Glucose Conjugation of Anthranilate by the Arabidopsis UGT74F2 Glucosyltransferase Is Required for Tryptophan Mutant Blue Fluorescence*

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Plant mutants with defects in intermediate enzymes of the tryptophan biosynthetic pathway often display a blue fluorescent phenotype. This phenotype results from the accumulation of the fluorescent tryptophan precursor anthranilate, the bulk of which is found in a glucose-conjugated form. To elucidate factors that control fluorescent tryptophan metabolites, we conducted a genetic screen for suppressors of blue fluorescence in the Arabidopsis trp1–100 mutant, which has a defect in the second enzymatic step of the tryptophan pathway. This screen yielded loss-of-function mutations in the UDP-glucosyltransferase gene UGT74F2. The bacterially expressed UGT74F2 enzyme catalyzed a conjugation reaction, with free anthranilate and UDP-glucose as substrates, that yielded the same fluorescent glucose ester compound as extracted from the trp1–100 mutant. These results indicate that sugar conjugation of anthranilate by UGT74F2 allows its stable accumulation in plant tissues. A highly related Arabidopsis enzyme UGT74F1 could also catalyze this reaction in vitro and could complement the ugt74F2 mutation when overexpressed in vivo. However, the UGT74F1 gene is expressed at a lower level than the UGT74F2 gene. Therefore, even though UGT74F1 and UGT74F2 have redundant conjugating activities toward anthranilate, UGT74F2 is the major source of this activity in the plant.

The Arabidopsis genome encodes over 100 predicted UDP-glucosyltransferase (UGT) genes (1). These genes are identified by a conserved amino acid motif in the carboxyl-terminal region of the protein sequence that binds the common UDP-glucose substrate molecule. UGT enzymes can form either glucose esters or glucosides with a wide range of substrate molecules. These enzymes serve a variety of important biological functions, including converting metabolically active molecules into inactive storage/transport forms or in some cases generating intermediates for the subsequent conversion into other metabolites. To understand the substrate specificity of the Arabidopsis UGTs, ninety of these enzymes have been expressed in bacteria and are being systematically tested for activity against a battery of potential substrate compounds. This strategy has identified specific Arabidopsis UGTs that can use the plant growth regulator indole-3-acetic acid (IAA) (2), various phenylpropanoid compounds (3), and various hydroxybenzoic acid compounds (4) as substrates in vitro. However, understanding the roles of specific UGTs in the plant is only in its early stages.

The previous in vitro screening strategy identified the UGT74F1 and UGT74F2 enzymes as being able to use benzoic acid, 2-hydroxybenzoic acid (salicylic acid), and 3-hydroxybenzoic acid as substrates for glucose conjugation (4). Here we report that UGT74F1 and UGT74F2 can also use the tryptophan precursor compound 2-amino benzoic acid (anthranilate) as a substrate for glucose ester conjugation both in vitro and in vivo. This finding has important implications for understanding the flux of metabolites in the tryptophan pathway.

The tryptophan pathway is of particular interest in plants, because it provides critical secondary metabolites, including the growth regulator IAA and indole glucosinolate defense compounds (5). Studies of this pathway have been greatly facilitated by the isolation of mutants with defects in tryptophan biosynthetic enzyme structural genes. A striking feature of some tryptophan mutants is a blue fluorescent phenotype under ultraviolet (UV) light, caused by accumulation of the first intermediate compound in the pathway, anthranilate (see Fig. 1). For example, the Arabidopsis mutant trp1–1, which has a defect in the second enzyme of the pathway, is strongly fluorescent throughout the plant (6). Biochemical analysis showed that the major fluorescent compound present in this mutant is an anthranilate-glucose conjugate.

Blue fluorescence due to tryptophan pathway mutations is a valuable reporter phenotype for probing tryptophan metabolism (7–9). This reporter phenotype has also been exploited to study factors that regulate cytosine methylation and gene silencing in plants (10–13). For example, a blue fluorescence Arabidopsis mutation, the weak trp1–100 allele in the gene encoding the second enzyme of the pathway (14, 15), was used to identify second site mutations that block the production of fluorescent compounds (7). In this work, the goal was to recover mutations that impair the synthesis of anthranilate. The suppressor mutations were therefore characterized for whether they conferred tryptophan auxotrophy. This screen yielded two trp4 loss-of-function alleles in the anthranilate synthase β subunit-encoding gene ASB1 (Fig. 1). Several other suppressor

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF498914.

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1 The abbreviations used are: UGT, UDP-glucosyltransferase; IAA, indole-3-acetic acid; Col, Columbia; WS, Wassilewskia; EST, expressed sequence tag; RT, reverse transcriptase; TUB, β-tubulin-4; GST, glutathione S-transferase; HPLC, high-performance liquid chromatography; ORF, open reading frame; SA, salicylic acid; ABA, abscisic acid.

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The tryptophan pathway in Arabidopsis. The series of enzymatic conversions that lead to the synthesis of tryptophan are represented by arrows, with the chemical structures of the first compound in the pathway, anthranilate, and its sugar-conjugated form shown. The trp1 mutations affect an anthranilate synthase (AS) β subunit gene, and the trp1 mutations affect the gene encoding phosphoribosylanthranilate isomerase (PAT).

Mutations isolated in this screen, but because they were not tryptophan auxotrophs they were not studied further.

To better understand the homeostasis of fluorescent metabolites in tryptophan mutants, we extended the previous trp1–100 fluorescence suppressor analysis with a focus on non-auxotrophic isolates. This study yielded a single major complementation group of suppressor mutations with loss-of-function defects in UGT74F2, which catalyzes glucose conjugation to anthranilate through an ester linkage. Our findings provide new insights into specificity and function for the large and important plant UGT enzyme family.

EXPERIMENTAL PROCEDURES

Mutant Isolation—The Columbia (Col) trp1–100 g1–1 strain was mutagenized by ethyl methane sulfonate as previously described (7). Seedlings were scored for fluorescence by plating on plant nutrient plus 0.5% sucrose (PNS) agar medium (16) and inspecting under short-wave UV light from a hand-held source at 2 weeks. This screen yielded the six alleles P155S, E206K, T341I, G344D, W364*, and W421*. The ugt74F2 alleles obtained from a previous screen were isolated by similar methods except that the agar medium contained supplements of tryptophan, phenylalanine, tyrosine, and para-aminobenzoic acid to aid the growth of aromatic amino acid auxotrophic seedlings (7). This selection yielded the four alleles G64D, Q159*, 11a, and Q327*. All the trp1–100 ugt74F2 alleles were back-crossed at least one time to trp1–100 prior to subsequent analysis.

Positional Cloning—The splice junction allele ugt74F2i1a was used for positional cloning of the locus. The Col trp1–100 g1–1 ugt74F2 strain was crossed to WS pat1E151K, and F2 progeny of the cross were scored for non-fluorescence, diagnostic of the wild type. For the 35S-UGT74F2 cDNA construct, 11 out of 38 transformants were fluorescent. For the 35S-UGT74F2 cDNA construct, 23 out of 31 transformants were fluorescent. We suspect that the non-fluorescent 35S-UGT74F2 and 35S-UGT74F1 isolates did not express the transgene sequences due to transgene silencing. Southern blot analysis of the 35S-UGT74F2 cDNA transgenic lines revealed that the non-complementing lines contained high copy numbers of the transgene, consistent with our hypothesis.

RNA Gel Blot Analysis—Plant RNA extractions, formaldehyde gel electrophoresis, transfer to nylon membranes, and hybridization with radiolabeled probes were performed as previously described (23). Probes were cDNA fragments from the indicated genes. The UGT74F2 (At2g43820) probe was an internal NcoI fragment. The UGT74F1 (At2g43840) probe was an internal EcoRV fragment. Neither of these probes was amplified by PCR. Blots were exposed to Xray film, and the autoradiographs were quantified by high stringency Southern blot analysis. The data were analyzed using IOD software. The UGT74F2 and UGT74F1 protein expression and enzyme assays.
mater. An analogous GST fusion construct was made for the UGT74F1 cDNA by similar methods. All constructs were verified by DNA sequencing. The constructs were transformed into the BL21(DE3) bacterial strain harboring a helper plasmid, JY2, that encodes both the AGA-specific argU tRNA and T7 lysozyme (26) for protein expression. Expression was induced by the addition of 0.4 mM isopropyl-1-thio-β-D-galactopyranoside to mid-logarithmic cultures of each strain grown in 2XYT broth (16 g of tryptone, 10 g of yeast extract, 5 g of sodium chloride per liter of water) at 17 °C. Cells were harvested after 16 h of induction. GST fusion proteins were purified from cell lysates using glutathione-coupled Sepharose gel according to the manufacturer’s instructions (Amersham Biosciences) and assessed by Coomassie Blue gel analysis. Enzyme activity assays were performed with 1 μg of purified protein for the conjugated and free anthranilate forms, respectively. The specific activity (μkat/mg) was calculated and Km (Sigma G4511) and upper limit of 15% error on individual measurements) was determined by Lineweaver-Burk plots.

Resolution of free and conjugated anthranilate was achieved over a range of 0.5–2.5 units of mM UDP-glucose. The Michaelis-Menten kinetics parameters were derived from Lineweaver-Burk plots. To estimate the upper limit of Km, substrate concentration was 0.5 μM. Reactions were incubated for 30 min at 30 °C before stopping by addition of 20 μl 240 mg/ml trichloroacetic acid. Reaction products were analyzed by TLC analysis using Silica Gel 60 TLC plates (VWR EM5721-7) using a 55:25:20 ethyl acetate:chloroform:formic acid mixture as mobile phase. Plant extracts for TLC analysis of fluorescent compounds were prepared as previously described (6). For mass spectrometry, fluorescent spots were scraped off of TLC plates, extracted with ethyl acetate, passed over a ZipTip C4 desalting system (Millipore Corp.) according to manufacturer’s instructions, and dried down. Electrospary ionization-mass spectrometry was performed at The Scripps Center for Mass Spectrometry facility. For analysis of conjugate linkages, conjugates were purified from TLC plates as described above. Conjugate treatments were as follows: TLC-purified fluorescent conjugate compounds were resuspended in water and incubated either in 10 mM Tris, pH 6.8, at 37 °C for 5 min (mock treatment), in 0.1 mM sodium hydroxide for 1 h at 25 °C before neutralization with 3 mM sodium acetate, or with 2.5 units of β-glucosidase in 50 mM sodium citrate, pH 5.0, at 37 °C for 5 min (β-glucosidase treatment), or with 2.5 units of a-glucosidase in 10 mM Tris-HCl, pH 6.8, at 37 °C for 5 min (a-glucosidase treatment). Commercially available β-glucosidase (Sigma G4511) and a-glucosidase (Sigma G3651) were used to test the nature of the linkage of the anthranilate glucosyltransferase activity.

Results

Isolation of UGT74F2 Mutations—The ugt74F2 mutations described here were isolated by screening ethyl methyl sulfonate-mutagenized Col trp1–100 seedlings for loss of cotyledon fluorescence (see “Experimental Procedures”). The trp1–100 mutation is caused by a serine to asparagine mutation at codon 15 of the PAT1 gene, which encodes phosphoribosylanthranilate transferase (15). This defect confers strong fluorescence in cotyledons and weaker fluorescence in adult plant tissues without any additional morphological or fertility defects (14). The trp1–100 fluorescence phenotype is caused by accumulation of glucose-conjugated anthranilate (6).

The suppressor screen yielded six independent trp1–100 ugt74F2 mutant isolates that were non-fluorescent both in seedling tissues (Fig. 2) and in adult rosette leaves but displayed weak residual fluorescence in silique tissue (data not shown). The residual silique fluorescence is likely to be due to the activity of an UGT74F2-related gene product UGT74F1 (see Fig. 7 results below). The ugt74F2 suppressor mutations in the trp1–100 background were found to be recessive when back-crossed to the trp1–100 parental strain, yielding fluorescent F1 heterozygous plants and segregating ~25% non-fluorescent seedlings in the F2 generation. The suppressor mutations comprised a single complementation group: pairwise crosses between mutant candidates yielded non-fluorescent F1 heterozygous seedlings. The trp1–100 ugt74F2 double mutants had no obvious morphological phenotypes.

We also received a set of non-auxotrophic suppressors of trp1–100 fluorescence that were recovered in the previous screen that yielded the trp4 mutations (7). These previously isolated mutants included an additional four alleles of ugt74F2, as determined by complementation crosses. One of these alleles displayed only a partial suppression of fluorescence in seedling and leaf tissues.

Positional Cloning of ugt74F2 Mutations—To understand the molecular nature of the trp1–100 suppressor mutants, we cloned the ugt74F2 suppressor locus by positional methods. For this analysis, we took advantage of a fluorescent trp1–100-like mutant that was isolated in the polymorphic strain background WS. The WS mutant carries a glutamic acid to lysine missense mutation of codon 151 of the PAT1 gene (WS pat1E151K). Similarly to trp1–100 mutation in the Col PAT1 gene, the WS pat1E151K allele conferred fluorescence without additional morphological defects. For mapping, a representative Col ugt74F2 trp1–100 (pat1S224N) double-mutant isolate was crossed to the WS pat1E151K mutant, and F2 progeny seedlings were examined for the suppressed non-fluorescent phenotype diagnostic of the homozygous ugt74F2 suppressor mutation. Approximately 25% of the F2 progeny of the mapping cross were non-fluorescent, showing that the ugt74F2 defect can suppress both pat1 mutations segregating in the cross.

Standard mapping analysis on a population of suppressed non-fluorescent F2 plants from the mapping cross revealed linkage to a locus on the lower arm of chromosome 2. Fine-structure mapping of this region on a population of 710 plants (1420 chromosomes) narrowed down the mutant locus to an interval containing ~70 predicted open reading frames (ORFs) (Fig. 3).

Within this interval, we focused on two ORFs encoding glucosyltransferases, UGT74F1 and UGT74F2 (1), as candidate
genes where loss-of-function mutations could block the accumulation of fluorescent glucose-conjugated anthranilate (Fig. 1). Although the UGT74F1 and UGT74F2 genes encode similar predicted protein products (Fig. 4), we identified UGT74F2 as the more likely candidate, because this gene is represented by cDNAs in the Arabidopsis expressed sequence tag (EST) collection, whereas the UGT74F1 gene is not.

We showed that the UGT74F2 gene is in fact the mutant locus with two approaches. First, we cloned and sequenced this gene from the ten mutant isolates, and found a coding sequence G:C to A:T transition mutation (Fig. 1). Although the UGT74F1 and UGT74F2 genes encode similar predicted protein products (Fig. 4), we identified UGT74F2 as the more likely candidate, because this gene is represented by cDNAs in the Arabidopsis expressed sequence tag (EST) collection, whereas the UGT74F1 gene is not.

Second, we found that the cloned UGT74F2 gene introduced on a transgene could complement the ugt74F2 trp1–100 double mutant to restore seedling fluorescence (Fig. 5). Fluorescence was complemented by either a genomic clone carrying UGT74F2 but no other complete ORFs or a cDNA of UGT74F2 driven from the strong constitutive Cauliflower Mosaic Virus 35S promoter (35S-UGT74F2 cDNA). Fluorescence was not complemented by a genomic clone of the related UGT74F1, even in transgenic lines that carried multiple copies of the transgene insert. These results indicate that UGT74F2 is the fluorescent suppressor locus. However, fluorescence was complemented by a 35S-UGT74F1 cDNA construct. This result suggests that the UGT74F1 gene product is capable of catalyzing the same reaction as the UGT74F2 gene product when expressed at sufficiently high levels.

Using sequence information for the trp1–100 mutation and the ugt74F2 splice junction mutation, we designed PCR-based strategies to follow both of these lesions through genetic crosses (see “Experimental Procedures”). The markers allowed us to identify progeny plants of a cross between wild type Col and the trp1–100 ugt74F2i1a splice mutant strain that had segregated the homozygous ugt74F2i1a mutation into an oth-
lower molecular weight degradation products for the UGT74F2 transcript (Fig. 6C). This loss of message stability is presumably due to nonsense-mediated decay (27). The remaining stop mutations, which occur nearer the end of the transcript, did not noticeably destabilize the message. There was also no effect on UGT74F2 message levels in either the weak missense allele ugt74F2G64D or a representative strong missense allele ugt74F2T341I. These results suggest that UGT74F2 transcription is not responsive to the product of the UGT74F2-catalyzed reaction.

To determine whether high ectopic expression of UGT74F2 might confer a fluorescent phenotype in wild type plants by channeling anthranilate into the conjugated form, we transformed Col with the SSS-UGT74F2 cDNA construct. None of 17 transformed plants displayed detectable fluorescence, regardless of transgene copy number (data not shown). This result suggests that there is not enough free anthranilate available in a wild type plant for even the overexpressed UGT74F2 enzyme to make a significant amount of conjugated product.

We also tested the expression patterns of the related gene UGT74F1 using RNA gel blot analysis. The UGT74F1 probe gave no signal in seedling RNA prepared from wild type Col, trp1-100, or trp1-100 ugt74F2 mutants, or from adult tissue RNAs prepared from wild type Col (data not shown). Moreover, the UGT74F1 probe gave no signal in RNA samples prepared from seedlings treated with the same battery of plant signaling molecules shown in Fig. 6B (data not shown). However, we were able to amplify a UGT74F1 cDNA using RT-PCR on template RNA prepared from silique tissue of the trp1-100 ugt74F2 mutant that displayed residual fluorescence. These results suggest that UGT74F1 is expressed but at a lower level than UGT74F2.

Functions of the UGT74F2 and UGT74F1 Proteins—The simplest explanation for our recovery of ugt74F2 mutations as suppressors of trp1-100 fluorescence is that the UGT74F2 protein is required to catalyze the conjugation of anthranilate into the form that stably accumulates in the trp1-100 mutant (Fig. 1). To test this hypothesis, we showed that the bacterially expressed UGT74F2 protein can indeed catalyze the glucose-conjugation of anthranilate in vitro.

UGT74F2 was expressed and purified as a GST fusion protein in Escherichia coli (Fig. 7A and see “Experimental Procedures”). The purified protein was capable of utilizing anthranilate and UDP-glucose as in vitro substrates to yield a product that co-migrated in thin layer chromatography (TLC) analysis with the fluorescent compound that accumulates in trp1-100 (Fig. 7B). In contrast, GST alone did not generate any detectable product in this assay. Both the in vitro reaction fluorescent product and the fluorescent compound from trp1-100 extracts were isolated from TLC plates and subjected to electrospray ionization-mass spectrometry analysis. This analysis revealed that the two compounds are identical and have a mass of 299, consistent with the structure of anthranilate conjugated to glucose (Fig. 1).

The fluorescent conjugated compound that accumulates in the trp1-1 mutant and a maize tryptophan mutant Bf-1 was previously characterized as an “anthranilate β-glucoside,” because it is cleaved by treatment with β-glucosidase (6, 28). However, this compound is more properly described as an anthranilate glucose ester (Fig. 1). To confirm the nature of the chemical linkage between anthranilate and glucose, we treated the fluorescent compound isolated from trp1-100 plants or from the in vitro reaction catalyzed by UGT74F2 with β-glucosidase, α-glucosidase, or base (NaOH) (Fig. 7C and data not shown). As previously reported (6), the compound was labile to β-glucosidase treatment but stable to α-glucosidase treatment.

![Fig. 6. Expression analysis of UGT74F2. A, RNA samples prepared from leaves (leaf), flowers and buds (flower), green siliques (silique), or roots (root) of adult wild type Col plants grown in soil, plus 10-day post-germination and aseptically grown whole Col seedlings (seedling), were used for gel blot analysis with a UGT74F2 probe. The ethidium bromide (EtBr)-stained gel is shown as a loading control. B, wild type Col seedlings were grown aseptically on PNS medium under glass plates for 10 days post-germination and then transferred to inducer-supplemented liquid PNS medium for a 6-h induction with aer-eration before RNA extraction. For each treatment, ~150 seedlings were transferred to 40 ml of liquid medium with the indicated concentration of inducer added: 20 µM methyl jasmonate (MeJa), 20 µM abscisic acid (ABA), 500 µM salicylic acid (SA), 20 µM IAA, 20 µM 6-benzylaminopurine (BAP), 20 µM gibberellic acid A1 (GA), or 20 µM 1-aminocyclopropane-1-carboxylic acid (ACC). The RNA samples were analyzed by gel blot with the UGT74F2 probe, or with a TUB probe as a gel loading control. C, the indicated strains were grown aseptically on agar medium and whole-seedling RNA was prepared at 10 days post-germination. Replicate blots were probed with UGT74F2 or TUB as a gel loading control.](http://www.jbc.org/content/374/12/6279.f6)

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These enzymatic assays indicate that anthranilate is conjugated to glucose through a β linkage. The compound was also labile to base treatment, diagnostic of the glucose ester linkage (3).

To understand the nature of the fluorescent compound that accumulates in the siliques of the otherwise non-fluorescent trp1-100 ugt74F2 plants, we subjected plant extracts made from these siliques to TLC analysis and found that the predominant fluorescent compound corresponded to the glucose ester form of anthranilate (data not shown). This result indicated that there is a residual conjugating activity present in trp1-100 ugt74F2 mutants. Because UGT74F1 is highly related to UGT74F2 (Fig. 4) and was capable of complementing the ugt74F2 mutant when overexpressed (Fig. 5), this enzyme was a likely candidate for the source of a secondary activity. We expressed UGT74F1 as a GST fusion in E. coli and assayed the enzyme in vitro under the same conditions used for UGT74F2 (Fig. 7). As for UGT74F2, UGT74F1 could use anthranilate and UDP-glucose as substrates to form the glucose ester conjugate. Given the similar in vitro activities of UGT74F1 and UGT74F2, we presume that only ugt74F2 mutations were isolated in the trp1-100 suppressor screen, because UGT74F2 is expressed at a higher level than UGT74F1 and is therefore predominantly responsible for anthranilate conjugation in the plant.

Both UGT74F1 and UGT74F2 displayed similar specific activities toward anthranilate (Table I). These values were within 2-fold of specific activities previously determined for both enzymes toward benzoic acid and for UGT74F1 toward salicylic acid (4). However, the specific activity of UGT74F2 toward anthranilate was ~10-fold higher than that previously determined toward salicylic acid (4).

Michaelis-Menten kinetic analysis of both enzymes revealed that UGT74F2 has at least 10-fold higher affinity toward anthranilate than UGT74F1 (Table I). In fact, for measurements of UGT74F2 enzyme kinetics we approached the lower detection limit of our HPLC system, so we were able to place only an upper limit on the value of $K_m$ (see “Experimental Procedures”). The estimate of $k_{cat}/K_m$ lower limit value shows UGT74F2 to be a better enzyme than UGT74F1 for glucose conjugation of anthranilate. The $K_m$ of UGT74F2 for anthranilate is similar to that previously determined for benzoic acid, but $k_{cat}/K_m$ is almost 5-fold higher (4), suggesting that anthranilate is a preferred substrate.

**DISCUSSION**

The blue fluorescent phenotype of plants with defects in tryptophan pathway enzymes is a useful reporter for the flux of tryptophan metabolites and the regulation of tryptophan pathway genes. The phenotype occurs due to the accumulation of tryptophan metabolites and the regulation of tryptophan pathway genes. The phenotype occurs due to the accumulation of tryptophan metabolites and the regulation of tryptophan pathway genes. The phenotype occurs due to the accumulation of tryptophan metabolites and the regulation of tryptophan pathway genes.
as the conjugated form, presumably because it is metabolized into non-fluorescent compounds. Thus, the UGT74F2 gene product might serve a specialized role of stabilizing free anthranilate when it accumulates in excess of the amounts found during normal flux of tryptophan biosynthesis. This stabilization could occur by rendering anthranilate non-reactive and/or by efficient localization of the conjugated form to a particular sub-cellular compartment. Although we do not know the metabolic fate of excess free anthranilate, this metabolism could potentially have deleterious consequences for the plant unless controlled by UGT74F2-mediated glucose conjugation.

Arabidopsis tryptophan biosynthetic enzymes carry predicted amino-terminal chloroplast-targeting sequences and have been shown to be imported and processed into isolated chloroplasts (29). The bulk of tryptophan biosynthesis is therefore thought to occur in the chloroplast, with subsequent transport of metabolites to other cellular compartments. The subcellular localization of UGT74F2 remains to be determined, but the protein lacks an amino-terminal extension with sequences diagnostic of chloroplast targeting. Thus, the UGT74F2 enzyme might be spatially separated from the site of anthranilate and tryptophan biosynthesis in the cell. In this scenario, only when anthranilate escapes from the chloroplast would it become available as a substrate for UGT74F2. Consistent with this model, overexpression of UGT74F2 is not sufficient to confer a fluorescent phenotype in wild type plants.

The UGT74F1-predicted protein is very similar to UGT74F2 (76% amino acid identity, Fig. 4), and both bacterially expressed enzymes can catalyze the glucose ester conjugation of anthranilate in vitro (Fig. 7). Furthermore, when UGT74F1 is overexpressed from a strong viral promoter rather than its own expression sequences, it can complement the ugt74F2 defect (Fig. 5). We presumably recovered mutations only in the UGT74F2 gene as suppressors of trp1–100 seedling fluorescence, because this gene is more abundantly expressed than UGT74F1 in seedling tissues (Fig. 6A and data not shown). However, it is likely that UGT74F1 is the source of residual anthranilate-conjugating activity in the siliques of trp1–100 ugt74F2 mutant plants where a low level of fluorescent-conjugated product still accumulates. It should therefore be possible to isolate ugt74F1 mutations as suppressors of the residual silique fluorescence. Such a suppressor screen would be the most facile means of generating a ugt74F1 ugt74F2 double mutant, because these genes lie only 4 kb apart (Fig. 3) and would be difficult to recombine from single-locus parents.

UGT74F1 and UGT74F2 were previously tested as part of a battery of ninety Arabidopsis glucosyltransferases for activity against benzoic acid and hydroxybenzoic acid compounds in vitro (4). Both enzymes, along with UGT75B1, displayed glucose ester conjugation activity against benzoic acid and 3-hydroxybenzoic acid. In addition, UGT74F1 and UGT74F2 uniquely displayed conjugating activity against SA, which is an important signaling molecule during plant pathogen defense responses (30). The levels of SA, both in the free and conjugated forms, rise in response to pathogen attack. The physiological consequences of sugar conjugation on the SA moiety are not known, but it has been proposed that this conjugation may serve as a detoxifying mechanism for the rapidly rising SA levels (31). Interestingly, UGT74F1 specifically formed the O-glucoside conjugate to the 2-hydroxy group of SA (4). UGT74F2 had lower activity toward SA and preferentially formed the glucose ester conjugate. This difference for in vitro activities advocates that UGT74F1 may be the predominant SA conjugating activity in vivo.

Given that UGT74F1 and UGT74F2 have both been implicated in conjugation of SA and benzoic acid, it is possible that these enzymes only utilize anthranilate as a substrate under exceptional conditions, such as the metabolic blocks found in some tryptophan pathway mutants, where anthranilate accumulates to high enough levels to become an effective substrate for sugar conjugation. However, in the case of UGT74F2, the high activity of the enzyme toward anthranilate (Table 1) suggests that it might have evolved to specifically discriminate this compound as a preferred substrate. Our work, together with previous results (4), indicates that UGT74F1 and UGT74F2 have partially overlapping profiles of benzoate compound substrates and therefore are likely to be partially redundant in the plant, with the specific contributions of each enzyme determined by its intrinsic activity and its expression profile in response to a particular substrate.

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