



Old Town Roads: routes of auxin biosynthesis across kingdoms

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Auxin is an important signaling molecule synthesized in organisms from multiple kingdoms of life, including land plants, green algae, and bacteria. In this review, we highlight the similarities and differences in auxin biosynthesis among these organisms. Tryptophan-dependent routes to IAA are found in land plants, green algae and bacteria. Recent sequencing efforts show that the indole-3-pyruvic acid pathway, one of the primary biosynthetic pathways in land plants, is also found in the green algae. These similarities raise questions about the origin of auxin biosynthesis. Future studies comparing auxin biosynthesis across kingdoms will shed light on its origin and role outside of the plant lineage.

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Introduction

A transmissible factor was first proposed to regulate root gravitropism in 1872 by Theophil Ciesielski [1]. A decade later Charles and Francis Darwin expanded on this idea and hypothesized that a mobile signal regulates plant photomorphogenesis [2]. The primary active auxin, indole-3-acetic acid (IAA), was later identified as this mobile signal and was characterized in the early 1940s (reviewed in Ref. [3]). Since that time, auxin has been implicated in plant cell division and expansion to drive embryogenesis, growth, and tissue differentiation. In addition to its endogenous role in plant development, natural and synthetic auxins or their precursors have been used for agricultural applications.

Synthesis and response to auxin are not limited to plants; indeed, organisms from across the kingdoms of life have been found to synthesize or respond to auxin. In each of these systems, a different primary route of IAA biosynthesis is utilized; however, for those organisms that IAA biosynthesis pathways have been elucidated, common themes of biosynthetic intermediates have arisen.

Some fungi can synthesize and/or respond to auxin. Indeed, the IAA molecule was originally identified in fermentation media growing yeast in 1934 [4]. Fungi alter their growth patterns in response to auxin. In *Saccharomyces cerevisiae* and other yeasts, auxin promotes cell expansion and negatively regulates cell division. In other fungal species, auxin induces spore germination (reviewed in Ref. [5]). Clearly, IAA biosynthesis and response are present in this kingdom.

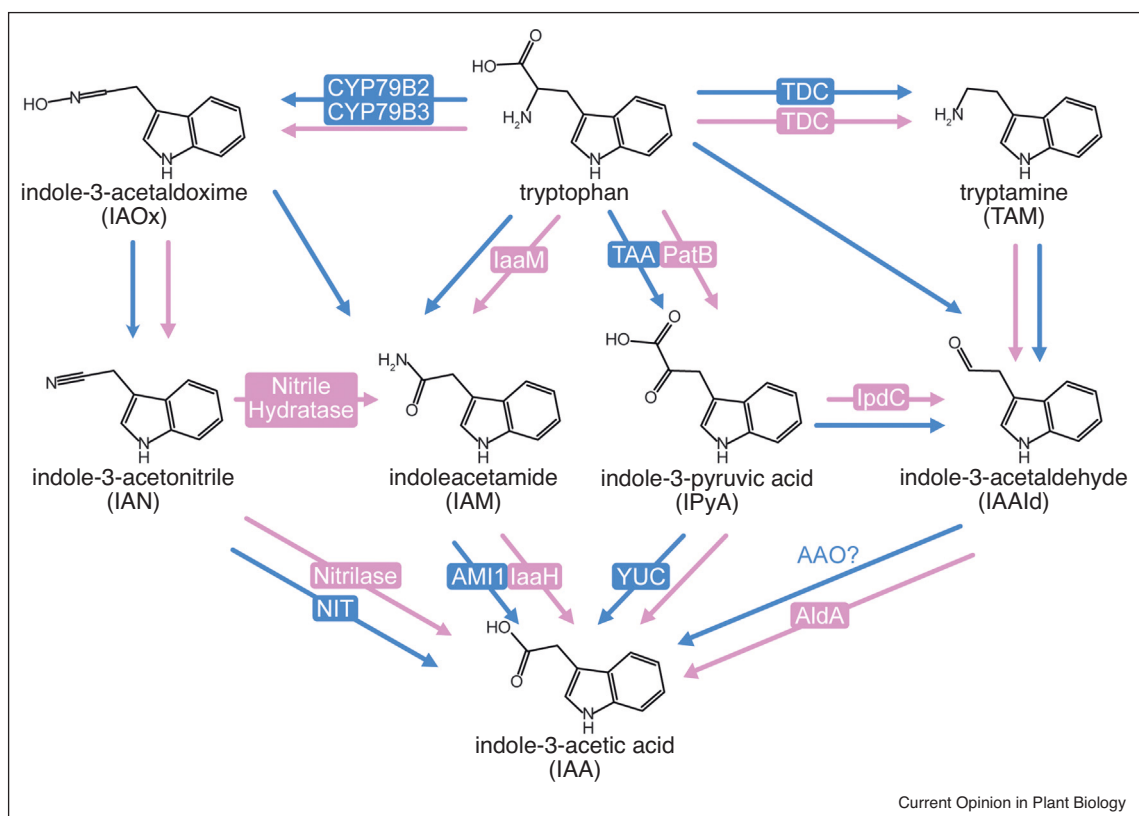
Auxin is also synthesized by some bacteria. For example, select plant pathogens rely on auxin to induce their cell growth and to increase pathogenicity (reviewed in Ref. [6]). In addition, bacteria in the rhizosphere may also rely on auxin synthesis to promote symbiotic relationships with plants [7,8]. Thus, in addition to plants and fungi, bacterial species may synthesize and respond to auxin.

Given the breadth of organisms making or responding to this small molecule, it raises the question of how and in which organisms is IAA synthesized. In plants, several enzymatic pathways result in IAA production through diverse intermediates that in turn can be conjugated to generate various storage forms (Reviewed in Ref. [9]) (Figure 1). Likewise, some bacteria use distinct pathways to generate IAA (reviewed in Refs. [6] and [7]) (Figure 1). In this review, we will focus on IAA production and metabolic mechanisms in both eukaryotes and prokaryotes.

Routes of IAA production in the land plant lineage

In land plants IAA is synthesized from a tryptophan precursor and flows through the indole-3-pyruvic acid (IPyA) biosynthetic pathway (Figure 1) [10[•],11–14]. The tryptophan aminotransferase (TAA) [11,12,14] family converts the amino acid tryptophan in IPyA, which is then metabolized by the YUCCA (YUC) [11,12,14] family of flavin monooxygenases in IAA. In both angiosperms and bryophytes, mutants defective in the IPyA pathway show severe developmental phenotypes, suggesting a conserved role of this pathway in IAA biosynthesis [11–13,15^{••},16].

Figure 1



Tryptophan-dependent IAA biosynthetic pathways in land plants and bacteria. Lines represent steps in the biosynthesis of IAA and its precursors in land plants (blue) and bacteria (pink). Enzymatic reactions that have been confirmed by genetic and biochemical analysis are provided in the appropriate steps. Unlabeled lines represent hypothesized steps in the pathway or steps for which genetic evidence is lacking. Many steps in the proposed pathways are currently unknown in both land plants and bacteria (reviewed in Refs. [6,7,9]).

The angiosperms show subfunctionalization of each of the core components in this pathway consistent with the transition to increased cell types and growth patterns [17]. In the liverwort *Marchantia polymorpha*, the core IPyA pathway consists of a single TAA and two YUC in contrast to the five TAA and eleven YUC enzymes in *Arabidopsis thaliana* (Table 1) [15^{••},18^{••}]. Functional conservation of the IPyA pathway in examined angiosperms and bryophytes suggests that this pathway is likely present across the plant lineage (please see Refs. [18^{••}] and [17] for phylogenetic analyses). The expansion of IPyA enzymes in flowering plants is consistent with published phylogenies of other auxin pathway components including auxin signaling. The suite of nuclear auxin signaling components in *Marchantia* evolved from a core set of five genes to over fifty in *Arabidopsis* [19,20,21^{••}]. Thus, *Marchantia* is an appealing system to study the evolution of auxin biosynthesis and catabolism in the context of nuclear auxin signaling.

TAA and YUC genes fall into distinct clades that have non-overlapping expression patterns and may regulate

different aspects of plant development [17]. The expression patterns of the TAA and YUC families suggest tissue specific roles for individual members. In *Arabidopsis* and *Marchantia*, expression of IPyA enzymes in specific tissue types is crucial for appropriate growth [10^{••},15^{••}]. Tracing the lineage of these distinct YUC and TAA enzymes back to *Physcomitrella* and *Marchantia* may help researchers understand the tissue specific functions of different classes of YUC and TAAs found in flowering plants.

Table 1

Number of known TAA and YUC family proteins identified in plant model species. Data compiled from Refs. [15^{••},17,63]

Species	Number of TAA enzymes	Number of YUC enzymes
<i>Marchantia polymorpha</i>	1	2
<i>Physcomitrella patens</i>	6	6
<i>Oryza sativa</i>	4	14
<i>Zea mays</i>	6	9
<i>Arabidopsis thaliana</i>	5	11

IPyA is not the only IAA precursor found in land plants (Figure 1) [9]. Indole-3-acetaldehyde (IAAld), indoleacetamide (IAM), indole-3-acetaldoximine (IAOx), and indole-3-acetonitrile (IAN) are endogenous molecules that when applied stimulate auxin responses [14,22–24]. However, it is not clear whether these intermediates contribute to auxin homeostasis, and in some cases, enzymes required in these potential pathways remain unidentified (Figure 1). For example, enzymes that convert tryptophan to IAAld and from IAAld to IAA remain unknown in flowering plants [14]. Aldehyde oxidases that convert IAAld to IAA have been proposed [25–27], but *Arabidopsis* lacking a co-factor required for aldehyde oxidase function fail to hyperaccumulate IAAld and show no auxin-related phenotypes, suggesting that these enzymes do not participate in IAAld to IAA conversion [14]. At this point, contributions of these specific biosynthetic pathways to the pool of IAA remain poorly understood. However, the presence of these precursors in charophytes suggests that they could represent ancestral routes to IAA [28,29].

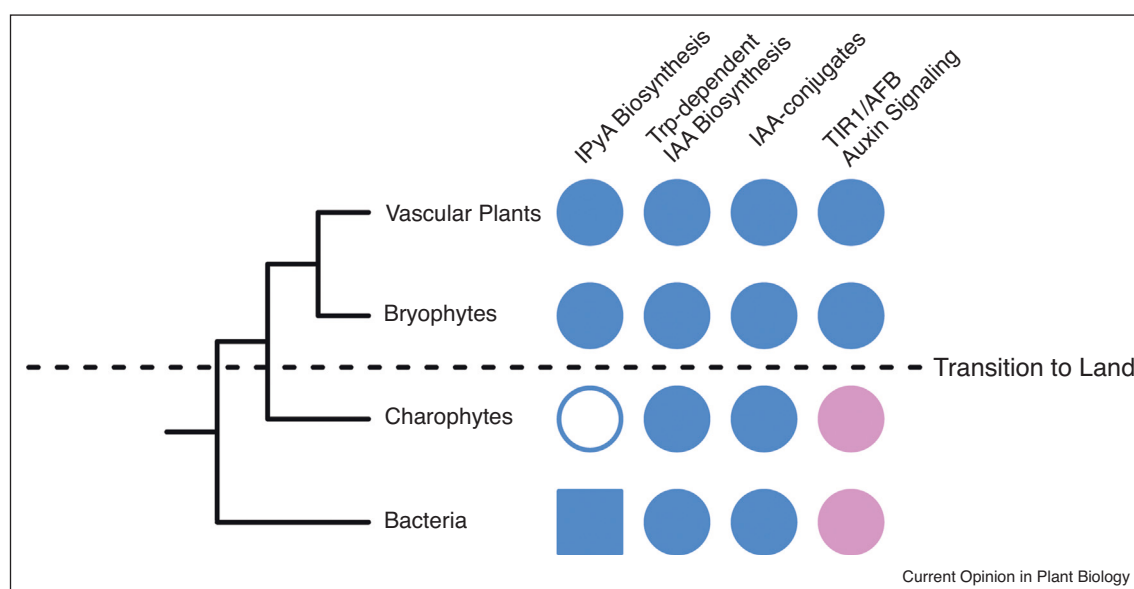
IAA production in green algae

Red, brown, and green algae produce auxin [28,30–33]. In addition, tested algal species respond to and alter their growth patterns in response to auxins and their antagonists, suggesting a role for auxin signaling in these organisms [34**]. These findings suggest that auxin production may have evolved in the last common ancestor of the all algal species.

The land plants diverged from their closest algal relatives, the charophytes or green algae, 514 to 470 million years ago [35]. Like the land plants, single and multicellular charophytes produce auxin via tryptophan-dependent pathways. However, charophytes lack components of the TIR1/AFB nuclear auxin signaling system, suggesting a distinct, ancestral role for auxin in these species (Figure 2) [21**]. The route of auxin biosynthesis in algae is less clear than in land plants. Whereas *YUC* genes have been identified in some algal species, the presence of *TAA* genes in these same species is debatable [18**,34**,36–39,40**]. These differences may be resolved with better genome sequences. Interestingly, the IPyA pathway is absent altogether from the charophyte seaweed *Ulva mutabilis*. This organism only encodes enzymes that function in the IAOx and IAAld pathways, suggesting that some species in the green algae lineage rely on alternative auxin biosynthetic pathways (Figure 1) [41].

These data suggest that IAA synthesis was present in the last common ancestor of all chlorophytic organisms, but the evolution of any specific pathway is less well understood. The putative absence of TAA from some charophytes raises the possibility that the direct conversion of tryptophan to IPyA was a crucial step for the transition to land along with the evolution of the TIR1/AFB auxin signaling pathway. However, the absence of TAA is based primarily on transcriptome sequencing which raises the possibility that TAAs may exist in the genomes of these species and were not expressed at the time of sampling

Figure 2



Auxin production predates TIR1/AFB auxin signaling. A simplified phylogeny showing the presence (blue) or absence (pink) of components of auxin signaling and production. Empty circles signify the presence of genes in the pathway but no other evidence. The square represents an altered IPyA pathway found in bacteria. The absence of the canonical TIR1/AFB pathway in the charophytes raises questions about the ancestral function of auxin in the green algae. For an in-depth review of land plant evolution, please see Ref. [64] and for information on the relationship and evolution of prokaryotes and algae, please see Ref. [65].

[36]. In studied algal species, IAA and IAM have been identified, but not IPyA, consistent with the absence of TAA [30]. This raises the possibility that the ancestral IPyA pathway is not one of the primary routes for IAA production in the charophytes as it is in the land plants. Genetic and metabolomic analysis will help determine the ancestral roles of TAA and YUC enzymes as well as which biosynthetic pathways are the primary source of IAA in the green algae.

Bacterial IAA biosynthesis

Tryptophan-dependent IAA biosynthesis has also been discovered outside of the plant lineage. In bacteria and fungi, which diverged from the plant lineage over a billion years ago [42], auxin plays important roles in plant pathogenesis and symbiosis [6]. Given auxin's role in regulating cell division and expansion, its use as a virulence factor is not surprising. Pathogen-derived IAA inhibits the host immune response and generates a more hospitable environment for the invading pathogen, sometimes by promoting uncontrolled cell growth [6]. Further, auxin is synthesized by nitrogen fixing bacteria that form symbiotic relationships with plants in the rhizosphere to promote cell division in roots [7,8].

Unlike in the charophytes, aminotransferases that convert tryptophan to IPyA have been characterized in some bacteria and fungi (Figure 1) [43,44]. IPyA is then converted to the IAAlD intermediate by decarboxylases before further conversion to IAA by a dehydrogenase (Figure 1) [7,43,45]. To date, no enzymes with YUC-like activity have been identified in IAA-producing bacteria, suggesting that direct conversion from IPyA to IAA is either absent or occurs by a distinct enzymatic mechanism.

The IAM pathway is a common route for IAA production in bacteria. In this pathway, a monooxygenase and hydroxylase, IaaM and IaaH respectively, convert tryptophan to IAM and then IAM to IAA (Figure 1) [46]. Interestingly, IAM is also found in flowering plants such as *Arabidopsis*, but the enzymes that convert tryptophan to IAM are currently unknown [9,47], although *Arabidopsis* genes that share sequence similarity with IaaH encode enzymes capable of converting IAM to IAA [48]. These distinct auxin biosynthesis routes suggest convergent evolution of auxin biosynthesis in bacteria and plants. The origin of bacterial auxin biosynthetic enzymes, and their relationship to the evolution of auxin biosynthesis in the plant lineage are not currently known (Figure 2).

Open questions and future directions

Auxin is vital to plants and influences the growth of many non-plant species. Auxin biosynthesis and metabolism have been of interest to the plant community since its discovery. Whereas advances have been made in understanding how auxin is synthesized, the origins of IAA biosynthesis in plants, the green algae, and plant

pathogens remain a mystery. It is not clear which IAA biosynthesis pathways in plants and bacteria are the result of a common origin, convergent evolution, or horizontal gene transfer (Figure 2).

In addition to IAA, auxin conjugates have been identified in the land plants, algae, and bacteria. These auxin conjugates are predicted to be storage forms and intermediates in auxin catabolism [30,49–53]. The evolution of auxin storage forms and their developmental roles have been investigated in the land plants, but their ancestral role in the algae and their roles in bacteria are less well understood. The roles of other IAA precursors are also not well understood. For example, indole-3-butyric acid (IBA) is a naturally occurring auxin precursor, but its synthesis is currently unknown (reviewed in Ref. [54]). Phenylacetic acid (PAA), a non-indole, naturally occurring, active auxin, is synthesized by TAA and YUC, but its function remains a mystery as no specific PAA-deficient mutants have been characterized [9,55]. IBA and PAA are found in plants as well as bacteria [7,9,56], suggesting potential roles across kingdoms. Studies of these auxins and auxin precursors in other organisms may shed light on their biosynthesis and their function.

Genomic and transcriptomic data from diverse species that span the plant lineage have been used to great effect to interrogate the evolution of the core nuclear auxin signaling system [21^{••},31,57–59]. This work has been used to identify the proto-ARF in *Chlorokybus atmophyticus* [57], a green alga, and has opened up new routes of study to understand the relationship between the transition from water to land in the plant lineage. Similar approaches can and should be taken with the known components of the various auxin biosynthetic pathways. Previous studies, like Smet *et al.* [60], can be updated and completed with newly released resources such as the 1KP project which may help resolve the origin of the IPyA pathway [61]. In addition, further study of gene families like the aldehyde oxidases proposed to convert IAAlD to IAA may lead to the identity of the enzymes responsible for this conversion *in vivo* [14,25–27].

Whereas genes encoding TAA and YUC enzymes have been identified in the green algae, their functions as IAA biosynthetic genes have not been tested [18^{••},62]. Genetic studies in algal species will help determine the ancestral state of the TAA family and its role in plant evolution as more TAA orthologs are identified in the green algae. Studies to identify potential routes of auxin biosynthesis in algae rely primarily on information gathered from the flowering plants [17] and may miss potential pathways not represented in the angiosperms. Algal species and non-flowering land plants like *Marchantia* may shed light on alternative auxin biosynthesis and catabolism pathways and aid in resolving their evolution. Further, blocking IAA biosynthetic pathways in the algae,

which seemingly lack the TIR1/AFB signaling pathway, will provide useful information on the function of auxin in those species – for example, is auxin used as a signaling molecule in these species? If so, for what purpose? Or, similar to microbes, do algae use IAA to alter growth of nearby plants? Resolving these questions may lend insight not only into IAA roles in algae, but may also uncover IAA signaling mechanisms present across organisms.

How pathogenic bacteria and symbionts evolved their auxin biosynthetic machinery remains unknown. Of note, not all bacteria rely on endogenous auxin biosynthesis to support their pathogenesis [6]. Comparative studies of closely related bacteria that do and do not display auxin production may increase our understanding of how auxin contributes to microbial functions and plant pathogenesis. Understanding evolutionary trajectories of auxin biosynthesis in plant and non-plant lineages could also identify novel biological roles for auxin. Further, determining the origin of the biosynthetic pathways found in both the plant and non-plant lineages and identifying species that respond to auxin will be crucial for understanding non-canonical auxin signaling across species.

Conclusions

Many organisms follow similar routes from tryptophan to the primary auxin IAA. The origin of these biosynthetic pathways remains a mystery. Further sequencing of non-model species, particularly those in the algal lineage, present an opportunity to study all aspects of auxin biosynthesis. The advent of gene editing technology like CRISPR will also increase the ability to test the function of putative IAA biosynthetic enzymes. However, the identification and classification of auxin precursors *in vivo* remains a rate limiting step. Improvements in identification and quantification of IAA precursors will aid researchers in testing hypotheses generated by genomic and genetic data and increase our understanding of the evolution of auxin biosynthesis and function.

Conflict of interest statement

Nothing declared.

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