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Indole-3-pyruvic acid regulates TAA1 activity, which plays a key role in coordinating the two steps of auxin biosynthesis

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Auxin biosynthesis involves two types of enzymes: the Trp aminotransferases (TAA/TARs) and the flavin monooxygenases (YUCCAs). This two-step pathway is highly conserved throughout the plant kingdom and is essential for almost all of the major developmental processes. Despite their importance, it is unclear how these enzymes are regulated and how their activities are coordinated. Here, we show that TAA1/TARs are regulated by their product indole-3-pyruvic acid (IPyA) (or its mimic KOK2099) via negative feedback regulation in *Arabidopsis thaliana*. This regulatory system also functions in rice and tomato. This negative feedback regulation appears to be achieved by both the reversibility of Trp aminotransferase activity and the competitive inhibition of TAA1 activity by IPyA. The K_m value of IPyA is 0.7 μM, and that of Trp is 43.6 μM; this allows IPyA to be maintained at low levels and prevents unfavorable nonenzymatic indole-3-acetic acid (IAA) formation from IPyA in vivo. Thus, IPyA levels are maintained by the push (by TAA1/TARs) and pull (by YUCCAs) of the two biosynthetic enzymes, in which TAA1 plays a key role in preventing the over- or under-accumulation of IPyA. TAA1 prefer Ala among various amino acid substrates in the reverse reaction of auxin biosynthesis, allowing TAA1 to show specificity for converting Trp and pyruvate to IPyA and Ala, and the reverse reaction.

auxin biosynthesis | Trp aminotransferase of *Arabidopsis* | feedback regulation | chemical biology

Significance

The plant hormone auxin (indole-3-acetic acid [IAA]) plays a central role in regulating plant growth and development, but the regulation of IAA biosynthesis is poorly understood. Here, we identified a key push and pull feedback mechanism, which governs auxin biosynthesis through the tryptophan aminotransferase TAA1. We demonstrate that novel inhibitors of TAA1 function in both monocots and eudicots and present a catalytic mechanism that explains both its substrate specificity and its inhibition.

The phytohormone auxin is important for almost all aspects of plant growth and development. The main natural auxin is indole-3-acetic acid (IAA). Spatiotemporal variation in IAA levels in plant tissues is crucial for cell division, elongation, and differentiation. Therefore, endogenous IAA levels must be strictly regulated. This process is mediated by multiple factors, including biosynthesis, metabolism, and transport. Whereas much is known about auxin metabolism, transport, and signaling systems, how auxin biosynthesis is regulated remains largely unknown (1, 2).

IAA biosynthesis that is dependent on L-tryptophan (Trp) is the major pathway of auxin biosynthesis and occurs via two steps (Fig. 1*A*). First, indole-3-pyruvic acid (IPyA), the major intermediate in this pathway, is produced from Trp in a reaction mediated by pyridoxal 5′-phosphate (PLP)-dependent Trp aminotransferases (TAA1/TARs) (3, 4). Second, YUCCAs (YUCs) convert IPyA into IAA (5, 6). This pathway was confirmed by chemical genetic studies of auxin (7, 8). The application of auxin biosynthesis inhibitors targeting either TAA1/TARs (9, 10) or YUCs (11) inhibited auxin biosynthesis and reduced endogenous IAA levels in *Arabidopsis thaliana*, confirming that auxin biosynthesis occurs via a two-step enzymatic process. However, how the endogenous activities of these two enzymes are regulated and coordinated is largely unknown.

Overexpressing *YUC* genes in plants leads to the overproduction of IAA (12), whereas overexpressing *TAA1*s does not lead to the overaccumulation of IAA (3–5). These findings led to the proposal that YUC proteins catalyze the rate-limiting step of the IPyA pathway (5, 12). However, it is not known why IAA does not overaccumulate in *TAA1/TAR*-overexpressing plants. Overexpressing *TAA1* would be expected to result in the overproduction of IPyA, thus leading to the overproduction of IAA, since IPyA is an unstable compound that can be nonenzymatically converted to other compounds, including IAA in aqueous solution. IPyA significantly overaccumulated in YUC-deficient mutants (5), but this increase was less than two-fold, which is milder than the overaccumulation of precursors in typical metabolic mutants. It is also unknown why YUC overexpressors overproduce IAA if IPyA production is not increased accordingly. Based on these findings, the balance between the two enzyme activities appears to be important for maintaining proper IPyA levels to prevent the overproduction of IAA. Here, we elucidated a mechanism that helps maintain proper IAA levels involving the negative feedback regulation of TAA1 activity by its product IPyA.

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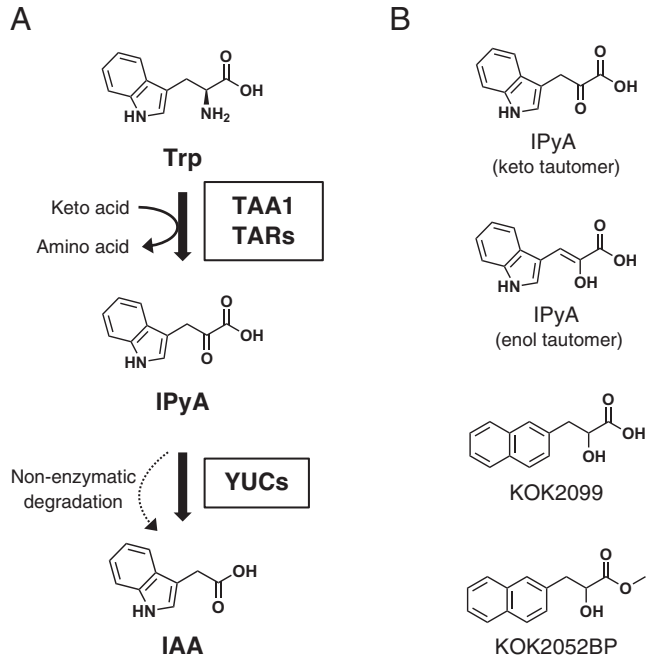

Fig. 1. The auxin biosynthesis pathway and chemical tools used in this study. (*A*) Auxin biosynthesis pathway in *Arabidopsis*. Enzyme names are shown in boxes. (*B*) Chemical structures of IPyA and its analogs used in this study.

Results and Discussion

IPyA and its Analog KOK2099 Inhibit Recombinant TAA1 Activity. Negative feedback inhibition of enzyme activity plays important roles in regulating metabolism, especially when it is undesirable for the product of the enzymatic reaction to accumulate excessively in vivo (13). We reasoned that IPyA might play a key role in regulating auxin biosynthesis, since the overaccumulation of IPyA in vivo would result in nonenzymatic IAA overproduction. In aqueous solution, IPyA exists as both keto and enol tautomers (Fig. 1*B*). To test our hypothesis, we generated the IPyA analog KOK2099 (Fig. 1*B*) and its methyl ester derivative KOK2052BP.

We assessed the inhibitory activity of KOK2099 in a TAA1 enzyme assay (*SI Appendix*, Fig. S1*A*). KOK2099 significantly inhibited recombinant TAA1 enzyme activity in vitro at 100 μM KOK2099 (Fig. 2*A*). We generated a Dixon plot of the effect of KOK2099 on recombinant TAA1 activity (Fig. 2*B*). This plot does not show that KOK2099 is a competitive inhibitor of Trp but suggests that the inhibitor employs complex inhibitory mechanisms.

Since the IPyA analog KOK2099 inhibited recombinant TAA1 enzyme activity, we tested whether IPyA would inhibit TAA1 activity. IPyA strongly inhibited TAA1 activity (Fig. 2*C*), suggesting that IPyA is a natural inhibitor of TAA1 and that there is a feedback inhibitory mechanism for TAA1 activity involving its product, IPyA (*SI Appendix*, Fig. S1*C*). However, in the Dixon plot of TAA1, IPyA did not show competitive or noncompetitive inhibition with Trp. The enzyme activity appeared to be saturated at high Trp or IPyA concentrations (Fig. 2*D*), suggesting that the mode of inhibition was complex under our experimental conditions.

If IPyA, the product of TAA1 enzyme activity, inhibits this enzyme (*SI Appendix*, Fig. S1*C*), removing IPyA from the enzyme reaction mixture should enhance the enzyme activity of TAA1 (*SI Appendix*, Fig. S1*D*). To test this hypothesis, we analyzed the enzyme activity of TAA1 in the presence of YUC. TAA1 enzyme activity (monitored by the amount of Trp consumption) was strongly enhanced in the presence of YUC (Fig. 3*A*). We then tested the activity of the inhibitor KOK2099 in a mixture of TAA1 and YUC (*SI Appendix*, Fig. S1*E*). KOK2099 strongly inhibited TAA1 activity (compare Fig. 3*C* to Fig. 2*A*). The Dixon plot of recombinant TAA1 shows that KOK2099 is a competitive inhibitor of TAA1 (Fig. 3*D*). In competitive inhibition, the K_m values change while V_{max} is constant for different Trp concentrations, resulting in the intersection of the lines at a single point in the plot. For comparison, we also tested the inhibitory activity of KOK2099 in a YUC enzyme reaction. KOK2099 did not significantly inhibit this enzyme reaction (Fig. 3*E*). These results indicate that KOK2099 functions as a mimic of IPyA and inhibits TAA1 enzyme activity as a competitive inhibitor.

We reassessed TAA1 enzyme activity in the presence or absence of YUC and measured its K_m and k_{cat} values (Table 1). TAA1 showed higher k_{cat} and K_m values, resulting in higher catalytic efficiency (k_{cat}/K_m) in the presence of YUC (Table 1). We also examined the Trp aminotransferase activities of FIB from rice (*Oryza sativa*) (14) and SlTAR2 from tomato (*Solanum lycopersicum*) (15). These enzyme activities were higher in the presence than in the absence of AtYUC10 (*SI Appendix*, Fig. S2*A*). Both IPyA and KOK2099 effectively inhibited the activities of FIB and SlTAR2 (*SI Appendix*, Fig. S2*B*). Therefore, the negative feedback regulatory mechanism of TAA1/TARs by IPyA or its analog, KOK2099, is conserved among a monocot and eudicots. The complex enzyme inhibition observed in Dixon plots of TAA1 (Fig. 2 *B* and *D*) can be explained by the presence of a combination of inhibitors. In Fig. 2*B*, the exogenously added inhibitor KOK2099 and the enzyme product IPyA inhibited TAA activity. By contrast, in Fig. 2*D*, exogenously added IPyA and the enzyme product IPyA inhibited TAA1 activity (the amount of the enzyme product IPyA that forms depends on the amount of exogenously added IPyA).

IPyA Levels are Maintained by a Key Push and Pull Feedback Mechanism. Based on these results, we reevaluated previous findings that overexpression of *YUC* genes, but not *TAA1*s, caused IAA to overaccumulate (3–5). We propose that in *TAA1* overexpressors, elevated endogenous IPyA levels inhibit TAA1 enzyme activity via a feedback regulatory mechanism, thereby preventing IPyA overproduction and leading to normal IAA biosynthesis and normal IAA levels (*SI Appendix*, Fig. S3*A*). In *YUC* overexpressors, high YUC enzyme activity reduces endogenous IPyA levels, thereby releasing the feedback inhibition of TAA1, leading to TAA1 activation and increased IAA production (*SI Appendix*, Fig. S3*B*). Therefore, the balance of enzyme activities between TAA1/TARs and YUCs is controlled by IPyA levels. Thus, proper IPyA levels are maintained by the push (by TAA1/TARs) and pull (by YUCs) of the two biosynthetic enzymes.

Reverse Enzymatic Reaction of TAA1. Aminotransferases generally catalyze reversible reactions (16, 17), but it is unknown whether TAA1/TARs have this ability. To gain insight into the regulatory mechanism of the Trp aminotransferase TAA1, we tested the reverse enzyme activity of TAA1 by evaluating if it could convert IPyA to Trp (Fig. 4*A*). This reverse enzyme activity was indeed functional for recombinant TAA1. IPyA was converted to Trp in the presence of TAA1, but not in the presence of heat-inactivated TAA1 (Fig. 4*B*). The K_m value of Trp in the forward reaction was 43.6 μM, whereas that of IPyA in the reverse reaction was 0.7 μM (Fig. 4*A*). Since Trp levels are usually higher than IPyA levels in vivo, the equilibrium of the enzyme reaction is tilted in the direction from Trp to IPyA to ensure IAA biosynthesis. However, our results suggest that if the level of IPyA becomes higher under limited YUC activity, the equilibrium of the TAA1

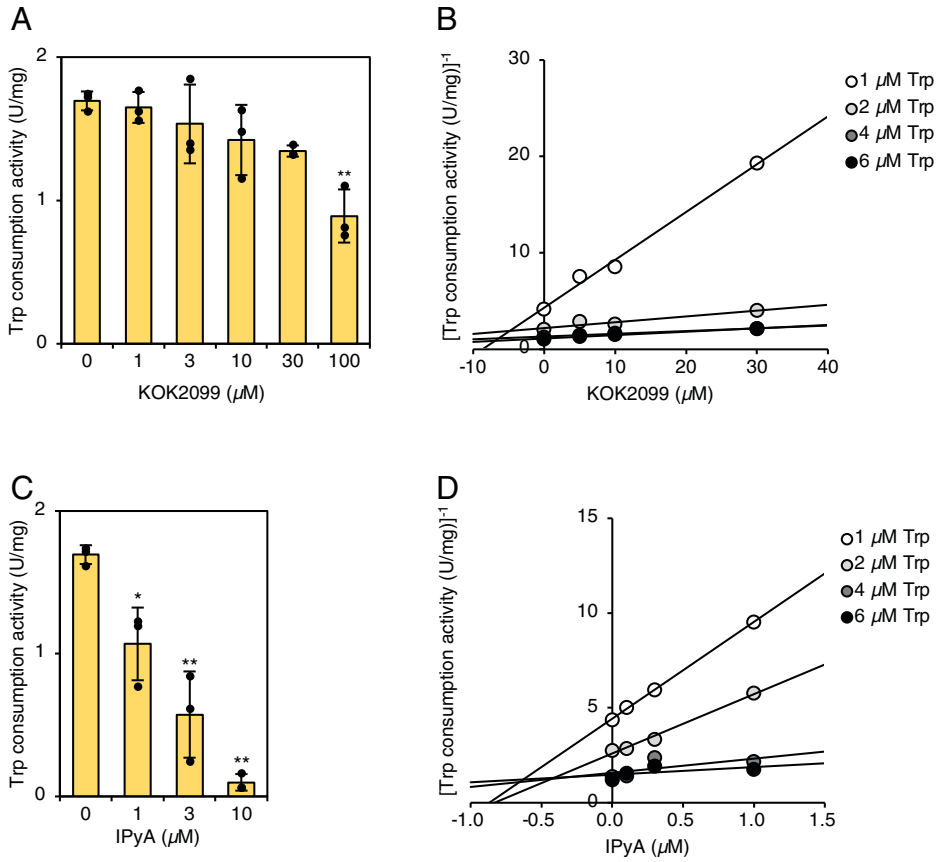

Fig. 2. Inhibition of recombinant TAA1 activity. Inhibition of TAA1 activity by KOK2099. Recombinant TAA1 activity was monitored by measuring the consumption of 10 μM Trp by HPLC with fluorescence detection. (*B*) Dixon plot of the inhibitory effect of KOK2099 on recombinant TAA1 activity at 1, 2, 4, and 6 μM Trp. (*C*) Inhibition of TAA1 activity by IPyA at 10 μM Trp. (*D*) Dixon plot of the inhibitory effect of IPyA on recombinant TAA1 activity at 1, 2, 4, and 6 μM Trp. Values are means ± SD (n = 3). Asterisk indicates a significant difference compared to mock treatment, as determined using the Dunnett's test (*P < 0.05, **P < 0.01).

enzyme reaction is affected. This reversibility of the enzyme reaction appears to be one of the molecular bases underlying the apparent feedback inhibition of TAA1 activity by IPyA. The K_m value of IPyA is much lower than that of any other known plant aminotransferase (16, 17), preventing the undesired accumulation of IPyA in vivo.

Substrate Specificity of TAA1 in the Reverse Reaction. Next, we focused on the substrate specificity of TAA1, which was previously examined in the presence of α-ketoglutarate and various amino acids (4). TAA1 efficiently uses multiple amino acids, such as phenylalanine (Phe), tyrosine (Tyr), and alanine (Ala), in addition to Trp. It is unclear why TAA1 shows such broad substrate specificity, since rigid substrate specificity is required for TAA1 to strictly regulate auxin biosynthesis. Given that TAA1 is an auxin biosynthetic enzyme, the efficient use of Phe and Tyr is reasonable: these compounds can serve as precursors of natural auxins (e.g., phenyl-3-acetic acid). By contrast, why TAA1 uses Ala has been unclear. Here, we analyzed the reverse reaction of TAA1 using various amino acids to identify the preferred amino acid substrate that is accepted in combination with the substrate IPyA. Ala was one of the best substrates in the reverse enzyme reaction of TAA1, and TAA1 only had stronger specificity for Tyr (Fig. 4*C*). Ala aminotransferase perfectly matches the reverse reaction of TAA1 (from Trp and Pyruvate to IPyA and Ala). Therefore, the substrate specificity of TAA1 is specific to mediate the aminotransferase reaction for auxin biosynthesis and its reverse reaction, the Ala aminotransferase reaction.

IPyA Analogs Strongly Inhibit Auxin Activity in *Arabidopsis* Seedlings. We tested the biological activities of the IPyA analogs in vivo in the model plant *Arabidopsis*. KOK2099 and KOK2052BP strongly inhibited primary root elongation and lateral root formation in *Arabidopsis* seedlings (*SI Appendix*, Fig. S4 *A* and *B*). This inhibition was recovered by treatment with exogenous IAA (*SI Appendix*, Fig S4 *D* and *E*), suggesting that KOK2099 and KOK2052BP inhibit auxin biosynthesis in vivo. We then measured endogenous IAA levels using LC-MS/MS. IAA levels were reduced in the presence of 30 μM KOK2099 or KOK2052BP compared to the control (*SI Appendix*, Fig. S4*F*): These compounds are among the strongest auxin biosynthesis inhibitors in vivo compared with previously reported inhibitors (18). Treatment with other IPyA analogs, SAK1019 and KOK3096 (*SI Appendix*, Fig. S5*A*), also inhibited recombinant TAA1 activity (*SI Appendix*, Fig. S5*B*), primary root elongation and lateral root formation (*SI Appendix*, Fig. S6 *A*–*C*), and the inhibition was recovered by IAA treatment (*SI Appendix*, Fig. S6 *D* and *E*). IAA level was reduced in the presence of 30 μM KOK3096 compared to the control (*SI Appendix*, Fig. S6*F*). Therefore, IPyA analogs function as strong auxin biosynthesis inhibitors in *Arabidopsis* seedlings.

Enzymatic Regulation of Auxin Biosynthesis via the IPyA pathway. Auxin levels are regulated both by auxin biosynthesis and catabolism. Both transcriptional and enzymatic regulation have been reported in the auxin catabolic pathways (19–23). For the regulation of auxin biosynthesis, it has been proposed that YUCCA enzymes catalyze the rate-limiting step of the IPyA pathway

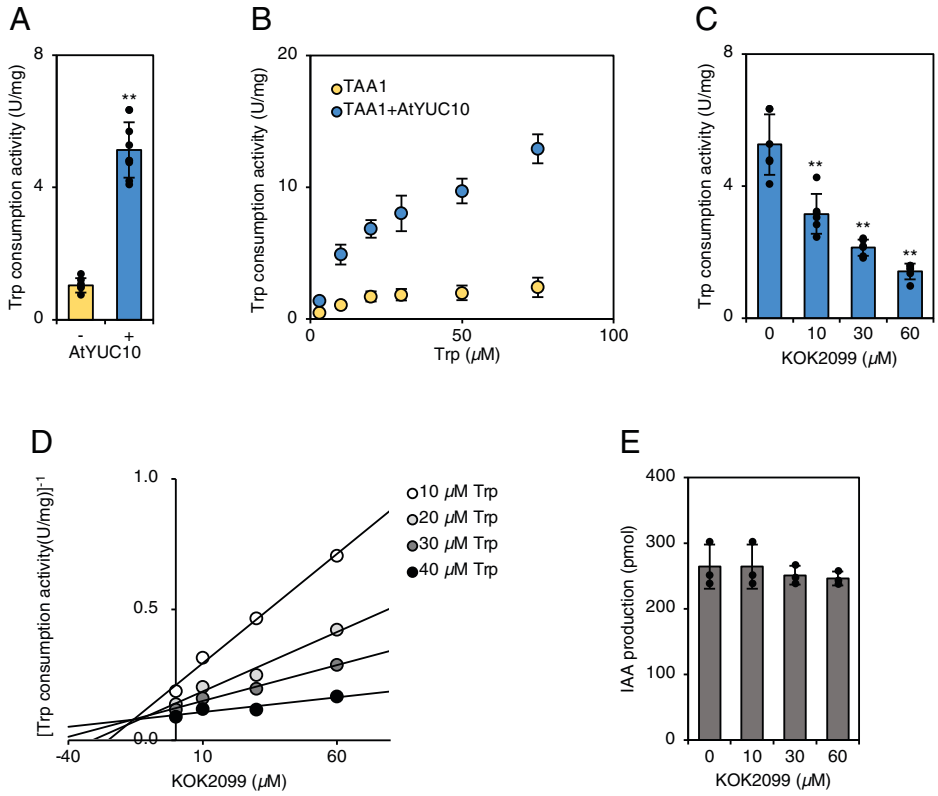

Fig. 3. Reevaluation of TAA1 enzyme activity in the presence of YUC. Activity of TAA1 enzyme in the presence or absence of AtYUC10 at 10 μM Trp. Values are means ± SD ($n \geq 6$). Asterisk indicates a significant difference compared to the absence of AtYUC10, as determined using the Welch's *t* test. (**$P < 0.01$) (*B*) TAA1 enzyme activity in the presence or absence of AtYUC10. Values are means ± SD ($n = 3$). (*C*) Inhibition of TAA1 activity by KOK2099 in the presence of AtYUC10 at 10 μM Trp. Consumption of Trp was analyzed by HPLC with fluorescence detection. Values are means ± SD ($n = 6$). Asterisk indicates a significant difference compared to mock treatment, as determined using the Dunnett's test. (*$P < 0.05$, **$P < 0.01$) (*D*) Dixon plot of the inhibitory effect of KOK2009 on recombinant TAA1 activity in the presence of AtYUC10 at 10, 20, 30 and 40 μM Trp. Values are means ± SD ($n = 3$). (*E*) Inhibition of recombinant AtYUC10 activity. AtYUC10 activity was monitored in the presence of KOK2099 at 3 μM IPyA. IAA production was monitored by HPLC with fluorescence detection. Values are means ± SD ($n = 3$). Significant differences compared to mock treatment were determined using the Dunnett's test.

(12). Transcriptional feedback regulation of YUC genes has been reported, while TAA1/TARs are regulated to a lesser extent by this way (24, 25). However, much less is known about the enzymatic regulation of auxin biosynthesis. Here, we developed KOK2099, an analog of IPyA, and found that it strongly inhibits auxin biosynthesis in vivo. Both KOK2099 and IPyA strongly inhibit TAA1 activity in vitro. Considering the molecular mode of action of KOK2099 based on the biochemical mechanisms of the Trp aminotransferase reaction, it is likely that KOK2099 enters the active site of TAA1 (E-PLP) as a Trp mimic. Trp forms a Schiff base (external aldimine) with TAA1 via its amino moiety (Fig. 5, *Left*), whereas KOK2099 lacks an amino moiety and therefore cannot form a Schiff base with this enzyme (Fig. 5, *Right*). Since KOK2099 cannot be a substrate of TAA1/TARs, it functions as a competitive inhibitor of TAA1/TARs (Fig. 3*D*). Based on this mechanism, we can also consider the mode of action of IPyA as an inhibitor of TAA1 via two possible mechanisms. First, IPyA could serve as a substrate of the reverse enzyme reaction, i.e., Ala aminotransferase activity (Fig. 5, *Left*). If IPyA levels increase, the equilibrium of the enzyme reaction can change. In the second mechanism, IPyA could serve as a Trp mimic and function as a competitive inhibitor in the forward enzyme reaction of TAA1/TARs (Fig. 5, *Right*). These findings suggest that IPyA has mixed complex inhibitory activity in the TAA1/TAR forward enzyme reaction (Fig. 5). Consistent with our findings, IPyA performed better than Trp in a docking experiment with TAA1 (4).

Table 1. K_m and k_{cat} values of TAA1 in the presence of YUC

	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m
TAA1	14.7	9.5	0.6
TAA1 + AtYUC10	43.6	97.1	2.2

Concluding Remarks One of the two steps of auxin biosynthesis involves the one-directional rate-limiting enzyme YUC, and the other employs the reversible regulatory enzyme Trp aminotransferase. TAA1 plays a key regulatory role in avoiding the over- or underaccumulation of IPyA. The different K_m values of Trp and IPyA allow low levels of IPyA to be maintained and prevent the unfavorable nonenzymatic IAA formation from IPyA in vivo. The substrate specificity of TAA1 is specific, allowing it to perform auxin biosynthesis and its reverse enzyme reaction, Ala aminotransferase. The IPyA-dependent regulation of Trp aminotransferase is also functional in rice and tomato, suggesting that this regulatory mechanism of auxin biosynthesis is conserved among monocots and eudicots.

Materials and Methods

Plant Materials and Growth Conditions. *Arabidopsis thaliana* (ecotype Col-0) was used as the wild type. Seeds were surface sterilized and grown on solid half-strength MS medium containing 1% sucrose and 0.8% agar (pH 5.8). Seedlings were grown vertically for 8 d under continuous light at 21 °C.

Plant Growth Conditions for Endogenous Auxin Measurement. *Arabidopsis* seedlings were grown on solid medium for 6 d under continuous light at

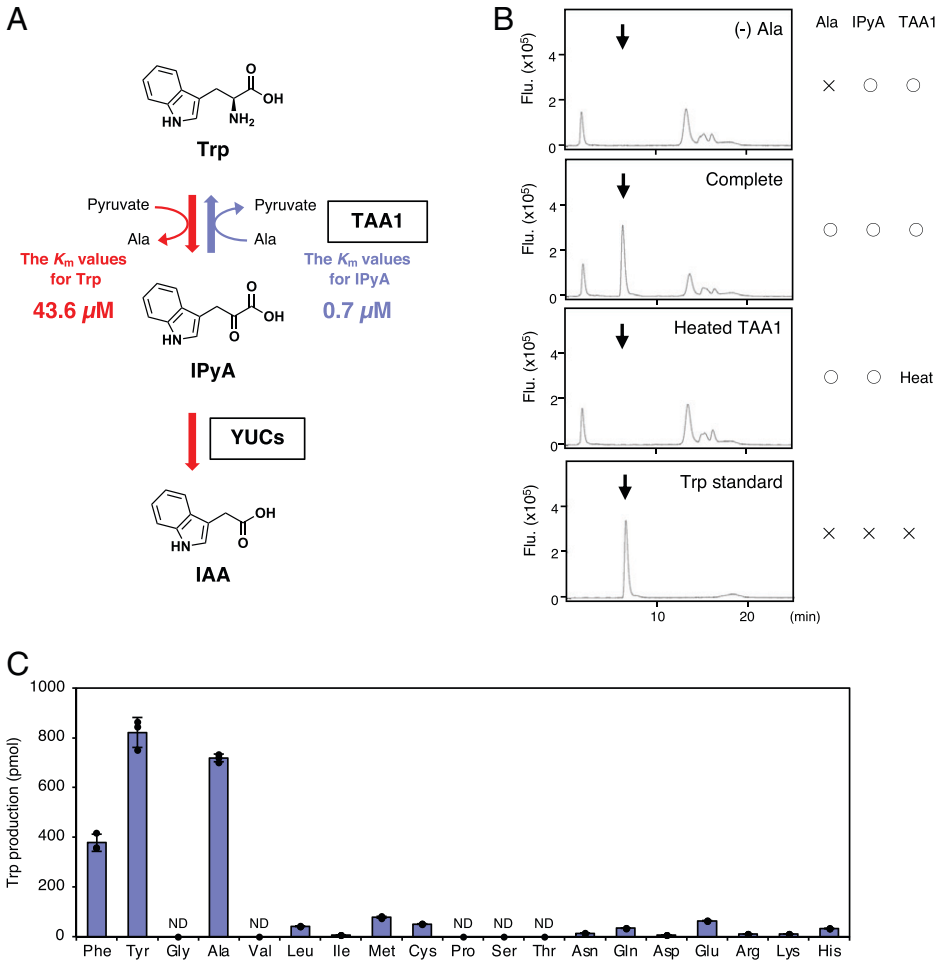

Fig. 4. TAA1 has reversible enzyme activity. (*A*) Reversibility of TAA1 enzyme activity with different K_m values in the forward and reverse enzyme reactions. (*B*) Reverse enzyme activity of TAA1. Enzyme assays were conducted in the presence of TAA1, IPyA, and Ala. The reaction product Trp was measured by HPLC with fluorescence detection. Enzyme reaction without Ala (*Top*), complete enzyme reaction (*Upper middle*), enzyme reaction with heat-inactivated enzyme (*Lower middle*), and Trp standard (*Bottom*). Arrows indicate peaks of Trp. (*C*) Substrate specificity of the reverse enzyme reaction of TAA1. Recombinant TAA1 enzyme was incubated with various amino acids in combination with IPyA. Production of Trp was analyzed by HPLC with fluorescence detection. Values are means ± SD ($n = 3$). ND indicates not detected.

21 °C and transferred to culture tubes containing liquid medium. The seedlings were incubated for 1 d with shaking and treated with or without inhibitors (dissolved in 1,000-fold concentrations of dimethyl sulfoxide [DMSO]) for 3 h. For mock treatment, 1/1,000 DMSO was added to the liquid culture medium. The seedlings were weighed, immediately frozen in liquid nitrogen, and stored at −80 °C until analysis of endogenous IAA levels. Endogenous IAA was measured according to Soeno et al. (18). IAA was extracted from the samples using the modified QuEChERS method (https://www.quechers.eu/) at 4 °C, purified using OASIS HLB and MCX cartridge columns (Waters Corporation, Milford, MA, USA), and analyzed by UPLC-MS/MS (ACQUITY Ultra-Performance Liquid Chromatography-TQ Detector, Waters) with an ACQUITY UPLC BEH C18 Column (Waters).

Preparation of Recombinant Enzymes. The coding sequences of TAA1/TARs or YUC enzymes were amplified from cDNA by PCR using the primers shown in *SI Appendix*, Table S1. The PCR products were introduced into the pCold I vector (Takara) and transformed into *E. coli* SoluBL21 cells (Genlantis), in which the chaperone vector pG-Tf2 (Takara) had been introduced. Expression and purification of the recombinant enzymes were performed as described previously (18).

Enzymatic Assays. A standard Trp aminotransferase activity assay was conducted using the borate buffer system, in which the enol tautomer of IPyA forms a IPyA-borate complex. This complex has strong absorption at 330 nm, allowing the detection of IPyA (*SI Appendix*, Fig. S6). However, this system cannot be used to test the activities of IPyA and its analog KOK2099 for enzyme regulation, since KOK2099 is also a diol compound and forms a complex with borate. Therefore, we developed an enzyme assay system for Trp aminotransferase using Tris·HCl buffer instead of borate buffer. As a result, we were successful to observe feedback inhibition by IPyA in Fig. 2*C*, as well as competitive inhibition in Fig. 3*D*. The assays for TAA1 or TARs were performed with a reaction mixture containing 50 mM Tris·HCl (pH 8.5), 10 μM Trp, 1 mM sodium pyruvate, 10 μM PLP, and 50 ng TAA1 or TARs. The AtYUC10 assay was performed in a reaction mixture containing 50 mM Tris·HCl (pH 8.5), 3 μM IPyA, 1 mM NADPH, 30 μM FAD, and 5 μg AtYUC10. The assays for the simultaneous reaction of TAA1 and AtYUC10 were performed in a reaction mixture containing 50 mM Tris·HCl (pH 8.5), 10 μM Trp, 1 mM sodium pyruvate, 1 mM NADPH, 10 μM PLP, 30 μM FAD, 10 ng TAA1, and 5 μg AtYUC10. All reactions were performed for 5 min at 35 °C and were stopped by adding 10 μL of 1 M HCl. The reaction products were analyzed by high-pressure liquid chromatography (HPLC; Hitachi) with a COSMOSIL 5C_{18}-MS- II column (Nacalai Tesque) with fluorescence detection (kex/kem = 280/355 nm). The Trp transamination activity of TAA1 or TARs was determined by measuring the consumption of Trp. The flavin monooxygenase activity of AtYUC10 was determined by measuring the amount of IAA produced. The inhibitory assay was performed by adding inhibitor to the reaction mixture. The inhibitor was dissolved in DMSO at 100 times the target concentration and added to the reaction mixture. For mock treatment, DMSO was added at the same concentrations. One unit of activity was defined as the amount of enzyme required to consume 1 μmol of Trp equivalent per minute.

IPyA Transamination Activity of TAA1. Two hundred microliters of reaction mixture containing 50 mM Tris·HCl (pH 8.5), 0.5 mM various amino acids,

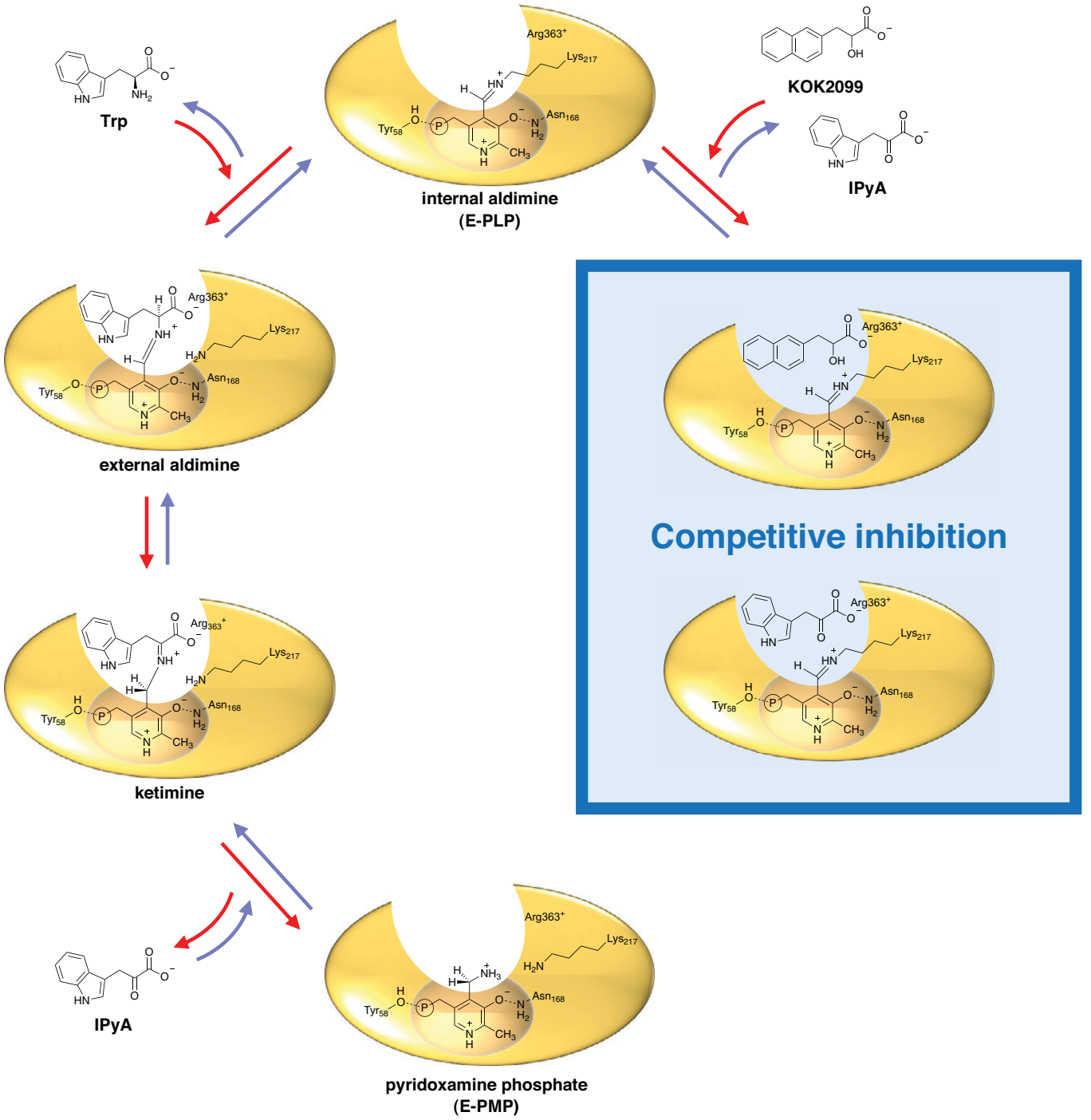

Fig. 5. Proposed scheme of the TAA1 enzyme reaction and its inhibitory mechanism. The mechanism of TAA1 aminotransferase-catalyzed conversion of Trp to IPyA is shown in the pathway on the *Left*. The substrate Trp is ligated to the active site of PLP-Lys217 aldimine (internal aldimine; E-PLP), followed by the formation of a Schiff base (external aldimine), and then via ketimine to give a Michaelis complex (pyridoxamine phosphate; E-PMP) with the release of IPyA. IPyA can be converted to Trp in the reverse flow. The molecular mechanism by which KOK2099 and IPyA serve as competitive inhibitors of TAA1 is shown in the *Right* side of the scheme. KOK2099 enters the active site of TAA1 (E-PLP) as a Trp mimic because the hydroxyl group of its side chain is smaller than the amino group of Trp. Since KOK2099 lacks an amino moiety, it cannot form a Schiff base with the enzyme and thus serves as a competitive inhibitor of Trp in the aminotransferase reaction of TAA1. IPyA enters the active site of the enzyme (E-PLP) as a Trp mimic. IPyA serves as a competitive inhibitor in the aminotransferase reaction of TAA1 because its side chain of the keto/enol moiety is smaller than the amino group of Trp and cannot form a Schiff base with the enzyme. The numbers of amino acid residues are given according to Tao et al. (4). The structure and ionic bonding mode of unliganded Schiff base (PLP-Lys217 aldimine; internal aldimine (E-PLP)), Michaelis Complex and pyridoxamine phosphate (E-PMP) were estimated based on Tao et al. (4) and Eliot and Kirsch (17).

10 μM IPyA, 10 μM PLP, and 0.5 μg TAA1 was incubated at 35 °C for 20 min. The K_m value for IPyA was measured in a reaction mixture containing 50 mM Tris·HCl (pH 8.5), 1 mM Ala, IPyA at a concentration of 0.1, 0.2, 0.4, 0.6, 0.8, or 1.2 μM, 10 μM PLP, and 50 ng TAA1. The reaction was performed for 1 min at 35 °C and was stopped by adding 10 μL of 1 M HCl. The production of Trp was measured by HPLC as described above. One unit of activity was defined as the amount of enzyme required to produce 1 μmol of Trp equivalent per minute.

Statistical Analysis. The following statistical tests were used to calculate the corresponding *P* values. For enzyme assay data, Welch's *t* tests were used for

pairwise comparisons, whereas Dunnett's tests were used to compare multiple sets of data to a control. For the other data, Welch's *t* tests were used for pairwise comparisons, whereas Steel's tests were used to compare multiple sets of data to a control.

Chemical Synthesis of Compounds and Characterizations. Described in *SI Appendix*, *Methods S1*.

Data Availability. All study data are included in the article and/or *SI Appendix*.

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