Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

3-Deoxy-D-*arabino*-heptulosonate 7-phosphate synthase as the gatekeeper of plant aromatic natural product biosynthesis

Ryo Yokoyama, Bailey Kleven, Anika Gupta, Yuer Wang and Hiroshi A. Maeda

Abstract

The shikimate pathway connects the central carbon metabolism with the biosynthesis of aromatic amino acids—L-tyrosine, L-phenylalanine, and L-tryptophan—which play indispensable roles as precursors of numerous aromatic phytochemicals. Despite the importance of the shikimate pathway-derived products for both plant physiology and human society, the regulatory mechanism of the shikimate pathway remains elusive. This review summarizes the recent progress and current understanding on the plant 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase (DAHP synthase or DHS) enzymes that catalyze the committed reaction of the shikimate pathway. We particularly focus on how the DHS activity is regulated in plants in comparison to those of microbes and discuss potential roles of DHS as the critical gatekeeper for the production of plant aromatic compounds.

Addresses

Department of Botany, University of Wisconsin–Madison, Madison, WI, 53705, USA

Corresponding authors: Maeda, Hiroshi A. (maeda2@wisc.edu); Yokoyama, Ryo (ryokoyama@wisc.edu)

Current Opinion in Plant Biology 2022, **67**:102219

This review comes from a themed issue on **Physiology and metabolism (2022)**

Edited by **Asaph Aharoni** and **Hiroshi A. Maeda**

For complete overview of the section, please refer the article collection - Physiology and metabolism (2022)

Available online 10 May 2022

https://doi.org/10.1016/j.pbi.2022.102219

Keywords

Primary metabolism, The shikimate pathway, Aromatic amino acids, 3-Deoxy-D-*arabino*-heptulosonate 7-phosphate synthase, Plant aromatic natural products, Allosteric regulation.

Abbreviations

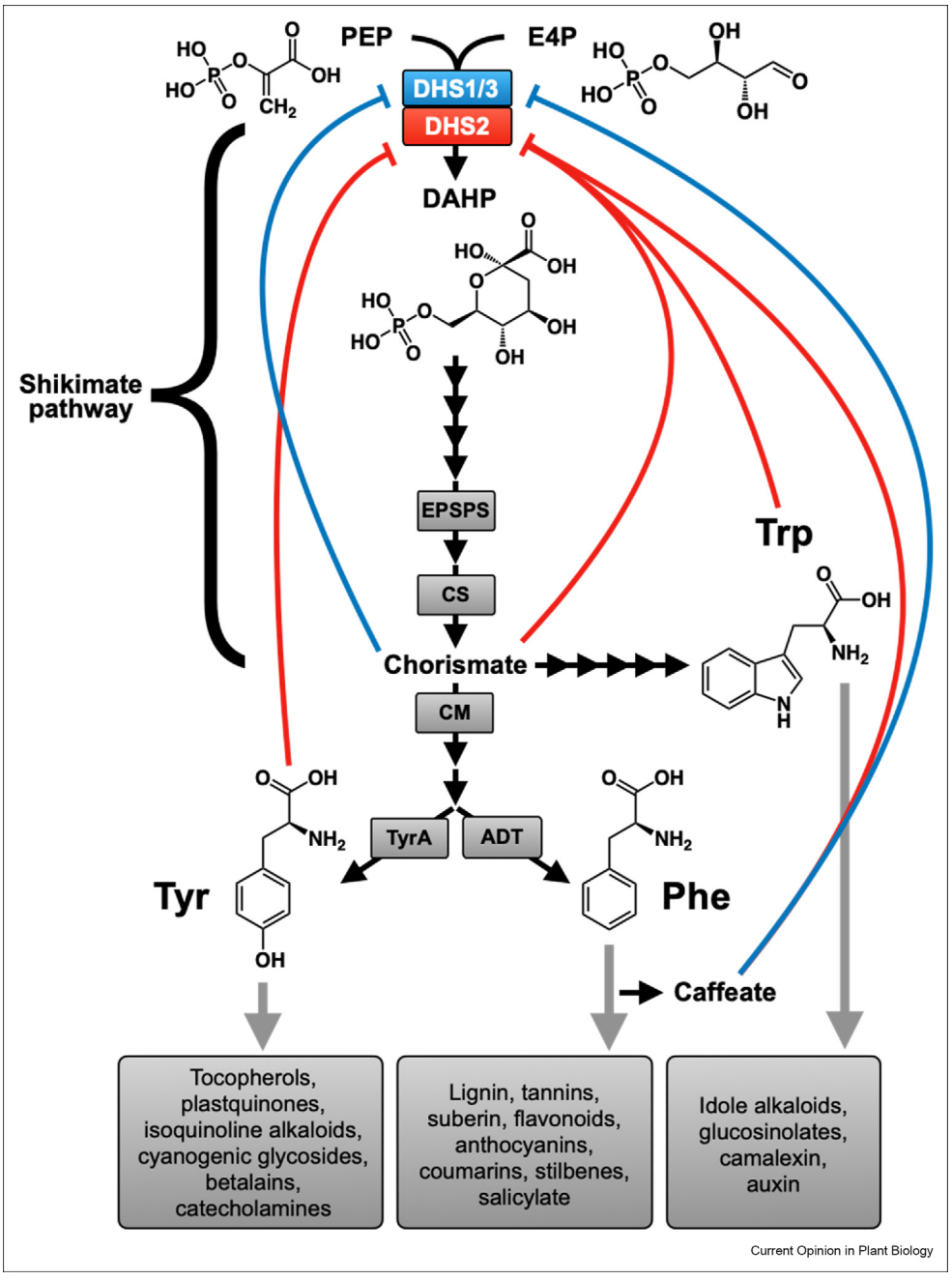
AAA, aromatic amino acid; ADT, arogenate dehydratase; CCA1, circadian clock associated 1; CM, chorismate mutase; CS, chorismate synthase; DAHP, 3-deoxy-D-*arabino*-heptulosonate 7-phosphate; DHS, 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase; DTT, 1,4-dithiothreitol; E4P, erythrose-4-phosphate; EPSPS, 5-enolpyruvyl-shikimate-3-phosphate synthase; ODO1, ODORANT1; PAL, phenylalanine ammonia-lyase; PEP, phospho*enol*pyruvate; Phe, L-phenylalanine; PIF4/5, phytochrome-interacting factor 4/5; TF, transcriptional factor; Trp, L-tryptophan; Tyr, L-tyrosine; TyrA, tyrosine arogenate dehydrogenase.

Introduction

The shikimate pathway is a primary metabolic pathway found in microbes and plants and connects the central carbon metabolism to the biosynthesis of aromatic amino acids (AAAs)—L-tyrosine (Tyr), L-phenylalanine (Phe), and L-tryptophan (Trp, Figure 1) [1–4]. AAAs are proteinogenic amino acids essential for protein synthesis in all organisms and are critical precursors in plants to synthesize numerous aromatic natural products, which is a hallmark of land plants, especially in angiosperms [4–6]. These aromatic phytochemicals play essential roles in plant development and adaptation and include phytohormones, defense compounds, and phenolic polymers, such as lignin, the second major biopolymer after cellulose (Figure 1) [7–9]. Therefore, plants must fine-tune the activity of the shikimate pathway to control the production of abundant and diverse aromatic chemicals and to orchestrate complex developmental and physiological processes. However, we still have limited knowledge about how plants regulate the shikimate pathway. Addressing this question is also a prerequisite for plant and microbial metabolic engineering of aromatic compounds, which are critical in our society as industrial materials, nutraceuticals, and pharmaceuticals [10].

The 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase (DHS, also known as DAHP synthase) catalyzes the first step of the seven enzymatic reactions in the shikimate pathway (Figure 1). Unlike well-characterized microbial DHSs, the regulation of plant DHS enzymes had been poorly understood until recently. This review summarizes emerging findings uncovering highly-complex regulatory mechanisms of plant DHS enzymes, which is likely important to meet the constantly-changing metabolic demand for the production of diverse AAA-derived metabolites in plants. The fundamental understanding of this key regulatory enzyme will be crucial for plant metabolic engineering and synthetic biology to efficiently produce various aromatic compounds in plants.

Figure 1



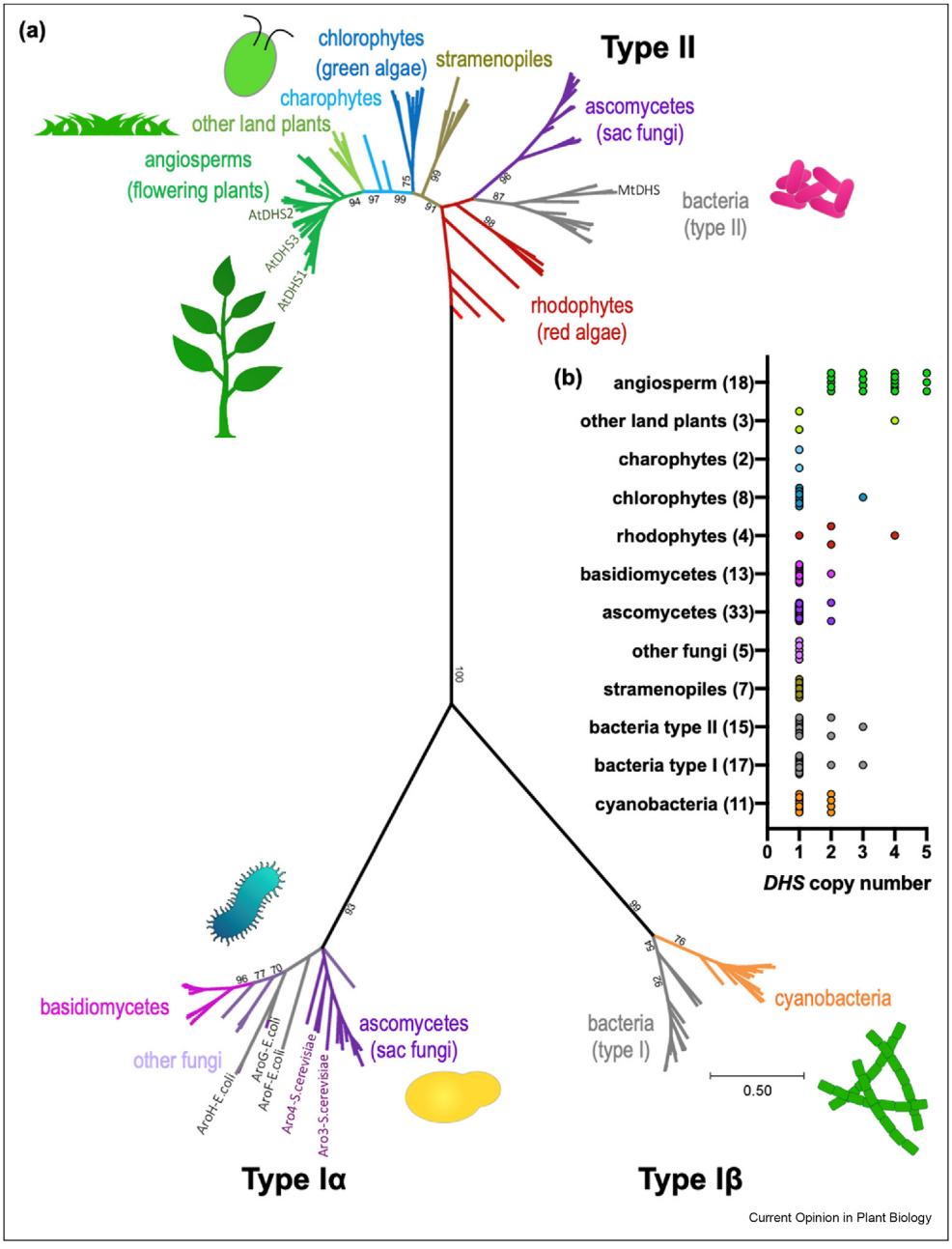
Schematic diagram of the shikimate pathway and AAA biosynthesis. Red and blue lines denote feedback regulation of Arabidopsis DHS2 and DHS1/3 isoforms, respectively. PEP, phospho*enol*pyruvate; E4P, erythrose-4-phosphate; Tyr, L-tyrosine; Phe, L-phenylalanine; Trp, L-tryptophan; DAHP, 3-deoxy-D-*arabino*-heptulosonate 7-phosphate; DHS, 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; CS, chorismate synthase; CM, chorismate mutase; ADT, arogenate dehydratase; TyrA, tyrosine arogenate dehydrogenase.

Multiple type II DHS isoforms present in angiosperms

DHS enzymes catalyze the aldol-type condensation of phospho*enol*pyruvate (PEP) and erythrose-4-phosphate (E4P), derived from glycolysis and the pentose phosphate pathways (e.g., the Calvin–Benson cycle in photosynthetic organisms), respectively, into the product 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP, Figure 1). DHSs utilize a divalent cation, such as manganese, cobalt, or iron, and typically form homotetramers [1–4,11]. DHSs can be categorized into type I and type II, and most microbes have type I enzymes, which can be further divided into type Iα and β

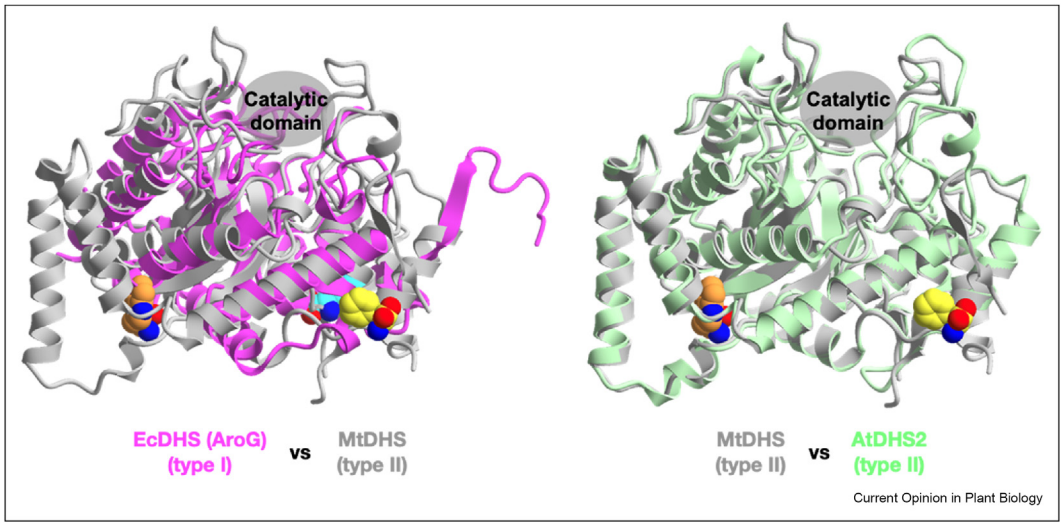
subfamilies, such as in *Escherichia coli* and cyanobacteria, respectively (Figure 2a) [11,12]. Type II DHSs are found in photosynthetic organisms and a limited number of fungi and bacteria (Figure 2a) [12–15]. Type I and II DHSs have low sequence identity (< 10%) but share a common structural fold (Figure 3), suggesting that all DHSs diverged from a common ancestor [11,12,15,16]. Plant DHSs form a condensed phylogenetic clade of type II DHSs (Figure 2a) and hence likely evolved from type II microbial DHSs, rather than

Figure 2



Evolution of type I and type II *DHS* genes from microbes and plants. **(a)** A phylogenetic tree of type I and type II *DHS* genes. DHS orthologs were first identified by BlastP searches with the amino acid sequence of AtDHS2, MtDHS (for type II DHSs), and *E. coli* AroF (for type I DHSs) as queries against NCBI RefSeq and Phytozome protein databases. All of the sequences obtained were then used to construct a tree of *DHS* genes using MEGA10.1.8 and are available as a FASTA file in Supplemental Data Set 1. The sequences were aligned by the ClustalW algorithm (Supplemental Data Set 2) and then constructed into a maximum-likelihood tree with 1000 bootstraps (Supplemental Data Set 3). Selected frequency values for important branches are shown. **(b)** The copy numbers of *DHS* genes in each organism that were counted from the phylogenetic tree data in this article and the previous study (Supplemental Data Set 4). The parenthesized number next to each taxonomic annotation indicates the number of species investigated in the taxon.

Figure 3



Structural comparisons of type I versus type II DHSs (left) and bacterial versus plant type II DHSs (right). EcDHS (AroG) and MtDHS monomer structures were obtained from Protein Data Bank (1KFL and 3KGF, respectively). The AtDHS2 structural model was predicted with the MtDHS structure as a template in SWISS-MODEL, and is available in Supplemental Data Set 5. The structures were visualized with Cuemol2. The Trp molecule bound to MtDHS and the Phe molecules bound to MtDHS and EcDHS are colored by orange, yellow and light blue, respectively.

acquisition of cyanobacterial type I DHSs through endosymbiosis [12]. While most microbes and algae have a single *DHS* gene, land plants, particularly angiosperms, have multiple DHS isoforms (Figure 2b) [13,14], coinciding with the evolution of diverse aromatic compounds, especially phenylpropanoids, produced in angiosperms [5,6]. Although plant DHS structures have not been solved, a predicted plant DHS model is structurally similar to microbial type II DHSs (Figure 3). Plant DHSs exhibit similar kinetic parameters to both type I and II microbial DHSs, with an exception of higher K_m toward E4P (Table 1) [14,16–21], suggesting that the overall DHS activity in plants likely depends on E4P availability from the pentose phosphate pathways, such as the Calvin–Benson cycle.

Highly-complex feedback regulation of plant DHS enzymes

Microbial DHSs from both type I and II groups are typically inhibited by AAAs [1,11]. For example, *E. coli* AroF, AroG, and AroH are inhibited by Tyr, Phe, and Trp, respectively [22]. These negative feedback inhibitions in type I DHSs are mediated through a single effector binding site, which is located away from the catalytic site and operates in an allosteric manner (Figure 3) [11,22]. The allosteric regulation of the type II *Mycobacterium tuberculosis* DHS (MtDHS) is more complex as its activity is synergistically inhibited by a combination of AAAs, which bind to more than one effector binding sites (Figure 3) [16]. According to structural and computational analyses of type I and II DHSs, effector binding likely leads to conformational changes that weaken the binding of E4P, but not PEP [11,23]. These AAA-mediated feedback inhibitions of microbial DHSs are critical in controlling the carbon flux into the shikimate pathway, as AAAs are the pathway "end products" to be used mainly for protein synthesis in most microbes [1,11].

Unlike in microbial studies, DHS activities detected from plant tissue extracts were found to be generally insensitive to AAAs [2,4,24–26], raising a decade-long question about the regulation of the plant DHSs [2–4]. Recently, biochemical characterization of the recombinant proteins of three *Arabidopsis thaliana* DHS isoforms (AtDHSs) revealed that the activity of AtDHS2, but not AtDHS1 or AtDHS3, is indeed inhibited by Tyr and Trp with IC_{50} of ~200 μM (Figure 1 and Table 1) [14], which is within the range of that of microbial DHSs [16,22]. Their lower concentrations did not inhibit AtDHS2 or other isoforms [27], consistent with reported physiological Tyr and Trp concentrations in plants [28]. The isoform-specific feedback regulation appear to operate also *in planta*, because i) DHS activity is also inhibited by Trp and Tyr, but not Phe, specifically in young seedlings where AtDHS2 is actively expressed and ii) the *dhs1* and *dhs3*, but not *dhs2*, knockout mutants of Arabidopsis are hypersensitive to exogenous Trp and Tyr treatments [14,27]. Notably, the Trp and Tyr-sensitivity of AtDHS2 is abolished when mixed with AtDHS1 or 3 [14], likely due to their heterocomplex formation. All AtDHSs and DHS activity from Arabidopsis and spinach leaf tissues are also strongly inhibited by chorismate and caffeate (IC_{50} of 50–100 μM), intermediates of the shikimate

Table 1

Representative kinetic parameters and inhibitors of microbial and plant DHS enzymes.

Type	Organism	Enzyme	K_m for PEP (mM)	K_m for E4P (mM)	Inhibitors	Reference
Type II	*Arabidopsis thaliana*	Recombinant protein (AtDHS1)	0.25	2.842	chorismate, caffeate	14
		Recombinant protein (AtDHS2)	0.36	1.755	Tyr, Trp, chorismate, caffeate	14
		Recombinant protein (AtDHS3)	0.706	1.55	chorismate, caffeate	14
	Spinacia oleracea (spinach)	Extract from leaf tissues	approx. 0.5–0.7	1.95		17
	Daucus carota (carrots)	Extract from cultured carrot cells	approx. 0.25–0.5	3.3		18
	Pseudomonas aeruginosa	Recombinant protein	0.018	0.028	Trp	19
	Mycobacterium tuberculosis	Recombinant protein	0.029	0.037	Phe + Trp	16
Type I	*Escherichia coli*	Recombinant protein (AroG)	0.035	0.25	Phe	20
	Saccharomyces cerevisiae	Recombinant protein (Aro4)	0.125	0.5	Tyr	21

and phenylpropanoid pathways, respectively (Figure 1 and Table 1) [14]. These findings revealed that plant DHSs are subjected to much more complex feedback regulation than their microbial counterparts. These isoform-specific, highly-complex DHS feedback regulations may reflect high and diverse metabolic demands of AAAs, since plants direct up to 30% of photosynthetically fixed carbons through the shikimate pathway toward high production of AAA-derived compounds, such as lignin and tannins [7,29].

Multiple-layers of plant DHS regulation

Besides the metabolite-mediated feedback inhibition, plant DHSs are subjected to additional layers of regulations that are associated with the functionality of the chloroplasts, where the shikimate pathway operates [4]. Unlike microbial DHSs, plant DHS enzymes require reducing agents, such as 1,4-dithiothreitol (DTT), for the detection of their *in vitro* activity [1,11,14,30]. The recombinant AtDHS1 protein can also be activated by co-incubation with thioredoxin *f* [30], one of the major thioredoxin isoforms in the chloroplasts [31]. Thus, the activity of the shikimate pathway is coordinated with the redox status of the plastids, likely to effectively utilize photosynthetically fixed carbon and energy for the production of AAAs and their derived products. Interestingly, unlike AtDHS1 or 3, AtDHS2 exhibits a minor but significant activity even in the absence of the reducing reagent [14]. Since *AtDHS2* is particularly upregulated in young seedlings and coexpressed with genes involved in plastid development rather than AAA metabolic genes [14], this redox-independent AtDHS2 activity may be critical in maintaining the shikimate pathway activity in rapidly-growing non-photosynthetic tissues, where a basal level of AAAs must be produced to support protein synthesis needed for growth even in the absence of photosynthesis.

Two distinct DHS activities that require manganese and cobalt (DHS-Mn and DHS-Co, respectively) have been isolated from many eudicot [17,18,26,32,33] and some monocot [33,34] species. The Mn- and Co-dependent DHS activities share similar kinetic parameters but are mainly detected from plastid and cytosolic fractions, respectively [33]. While the shikimate and AAA biosynthetic pathways are dominantly located in the plastids [4], recent studies have proposed cytosolic Tyr and Phe production that is mediated by a complete set of cytosolic Tyr/Phe biosynthetic enzymes from chorismate, such as cytosolic chorismate mutase 2 (CM2) [35–37]. Although it is still under debate if chorismate is produced in cytosol or exported from plastids by unknown transporters [36,37], DHS-Co might participate in a cytosolic production of chorismate. However, the physiological roles and the identities of cytosolic DHS-Co activity and shikimate pathway remain undescribed.

The Clp protease system mediates ATP-dependent proteolysis that selectively targets certain stromal proteins (e.g. chlorophyll biosynthetic enzymes) to maintain protein homeostasis in the plastids [38]. A recent biochemical study reported that Arabidopsis ClpS, a subunit of the Clp complex, interacts with all three AtDHSs as well as chorismate synthase (CS), the last enzyme in the shikimate pathway (Figure 1), suggesting that DHSs and CS are likely targeted for proteolysis by the Clp protease machinery [39]. However, it remains to be tested genetically if the DHS and CS degradation by Clp specifically affects AAA production *in planta*.

Earlier studies found that plant DHS activity is induced in response to environmental stimuli, such as wounding and elicitor treatments [40–42]. This is likely due to transcriptional regulation of certain *DHS* genes, as summarized in Table 2 [43–53]. For instance, *AtDHS1* is highly expressed after wounding and *Pseudomonas syringae* infection, and *DHS2* from *Solanum lycopersicum* is induced by elicitor treatments, whereas other *DHS* isoforms are generally constitutively expressed (Table 2) [43,46]. The stress-induced *DHS* upregulation

Table 2

A summary of studies that reported *DHS* transcriptional upregulation.

Organism	Tissue	Stress/treatment	Correlated transcriptional factors	Upregulated *DHS* isoform(s)	Other upregulated gene(s)	Reference
Solanum tuberosum	Tuber and leaf	Wounding	Not tested	*StDAHPSs*	*PAL*	41
Solanum lycopersicum	Fruit pericarp, root, and stem	Wounding	Not tested	*SlDAHPSs*	Not tested	41
Arabidopsis thaliana	Rossette leaf	Wounding	Not tested	*AtDHS1*	*AtPAL*	43
Arabidopsis thaliana	Rossette leaf	Wounding	Not tested	*AtDHS2*	Not tested	44
Solanum tuberosum	Tuber	Wounding	Not tested	*StshkA*	Not tested	45
Arabidopsis thaliana	Rossette leaf	*Pseudomonas syringae*	Not tested	*AtDHS1*	Not tested	43
Solanum lycopersicum	Cell suspension culture	Fungal elicitors	Not tested	*DHS2*	*PALs*, *SK*, *EPSPS*, and *CS1*	46
Solanum lycopersicum	Leaf	*Phytophthora infestans*	Not tested	*DHS2*	*EPSPS* and *CS1*	46
Catharanthus roseus	Cell suspension culture	UV-B irradiation	Not tested	*CrDHS1*	Not tested	47
Arabidopsis thaliana	Whole seedling	Diurnal cycle	*AtCCA1*	*AtDHS1*	*SK1* and *CS*	48
Picea glauca	Xylem and bark/phloem	Diurnal cycle	*PtMYB1 and Pt/PgMYB8*	*PgDHS2*	*PgSHM4*, *PgMEE58*, *PgCM1*, *PgCOMT1*, *PgHCT*, *Pg4CL2*, *PgPRR2*, and Lipase/thio.	49
Solanum lycopersicum	Fruit	Heterologous expression of *PhODO1*	*PhODO1*	*DAHPS*	*DHQS*, *SK*, *EPSPSs*, *CS1*, *CM*, *ADTs/PDTs*, 4CLs, *PALs*, *CCR*, *CCoAOMTs*, *C4Hs*, *THTs*, *HQT*, *CHS*, putative *AspAT*, *PAT*, and UDP-xylose phenolic glycosyltransferase	50
Solanum lycopersicum	Fruit	Heterologous expression of *AtMYB12*	*AtMYB12*	*SlDAHPS*	*SlG6PD, SlPGLS, Sl6PGD1, Sl6PGD2, SlRpi, SlRpe, SlTKT, SlTALDO1, SlSUS1, SlPFK1, SlGADPH, SlGADPHL, SlPGM, SlENO, SlDHQS*, *SlDHQD*, *SlSHD*, *SlSK*, *SlEPSPS1*, *SlEPSPS2*, *SlCS*, *SlCM1*, *SlPAT*, *SlADT*, *SlPAL5A*, *SlPAL5B*, *SlPAL5C*, *SlPAL5D*, *SlPAL*, *Sl4CL*, *Sl4CL-like*, *SlCHS-1*, *SlCHS-2*, *SlCHS*, *SlCHIL*, *SlF3H*, *SlFLS*, *SlDFRL1*, *SlDFRL2*, *SlANS*, *Sl3GT*, *SlHCT*, and *SlC3H*	51
Solanum lycopersicum	Fruit pericarp	Overexpression of *SlMIXTA-like*	*SlMIXTA-like*	*SlDAHPS*	*SlSUS1*, *SlHK*, *SlGPI*, *SlPFP*, *SlTPI*, *SlPFK1*, *SlENO*, *SlPGLS1*, *SlPGLS3*, *SlPGD1*, *SlRpe*, *SlDHQS*, *SlDHQD*, *SlSHD*, *SlSK*, *SlEPSPS*, *SlCS*, *SlCM*, *SlC4H*, *SlPSY*, *SlPDS*, *SlZDS*, *SlLYCB*, *SlPAL5A*, *SlPAL5B*, *SlPAL5C*, and *4CL*	52
Picea glauca	Plantlet	Heterologous expression of *Pinus taeda MYB1 and MYB8*	*PtMYB1* and *PtMYB8*	*PgDHS2*	Of 66 co-expressed genes common to PtMYB1, PtMYB8, and PtMYB14 transgenics, 20% linked to flavonoid and phenylpropanoid biosynthesis.	49
Petunia hybrida	Flower petal	RNAi suppression of *PhADT1*	Not tested	*PhDAHPS*	*PhEPSPS* and *PhCM*	53

Table 3

A summary of studies that reported direct/indirect DHS manipulation for altered accumulation of plant specialized metabolites.

Organism	Tissue	Genetic manipulation	Regulated endogenous DHS isoform(s)	Metabolite phenotype	Reference
Petunia hybrida	Flower petal	RNAi suppression of *PhODO1*	*PhDAHPS*	Reduced emissions of volatile benzenoids	56
Solanum lycopersicum	Fruit	Heterologous expression of *PhODO1*	*DAHPS*	Reduced Phe and methionine levels and benzaldehyde and 2-phenylacetonitrile emission. Increased phenylpropanoids, such as ferulic acid and coniferaldehyde.	50
Solanum lycopersicum	Fruit	Heterologous expression of *AtMYB12*	*SlDAHPS*	Reduced sucrose, glucose, fructose, myo-inositol, Phe, and shikimic acid. Increased glycine, valine, leucine, citric acid, succinic acid, quinic acid, Trp, Tyr, chlorogenic acid, and flavonols.	51
Populus tremula x *Populus tremuloides* and *Populus tremula* x *Populus alba*	Leaf	Overexpression of *MYB182*	*PtDAHPS*, *PtDAHPS1*, and *PtDAHPS2*	Reduced anthocyanins and proanthocyanidins.	63
Populus tremula x *Populus tremuloides*	Leaf	Overexpression of *MYB165*	*PtDAHPSs*	Reduced anthocyanins, proanthocyanidins, salicortin, tremulacin, cis-3-coumaroyl-quinate, trans-3-coumaryl-quinate, caffeoyl-quinate. Increased Phe, Trp, Tyr, valine, isoleucine, leucine, histidine, and glutamine.	64
Nicotiana tabacum	Whole seedling	Heterologous expression of *Camellia sinensis MYB4a*	*NtAROF*	Reduced total lignin, rutin, chlorogenic acid, and Phe.	65
Arabidopsis thaliana	Stem	Quadruple T-DNA insertional mutant of *AtMYB20/AtMYB42/AtMYB43/AtMYB85*	*AtDHS3*	Reduced G- and S-lignin subunits and total lignin content. Increased Phe, anthocyanin, kaempferol 3-*O*-[6″-*O*-(rhamnosyl)glucoside] 7-*O*-rhamnoside, kaempferol 3-*O*-glucoside 7-*O*-rhamnoside, and kaempferol 3-*O*-rhamnoside 7-*O*-rhamnoside.	66
Solanum lycopersicum	Fruit pericarp	Overexpression of *SlMIXTA-like*	*SlDAHPS*	Reduced rutin. Increased anthranilic acid, tryptamine, *p*-coumaric acid, *p*-coumaraldehyde, dihydroquercetin, coniferaldehyde, chlorogenic acid, caffeic acid, ferulic acid, vanillin, scopoletin, feruloyl quinic acid, ferulic acid hexoside, vanillic acid, lycopene, and β-carotene.	52
Petunia hybrida	Flower petal	RNAi suppression of *PhADT1*	*PhDAHPS*	Reduced shikimate, Phe, and Trp levels, and decreased emissions of all phenylpropanoids/benzenoids.	53
Petunia hybrida	Flower petal	Heterologous expression of *Escherichia coli* feedback-insensitive $AroG_{175}$ and/or overexpression of $PhADT1^{R353A}$	Not tested	Increased Phe level and Phe-derived volatile emission.	55
Arabidopsis thaliana	Flower and aerial tissue of seedling	Heterologous expression of *Escherichia coli* feedback-insensitive $AroG_{175}$	Not tested	Increased shikimate, prephenate, Phe, phenylacetonitrile, homogentisate, alanine, 4-hydroxybenzoate, Trp, coumarate hexose (I), coumarate hexose (II), ferulate hexose (I), ferulate hexose (II), ferulate derivative (I), ferulate derivative (II), sinapoyl hexose, sinapic acid, sinapyl alcohol, coniferin, kaempferol deoxyhexose, kaempferol deoxyhexose−deoxyhexose, hydroxybenzoate hexose, 2-phenylethyl glucosinolate, 4-*O*-methoxyindole glucosinolate, and 12-hydroxyjasmonate-hexose. In floral organs, increased total lignin content and H and G monomer content.	60
Solanum lycopersicum	Fruit	Heterologous expression of *Escherichia coli* feedback-insensitive $AroG_{175}$	Not tested	Increased Phe and Trp.	61
			Not tested		61

(*continued on next page*)

Table 3. (*continued*)

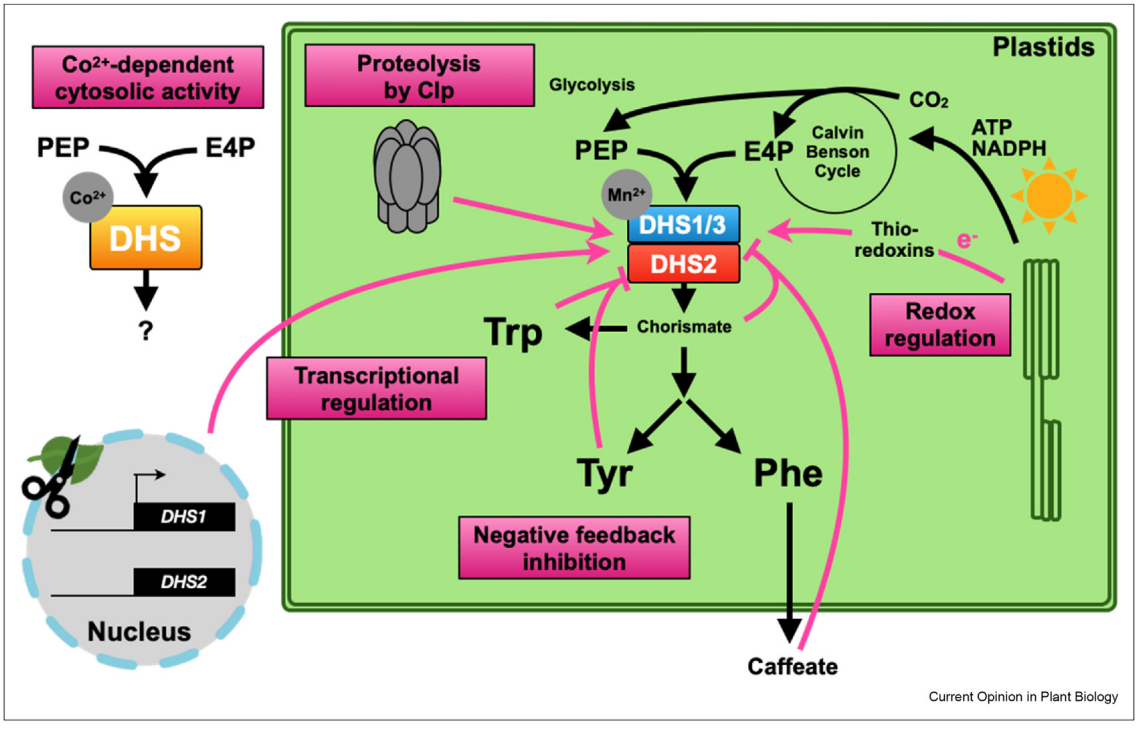
Organism	Tissue	Genetic manipulation	Regulated endogenous DHS isoform(s)	Metabolite phenotype	Reference
Solanum lycopersicum	Aerial tissue of fruit peel or flesh	Heterologous expression of *Escherichia coli* feedback-insensitive $AroG_{209}$		In fruit peel, increased raffinose, trehalose, cellobiose, guanosine, fumaric acid, shikimic acid, prephenic acid, Trp, Phe, Tyr, coumaric acid hexose (II), *p*-coumaric acid, ferulic acid hexose, tricaffeoylquinic acid, 3-caffeoylquinic acid, 4-caffeoylquinic acid, naringenin chalcone hexose, quercetin-hexose-deoxyhexose-pentose-*p*-coumarate, and decreased lycopene, phytofluene, phytoene, and putrescine. In fruit flesh, increased maltose, raffinose, trehalose, cellobiose, dehydrolycoperoside G, F, A, shikimic acid, prephenic acid, Phe, Trp, Tyr, *p*-coumaric acid, coumaric acid derivative I, kaempferol glucose rhamnose, and decreased lycopene, phytofluene, phytoene, asparagine, putrescine, caffeic acid hexose (I), and caffeic acid hexose (II). In whole fruit, increased benzaldehyde, phenylacetaldehyde, 2-phenylacetaldehyde, phenyl acetic acid and decreased limonene, β-ionone, geranylacetone, and eugenol.	
Nicotiana tabacum	Leaf	Heterologous expression of *Escherichia coli* feedback-insensitive $AroG_{175}$	Not tested	Increased total polyphenol content, quinate, shikimate, phenylpyruvate, Tyr, Phe, Trp, phenylethylamine, phenyllactate, benzylglucopyranoside, 4-hydroxybenzoate, and phenylethylamine.	62
Nicotiana tabacum	Leaf	*Phelipanche egyptiaca* infection of *N. tabacum* with heterologous expression of *Escherichia coli* feedback-insensitive $AroG_{175}$	Not tested	In tobacco roots, increased Trp, Phe, Tyr, quinic acid, caffeic acid, ferulic acid, and chlorogenic acid. In *P. egyptiaca* attached to the roots of these plants, increased Trp, caffeic acid, and coumaric acid, and decreased Tyr and sinapinic acid.	62
Vitis vinifera	Cell suspension culture	Heterologous expression of *Escherichia coli* feedback-insensitive $AroG_{175}$	Not tested	Increased shikimate, phenylpyruvate, Tyr, Phe, 3-hydroxy phenylacetate, *p*-coumarate, dihydroquercetin, hydroquinone, resveratrol (trans), 4-hydroxyphenyl β glucopiranoside, and 4-hydroxy benzoate. Reduced delphinidin, malvidin, and petunidin.	67
Petunia hybrida	Leaf and flower	Heterologous expression of *Escherichia coli* feedback-insensitive $AroG_{175}$ or $AroG_{209}$	Not tested	Increased Phe, Trp, Tyr, phenylpyruvate, 3-hydroxy cinnamate, trans coumarate, caffeate, ferulate, sinapate, vanillate, salycilate, salicin, 4-hydroxybenzoate, 4-hydroxyphenyl-β-glucopyranosid, tyrosol, *p*-cresol, 4-hydroxy-3-methoxy-mandelate, phenylacetaldehyde, 2-phenylethanol, benzaldehyde, benzyl alcohol, benzoate, benzyl acetate, methyl benzoate, benzyl benzoate, and total benzenoid-phenylpropanoid volatile content.	68
Petunia hybrida	Leaf	Heterologous expression of *Escherichia coli* feedback-insensitive $AroG_{175}$	Not tested	Increased shikimate, prephenate, phenylpyruvate, Phe, Trp, Tyr, caffeate, ferulate, *p*-coumarate, sinapic acid, benzyl alcohol, 1-benzylglucopyranoside, 4-hydroxybenzoate, benzene 1,2,3-triol, 3,4-hydroxyphenyllactate, indole 3-pyruvate, indole 3-lactate, α-tocopherol, phyllodihydroquinone, pABA, salicylate glucopyranoside, hydroquinone, hydroquinone β-D-glucopyranose, rosmarinate, and 3-phenyllactate.	69
Petunia hybrida	Leaf	*Botrytis cinerea* infection of *P. hybrida* with heterologous expression of *Escherichia coli* feedback-insensitive $AroG_{175}$	Not tested	Reduced reactive oxygen species (ROS) accumulation.	69

potentially promotes the production of AAA-derived metabolites likely in a jasmonic acid-dependent manner [54], to cope with various stresses. Suppression of an arogenate dehydratase (ADT) that synthesizes Phe (Figure 1) also upregulates *DHS* (an ortholog of *AtDHS3*), as well as *5-enolpyruvylshikimate-3-phosphate synthase* (*EPSPS*) and *CM*, genes in *Petunia hybrida* flowers [53], suggesting that a shortage of AAAs can induce expression of *DHS* genes. More recently, overexpression of a deregulated ADT in petunia flowers led to an unexpected reduction in Phe and Phe-derived volatiles, which can be partially recovered by additional expression of the plastid AAA transporter, deregulated bacterial DHS, or D-ribulose-5-phosphate 3-epimerase in the pentose phosphate pathway (Table 3) [55]. Conversely, the hyper-accumulation of Phe, along with Tyr and Trp, caused by reduced Phe ammonia-lyase (PAL) activity did not alter *DHS* and other shikimate pathway gene expression, although the shikimate pathway flux was reduced [55]. These results together suggest that elevated AAAs, especially in the plastids, leads to post-transcriptional down-regulation of the shikimate pathway, which potentially includes DHS inhibition by the accumulated Tyr and Trp and/or pathway intermediates (e.g. chorismate) [14].

Transcription of the shikimate pathway genes, including *AtDHS1*, is subjected to phytochrome B-mediated circadian oscillation involving multiple transcriptional factors (TFs), such as circadian clock associated 1 (CCA1) (Table 2) [48]. The expression of *DHS*, *EPSPS*, and *CM*, *PAL1*, and *PAL2* genes is also regulated by ODORANT1 (ODO1), a MYB TF that positively regulates benzenoid volatile biosynthesis in petunia [56]. Interestingly, overexpression of petunia *ODO1* in tomato fruits elevated the expression of *DHS*, *EPSPS* and *CM* genes, though without an increase in AAAs and Phe-derived flavor volatiles (Table 3) [50]. In contrast, *AtMYB12* expression in tomato fruits directly activated the promoters of *DHS* and other upstream and downstream genes (e.g., *PAL*) and enhanced total flavonol production (Table 3) [51]. MYB TFs are likely involved in regulating *DHS* expression by binding to its AC promoter *cis*-elements, as demonstrated for PgMYB8 and PgMYB15 in white spruce (*Picea glauca*) (Table 2) [49]. The next challenge is to understand how different TFs coordinate the expression of *DHS* and other pathway genes through direct and indirect binding to their promoters.

Taken together, the entry reaction of the shikimate pathway, catalyzed by DHSs, is controlled by at least five different mechanisms (Figure 4). Such complex post-translational regulatory systems, seen in plant DHSs, have not been discovered in the other shikimate pathway enzymes so far, with an exception of the

Figure 4



Multiple regulations of plant DHS activity, as indicated by pink boxes and lines. The identitiy of cytosolic Co^{2+}-dependent DHS activity (orange box) is currently unknown. PEP, phospho*enol*pyruvate; E4P, erythrose-4-phosphate; Tyr, tyrosine; Phe, phenylalanine; Trp, tryptophan.

potential CS degradation by the Clp complex. These multi-layer regulatory mechanisms suggest that plant DHSs act as the "gatekeeper" of the shikimate pathway, which integrates multiple signals, especially in the plastids, to control carbon allocation toward biosynthesis of various aromatic natural products under different conditions.

Modulating DHS for aromatic compound production through plant synthetic biology

In microbes, many feedback-resistant DHSs have been generated based on enzyme structural analyses followed by site directed mutagenesis [11,16,57,58] and widely utilized to eliminate the DHS allosteric regulations and to overproduce targeted aromatic compounds [59]. Heterologous expression of deregulated microbial DHSs, such as a feedback-resistant *E. coli AroG* but not wild-type *AroG*, enhanced accumulation of shikimate, Phe, Trp, and broad classes of AAA-derived specialized metabolites in Arabidopsis [60] and crops, such as *S. lycopersicum* fruit and *Nicotiana tabacum* (Table 3) [61,62]. The mutated AroG-expressing tobacco showed enhanced tolerances to salt stress and a parasitic plant *Phelipanche egyptiaca* [62], although additional characterization is needed to establish the underlying mechanisms. In general, studies that directly or indirectly manipulated *DHS* expression reported altered accumulation of phenylpropanoid compounds in plants, as summarized in Table 3 [50–53,55,56,60–69]. These studies suggest that the DHS-catalyzed reaction is a major limiting step of the shikimate pathway in plants, which can be bypassed by introducing a deregulated, exogenous DHS. A better understanding of plant DHS enzyme structure and regulatory mechanisms will enable the deregulation of endogenous plant DHSs by base editing [70]. To further enhance the overall shikimate pathway activity, we have to consider other factors, such as increasing the availability of DHS substrates, especially E4P, given that plant DHSs have a higher K_m toward E4P and that the activity of the pentose phosphate pathway enzymes, such as transketolase and D-ribulose-5-phosphate 3-epimerase, impacts AAA production in plants [14,55,71]. Metabolic control analyses [60–62] in plants that are deregulated at the DHS step will also highlight additional bottlenecks in the pathway. Coordinated upregulation of the shikimate pathway genes, together with the upstream and downstream genes, is also important for enhanced carbon flux through the shikimate and downstream natural product pathways, as demonstrated by AtMYB12 expression in tomato fruits [51].

Conclusion and future perspective

Plant DHS activity is controlled by multiple regulatory networks of complex metabolite-mediated feedback inhibition, redox regulation within the plastids, cofactor/substrate availability, and transcriptional regulation (Figure 4). These intricate regulatory systems of plant DHSs are likely important for optimizing carbon allocation from the central carbon metabolism through the shikimate pathway to produce a variety of aromatic phytochemicals under different conditions. Considering the plant's natural ability to synthesize abundant and diverse aromatic compounds from CO_2 using sunlight energy, plants provide a promising sustainable platform to produce numerous aromatic chemicals, which are in high demand but are currently extracted primarily from fossil fuels [72]. Although further biochemical, genetic, and metabolomic studies are needed to address a number of remaining questions, as listed in BOX1, answering these questions will enable rational engineering of the shikimate pathway to construct plant chemical platforms for efficient and sustainable production of various aromatic compounds.

Funding sources

This work was supported by the National Science Foundation [MCB-1818040, IOS-1836824] and the USDA National Institute of Food and Agriculture [2020-67013-30898] to H.A.M. R.Y. was financially supported in part as the postdoctoral fellows of the Japanese Society for the Promotion of Science (JSPS) and the Uehara Memorial Foundation [grant nos. 2017-2444 and 201,730,074, respectively]. A.G. was financially supported from the Hilldale

BOX1. Outstanding questions regarding the function and regulation of plant DHS enzymes.

- What are the roles of different DHS isoforms having distinct transcriptional and post-transcriptional regulations?
- Which metabolites that inhibit the plant DHS activity play critical roles in controlling the overall flux through the shikimate pathway in plants?
- Which amino acid residue(s) of DHS determines its sensitivity to the various effector molecules?
- What are the roles and identities of Co^{2+}-dependent DHS activity detected in plant tissues?
- What is the significance of redox, cofactor, and Clp-mediated DHS regulation *in planta*?
- How do plants differentially regulate *DHS* gene expression in the response to metabolic demands from the downstream aromatic natural product biosynthesis in each tissue?
- What are the upstream signals and TF networks that regulate the expression of *DHS* genes?
- How are these multi-layered DHS regulations coordinated under different conditions and with other shikimate pathway genes and enzymes?

undergraduate research fellowship at University of Wisconsin-Madison.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank the members of Maeda lab for providing valuable feedback on the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pbi.2022.102219.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Bentley R: **The shikimate pathway–a metabolic tree with many branches**. *Crit Rev Biochem Mol Biol* 1990, **25**:307–384.

2. Herrmann KM: **The shikimate pathway: early steps in the biosynthesis of aromatic compounds**. *Plant Cell* 1995, **7**: 907–919.

3. Tzin V, Galili G: **New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants**. *Mol Plant* 2010, **3**:956–972.

4. Maeda H, Dudareva N: **The shikimate pathway and aromatic amino acid biosynthesis in plants**. *Annu Rev Plant Biol* 2012, **63**:73–105.

5. Tohge T, Watanabe M, Hoefgen R, Fernie AR: **The evolution of phenylpropanoid metabolism in the green lineage**. *Crit Rev Biochem Mol Biol* 2013, **48**:123–152.

6. Maeda HA, Fernie AR: **Evolutionary history of plant metabolism**. *Annu Rev Plant Biol* 2021, **72**:185–216.

7. Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W: **Lignin biosynthesis and structure**. *Plant Physiol* 2010, **153**: 895–905.

8. Kliebenstein DJ: **Plant defense compounds: systems approaches to metabolic analysis**. *Annu Rev Phytopathol* 2012, **50**:155–173.

9. Zhao Y: **Auxin biosynthesis: a simple two-step pathway converts tryptophan to indole-3-acetic acid in plants**. *Mol Plant* 2012, **5**:334–338.

10. Maeda HA: **Harnessing evolutionary diversification of primary metabolism for plant synthetic biology**. *J Biol Chem* 2019, **294**: 16549–16566.

11. * Jiao W, Lang EJ, Bai Y, Fan Y, Parker EJ: **Diverse allosteric componentry and mechanisms control entry into aromatic metabolite biosynthesis**. *Curr Opin Struct Biol* 2020, **65**: 159–167.
The authors identified a mutation that deregulates the negative feedback inhibition of *Mycobacterium tuberculosis* type II DHS without affecting its interaction with effector molecules. This mutation will be useful for type II DHS engineering.

12. Richards TA, Dacks JB, Campbell SA, Blanchard JL, Foster PG, McLeod R, Roberts CW: **Evolutionary origins of the eukaryotic shikimate pathway: gene fusions, horizontal gene transfer, and endosymbiotic replacements**. *Eukaryot Cell* 2006, **5**: 1517–1531.

13. Tohge T, Watanabe M, Hoefgen R, Fernie A: **Shikimate and phenylalanine biosynthesis in the green lineage**. *Front Plant Sci* 2013, **4**:62.

14. * Yokoyama R, de Oliveira MVV, Kleven B, Maeda HA: **The entry reaction of the plant shikimate pathway is subjected to highly complex metabolite-mediated regulation**. *Plant Cell* 2021, **33**: 671–696.
This study demonstrates that Arabidopsis DHSs were regulated by at least five downstream metabolites with distinct sensitivities among three isoforms. These findings uncover more complex feedback regulation of the shikimate pathway in plants than in microbes.

15. Webby CJ, Baker HM, Lott JS, Baker EN, Parker EJ: **The structure of 3-Deoxy-d-arabino-heptulosonate 7-phosphate synhase from *Mycobacterium tuberculosis* reveals a common catalytic scaffold and ancestry for type I and type II enzymes**. *J Mol Biol* 2005, **354**:927–939.

16. Webby CJ, Jiao W, Hutton RD, Blackmore NJ, Baker HM, Baker EN, Jameson GB, Parker EJ: **Synergistic allostery, a sophisticated regulatory network for the control of aromatic amino acid biosynthesis in *Mycobacterium tuberculosis***. *J Biol Chem* 2010, **285**:30567–30576.

17. Doong RL, Gander JE, Ganson RJ, Jensen RA: **The cytosolic isoenzyme of 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase in *Spinacia oleracea* and other higher plants: extreme substrate ambiguity and other properties**. *Physiol Plantarum* 1992, **84**:351–360.

18. Suzuki N, Sakuta M, Shimizu S: **Purification and characterization of a cytosolic isozyme of 3-Deoxy-D-arabino-heptulosonate 7-phosphate synthase from cultured carrot cells**. *J Plant Physiol* 1996, **149**:19–22.

19. Sterritt OW, Kessans SA, Jameson GB, Parker EJ: **A pseudoisostructural type II DAH7PS enzyme from *Pseudomonas aeruginosa*: alternative evolutionary strategies to control shikimate pathway flux**. *Biochemistry* 2018, **57**:2667–2678.

20. Xu J, Hu C, Shen S, Wang W, Jiang P, Huang W: **Requirement of the N-terminus for dimer formation of phenylalanine-sensitive 3-deoxy-D-arabino-heptulosonate synthase AroG of *Escherichia coli***. *J Basic Microbiol* 2004, **44**: 400–406.

21. Schnappauf G, Hartmann M, Künzler M, Braus GH: **The two 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase isoenzymes from *Saccharomyces cerevisiae* show different kinetic modes of inhibition**. *Arch Microbiol* 1998, **169**: 517–524.

22. Shumilin IA, Kretsinger RH, Bauerle RH: **Crystal structure of phenylalanine-regulated 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase from *Escherichia coli***. *Structure* 1999, **7**: 865–875.

23. Jiao W, Hutton RD, Cross PJ, Jameson GB, Parker EJ: **Dynamic cross-talk among remote binding sites: the molecular basis for unusual synergistic allostery**. *J Mol Biol* 2012, **415**: 716–726.

24. Huisman OC, Kosuge T: **Regulation of aromatic amino acid biosynthesis in higher plants. II. 3-Deoxy-arabino-heptulosonic acid 7-phosphate synthetase from cauliflower**. *J Biol Chem* 1974, **249**:6842–6848.

25. Pinto JE, Suzich JA, Herrmann KM: **3-Deoxy-d-arabino-Heptulosonate 7-phosphate synthase from potato tuber (*Solanum tuberosum* L.)**. *Plant Physiol* 1986, **82**:1040–1044.

26. Sharma R, Bhatnagar RK, Sarin NB: **Differential expression of DAHP synthase and chorismate mutase isozymes during rhizogenesis of *Brassica juncea* cultured in vitro**. *Physiol Plantarum* 1993, **88**:281–286.

27. Kanaris M, Poulin J, Shahinas D, Johnson D, Crowley VM, Fucile G, Provart N, Christendat D: **Elevated tyrosine results in the cytosolic retention of 3-deoxy-d-arabino-heptulosonate 7-phosphate synthase in *Arabidopsis thaliana***. *Plant J* 2022, **109**:789–803, https://doi.org/10.1111/tpj.15590.

28. Farre EM, Fernie AR, Willmitzer L: **Analysis of subcellular metabolite levels of potato tubers (*Solanum tuberosum*)**

displaying alterations in cellular or extracellular sucrose metabolism. *Metabolomics* 2008, **4**:161–170.

29. Razal RA, Ellis S, Singh S, Lewis NG, Towers GHN: **Nitrogen recycling in phenylpropanoid metabolism**. *Phytochemistry* 1996, **41**:31–35.

30. Entus R, Poling M, Herrmann KM: **Redox regulation of Arabidopsis 3-Deoxy-d-arabino-Heptulosonate 7-phosphate synthase**. *Plant Physiol* 2002, **129**:1866–1871.

31. Yoshida K, Yokochi Y, Hisabori T: **New light on chloroplast redox regulation: molecular mechanism of protein thiol oxidation**. *Front Plant Sci* 2019, **10**:1534.

32. Rubin JL, Jensen RA: **Differentially regulated isozymes of 3-Deoxy-d-arabino-Heptulosonate-7-Phosphate synthase from seedlings of *Vigna radiata* [L.] wilczek**. *Plant Physiol* 1985, **79**: 711–718.

33. Ganson RJ, D'Amato TA, Jensen RA: **The two-isozyme system of 3-Deoxy-D-arabino-Heptulosonate 7-phosphate synthase in *Nicotiana silvestris* and other higher plants**. *Plant Physiol* 1986, **82**:203–210.

34. Graziana A, Boudet AM: **3-Deoxy-d-arabino heptulosonate 7-phosphate synthase from *Zea mays*: general properties and regulation by tryptophan**. *Plant Cell Physiol* 1980, **21**:793–802.

35. Eberhard J, Ehrler TT, Epple P, Felix G, Raesecke HR, Amrhein N, Schmid J: **Cytosolic and plastidic chorismate mutase isozymes from *Arabidopsis thaliana*: molecular characterization and enzymatic properties**. *Plant J* 1996, **10**: 815–821.

36. Lynch JH, Dudareva N: **Aromatic amino acids: a complex network ripe for future exploration**. *Trends Plant Sci* 2020, **25**: 670–681.

37. Qian Y, Lynch JH, Guo L, Rhodes D, Morgan JA, Dudareva N: **Completion of the cytosolic post-chorismate phenylalanine biosynthetic pathway in plants**. *Nat Commun* 2019, **10**:15.

38. Nishimura K, van Wijk KJ: **Organization, function and substrates of the essential Clp protease system in plastids**. *Biochim Biophys Acta* 2015, **1847**:915–930.

39. Nishimura K, Asakura Y, Friso G, Kim J, Oh S-H, Rutschow H, Ponnala L, van Wijk KJ: **ClpS1 is a conserved substrate selector for the chloroplast Clp protease system in Arabidopsis**. *Plant Cell* 2013, **25**:2276–2301.

40. McCue KF, Conn EE: **Induction of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase activity by fungal elicitor in cultures of *Petroselinum crispum***. *Proc Natl Acad Sci U S A* 1989, **86**:7374–7377.

41. Dyer WE, Henstrand JM, Handa AK, Herrmann KM: **Wounding induces the first enzyme of the shikimate pathway in Solanaceae**. *Proc Natl Acad Sci U S A* 1989, **86**:7370–7373.

42. Muday GK, Herrmann KM: **Wounding induces one of two isoenzymes of 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase in *Solanum tuberosum* L**. *Plant Physiol* 1992, **98**: 496–500.

43. Keith B, Dong XN, Ausubel FM, Fink GR: **Differential induction of 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase genes in *Arabidopsis thaliana* by wounding and pathogenic attack**. *Proc Natl Acad Sci U S A* 1991, **88**:8821–8825.

44. Raipuria RK, Kumar V, Guruprasad KN, Bhat SR: ***Arabidopsis thaliana* DHS2 (*AT4G33510*) gene promoter is highly wound responsive and requires a part of the first exon sequences for its function**. *J Plant Biochem Biotechnol* 2018, **27**:241–247.

45. Jones JD, Henstrand JM, Handa AK, Herrmann KM, Weller SC: **Impaired wound induction of 3-Deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase and altered stem development in transgenic potato plants expressing a DAHP synthase antisense construct**. *Plant Physiol* 1995, **108**: 1413–1421.

46. Görlach J, Raesecke HR, Rentsch D, Regenass M, Roy P, Zala M, Keel C, Boller T, Amrhein N, Schmid J: **Temporally distinct accumulation of transcripts encoding enzymes of the prechorismate pathway in elicitor-treated, cultured tomato cells**. *Proc Natl Acad Sci Unit States Am* 1995, **92**: 3166–3170.

47. Ramani S, Patil N, Jayabaskaran C: **UV-B induced transcript accumulation of DAHP synthase in suspension-cultured *Catharanthus roseus* cells**. *J Mol Signal* 2010, **5**:13.

48. Sharkhuu A, Narasimhan ML, Merzaban JS, Bressan RA, Weller S, Gehring C: **A red and far-red light receptor mutation confers resistance to the herbicide glyphosate**. *Plant J* 2014, **78**:916–926.

49. Bomal C, Duval I, Giguère I, Fortin É, Caron S, Stewart D, Boyle B, Séguin A, MacKay JJ: **Opposite action of R2R3-MYBs from different subgroups on key genes of the shikimate and monolignol pathways in spruce**. *J Exp Bot* 2014, **65**:495–508.

50. Dal Cin V, Tieman DM, Tohge T, McQuinn R, de Vos RCH, Osorio S, Schmelz EA, Taylor MG, Smits-Kroon MT, Schuurink RC, *et al.*: **Identification of genes in the phenylalanine metabolic pathway by ectopic expression of a MYB transcription factor in tomato fruit[W]**. *Plant Cell* 2011, **23**: 2738–2753.

51. Zhang Y, Butelli E, Alseekh S, Tohge T, Rallapalli G, Luo J, Kawar PG, Hill L, Santino A, Fernie AR, *et al.*: **Multi-level engineering facilitates the production of phenylpropanoid compounds in tomato**. *Nat Commun* 2015, **6**:8635.

52. Ying S, Su M, Wu Y, Zhou L, Fu R, Li Y, Guo H, Luo J, Wang S, Zhang Y: **Trichome regulator SlMIXTA-like directly manipulates primary metabolism in tomato fruit**. *Plant Biotechnol J* 2020, **18**:354–363.

53. Maeda H, Shasany AK, Schnepp J, Orlova I, Taguchi G, Cooper BR, Rhodes D, Pichersky E, Dudareva N: **RNAi suppression of *Arogenate Dehydratase1* reveals that phenylalanine is synthesized predominantly via the arogenate pathway in petunia petals**. *Plant Cell* 2010, **22**: 832–849.

54. Devoto A, Ellis C, Magusin A, Chang H-S, Chilcott C, Zhu T, Turner JG: **Expression profiling reveals COI1 to be a key regulator of genes involved in wound- and methyl jasmonate-induced secondary metabolism, defence, and hormone interactions**. *Plant Mol Biol* 2005, **58**:497–513.

55. ** Yoo H, Shrivastava S, Lynch JH, Huang X-Q, Widhalm JR, Guo L, Carter BC, Qian Y, Maeda HA, Ogas JP, *et al.*: **Overexpression of arogenate dehydratase reveals an upstream point of metabolic control in phenylalanine biosynthesis**. *Plant J* 2021, **108**:737–751.
This study characterized transgenic petunia flowers expressing a deregulated ADT, demonstrating that the regulation of DHS activity, as well as transcriptional control of the pentose phosphate pathway activity, are key processes for Phe production

56. Verdonk JC, Haring MA, van Tunen AJ, Schuurink RC: ***ODORANT1* regulates fragrance biosynthesis in petunia flowers**. *Plant Cell* 2005, **17**:1612–1624.

57. Hu C, Jiang P, Xu J, Wu Y, Huang W: **Mutation analysis of the feedback inhibition site of phenylalanine-sensitive 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase of *Escherichia coli***. *J Basic Microbiol* 2003, **43**:399–406.

58. Jiao W, Fan Y, Blackmore NJ, Parker EJ: **A single amino acid substitution uncouples catalysis and allostery in an essential biosynthetic enzyme in *Mycobacterium tuberculosis***. *J Biol Chem* 2020, **295**:6252–6262.

59. Noda S, Kondo A: **Recent advances in microbial production of aromatic chemicals and derivatives**. *Trends Biotechnol* 2017, **35**:785–796.

60. Tzin V, Malitsky S, Zvi MMB, Bedair M, Sumner L, Aharoni A, Galili G: **Expression of a bacterial feedback-insensitive 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase of the shikimate pathway in Arabidopsis elucidates potential metabolic bottlenecks between primary and secondary metabolism**. *New Phytol* 2012, **194**:430–439.

61. Tzin V, Rogachev I, Meir S, Moyal Ben Zvi M, Masci T, Vainstein A, Aharoni A, Galili G: **Tomato fruits expressing a bacterial feedback-insensitive 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase of the shikimate pathway**

possess enhanced levels of multiple specialized metabolites and upgraded aroma. *J Exp Bot* 2013, **64**:4441–4452.

62. ** Oliva M, Guy A, Galili G, Dor E, Schweitzer R, Amir R, Hacham Y: **Enhanced production of aromatic amino acids in tobacco plants leads to increased phenylpropanoid metabolites and tolerance to stresses**. *Front Plant Sci* 2021, **11**:2110.
Heterologous expression of a bacterial deregulated DHS in tobacco elevates production of AAA and aromatic natural products, enhancing stress tolerance. These results indicate that deregulation of the shikimate pathway is promising for metabolic engineering of aromatics.

63. Yoshida K, Ma D, Constabel CP: **The MYB182 protein downregulates proanthocyanidin and anthocyanin biosynthesis in poplar by repressing both structural and regulatory flavonoid genes**. *Plant Physiol* 2015, **167**:693–710.

64. Ma D, Reichelt M, Yoshida K, Gershenzon J, Constabel CP: **Two R2R3-MYB proteins are broad repressors of flavonoid and phenylpropanoid metabolism in poplar**. *Plant J* 2018, **96**:949–965.

65. Li M, Li Y, Guo L, Gong N, Pang Y, Jiang W, Liu Y, Jiang X, Zhao L, Wang Y, *et al.*: **Functional characterization of tea (*Camellia sinensis*) MYB4a transcription factor using an integrative approach**. *Front Plant Sci* 2017, **8**:943.

66. Geng P, Zhang S, Liu J, Zhao C, Wu J, Cao Y, Fu C, Han X, He H, Zhao Q: **MYB20, MYB42, MYB43, and MYB85 regulate phenylalanine and lignin biosynthesis during secondary cell wall formation**. *Plant Physiol* 2020, **182**:1272–1283.

67. Manela N, Oliva M, Ovadia R, Sikron-Persi N, Ayenew B, Fait A, Galili G, Perl A, Weiss D, Oren-Shamir M: **Phenylalanine and tyrosine levels are rate-limiting factors in production of health promoting metabolites in *Vitis vinifera* cv. Gamay Red cell suspension**. *Front Plant Sci* 2015, **6**:538.

68. Oliva M, Ovadia R, Perl A, Bar E, Lewinsohn E, Galili G, Oren-Shamir M: **Enhanced formation of aromatic amino acids increases fragrance without affecting flower longevity or pigmentation in *Petunia × hybrida***. *Plant Biotechnol J* 2015, **13**:125–136.

69. Oliva M, Hatan E, Kumar V, Galsurker O, Nisim-Levi A, Ovadia R, Galili G, Lewinsohn E, Elad Y, Alkan N, *et al.*: **Increased phenylalanine levels in plant leaves reduces susceptibility to *Botrytis cinerea***. *Plant Sci* 2020, **290**:110289.

70. Molla KA, Sretenovic S, Bansal KC, Qi Y: **Precise plant genome editing using base editors and prime editors**. *Native Plants* 2021, **7**:1166–1187.

71. Henkes S, Sonnewald U, Badur R, Flachmann R, Stitt M: **A small decrease of plastid transketolase activity in antisense tobacco transformants has dramatic effects on photosynthesis and phenylpropanoid metabolism**. *Plant Cell* 2001, **13**:535–551.

72. U.S. DEPARTMENT OF ENERGY: *Accelerating breakthrough innovation in carbon capture*. Utilization; 2017. and Storage.