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The Biosynthetic Pathways for Shikimate and Aromatic Amino Acids in *Arabidopsis thaliana*

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The aromatic amino acids phenylalanine, tyrosine and tryptophan in plants are not only essential components of protein synthesis, but also serve as precursors for a wide range of secondary metabolites that are important for plant growth as well as for human nutrition and health. The aromatic amino acids are synthesized via the shikimate pathway followed by the branched aromatic amino acid metabolic pathway, with chorismate serving as a major branch point intermediate metabolite. Yet, the regulation of their synthesis is still far from being understood. So far, only three enzymes in this pathway, namely, chorismate mutase of phenylalanine and tyrosine synthesis, tryptophan synthase of tryptophan biosynthesis and arogenate dehydratase of phenylalanine biosynthesis, proved experimentally to be allosterically regulated. The major biosynthesis route of phenylalanine in plants occurs via arogenate. Yet, recent studies suggest that an alternative route of phynylalanine biosynthesis via phenylpyruvate may also exist in plants, similarly to many microorganisms. Several transcription factors regulating the expression of genes encoding enzymes of both the shikimate pathway and aromatic amino acid metabolism have also been recently identified in Arabidopsis and other plant species.

INTRODUCTION

The aromatic amino acids (AAA), phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp) (Fig. 1), are central molecules in plant metabolism. Besides their function as building blocks of proteins, the three AAA serve as precursors for a variety of plant hormones, such as auxin and salicylate, as well as for a very wide range of aromatic secondary metabolites with multiple biological functions and biotechnological value in the health promoting, medical and food industries (Bartel, 1997; Vogt, 2010). The AAA of plants are also essential nutritive compounds in the diets of humans and monogastric livestock, which are unable to synthesize them (Li and Last, 1996; Galili et al., 2002). Additionally, the shikimate pathway enzyme 5-enolpyruvylshikimate-3-phospate synthase (EPSP synthase) is the target of the glyphosate herbicide, and non-plant EPSP synthase provides the herbicide-resistance trait in a number of commercial transgenic crops (Duke and Powles, 2008). These important properties account for the major motivation to elucidate the regulation of the shikimate and AAA biosynthesis pathways in plants.

The biosynthesis of AAA from core primary metabolism initiates via the shikimate pathway, leading to the synthesis of chorismate (Fig. 2). Chorismate is the initial branch point metabolite in the synthesis of all three AAA (Fig. 2) and the wide range of aromatic secondary metabolites derived from it (Gilchrist and Kosuge, 1980; Herrmann, 1995). Hence, the shikimate and AAA biosynthesis pathways also represent a major regulatory link of primary and secondary metabolism in plants.

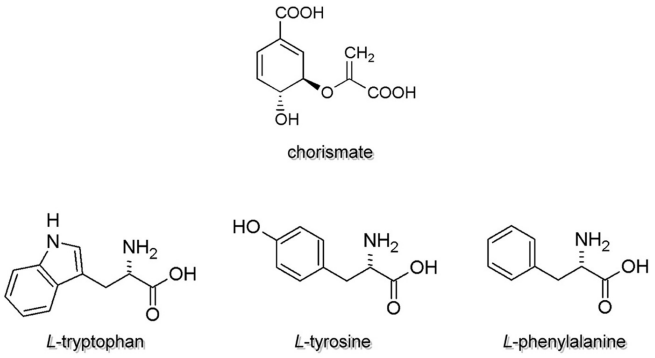

Figure 1. Structures of chorismate and the three aromatic amino acids.

Despite the extreme significance of the AAA to the life cycles of plants, the regulation their biosynthesis via the shikimate and AAA biosynthesis pathways has been largely ignored and even not reviewed in the last decade. Yet, these biosynthesis pathways have been re-visited in recent years by a number of studies. The present review focuses on new insights into the regulation of AAA biosynthesis, which are based on: (i) recent studies, focusing mainly on Phe and to a smaller extent also on Tyr and Trp biosyn-

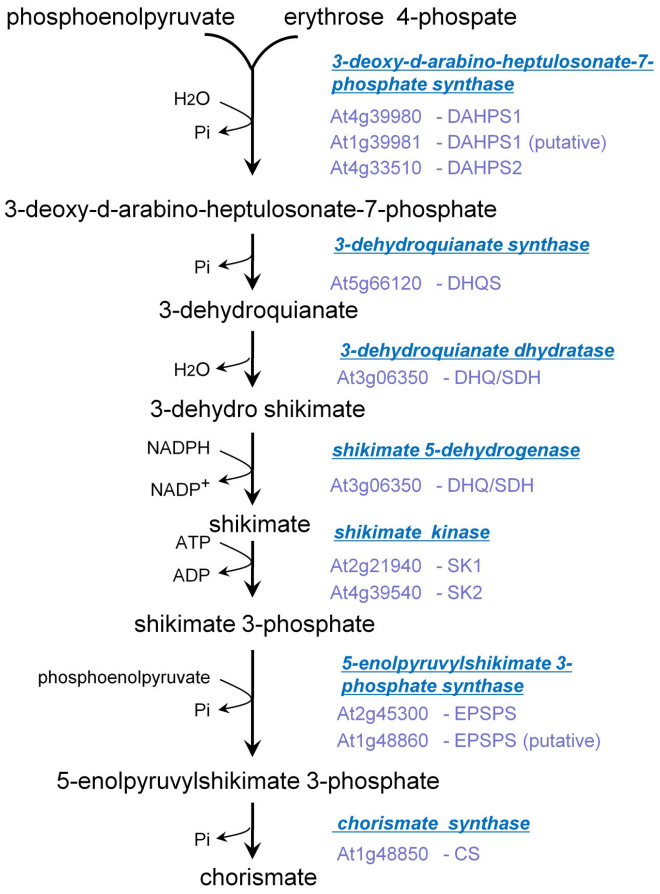

Figure 2. The shikimate pathway. Enzymes involved in the biosynthesis of chorismate.

thesis; and (ii) gene sequence data generated from the sequencing of the entire *Arabidopsis thaliana* (Arabidopsis) genome. A more extensive background on the biochemistry of the shikimate and AAA biosynthesis pathways is available in the following outstanding and most recent reviews dating to the years 1995 and 1999 (Herrmann, 1995; Herrmann and Weaver, 1999).

THE SHIKIMATE PATHWAY

The shikimate pathway, also known as the chorismate biosynthesis pathway, converts two metabolites, phosphoenolpyruvate (PEP) of the glycolysis pathway and erythrose 4-phosphate (E4-P) of the non-oxidative branch of the pentose phosphate pathway, into chorismate (Fig. 2). Genes encoding enzymes of the entire shikimate pathway have been identified in Arabidopsis and other plant species, mostly due to their homology to shikimate pathway genes from microbial organisms. The conversion of PEP and E4-P to chorismate comprises seven reactions catalyzed by six enzymes. The first enzyme of the shikimate pathway is 3-deoxy-d-arabino-heptulosonate-7-phosphate synthase (DAHPS) (EC 2.5.1.54) converting PEP and E4-P into 3- dehydroquaianate (Fig. 2). Arabidopsis plants possess two known DAHPS genes: *AtDAHPS1* (At4g39980) and *AtDAHPS2* (At4g33510) in addition to one putative gene (At1g22410) with high similarity to *AtDAHPS1*. Expression of *AtDAHPS1* in *Escherichia coli* showed that this enzyme requires Mn^{2+} and reduced thioredoxin (TRX) for activity, thereby, linking carbon flow into the shikimate pathway to electron flow from photosystem I (Entus et al., 2002). Despite the metabolic importance of DAHPS as a branch point metabolite converting primary carbon metabolism into the shikimate pathway, it is still unknown whether this enzyme serves as a major regulator of flux between primary and secondary metabolism in plants. DAHPS activity may however be central to the ability of the shikimate pathway to compete for PEP and E4-P with glycolysis as well as with the non-oxidative pentose phosphate pathway (Fig. 2).

The second enzyme of the shikimate pathway is 3-dehydroquinate synthase (DHQS; EC 4.2.3.4; At5g66120), which converts 3-deoxy-d-arabino-heptulosonate-7-phosphate into 3-dehydroquinate (Fig. 2). The third and fourth enzymatic steps are catalyzed by the bi-functional enzyme 3-dehydroquinate dehydratase/shikimate 5-dehydrogenase (DHQ/SDH; EC 4.2.1.10 and EC 1.1.1.25) (At3g06350), leading to the formation of shikimate (Fig. 2). This bifunctional enzyme has been characterized in tomato (*Solanum lycopersicum*) (Bischoff et al., 2001) and tobacco (*Nicotiana tabacum*) (Bonner and Jensen, 1994). A recent study showed that the Arabidopsis *AtDHQ/SDH* gene is required for female gametophyte development and function (Pagnussat et al., 2005). The crystal structure of Arabidopsis DHQ/SDH with shikimate bound at the SDH site and tartrate at the DHQ site has recently been elucidated (Singh and Christendat, 2006). The interactions observed in the DHQ-tartrate complex reveal a conserved mode for substrate binding between the plant and microbial DHQ dehydratase family of enzymes. The arrangement of the two functional domains of this enzyme suggests that the control of metabolic flux through the shikimate pathway is achieved by increasing the effective concentration of the intermediate substrate, 3-dehydroshikimate, through the proximity of the two sites (Singh and Christendat, 2006). While Arabidopsis plants possess only a single *AtDHQ/SDH* gene, tobacco plants possess two genes. RNAi-mediated suppression of either of the two tobacco *DHQ/SDH-1* and *NtDHQ/SDH-2* genes caused differential steady state levels of the pathway substrates dehydroquinate and shikimate (Ding et al., 2007).

The fifth enzymatic step of the shikimate pathway is catalyzed by shikimate kinase (SK) (EC 2.7.1.71), which converts shikimate to shikimate 3-phosphate (Fig. 1). Arabidopsis plants possess two SK isoforms: AtSK1 (At2g21940) and AtSK2 (At4g39540) as well as two additional *SK-like* genes that arose from an ancestral plant *SK* gene duplicates, but lost their SK activity (Fucile et al., 2008). It has been suggested that these two genes may have evolved a new enzymatic function that is not related to the shikimate pathway (Fucile et al., 2008). Several lines of evidence suggest that plant SK acts as a regulatory step for the shikimate pathway, facilitating metabolic flux towards specific pools of secondary metabolite. These include: (i) a rapid induction of plant SK transcripts by fungal elicitors (Gorlach et al., 1995); (ii) a significant sensitivity of plant SK activity to cellular ATP energy charge; and (iii) the differential expression of the three rice (*Oryza sativa*) SK genes in specific developmental stages and in response to biotic stress (Kasai et al., 2005).

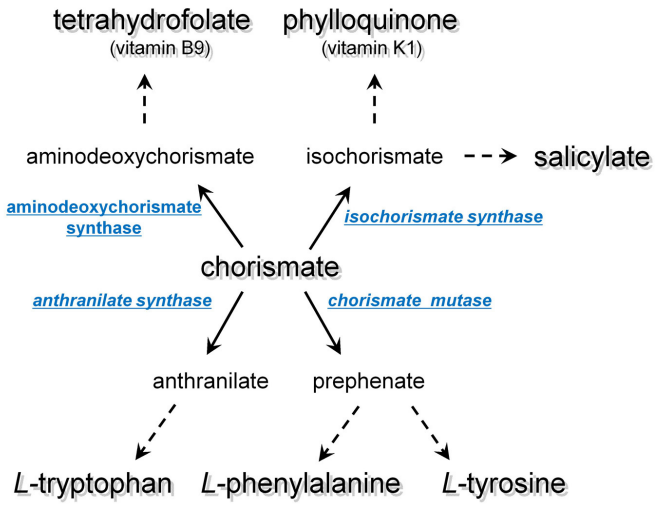

Figure 3. Chorismate, a central branch point metabolite in the synthesis of aromatic amino acids and secondary metabolites. First enzymes involved in several secondary pathways derived from chorismate.

The sixth enzymatic step of the shikimate pathway is catalyzed by 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) (CE 2.5.1.19), which leads to the synthesis of enolpyruvylshikimate 3-phosphate (EPSP) (Fig. 1). The Arabidopsis EPSPS is encoded by one functional gene (At2g45300) and perhaps also by a second putative gene (At1g48860) (Klee et al., 1987). This enzyme has been broadly studied for the last ~30 years (for review see Duke and Powles, 2008) due to its association with resistance to the herbicide N-phosphonomethylglycine (glyphosphate, an analog of phosphoenylpyruvate), which is the basis for the Roundup-Ready transgenic crops (Singer and McDaniel, 1985; Smart et al., 1985; Stalker et al., 1985). The native plant EPSPS is competitively inhibited by the herbicide glyphosphate, the consequence of which is a diminished flux of the shikimate pathway (Healy-Fried et al., 2007).

The final step in the shikimate pathway is catalyzed by chorismate synthase (CS) (CE 4.2.3.5), which converts EPSP to chorismate (Fig. 2). This enzyme was first characterized in *Corydalis semoervirens* (Schaller et al., 1991) and is proposed to have been derived from a common ancestor for bacteria, plants and fungi (Macheroux et al., 1999). Arabidopsis possesses a single CS gene (At1g48850), in contrast to tomato plants, which possess two differentially expressed CS genes, termed *LeCS1* and *LeCS2* (Gorlach et al., 1993).

CHORISMATE, A CENTRAL BRANCH POINT METABOLITE IN THE SYNTHESIS OF AROMATIC AMINO ACIDS AND SECONDARY METABOLITES

Chorismate, the terminal metabolite of the shikimate pathway serves as the initiator metabolite for the synthesis of the three AAA (Fig. 3) and hence also for the various aromatic secondary metabolites derived from them. Yet, chorismate also serves one of the initiator substrate of the synthesis of a number of other aromatic metabolites, many of which are likely to be still unknown. Some examples of chorismate-derived metabolites are: (i) chorismate is one of the precursor metabolites for the synthesis of tetrahydrofolate (vitamin B9; also commonly termed folate), serving as the substrate of the aminodeoxychorismate synthase (Fig. 3) (Basset et al., 2004; Waller et al., 2010); (ii) chorismate is converted to isochorismate by isochorismate synthase (Wildermuth et al., 2001) on route to the production of salicylate (SA) (Fig. 3) (Garcion et al., 2008); and (iii) chorismate also serves the precursor metabolite for the synthesis of phylloquinone (vitamin K1) and many other plant pigments (Gross et al., 2006; Kim et al., 2008). Hence, chorismate is one of the central branch point metabolites in plant cells.

THE BIOSYNTHESIS NETWORK OF THE THREE AROMATIC AMINO ACIDS PHE, TYR AND TRP

The unsolved pathway of Phe biosynthesis: two possible metabolic routes using arogenate or phenylpyruvate as intermediates

The first committed step of Phe biosynthesis from chorismate is catalyzed by chorismate mutase (CM) (CE 5.4.99.5), which converts chorismate to prephenate (Fig. 4). Three CM genes have so far been described in Arabidopsis, namely *AtCM1* (At3g29200), *AtCM2* (At5g10870) and *AtCM3* (At1g69370) (Mobley et al., 1999). The three genes are differentially expressed in various tissues and the expression of only *AtCM1* is induced by various elicitors and pathogens (Mobley et al., 1999; Ehlting et al., 2005). The activities of the three Arabidopsis CM isoforms were demonstrated by complementing *E. coli* and yeast CM-deficient strains (Eberhard et al., 1993; Eberhard et al., 1996). The activities of AtCM1 and AtCM3 are inhibited by Phe and Tyr, whereas the activity of AtCM2 appears to be insensitive to these amino acids (Eberhard et al., 1996). The final two enzymatic steps converting prephenate to Phe in plants are still not entirely elucidated. The major route involves the conversion of chorismate via arogenate to Phe, catalyzed by respective enzymes prephenate aminotransferase (PAT) (CE 2.6.1.79) and arogenate dehydratase (ADT) (CE 4.2.1.49) (Cho et al., 2007; Yamada et al., 2008; Maeda et al., 2010)(Fig. 4). Yet, it is still not clear whether plants can also convert chorismate to Phe via phenylpyruvate (PPY), using enzymes with prephenate dehydratase (PDT) and Phe aminotransferase activities (Fig. 4) in a similar manner to *E. coli* and various other microorganisms. A PAT enzymatic activity, converting prephenate into arogenate (Fig. 4), has been reported in plants (Siehl et al., 1986; De-Eknamkul and Ellis, 1988). Yet, no plant gene encoding such an activity has so far been reported. An *in silico* data mining approach identified six putative ADT genes in Arabidopsis, namely, ADT1 (At1g11790), ADT2 (At3g07630), ADT3 (At2g27820), ADT4 (At3g44720), ADT5 (At5g22630) and ADT6 (At1g08250). Biochemical characterization of the recombinant enzymes encoded by these six Arabidopsis genes suggested that all of them possess arogenate dehydratase activity, converting arogenate into Phe (Fig. 4). Yet, three of them (ADT1, ADT2 and ADT6) can also utilize prephenate as a substrate and convert it to PPY (Fig. 4), even though they exhibit a preference for arogenate (Cho et al., 2007). A rice 5-methyl-Trp resistant mutant, called *Mtr1*, which over-accumulates Phe, Trp and several phenylpropanoids,

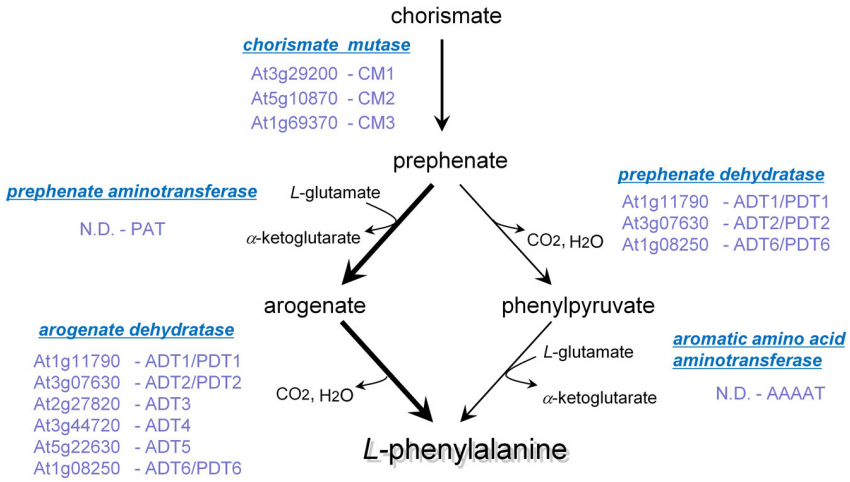

Figure 4. The pathway of Phe biosynthesis. Enzymes involved in the biosynthesis of Phe. N.D. not detected in Arabidopsis plants.

appeared to result from a point mutation in a gene encoding an enzyme possessing both ADT and PDT activities, rending these activities insensitive to feedback inhibition by Phe (Yamada et al., 2008). Nevertheless, similar to the Arabidopsis enzymes that can utilize both ADT and PDT substrates, this rice enzyme possessed a preference to arogenate, implying that it functions primarily as an ADT. Recently, three genes encoding ADT enzymes were identified in petunia (*Petunia hybrida*). Similar to the Arabidopsis ADT isozymes, the three petunia ADT isozymes preferentially use arogenate as a substrate, but can also use prephenate as a substrate at a much lower efficiencies, supporting the hypothesis of preferential utilization of the arogenate route rather than the PPY route for Phe biosynthesis in plants (Maeda et al., 2010). However, feeding shikimate into petunia petals with suppressed expression of ADT1 (the major ADT enzyme in petunia) led to the accumulation of prephenate and PPY and also to partial recovery of the reduced Phe level, strongly indicating that petunia plants can also synthesize Phe via the PPY route.

To study the consequence of producing PPY in plants by metabolic engineering, we have recently expressed a bacterial *PheA* gene encoding a bi-functional CM/PDT enzyme that converts chorismate via prephenate to PPY (Tzin et al., 2009). These Arabidopsis plants had a significant increase in the level of Phe, with no increase in the level of PPY. Although it is likely that a considerable amount of the prephenate, produced by the CM activity of the bacterial CM/PDT enzyme, was converted via arogenate to Phe using the ADT enzyme (Fig. 4), the fact that these plants showed no increased level of PPY suggests that Arabidopsis apparently possesses an endogenous AAAT activity that can use PPY as a substrate and covert it to Phe (Tzin et al., 2009) (Fig. 4). Yet, no gene encoding an aromatic amino acid aminotransferase (AAAAT) (CE 2.6.1.57) that can specifically convert PPY into Phe has so far been identified in plants. Hence, taken together, the studies described above imply that plants use primarily the arogenate route for the synthesis of Phe, although some minor function of the PPY route in Phe biosynthesis cannot be ruled out. This is also supported by the observation that a number of plants species contain PPY, which also serves as a precursor for a number of secondary metabolites such as phenylacetaldehyde, 2-phenylethanol and 2-phenylethyl b-d-glucopyranoside (Watanabe et al., 2002; Kaminaga et al., 2006).

The pathway of Tyr biosynthesis

The major route of Tyr biosynthesis initiates from chorismate, using the same first two enzymes of Phe biosynthesis, namely CM and PAT, to produce arogenate (Fig. 4 and 5). Arogenate is then converted into Tyr by arogenate dehydrogenase (TyrA) (CE 1.3.1.43) (Fig. 5). TyrA activity has been demonstrated in tobacco (Gaines et al., 1982), maize (Byng et al., 1981), sorghum (Connelly and Conn, 1986) and Arabidopsis (Rippert and Matringe, 2002b). In Arabidopsis plants, two genes encoding TyrA enzymes were identified: TyrA1 (At5g34930) and TyrA2 (At1g15710) (Rippert and Matringe, 2002b, a; Rippert et al., 2009).

A second possible route of Tyr biosynthesis has also been suggested, which includes the conversion of prephenate to p-hydroxyphenylpyruvate (p-hydroxyPPY) by prephenate dehydrogenase (PDH) (CE 1.3.1.43), which may be catalyzed by TyrA2 (Rippert and Matringe, 2002b). Subsequently, p-hydroxyPPY converts to Tyr by a broad range AAAAT (Fig. 5). Nevertheless, at a non-saturating concentration of prephenate, TyrA2 enzyme activity is 2000 times less efficient in catalyzing the reaction with prephenate than with arogenate (Rippert and Matringe, 2002a), and therefore the possible existence of this alternative route for Tyr biosynthesis using PDH is still in doubt.

The pathway of Trp biosynthesis

The first committed step of Trp biosynthesis includes a transfer of an amino group of glutamine to chorismate to generate anthranilate and pyruvate, catalyzed by anthranilate synthase (AS) (CE 4.1.3.27) (Fig. 6). Purified plant AS holoenzymes are believed to

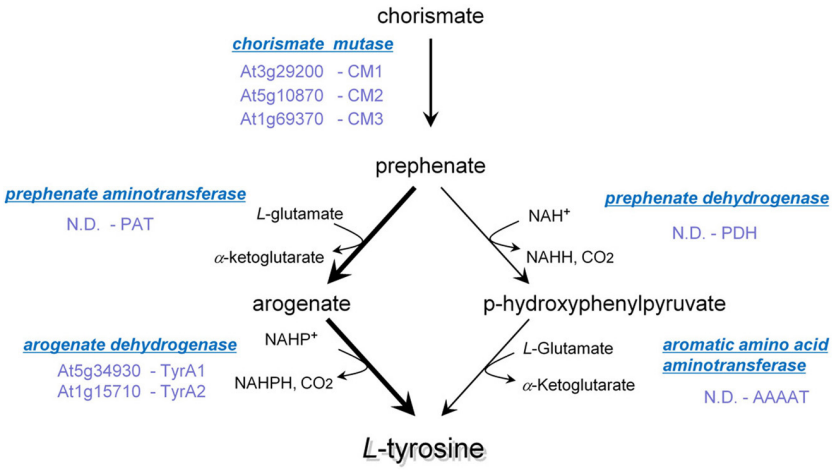

Figure 5. The pathway of Tyr biosynthesis. Enzymes involved in the biosynthesis of Tyr. N.D. not detected in Arabidopsis plants.

be heterotetramers composed of two alpha and two beta subunits (Niyogi et al., 1993; Poulsen et al., 1993). The Arabidopsis genome possesses two functional genes encoding the AS alpha subunit, ASa1 (At5g05730) and ASa2 (At2g29290), as well as a single functional ASb1 gene (At1g25220) encoding the AS beta subunit. In addition, two other genes were putatively assigned as encoding ASa subunits (At2g28880 and At3g55870) and additional five genes were putatively assigned as encoding ASb subunits (At5g57890, At1g25155, At1g24807, At1g24909 and At1g25083). Interestingly, four of the putative genes encoding ASb are located on one cluster on chromosome 1 (for more details see http://www.plantcyc.org). The alpha subunit possesses the catalytic activity and the beta subunit possesses an aminotransferase activity, which transfers an amino group from glutamine to the alpha subunit. AS activity in plants is feedback inhibited by Trp through binding of Trp to the alpha subunit. Expression of AS genes encoding feedback-insensitive enzymes in a variety of plant species generally increases the production of free Trp and secondary metabolites derived from it (Li and Last, 1996; Tozawa et al., 2001; Hughes et al., 2004). The *trp4* mutation in the gene encoding the Arabidopsis ASb1 subunit suppresses accumulation of the product of this enzyme, anthranilate (Niyogi et al., 1993). Anthranilate possesses a strong blue fluorescence under UV light, which has been utilized as a phenotypic marker for indentifying Arabidopsis mutants in the Trp biosynthesis enzymes (Rose et al., 1992; Radwanski et al., 1995).

The second enzyme in the Trp biosynthesis pathway is anthranilate phosphoribosylanthranilate transferase (PAT1) (CE 2.4.2.18; At5g17990), which converts anthranilate and phosphoribosylpyrophosphate into phosphoribosylanthranilate and inorganic pyrophosphate (Fig. 6).

The third enzyme in the Trp biosynthesis pathway is phosphoribosylanthranilate isomerase (PAI) (CE 5.3.1.24), which converts phosphoribosylanthranilate into l-(O-carboxyphenylamino)-l-deoxyribulose-5-phosphate (CDRP) (Fig. 6). Arabidopsis possesses three genes encoding PAI isoforms; PAI1 (At1g07780), PAI2 (At5g05590) and PAI3 (At1g29410).

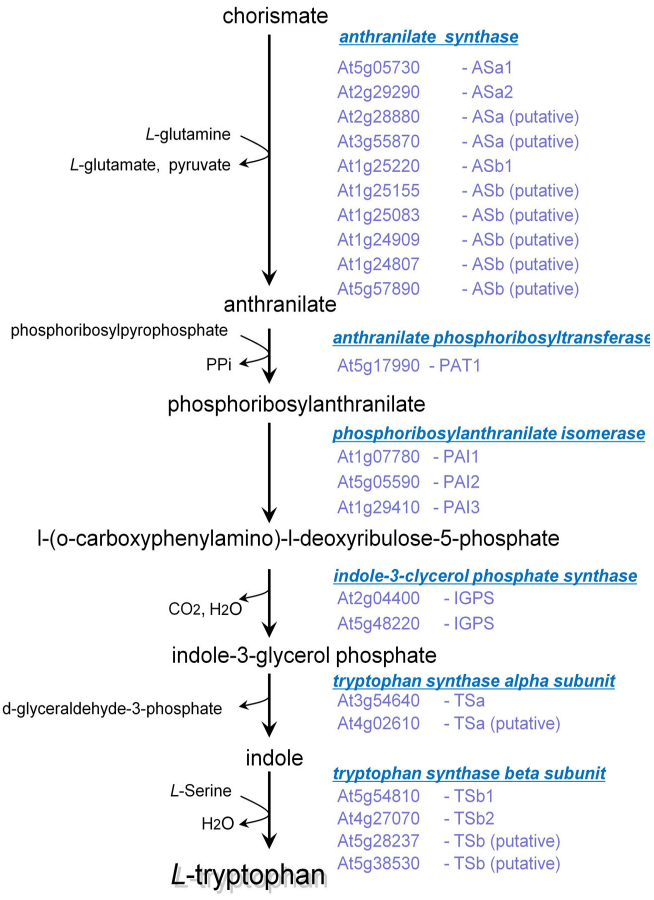

Figure 6. The pathway of Trp biosynthesis. Enzymes involved in the biosynthesis of Trp.

The fourth enzyme of Trp biosynthesis is indole-3-glycerol phosphate synthase (IGPS) (EC 4.1.1.48), which catalyzes the conversion of 1-(O- carboxyphenylamino)-1-deoxyribulose-5-phosphate to indole-3-glycerol phosphate (Li et al., 1995a). Arabidopsis plants possess one gene encoding a functional IGPS (AT2G04400) and also a second gene (AT5G48220) encoding a putative IGPS (Li et al., 1995a). IGPS is an important enzyme in the biosynthesis of Trp and the hormone indole-3-acetic acid (IAA; auxin) because it is the only known enzyme that catalyzes the formation of the indole ring. Quantitative comparison of the relative levels of Trp and IAA content in different Arabidopsis Trp biosynthesis mutants as well as in transgenic plants expression an IGPS antisense construct indicates that indole-3-glycerol phosphate is the branch-point metabolite for a *de novo* Trp-independent IAA biosynthesis in Arabidopsis (Ouyang et al., 2000). Interestingly, in both fungi and bacteria, IGPS is synthesized as a fusion protein containing one or two other enzymes of the Trp biosynthesis pathway (Li et al., 1995b). However, in plants IGPS generally appears as a mono-functional enzyme based on its cDNA sequence and functional complementation analysis (Li et al., 1995a).

The last two steps in the Trp biosynthesis are catalyzed by Trp synthase (TS) (CE 4.2.1.20), which includes both alpha (TSa) and beta (TSb) subunits. Indole-3-glycerol phosphate is cleaved by TSa to indole and glyceraldehyde-3-phosphate (α-reaction). Then, indole is transported to TSb, which catalyzes its condensation with serine (β-reaction) to produce Trp (Miles, 2001; Weber-Ban et al., 2001). Arabidopsis possesses at least one functional gene encoding TSa (At3g54640). Yet, a gene encoding a putative TSa homolog (At4g02610), also named indole synthase, was identified and characterized in Arabidopsis. Indole synthase possesses ~65% amino acid sequence identity to TSa (Zhang et al., 2008). Arabidopsis possess two genes encoding functional TSb subunits, namely, TSb1 (At5g54810) and TSb2 (At4g27070), as well as two additional genes encoding putative TSb subunits (At5g28237 and At5g38530). The function of TSa1 and TSb1 was demonstrated by the facultative Trp auxotroph mutants, *trp3* and *trp2*, respectively (Last et al., 1991), and it was suggested that the TSa1 and TSb1 subunits form an active heterodimer (Radwanski et al., 1995). The Arabidopsis gene encoding TSa1 was cloned by functional complementation of an *E. coli* mutant and suggested to function as a monomer (Bohlmann et al., 1995; Radwanski and Last, 1995; Radwanski et al., 1995). Yet, whether TS activity operates as a monomer or as a multi-enzyme complex is still not clear (Kriechbaumer et al., 2008).

TRANSCRIPTIONAL AND POST TRANSCRIPTIONAL REGULATON OF THE SHIKIMATE PATHWAY AND AROMATIC AMINO ACID METABOLISM

Transcriptional regulation

Transcriptional regulation of the shikimate pathway and aromatic amino acid metabolism in plants has so far not been studied extensively. The expression of *DAHPS* encoding the first enzyme of the shikimate pathway (Fig. 2) is induced by physical wounding and methyl-jasmonate (Devoto et al., 2005; Yan et al., 2007), infiltration with pathogenic *Pseudomonas syringae* strains (Keith et al., 1991), redox state (Entus et al., 2002) and abscisic acid (Leonhardt et al., 2004; Catala et al., 2007). The expression of the gene encoding EPSPS is induced in response to infection by the necrotrophic fungal pathogen *Botrytis cinerea* (Ferrari et al., 2007) and by sulfate starvation (Nikiforova et al., 2003). Fungal elicitors also rapidly stimulate the production of mRNA of SK (Gorlach et al., 1995). Ozone treatment induces a significant part of the shikimate pathway genes in tomato (Bischoff et al., 1996; Bischoff et al., 2001), tobacco (Janzik et al., 2005) and in the European beech (*Fagus sylvatica*) (Betz et al., 2009). Oligogalacturonides that are released from plant cell walls upon infection with of the *Botrytis cinerea* pathogen stimulate a number of genes encoding enzymes of the shikimate and AAA biosynthesis pathways, as well as genes encoding enzyme of secondary metabolites derived from the AAA (Ferrari et al., 2007). The expression of the three Arabidopsis genes encoding the three PAI isoforms (Fig. 6) is differentially regulated under normal growth conditions, with PAI1 and PAI3 showing ~10-fold higher expression level than PAI2 (He and Li, 2001). Expression of these three PAI genes also respond differentially to environmental stresses, such as UV irradiation and treatment with the abiotic elicitor silver nitrate in a tissue- and cell-type-specific manner (Li et al., 1995b; He and Li, 2001). Deletion of the Arabidopsis gene encoding PAI1 causes some abnormal growth (He and Li, 2001) which indicates its predominant importance in Trp biosynthesis. Interestingly, the Arabidopsis PAI gene family is regulated by methylation in the Wassilewskija, but not Columbia ecotypes (Bender and Fink, 1995; Melquist et al., 1999). The PAI genes of Wassilewskija contain inverted repeats, which provide a trigger for their methylation (Bender and Fink, 1995; Melquist et al., 1999; Bartee and Bender, 2001; Melquist and Bender, 2003).

The Arabidopsis gene encoding IGPS of Trp biosynthesis (Fig. 6) is regulated by the hormones jasmonate (Sasaki-Sekimoto et al., 2005; Dombrecht et al., 2007) and salicylate (Rajjou et al., 2006), and also in seeds and seedlings by various defense mechanisms (Job et al., 2005; Chibani et al., 2006). In addition, expression of the Arabidopsis gene encoding PAT1 is apparently controlled by regulatory elements located inside introns, as inclusion of introns was shown to enhance the expression of PAT1-GUS fusion constructs that were stably transformed into Arabidopsis (Rose and Beliakoff, 2000).

Recently, in the frame of the AtGenExpress project, the response of the global Arabidopsis transcriptome to a variety of abiotic and biotic stresses was studied in roots and shoots, using the Affymetrix ATH1 microarray (NASC; http://affymetrix.arabidopsis.info/) (Kilian et al., 2007). The database of these experiments was used in a bioinformatics study to analyze of the response of genes encoding biosynthesis enzymes as well as enzymes responsible for the first catabolic enzymes of the different amino acid in a variety of amino acid metabolic pathways (Less and Galili, 2008). The results showed that genes encoding amino acid catabolic enzymes principally respond in shorter time periods and are much more sensitive to abiotic stresses than genes encoding biosynthetic (allosteric and non-allosteric) enzymes. These responses also operated in a pathway-specific manner in response to different stress conditions (Less and Galili, 2008). These results imply that the catabolic genes play major regulatory roles in amino acid metabolism upon exposure to these stresses (Less and Galili, 2008). Interestingly, the Trp and Phe/Tyr branches of the AAA biosynthesis pathway responded

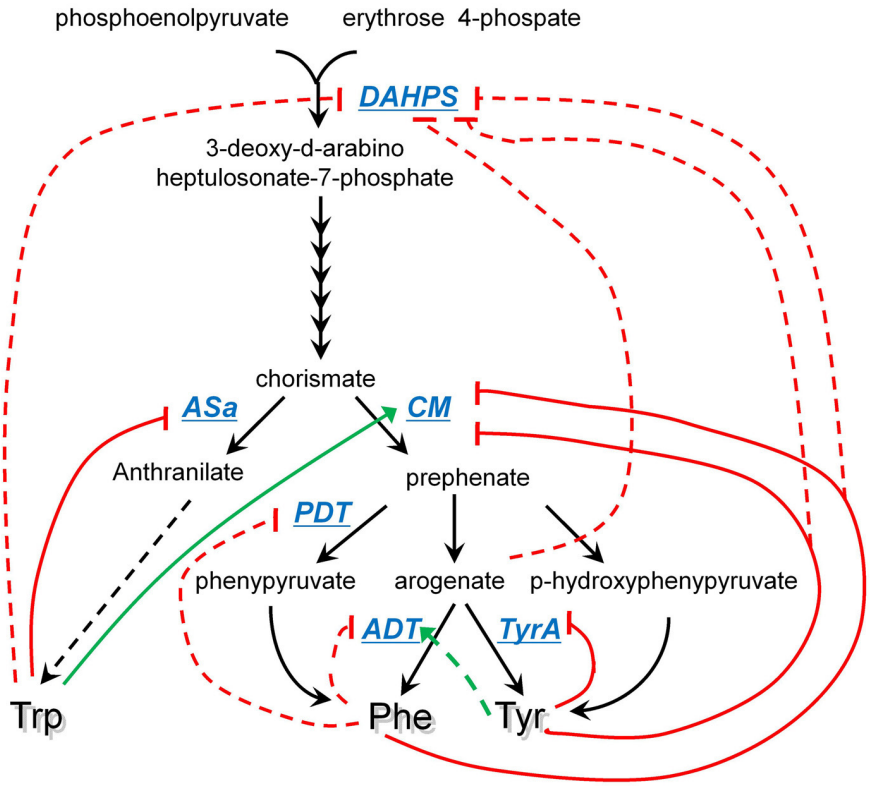

Figure 7. Post-transcriptional regulation of the shikimate pathway and aromatic amino acid metabolism. Key enzymes and metabolites are shown. Known allosteric regulation by compounds within the pathway is shown, activation with a green arrow, inhibition with a red line and bar, and putative allosteric inhibition with a dashed red line. DAHPS, 3-deoxy-d-arabino-heptulosonate-7-phosphate synthase; ASa, anthranilate synthase alpha subunit; CM, chorismate mutase; PDT, prephenate dehydratase ; ADT, arogenate dehydratase; TyrA, arogenate dehydrogenase.

differently to UV-B stress. In the Trp biosynthesis pathway, UV-B stress stimulated the expression of the genes encoding both the biosynthesis enzymes and the catabolic enzymes CYP79B2 and CYP79B3. In contrast, this stress did not affect the expression of the genes encoding the biosynthesis enzymes of the Phe/Tyr branch, while it stimulated the expression of only the gene encoding the Tyr catabolism enzyme Tyr-aminotransferase, but not the Phe catabolism enzyme Phe-ammonia lyase (PAL). Interestingly, exposure of Arabidopsis plants to various stresses, including amino acid starvation, as well as to treatments with the oxidative stress-inducing herbicide acifluorfen and the abiotic elicitor alpha-amino butyric acid, also induce the expression of genes encoding Trp biosynthesis enzymes (Zhao et al., 1998). Overexpression of members of two clades of Arabidopsis genes, encoding “altered Trp regulation1” [ATR1]-like and MYB28-like transcription factors in transgenic Arabidopsis stimulates the expression of specific genes belonging to both the shikimate and Trp biosynthesis pathways, as well as genes encoding enzymes of Trp-derived secondary metabolites (Malitsky et al., 2008). Similar results were also obtained upon expression of the petunia *ODORANT1* gene, encoding a R2R3-type *MYB* transcription factor in petunia flowers (Colquhoun et al., 2010). Down-regulation of *ODORANT1* in transgenic petunia plants strongly reduced the abundance of transcripts and metabolites from the shikimate pathway (Verdonk et al., 2003). A functional homolog of *ODORANT1* was not yet been identified in Arabidopsis.

Post-translational regulation by enzyme feedback-inhibition loops

The activities of DAHPS enzymes (the first enzymatic step of the shikimate pathway; see Fig. 2) from various microorganisms are generally regulated by allosteric feedback inhibition by the different AAA (Byng et al., 1983; Knaggs, 2001). In contrast, there is no published evidence showing that plant DAHPS enzymes are strongly allosterically inhibited *in vivo* by any of the AAA, and it is generally assumed that DAHPS activity in higher plants is not subject to a major allosteric control (Gilchrist and Kosuge, 1980; Herrmann and Weaver, 1999). Yet, the *in vitro* activities of DAHPSs from different plant species are weakly inhibited by Trp (Graziana and Boudet, 1980; Rubin and Jensen, 1985) and Tyr (Reinink and Borstap, 1982), or even can also be weakly activated by either Trp or Tyr (Suzich et al., 1984; Pinto et al., 1986) (Fig.7). The activity of *Vigna radiate* (bean) DAHPS is weakly inhibited by prephenate and arogenate, the precursor metabolites of Phe and Tyr biosynthesis (Fig. 7) (Rubin and Jensen, 1985), but whether this is due to inhibition of enzyme level or activity is

still unknown (Herrmann, 1995). It has also been suggested that the *Petroselinum crispum* (parsley) DAHPS activity results from several different isoforms, whose activities may be dependent on Mn^{2+} or Co^{2+} ions (McCue and Conn, 1989; Gorlach et al., 1993). In addition, the Mn^{2+}-dependent regulation of DAHPS activity by arogenate was proposed as one of the key circuits in the overall pattern of allosteric control for the entire network of the shikimate and AAA biosynthesis (Doong et al., 1992; Doong et al., 1993). All in all, the above results imply that the shikimate pathway in plants is mostly regulated at the gene expression level rather than by post-translational controls.

The Regulation of AAA biosynthesis from chorismate by feedback inhibition loops is primarily associated with: (i) the branch point enzymes AS and CM, which utilize the substrate chorismate; (ii) the branch point enzyme ADT catalyzing the final step in Phe biosynthesis; and (iii) the branch point enzyme TyrA catalyzing the final step of Tyr biosynthesis (Fig. 7). AS, the first enzyme specific for Trp biosynthesis, is feedback-inhibited by Trp (Fig. 7). Arabidopsis *trp5* mutants, producing an ASa1 subunit that is insensitive to feedback inhibition by Trp, were isolated in the 1990s either by screening for accumulation of the intermediate metabolite anthranilate (through measuring its fluorescent properties) or by resistance to toxic Trp analogs, such as 6-methyltryptophan (Kreps et al., 1996; Li and Last, 1996). CM, the first specific enzyme for Phe and Tyr biosynthesis, is normally feedback inhibited by Phe and Tyr and induced by Trp (Eberhard et al., 1996) (Fig. 7). To investigate the enzymatic properties of the three Arabidopsis CM isoforms, the Arabidopsis *CM1*, *CM2* or *CM3* cDNAs were expressed in yeast (Mobley et al., 1999). The activities of both the CM1 and CM3 isozymes were feedback inhibited by Phe and Tyr, while stimulated by Trp. In contrast, CM2 activity was insensitive to feedback inhibition by any of the AAA (Mobley et al., 1999). The activity of TyrA, the last enzyme of Tyr biosynthesis, is feedback inhibited by Tyr (Fig. 7) in Arabidopsis (Rippert and Matringe, 2002b) and *Sorghum bicolor* (Connelly and Conn, 1986). In addition, ADT activity from tobacco, spinach, and *S. bicolor* was shown to be positively regulated by Tyr and negatively regulated by Phe (Jung et al., 1986; Siehl and Conn, 1988). However, the allosteric regulation has not yet been characterized in Arabidopsis plants (Cho et al., 2007). In addition, the rice ADT is negatively regulated by Phe (Yamada et al., 2008), while its potential regulation by Tyr has not yet been elucidated.

Comparison of these feedback regulation loops shows that the flux from chorismate towards Phe and Tyr biosynthesis is generally significantly stronger than the flux towards Trp biosynthesis, and the flux from arogenate towards Phe biosynthesis is significantly stronger than that into Tyr biosynthesis (Rippert et al., 2004) (Fig. 7). This may also reflect the fact that Phe produces a significantly larger variety of secondary metabolites than Tyr and Trp.

The enzymes of the shikimate and AAA biosynthesis pathways are generally synthesized as precursors containing a plastid transit peptide that directs them to the plastid, the organelle in which these two essential pathways operate (Mustafa and Verpoorte, 2005; Weber et al., 2005; Zybailov et al., 2008). However, the intra-cellular localization of two enzymes, CM2 and ADT3, is still under some debate. Sub-cellular fractionation analysis suggested that the tobacco CM2 isozyme is localized in the cytosol (d'Amato et al., 1984), but whether this polypeptide indeed possesses CM activity has not been confirmed. Although *in vitro* studies showed that AtCM2 possesses CM activity (Eberhard et al., 1993; Eberhard et al., 1996), the physiological significance of AtCM2 still remains questionable (Rippert et al., 2009). In addition, an Arabidopsis polypeptide termed PDT1 (which corresponds to the ADT3 isozyme of Phe biosynthesis, characterized by Cho et al. 2007), was suggested to be a component of the heterotrimeric G-protein complex that is associated with the plasma membrane (Warpeha et al., 2006). This observation is in contrast to a more recent report, using an *in situ* microscopy analysis, which showed that all of the Arabidopsis ADT isozymes are localized in the plastid (Rippert et al., 2009). Thus, the current dogma is that all ADT isozymes are generally localized to the plastid, although it cannot be ruled out that under specific growth stages or physiological conditions, ADT3 may also be associated with other complexes before it is post-translationally transported into the plastid.

Influence of genetic, metabolic and environmental factors on the regulation of AAA metabolism

Several mutants and transgenic plants with modified shikimate and AAA biosynthesis pathways were used to elucidate the regulation of the biosynthesis of the three AAA. A rice 5-methyl Trp-resistant mutant (Mtr1), apparently encoding a feedback-insensitive PDT/ADT, was shown to over-accumulate Phe and Trp in both callus tissue and leaves (Wakasa and Widholm, 1987; Yamada et al., 2008). Expression of a bacterial *PheA** gene, encoding a bifunctional CM/PDT enzyme that is feedback insensitive to Phe, in transgenic Arabidopsis plants, caused: (i) significant increases in the levels of Phe as well as a number of Phe-derived and Tyr-derived secondary metabolites; and (ii) significant decreases of Trp-derived secondary metabolites (Tzin et al., 2009). This implied a regulatory cross-interaction between the biosynthesis fluxes of the three AAA from chorismate, which also influence the rates of their conversion into various secondary metabolites. An Arabidopsis double mutant lacking PAL1 and PAL2 activities has an ~100-fold increase in Phe and a 4-fold increase in Trp levels (Rohde et al., 2004). This *pal1* and *pal2* double mutant also influences the transcription of genes associated with the AAA biosynthesis network as well as genes associated phenylpropanoid secondary metabolites (Rohde et al., 2004). Arabidopsis and rice mutants with a feedback-insensitive ASa of Trp biosynthesis generally accumulate Trp, but not Phe or Tyr (Kreps et al., 1996; Li and Last, 1996; Bender and Fink, 1998; Tozawa et al., 2001; Ishihara et al., 2006). Exposure of Arabidopsis seedlings to sulfate starvation triggers an increase in the level of shikimate as well as the Phe and Trp and secondary metabolites derived from them (Nikiforova et al., 2003; Nikiforova et al., 2004; Nikiforova et al., 2006).

CATABOLISM OF THE AROMATIC AMINO ACIDS INTO SECONDARY METABOLITES

Phe catabolism

Phe serves as a precursor for a large family of secondary metabolites. The major group of these secondary metabolites is the phenylpropanoids, whose biosynthesis is initiated by the activ-

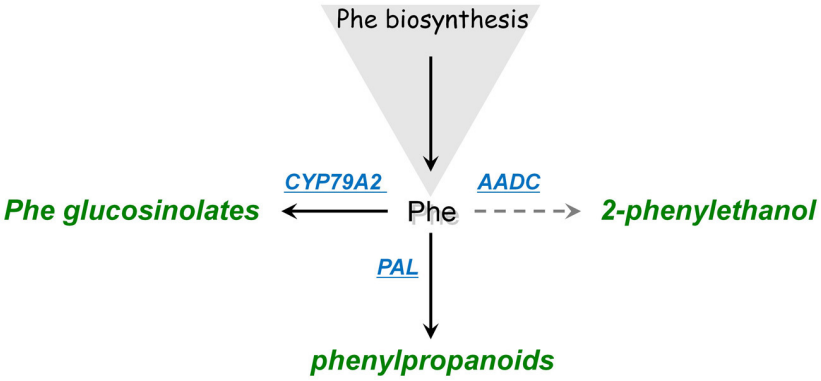

Figure 8. Phe catabolism. Only the first enzymes involved in several secondary metabolism pathways derived from Phe are indicated. A putative pathway in Arabidopsis is marked with a dashed grey line. PAL, Phe-ammonia lyase; AADC, aromatic amino acid decarboxylase.

ity of Phe-ammonia lyase (PAL) (CE 4.3.1.5) (Fig. 8). Arabidopsis possesses four genes encoding the PAL1-PAL4 isozymes (At2g37040, At3g53260, At5g04230 and At3g10340, respectively). The phenylpropanoids possess multiple functions, particularly protecting against various abiotic and biotic stresses, and their production is generally stimulated by such stresses (Dixon and Paiva, 1995; Dixon, 2001; Casati and Walbot, 2005). The transcription of the PAL genes is generally highly regulated by biotic and abiotic stresses, as well as by conditions that demand increased production of the cell wall component lignin in various tissues (Anterola and Lewis, 2002). Genetic mutations that affect the production of PAL generally cause significant alteration in the levels of many phenylpropanoids (Shadle et al., 2003; Rohde et al., 2004). The major sub-groups of phenylpropanoids include the flavonoids, the lignin cell wall components, and the anthocyanins. The metabolite composition of the phenylpropanoids, as well as genes encoding enzymes and regulatory proteins associated with their synthesis, have been recently discussed in several excellent reviews, examples of which are (Weisshaar and Jenkins, 1998; Pichersky and Gang, 2000; D'Auria and Gershenzon, 2005; Boudet, 2007; Vogt, 2010). Some decisive steps in phenylpropanoid biosynthesis were resolved only recently, such as the 2-hydroxylation involved in coumarate biosynthesis (Kai et al., 2008). In addition, genomics approaches revealed new organ-specific pathways, such as the formation of tapetum-specific trisacyl-polyamine conjugates of Arabidopsis flower buds (Alves-Ferreira et al., 2007; Ehlting et al., 2008; Fellenberg et al., 2009; Matsuno et al., 2009). The fine regulation of phenylpropanoid biosynthesis is achieved by combinatorial actions of transcription factors, expressed in a spatially and temporally controlled manner as exemplified in the following reports: (Ramsay and Glover, 2005; Lepiniec et al., 2006; Stracke et al., 2007). A group of volatile compounds, including methylbenzoate, phenylethylacetate and isoeugenol, is also among the phenylpropanoids produced by PAL (Verdonk et al., 2003; Schuurink et al., 2006; Wildermuth, 2006; Ben Zvi et al., 2008).

Another class of sulfur-rich Phe-derived secondary metabolites includes the Phe-glucosinolates, whose basic skeleton consists of a b-thioglucose residue, an N-hydroxyiminosulfate moiety and a variable side chain (Reichelt et al., 2002). Phe-glucosinolates are generally not widespread in Arabidopsis, but some Arabidopsis ecotypes do synthesize these compounds, such as phenylethylglucosinolate in the leaves (Mikkelsen et al., 2004) and benzoyloxyglucosinolates in seeds (Kliebenstein et al., 2007). The committing gene in the biosynthesis of Phe-glucosinolates is the cytochrome P450, *CYP79A2* (At5g05260), encoding an N-hydroxylase (CE 1.14.13) (Fig. 8) that converts Phe into phenylacetaldoxime, the precursor of benzylglucosinolate (Wittstock and Halkier, 2000).

Some plant species also produce the volatile Phe-derived secondary metabolite 2-phenylethanol (Facchini et al., 2000; Watanabe et al., 2002; Baldwin et al., 2004; Kaminaga et al., 2006; Tieman et al., 2006; Gonda et al., 2010). However, 2-phenylethanol is produced in flowers and/or fruits of specific plants, such as petunia, rose and tomato, and so far this volatile has not been detected in Arabidopsis.

Tyr catabolism

Tyr serves as a precursor of several families of secondary metabolites, including tocochromanols (vitamin E), plastoquinones, isoquinoline alkaloids and non-protein amino acids, and it has also been speculated that Tyr may also lead to the production of some phenylpropanoids (Fig. 9). The tocochromanols, which include both tocopherols and tocotrienols, are essential antioxidants in the diets of human and farm animals (Schneider, 2005; Della-Penna and Pogson, 2006; Mene-Saffrane and Dellapenna, 2009). The first committed enzyme of tocochromanols biosynthesis from Tyr is Tyr-aminotransferase (CE 2.6.1.5) (At5g53970) (Lopukhina et al., 2001), which produces p-hydroxyPPY (Fig. 9) (Norris et al., 1995; Garcia et al., 1999). It has been suggested that p-hydroxyPPY can also be synthesized from prephenate via an alternative biosynthesis pathway (Fig. 5) (Rippert and Matringe, 2002b; Rippert et al., 2004). If such a pathway indeed naturally exists, then p-hydroxyPPY can also be used for tocochromanols biosynthesis, bypassing Tyr (Fig. 5).

The Tyr catabolism pathway also produces isoquinoline alkaloids, which represent a large, diverse group of natural products found in ~20% of all plant species (Facchini et al., 2004). In Arabi-

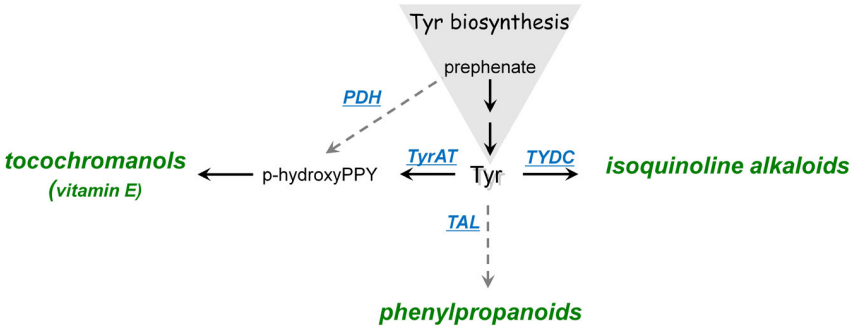

Figure 9. Tyr catabolism. Only the first enzymes involved in several secondary metabolism pathways derived from Tyr are indicated. Putative pathways in Arabidopsis are marked with dashed grey lines. TyrAT, Tyr-aminotransferase; TYDC, Tyr/L–dopa decarboxylase; PDH, prephenate dehydrogenase; TAL, Tyr-ammonia lyase.

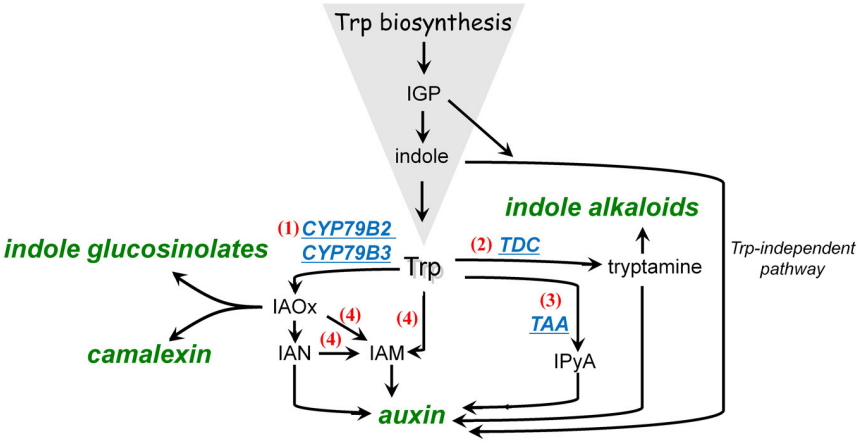

Figure 10. Trp catabolism. Only the first enzymes involved in several secondary metabolism pathways derived from Trp are indicated. The numbers within the Trp catabolism pathway indicate: 1) the indole-3-acetaldoxime (IAOx) pathway catalyzed by two cytochrome P450s (CYP79B2 and CYP79B3); 2) the tryptamine (*YUCCA*) pathway catalyzed by Trp decarboxylase (TDC); 3) the indole-3-pyruvate (IPyA) pathway catalyzed by Trp aminotransferase (TAA); and 4) the indoleacetamide (IAM) pathway which initiates directly from Trp via either IAOx or indole-3-acetonitrile (IAN).

dopsis, Tyr is also catabolized into tyramine by Tyr/L–dopa decarboxylase (TYDC) (EC 4.1.1.25), which is encoded by two genes (At2g20340, At4g28680). Tyramine is a precursor for benzyl-isoquinoline alkaloids, as well as cell wall-bound hydroxycinnamic acid amides (Facchini et al., 2000). It has been suggested that tyramine in involved in the Arabidopsis defense response (Trezzini et al., 1993).

Even though phenylpropanoids are classically synthesized from Phe, it has also been proven that in several plant species the second metabolite of the phenylpropanoid pathway, namely coumarate, can also be synthesized directly from Tyr by Tyr ammonia-lyase (TAL) (EC 4.3.1.) (Neish, 1961; Beaudoin-Eagan and Thorpe, 1985; Guerra et al., 1985; Rosler et al., 1997; Khan et al., 2003; MacDonald and D'Cunha, 2007). All four isoforms of Arabidopsis PAL, the first enzyme of phenylpropanoid biosynthesis from Phe (Fig. 8), exhibit higher affinity for Phe than for Tyr (Cochrane et al., 2004). However, a point mutation in the Arabidopsis gene encoding the PAL1 isoform resulted in a lower PAL activity and a compensatory increase in TAL activity (Watts et al., 2006), supporting the potential use of TAL in the phenylpropanoid biosynthesis pathway.

Trp catabolism

Trp is catabolized into many indole-containing secondary metabolites, such as indole-3-acetic acid (IAA, auxin) (Ostin et al., 1998; Davies, 2004), indole glucosinolates (Halkier, 1999), phytoalexins (Pedras et al., 2000), terpenoid indole alkaloids (De Luca and St Pierre, 2000; Facchini et al., 2004), and tryptamine derivatives (Facchini et al., 2000) (Fig. 10). Auxins are some of the key metabolites synthesized from Trp. However, the biosynthetic pathway(s) leading to IAA, the main auxin metabolite, are not well understood. Although there is good evidence that IAA is synthesized from Trp (Gibson et al., 1972; Wright et al., 1991; Tsurusaki et al., 1997), several different routes of IAA biosynthesis from Trp

Table 1. *Arabidopsis thaliana* genetic loci and enzyme activities mentioned in this review.

Pathway	Step	Gene name	AGI locus ID	CE	Proven or predicted activities in A.thaliana
Shikimate	1	DAHPS1	At4g39980	2.5.1.54	3-Deoxy-D-arabino-heptulosonate-7-phosphate synthase
Shikimate	1	DAHPS1	At1g39981	2.5.1.54	3-Deoxy-D-arabino-heptulosonate-7-phosphate synthase (putative)
Shikimate	1	DAHPS2	At4g33510	2.5.1.54	3-Deoxy-D-arabino-heptulosonate-7-phosphate synthase
Shikimate	2	DHQS	At5g66120	4.2.3.4	3-Dehydroquinate Synthase
Shikimate	3	DHQ/SDH	At3g06350	4.2.1.10	3-Dehydroquinate dehydratase//Shikimate 5-dehydrogenase
Shikimate	4	DHQ/SDH	At3g06350	1.1.1.25	3-Dehydroquinate dehydratase//Shikimate 5-dehydrogenase
Shikimate	5	SK1	At2g21940	2.7.1.71	Shikimate Kinase
Shikimate	5	SK2	At4g39540	2.7.1.71	Shikimate Kinase
Shikimate	6	EPSPS	At2g45300	2.5.1.19	5-Enolpyruvylshikimate 3-phosphate Synthase
Shikimate	6	EPSPS	At1g48860	2.5.1.19	5-Enolpyruvylshikimate 3-phosphate Synthase (putative)
Shikimate	7	CS	At1g48850	4.2.3.5	Chorismate Synthase
Aromatic amino acids					
Phe/Tyr	1	CM1	At3g29200	5.4.99.5	Chorismate Mutase
Phe/Tyr	1	CM2	At5g10870	5.4.99.5	Chorismate Mutase
Phe/Tyr	1	CM3	At1g69370	5.4.99.5	Chorismate Mutase
Phe option 1:					
Phe - Arogenate route	2	PAT	-	2.6.1.79	Prephenate Aminotransferase
Phe - Arogenate route	3	ADT1	At1g11790	4.2.1.49	Arogenate Dehydratase
Phe - Arogenate route	3	ADT2	At3g07630	4.2.1.49	Arogenate Dehydratase
Phe - Arogenate route	3	ADT3	At2g27820	4.2.1.49	Arogenate Dehydratase
Phe - Arogenate route	3	ADT4	At3g44720	4.2.1.49	Arogenate Dehydratase
Phe - Arogenate route	3	ADT5	At5g22630	4.2.1.49	Arogenate Dehydratase
Phe - Arogenate route	3	ADT6	At1g08250	4.2.1.49	Arogenate Dehydratase
Phe option 2:					
Phe - Phenylpyruvate route	2	ADT1	At1g11790	4.2.1.49	Arogenate Dehydratase//Prephenate Dehydratase
Phe - Phenylpyruvate route	2	ADT2	At3g07630	4.2.1.49	Arogenate Dehydratase//Prephenate Dehydratase
Phe - Phenylpyruvate route	2	ADT6	At1g08250	4.2.1.49	Arogenate Dehydratase//Prephenate Dehydratase
Phe - Phenylpyruvate route	3	AAAAT	-	2.6.1.57	Aromatic Amino Acid Aminotransferase
Tyr option 1:					
Tyr - Arogenate route	2	PAT	-	2.6.1.79	Prephenate Aminotransferase
Tyr - Arogenate route	3	TyrA1, ADS1	At5g34930	1.3.1.43	Arogenate Dehydrogenase
Tyr - Arogenate route	3	TyrA2, ADS2	At1g15710	1.3.1.43	Arogenate Dehydrogenase
Tyr option 2:					
Tyr - p-Hydroxyphenylpyruvate route	2	PDH	-	1.3.1.43	Prephenate Dehydrogenase
Tyr - p-Hydroxyphenylpyruvate route	3	AAAAT	-	2.6.1.57	Aromatic Amino Acid Aminotransferase
Trp					
Trp	1	ASa1, AMT1, TRP5	At5g05730	4.1.3.27	Anthranilate Synthase alpha subunit 1
Trp	1	ASa2	At2g29290	4.1.3.27	Anthranilate Synthase alpha subunit 2
Trp	1	ASa	At2g28880	4.1.3.27	Anthranilate Synthase alpha subunit (putative)
Trp	1	ASa	At3g55870	4.1.3.27	Anthranilate Synthase alpha subunit (putative)
Trp	1	ASb1, TRP4	At1g25220	4.1.3.27	Anthranilate Synthase beta subunit 1
Trp	1	ASb	At1g25155	4.1.3.27	Anthranilate Synthase beta subunit (putative)
Trp	1	ASb	At1g25083	4.1.3.27	Anthranilate Synthase beta subunit (putative)
Trp	1	ASb	At1g24909	4.1.3.27	Anthranilate Synthase beta subunit (putative)
Trp	1	ASb	At1g24807	4.1.3.27	Anthranilate Synthase beta subunit (putative)
Trp	1	ASb	At5g57890	4.1.3.27	Anthranilate Synthase beta subunit (putative)
Trp	2	PAT1, TRP1	At5g17990	2.4.2.18	Anthranilate Phosphoribosyltransferase
Trp	3	PAI1	At1g07780	5.3.1.24	Phosphoribosylanthranilate Isomerase
Trp	3	PAI2	At5g05590	5.3.1.24	Phosphoribosylanthranilate Isomerase
Trp	3	PAI3	At1g29410	5.3.1.24	Phosphoribosylanthranilate Isomerase
Trp	4	IGPS	At2g04400	4.1.1.48	Indole-3-Glycerol Phosphate Synthase
Trp	4	IGPS	At5g48220	4.1.1.48	Indole-3-Glycerol Phosphate Synthase (putative)
Trp	5	TSa, TRP3	At3g54640	4.2.1.20	Tryptophan Synthase alpha subunit

(continued on next page)

Table 1. *(continued)*

Pathway	Step	Gene name	AGI locus ID	CE	Proven or predicted activities in A.thaliana
Trp	5	TSa, INS	At4g02610	4.2.1.20	Tryptophan Synthase alpha subunit (putative)
Trp	6	TSb1, TRP2	At5g54810	4.2.1.20	Tryptophan Synthase beta 1 subunit
Trp	6	TSb2	At4g27070	4.2.1.20	Tryptophan Synthase beta 2 subunit
Trp	6	TSb	At5g28237	4.2.1.20	Tryptophan Synthase beta subunit (putative)
Trp	6	TSb	At5g38530	4.2.1.20	Tryptophan Synthase beta subunit (putative)
Phe catabolism (only representative enzyme)					
Phenylpropanoids		PAL1	At2g37040	4.3.1.5	Phe-Ammonia-Lyase1
Phenylpropanoids		PAL2	At3g53260	4.3.1.5	Phe-Ammonia-Lyase2
Phenylpropanoids		PAL3	At5g04230	4.3.1.5	Phe-Ammonia-Lyase3
Phenylpropanoids		PAL4	At3g10340	4.3.1.5	Phe-Ammonia-Lyase4
Phe-glucosinolates		CYP79A2	At5g05260	1.14.13.-	cytochrome P450 CYP79A2
Tyr catabolism (only representative enzyme)					
Tocochromanols		TyrAT	At5g53970	2.6.1.5	Tyr-Aminotransferase
Benzyl-isoquinoline alkaloids		TYDC	At2g20340	4.1.1.25	Tyr/L–Dopa Decarboxylase
Benzyl-isoquinoline alkaloids		TYDC	At4g28680	4.1.1.25	Tyr/L–Dopa Decarboxylase
Trp catabolism (only representative enzyme)					
Auxin - indole-3-acetaldoxime (IAOx) pathway//Camalexin//Indole glucosinolate		CYP79B2	At4g39950	1.14.13.-	cytochrome P450s CYP79B2
Auxin - indole-3-acetaldoxime (IAOx) pathway//Camalexin//Indole glucosinolate		CYP79B3	At2g22330	1.14.13.-	cytochrome P450s CYP79B3
Auxin - indole-3-pyruvic acid (IPyA) pathway		TAA	At1g70560	2.6.1.1	Trp Aminotransferase

have been proposed (Fig. 10) (Strader and Bartel, 2008; Quittenden et al., 2009). These include: 1) the indole-3-acetaldoxime (IAOx) pathway catalyzed by two cytochrome P450s (CE 1.14.13) (CYP79B2 and CYP79B3; At4g39950 and At2g22330) (Hull et al., 2000; Bartel et al., 2001); 2) the tryptamine (*YUCCA*) pathway catalyzed by Trp decarboxylase (TDC) (CE 4.1.1.28) (Facchini et al., 2000; Quittenden et al., 2009); 3) the indole-3-pyruvate (IPyA) pathway catalyzed by Trp aminotransferase (TAA)(CE 2.6.1.1) (At1g70560) (Stepanova et al., 2008; Tao et al., 2008); and 4) the indoleacetamide (IAM) pathway which initiates directly from Trp via either IAOx or indole-3-acetonitrile (IAN) (Pollmann et al., 2002). In addition, a possible additional, Trp-independent pathway of IAA biosynthesis directly from indole has been proposed (Normanly et al., 1993; Radwanski et al., 1996).

Another important group of secondary metabolites derived from Trp includes the glucosinolates, which are amino acid-derived natural plant products containing a thio-Glc moiety and a sulfonate moiety bound to an oxime function (Halkier and Gershenzon, 2006). They are implicated in plant-insect and plant-pathogen interactions, and also recently attracted attention as cancer-preventive agents in humans (Halkier, 1999). Glucosinolates are found almost exclusively in the Brassicales and have been widely studied in Arabidopsis and in other species of the *Brassicaceae* family (Rask et al., 2000; Reichelt et al., 2002; Yatusevich et al., 2009). The IAOx, described above is also channeled by the oxime-metabolizing CYP83B1 enzyme into the biosynthetic pathway of indole glucosinolates (Naur et al., 2003).

The Trp catabolic pathway also synthesizes camalexin, the major indolic phytoalexin in Arabidopsis accumulating upon infection with plant pathogens and abiotic elicitors (Zhao and Last, 1996; Bottcher et al., 2009). Camalexin originates from IAOx (Fig. 10) (Hull et al., 2000; Mikkelsen et al., 2000; Zhao et al., 2002). In addition, the Trp catabolic pathway also leads to the synthesis of indole alkaloids via tryptamine (Fig. 10). One example of the Trp-derived indole alkaloids is vindoline, an important metabolite in human health (Facchini et al., 2000; Facchini et al., 2004; Malitsky et al., 2008; Sugawara et al., 2009). However, indole alkaloids are generally not found in Arabidopsis.

FUTURE PROSPECTS

The entire set of genes and enzymes associated with the shikimate pathway have been elucidated (Table 1). However, elucidation of the regulation of this pathway is still in its infancy, requiring future studies. Even though, there were significant discoveries associated with genes and enzymes of the biosynthesis of the AAA in recent years, there are still missing links and debates about some key regulatory steps. The major route of Phe biosynthesis occurs through arogenate, but gene(s) encoding prephenate aminotransferase have yet to be identified. In addition, due to the fact that some plant arogenate dehydratase isozymes also possess residual prephenate dehydrate activities, as well as the observation that plants apparently possess aminotransferase activity that can convert PPY into Phe, one cannot rule out a minor contribution of a bacterial-like PPY route to Phe biosynthesis in plants. In addition, future studies should identify whether arogenate is the precursor for Tyr biosynthesis or whether an alternative bacterial-like route of Try biosynthesis via p-hyroxyPPY also exists. The regulation of the flux balance in the conversion of chorismate into

Trp and Phe/Tyr has already been extensively studied. Yet, the flux balance regulating the conversion of arogenate into either Phe or Tyr is still unknown, requiring future studies. Although relatively old studies suggest the presence of several enzyme feedback inhibition loops within the AAA biosynthesis pathway, some studies provide clues for additional ones (Fig. 7), which require future confirmation. Finally, a number of transcription factors have been proven to control different steps in the biosynthesis of AAA and secondary metabolites derived from them. Yet, it is likely that these do not represent the full set and additional studies are required to address this issue. Interestingly, some transcription factors regulate genes encoding both primary and secondary metabolism associated with the AAA, and an exciting prospect for future research would to test whether the primary metabolism enzymes regulated by these transcription factors represent key regulatory enzymes connecting primary and secondary metabolism.

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