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Update on Hormones

Rethinking Auxin Biosynthesis and Metabolism

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Charles Darwin cited the 1871 Ph.D. thesis of Theophil Ciesielski when he postulated in 1880 that a "transmitted influence" present in the tip of plant shoots was responsible for gravitropism. Both Darwin and Ciesielski had realized that the influence was affecting growth differentially. This influence was given the name auxin more than 50 years later and subsequently any compound that promoted growth in specific bioassays was defined as an auxin. The chemical structure of the primary plant auxin, IAA (Fig. 1), has been known since the 1930s to be a 3-substituted indole like Trp. Since that time, the prevailing theory has been that IAA is derived from Trp. However, due to lack of convincing evidence, the biosynthetic pathway for IAA in plants is still undefined. Within the last 10 years, development of precise quantitative methods, good model systems for in vivo analysis, and mutants altered in IAA metabolism have resulted in substantial progress in our understanding of IAA biosynthesis. This review focuses on the new and more complex picture of IAA biosynthesis that has emerged as a result of recent experiments.

THE TROUBLE WITH TRP

In the late 1940s and 1950s, studies showed that labeled and unlabeled Trp applied to various plant tissues was converted to IAA. The ensuing biochemical analysis of over 20 different species of plants led to the conclusion that IAA is derived from Trp, albeit through several possible pathways (reviewed by Nonhebel et al., 1993). Predicted intermediates have been shown to incorporate label from Trp and to be present as native compounds in plants. Enzyme activities that catalyze the interconversion of specific intermediates have been identified, and in some cases the genes encoding these enzyme activities have been cloned (De Luca et al., 1989; Bartel and Fink, 1994; Bartling et al., 1994).

Biochemical studies carried out with tissue segments or plant extracts disrupt compartmentalization; therefore, the enzymes that can catalyze the interconversion of Trp to IAA in vitro may, in fact, never come into contact with the required intermediates in intact cells. So, although many studies have demonstrated the competence of plants to convert Trp to IAA, the physiological relevance of the hypothetical pathways remains questionable. Over the past four decades, the assertion that Trp is the precursor to IAA has been questioned several times. Trp is present in vast excess to IAA, thus the incorporation of label from Trp into IAA amounts to only a meager few percent. In early experiments, minimal attempts were made to quantify the total amount of IAA produced, thereby making it difficult to determine if the low level of conversion was physiologically meaningful. Furthermore, many of the experiments were not carried out under aseptic conditions. Since it has been well established that microbes convert Trp to IAA, the argument has been made that bacteria associated with the plants were actually responsible for the observed conversion of Trp to IAA. Perhaps most troubling for the interpretation of these studies is the observation that Trp is readily converted to IAA nonenzymatically upon routine handling in the laboratory. Although these challenges to the prevailing theory of IAA biosynthesis have been noted periodically over the last 40 years, they have been inexplicably dismissed and the hypothesis that Trp is the primary precursor to IAA has persisted.

STABLE ISOTOPE DILUTION GC-MS SETS A NEW STANDARD FOR ANALYZING IAA BIOSYNTHESIS

A critical component for the study of IAA biosynthesis is the ability to accurately quantify the precursors, intermediates, and end products involved. IAA is present in very low abundance and requires more sensitive detection methods than those used for the study of a major metabolic pathway. Isotope dilution analysis coupled with MS has a long history of solving difficult analytical problems in biology and biochemistry. Stable isotope dilution analysis is based on the principle that a heavy-labeled compound (e.g. ^{2}H-, ^{15}N-, or ^{13}C-labeled compounds) of known amount added to a plant extract will behave analogously to the unlabeled endogenous compound throughout the isolation procedure. Since the amount of labeled compound is known, determining the ratio of labeled to unlabeled compound with GC-MS reveals the amount of endogenous, unlabeled compound. Furthermore, the added specificity obtained by monitoring individual ions lessens the emphasis on purity and yield during isolation of the compound to be measured. The application of this technique to IAA

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Abbreviations: GST, glutathione-*S*-transferase; IAN, indole acetonitrile; IBA, indole-3-butyric acid; TDC, tryptophan decarboxylase.

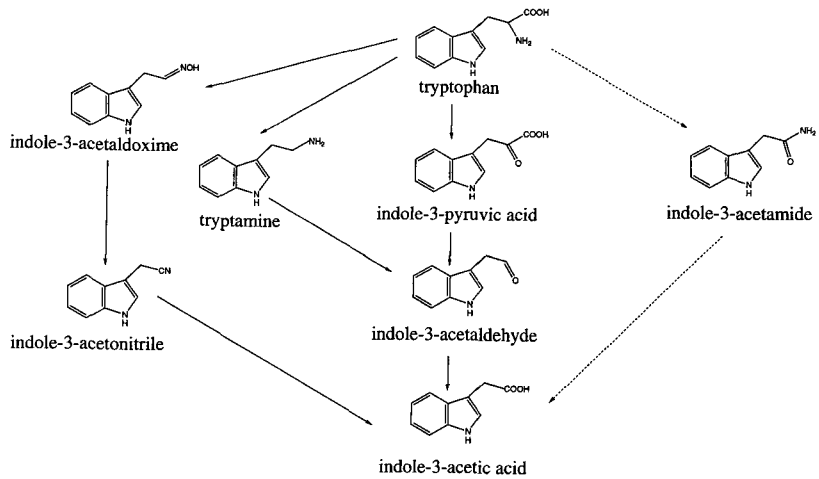

Figure 1. Proposed routes of IAA biosynthesis from Trp. The pathway utilized by microbes is indicated by dashed arrows. Reviewed by Nonhebel et al. (1993).

biosynthesis has increased in the last decade as a result of two important advances. The first was the availability of a reliable internal standard. IAA with ^{13}C substituted at all six of the benzene ring carbons is stable, can be readily distinguished from endogenous IAA by MS, and behaves like the endogenous IAA during purification. Second, significant improvement has been made in the speed with which one can isolate IAA from small amounts of tissue. Good yields can be obtained from as little as 100 mg of tissue in less than 1 d (see Wright et al., 1991; Normanly et al., 1993, and refs. therein). As a consequence, the measurement of IAA levels (and for that matter intermediates in the pathway of IAA synthesis) is now feasible as a routine laboratory procedure.

Stable isotopes are also useful for establishing a precursor-to-product relationship between any two compounds that will incorporate isotope as a result of de novo synthesis. For example, in the case of IAA biosynthesis, if Trp were the sole precursor to de novo-synthesized IAA, then the percent incorporation of isotope into Trp from a labeled Trp precursor (e.g. [^{15}N]anthranilate; see Fig. 2) would be expected to be greater than or equal to the percent incorporation of isotope into IAA. If the percent incorporation of isotope into IAA is greater than that of Trp, there has to be another precursor available to synthesize IAA, in a manner that bypasses Trp, to account for the higher level of isotopic enrichment. This approach provides a means to assess the significance of any compound postulated to be a precursor to IAA.

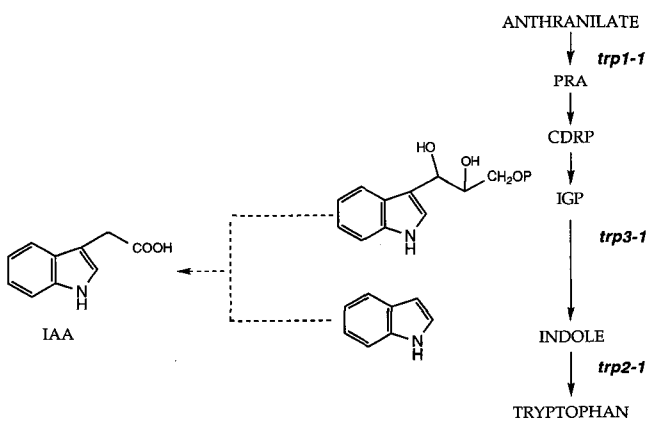

Figure 2. Trp biosynthetic pathway. PRA, *N*-Phosphoribosylanthranilate; CDRP, 1-(*O*-carboxyphenylamino)-1-deoxyribulose phosphate; IGP, indole-3-glycerol phosphate; trp, Trp.

QUANTITATIVE IN VIVO LABELING STUDIES PROVIDE INSIGHT

Aseptic cultures of the aquatic monocot *Lemna gibba* (duckweed) are ideal for stable isotope labeling studies because the entire underside of the plant readily takes up labeled compounds from the surrounding media. Using *Lemna*, the experiments by Baldi et al. (1991) were the first to quantitatively address the question of whether Trp is the precursor to IAA in vivo in intact, growing plants. Their results completely contradicted the then-prevailing theories about IAA biosynthesis (Baldi et al., 1991). *Lemna* fed [^{15}N]Trp, to the extent that 98% of the Trp pool was labeled, incorporated very little ^{15}N into IAA over a period of 5 d. Feeding *Lemna* unlabeled Trp in vast excess had no effect on IAA levels either.

Deuterium oxide, or heavy water, is an excellent compound for labeling studies, since water is generally freely accessible to all compartments of the plant. Deuterium exchange will occur early in the shikimate pathway prior to the synthesis of anthranilate; therefore, knowledge of the precursors and intermediates in IAA biosynthesis is not required. This technique has played a critical role in a number of recent studies on de novo IAA biosynthesis (Baldi et al., 1991; Cooney and Nonhebel, 1991; Wright et al., 1991; Bandurski et al., 1992). In one such study, dark-grown maize seedlings fed deuterium oxide incorporated deuterium into Trp but not into IAA over a 7-d period. This result indicates that synthesis of the two compounds is separable over time (Bandurski et al., 1992).

MUTANTS REVEAL A TRP-INDEPENDENT IAA BIOSYNTHETIC PATHWAY

Because of a lack of auxin biosynthetic mutants, a genetic approach to the problem of IAA biosynthesis has not been

feasible in the past. Although such mutants still have not been identified, analysis of recently identified Trp auxotrophs resulted in some surprising new data that have dramatically changed our view of IAA biosynthesis. Maize and *Arabidopsis thaliana* Trp mutants clearly showed that IAA biosynthesis can proceed without Trp and that a previously unknown IAA biosynthetic pathway likely branches from the Trp biosynthetic pathway at indole or indole-glycerol phosphate (Fig. 2).

Orange pericarp maize is a double mutant defective in both Trp synthase β genes. In vivo labeling with [^{15}N]anthranilate revealed that this mutant does not synthesize Trp ([^{15}N]Trp was not detected) but does synthesize IAA to levels 50-fold above those of wild type. Feeding deuterium-labeled Trp to the mutant did not result in any incorporation of deuterium into IAA, providing strong evidence for a Trp-independent IAA biosynthetic pathway (Wright et al., 1991).

The *trp2* mutant of *Arabidopsis* is defective in one of two Trp synthase β genes and is a Trp auxotroph in conditions of high light intensity. The *trp3* mutant is defective in Trp synthase α and the *trp1-1* mutant is defective in anthranilate phosphoribosyl transferase activity (Last, 1993). Like the *orange pericarp* mutant, both *trp2* and *trp3* mutants accumulate IAA (38- and 19-fold above wild-type levels, respectively) (Normanly et al., 1993). The *trp2* mutant also accumulates indole. Double labeling of the *trp2* mutant with [^{15}N]anthranilate and deuterated Trp revealed that significantly more ^{15}N was incorporated into IAA than into Trp, which argues against Trp being a precursor. The amount of deuterium incorporated into IAA from deuterated Trp was not significantly above the background nonenzymatic conversion of Trp to IAA.

Although in vivo labeling experiments with *Lemna*, maize, and *Arabidopsis* have uncovered IAA biosynthetic pathways that were not detected by previous methodologies, Trp as a precursor to IAA is certainly not eliminated by these results. *Phaseolus vulgaris* seedlings have proven to be another good model system with which to study IAA biosynthesis. In vivo labeling studies of bean seedlings clearly demonstrated that essentially all of the IAA is derived from Trp (Bialek et al., 1992).

MULTIPLE, DEVELOPMENTALLY REGULATED IAA BIOSYNTHETIC PATHWAYS

Careful investigation into the role played by IAA biosynthesis in somatic embryogenesis has yielded very intriguing information about IAA biosynthesis. Carrot cells, cultured in the presence of the synthetic auxin 2,4-D, proliferate in an undifferentiated state, whereas removal of 2,4-D induces somatic embryogenesis. In this system, IAA is synthesized at all times; however, two different biosynthetic pathways are utilized in a developmentally or 2,4-D-regulated manner. Cells proliferating in the presence of 2,4-D synthesized IAA from Trp. Once 2,4-D was removed and the cells were undergoing embryogenesis, Trp was no longer utilized as a precursor and instead IAA was produced via a Trp-independent pathway (Michalczuk et al., 1992). This is the first clear demonstration of multiple IAA biosynthetic pathways in a single plant type, and it indicates a greater degree of regulatory complexity than has been previously presumed.

There is evidence for multiple pools of Trp throughout the cell and IAA biosynthesis has been shown to occur in chloroplasts as well as in the cytoplasm (reviewed by Nonhebel et al., 1993). For this reason it has been argued that lack of incorporation of label from Trp into IAA could be due to the inability of the amino acid to localize to the site of IAA biosynthesis. The experiments carried out in the carrot system demonstrated that deuterated Trp labels plastids and cytoplasm with similar efficiency (Michalczuk et al., 1992), an important point for interpreting in vivo labeling studies.

Although recent work has provided an exciting impetus to the field of IAA biosynthesis, we are still left with the same question: how do plants make IAA? Both the maize endosperm and carrot cell culture systems will be useful in the characterization of the novel Trp-independent pathways. Recently, Bandurski and co-workers demonstrated the suitability of immature maize endosperm as a model system to study IAA biosynthesis. In this in vitro system radioactive indole was converted into IAA, indicating that all of the enzymes required for IAA biosynthesis must be present (Rekoslavskaya and Bandurski, 1994). Furthermore, Trp does not appear to be the precursor to IAA, since the yield of radioactive IAA from labeled indole was not reduced by the addition of unlabeled Trp (Jensen and Bandurski, 1994). These results are consistent with the in vivo data from the *orange pericarp* mutant.

Those systems in which Trp is confirmed to be the precursor to IAA can now be examined much more quantitatively with isotope dilution analysis to determine which of the previously postulated pathways from Trp are utilized. Below is a summary of those pathways (see Fig. 1).

The Indole-3-Pyruvate Pathway

Nonhebel and Cooney used isotope dilution analysis to establish that indole-3-pyruvate is present as a natural component of tomato (Cooney and Nonhebel, 1991; Nonhebel et al., 1993). In deuterium-labeling studies with seedlings, indole-3-pyruvate incorporated more deuterium than IAA, which would be expected of a precursor to IAA. Because incorporation of deuterium into Trp was lower than into indole-3-pyruvate, Nonhebel and Cooney invoke Trp compartmentation and selective utilization of Trp pools to explain the observed labeling patterns. In light of recent results in other systems, however, their data are not inconsistent with a Trp-independent IAA biosynthetic pathway. Although enzyme activities for each step in the indole pyruvate pathway have been identified in plants, and the genes encoding indole-pyruvate decarboxylase have been isolated from *Enterobacter cloacae* (Koga et al., 1991) and *Azospirillum brasilense* (Costacurta et al., 1994), none of the plant genes encoding these enzymes has been cloned.

The IAN Pathway

IAN and other components of the IAN pathway have been found primarily in the Brassicacae; therefore, this pathway has not been considered to be of general importance. Trp is converted to indole-3-acetaldoxime by an enzyme activity that has been detected in several plant species (Ludwig-Müller and Hilgenberg, 1988). Indole-3-acetaldoxime conversion to indole-3-acetonitrile has been demonstrated in plasma membranes of Chinese cabbage (Ludwig-Müller and Hilgenberg, 1990). The nitrilase that converts indole-3-acetonitrile to IAA has been cloned in *Arabidopsis* (Bartel and Fink, 1994; Bartling et al., 1994), where there are four genes that show differential expression (Bartel and Fink, 1994). Because *Arabidopsis* is particularly amenable to molecular genetic analysis, this pathway has received renewed interest. Although earlier studies suggest that IAN is derived from Trp, IAN accumulates in the *Arabidopsis trp2* mutant, indicating that it could be derived independently of Trp (Normanly et al., 1993).

The majority of IAA in plants is conjugated to sugars, peptides, amino acids, or *myo*-inositol via ester or amide linkages. The identities of IAA conjugates are at present unknown in most plant species, so in order to quantify ester-linked and amide-linked IAA, extracts are treated with base to cleave the conjugates and yield free IAA, which is then fractionated on HPLC. The Brassicacae produce a large variety and quantity of indole compounds, IAN and indoleglucosinolates among them. These compounds present a challenge in the quantitation of IAA, since they can be potentially converted nonenzymatically to IAA in alkaline conditions. Therefore, when measuring conjugated IAA in *Arabidopsis* or other Brassicacae, it is necessary to account for the "background" levels of IAA that are derived from nonenzymatic conversion of IAN. This entails adding ^{13}C-labeled IAN as an internal standard to determine the amount of IAN in the sample under conditions of neutral pH, then subtracting the ^{13}C-labeled IAA and corresponding amounts of unlabeled IAA that result from breakdown of IAN (Normanly et al., 1993). Selective hydrolysis applied to members of the Brassicacae without correcting for IAN conversion is subject to significant error. Under the standard conditions used for extraction of conjugated IAA, indolemethylglucosinolate does not convert to IAA. Knowing the identity of IAA conjugates in *Arabidopsis* would simplify quantitation enormously, since they could then be analyzed directly.

The Tryptamine Pathway

The first step in a third pathway postulated for the conversion of Trp to IAA involves the decarboxylation of Trp to tryptamine by way of TDC. The gene encoding this enzyme has been isolated from *Catharanthus roseus* (De Luca et al., 1989) and tryptamine has been identified as a native compound in tomato by GC-MS (Cooney and Nonhebel, 1991). Transgenic tobacco expressing the *C. roseus* TDC gene under control of the cauliflower mosaic virus 35S promoter accumulated tryptamine but not IAA (Songstad et al., 1990). This appears to negate a role for tryptamine in IAA biosynthesis, although it could be argued that the 35S promoter did not direct expression of TDC in a manner that was temporally and spatially compatible with the other enzymes in this pathway. Deuterium labeling ruled out tryptamine as an intermediate in tomato (Cooney and Nonhebel, 1991), and tryptamine is not universally present in plants; thus, a tryptamine pathway may not be widespread.

D-Trp versus L-Trp

Radiolabeling studies, together with the observation that D-Trp stimulated seedling growth more effectively than L-Trp, led several laboratories to the hypothesis that the D- rather than the L-isomer of Trp is used as the IAA precursor (see Baldi et al., 1991). This theory was supported by the report that 4-Cl-Trp, the expected precursor to 4-Cl-IAA found in pea, also occurred in the D-form (see Sakagami et al., 1993). Baldi et al. (1991) carried out a careful labeling study using *Lemna* as a model system to test this premise and found no evidence for such a pathway. The *Lemna* experiments were performed under aseptic conditions, and uptake of both D- and L-forms of Trp from the medium occurred rapidly. Even after several days, the D-[^{15}N]Trp taken up from the medium was not converted into [^{15}N]IAA, although there was a several hundred-fold enrichment of the D-Trp pool. In addition, only low levels of L-Trp conversion were observed, and this L-[^{15}N]Trp to [^{15}N]IAA labeling occurred without detectable labeling of the D-Trp pool (Baldi et al., 1991).

Conversion of *N*-malonyltryptophan (found in vivo, in both the L- and D-Trp forms) to indole-3-acetaldoxime and then to IAA has been proposed as another route to IAA. However, Ludwig-Müller and Hilgenberg (1989) showed that whereas *N*-malonyltryptophan was converted, it was *N*-malonyl-L-tryptophan that was the substrate for this reaction. An additional set of data that is not widely known but that also sheds light on this area shows that 4-Cl-Trp, the expected precursor to 4-Cl-IAA in pea seeds, occurs primarily in the L-form. Contrary to previous reports, only about 2% of 4-Cl-Trp is in the D-form and the bulk of 4-Cl-Trp is in the L-isomer (Sakagami et al., 1993). These results suggest that only L-Trp can be converted into IAA.

OTHER AUXINS

Although IAA was the first auxin isolated and is the major auxin, other compounds with auxin activity occur in plants as well. Most of these compounds are active only at higher concentrations than IAA and their role in growth remains largely unknown. IBA and 4-Cl-IAA are two indolic auxins other than IAA with significant biological activity. IBA has recently been positively identified in plants by GC-MS (Epstein and Ludwig-Müller, 1993). The role of IBA in plant growth regulation is unknown, although it is implicated in root formation and widely used commercially for induction of adventitious rooting. The interconversion of IBA and IAA occurs in plants invoking a mechanism of chain lengthening and β-oxidation, analo-

gous to that occurring in fatty acid biosynthesis. Nothing is known about the regulation of such reactions.

A highly active halogenated indole auxin, 4-Cl-IAA, has been identified in a number of plants, mainly members of the Fabaceae, but also in pine seeds (Ernstsen and Sandberg, 1986, and refs. therein). In bioassays, 4-Cl-IAA has been shown to have up to 10 times the biological activity of IAA. 4-Cl-IAA occurs as the methyl ester in many of the plants examined, although 4-Cl-IAA aspartate and its monomethyl ester have also been described. As with IBA, a clear physiological role for 4-Cl-IAA has not been established, although the recent report of its activity in the stimulation of pod growth in deseeded pea, where other auxins are weak or inactive, and its presence in seeds and pod tissue suggest a function in pod development (Ozga et al., 1993).

WHY NO AUXIN AUXOTROPHS?

Mutants defective in biosynthesis of such a major hormone might very well be inviable. The discovery of multiple IAA biosynthetic pathways provides an additional explanation for the lack of auxin auxotrophs. The interdependence of these pathways may be the real reason for the lack of auxin biosynthetic mutants. Are these pathways separable in space and time? Would a block in one pathway result in compensation by another pathway? Data regarding these issues are accumulating as a consequence of the newly available genetic and quantitative tools, but explanations are still only working hypotheses.

IS IAA CONJUGATION A KEY POINT OF REGULATION?

In mutants or transgenic plants that accumulate IAA, the levels of free IAA generally remain normal while conjugated IAA accumulates. This leads to the speculation that free IAA is the biologically active form and that conjugation of IAA is a control mechanism for hormone levels. The IAA-conjugating and -deconjugating enzymes are therefore of great interest from a regulatory standpoint. Hangarter and Good (1981) showed that auxin conjugates could be used as "slow-release" forms of IAA in plant tissue cultures. They attributed differences in physiological activity and persistence to the slow hydrolysis of IAA amino acid conjugates, although hydrolysis rates were not measured. The in vivo hydrolysis of IAA amino acid conjugates was studied by applying radioactive conjugates to bean stems and measuring the release of free IAA by reverse isotope dilution analysis (Cohen et al., 1988, and refs. therein). The rate of hydrolysis was found to correlate positively with stem bending. Conjugate hydrolysis has been extremely difficult to reproduce in vitro, thereby hindering the isolation of these enzymes. An extract capable of hydrolyzing IAA-amino acid conjugates was prepared from bean tissue (Cohen et al., 1988), but the activity was too labile for purification. Kuleck and Cohen (1992) reported the isolation of a similar, also labile, enzymatic activity from carrot cell cultures. This enzyme showed specificity for IAA-Ala, IAA-Phe, and related amino acid conjugates. A report that the protein encoded by the *Agrobacterium rhizogenes rolB* gene might catalyze the hydrolysis of auxin conjugates has now been shown to be incorrect (Nilsson et al., 1993).

The pathways for auxin conjugation have been studied most extensively in the endosperm of maize kernels (for review, see Bandurski et al., 1994), where esters among IAA and inositol, Glc, inositol glycosides, and glucans account for essentially all of the IAA present. Enzymes that catalyze the following reactions have been described, and in some cases at least partially purified:

$$\text{IAA} + \text{UDP-Glc} \leftrightarrow \text{1-}O\text{-IAA-Glc} + \text{UDP}$$

$$\text{1-}O\text{-IAA-Glc} + \textit{myo}\text{-inositol} \rightarrow \text{IAA-}\textit{myo}\text{-inositol} + \text{Glc}$$

$$\text{IAA-}\textit{myo}\text{-inositol} + \text{UDP-Gal} \rightarrow \text{IAA-}\textit{myo}\text{-inositol-Gal} + \text{UDP}$$

$$\text{IAA-}\textit{myo}\text{-inositol} + \text{UDP-Ara} \rightarrow \text{IAA-}\textit{myo}\text{-inositol-Ara} + \text{UDP}$$

$$\text{1-}O\text{-IAA-Glc} \rightarrow \text{IAA} + \text{Glc}$$

$$\text{IAA-}\textit{myo}\text{-inositol} + \text{Glc} \rightarrow \text{6-}O\text{-IAA-Glc}$$

$$\text{6-}O\text{-IAA-Glc} \rightarrow \text{IAA} + \text{Glc}$$

In addition, in vitro evidence exists for the conversion of IAA-*myo*-inositol-Ara and IAA-*myo*-inositol-Gal back to IAA-*myo*-inositol. The first gene for a plant enzyme involved in IAA metabolism to be cloned is the maize gene for the IAA-Glc synthase (Szerszen et al., 1994). Tobacco plants overexpressing this gene showed reduced apical dominance and weak geotropism but normal flowering.

A novel peptide conjugate has been identified in bean seedlings (Bialek and Cohen, 1986). Antibodies raised against the 18-amino acid peptide can be used to determine the prevalence of this conjugate in other plant species. Cloning the gene encoding this peptide will be useful for investigating the role of protein-IAA conjugates in plants and the regulation of peptide conjugation.

A number of IAA-induced genes show significant homology to GST. A 25-kD polypeptide with significant homology to tobacco and maize GST was isolated from *Hyoscyamus muticus* based on its binding to azido-IAA (Bilang et al., 1993). This protein had GST activity and in fact could be purified based on its affinity for glutathione. In competition assays active auxins were able to inhibit labeling of this GST by azido-IAA, giving rise to speculation that GST may play a role in IAA metabolism. It has been speculated that GST facilitates the formation of the conjugate IAA-Glc via an IAA-CoA intermediate. The recent isolation and cloning of the IAA-Glc synthase precludes this possibility, however, since this enzyme does not utilize IAA-CoA as an intermediate and the gene has homology to a Glc transferase (Szerszen et al., 1994).

IAA TURNOVER

The level of IAA available to mediate a biological response in a cell at any given time is regulated by a number

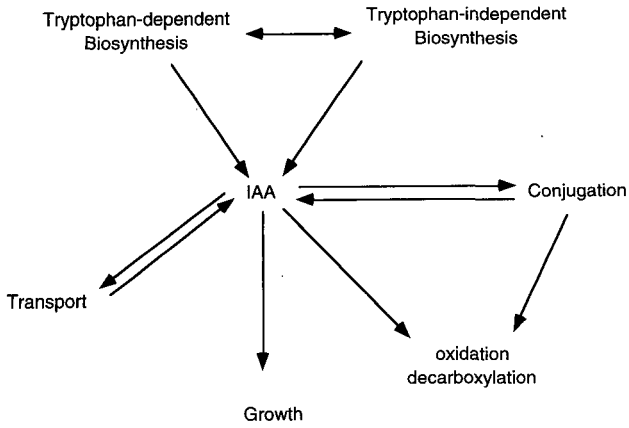

Figure 3. Factors affecting IAA levels in plant cells.

of factors: biosynthesis, conjugation, transport, and degradation (Fig. 3). While biosynthesis and conjugation have been the focus of intense study with recent breakthroughs, degradation or "turnover" has been largely ignored until recently. The isolation of α-methyl-Trp-resistant lines of *Lemna* is providing new insight into the importance and dynamic nature of IAA turnover (Tam et al., 1995). In these lines, anthranilate synthase is resistant to feedback inhibition by Trp, which results in Trp accumulation. Indole and indole-3-glycerol phosphate might also be expected to accumulate and lead to increased IAA levels, yet GC-MS analysis showed only a slight increase in free IAA and no change in conjugated IAA levels. Most interestingly, the half-life of IAA was 1 h, 10 times faster than that of wild type. Somehow, degradation of IAA has increased in response to the greater flux of metabolites through the Trp biosynthetic pathway. With the sensitivity afforded to IAA analysis by GC-MS, it should now be possible to make a thorough examination of IAA turnover as a function of developmental and environmental state.

Whatever Happened to IAA Oxidase?

It is also important to note that our ideas concerning IAA catabolism have recently undergone substantial revision. IAA catabolism was thought to occur primarily through the action of IAA oxidase, a companion activity to most peroxidases of plant origin. This concept has now received serious challenge in that (a) the products of "IAA oxidase" (noted by the loss of the carboxyl carbon) do not appear to be present in plants in significant amounts (Ernstsen et al., 1987), and (b) experiments with transgenic plants show no change in IAA levels even with a 10-fold increase in peroxidase expression or a 90% decrease in peroxidase levels (Lagrimini, 1991).

Recent data provide evidence for two nondecarboxylative oxidation routes. In the first, IAA is oxidized to oxindole-3-acetic acid and subsequently glycosylated through an added 7-OH. An enzyme from maize has been isolated that oxidizes IAA at the 2 position in a nondecarboxylating manner, and this product, oxindole-3-acetic acid, is found in plants in quantities similar to that of IAA (Reinecke, 1990). In a second pathway, IAA is conjugated to aspartate and subsequently oxidized (Tsurumi and Wada, 1990). The resultant oxindole-3-acetylaspartate can either be further oxidized to the 3-hydroxy derivative or hydrolyzed back to oxindole-3-acetic acid (Tuominen et al., 1994). Recent progress in this area highlights the need to study turnover as a component of IAA regulation.

CONCLUSIONS

Within the last 10 years the basic assumptions about how indolic auxin compounds are made and degraded in plants have changed. These changes have created new challenges to our understanding of how auxins regulate development in plants and how, in turn, development regulates the rates of production, rates of degradation, type of indolic auxin, and even the pathways that will be used to produce these compounds.

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