Shikimate Pathway and Aromatic Amino Acid Biosynthesis

Advanced article

Article Contents

Online posting date: 15th August 2012

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Based in part on the previous version of this eLS article 'Shikimate Pathway: Aromatic Amino Acids and Beyond' (2001) by Klaus Herrmann and Robert Entus.

The shikimate pathway consists of seven enzymatic reactions whose end product chorismate is the precursor for the synthesis of the aromatic amino acids Phe, Tyr and Trp. In fungi and plants, chorismate is a precursor for many specialised metabolites (i.e. secondary metabolites) that play an important role in the plant's interaction with its environment. The shikimate pathway and aromatic amino acid biosynthesis have been extensively studied in a variety of microorganisms, fungi and plants. Furthermore, the dual involvement of the shikimate and aromatic amino acid biosynthesis pathways in central and specialised metabolism still raises major questions regarding the genes and enzymes involved, and their control, their evolutionary origins and coordinated regulation with genes of associated pathways in response to altered environmental conditions and diverse developmental programs.

Introduction

The shikimate pathway is named after its central intermediate, shikimic acid, which was first isolated from fruits of aniseed (*Illicium anisatum*) in 1885 and was named after the Japanese name of the plant *shikimi-no-ki*. In turn, the shikimic acid (shikimate) has given rise to the common name of the pathway, which is also known as the chorismate biosynthesis pathway. The seven enzymatic steps of the shikimate pathway convert the two metabolites, phosphoenolpyruvate (PEP) of the glycolysis pathway and erythrose 4-phosphate of the nonoxidative branch of the pentose phosphate pathway, into chorismate (**Figure 1**). Major metabolic routes use chorismate for the synthesis of the three aromatic amino acids, and additional minor metabolic routes lead to the synthesis of various specialised metabolites, such as isochorismate, *p*-amino- and *p*-hydroxybenzoate (**Figure 2**).

The shikimate and aromatic amino acid biosynthesis pathways operate in bacteria, fungi and plants, but not in humans or animals. Therefore, Phe and Trp are considered essential nutritive compounds in the diets of humans and their monogastric livestock, which are unable to synthesise them. This fact renders the shikimate and aromatic amino acid biosynthesis pathways important targets for the design of novel drugs and has also motivated the establishment of a new database called 'The ShiKimate Pathway DataBase (SKPDB),' a relational database applied to the study of shikimate pathway enzymes in microorganisms and plants (http://lsbzix.rc.unesp.br/skpdb/) (Arcuri *et al*., 2010). However, Tyr is exceptional and is not considered an essential amino acid since it is derived from Phe by one hydroxylation step which occurs in animals (Pribat *et al*., 2010).

In most bacteria, the major purpose of the shikimate pathway is to provide aromatic amino acids for protein synthesis. This pathway has been extensively explored in bacteria, partly because of the biotechnological significance of this metabolic pathway in the food and drug industry, for example, production of the anti-Parkinson's drug (L-dopa) and the low-calorie sweetener Aspartame. In plants, elucidation of the regulation of the metabolic pathways converting chorismate into the aromatic amino acids is essential since this pathway fulfills a vast array of various specialised metabolites and regulatory roles in plant development and interactions with the environment. For example, stilbenes, coumarins and isoflavonoids are phytoalexins produced by diseased plants; flavonoids serve

eLS subject area: Plant Science

How to cite:
Tzin, Vered; Galili, Gad; and Aharoni, Asaph (August 2012) Shikimate Pathway and Aromatic Amino Acid Biosynthesis. In: eLS. John Wiley & Sons, Ltd: Chichester.
DOI: 10.1002/9780470015902.a0001315.pub2

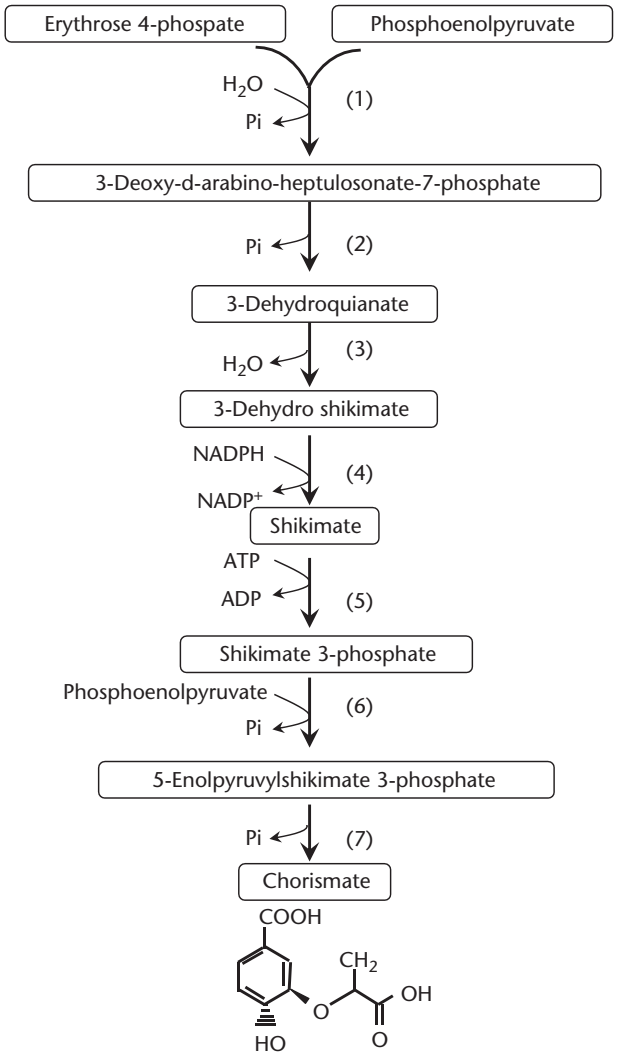

Figure 1 The shikimate pathway. Numbers indicate individual enzymes. Their names, substrates and products are given in **Table 1**.

as UV irradiation protectants and signals in interactions with symbionts; and acetosyringone and salicylate are involved in plant–pathogen interactions. **See also**: Evolution of Secondary Plant Metabolism; Plant Defences against Herbivore Attack

The importance of the shikimate pathway is demonstrated by the estimation that ~30% of the carbon fixed by plants flows through this pathway (Haslam, 1993). It is estimated that lignins, the most abundant plant biopolymers incorporated in the cell wall which are derived from the shikimate pathway, account for approximately 30% of the organic carbon in the biosphere. The regulation of lignin biosynthesis has been investigated in depth recently due to an increasing demand to reduce this polymer content for efficient extraction of various biofuel products synthesised in plants. **See also**: Lignins; Secondary Cell Walls

The Shikimate Pathway

Key enzymes and their intracellular localisation

The shikimate pathway possesses seven enzymatic steps that altogether convert phosphoenolpyruvate (PEP) and erythrose 4-phosphate into chorismate (a schematic drawing of the shikimate pathway, the names of the corresponding enzymes and the reactions that they catalyse are given in **Table 1** and **Figure 1**). The most studied enzymes in this pathway are the first enzyme, 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DAHPS) and the sixth enzyme, 5-enol-pyruvoylshikimate 3-phosphate synthase (EPSPS). Bacterial DAHPSs are generally feedback-inhibited by the different aromatic amino acids. Genetic manipulation of DAHPS, particularly rendering this enzyme insensitive to feedback inhibition by Phe, was performed in bacteria (Hu *et al*., 2003) and fungi (Luttik *et al*., 2008), which caused an increased production of the three aromatic amino acids as well as plant specialised metabolites derived from them. In contrast to bacteria and fungi, the allosteric regulation of plant DAHPS is still questionable (Gilchrist and Kosuge, 1980; Herrmann and Weaver, 1999). Yet, *in vitro* activities of DAHPS from different plant species are weakly inhibited by Trp (Graziana and Boudet, 1980; Rubin and Jensen, 1985) and Tyr (Reinink and Borstap, 1982), or weakly activated by either Trp or Tyr (Suzich *et al*., 1984; Pinto *et al*., 1986). In addition, the *Vigna radiate* (bean) DAHPS activity is weakly inhibited by prephenate and arogenate, the precursors of Phe and Tyr biosynthesis (Rubin and Jensen, 1985); whether this is due to inhibition of enzyme level or activity is still unknown (Herrmann, 1995; Tzin and Galili, 2010).

The growth of plants is generally strongly inhibited by the broad-spectrum systemic herbicide glyphosate (N-(phosphonomethyl)-glycine), commercially known as Roundup. This chemical serves as a broad-spectrum herbicide for killing weeds growing in fields cultivated by crop plants and was commercially introduced in the 70s. It was found that glyphosate is a transition state analog of PEP, one of the substrates for EPSPS (**Figure 1**), and it is the only herbicide that targets EPSPS. Since its mode of action was discovered, various genetic engineering and biotechnology approaches producing glyphosate-resistant (GR) crops were tried with limited success until the CP4 gene, a homolog gene of *Agrobacterium sp*., was found to encode a GR form of EPSPS. When this CP4 gene was placed into the genome of certain crops, high levels of GR were expressed. The CP4 gene is responsible for glyphosate resistance in most commercial GR crops. In addition to the CP4 gene, a gene from *Ochrobactrum anthropi* encoding glyphosate oxidoreductase (an enzyme of glyphosate degradation) was employed to contribute to resistance in canola. For maize, the EPSPS has been altered by site-directed mutagenesis of a maize gene to provide a form of GR EPSPS that is used in some GR maize varieties (Duke

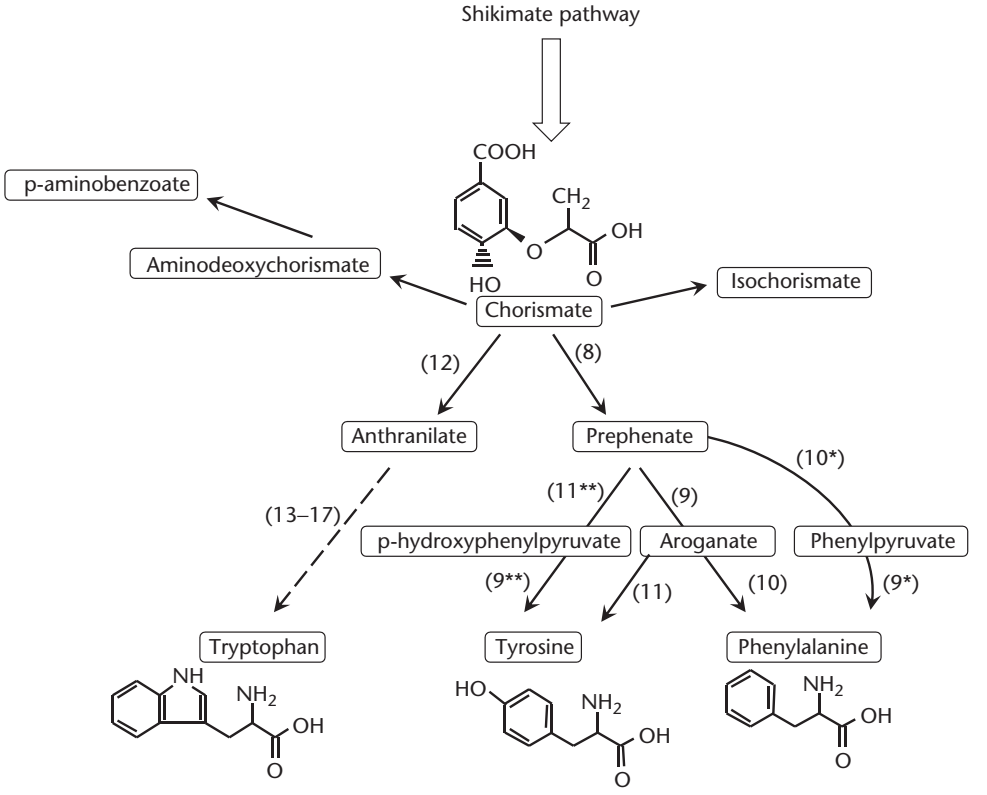

Figure 2 The aromatic amino acid biosynthesis. Numbers indicate individual enzymes. Their names, substrates and products are given in **Table 1**.

and Powles, 2008; Pollegioni *et al*., 2011). Other enzymes associated with photosynthesis and photorespiration redox homeostasis are considered secondary glyphosate targets (Vivancos *et al*., 2011).

In bacteria and fungi, enzymes of the shikimate pathway are localised in the cytoplasm, whereas in plants the enzymes of this pathway are generally localised in the plastid. The plants' proteins are synthesised as precursors containing a plastid transit peptide that directs them to the organelle. The substrates of the shikimate pathway are also localised in the plastid, but whether these substrates are synthesised inside the plastid or in another intracellular compartment and subsequently transported into the plastid is still largely unknown.

Shikimic acid is an essential metabolite for chorismate biosynthesis and lignin

Shikimic acid is an intermediate metabolite of the shikimate pathway (product of step 4 catalysed by shikimate 5-dehydrogenase) whose steady-state level has been shown to rise upon various manipulations of the shikimate pathway genes in plants. Shikimic acid was also accumulated in GR EPSPS plants, which catalysed the downstream reaction of the shikimic acid (Pline *et al*., 2002; Mueller *et al*., 2003; Buehring *et al*., 2007). Contrarily, RNAi-mediated suppression of the bifunctional shikimate pathway enzyme DHQ/SDH (steps 3 and 4) had shown accumulation rather than reduction in the level of shikimic acid in transgenic tobacco plants (Ding *et al*., 2007). Shikimic acid is also a substrate for the hydroxycinnamoyl-CoA shikimic acid/quinic acid hydroxycinnamoyltransferase (HCT) enzyme, which contributes significantly to lignification of plant leaves (Hoffmann *et al*., 2004). These findings suggest that Shikimic acid is an essential metabolite whose changing levels may indicate alterations in the metabolic status of the plant as well as the regulatory complexity of the whole shikimate pathway. **See also**: Lignins; Secondary Cell Walls

Evolutionary origins of the eukaryotic shikimate pathway

The shikimate pathway is an ancient eukaryotic pathway that has been subjected to diverse evolutionary pressures. The seven enzymes of the shikimate pathway and the metabolic intermediates in the biosynthesis of chorismate appear to be universal for all organisms that possess this pathway. However, the structures of the enzymes and the cell compartments in which they are located show significant phylogenetic divergence. In *Escherichia coli* and other prokaryotes, all enzymes are monofunctional and their corresponding genes are widely scattered over the genetic map. However, in plants, reactions 3 and 4 are catalyzed by

Table 1 The enzymes of the shikimate and aromatic amino acid biosynthesis pathways

	Step	CE	Enzyme	Substrate 1	Substrate 2/ Cofactor	Product 1	Product 2
Shikimate							
1	2.5.1.54	3-Deoxy-D-arabino-heptulosonate-7-phosphate synthase	Phosphoenolpyruvate	Erythrose 4-phosphate	→	3-Deoxy-D-arabino-heptulosonate 7-phosphate	Pi
2	4.2.3.4	3-Dehydroquinate Synthase	3-Deoxy-D-arabino-heptulosonate 7-phosphate	–	→	3-Dehydroquinate	Pi
3	4.2.1.10	3-Dehydroquinate dehydratase	3-Dehydroquinate	–	→	3-Dehydroshikimate	H_2O
4	1.1.1.25	Shikimate 5-dehydrogenase	3-Dehydroshikimate	NADPH	→	Shikimate	$NADP^+$
5	2.7.1.71	Shikimate Kinase	Shikimate (Shikimic acid)	ATP	→	Shikimate 3-phosphate	ADP
6	2.5.1.19	5-Enolpyruvylshikimate 3-phosphate Synthase	Shikimate 3-phosphate	Phosphoenolpyruvate	→	5-Enolpyruvoylshikimate 3-phosphate	Pi
7	4.2.3.5	Chorismate Synthase	5-Enolpyruvoylshikimate 3-phosphate		→	Chorismate	Pi
Aromatic amino acids							
8	5.4.99.5	Chorismate Mutase	Chorismate	–	→	Prephenate	–
Phe route 1							
9	2.6.1.79	Prephenate Aminotransferase	Prephenate	Glutamate	→	Arogenate	a-ketoglutarate
10	4.2.1.49	Arogenate Dehydratase	Arogenate		→	Phe	H_2O, CO_2
Phe route 2							
10*	4.2.1.49	Prephenate Dehydratase	Prephenate		→	Phenylpyruvate	H_2O, CO_2
9*	2.6.1.57	Aromatic Amino Acid Aminotransferase	Phenylpyruvate	Glutamate	→	Phe	a-ketoglutarate
Tyr route 1							
9	2.6.1.79	Prephenate Aminotransferase	Prephenate	Glutamate	→	Arogenate	a- ketoglutarate
11	1.3.1.43	Arogenate Dehydrogenase	Arogenate	NADP +	→	Tyr	NADPH
Tyr route 2							
11**	1.3.1.43	Prephenate Dehydrogenase	Prephenate	NAD +	→	p-Hydroxyphenylpyruvate	NADH
9**	2.6.1.57	Aromatic Amino Acid Aminotransferase	p-Hydroxyphenylpyruvate	Glutamate	→	Tyr	a-ketoglutarate
Trp							
12	4.1.3.27	Anthranilate Synthase	Chorismate	Glutamine	→	Anthranilate	Pyruvate, Glutamate

13	2.4.2.18	Anthranilate Phosphoribosyltransferase	Anthranilate	Phosphoribosyl pyrophosphate	→	Phosphoribosyl anthranilate	PPi
14	5.3.1.24	Phosphoribosyl anthranilate Isomerase	Phosphoribosyl anthranilate	–	→	1-(*o*-Carboxyphenylamino)-1-deoxyribulose 5-phosphate	
15	4.1.1.48	Indole-3-Glycerol Phosphate Synthase	1-(*o*-Carboxyphenylamino)-1-deoxyribulose 5-phosphate	–	→	Indole-3-Glycerol Phosphate	H_2O, CO_2
16	4.2.1.20	Tryptophan Synthase alpha subunit	Indole-3-Glycerol Phosphate	–	→		glyceraldehyde 3-phosphate
17	4.2.1.20	Tryptophan Synthase beta subunit	Indole	Serine	→	Tryptophan	H_2O

a bifunctional enzyme (DHQ/SDH) complex. In *Toxoplasma gondii*, a species of parasitic protozoa, a single gene named *AroM* encodes a five-enzyme pentafunctional protein (enzymatic steps 2–6) in the shikimate pathway (Richards *et al*., 2006).

In plants, genes of the shikimate pathway do not originate from a single prokaryotic ancestor of cyanobacterial origin, but are likely derived from at least three different sources. The phylogenies suggest that two of the genes were derived from the cyanobacterial plastid progenitor genome. The other five shikimate pathway genes were obtained from a minimum of two other eubacterial genomes. These would have been acquired by endosymbiotic and horizontal gene transfer (for more information on this, see Richards *et al*., 2006). Alternatively, a portion of the shikimate pathway proteins targeted to plastids seems to have evolved from their host eukaryotic genome rather than through acquisition from cyanobacteria. Following the endosymbiotic event, new shikimate pathway enzymes containing plastid transit peptides (targeting the proteins from the cytosol into plastids), as well as metabolite transport mechanisms, have been evolved. Therefore, the shikimate enzymes of higher plants are generally synthesized as precursors containing a transit peptide that directs them to the plastid. Interestingly, the unicellular flagellate *Euglena gracilis* possesses two DAHPS class II enzymes that are differentially expressed during light-induced chloroplast development, and it is known to possess both a cytosol- and a plastid-associated shikimate pathway.

Aromatic amino acid biosynthesis

The synthesis of the aromatic amino acids Phe, Tyr and Trp begins from chorismate, the terminal metabolite of the shikimate pathway, which also serves as an initiator substrate for the synthesis of a number of other aromatic metabolites, such as tetrahydrofolate (vitamin B9, also commonly termed folate), the plant hormone salicylate and phylloquinone (vitamin K1). A schematic drawing of the aromatic amino acid biosynthesis pathways, the names of the corresponding enzymes and the reactions they catalyse are presented in **Table 1** and **Figure 2**.

The first committed enzyme of Phe and Tyr biosynthesis from chorismate is chorismate mutase (CM; **Table 1**, step 8), which converts chorismate to prephenate. Microorganisms use at least two different metabolic routes for the synthesis of the aromatic amino acid Phe from prephenate: one via the intermediate metabolite phenylpyruvate and the second via the intermediate metabolite arogenate. Some cyanobacteria, coryneform bacteria and spore-forming actinomycetes use mostly the arogenate route as the major substrate to synthesise Phe.

In contrast to microorganisms, the metabolic route from chorismate to Phe in plants is still not entirely known. Recent studies in several plant species provided lines of evidence suggesting that plants synthesise Phe primarily via the arogenate route. For example, genes encoding prephenate aminotransferase were identified and shown to direct carbon flux from prephenate towards arogenate (Dal Cin *et al*., 2011; Maeda *et al*., 2011), and genes encoding arogenate dehydratase were shown to direct carbon flux from arogenate towards Phe (Cho *et al*., 2007; Yamada *et al*., 2008; Graindorge *et al*., 2010; Maeda *et al*., 2010). This data suggested that the arogenate pathway is predominant in plant phe biosynthesis (Cho *et al*., 2007; Yamada *et al*., 2008; Graindorge *et al*., 2010; Maeda *et al*., 2010). Nevertheless, a number of plant species contain phenylpyruvate, which also serves as a precursor for a number of specialised metabolites such as phenylacetaldehyde and 2-phenylethanol (Watanabe *et al*., 2002; Kaminaga *et al*., 2006; **Figure 3**). It has been suggested that phenylpyruvate can also serve as an intermediate metabolite in Phe biosynthesis in plants (Tzin *et al*., 2009), although such an alternative route is a minor route compared to the major route via arogenate. The potential existence of a metabolic route via phenylpyruvate was hypothesised based on a study showing that expression of a bacterial *PheA* gene encoding a bifunctional enzyme chorismate mutase/prephenate dehydratase, which converts chorismate via prephenate into phenylpyruvate in *Arabidopsis* plants, resulted in a major increase in Phe levels (Tzin *et al*., 2009). **See also**: Plant Volatiles

In plants and some bacteria, the major route for Tyr biosynthesis is initiated from chorismate and uses the same first two enzymes of Phe biosynthesis to produce arogenate (**Figure 2**). Arogenate is converted into Tyr by arogenate dehydrogenase, whose activity has been demonstrated in tobacco, maize, sorghum and *Arabidopsis* (Rippert and Matringe, 2002; Rippert *et al*., 2009). Similar to the Phe biosynthesis route in plants, an alternative biosynthetic route for Tyr has also been suggested to occur in plants, which includes the conversion of prephenate to *p*-hydroxyphenylpyruvate (Rippert and Matringe, 2002). In plants, the enzymes of the Phe and Tyr biosynthesis pathways are generally localised in the plastid (Mustafa and Verpoorte, 2005; Weber *et al*., 2005; Zybailov *et al*., 2008; Rippert *et al*., 2009) with two potential exceptions: CM isoform 2 in *Arabidopsis* and arogenate dehydratase isoform 3 in tobacco were suggested to be located in the cytosol. The physiological significance of these potential cytosolic isoforms is still an enigma (Rippert *et al*., 2009).

The first committed step of Trp biosynthesis is anthranilate synthase (AS; **Table 1**, step 12), which is probably the most studied enzyme of this pathway due to its structure and allosteric regulation by Trp. In plants, AS forms heterotetramers composed of two alpha and two beta subunits (Niyogi *et al*., 1993). Expression of mutated genes encoding feedback-insensitive AS enzymes in a variety of plant species generally increases the production of free Trp and specialised metabolites derived from it (Hughes *et al*., 2004). All the enzymes of the Trp biosynthesis pathway are generally synthesised as precursors containing a plastid transit peptide, implying their localisation in this organelle (Mustafa and Verpoorte, 2005; Weber *et al*., 2005; Zybailov *et al*., 2008).

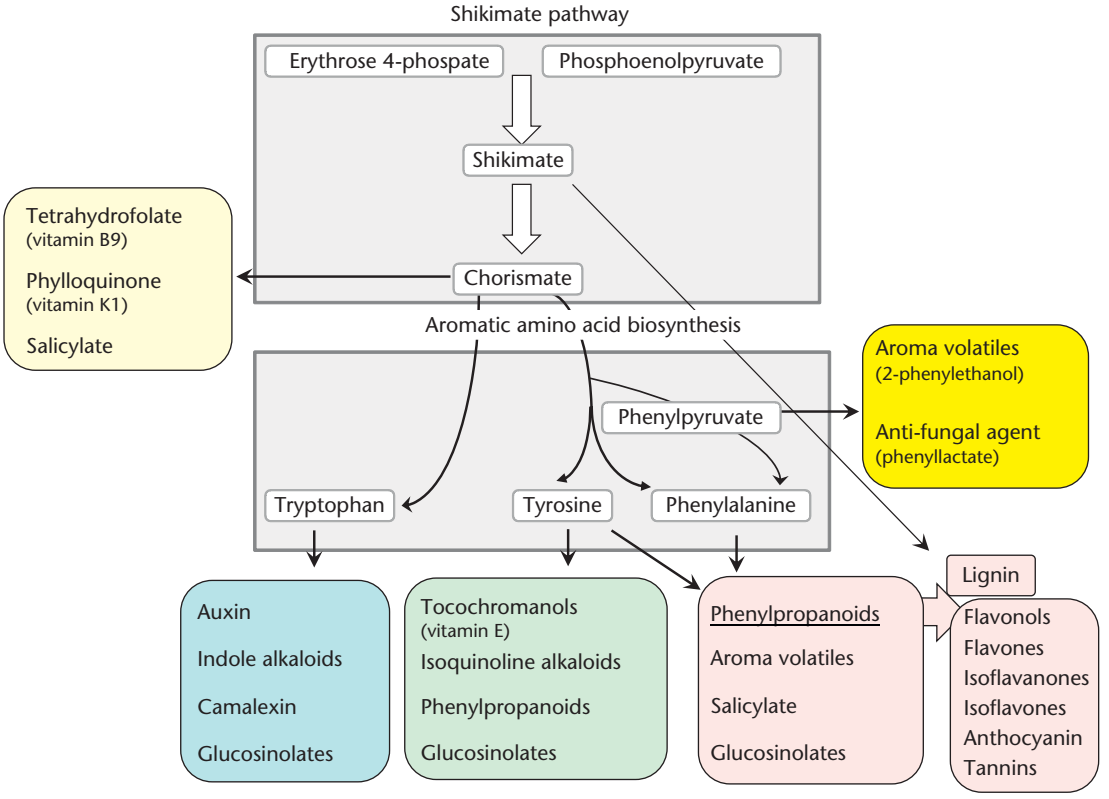

Figure 3 Major classes of specialised metabolites derived from shikimate, chorismate, Phe, Tyr and Tryptophan.

Nematodes secrete a chorismate mutase enzyme as part of their interaction with plants

The complete sets of metabolic enzymes for synthesis of the aromatic amino acid pathway do not exist in animals, except for a secreted form of chorismate mutase (CM) that is present in the root-knot nematode and potato cyst nematode. These organisms specifically express CM genes in the esophageal glands (Popeijus *et al*., 2000; Bekal *et al*., 2003). Transgenic expression of the *Meloidogyne javanica* nematode *MjCM1* gene in plant roots suppresses lateral root formation and the development of the vascular system, which can be rescued by exogenous application of auxin, suggesting that the expression of *MjCM1* reduces auxin levels (Doyle and Lambert, 2003). Since chorismate is also a precursor for the synthesis of the plant hormones auxin and salicylate, the expression of the *MjCM1* in plant cells apparently competitively reduces the fluxes towards: (1) the synthesis of Trp and its downstream hormone auxin; and (2) the synthesis of salicylate directly from chorismate. Because plants contain the shikimate pathway and nematodes have only a secreted form of CM, this nematode enzyme is thought to be involved in the alteration of the plant's pathway upon infection by the nematode (Davis *et al*., 2008).

Regulation of the shikimate and aromatic amino acid biosynthesis pathways

Transcriptional regulation

In plant genes from the shikimate pathway, aromatic amino acid biosynthesis and phenylpropanoids are regulated by transcription factors (TFs). Here we describe only a few of these TFs R2R3-MYB family: (1) *ODORANT1*, R2R3-MYB TFs that were discovered in *Petunia* (*Petunia hybrid*) flowers, regulates the production of volatile benzenoids through activating various genes, including those encoding enzymes of the shikimate and aromatic amino acid biosynthesis: *DAHPS* (step 1), *EPSPS* (step 6) and *CM* (step 8) (Schuurink *et al*., 2006). Ectopic expression of the petunia *ODORANT1* in tomato induced several genes encoding shikimate and aromatic amino acid biosynthesis, as well as facilitated discovery of the tomato gene encoding prephenate aminotransferase (step 9/9*), which converts prephenate to arogenate (Dal Cin *et al*., 2011); (2) *EMISSION OF BENZENOIDS II* (*EOBII*), a regulatory factor of phenylpropanoid volatile biosynthesis that was found to be flower-specific and temporally and spatially associated with scent production/emission. Up/down regulation of *EOBII* in petunia affected transcript levels of several biosynthetic floral scent-related genes encoding enzymes from the phenylpropanoid pathway that are directly involved in

the production of these volatiles and enzymes from the shikimate pathway that determine substrate availability (Spitzer-Rimon *et al*., 2010); (3) *AtMYB15* in *Arabidopsis* is an early wounding-inducible gene and its overexpression in transgenic plants resulted in elevated expression of almost all the genes involved in the shikimate pathway (Chen *et al*., 2006); and (4) *PRODUCTION OF ANTHOCYANIN PIGMENT1 (PAP1)* in *Arabidopsis* regulates the expression of several genes encoding to the shikimate pathway and key phenylpropanoids (Borevitz *et al*., 2000).

In yeast, most structural genes are regulated by the transcriptional activator GCN4, also named the 'mechanism of general control.' This is a regulator of the general amino acid control network, which couples transcriptional activation to amino acid starvation of numerous structural genes in multiple amino acid biosynthetic pathways, including the shikimate pathway and amino acid biosynthesis. A yeast strain deficient in the regulatory transcriptional system of amino acid biosynthesis is unable to survive in the presence of high amounts of Phe and Tyr (Gientka and Duszkiewicz-Reinhard, 2009).

In several bacterial species, the genes encoding Phe and Tyr biosynthesis enzymes are located in adjacent regions on the chromosome, distinct from the location of the genes encoding the Trp-operon biosynthesis pathway. The Trp-operon encodes five structural genes and a promoter which binds to RNA polymerase and an operator which blocks transcription when bound to the protein synthesised by the repressor gene (trp R) that binds to the operator (Ikeda, 2006; Sprenger, 2006). The Trp-operon was the first operon described, and it was discovered in 1953 by Jacques Monod and colleagues in *E. coli*. The Trp-operon is negatively regulated by Trp. The repression is mediated by the RNA polymerase transcribing the genes in the operon, and the Trp-operon contains a leader peptide and an attenuator sequence which allows graded regulation (Merino *et al*., 2008).

Post-translationional regulation

The synthesis of a large part of amino acids is regulated by post-translational feedback inhibition loops. Hence, enzymes from both the shikimate pathway and aromatic amino acid biosynthesis are subject to post-translational regulation. *E. Coli*, for instance, possesses three different DAHPS isoforms, encoded by the *AroF, AroG* and *AroH* genes, which are feedback-inhibited by Tyr, Phe and Trp, respectively (Hu *et al*., 2003; Luttik *et al*., 2008). In plants, feedback-inhibition regulation of the DAHPS enzymes by any of the aromatic amino acids is still questionable as described above. A recent study revealed that ectopic expression of the *E. coli DAHPS* gene (AroG) with Phe-feedback-insensitive in *Arabidopsis* presents increased levels of shikimic acid, prephenate and aromatic amino acids, as well as induction of broad classes of specialised metabolites including phenylpropanoids, glucosinolates, auxin and other hormone conjugates (Tzin *et al*., 2012).

Taken together with extensive biochemical bioinformatics studies on amino acid biosynthesis, it is implied that amino acid biosyntheses are predominantly regulated by post-translational allosteric feedback loops, whereas amino acid catabolism is principally regulated at the transcriptional level (Less and Galili, 2008). Several of the aromatic amino acid biosynthesis enzymes are allosterically regulated: (1) *Arabidopsis* CM (**Table 1**, step 8) isoforms, each of the AtCM cDNAs were expressed in yeast. The activities of both AtCM1 and AtCM3 isozymes, localised in the plastid, were feedback-inhibited by Phe and Tyr, while stimulated by Trp. AtCM2, the only CM isoform that has been localised in the cytosol, was insensitive to allosteric regulation by any of the aromatic amino acids (Mobley *et al*., 1999); (2) dehydratase/prephenate dehydratase (**Table 1**, step 10/10*) which is positively regulated by Tyr and negatively regulated by Phe. A similar regulatory mechanism was also found in tobacco, spinach, *Sorghum bicolor* and recently in rice (Yamada *et al*., 2008); (3) arogenate dehydrogenase (**Table 1**, step 11/11**); this enzyme is regulated by feedback inhibition of Tyr in *Arabidopsis* (Rippert and Matringe, 2002) and Sorghum bicolor (Connelly and Conn, 1986); and (4) AS alpha-subunit (**Table 1**, step 12). Feedback-insensitive AS alpha-subunit in *Arabidopsis* and rice mutants generally accumulates Trp, but not Phe or Tyr (Tozawa *et al*., 2001; Ishihara *et al*., 2006).

In bacteria, enzymes which convert chorismate were evolved to be allosterically regulated by the pathway end products: Phe inhibits CM/PDT (**Table 1**, steps 8 and 9*); Tyr inhibits CM/PDH (**Table 1**, steps 8 and 11**); and Trp inhibits AS/PAT (**Table 1**, steps 12 and 13) in *E. coli* (Ikeda, 2006; Sprenger, 2006).

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