesis plays an essential role in many developmental processes. Determining the molecular mechanisms of auxin biosynthesis may provide new tools for solving difficult plant development questions, defining the roles of auxin in plant development, understanding auxin transport, and studying the mechanisms of auxin in regulating plant development.

Auxin biosynthesis in plants is fairly complex. Multiple pathways have been postulated that contribute to de novo auxin biosynthesis (Fig. 1). Although different plant species may have unique strategies and modifications to optimize their metabolic pathways, it would seem reasonable that plants would share evolutionarily conserved core mechanisms for auxin biosynthesis because IAA is a fundamental substance in the plant life cycle.

In this review, the focus is on assessing the consensus pathway for auxin biosynthesis, and the genes and metabolites...
involved in auxin biosynthesis in the plant kingdom are summarized.

2. Multiple pathways postulated for auxin biosynthesis in plants

Two major pathways for IAA biosynthesis have been proposed in plants: the tryptophan (Trp)-independent and Trp-dependent pathways (Woodward and Bartel, 2005; Chandler, 2009; Normany, 2010; Zhao, 2010). In Trp-independent IAA biosynthesis, indole-3-glycerol phosphate or indole is the likely precursor, but little is known about the biochemical pathway to IAA (Ouyang et al., 2000; Zhang et al., 2008) (Fig. 1).

In Trp-dependent IAA biosynthesis, several pathways have been postulated (Woodward and Bartel, 2005; Pollmann et al., 2006a; Chandler, 2009; Mano et al., 2010; Normany, 2010; Zhao, 2010): (i) the indole-3-acetamide (IAM) pathway; (ii) the indole-3-pyruvic acid (IPA) pathway; (iii) the tryptamine (TAM) pathway; and (iv) the indole-3-acetaldoxime (IAOX) pathway (Fig. 1). Genes that have been suggested to participate in IAA biosynthesis in plants and their respective functions are summarized in Table 1. Although auxin IAA was the first plant hormone identified, little is known regarding the genetic basis of the key enzymes involved in the IAA biosynthetic pathway(s), and it is unclear whether all pathways exist in all plant species.
3. Tryptophan is synthesized in the chloroplast

Trp is synthesized from chorismate via indole-3-glycerol phosphate in the chloroplast (Radwanski and Last, 1995) (Fig. 1). The \textit{ASA}1 and \textit{ASA}2 genes of \textit{Arabidopsis thaliana} encode the \textalpha-subunit of anthranilate synthase, the enzyme catalysing the first reaction of the tryptophan biosynthetic pathway. Both predicted proteins have putative chloroplast transit peptides at their N-termini (Niyogi and Fink, 1992; Bohlmann et al., 1996). The \textit{IGS} gene of \textit{A. thaliana} encodes indole-3-glycerol phosphate synthase, which catalyses the conversion of 1-(\textO-carboxyphenylamino)-1-deoxy-ribulose-5-phosphate to indole-3-glycerol phosphate, a branch point compound in the Trp-independent IAA biosynthetic pathway (Li et al., 1995; Ouyang et al., 2000). The deduced \textit{Arabidopsis} IGS protein also has a putative chloroplast target sequence of \textasciitilde 80 amino acid residues at the N-terminus (Li et al., 1995).

Trp synthase is a typical heterotetrameric \textalpha_2\textbeta_2 complex consisting of Trp synthase \textalpha (\textit{TS}A1) and Trp synthase \textbeta (\textit{TS}B) subunits, encoded by \textit{TS}A1 and \textit{TS}B1, 2 genes, respectively. \textit{Arabidopsis thaliana} has two closely related, non-allelic tryptophan synthase \textbeta genes (\textit{TS}B1 and \textit{TS}B2), each containing a chloroplast target sequence at the N-terminus (Last et al., 1991). Trp is used to produce many indole-containing substances in plants, such as IAA, indole glucosinolates, phytoalexins, and TAM derivatives.

4. The indole-3-acetamide pathway

4.1. Studies on hairy roots led to the discovery of a new route in IAA biosynthesis

The IAA biosynthetic pathway via IAM was thought to be a bacteria-specific pathway because no evidence for this pathway had been found in plants. The plant pathogen \textit{Agrobacterium rhizogenes} harbours a large root-inducing (Ri) plasmid and generates hairy-root disease, which is a bacteria-specific pathway because no evidence for this pathway had been found in plants. The plant pathogen \textit{Agrobacterium rhizogenes} harbours a large root-inducing (Ri) plasmid and generates hairy-root disease, which is characterized by root proliferation from the infection site. A portion of the Ri plasmid, designated the T-DNA, is transferred to the host plant cell, integrated into the plant genome, and expressed in polyadenylated mRNA (Moore et al., 1979; White and Nester, 1980; Chilton et al., 1982). Hairy roots can grow aseptically in phytohormone-free media (Mano et al., 1986, 1989; Mano, 1993).

In hairy roots, IAA is synthesized from Trp by a two-step reaction as a result of the expression of the integrated genes \textit{aux1} (also referred to as \textit{iaaM1/m}t\textit{ms1}) and \textit{aux2} (also referred to as \textit{iaaH1/m}t\textit{ms2}) of Ri TR-DNA (Yamada et al., 1985; Camilleri and Jouanin, 1991; Gaudin et al., 1993; Casanova et al., 2005). The auxin biosynthetic pathway catalysed by the \textit{aux1} and \textit{aux2} genes product is similar to that in \textit{Agrobacterium tumefaciens} and \textit{Pseudomonas syringae} (Comai and Kosuge, 1982; Schroder et al., 1984; Thomasow et al., 1984; Yamada et al., 1985; Camilleri and Jouanin, 1991). \textit{Trp} is first converted to \textit{IAM} by the enzyme tryptophan-2-monoxygenase encoded by the \textit{aux11iaaM1/m}t\textit{ms1} gene (Yamada et al., 1985; Camilleri and Jouanin, 1991; Gaudin et al., 1993) (Fig. 1). Then, IAM is converted to IAA by indole-3-acetamide hydrolase encoded by the \textit{aux21iaaH1/m}t\textit{ms2} gene (Yamada et al., 1985; Camilleri and Jouanin, 1991; Gaudin et al., 1993; Nemoto et al., 2009a, b; Mano et al., 2010).

Tobacco (\textit{Nicotiana tabacum}) Bright Yellow-2 (BY-2) cells proliferate rapidly, exhibiting considerable homogeneity, like meristematic cells (Nagata et al., 1992), and requiring only auxin for culture. Thus, a transgenic BY-2 cell line transformed with the Ri plasmid is an excellent tool for the investigation of the role of auxin in the plant cell division that takes place in the meristem. Based on the finding that the overexpression of the \textit{aux1} gene of the Ri plasmid allowed BY-2 cells to grow rapidly in the absence of auxin (Nemoto et al., 2009a, c), the indole-3-acetamide hydrolase gene was recently isolated from \textit{Nicotiana} sp. and designated \textit{NtA}M\textit{I}1 (Nemoto et al., 2009b). The \textit{NtA}M\textit{I}1 gene consists of 1278 bp, encoding a putative protein of 425 amino acids with a calculated molecular weight of 45052 (GenBank accession number AB457638), and it is the same length as the \textit{AtA}M\textit{I}1 gene (GenBank accession number NM100769) isolated from \textit{A. thaliana} based on the sequence of the \textit{Agrobacterium aux2} gene (Pollmann et al., 2003) (Table 1).

4.2. The \textit{AtA}M\textit{I}1 and \textit{NtA}M\textit{I}1 genes encode indole-3-acetamide hydrolase, which functions in the cytoplasm

Expression vectors to produce the \textit{AtA}M\textit{I}1 or \textit{NtA}M\textit{I}1 fusion protein in \textit{Escherichia coli} were constructed to confirm that the gene products have the enzyme activity. Fusion proteins of \textit{AtA}M\textit{I}1 (Pollmann et al., 2003) and \textit{NtA}M\textit{I}1 (Nemoto et al., 2009b) have the enzyme activity converting IAM to IAA, indicating that these \textit{A}M\textit{I}1 genes encode indole-3-acetamide hydrolase (Fig. 1, Table 1). A specific serine residue, Ser137 of \textit{AtA}M\textit{I}1 and Ser136 of \textit{NtA}M\textit{I}1, is essential for \textit{A}M\textit{I}1 enzymatic activity (Neu et al., 2007; Lehmann et al., 2010; Mano et al., 2010). By experiments using fusion proteins of \textit{A}M\textit{I}1–green fluorescent protein (GFP), the \textit{A}M\textit{I}1 protein was confirmed to be located in the cytoplasm of plant cells (Pollmann et al., 2006b).

4.3. IAM, as a metabolic intermediate of auxin biosynthesis, is detected in every plant species

IAM has been detected in many plants including the fruits of \textit{Citrus unshiu} (Takahashi et al., 1975), the hypocotyls of Japanese cherry (Saotome et al., 1993), and aseptically grown squash seedlings (Rajagopal et al., 1994), tobacco (Lemcke et al., 2000), \textit{Arabidopsis} (Pollmann et al., 2002; Sugawara et al., 2009), maize, rice, and tobacco (Sugawara et al., 2009). The levels of IAM are \textasciitilde 10 ng g\textsuperscript{1} fresh weight (FW) in 2-week-old seedlings (Sugawara et al., 2009) or \textasciitilde 3.5 ng g\textsuperscript{1} FW in 2-week-old plants to \textasciitilde 130 pg g\textsuperscript{1} FW in fully grown rosettes (Pollmann et al., 2002) in \textit{Arabidopsis}. The levels of IAM are 156\textpm 60 pmol g\textsuperscript{1} FW in untransformed tobacco (Lemcke et al., 2000), \textasciitilde 3 ng g\textsuperscript{1} FW in 2-day-old coleoptiles of rice, \textasciitilde 11 ng g\textsuperscript{1} FW in 3-day-old coleoptile
tips of maize, and \(\sim 1\) ng g\(^{-1}\) FW in 2-month-old shoot apexes of tobacco (Sugawara et al., 2009).

Indole-3-acetamide hydrolase activity has been detected in rice cells (Kawaguchi et al., 1991; Arai et al., 2004) and in a crude extract from young fruits of Poncirus trifoliata (Kawaguchi et al., 1993). Cell-free extracts from various plant species, including Arabidopsis, cauliflower, maize, potato, sunflower, tobacco, tomato, and white mustard, can convert Trp into IAM, which is further converted to IAA (Pollmann et al., 2009). These findings indicate that IAM is incorporated into plant cells and converted into IAA by the AMI1 gene product.

4.4. IAM is incorporated into plant cells and converted to IAA by the AMI1 gene product

To examine which compound(s) is a precursor of IAA, BY-2 cells were cultured in LS solid medium containing Trp, IAM, indole-3-acetonitrile (IAN), and indole-3-acetaldehyde (IAD). These feeding experiments showed that BY-2 cells can grow in LS media containing \(10^{-8}\) M IAM or \(10^{-5}\) M IAA, but cannot grow in Trp-, IAN-, or IAD-containing media (Nemoto et al., 2009). It was confirmed by chemical analysis that the incorporated IAM was converted into IAA in BY-2 cells (K. Nemoto et al., unpublished data). IAM and IAD may not be efficiently incorporated into plant cells and/or may not be converted into IAA in plant cells.

Overexpression of the \(NtAMII\) gene allowed BY-2 cells to proliferate at lower concentrations of IAM, whereas RNA interference (RNAi)-mediated suppression of \(NtAMII\) severely inhibited plant cell division in IAM-containing medium. These \(in\) \(vivo\) experiments using transgenic plant cells show that IAM is incorporated into plant cells and converted into IAA by the \(AMII\) gene product, and also show that IAM itself does not possess auxin activity (Nemoto et al., 2009).

4.5. The AMI1 family is widely distributed in the plant kingdom

By combining a homology search with a phylogenetic tree analysis, it is possible to clarify relationships between sequences and to determine genetic distances. The nucleotide sequence for the \(NtAMII\) coding region was submitted to the DDBJ/GenBank/EMBL databases, and DNA alignment was determined by a FASTA search of all sequences, including expressed sequence tags (ESTs) (Mano et al., 2010). Phylogenetic analysis with the nucleotide sequences using ClustalW and the Neighbor-Joining (NJ) method showed that sequences homologous to the \(NtAMII\) gene

### Table 1. Plant genes thought to be involved in IAA biosynthesis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Orthologue</th>
<th>Gene product (localization)</th>
<th>Plant species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAM pathway</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMI1</td>
<td>AtAMI1</td>
<td>Indole-3-acetamide hydrolase (cytoplasm)</td>
<td>Arabidopsis thaliana</td>
<td>Pollmann et al. (2003); Neu et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>NtAMI1</td>
<td>Indole-3-acetamide hydrolase (cytoplasm)</td>
<td>Nicotiana tabacum</td>
<td>Nemoto et al. (2009b); Mano et al. (2013)</td>
</tr>
<tr>
<td>IPA pathway</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAA1</td>
<td>TAA1</td>
<td>Tryptophan aminotransferase (cytoplasm)</td>
<td>Arabidopsis thaliana</td>
<td>Stepanova et al. (2008); Tao et al. (2008)</td>
</tr>
<tr>
<td>TIR2</td>
<td>TIR2</td>
<td>Tryptophan aminotransferase (??)</td>
<td>Arabidopsis thaliana</td>
<td>Yamada et al. (2009)</td>
</tr>
<tr>
<td>TAR1</td>
<td>TAR1</td>
<td>Tryptophan aminotransferase (?)</td>
<td>Arabidopsis thaliana</td>
<td>Stepanova et al. (2008)</td>
</tr>
<tr>
<td>AAO1</td>
<td>zmAO-1</td>
<td>Aldehyde oxidase (cytoplasm)</td>
<td>Zea mays</td>
<td>Sekimoto et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>atAO-1</td>
<td>Aldehyde oxidase (cytoplasm)</td>
<td>Arabidopsis thaliana</td>
<td>Sekimoto et al. (1998)</td>
</tr>
<tr>
<td>YUC</td>
<td>AtYUC1,2,4,6</td>
<td>Flavin monoxygenase-like enzyme (?)</td>
<td>Arabidopsis thaliana</td>
<td>Mashiguchi et al. (2011); Stepanova et al. (2011); Won et al. (2011)</td>
</tr>
<tr>
<td>TAM pathway</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDC</td>
<td>TDC</td>
<td>Tryptophan decarboxylase (cytoplasm)</td>
<td>Catharanthus roseus</td>
<td>De Luca et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>TDC</td>
<td>Tryptophan decarboxylase (?)</td>
<td>Camptotheca acuminata</td>
<td>López-Meyes and Nessler (1997)</td>
</tr>
<tr>
<td></td>
<td>OpTDC</td>
<td>Tryptophan decarboxylase (?)</td>
<td>Ophiorhiza pumila</td>
<td>Yamazaki et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>TDC</td>
<td>Tryptophan decarboxylase (?)</td>
<td>Oryza sativa</td>
<td>Ueno et al. (2003)</td>
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<tr>
<td></td>
<td>TDC</td>
<td>Tryptophan decarboxylase (?)</td>
<td>Oryza sativa</td>
<td>Kang et al. (2007)</td>
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<td>YUC (YUCCA)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FZY</td>
<td>FZY</td>
<td>Flavin monoxygenase-like enzyme (?)</td>
<td>Arabidopsis thaliana</td>
<td>Zhao et al. (2001)</td>
</tr>
<tr>
<td>OsYUC1</td>
<td>OsYUC1</td>
<td>Flavin monoxygenase-like protein (?)</td>
<td>Petunia hybrida</td>
<td>Tobena-Santamaria et al. (2002)</td>
</tr>
<tr>
<td>sp1</td>
<td>sp1</td>
<td>YUCCA-like flavin monoxygenase (?)</td>
<td>Oryza sativa</td>
<td>Yamamoto et al. (2007)</td>
</tr>
<tr>
<td>SIFZY</td>
<td>SIFZY</td>
<td>No conversion of TAM to N-hydroxyTAM (?)</td>
<td>Solanum lycopersicum</td>
<td>Gallavotti et al. (2008)</td>
</tr>
<tr>
<td>ToFZY</td>
<td>ToFZY</td>
<td>YUCCA-like flavin monoxygenase (?)</td>
<td>Solanum lycopersicum</td>
<td>Exposito-Rodriguez et al. (2011)</td>
</tr>
<tr>
<td>PsYUC-like</td>
<td></td>
<td>No conversion of TAM to N-hydroxyTAM (?)</td>
<td>Pisum sativum</td>
<td>Tivendale et al. (2010)</td>
</tr>
<tr>
<td>OAO</td>
<td>OpTDC</td>
<td>Tryptophan decarboxylase (?)</td>
<td>Ophiorhiza pumila</td>
<td>Yamazaki et al. (2003)</td>
</tr>
</tbody>
</table>

(Amended)
can be classified into two major groups, AMI1 and Toc64, and also showed that the AMI1 gene is widespread in the plant kingdom in both monocots and dicots (Mano et al., 2010).

For this review, the amino acid sequence of the NtAMI1 protein was newly submitted to DDBJ/GenBank/EMBL, and amino acid sequence alignment was determined by a FASTA search of all sequences in the database (Protein Similarity Search of EMBL-EBI). After excluding redundancy caused by the same proteins having different accession numbers, a phylogenetic tree was newly constructed by ClustalW2 and the NJ method using the tools of the EMBL-EBI program (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Additionally, Arabidopsis lyrata and Oryza sativa subsp. indica were excluded because they are the allied species of A. thaliana and O. sativa subsp. japonica, respectively. The moss Physcomitrella patens was also excluded because moss is not a higher plant.

The phylogenetic tree shows that the AMI1 protein is evolutionarily different from the ‘translocon on the outer membrane of chloroplasts of the 64 kDa’ (Toc64) protein (Sohrt and Soll, 2000; Chew et al., 2004; Qbadou et al., 2007; Schlegel et al., 2007) (Fig. 2), which is an integral membrane protein of the outer envelope of chloroplasts and mitochondria that consists of an inactive amidase domain and three tetratricopeptide repeat (TPR) motifs at the C-terminal end (Sohrt and Soll, 2000; Lee et al., 2004; Schlegel et al., 2007; Kalanon and McFadden, 2008).

Plants belonging to the AMI1 family were tobacco, tomato, grape, poplar, and Arabidopsis in dicots, and maize, sorghum, rice, and wheat in monocots (Fig. 2). The phylogenetic tree also shows that the AMI1 protein is widespread in the plant kingdom (Mano et al., 2010) (Fig. 2). The IAM pathway can be a consensus pathway for auxin biosynthesis.

4.6. How is IAM synthesized in plants?

IAM is present in many plants, as described above. Cell-free extracts from various plant species can convert Trp into IAM, which is further converted to IAA (Pollmann et al., 2009). Bioinformatics studies also suggest that the IAA biosynthetic pathway via IAM is widespread in the plant kingdom (Mano et al., 2010) (Fig. 2).

So, how is IAM synthesized? Two possibilities are (i) that IAM is synthesized via indole-3-glycerol phosphate in the Trp-independent pathway; and (ii) that IAM is synthesized via Trp by the tryptophan-2-monooxygenase encoded by the gene homologous to the bacterial aux1 gene. The search for sequences homologous to the aux1 gene, however, has
Table 2. Abbreviations used in the phylogenetic tree

<table>
<thead>
<tr>
<th>Abbreviation in EMBL-EBI</th>
<th>Scientific name</th>
<th>Common name</th>
<th>Abbreviation in EMBL-EBI</th>
<th>Scientific name</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEGTA</td>
<td>Aegilops tauschii</td>
<td>Tausch's goatgrass</td>
<td>9LILI</td>
<td>Triglochin maritima</td>
<td>Seaside arrowgrass</td>
</tr>
<tr>
<td>ALLCE</td>
<td>Allium cepa</td>
<td>Onion</td>
<td>LOTJA</td>
<td>Lotus japonicus</td>
<td>Miyakogusa</td>
</tr>
<tr>
<td>ALLCG</td>
<td>Allium cepa var. aggregatum</td>
<td>Shallot</td>
<td>MAIZE</td>
<td>Zea mays</td>
<td>Maize</td>
</tr>
<tr>
<td>ALLSA</td>
<td>Allium sativum</td>
<td>Garlic</td>
<td>MANES</td>
<td>Manihot esculenta</td>
<td>Cassava</td>
</tr>
<tr>
<td>ALLTU</td>
<td>Allium tuberosum</td>
<td>Garlic chives</td>
<td>ORYSA</td>
<td>Orzyza sativa</td>
<td>Rice</td>
</tr>
<tr>
<td>ARAHY</td>
<td>Arachis hypogaea</td>
<td>Peanut</td>
<td>ORYSJ</td>
<td>Orzyza sativa subsp. japonica</td>
<td>Rice</td>
</tr>
<tr>
<td>ARATH</td>
<td>Arabidopsis thaliana</td>
<td>Mouse-ear cress</td>
<td>PAPSO</td>
<td>Papaver somniferum</td>
<td>Opium poppy</td>
</tr>
<tr>
<td>BRACM</td>
<td>Brassica campestris</td>
<td>Field mustard</td>
<td>PEA</td>
<td>Pisum sativum</td>
<td>Garden pea</td>
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<tr>
<td>BRANA</td>
<td>Brassica napus</td>
<td>Rape</td>
<td>PETCR</td>
<td>Petroselinum crispuum</td>
<td>Parsley</td>
</tr>
<tr>
<td>BRAOA</td>
<td>Brassica oleracea var. alboglabra</td>
<td>Chinese kale</td>
<td>PETHY</td>
<td>Petunia hybridis</td>
<td>Petunia</td>
</tr>
<tr>
<td>BRAOB</td>
<td>Brassica oleracea var. botrytis</td>
<td>Cauliflower</td>
<td>POPTR</td>
<td>Populus trichocarpa</td>
<td>Western balsam poplar</td>
</tr>
<tr>
<td>BRAOL</td>
<td>Brassica oleracea</td>
<td>Wild cabbage</td>
<td>RAUVE</td>
<td>Rauvolfia verticillata</td>
<td>Common devil-pepper</td>
</tr>
<tr>
<td>BRARC</td>
<td>Brassica rapa subsp. chinensis</td>
<td>Pak-choi</td>
<td>RICCO</td>
<td>Ricinus communis</td>
<td>Castor bean</td>
</tr>
<tr>
<td>BRARP</td>
<td>Brassica rapa subsp. pekinensis</td>
<td>Chinese cabbage</td>
<td>ROSDA</td>
<td>Rosa damascena</td>
<td>Damask rose</td>
</tr>
<tr>
<td>CAMAC</td>
<td>Campthotheca acuminata</td>
<td>Happy tree</td>
<td>ROSHC</td>
<td>Rosa hybrid cultivar</td>
<td></td>
</tr>
<tr>
<td>CAPAN</td>
<td>Capsicum annuum</td>
<td>Bell pepper</td>
<td>SINAL</td>
<td>Sinapis alba</td>
<td>White mustard</td>
</tr>
<tr>
<td>CATRO</td>
<td>Catharanthus roseus</td>
<td>Madagascar periwinkle</td>
<td>SOLLCC</td>
<td>Solanum lycopersicum</td>
<td>Tomato</td>
</tr>
<tr>
<td>CIMRA</td>
<td>Cimicifuga racemosa</td>
<td>Black cohosh</td>
<td>SORBI</td>
<td>Sorghum bicolor</td>
<td>Sorghum</td>
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<tr>
<td>CITSI</td>
<td>Citrus sinensis</td>
<td>Sweet orange</td>
<td>SOYBEY</td>
<td>Glycine max</td>
<td>Soybean</td>
</tr>
<tr>
<td>9GENT</td>
<td>Ophiopogon prostrata</td>
<td></td>
<td>THEHA</td>
<td>Thellungiella halophila</td>
<td>Salt cress</td>
</tr>
<tr>
<td>HORSP</td>
<td>Hordeum spontaneum</td>
<td>Wild Barley</td>
<td>THLFG</td>
<td>Thalictrum flavum subsp. glaucum</td>
<td>Yellow meadow rue</td>
</tr>
<tr>
<td>HORVD</td>
<td>Hordeum vulgare var. distichum</td>
<td>Two-rowed barley</td>
<td>TOBAC</td>
<td>Nicotiana tabacum</td>
<td>Common tobacco</td>
</tr>
<tr>
<td>HORVU</td>
<td>Hordeum vulgare</td>
<td>barley</td>
<td>VITVI</td>
<td>Vitis vinifera</td>
<td>Grape</td>
</tr>
<tr>
<td>LACSA</td>
<td>Lactuca sativa</td>
<td>Garden lettuce</td>
<td>WHEAT</td>
<td>Triticum aestivum</td>
<td>Wheat</td>
</tr>
</tbody>
</table>

* The red letters show monocotyledonous plants and the black letters show dicotyledonous plants.

not found a candidate. The plant indole-3-acetamide hydrolase group is phylogenetically distantly related to the bacterial indole-3-acetamide hydrolase group (Mano et al., 2010). In the case of tryptophan-2-monoxygenase, the plant enzyme may be too divergent from the bacterial counterparts to be recognizable, based on the homology search. It would be desirable to isolate genes related to IAM synthesis in plants, the gene encoding tryptophan-2-monoxygenase, or novel genes in a Trp-independent pathway.

5. The indole-3-pyruvic acid pathway

5.1. The TAA1 gene encodes an alliinase-related protein with Trp aminotransferase activity functioning in the cytoplasm

The *TAA1* (*TRYPHTOPHAN AMINOTRANSFERASE of ARABIDOPSIS*) gene, which encodes an aminotransferase that converts Trp to IPA (Fig. 1), was isolated by two groups based on the characterization of mutants that were defective in shade avoidance (Tao et al., 2008) and in ethylene responses (Stepanova et al., 2008) (Table 1). Mutations in *TAA1* alone lead to a dramatic reduction in free IAA levels, suggesting that IPA-dependent IAA biosynthesis is an important pathway for the biosynthesis of free IAA. Within 1 h after transferring *Arabidopsis* seedlings from white light to shade, the levels of free IAA increased in the wild type due to an increase in the rate of IAA biosynthesis; in contrast, IAA levels were reduced in mutant seedlings lacking the TAA1 protein, and there was no significant change in IAA levels in response to shade (Tao et al., 2008). These findings indicate that *TAA1* is required for the rapid increase in auxin levels through de novo IAA biosynthesis upon exposure to shade.

Overexpression of *TAA1* under the control of the cauliflower mosaic virus (CaMV) 35S promoter does not cause auxin overproduction phenotypes and also does not enhance hypocotyl elongation in the shade, suggesting that TAA1 is unlikely to be a rate-limiting enzyme in auxin biosynthesis (Stepanova et al., 2008; Tao et al., 2008). The TAA1 protein was confirmed to be located in the cytoplasm of plant cells by experiments using transgenic lines overexpressing TAA1–yellow fluorescent protein (YFP) and TAA1–GFP fusion proteins (Stepanova et al., 2008; Tao et al., 2008).

5.2. The *TIR2* gene is identical to the *TAA1* gene

Molecular characterization revealed that the *TIR2* (*TRANSPORT INHIBITOR RESPONSE 2*) gene was identical to the *TAA1* gene (Yamada et al., 2009) (Fig. 1, Table 1). The *tir2* mutant, which is resistant to the auxin transport inhibitor N-1-naphthylphthalamic acid (NPA), has a short hypocotyl. This phenotype can be rescued by IPA and IAA, suggesting that *TIR2* may be involved in the auxin biosynthetic pathway. Overexpression of *TIR2* does
not result in growth defects and the plants display normal sensitivity to exogenous Trp, suggesting that increasing endogenous IPA levels does not result in the synthesis of more IAA (Yamada et al., 2009).

5.3. 
TAA1 protein, which belongs to the superfamily of the α class of pyridoxal-5'-phosphate (PLP)-dependent enzymes, possesses Trp aminotransferase activity that is stimulated by the presence of PLP in the reaction mixture. Examination of substrate specificity showed that the purified TAA1 protein uses L-Trp, but not D-Trp, as a substrate and also uses L-Phe, Tyr, Leu, Ala, Met, and Gln as substrates. In vitro production of IPA by the TAA1 protein was confirmed using liquid chromatography–mass spectrometry (LC/MS) (Tao et al., 2008). Purified recombinant glutathione S-transferase (GST)–TAA1 protein also showed aminotransferase activity, and IPA is confirmed as the main product of the GST–TAA1 enzymatic activity by HPLC–MS ion chromatogram and ion mass spectrum (Stepanova et al., 2008).

5.4. Four genes closely related to TAA1 in the Arabidopsis genome

The genes which are closely related to TAA1 in the Arabidopsis genome have been found and referred to as ‘TRYPTOPHAN AMINOTRANSFERASE RELATED 1 to 4’ (TAR1–TAR4). The TAR1 gene is expressed in seedlings at very low levels (~500 times lower than TAA1). A whole-gene translational fusion of TAR1 with GFP failed to reveal GFP fluorescence in etiolated transgenic seedlings (Stepanova et al., 2008).

5.5. Protein structures of TAA1/TIR2 and TAR1–TAR4

The TAA1/TIR2 gene is one of a five-member gene family in Arabidopsis: TAA1 and TAR1–TAR4 genes (Stepanova et al., 2008). TAA1/TIR2 protein does not contain the N-terminal extension, consistent with the finding that the TAA1 protein is localized to the cytoplasm (Stepanova et al., 2008; Tao et al., 2008). TAR1 protein also does not contain the N-terminal extension (Stepanova et al., 2008).

On the other hand, TAR2–TAR4 proteins contain an N-terminal extension, predicted to be a signal peptide, suggesting that these proteins may function in the vacuole, similar to the onion or garlic alliinases (Stepanova et al., 2008). These findings show that the function of TAR2–TAR4 proteins is different from that of TAA1/TIR2 protein.

5.6. TAA1/TIR2 has only a small family in the plant kingdom

A phylogenetic tree was constructed by ClustalW2 and the NJ method using the tools of the EMBL-EBI program (http://www.ebi.ac.uk/Tools/msa/clustalw2/) by submitting the amino acid sequence of TAA1 protein, as described in the previous section. The phylogenetic tree shows that TAA1/TIR2 has only a small family in the plant kingdom (Fig. 3, Table 2). TAR1 (accession number Q9LR29) may be a paralogue of TAA1/TIR2. The TAA1/TIR2 protein (391 amino acids), having Trp aminotransferase activity (EC 2.6.1.27), is different from an alliin lyase (EC 4.4.1.4); such as TAR2 (accession number Q94A02, 440 amino acids). Interestingly, Thellungiella halophila (salt cress; accession number E4MXR3) is a close relative of A. thaliana; analysis of ESTs reveals 90–95% nucleotide identity between Arabidopsis and Thellungiella in transcripts for well-known housekeeping genes (Zhu, 2001; Taji et al., 2010). Thus, TAA1/TIR2 may be an Arabidopsis-specific gene.

The vanishing tassel2 (vt2) gene has been isolated from maize, but the enzymatic activity of the vt2 gene product is unknown (Phillips et al., 2011). Although these authors suggested that vt2 encodes a co-orthologue of the TAA1/TAR1/TAR2 genes of Arabidopsis, based on positional cloning and phylogenetic analyses, the vt2 gene product (accession number F2FB37, 530 amino acids) contains an N-terminal signal peptide, like the TAR2–TAR4 proteins that function in the vacuole. Accession number F2FB37 was not included in the best-scoring candidates by a FASTA search submitting TAA1 amino acid sequence; thus, the vt2 protein appears different from TAA1/TIR2.

5.7. It is not known whether IPA is converted into IAD in plants

In bacteria, two pathways for IAA biosynthesis are widespread: the IAM pathway (Trp→IAM→IAA) and the IPA pathway (Trp→IPA→IAD→IAA). Most phytopathogens, such as A. rhizogenes, A. tumefaciens, and P. syringae pv. savastanoi, use the IAM pathway to synthesize IAA, as described above (Schroder et al., 1984; Thomashow et al., 1984; Yamada et al., 1985; Gaudin and Jouanin, 1995; Casanova et al., 2005), whereas the IPA pathway is found in plant growth-promoting rhizobacteria species, including Azospirillum brasiliense, Enterobacter cloacae, and Pseudomonas putida (Koga et al., 1991; Costacurta et al., 1994; Patten and Glick, 2002).

In the bacterial IPA pathway, the precursor Trp is converted to IPA by Trp aminotransferase, and IPA is then converted to indole-3-acetaldehyde (IAD) by indole-3-pyruvate decarboxylase. IAA is produced after oxidation of IAD by indole-3-acetaldehyde oxidase. However, it is unknown whether IPA is converted into IAD in plants, as neither the gene nor the enzyme has yet been isolated (Fig. 1). Is the IPA pathway widespread in the plant kingdom?

There are few reports concerning the metabolic intermediates of the IPA pathway. IPA has been only detected in Arabidopsis seedlings; levels varied from 4 ng g⁻¹ to 13 ng g⁻¹ (Tam and Normanly, 1998). IAD has only been detected in pea (Pisum sativum); ~20 ng g⁻¹ FW in roots, 4 ng g⁻¹ FW in the apical bud, 1.2 ng g⁻¹ FW in the leaf, and 0.7 ng g⁻¹ FW in the internode (Quittenden et al., 2009).
5.8. The aldehyde oxidases have a broad substrate specificity

Aldehyde oxidases have been identified in cucumber (Bower et al., 1978), maize (Koshiba and Matsuyama, 1993; Koshiba et al., 1996), and Arabidopsis (Seo et al., 1998). Higher activity of an aldehyde oxidase has also been measured in the auxin-overproducing superroot1 (sur1) mutant of A. thaliana. Arabidopsis has three isoforms (AO1–AO3), and one of them (AO1) shows a higher substrate preference for indole-3-aldehyde and abscisic aldehyde (Seo et al., 1998). The indole-3-acetaldehyde oxidase activity has also been detected in the AO1 sample (Seo et al., 1998).

Generally, aldehyde oxidases are characterized by broad substrate specificity and play an important role in many developmental processes as well as in a variety of abiotic and biotic stress responses (Mendel and Hansch, 2002; Mauch-Mani and Mauch, 2005; Mendel and Bittner, 2006; Verslues and Bray, 2006). It seems likely that plant aldehyde oxidases are also involved in several metabolic reactions as well as the conversions of abscisic aldehyde to abscisic acid, indole-3-acetaldehyde to IAA, and benzaldehyde to benzoic acid, as detected in maize aldehyde oxidase (Koshiba et al., 1996).

Corresponding cDNAs have been isolated from maize (zmAO-1 and zmAO-2) and Arabidopsis (atAO-1, -2, -3, and -4) (Sekimoto et al., 1997, 1998) (Table 1). It has been suggested that the product of the AAO3 gene (formerly called atAO-3) is abscisic aldehyde oxidase (Seo et al., 2000a, b). Three cDNAs encoding aldehyde oxidase proteins in P. sativum (PsAO1, PsAO2, and PsAO3) have been isolated based on an RT-PCR (reverse transcription-polymerase chain reaction) strategy, although the enzymatic activity of these gene products is unknown (Zdunek-Zastocka, 2008).

Abscisic aldehyde is the native precursor of the plant hormone abscisic acid, which is involved in many aspects of plant growth and development, including adaptation to a variety of environmental stresses (Mauch-Mani and Mauch, 2005; Mendel and Bittner, 2006; Verslues and Bray, 2006). Aldehyde oxidases in plants are essential for many physiological processes that require the involvement of abscisic acid and perhaps also of auxins. It is necessary to determine which gene encodes indole-3-acetaldehyde oxidase.

5.9. The aldehyde oxidase family

Aldehyde oxidases (EC 1.2.3.1) are a group of structurally conserved cytosolic proteins, represented in both the animal and plant kingdoms, and they constitute a subfamily of molybdo-flavoenzymes. These enzymes require a molybdopterin cofactor and flavin adenine dinucleotide for catalytic activity, and they catalyse the hydroxylation of N-heterocycles and the oxidation of aldehydes to the corresponding acid (Mendel, 2007, 2011).

A phylogenetic tree shows that plant aldehyde oxidases belong to a multigene family (Fig. 4, Table 2). These
enzymes may possess a broad substrate specificity; thus, it is not easy to estimate which group(s) may be involved in the conversion of IAD to IAA by analysing relationships between sequences.

6. The tryptamine pathway

6.1. The TDC gene encodes Trp decarboxylase which is widespread in the plant kingdom

Tryptophan decarboxylase (TDC; EC 4.1.1.28) is a cytosolic enzyme that converts Trp to TAM (Fig. 1), which is a protoalkaloid in an early step of the terpenoid indole alkaloid biosynthetic pathway (Di Fiore et al., 2002). TDC is well characterized at the molecular and biochemical level, and TDC genes have been isolated from Catharanthus roseus (De Luca et al., 1989), Camptotheca acuminata (Lopez-Meyer and Nessler, 1997), Ophiorrhiza pumila (Yamazaki et al., 2003), and rice (Ueno et al., 2003; Kang et al., 2007) (Table 1).

A phylogenetic tree was constructed by ClustalW2 and the NJ method using the tools of the EMBL-EBI program (http://www.ebi.ac.uk/Tools/msa/clustalw2/) by submitting the amino acid sequence of TDC protein of C. roseus, as described above (Fig. 5, Table 2). Plants belonging to the TDC group were Apocynaceae (C. roseus and Rauvolfia verticillata), Solanaceae (Capsicum annuum), Cornaceae (Camptotheca acuminata), Rubiaceae (Ophiorrhiza pumila and Ophiorrhiza prostrata), and Ranunculaceae (Cimicifuga racemosa) in dicots, and rice, wheat, and barley in monocots. The phylogenetic tree shows that the TDC protein is widespread in the plant kingdom (Fig. 5).

6.2. Tryptamine is regarded as the intermediate in indole alkaloid and serotonin biosynthesis

These TDC genes have been functionally characterized to participate in indole alkaloid and serotonin biosynthesis (Fig. 1). Transgenic tobacco plants overexpressing the TDC gene of C. roseus accumulated very high levels of TAM, whereas IAA levels were unaffected (Songstad et al., 1990). Transgenic rice plants overexpressing the TDC gene showed a normal phenotype and contained 25-fold and 11-fold higher serotonin in the leaves and seeds, respectively, than did wild-type plants (Kang et al., 2007).

TDC activity in rice plants may be implicated in the production of TAM-derived metabolites, resulting in sekiguchi lesions or serotonin derivatives. Rice sekiguchi lesion (sl) mutants accumulate TAM, whereas IAA levels were unaffected (Songstad et al., 1990). Transgenic rice plants overexpressing the TDC gene showed a normal phenotype and contained 25-fold and 11-fold higher serotonin in the leaves and seeds, respectively, than did wild-type plants (Kang et al., 2007).

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6.3. The YUCCA gene family

It has also been proposed that the TAM pathway is one of the IAA biosynthetic pathways (Fig. 1). The YUCCA gene, which encodes a flavin monoxygenase-like enzyme that appears to oxidize TAM to N-hydroxytryptamine, has been isolated from Arabidopsis thaliana (Zhao et al., 2001). Orthologous genes of YUCCA have been found in other plants, including...
petunia (FZY) (Tobena-Santamaria et al., 2002), rice (OsYUCCA1–OsYUCCA7) (Yamamoto et al., 2007), maize (spin1) (Gallavotti et al., 2008), pea (PsYUC-like) (Tivendale et al., 2010), and tomato (ToFZY) (Exposito-Rodriguez et al., 2011) (Table 1).

A phylogenetic tree shows that YUCCA proteins belong to a multigene family (Fig. 6, Table 2). YUC1 is a member of an Arabidopsis flavin monooxygenase-like protein clade that includes 11 members (YUC1–YUC11), a subset of which appears to have overlapping functions (Zhao et al., 2001; Cheng et al., 2006, 2007). The enzymes Arabidopsis AtYUC1 and AtYUC6, tomato ToFZY1, and maize ZmYUC have been reported to catalyse the conversion of TAM to N-hydroxytryptamine (Zhao et al., 2001; LeClere et al., 2010; Exposito-Rodriguez et al., 2011).

6.4. A phantom compound: N-hydroxytryptamine

Transgenic rice plants overexpressing OsYUCCA1 (= OsYUC1) exhibit increased IAA levels and characteristic auxin overproduction phenotypes, whereas plants expressing antisense OsYUC1 cDNA display defects that are similar to those of rice auxin-insensitive mutants (Yamamoto et al., 2007). Also, FZY overexpression results in increased IAA levels and in an auxin overproduction phenotype in transgenic petunia (Tobena-Santamaria et al., 2002). Based on these results, it has been suggested that the TAM pathway to IAA is widespread in both dicots and monocots.

On the other hand, it has recently been reported that YUC (YUCCA) may not catalyse the conversion of TAM to N-hydroxytryptamine (Tivendale et al., 2010; Ross et al., 2011). Tivendale et al. indicated that there were major inconsistencies between the mass spectra reported for the reaction products, and indicated that the conclusions of Zhao et al. (2001), which were based on in vitro assays followed by mass spectrometry or HPLC analyses, need to be confirmed (Tivendale et al., 2010). They also point out that Zhao et al. made no firmer statement than that their mass spectra were consistent with N-hydroxytryptamine.

The compound N-hydroxytryptamine is relatively unknown, with no report of its presence in plants to date. Based on the mass spectral data for authentic N-hydroxytryptamine, 5-hydroxytryptamine (serotonin), and TAM, Tivendale et al. (2010) concluded that at least some of the published mass spectral data for the YUC in vitro product are inconsistent with N-hydroxytryptamine and that there is now a significant possibility that the product obtained by Zhao et al. was not N-hydroxytryptamine (Tivendale et al., 2010). Zhao (2010) also has noted that further investigations are needed to determine whether TAM is the in vivo substrate for YUCs because flavin monooxygenases are known to have broad substrate specificities in vitro.

6.5. Re-examining the function of YUCCA genes

It has been thought that the function of YUCCA genes should be re-examined, because there are some inconsistencies in the data. TAM is present in barley (Hordeum vulgare) (Schneider and Wightman, 1974), tomato (Solanum lycopersicum) (Cooney and Nonhebel, 1991), rice (O. sativa) (Ishihara et al., 2008), pea (P. sativum) (Quittenden et al., 2009), and Arabidopsis (Sugawara et al., 2009). However, N-hydroxytryptamine was not detected in pea roots, and 14C-labelled TAM, incorporated rapidly in pea roots, was...
not converted to $[^{14}C]N$-hydroxytryptamine (Quittenden et al., 2009). $[^{14}C]$-labelled TAM was converted to $[^{14}C]N$-acetyltryptamine in pea roots, which is not involved in IAA biosynthesis (Quittenden et al., 2009). TAM is not metabolized to IAA in pea seeds, although a $PsYUC$-like gene is strongly expressed in these organs (Tivendale et al., 2010). Can YUCCA convert TAM to $N$-hydroxytryptamine?

Based on the data showing that the developmental defects of $yuc1yuc4$ and $yuc1yuc2yuc6$ in Arabidopsis were rescued by tissue-specific expression of the bacterial auxin biosynthesis gene $iaaM$ but not by application of exogenous auxin, Cheng et al. (2006) have suggested that spatially and temporally regulated auxin biosynthesis by the $YUC$ genes is essential for the formation of floral organs and vascular tissues. Note that the bacterial $iaaM$ gene, which is the same as the $aux1/ims1$ gene, encodes the enzyme tryptophan-2-monooxygenase, which converts Trp to IAM (Huffman et al., 1984; Thomashow et al., 1984; Offringa et al., 1986; Nemoto et al., 2009; Mano et al., 2010) but does not convert TAM to $N$-hydroxytryptamine. The substance synthesized by the $iaaM$ gene product is IAM; thus, the interpretation by Cheng et al. (2006) that loss-of-function $yuc$ mutants can be rescued by the $iaaM$ gene does not seem to make sense.

Combining these findings, there is currently insufficient evidence to consider $N$-hydroxytryptamine as an intermediate in IAA biosynthesis, and the YUCCA function and the TAM pathway to IAA remain poorly understood.

7. The indole-3-acetaldoxime pathway

7.1. Cytochrome P450 enzymes CYP79B2 and CYP79B3 convert Trp to indole-3-acetaldoxime

IAOX is synthesized from Trp by two homologous cytochrome P450 enzymes, CYP79B2 and CYP79B3 (Fig. 1), which contain a chloroplast transit peptide at the N-terminus. Both enzymes are predicted to be targeted to the chloroplast (Hull and Celenza, 2000; Hull et al., 2000; Mikkelsen et al., 2000). These enzymes for the formation of IAOX have only been conclusively demonstrated in Arabidopsis (Hull et al., 2000; Mikkelsen et al., 2000) and Brassica (Kindl, 1968; Ludwig-Müller and Hilgenberg, 1988).
7.2. The CYP79B family is restricted to only Brassicaceae species

Two Arabidopsis genes, CYP79B2 and CYP79B3, have been isolated and characterized (Hull and Celenza, 2000; Hull et al., 2000; Mikkelsen et al., 2000) (Table 1). The CYP79B gene family has only been identified in Arabidopsis, Brassica napus, and Sinapis alba (Bak et al., 1998). A phylogenetic tree shows that the CYP79B protein family (= Trp-specific P450 enzymes) is also restricted to only Brassicaceae species (Fig. 7, Table 2), indicating that IAOX-dependent IAA biosynthesis is not a common pathway in plants.

7.3. IAOX is found only in the Brassicaceae

IAOX was first isolated from Brassica oleracea (Kindl, 1968) and identified by mass spectrometry from extracts of Brassica campestris (Ludwig-Müller and Hilgenberg, 1988), and has been recently found in Arabidopsis (Sugawara et al., 2009). The level of IAOX in Arabidopsis plants was ~1.7 ng g\(^{-1}\) FW by liquid chromatography–electrospray ionization-mass/mass spectrometry (LC-ESI-MS/MS) analysis. However, IAOX has not been found in tomato (Cooney and Nonhebel, 1991) or pea (Quittenden et al., 2009). Similarly, IAOX has not been detected in rice, maize, or tobacco (Sugawara et al., 2009). Although tobacco plants do not have detectable levels of IAOX (limit of detection ~10 pg g\(^{-1}\) FW), transgenic tobacco plants expressing either the CYP79B2 or CYP79B3 genes from Arabidopsis under the CaMV 35S promoter accumulate a large amount of IAOX in the range of 1–9 mg g\(^{-1}\) FW (Nonhebel et al., 2011). This biochemical evidence that IAOX is not found in plants other than Brassicaceae also indicates that IAOX-dependent IAA biosynthesis is not a common but rather a species-specific pathway in plants (Sugawara et al., 2009).

7.4. IAOX is a metabolic intermediate in indole glucosinolate and camalexin biosynthesis

IAOX is well known as an intermediate in the synthesis of plant secondary metabolites, such as indole glucosinolates, the alkaloid camalexin, and IAN, in Brassicaceae species including Arabidopsis (Hansen and Halkier, 2005; de Vos et al., 2008; Mikkelsen et al., 2009) (Fig. 1). The level of IAOX in wild-type Arabidopsis is several orders of magnitude lower than the amounts of the end-products camalexin or indole glucosinolates, as well as IAN. It is also significantly lower than that of IAM and IAA in Arabidopsis (Sugawara et al., 2009).

IAOX is channelled into indole glucosinolate biosynthesis by the cytochrome P450 CYP83B1 (Barlier et al., 2000; Bak et al., 2001) and into camalexin biosynthesis by the cytochrome P450 CYP71A13 (Nafisi et al., 2007; Mikkelsen et al., 2009) (Fig. 1). The cytochrome P450 CYP71B15 is essential for the final step in camalexin biosynthesis (Schuhegger et al., 2006; Bottcher et al., 2009). Camalexin is a characteristic phytoalexin of Arabidopsis and is synthesized only under inducing conditions such as infection by plant pathogens. Thus, IAOX is presumed to be funnelled into IAN and camalexin under inducing conditions (Glawischnig, 2007) (Fig. 1).

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Fig. 7. Phylogenetic analysis of the deduced amino acid sequences of CYP79B2 proteins. The amino acid sequence of the Arabidopsis CYP79B2 protein was submitted to DDBJ/GenBank/EMBL, and a phylogenetic tree was constructed, as described in the legend of Fig. 2. The scale bar indicates 0.1 substitution per amino acid.
7.5. CYP79B is involved primarily in indole glucosinolate and camalexin biosynthesis

CYP79B2 is induced in response to infection by pathogens (Hull et al., 2000), is wound inducible, and is expressed in leaves, stem, flowers, and roots, with the highest expression in roots (Mikkelsen et al., 2000). The CYP79B2 expression is adapted for camalexin formation, while the main function of CYP79B3 is in indole glucosinolate biosynthesis (Glawischnig, 2007). Double knock-out Arabidopsis mutants of CYP79B2 and CYP79B3 genes do not have any detectable indole glucosinolates or camalexin, and also have drastically reduced levels of IAN (Zhao et al., 2002; Glawischnig et al., 2004), whereas the IAA content of the mutants is almost the same as in the wild-type strain under normal conditions (Zhao et al., 2002). On the other hand, the transgenic Arabidopsis lines overexpressing the CYP79B2 gene have significantly elevated levels of indole glucosinolates and IAN (Mikkelsen et al., 2000; Zhao et al., 2002), although the overexpressors show a normal content of IAA (Zhao et al., 2002). These findings indicate that CYP79B is involved in indole glucosinolate and camalexin biosynthesis (Mikkelsen et al., 2000).

7.6. IAN is directly formed by dehydration of IAOX catalysed by CYP71A13

IAN has also been proposed as an intermediate in IAA biosynthesis via indole glucosinolate metabolism. It was thought that IAN was an enzymatic breakdown product of indole glucosinolate, induced upon tissue damage (Halkier and Gershenzon, 2006). However, Nafisi et al. (2007) demonstrated that (i) CYP71A13 enzyme converted IAOX to IAN in vitro; (ii) exogenously supplied IAN restored camalexin production in Arabidopsis CYP71A13-defective mutants; and (iii) expression of CYP79B2 and CYP71A13 genes in Nicotiana benthamiana, which does not normally produce IAOX or IAN, resulted in the conversion of Trp to IAN. Based on these results, the authors concluded that CYP71A13 catalyses the conversion of IAOX to IAN in camalexin biosynthesis in Brassicaceae species (Nafisi et al., 2007). IAN is subsequently converted to camalexin by the cytochrome P450 CYP71B15 in Brassicaceae species (Bottcher et al., 2009) (Fig. 1). Thus, the IAA biosynthesis pathway via IAOX and IAN is somewhat unclear even in the Brassicaceae species.

7.7. IAOX and IAN in the plant kingdom: ‘To be or not to be: that is the question.’

As described above, IAOX is not present in plants other than indole glucosinolate-producing plants such as Brassicaceae species, indicating that IAOX-dependent IAA biosynthesis is not a common pathway in plants. Although the exact mechanism(s) by which IAOX is converted to IAA remains unclear, IAN and/or IAD have been suggested as potential intermediates for IAA biosynthesis (Barlier et al., 2000; Bak et al., 2001). Based on early reports of the cell-free conversion of IAOX to IAA via IAD in extracts of Brassica (Mahadevan, 1963; Rajagopal and Larsen, 1972; Helmlinger et al., 1987), IAOX has long been a subject of interest as a possible precursor of IAD. However, no enzyme or gene has yet been identified in the IAOX→IAA pathway. Thus, the IAOX→IAA pathway has been left out of Fig. 1.

IAN has not been detected in rice, maize, tobacco, or pea (Quittenden et al., 2009; Sugawara et al., 2009), although it is present at the level of 9720 ng g⁻¹ FW in Arabidopsis seedling extracts (Sugawara et al., 2009). IAN has been recognized as a phytoalexin, an inducible metabolite involved in defence responses against fungal attack, in Brassica juncea (Pedras et al., 2002). There is no evidence of the conversion of [³H]IAOX to [³H]IAN in feeding experiments using pea (Quittenden et al., 2009). These findings indicate that IAN is not a common intermediate in IAA biosynthesis in plants.

7.8. Arabidopsis nitrilases are involved primarily in the conversion to carboxylic acids of nitriles derived from indole glucosinolates

It has been thought that plant nitrilases convert IAN to IAA. Although not widely recognized, this view has changed considerably in recent years (Piotrowski, 2008). The conclusion is that it seems unlikely that plant nitrilases participate in the conversion of IAN to IAA.

Nitrilase genes (ArNIT1–ArNIT4) have been isolated from Arabidopsis (Bartling et al., 1992, 1994; Bartel and Fink, 1994; Hillebrand et al., 1996, 1998) (Fig. 1, Table 1). ArNIT1, 2, and 3 gene products were thought to participate in the conversion of IAN to IAA (Bartling et al., 1992; Bartel and Fink, 1994). However, these three nitrilases have been found to have a strong substrate preference towards phenylpropionitrile, allyleyamide, phenylthio acetonitrile, and methylthio acetonitrile (Vorwerk et al., 2001). IAN hydrolysis by these nitrilases in vitro is inefficient. The preferred substrates are either naturally occurring substrates, which may originate from glucosinolate breakdown, or close relatives of these. Thus, a major function of AtNIT1, AtNIT2, and AtNIT3, appears to be the conversion to carboxylic acids of nitriles derived from indole glucosinolate turnover or degradation (Vorwerk et al., 2001).

The ArNIT4 gene product hydrolyses β-cyanoalanine to aspartic acid and ammonia (Piotrowski et al., 2001; Janowitz et al., 2009). β-Cyanoalanine is synthesized as an intermediate during cyanide detoxification and is also produced as a defensive compound against herbivory (Ressler et al., 1997; Janowitz et al., 2009). AtNIT4 may represent an important detoxification mechanism in A. thaliana, hydrolysing β-cyanoalanine to non-toxic products (Howden et al., 2009).

7.9. Maize nitrilases may function in the detoxification of β-cyanoalanine in vivo

Although IAOX and IAN have not been detected in rice, maize, or tobacco (Sugawara et al., 2009), an in vitro activity of the ZmNIT2 gene product that converts IAN to IAA has been reported (Park et al., 2003; Kriechbaumer...
Maize has two nitrilase genes, \textit{ZmNIT1} and \textit{ZmNIT2} (Park et al., 2003) (Table 1). Expression vectors to produce the \textit{ZmNIT1} or \textit{ZmNIT2} fusion proteins in \textit{E. coli} were constructed to confirm that the gene products had the enzyme activity \textit{in vitro}. \textit{ZmNIT1} did not show the activity toward the tested nitrile compounds including IAN (Park et al., 2003), but only hydrolysed \(\beta\)-cyanoalanine (Kriechbaumer et al., 2007). \textit{ZmNIT2} showed high activity toward IAN, 3-phenylpropionitrile, allylcyanide, methylthioacetonitrile, and 4-phenylbutyronitrile, which was hydrolysed most rapidly (Park et al., 2003).

\textit{ZmNIT1/ZmNIT2} heteromers have been shown to participate in cyanide detoxification via \(\beta\)-cyanoalanine turnover, although \textit{ZmNIT2} homomers can hydrolyse IAN to IAA \textit{in vitro} (Kriechbaumer et al., 2007). \textit{ZmNIT1} and \textit{ZmNIT2} share 75\% sequence identity at the amino acid level, and \textit{ZmNIT2} shows relatively high homology to \textit{Arabidopsis AtNIT4} (Park et al., 2003). A phylogenetic tree showed that \textit{ZmNIT1} and \textit{ZmNIT2} are nearer to \textit{AtNIT4} than to the group of \textit{AtNIT1/AtNIT2/AtNIT3} (Fig. 8, Table 2).

Several members of the Gramineae, such as maize \textit{ZmNIT1/2} and rice \textit{OsNIT4}, belong to the nitrilases of the \textit{AtNIT4} group (Fig. 8). Nitrilases belonging to the \textit{AtNIT4} family may have a different and more general function and are apparently not associated with IAA biosynthesis (Piotrowski et al., 2001). It has recently been shown that the \textit{AtNIT4} homologue of \textit{Sorghum bicolor} must form heteromeric complexes in order to have high activity with \(\beta\)-cyanoalanine (Jenrich et al., 2007). \textit{OsNIT4} and tobacco \textit{TNIT4/A/B}, which are homologues of \textit{AtNIT4}, have high substrate specificity for \(\beta\)-cyanoalanine but do not hydrolyse IAN to IAA (Piotrowski et al., 2001). \textit{ZmNIT2} protein may form the heteromer by associating with \textit{ZmNIT1} protein and participate in the detoxification of \(\beta\)-cyanoalanine \textit{in vivo}.

Thus, recent works have shown that nitrilases are involved in the process of cyanide detoxification, in the catabolism of cyanogenic glycosides, and presumably in the catabolism of glucosinolates. It seems unlikely that the indole-3-acetaldoxime pathway via IAOX and IAN is involved in IAA biosynthesis.

8. The redefined \textit{TAA1–YUC} pathway

After the submission of this review article, several papers showing that the \textit{YUC} family functions in the conversion of IPA to IAA in \textit{A. thaliana} were published (Mashiguchi et al., 2011; Won et al., 2011; Stepanova et al., 2011). The genetic, enzymatic, and metabolite-based evidence indicated that \textit{TAA} and \textit{YUC} families function in the same auxin biosynthetic pathway in \textit{Arabidopsis}. It was proposed that the \textit{TAA1–YUC} pathway is ‘the main auxin biosynthesis pathway in \textit{Arabidopsis’} (Mashiguchi et al., 2011).

8.1. The \textit{TAA} family mainly produces IPA from Trp in \textit{Arabidopsis}

In the Trp-auxotroph \textit{trp1-1} mutants of \textit{Arabidopsis} cultured in liquid media containing \([\text{\textsuperscript{13}C}_{11},\text{\textsuperscript{15}N}_2]\text{Trp}, \text{Trp} was efficiently converted to IPA as well as IAD (Mashiguchi et al., 2011). To test which compound is mainly produced from Trp in \textit{Arabidopsis}, estradiol-inducible \textit{TAA1} overexpression (\textit{TAA1ox}) plants were used in feeding experiments. The IPA content was increased drastically in \textit{TAA1ox} plants, whereas the IAD content did not show a significant change relative to that in vector control plants, indicating that IAD is most probably not implicated in the IPA pathway but in another Trp-dependent pathway (Mashiguchi et al., 2011). These results, together with the evidence that \textit{TAA1} protein possesses Trp aminotransferase

Fig. 8. Phylogenetic analysis of the deduced amino acid sequences of \textit{NIT2} proteins. The amino acid sequence of the \textit{Arabidopsis} \textit{NIT2} protein was submitted to DDBJ/GenBank/EMBL, and a phylogenetic tree was constructed, as described in the legend of Fig. 2. The scale bar indicates 0.1 substitution per amino acid.
activity (Tao et al., 2008; Stepanova et al., 2008) as described above, show that the TAA family mainly produces IPA from Trp in Arabidopsis.

L-Kynurenine (Kyn), which inhibits ethylene responses by decreasing ethylene-induced auxin biosynthesis in A. thaliana root tissues, effectively and selectively bound to the substrate pocket of TAA1/TAR proteins (He et al., 2011). The treatment of this potent inhibitor of in vivo TAA1/TAR activity blocked all of the high auxin phenotypes of YUC1ox plants (Stepanova et al., 2011). These results suggested that the high auxin phenotypes of YUC1ox require the function of TAA1/TARs genes, and also suggested the linear action model for TAA1/TARs and YUCs genes (Stepanova et al., 2011).

8.2. Revision in the function of YUC genes: YUC proteins convert IPA to IAA

Fusion protein of GST–YUC2 heterologously expressed in E. coli converted IPA to IAA in vitro in an NADPH-dependent manner, but did not convert IPA to IAD (Mashiguchi et al., 2011). The production of IAA was confirmed by LC-ESI-MS/MS. TAM was not a substrate of GST–YUC2 in this assay condition, indicating that the YUC family is implicated in the conversion of IPA to IAA in Arabidopsis (Mashiguchi et al., 2011).

8.3. Synergistic interactions between TAA and YUC in IAA biosynthesis of Arabidopsis

The IPA content was 33% reduced in estradiol-inducible YUC6 overexpression plants (YUC6ox) of Arabidopsis relative to that in vector control plants, whereas the IAA content was 34–47% increased in YUC6ox plants (Mashiguchi et al., 2011). The IPA content in double knock-out Arabidopsis mutants of TAA1 and TAR2 genes, wei8 tar2-2, was reduced dramatically, and the wei8 tar2-2 mutants showed severe growth. In contrast, the yuc1 yuc2 yuc6 triple mutants had an elevated content of IPA (Mashiguchi et al., 2011; Won et al., 2011). Interestingly, the yuc1 yuc4 wei8 tar2 quadruple mutants did not make any hypocotyls and roots, although the juvenile plants of wei8 tar2-1 were similar to plants of yuc1 yuc4 mutants (Won et al., 2011). The IAA content in the quadruple yuc1246/1 mutants was nearly as low as in wei8-2 tar2-1 mutants, and was much lower compared with that in the wild-type plants (Stepanova et al., 2011). The IAA content was 44% increased in YUC6ox plants relative to that in vector control plants, and it was 146% increased in TAA1 YUC6 co-overexpression plants (TAA1ox YUC6ox) of Arabidopsis (Mashiguchi et al., 2011).

These results showing that the taa mutants were partially IPA deficient and the yuc mutants accumulated IAA indicated that TAAs are responsible for converting Trp to IPA, and also indicated that YUC and TAA work in the same pathway in Arabidopsis. In future, it must be revealed whether or not the TAA1–YUC pathway is widely distributed in the plant kingdom.

8.4. Different mechanisms for auxin biosynthesis in YUC genes and the iaaM gene

In some plant pathogenic bacteria, Trp is first converted to IAM by the enzyme tryptophan-2-monoxygenase encoded by the auxI/iaaMtlms1 gene (Yamada et al., 1985; Camilleri and Jouanin, 1991; Gaudin et al., 1993), as described above. Cheng et al. (2006) showed that expression of the bacterial auxin biosynthesis gene iaaM rescued yuc mutant phenotypes, as discussed above. Overexpression of the iaaM gene led to the typical auxin overproduction phenotypes in both wild-type and wei8 tar2 mutants of Arabidopsis (Won et al., 2011). Together with the results showing that the iaaM gene also partially rescued the defects of wei8 tar2 phenotypes at juvenile and adult stages, the authors indicated that YUC genes and iaaM genes probably use different mechanisms for auxin biosynthesis in Arabidopsis (Won et al., 2011). Overexpression of the auxI/iaaMtlms1 gene of the Ri plasmid allowed tobacco BY-2 cells to grow in the absence of auxin (Nemoto et al., 2009a, c), as described earlier in this review. These results indicate that the production of IAM in plant cells is important in IAA biosynthesis.

9. Conclusions and perspectives

Plants would be expected to share evolutionarily conserved core mechanisms for auxin biosynthesis because IAA is a fundamental substance in the plant life cycle, although different plant species may have unique strategies and modifications to optimize their metabolic pathways. Biochemical and molecular biological findings and bioinformatics studies indicate that the best candidate for the major pathway of IAA biosynthesis is the IAM pathway and/or the IPA pathway.

In the IAM pathway, indole-3-acetamide hydrolase, encoded by the AMII gene, is widely distributed in the plant kingdom. On the other hand, in the IPA pathway, TAA1/TIR2, which participates in the conversion of Trp to IPA, may be a Brassicaceae-specific enzyme. In the TAM pathway, the function of YUCs is ambiguous. The IAOX pathway is a Brassicaceae species-specific pathway that may be involved in the synthesis of plant secondary metabolites, such as indole glucosinolates and the alkaloid camalexin.

For the next step in advancing our understanding, it must be revealed whether or not the TAA1–YUC pathway is widely distributed in the plant kingdom. Additionally, the gene(s) functioning in IAM biosynthesis must be identified. By analysing the expression of the IAM biosynthesis gene(s), together with the AMII gene, it will be possible finally to determine how, when, and where auxin is synthesized in plants.

Acknowledgements

This work was supported, in part, by a Grant-in-Aid for Challenging Exploratory Research from the Japan Society for the Promotion of Science (grant no. 23658096 to YM).
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