Arabidopsis cytochrome P450s that catalyze the first step of tryptophan-dependent indole-3-acetic acid biosynthesis

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Communicated by Gerald R. Fink, Whitehead Institute for Biomedical Research, Cambridge, MA, December 23, 1999 (received for review December 10, 1999)

Plants synthesize numerous secondary metabolites that are used as developmental signals or as defense against pathogens. Tryptophan (Trp)-derived secondary metabolites include camalexin, indole glucosinolates, and indole-3-acetic acid (IAA); however, the steps in their synthesis from Trp or its precursors remain unclear. We have identified two *Arabidopsis* cytochrome P450s (CYP79B2 and CYP79B3) that can convert Trp to indole-3-acetaldoxime (IAOx), a precursor to IAA and indole glucosinolates.

auxin | metabolism | glucosinolates

Plants are notable in that they make numerous secondary metabolites derived from amino acids and nucleotides (1, 2). Many of these secondary metabolites have roles in plant development and defense against pathogens. Tryptophan (Trp) and its indolic precursors are the sources of indole-3-acetic acid (IAA), certain glucosinolates, and phytoalexins (3). Combined molecular genetic and biochemical approaches have begun to unravel the pathways that are used to synthesize indolic secondary compounds (4, 5). Here we focus on two of the compounds that can be derived from Trp: IAA and indole glucosinolates.

IAA is the major naturally occurring auxin, and its role in plant development has been well documented (ref. 6 and references therein). Labeling studies have demonstrated that IAA can be derived from both Trp-independent and Trp-dependent pathways (reviewed in ref. 4). 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. Several Trp-dependent pathways have been proposed; however, it is not clear whether all pathways exist in every plant species (4). In recent years, two Trp-dependent pathways have become generally accepted as occurring in plants: (*i*) the indole-3-acetonitrile (IAN) pathway [Trp → indole-3-acetaldoxime (IAOx) → IAN → IAA] and (*ii*) the indole pyruvic acid (IPA) pathway (Trp → IPA → indole-3-acetaldehyde → IAA) (4). Although there appears to be redundancy in IAA biosynthesis, evidence suggests that Trp-dependent and Trp-independent IAA biosynthesis are differentially regulated (7–9).

Indole glucosinolates are members of the glucosinolate family (mustard seed oils) of compounds found in *Brassica* species (1, 10, 11). Glucosinolates are sequestered in the vacuole in ''inactive'' forms. Upon tissue damage, glucosinolates are cleaved by myrosinases into unstable glucosinolate aglycones, which spontaneously break down into isothiocyanates and other potentially toxic compounds. These derivatives may function in defense against insects and fungi, although roles as insect attractants also have been reported (1, 12). In addition, some glucosinolate-breakdown products have anticancer activities (13). All glucosinolates are sulfur-containing compounds that have a common structure linked to an R group derived from an amino acid. Enzymatic activities have been described for the steps in glucosinolate biosynthesis, but the genes encoding these activities have yet to be identified (1, 10).

We describe here the isolation and characterization of two *Arabidopsis* genes, *CYP79B2* and *CYP79B3*, that encode cytochrome P450s predicted to *N*-hydroxylate amino acids. These two genes, when expressed in *Escherichia coli*, can specifically convert Trp to IAOx *in vitro*. We also show that *CYP79B2*, when overexpressed in *Arabidopsis* or yeast, confers resistance to toxic analogs of Trp. In addition, *CYP79B2* is expressed in response to bacterial pathogens in a manner similar to Trp biosynthetic genes. Taken together, our data suggest a model in which *CYP79B2* and *CYP79B3* metabolize Trp to IAOx that can be used for either IAA or indole glucosinolate biosynthesis.

Materials and Methods

Selection of *Arabidopsis* cDNAs in Yeast and Plasmid Construction. Unless noted, all yeast and *E. coli* growth and molecular biological techniques followed standard methods (14). Restriction enzymes were purchased from New England Biolabs; [α-^{32}P]dCTP was from NEN; oligonucleotides were from Genemed Biotechnologies (South San Francisco, CA); chemicals were purchased from Sigma. *E. coli* strains expressing *CYP79B2* or *CYP79B3* were grown at 30°C to maintain plasmid stability.

CYP79B2 was isolated as follows. An *Arabidopsis* cDNA expression library (15) was transformed into a diploid yeast strain constructed by crossing L5140 × L5141 (a gift from G. R. Fink, Whitehead Institute for Biomedical Research, Cambridge, MA). L5140 = *MATa ura3–52 leu2–3,112 his3*Δ*200 trp1*Δ*63 ade2 GAL*. L5141 = *MAT*α *ura3–52 leu2–3,112 his3–11,15 lys2* Δ *201 ade2 GAL*. Approximately 200,000 transformants were screened on complete minimal medium lacking uracil and containing 2% galactose, 200 μM 5-fluoroindole (5FI), and 2 mg/liter Trp (instead of the standard 20 mg/liter Trp). 5FI-resistant clones, which conferred galactose-dependent 5FI-resistant growth in yeast, were recovered by transformation into *E. coli* strain DH5α. cDNA clones were subcloned with *Eco*RI into pBluescript KS(+) (Stratagene), sequenced, and determined to be identical to each other and to *CYP79B2* (GenBank accession no. ATT5J17.120). One of these subclones was in frame with the *lacZ* gene carried on pBluescript KS(+) and was used for isopropyl β-D-thiogalactoside (IPTG)-inducible expression in *E. coli* and assayed for Trp-metabolizing activity in Fig. 2*C*.

CYP79B3 cDNAs were cloned as follows. Oligonucleotide primers AO18 (5′-ACGACCAAGTCAAGTCTCGGAATG-

Abbreviations: Trp, tryptophan; IAA, indole-3-acetic acid; IAN, indole-3-acetonitrile; IAOx, indole-3-acetaldoxime; 5FI, 5-fluoroindole; 5FT, 5-fluoroindole; 5MA; 5-methylanthranilate; 5MT, methyltryptophan.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. ATT5J17.120 and AC006592.4).

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Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.040569997. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.040569997

3′) and AO19 (5′-GGATCACGTGAGTGTTTCCTAGAC-3′) were used in a PCR on *Arabidopsis* cDNAs to create a 286-bp fragment identical to the 5′ end of *CYP79B3* (GenBank accession no. AC006592.4). This fragment was used to screen an *Arabidopsis* λ YES cDNA library (15). Five positive clones were isolated, and the two longest clones were subcloned with *Eco*RI into pBluescript KS(+). One of these contained the entire coding region whereas the other lacked the first 8 bp. The incomplete cDNA was in frame with the *lacZ* gene of pBluescript KS(+) and was used for IPTG-inducible expression in *E. coli* and assayed for Trp-metabolizing activity in Fig. 2*C*.

Modification of the 5′ end of the *CYP79B2* cDNA was performed by replacing the first 90 bp of the coding region with a sequence encoding the first 14 aa of CYP17A1 (16). This sequence was encoded by the complementary oligonucleotides AO10 (5′-TCGAGCATATGGCTCTGTTATTAGCAGTTTTTCA-3′) and AO11 (5′-AGCTTGAAAAACTGCTAATAACAGAGCCATATGC-3′). These oligonucleotides, after annealing, create an *Xho*I site at the 5′ end of *CYP79B2* and a *Hin*dIII site at the 3′ end. The annealed oligonucleotides were cloned into the 5′ end of *CYP79B2* carried in pBluescript KS(+) by using *Xho*I and *Hin*dIII. *CYP79B2mod* then was subcloned into pCWori+ by using the *Nde*I site (contained within the annealed oligonucleotides) and an *Xba*I site at the 3′ end of the gene.

For expression in plants, the *CYP79B2* cDNA was subcloned by using *Eco*RI into the pBICaMV expression vector. pBICaMV is derived from pBI121 (CLONTECH) in which the original *Eco*RI site was destroyed and the annealed oligonucleotides (5′-CCGGGTACCGAATTCGAGCT-3′ and 5′-CGAATTCGGTAC-3′) were cloned into the *Xma*I and *Sac*I sites.

Assay for Trp-Metabolizing Activity. Expression of *CYP79B2mod* is under the control of the *tactac* promoter in the plasmid pCWori$^+$ and is inducible by IPTG (17). Membrane extracts were prepared from *E. coli* expressing either CYP79B2mod or a non-P450 cDNA as follows. An *E. coli* culture was grown overnight in LB containing 100 μg/ml Ap, diluted 1:100 into Terrific Broth containing 100 μg/ml Ap, and grown at 37°C at 225 rpm. The culture was grown to an A_{550} of 0.9, 1 mM IPTG and 80 μg/ml δ-aminolevulonic acid was added, and incubation was continued at 30°C and 190 rpm for 72 h. The cells were collected by centrifugation in a Sorvall SH3000 rotor at 4,000 rpm for 15 min at 4°C, washed twice with 4°C Mops buffer (100 mM Mops/10% glycerol/0.2 mM DTT/1 mM EDTA, adjusted to pH 7.3 with NaOH), and resuspended in Mops buffer before sonication on ice in a Fisher Scientific 550 Sonic Dismembrator for 30 × 2-s bursts on setting 3. The membranes were collected by centrifugation at 31,000 rpm in a Beckman Ti60 rotor for 30 min at 4°C, and the membrane proteins were solubilized in 4°C Mops buffer containing 0.5% CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate) by slow rocking on ice for 2 h. The sample was centrifuged again at 31,000 rpm in a Beckman Ti60 rotor for 30 min at 4°C, and the supernatant containing the solubilized membranes was frozen in aliquots. For assays, thawed extracts then were reconstituted with dilaurylphosphatidylcholine, mixed with purified rat NADPH-cytochrome P450 reductase, NADPH, and 750 μM [^{14}C]L-Trp (side chain 3-^{14}C, ≈300,000 dpm; American Radiolabeled Chemicals, St. Louis) (Fig. 2*A*) or 750 μM nonradioactive Trp (Fig. 2 *B* and *C*), and incubated at 30°C for various times. The indolic compounds were extracted with ethyl acetate, evaporated, and applied onto a silica gel 60 F_{254} TLC plate. The compounds were separated in ethyl acetate/chloroform/formic acid (55:35:10). Plates were air-dried and compounds were visualized by autoradiography (Fig. 2*A*) by using a Bio-Rad Molecular Imager or by UV epifluorescence (Fig. 2 *B* and *C*) using a Bio-Rad Fluor-S imager.

IAOx was synthesized as described previously (18, 19). GC-MS analysis of the synthesized IAOx confirmed its identity as represented by ions at m/z 174 and 130 (20).

Transgenic Plants Expressing CYP79B2. *Arabidopsis* (Col-0 ecotype) were transformed (21) with pBICaMV carrying the *CYP79B2* cDNA (see above). Two homozygous transgenic lines were selected that showed the highest level of *CYP79B2* mRNA expression (data not shown).

Northern Blot Analysis. RNA samples prepared at the times indicated from *Pseudomonas syringae* pv *maculicola* (ES4326)-infected *Arabidopsis* were described previously (22). Ten micrograms of total RNA per sample was used to prepare a Northern blot that was probed with either a ^{32}P-labeled *CYP79B2* cDNA fragment or an *Arabidopsis* β-tubulin gene (14).

Results

Isolation of *CYP79B2*. IAA and indole glucosinolates are both derived from Trp or its immediate precursors. However, no gene has been cloned from *Arabidopsis thaliana* whose product can convert Trp or indole to any of the proposed intermediates in IAA or indole glucosinolate biosynthesis. Therefore, we designed a yeast screen to identify *Arabidopsis* cDNAs encoding enzymes capable of indole or Trp metabolism. This screen took advantage of the toxicity to yeast of an analog of indole, 5FI. In yeast, 5FI is converted to 5-fluorotryptophan (5FT) by tryptophan synthase β. 5FT is toxic to yeast for two reasons: first, 5FT gets incorporated into proteins and disrupts protein function, and second, 5FT mimics Trp in its ability to feedback-inhibit anthranilate synthase, thus shutting down further Trp biosynthesis (23).

We screened ≈200,000 *Arabidopsis* cDNAs and identified one clone that conferred 5FI resistance upon retransformation into yeast (Fig. 1*A*). DNA sequencing revealed that this cDNA was identical in part to a previously described cDNA fragment called *CYP79B2* based on its similarity to the Sorghum cytochrome P450, CYP79A1 (25). A comparison of the deduced amino acid sequence of the newly isolated *CYP79B2* clone and CYP79A1 is shown in Fig. 1*B*. Our functionally isolated *CYP79B2* cDNA is full-length based on comparison with the recently sequenced *CYP79B2* genomic region (Arabidopsis Genome Initiative, http://www.arabidopsis.org/agi.html) and with CYP79A1 and other cytochrome P450 sequences. CYP79A1 and CYP79B2 are approximately 49% identical and both contain a predicted N-terminal transmembrane domain found in all cytochrome P450s. However, based on the amino acid composition of the N-terminal 22 aa, CYP79B2 contains a chloroplast transit peptide (26) that is not found in CYP79A1 (27).

CYP79B2 Metabolizes Trp to IAOx *in Vitro*. CYP79A1 is the cytochrome P450 most similar to CYP79B2 whose activity is known. CYP79A1 converts tyrosine to *p*-hydroxyphenylacetaldoxime (27). However, we hypothesized that CYP79B2 would use tryptophan (not tyrosine) as a substrate for the following reasons. First, a *trp1* auxotrophic yeast strain expressing *CYP79B2* requires more exogenous Trp than does the same yeast strain carrying the vector without the *CYP79B2* gene (data not shown). This suggests that CYP79B2 depletes Trp away from its use in protein synthesis. Second, *CYP79B2* expression in yeast does not confer resistance to m-fluorotyrosine (data not shown), suggesting that tyrosine is not a substrate for CYP79B2.

To identify the substrate for CYP79B2 biochemically, we tested the *in vitro* activity of CYP79B2 by expressing an N-terminally modified form of CYP79B2 (CYP79B2mod) in *E. coli*. This modification removes the predicted chloroplast transit peptide and substitutes a different transmembrane domain for the putative transmembrane domain of CYP79B2. This modification enables efficient expression of P450s in *E. coli* (16).

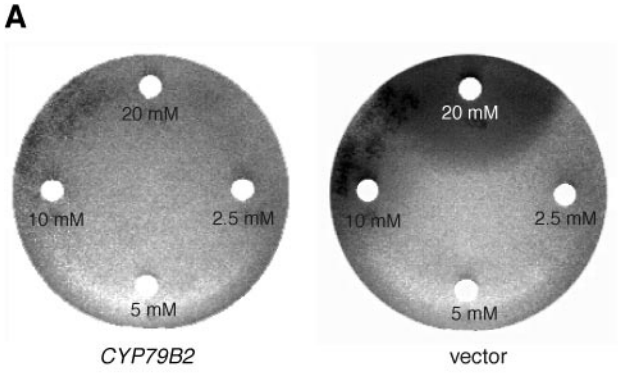

Fig. 1. Identification of *CYP79B2* and *CYP79B3*. (*A*) Yeast expressing *CYP79B2* are resistant to 5FI. A saturated culture (0.1 ml) of diploid strain L5140 × L5141 carrying either *CYP79B2* or the library vector was mixed with 3.7 ml of molten 0.7% agar and plated onto complete minimal medium lacking uracil and containing 2% galactose and 2 mg/liter Trp. After the top agar had solidified, sterile filter disks were placed onto the surface and 10 μl of the indicated concentration of 5FI was pipetted onto the filter. Yeast plates were incubated for 48 h and photographed. (*B*) Alignments of deduced amino acid sequences of the CYP79B2 and CYP79B3 proteins and the Sorghum CYP79A1 protein. Sequences were aligned with the program MEGALIGN (DNAstar, Madison, WI) by using the Clustal method (24). Amino acid residues identical in at least two of three sequences are boxed, and hyphens indicate gaps introduced to maximize alignment. GenBank accession numbers for the previously identified sequences are: *CYP79B2*, ATT5J17.120; *CYP79B3*, AC006592.4; and *CYP79A1*, U32624. Sequences for the newly isolated cDNAs of *CYP79B2* and *CYP79B3* are identical to the previously identified sequences.

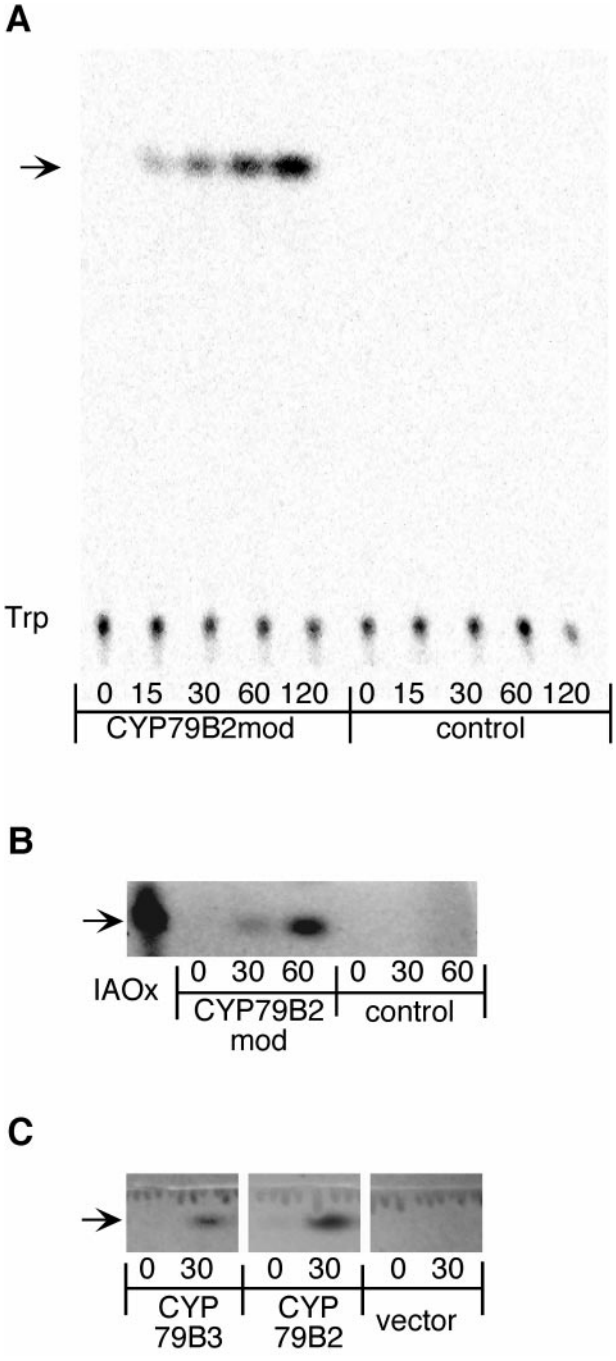

Fig. 2. CYP79B2 and CYP79B3 convert Trp to IAOx. (*A*) CYP79B2 metabolizes Trp. *E. coli* membrane extracts carrying *CYP79B2mod* or a non-P450 cDNA (control) were assayed for Trp-metabolizing activity as described in *Materials and Methods*. Radioactive compounds derived from [^{14}C]Trp were detected by using a Bio-Rad Molecular Imager. The positions of Trp and the reaction product (arrow) are indicated. Reaction times are indicated in minutes. (*B*) IAOx comigrates with the CYP79B2mod reaction product. Similar samples as in *A* were assayed for Trp-metabolizing activity except that nonradioactive Trp was substituted for [^{14}C]Trp. Indolic compounds were visualized by UV epifluorescence by using a Bio-Rad Fluor-S imager. Positive images were inverted into negative images by using Adobe PHOTOSHOP LE 3.0. Images were cropped to exclude nonessential portions. IAOx was loaded as a standard. Reaction products are indicated by an arrow, and reaction times are indicated in minutes. (*C*) CYP79B3 has an activity similar to CYP79B2. Membrane extracts prepared from *E. coli* expressing CYP79B3, CYP79B2, or the pBluescript KS(+) vector were assayed for Trp-metabolizing activity as in *A*, and the reactions were visualized as in *B*. Reaction products are indicated by an arrow, and reaction times are indicated in minutes.

Membrane extracts prepared from *E. coli* expressing either CYP79B2mod or a non-P450 cDNA were assayed for Trp-metabolizing activity, and indolic compounds were separated by TLC. As seen in Fig. 2*A*, a novel product accumulates in a time-dependent manner only in the reactions containing the CYP79B2mod extract. When extracts were prepared from uninduced *E. coli* carrying CYP79B2mod, no products were detected (data not shown). In addition, when [^{14}C]tyrosine or [^{14}C]indole was substituted for [^{14}C]tryptophan, no novel spots were detected (data not shown). These results suggest that CYP79B2 is specific for Trp; strict substrate specificity also has been observed for CYP79A1 (28). Two lines of evidence suggest that the product generated by CYP79B2 in *E. coli* extracts is IAOx. First, the R_f value of the product is identical to the

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published value for IAOx (data not shown; ref. 19). Second, the product comigrates with partially purified IAOx (Fig. 2*B*).

Based on the structure of other cytochrome P450 proteins, the conserved cysteine residue required for heme binding and P450 function is cysteine 477 (C477) (29). To confirm that the CYP79B2 cytochrome P450 activity observed in *E. coli* extracts is responsible for the 5FI resistance conferred by CYP79B2 expressed in yeast, we created a mutant form of CYP79B2mod in which C477 was changed to an alanine (data not shown). This mutant allele of *CYP79B2* showed no activity when expressed in *E. coli* and failed to confer 5FI resistance in yeast (data not shown). This result indicates that the P450 activity responsible for Trp metabolism *in vitro* is also responsible for the 5FI resistance observed in yeast.

A Second *Arabidopsis* Gene also Converts Trp to IAOx. A database search revealed that *Arabidopsis* contains a second sequence predicted to encode a protein with 85% amino acid identity to CYP79B2 called CYP79B3 (Fig. 1*B*). We cloned a *CYP79B3* cDNA and expressed CYP79B3 in *E. coli*. The extract then was assayed for IAOx-forming activity as before except nonradioactive Trp was used in the assay. As seen in Fig. 2*C*, CYP79B3 also can catalyze the conversion of Trp to IAOx.

***CYP79B2* Overexpression in Plants Confers Resistance to Toxic Trp Pathway Analogs.** Because *CYP79B2* expression in yeast confers resistance to 5FI and 5FT, we predicted that overexpression of *CYP79B2* in plants would make plants resistant to these and other Trp pathway analogs. Therefore, we constructed Columbia ecotype (Col-0) *Arabidopsis* overexpressing *CYP79B2* from the cauliflower mosaic virus 35S promoter. Two independently derived transgenic lines with increased *CYP79B2* mRNA levels (data not shown) were tested for their sensitivity to toxic analogs of Trp [5-methyltryptophan (5MT)] or of the Trp biosynthetic intermediates anthranilate [5-methylanthranilate (5MA)] and indole (5FI). Fig. 3 shows the growth of one of these lines compared with Col-0. When grown on unsupplemented medium (Fig. 3*A*), Col-0 and the *35S-CYP79B2* plants grew similarly. However, *35S-CYP79B2* plants were less sensitive than Col-0 to the toxic effects of 5MA, 5MT, or 5FI (Fig. 3 *B–D*).

***CYP79B2* Expression Is Pathogen-Induced.** Because genes involved in Trp biosynthesis and its secondary metabolites are induced in response to pathogens (22, 31, 32), we tested whether *CYP79B2* mRNA expression is induced in response to bacterial infection. A Northern blot was prepared from Col-0 *Arabidopsis* leaves that were infected for various times with either the virulent strain *P. syringae* pv. *maculicola* (ES4326) or the avirulent strain *P. syringae* pv. *tomato* (MM1065) (22). We observed induction of the CYP79B2 mRNA levels 12.5 h after infection by virulent strain (Fig. 4), but not in a mock infection or with the avirulent strain (data not shown). In the mock infection and avirulent strain infection, *CYP79B2* mRNA was not detectable at any time point. The increase in expression after 12.5 h of infection with the virulent strain is similar to the induction seen for the *ASA1* and *ASB1* genes of *Arabidopsis* (22). These genes encode, respectively, α- and β-subunits of *Arabidopsis* anthranilate synthase, the first committed step in Trp biosynthesis. In the analysis of *ASA1* and *ASB1*, a modest induction was seen after infection by an avirulent strain (22). We did not see induction of *CYP79B2* under this condition; however, this could be because *CYP79B2* expression was below the level of detection. Nonetheless, that *CYP79B2* expression parallels that of *ASA1* and *ASB1* suggests that there is coordinate regulation between Trp biosynthetic genes and genes that encode Trp-metabolizing enzymes.

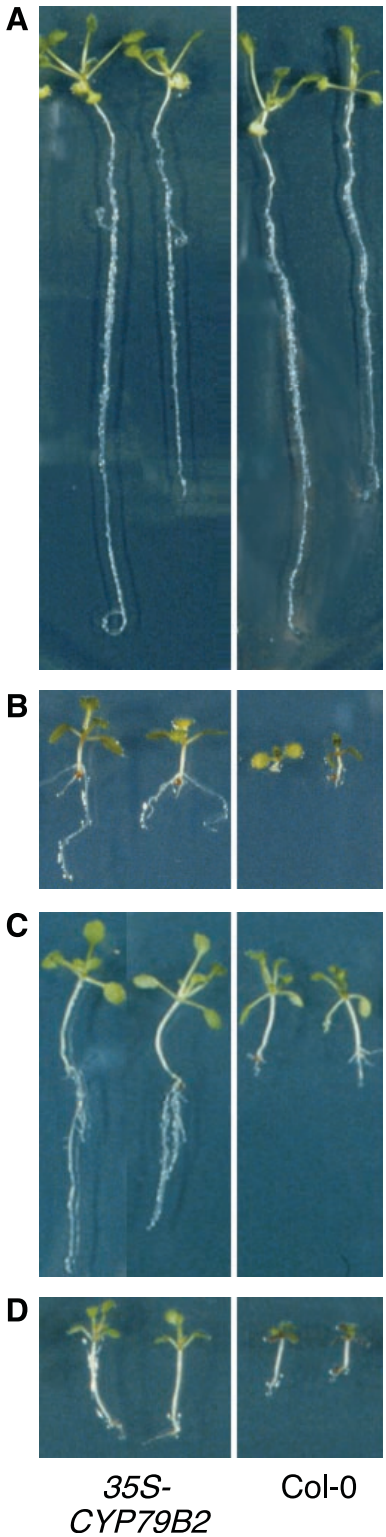

Fig. 3. *35S-CYP79B2* plants are resistant to toxic analogs of Trp and its precursors. *35S-CYP79B2* and Col-0 plants were germinated and grown for 10 days on PNS medium (30) containing no supplement (*A*), 10 μM 5MA (*B*), 15 μM 5MT (*C*), or 10 μM 5FI (*D*). Plants were positioned horizontally on fresh, unsupplemented PNS medium for photography.

Discussion

We have cloned and characterized two *Arabidopsis* genes, *CYP79B2* and *CYP79B3*, that encode cytochrome P450s capable

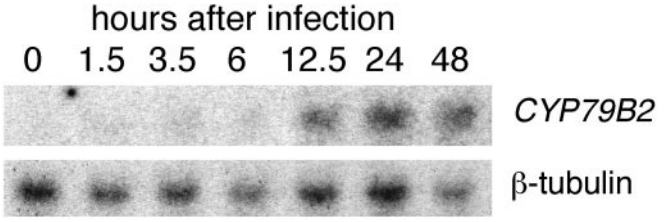

Fig. 4. Induction of the *CYP79B2* mRNA by a virulent bacterial pathogen. RNA samples were prepared at times indicated after *Arabidopsis* was infected with *P. syringae* pv *maculicola* (ES4326). A Northern blot prepared from these RNA samples was probed with the *CYP79B2* cDNA or β-tubulin. The blot was exposed for 7 days (*CYP79B2*) or 24 h (β-tubulin).

of converting Trp to IAOx. IAOx has been proposed as an intermediate for both IAA and indole glucosinolate biosynthesis (see below). Previous reports suggested that in Chinese Cabbage (also a member of the *Brassicaceae*), the conversion of Trp to IAOx is catalyzed by a plasma membrane-associated peroxidase (20), although earlier reports suggested a cytochrome P450-like activity (33). Whether a peroxidase capable of converting Trp to IAOx exists in *Arabidopsis* in addition to CYP79B2 and CYP79B3 is unknown. Besides an *in vitro* Trp-metabolizing activity, several lines of evidence are consistent with CYP79B2 and CYP79B3 having a role in Trp metabolism in plants. First, *CYP79B2*, when overexpressed in plants, confers resistance to toxic Trp pathway analogs (discussed below). Second, *CYP79B2* mRNA expression is induced in response to pathogens in a manner similar to other Trp biosynthetic genes (22). Third, both proteins are predicted to be targeted to the chloroplast, which is the site of Trp biosynthesis (25, 34).

Why does CYP79B2 confer resistance to toxic analogs of Trp when expressed in yeast or when overexpressed in plants? Trp analogs such as 5FT are toxic because they disrupt protein function and feedback-inhibit anthranilate synthase (23). Therefore, any mechanism that increases the ratio of Trp:5FT should relieve toxicity. For example, *Arabidopsis* strains carrying feedback-resistant alleles of anthranilate synthase or mutants that cause increased transcription of the *ASA1* gene confer resistance to toxic Trp analogs (35, 36). From our *in vitro* data, CYP79B2 can metabolize Trp and Trp analogs to IAOx or IAOx analogs (Fig. 2*A* and data not shown). Therefore, overexpression of *CYP79B2* in plants or yeast is predicted to result in a decrease in the concentrations of both Trp and the toxic analogs of Trp and reduce feedback inhibition of anthranilate synthase. An increase in anthranilate synthase activity would cause increased production of Trp, resulting in a higher ratio of Trp:5FT than found in wild-type *Arabidopsis* or yeast. Thus, increased conversion of Trp to IAOx by CYP79B2 should result in resistance to toxic Trp pathway analogs.

What is the role of IAOx in plants? In *Brassicaceae*, IAOx has been proposed as an intermediate for Trp-dependent IAA biosynthesis via the IAN pathway (Fig. 5). CYP79B2 and CYP79B3 therefore are prime candidates for the enzymes that catalyze the first step in this pathway in *Arabidopsis*. Although there is contradictory evidence as to whether IAN is derived

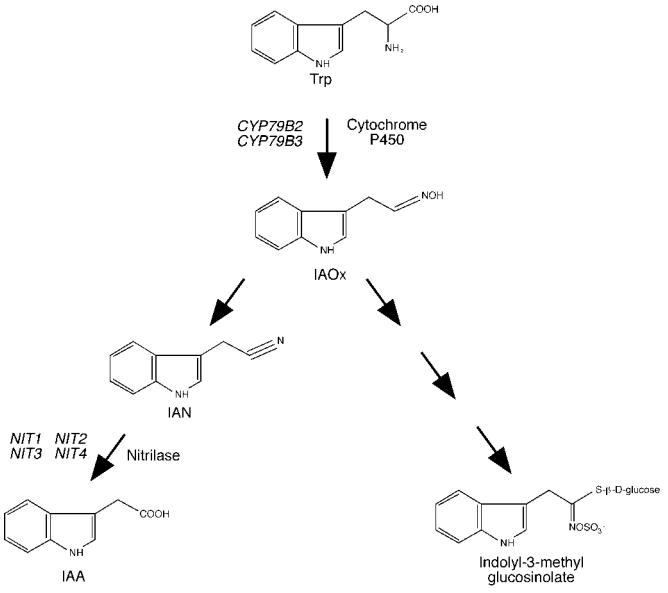

Fig. 5. Trp secondary metabolism derived from IAOx. Cloned genes and corresponding activities for the conversion of Trp to IAOx and for conversion of IAN to IAA are shown. The steps for glucosinolate biosynthesis and other indole glucosinolates are described in ref. 1.

from Trp (37, 38), discovery of CYP79B2 and CYP79B3 suggests that at least a portion of IAN could be Trp-derived via an IAOx intermediate. In addition, the *CYP79B2* and *CYP79B3* genes will be useful in clarifying the roles of the four *Arabidopsis* nitrilase genes (Fig. 5; refs. 31 and 39), whose *in vivo* role in IAA biosynthesis remains unclear (35, 40).

IAOx is also the likely intermediate for indole glucosinolates (Fig. 5). Glucosinolates are derived from oxime derivatives of amino acids; therefore, indole glucosinolates should be derived from Trp via IAOx (10). Thus, *CYP79B2* and *CYP79B3* encode enzymes that make IAOx, which could be used for IAA and/or indole glucosinolate biosynthesis, suggesting a common mode of regulation for these two pathways.

The elucidation of the different IAA biosynthetic pathways is critical to understanding the roles of IAA in plant development. In addition, glucosinolates have been implicated in plant/pathogen interactions, but their function remains unclear. The identification of CYP79B2 and CYP79B3 now provides tools to examine the biosynthesis and functions of these two important Trp secondary metabolites.

We thank M. Waterman for pCWori+; J. Normanly, K. Goss, and D. J. Waxman for help with cytochrome P450 assays; R. Laursen for help with IAOx synthesis; K. K. Niyogi for mRNA; T. Gilmore and R. DiDonato for critical comments on the manuscript; and B. Shea for insightful discussions. A.K.H. was supported in part by a grant from the Sweden-America Foundation. R.V. was supported by National Science Foundation Research Experiences for Undergraduates Grant DBI-9605157. This work was supported in part by National Science Foundation Grant IBN-9514096.

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