The Plant Cell, Vol. 13, 1, January 2001, www.plantcell.org © 2001 American Society of Plant Physiologists

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New Insights into Auxin Biosynthesis

It is well known that auxins, primarily indole-3-acetic acid (IAA), exert control over many important developmental processes in plants, including cell division and cell expansion, vascular tissue differentiation, root initiation, apical dominance, gravitropic and phototropic responses, flowering, fruit ripening, leaf senescence, and abscission of leaves and fruit. Despite the wealth of knowledge regarding the physiological roles and importance of auxin in controlling plant development, the biosynthetic pathway to IAA remains enigmatic, perhaps due to the existence of multiple pathways and, possibly, functional redundancies among various participating enzymes (Bartel, 1997; reviewed in Normanly and Bartel, 2000). Further complicating the issue, most of the IAA in plants is conjugated (a portion reversibly) to a variety of amino acids, peptides, and sugars. The starting point for IAA synthesis is in the Trp biosynthetic pathway, and in recent years it has become generally accepted that there exists a "Trp-independent" pathway (branching off from the Trp biosynthetic pathway at a Trp precursor, such as indole) as well as a Trp-dependent pathway. The notion of a Trp-independent pathway is based, in part, on the observation that mutants of Arabidopsis with reduced levels of Trp synthase have increased amounts of IAA conjugates (Normanly et al., 1997). However, Müller and Weiler (2000a) have now cast doubt on the existence of a Trp-independent pathway to IAA in Arabidopsis. Using isotope labeling, they showed that the apparent increase in IAA conjugates in Trp synthase mutants in fact results from degradation of indole-3-glycerophosphate, the IAA (and Trp) precursor that hyperaccumulates in the mutants, suggesting that the Trp-independent pathway to IAA may be an artifact (Müller and Weiler, 2000a). Also, Glawischnig et al. (2000) have shown definitively that a Trp-dependent pathway to IAA predominates in maize endosperm tissue; they could find no evidence of a Trp-independent pathway in this system. These observations may explain why there is a paucity of information about biochemical steps involved in the putative Trp-independent pathway (see Normanly and Bartel, 2000), and they put Trp back at center stage as the primary precursor in IAA biosynthesis.

Multiple pathways to IAA still remain under the rubric of "Trp-dependent" IAA pathway. Hull et al. (2000) cite two routes from Trp to IAA as being generally accepted to occur in plants, one leading from Trp through indole-3-acetaldoxime (IAOx) and indole-3-acetonitrile (IAN) to IAA, and one proceeding from Trp via indole acetaldehyde (IAAld) to IAA, but definitive biochemical evidence is lacking and it is unknown if one of these (or some variation) is the principal pathway in vivo. This group identified two cytochrome P450 enzymes, CYP79B2 and CYP79B3, that catalyze the formation of IAOx from Trp. They proposed that the IAOx thus formed could be used in either IAA or indole glucosinolate biosynthesis (Hull et al., 2000). In this issue of *The Plant Cell*, **Bak et al. (pages 101–111)** offer further insight into the route to IAA and glucosinolate biosynthesis through IAOx.

CYP83B1: AT THE METABOLIC BRANCH POINT BETWEEN IAA AND INDOLE GLUCOSINOLATE BIOSYNTHESIS

Several years ago, Winkler et al. (1998) developed a program to systematically isolate lines of Arabidopsis with T-DNA insertional mutations in individual cytochrome P450 genes. Cytochrome P450 monooxygenases (P450s) are heme-thiolate, membrane-localized enzymes involved in the oxidative metabolism of lipophilic substrates in a wide range of secondary metabolic pathways, including the biosynthesis of phenylpropanoids, alkaloids, terpenoids, glucosinolates and cyanogenic glycosides, phytoalexins, and various plant hormones (reviewed in Chapple, 1998). Approximately 250 P450s have been identified in the Arabidopsis genome; the function of the vast majority of these is unknown. One of the P450 T-DNA insertional mutants identified, named *rnt1-1*, showed a phenotype similar to auxin-overproducing mutants such as *sur1* and *sur2* (described in Delarue et al., 1998), including an elongated hypocotyl, epinasty of the cotyledons, and a proliferation of lateral roots. Bak et al. (2001) show that *RNT1-1* encodes the P450 CYP83B1, which converts IAOx to its corresponding *aci*-nitro compound, the first step in indole glucosinolate biosynthesis. This represents a metabolic branch point between IAA and indole glucosinolate biosynthesis in Arabidopsis, and CYP83B1 is found to play a role in regulating auxin homeostasis. In the absence of CYP83B1 activity, IAOx is converted to IAA, which hyperaccumulates in the *rnt1-1* mutant and gives rise to the classic auxin overproduction phenotype. The *RNT1-1* gene was thought to be an allele of *SUR2*, based on physical map data and phenotypic analysis. In another report published this month in *Proceedings of the National Academy of Sciences*, Barlier et al. (2001) confirm that the Arabidopsis *SUR2* gene encodes CYP83B1. The work of Bak et al. (2001) and Barlier et al. (2001) establish the importance of CYP83B1 as a

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regulatory enzyme in the IAA biosynthetic pathway in Arabidopsis.

IAN AND IAALD: MYSTERY PLAYERS

So how is IAOx converted to IAA? Is there more than one route to IAA via IAOx? And is there a Trp-dependent pathway that does not involve IAOx? As mentioned above, it is often assumed that the conversion of IAOx to IAA proceeds through IAN. IAN can be converted to IAA through the action of nitrilases, the distribution of which appears to be limited to three families (Cruciferae, Graminae, and Musaceae), although it is possible that other plants have similar enzymes that catalyze this reaction. Three nitrilases from Arabidopsis (*NIT1* to *NIT3*) are capable of converting IAN to IAA, and the Arabidopsis *nit1* mutant is insensitive to exogenous IAN, which in wild-type seedlings has effects similar to those of exogenously applied IAA (Normanly et al., 1997). The *nit2* and *nit3* mutants retain IAN sensitivity, suggesting that they do not function in the conversion of IAN to IAA in vivo. A fourth nitrilase, *NIT4*, does not accept IAN as a substrate and probably does not play a role in IAA biosynthesis (Piotrowski et al., 2000). *nit1* mutants do not have a dramatic phenotype in the absence of IAN, and they have levels of endogenous IAN and free and conjugated IAA comparable to wild-type plants, suggesting the existence of another pathway to IAA that does not involve IAN (functional redundancy in the *NIT* genes does not adequately explain the phenotype, because *nit1* mutants are completely insensitive to exogenous IAN). Interestingly, the work of Bak et al. (2001) suggests that IAN is not a direct metabolite of IAOx. *rnt1-1/sur2* mutant seedlings overproduce IAA as a result of a block in the branch point from IAOx to indole glucosinolate biosynthesis. The *nit1-1* mutants are blocked in the conversion of IAN to IAA (i.e., they are insensitive to exogenous IAN). Thus, the *rnt1-1* mutant phenotype should be mitigated in the *nit1-1* background if IAN is the principal metabolite of IAOx en route to IAA. However, the authors found that this was not the case: the *rnt1-1* phenotype was not altered in the *nit1-1* background, suggesting that IAOx may be converted to IAA via another route.

IAAld may be a good candidate for an intermediate between IAOx and IAA: instead of conversion to IAN, IAOx could be reduced to an imine followed by pH-dependent hydrolysis to IAAld, which can be converted to IAA via aldehyde oxidase. The Arabidopsis *sur1* auxin-overproducing mutant was found to have a higher than wild-type level of aldehyde oxidase activity, which was shown to be capable of converting IAAld to IAA (Seo et al., 1998). Barlier et al. (2001) found that IAAld accumulates to high levels in the *sur2* mutant, but labeling experiments showed only minute amounts of label going from IAAld to IAA. They suggest that IAOx is converted to IAA via IAN and that hydrolysis of IAOx to IAAld is essentially a dead-end side path. This view is supported by the observation that the *sur2* phenotype is restored to wild type by low pH; at low pH, even more IAOx would be converted to IAAld instead of IAA, alleviating the overproduction of IAA (Barlier et al., 2001). However, the fact that IAAld is converted only very slowly to IAA in the *sur2* mutant does not preclude its being a normal intermediate in IAA biosynthesis in wild-type plants. As Müller and Weiler (2000a) point out, metabolism in a mutant carrying a block in a biosynthetic pathway may be severely altered from the wild type, and it cannot be taken for granted that blockade of a pathway through mutation will reveal accurate information about pathways normally functional in the wild type. There is still a lot of evidence that the biosynthesis of IAA from Trp proceeds through IAN (Müller and Weiler, 2000a, 2000b; Barlier et al., 2001). Müller and Weiler (2000b) recently characterized an "IAA synthase enzyme complex," a 160-kD complex capable of catalyzing the conversion of Trp to IAA in vitro. The complex contained nitrilase immunoreactivity (although only a fraction of that found in the total crude extract) but no immunoreactivity against aldehyde oxidases, again suggesting that the conversion of Trp to IAA proceeds through IAN. The answer to this mystery awaits complete enzymatic characterization of the steps from IAOx to IAA.

SUR1: ANOTHER KEY PLAYER?

The *sur1* mutant is also of great interest to this story. The *SUR1* gene encodes a protein with high similarity to tyrosine aminotransferase, whose role in auxin biosynthesis remains unknown (Golparaj et al., 1996). The phenotype of *sur1 sur2* double mutants suggests an additive effect of the two mutations. Tyrosine aminotransferase may catalyze the transamination of both dicarboxylic and aromatic amino acids and may have other substrates than tyrosine in vivo, including Trp. As mentioned above, the *sur1* mutant exhibits high levels of aldehyde oxidase activity, which is capable of converting IAAld to IAA. Thus, it will be important to determine the function of the *SUR1* gene in the regulation of auxin homeostasis.

It also will be important to determine how flux through IAOx is regulated in noncruciferous plants, which lack significant glucosinolate production. Bak et al. (2001) suggest that the glucosinolate pathway may have evolved from the IAA biosynthetic pathway independently of the evolution of cyanogenic glycoside biosynthesis. Glucosinolates, which are found primarily in cruciferous and other plants of the order Capparales, are sulfur-containing com-

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pounds that are sequestered in the vacuole. Upon tissue damage, glucosinolates are released and converted into isothiocyanates and other potentially toxic substances, which may function in plant defense against various insect pests and/or fungal pathogens. Cyanogenic glycosides, in contrast to glucosinolates, are widespread among many diverse plant species. Perhaps other branch points exist between IAA and cyanogenic glycoside biosynthesis that have important roles in the regulation of IAA homeostasis. Interestingly, the cyanogenic glycoside dhurrin is derived from tyrosine through the sequential action of the P450s CYP79A1 and CYP71E1 in Sorghum (Kahn et al., 1999), and Paquette et al. (2000) consider CYP71E1 to be related to the CYP83 class of P450s. It is intriguing to speculate that *SUR1* occupies a branch point of an alternative Trp-dependent pathway involving IAAld or a step in a pathway from IAOx to IAA via IAAld.

Nancy A. Eckardt
News and Reviews Editor

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