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# Two genetically discrete pathways convert tryptophan to auxin: more redundancy in auxin biosynthesis

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**The answer to the simple question of how plants make auxin has proven to be inordinately complex. Recent *in planta* studies in *Arabidopsis* have uncovered additional complexity in auxin biosynthesis. Two distinct pathways from tryptophan to the intermediate indoleacetaldoxime were identified. Genic, as well as functional redundancy, appear to be characteristic for auxin biosynthesis and plants might have evolved many different solutions for making and regulating auxin.**

It has taken scientists >100 years to make marked progress in answering the question of how plants make auxin, a hormone that is not only essential for growth but also plays important roles in many developmental processes and in environmental responses. We cannot begin to ask crucial questions of agricultural significance about how auxin biosynthesis is regulated until the process is defined. Recently, Yunde Zhao and colleagues [1] have defined a key step in the biosynthesis of auxin from tryptophan. These results reveal a complex situation where two genetically discrete pathways operating in a single plant both start from the same precursor and result in the formation of the same signal messenger. These results are an impressive example of why it has taken so long to understand such an essential process.

## New pathway defined

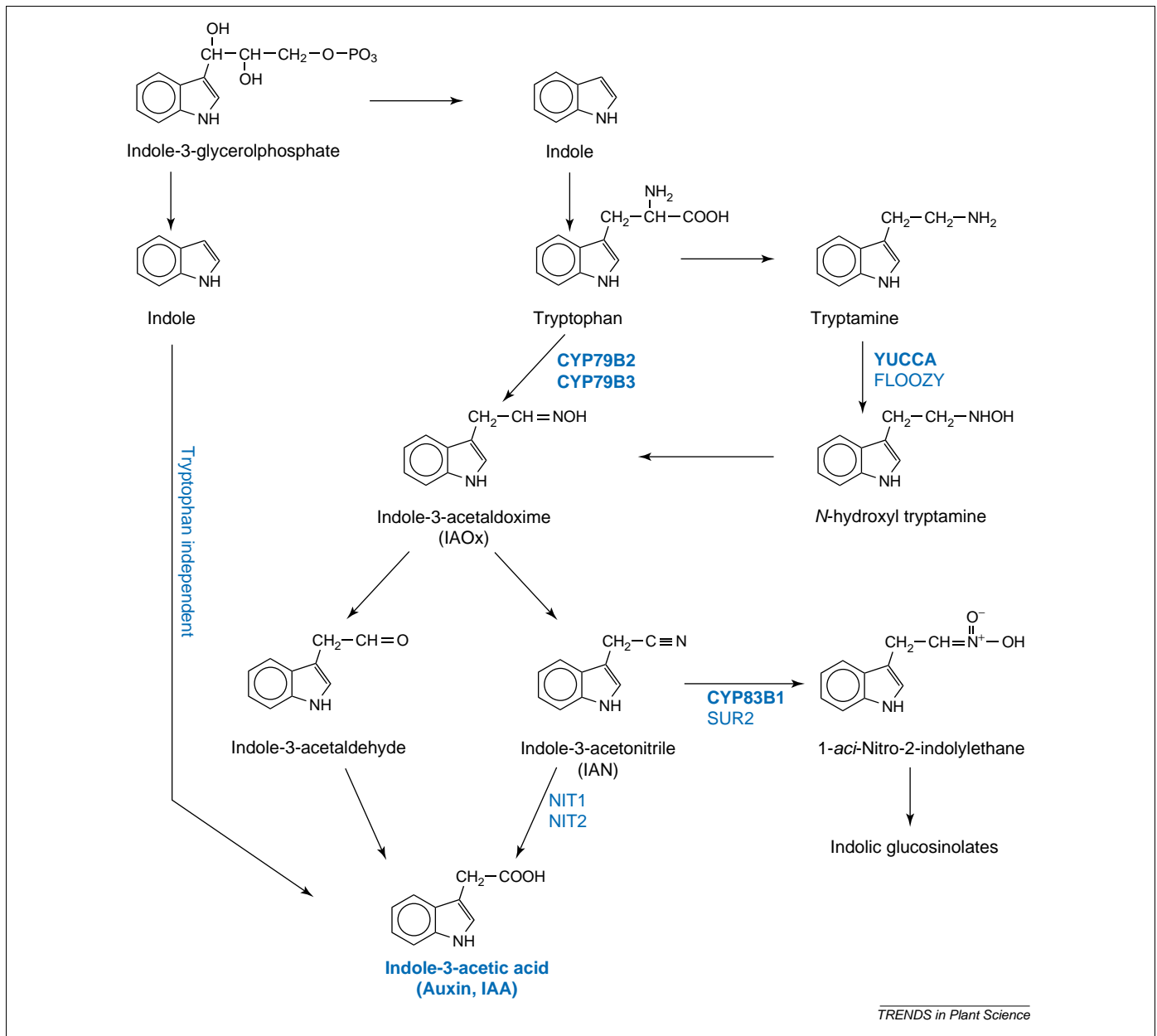
The classical genetic approach, screening for auxin-deficient mutants, yielded few positive hits. This was attributed either to such mutations being lethal or that multiple biosynthetic pathways and/or functional redundancy [2] in multiple genes in the same pathway resulted in silent mutations. Two different biosynthetic pathways are involved in indole-3-acetic acid (IAA) biosynthesis, one which uses tryptophan as a precursor, and another, discovered about ten years ago [3,4], which bypasses tryptophan and uses indole as a precursor for IAA

biosynthesis. Using gain-of-function approaches in *Arabidopsis*, redundant pathways have now been defined within the tryptophan-dependent pathway. Activation tagging resulted in the identification of the YUCCA gene, which encodes a novel flavin monooxygenase that catalyzes the rate-limiting N-hydroxylation of tryptophan to create N-hydroxyl tryptamine [5]. This important advance defined a new pathway that had not been predicted for indole-3-acetic acid (IAA) biosynthesis from tryptophan. The identification of two *Arabidopsis* YUCCA paralogs extends the idea of redundancy to the gene level within a single pathway.

As with other Cruciferae species, *Arabidopsis* contains a class of secondary compounds known as glucosinolates that because of their flavor and medicinal properties have attracted much interest in their mode of production within plants. Recent attention has focused on the role of cytochrome P450s in the reactions leading to indolic glucosinolates [6,7]. Overexpression of *superroot2* (*sur2*) [8], an *Arabidopsis* gene encoding the cytochrome P450 CYP83B1, or overexpression of a related cytochrome P450, CYP83A1, results in increased indolic glucosinolate levels, but the morphological phenotype is consistent with underproduction of IAA. Mutations of *sur2* cause increased adventitious rooting and epinasty, consistent with IAA overproduction. It has been suggested that CYP83B1 serves as a branch point between IAA and indolic glucosinolate biosynthesis, but functions downstream from the most likely branch point from IAA production [9] where indoleacetaldoxime (IAOx) is an intermediate (Fig. 1).

The conversion of tryptophan to IAOx was already known to be catalyzed by two other cytochrome P450s, CYP79B2 and CYP79B3 in *Arabidopsis*, but whether IAA or only glucosinolates were produced from IAOx was unclear [10]. The CYP83B1 and CYP83B2 results suggested that supplying IAOx for conversion to 1-acetyl-2-indolyl-ethane, the committed step of indolic glucosinolate biosynthesis, was the most likely role for

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**Fig. 1.** Summary of reactions involving CYP79B2, CYP79B3, YUCCA and CYP83B1 leading to the biosynthesis of indole-3-acetic acid (IAA). IAA biosynthesis independent of tryptophan from free indole has been shown using tryptophan auxotrophic mutants of maize and *Arabidopsis* [3]. Biosynthesis from indole-3-pyruvate through indole-acetaldehyde to IAA is not shown, but is also thought to be active based on the presence of indole-3-pyruvate in *Arabidopsis* [18]. Abbreviations: NIT, nitrilase; SUR2, SUPERROOT2.

these cytochrome P450s [7]. However, a detailed analysis of CYP79B2 and CYP79B3 function relative to YUCCA places these two proteins in the middle of another pathway using IAOx in *Arabidopsis* for the production of IAA from tryptophan [1].

A convergence of approaches produced these unexpected results. Overexpression of CYP79B2 yielded plants with an IAA-overproduction phenotype, elevated IAA levels and increased expression of IAA-inducible genes similar to that seen in *yucca* overexpressors, but different from that obtained when IAA is supplied in the culture media. Both CYP79B2 overexpression lines and *yucca* overexpressors respond to tryptophan supplementation by increased adventitious root formation and are resistant to toxic analogs of tryptophan. Wild-type seedlings do not respond

to tryptophan supplementation. Consistent with the concept of functional redundancy, single knockouts of CYP79B2 or CYP79B3 had no profound phenotype. However, double mutants displayed the shorter petioles and smaller leaves associated with decreased auxin, as well as a decrease in indoleacetonitrile (IAN) levels. IAN is thought to be the next intermediate in this pathway to IAA (Fig. 1). The story is further complicated by the temperature dependence of the effect on IAA levels in these mutants. IAA levels are essentially the same as wild type at 21 °C but are significantly reduced at 26 °C. Temperature has been shown to affect the IAA biosynthetic pathway chosen by other plant species [11] and a temperature effect on IAA levels in *Arabidopsis* has been noted previously [12].

### Locations within the cell raise prospects

CYP79B2 and CYP83B1 are differentially localized within the cell. CYP79B2 is chloroplastic and CYP83B1 resides in the endoplasmic reticulum (ER). YUCCA appears to be cytoplasmic. The disparate localizations for these enzymes rule out their involvement in an IAA-synthase enzyme complex such as that postulated by Axel Müller and Elmar Weiler based on antibody pull-down studies [13], but do provide some insight as to what the cell is doing with this redundancy. The differential subcellular localizations suggest that a great deal of internal indolic trafficking is involved in the use and control of these pathways, and is consistent with isotopic labeling studies that showed that tryptophan-dependent and independent IAA biosynthesis does not occur within the same subcellular compartment [14]. Metabolic trafficking seems to be a prerequisite if CYP79B2 and CYP83B1 are functioning together in the same pathway, but this was not suggested by the labeling experiments. Previous reports of KDEL-like ER retention sequences on IAA-amino acid hydrolases [15] and on an auxin binding protein, ABP1 [16], had been difficult to understand, but as more data become available, the cellular biology of auxin metabolism might become key to our next level of understanding.

### Different solutions for different plants

Several important conclusions are possible from the CYP79B2 and CYP79B3 results, and these in turn raise new questions. At least two routes for IAA biosynthesis from tryptophan are active in *Arabidopsis*. We can also conclude that multiple pathways arising from different precursors, as well as functional redundancy within a single pathway, is characteristic of IAA biosynthesis in *Arabidopsis*, although other plant species appear to have evolved different solutions to the problems of making IAA. FLOOZY, a YUCCA-like flavin monooxygenase from petunia is, for example, apparently a single-copy gene [17], and no orthologs of CYP79B2 and CYP79B3 have been found in rice, suggesting that there are different biosynthetic pathways in monocots.

We are now beginning to assign specific genes to a function in the tryptophan-dependent IAA biosynthesis pathway. This work allows us to predict what the function of other genes in the pathway might be. Although these are major advances, the enormity of the problem of integrating this pathway with an entirely different pathway that does not involve tryptophan suggests that plant molecular geneticists still have much more work to do. Strong biochemical and genetic evidence for IAA biosynthesis from indole or indole-3-glycerolphosphate independent of tryptophan needs to be followed up with gene discovery to define the reactions involved in this process. Such advances would then allow the relationship between several pathways to be analyzed, which is necessary to

understand the complexity in developmental and environmental signaling processes that are transduced through altered auxin levels.

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