Trends in Biotechnology

CellPress REVIEWS

Review

Plant Aromatic Prenyltransferases: Tools for Microbial Cell Factories

Wouter J.C. de Bruijn [1,4,5] Mark Levisson [2,5] Jules Beekwilder [3] Willem J.H. van Berkel [1,4] and Jean-Paul Vincken [1,4,*]

In plants, prenylation of aromatic compounds, such as (iso)flavonoids and stilbenoids, by membrane-bound prenyltransferases (PTs), is an essential step in the biosynthesis of many bioactive compounds. Prenylated aromatic compounds have various health-beneficial properties that are interesting for industrial applications, but their exploitation is limited due to their low abundance in nature. Harnessing plant aromatic PTs for prenylation in microbial cell factories may be a sustainable and economically viable alternative. Limitations in prenylated aromatic compound production have been identified, including availability of prenyl donor substrate. In this review, we summarize the current knowledge about plant aromatic PTs and discuss promising strategies towards the optimized production of prenylated aromatic compounds by microbial cell factories.

Highlights

Prenylation of aromatic compounds is an essential modification to enhance functionality of plant secondary metabolites, and many prenylated aromatic compounds possess health-beneficial bioactivities.

A comprehensive overview of characterized plant aromatic PTs shows that most of these enzymes possess strict substrate- and regio-specificities.

Knowledge gaps include the absence of high-resolution plant aromatic PT structures and lack of characterized plant aromatic *O*-PTs.

Microbial cell factories expressing plant aromatic PTs have potential for biotechnological production of valuable bioactive compounds.

Improving prenyl donor supply, and colocalization of plant aromatic PTs and substrates, have been identified as the current challenges that need to be addressed to increase prenylation yields.

Plant Aromatic PTs Are Essential in Secondary Metabolism

Plants produce **secondary metabolites** (also called **specialized metabolites**, see Glossary), such as phenolic compounds, terpenes, or alkaloids, that serve a wide variety of functions ranging from colorant to odorant to chemical defense compound. Thereby, secondary metabolites play an integral role in the survival and disease resistance of many plant species, including those of agricultural importance [1,2]. To synthesize secondary metabolites with such diverse functionalities, plants possess an assortment of enzymes to perform structural modifications of the different building blocks. One such modification that is of particular interest is prenylation, which refers to attachment of an **isoprenoid** moiety, most commonly a **prenyl** group (C5). The term prenylation is also used in a wider context to refer to substitution with longer isoprenoid units, like geranyl- (C10) or farnesyl-groups (C15) [3]. The structures of these isoprenoids, along with their biosynthesis, are described in more detail in Box 1.

Prenylation of aromatic substrates is known to enhance their bioactivity and is an essential step in the biosynthesis of biologically active secondary metabolites, such as vitamin E, cannabinoids, hop acids, and prenylated (iso)flavonoids and stilbenoids (Box 2) [3,4]. Prenylated (iso)flavonoids and stilbenoids have been investigated extensively in plants of the legume family (Leguminosae or Fabaceae). These secondary metabolites have been found to possess a range of bioactivities, amongst others, antimicrobial, antitumor, cancer preventive, (anti-)estrogenic, vasodilatory, and antiallergic activities [5,6]. Thereby, these prenylated aromatic compounds are valuable for several industries. Their antimicrobial properties, for example, make prenylated (iso)flavonoids and stilbenoids interesting for use as **natural preservatives** in food and feed [7]. Additionally, natural products with antitumor and cancer preventive activities are potential **lead compounds** for drug development in the pharmaceutical industry [8].

[1]Laboratory of Food Chemistry, Wageningen University & Research, Bornse Weilanden 9, 6708 WG, Wageningen, Netherlands
[2]Laboratory of Plant Physiology, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB, Wageningen, Netherlands
[3]Wageningen Plant Research, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB, Wageningen, Netherlands
[4]Laboratory of Food Chemistry LinkedIn: https://www.linkedin.com/company/fchwur/
[5]These authors contributed equally as first authors

*Correspondence: jean-paul.vincken@wur.nl (J.-P. Vincken).

Box 1. Isoprenoid Biosynthesis – MVA and MEP Pathways

In order to perform their catalytic activity, aromatic PTs require the presence of two substrates: the acceptor substrate (aromatic compound) and the donor substrate (isoprenoid pyrophosphate). Isoprenoid pyrophosphates can be synthesized via two distinct biosynthetic pathways, the mevalonate and the non-mevalonate pathway (Figure I).

The mevalonate pathway, or MVA pathway, is responsible for isoprenoid biosynthesis in the majority of organisms, including some Gram-positive bacteria, yeasts, animals, and plants. It starts from condensation of two molecules of acetyl-CoA to form acetoacetyl-CoA, to which another molecule of acetyl-CoA is added to obtain 3-hydroxy-methylglutaryl-CoA (HMG-CoA). HMG-CoA is reduced to MVA, which then undergoes two consecutive phosphorylations to form mevalonate-5-pyrophosphate (MVAPP), via mevalonate-5-phosphate (MVAP). MVAPP undergoes decarboxylation to form isopentenyl pyrophosphate (IPP). Recently, an alternative to the last two steps of the MVA pathway has been discovered in which MVAP can be converted to IPP via isopentenyl phosphate. In plants, these reactions occur in the ER, in peroxisomes, and in the cytosol [106,107].

The non-mevalonate pathway, or 2-*C*-methylerythritol 4-phosphate (MEP) pathway, is used by a smaller group of organisms, including most Gram-negative bacteria, cyanobacteria, green algae, and plants. Plants are unique in this respect because they can utilize MVA and MEP pathways to biosynthesize isoprenoids. The MEP pathway starts with the condensation of pyruvic acid and D-glyceraldehyde 3-phosphate to form 1-deoxy-D-xylulose 5-phosphate (DXP). DXP is reduced and rearranged into MEP, which is then converted to 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol (CDP-ME). A consecutive phosphorylation yields 2-phospho-4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol (CDP-MEP), which is cyclized to form 2-*C*-methyl-D-erythritol-2,4-cyclodiphosphate (MEcPP). MEcPP undergoes two consecutive reductions, resulting in a mixture of IPP and its isomer dimethylallyl pyrophosphate (DMAPP), via 4-hydroxy-3-methylbut-2-enyl diphosphate (HMB-PP). In plants, all of these reactions take place in plastids [107].

IPP and DMAPP can be interconverted by IPP isomerase, however, most aromatic PTs of the UbiA superfamily cannot utilize IPP. DMAPP can undergo consecutive chain elongations, by condensation with IPP, to form geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP), and longer chain isoprenoid pyrophosphates. Besides their function as donor substrates for prenylation of aromatic substrates, these isoprenoid pyrophosphates serve as precursors for the biosynthesis of a wide variety of primary and secondary metabolites such as sterols, carotenoids, and chlorophylls [107].

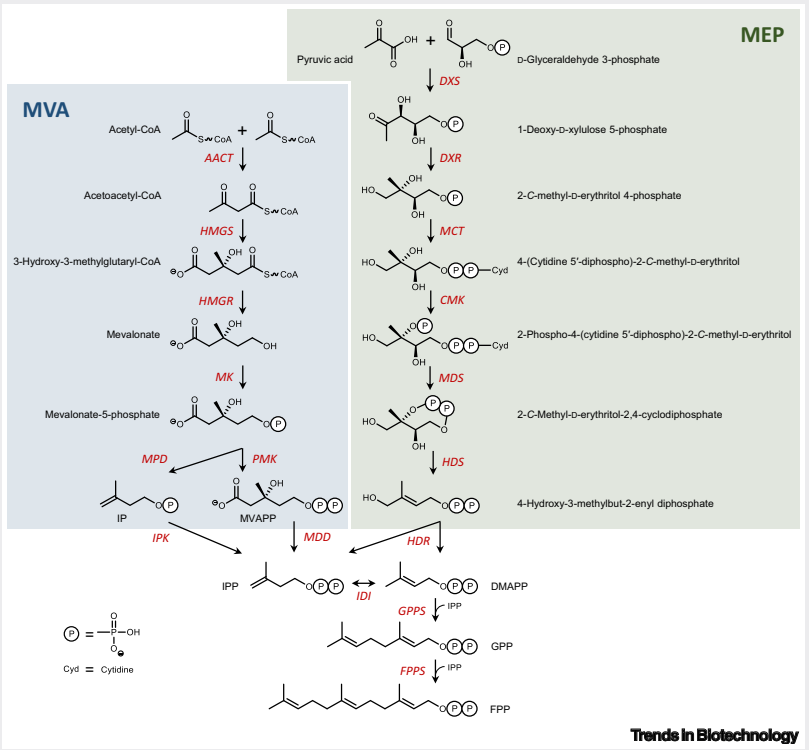

Figure I. Isoprenoid Biosynthesis via the Mevalonate (MVA, Left) and 2-*C*-Methylerythritol 4-Phosphate (MEP, Right) Pathways. Abbreviations: AACT, acetoacetyl-CoA thiolase; CMK, 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol kinase; CoA, coenzyme A; DMAPP, dimethylallyl pyrophosphate; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; FPP, farnesyl pyrophosphate; FPPS, FPP synthase GPP, geranyl pyrophosphate; GPPS, GPP synthase; HDR, 4-hydroxy-3-methylbut-2-enyl diphosphate reductase; HDS, 4-hydroxy-3-methylbut-2-enyl diphosphate synthase; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase; IDI, isopentenyl diphosphate isomerase; IP, isopentenyl phosphate; IPK, IP kinase; IPP, isopentenyl pyrophosphate; MCT, 2-*C*-methyl-D-erythritol 4-phosphate cytidylyltransferase; MDD, MVAPP decarboxylase; MDS, 2-*C*-methyl-D-erythritol-2,4-cyclodiphosphate synthase; MK, mevalonate kinase; MPD, mevalonate-5-phosphate decarboxylase; MVAPP, mevalonate-5-pyrophosphate; PMK, phosphomevalonate kinase.

Box 2. Bioactive Prenylated Aromatic Compounds from Plants

Plants produce a wide array of compounds that are well known due to their functionality in food or bioactivity in humans, for which prenylation of aromatic substrates is an essential step in the biosynthesis (Figure I).

Hop α acids (humulones) and β acids (lupulones) possess antibacterial properties against Gram-positive bacteria, thereby contributing to microbial stability in beer. Additionally, derivatives of the α acids, that is, iso-α acids, give bitter taste to beer and contribute to beer foam stability [108]. The prenylated isoflavone 8-prenylnaringenin and prenylated chalcone xanthohumol, also produced in hops, possess a wide variety of bioactivities such as antimicrobial, anti-inflammatory, anticancer, antioxidant, and estrogenic activity [109]. Cannabinoids, exemplified by cannabidiol and Δ^9-tetrahydrocannabinol (THC) in Figure I, are responsible for the medicinal properties of cannabis, which can, amongst others, be applied for chronic pain management, and for treatment of anxiety-related disorders and kidney disease [110]. However, THC is best known for its psychotropic activity and is therefore most commonly associated with drug use. Vitamin E has an excellent reputation due to its antioxidant activity and its function as an essential micronutrient for humans, besides many other beneficial bioactivities. Vitamin E is the collective name for the fat-soluble compounds tocopherols and tocotrienols, which are produced by plants [111].

As exemplified by humulones and lupulones, modification after or upon prenylation can lead to changes in the acceptor substrate structure; for example, leading to loss of aromaticity. In other cases, such as tocopherol, cannabidiol, and THC, the structure of the isoprenoid moiety itself is modified after attachment to the acceptor substrate (Figure I).

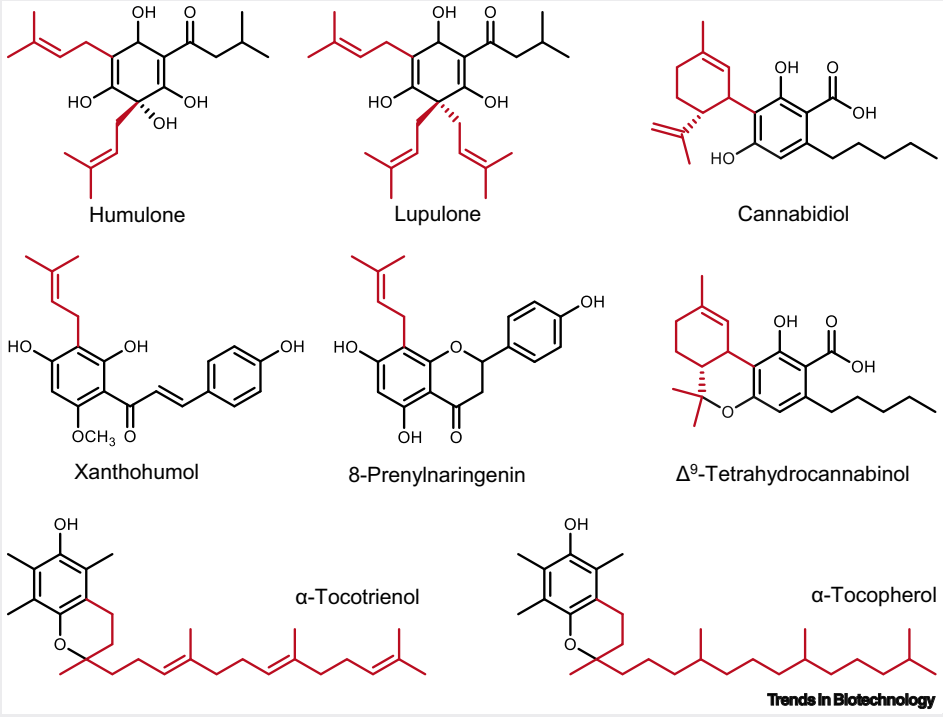

Figure I. Examples of Bioactive Prenylated Compounds. Several well-known bioactive compounds are shown, for which prenylation of aromatic substrates is an essential step in the biosynthesis. The (modified) isoprenoid moieties are shown in red.

Glossary

Chemoenzymatic synthesis: use of enzymes to catalyze chemical reactions for the synthesis of organic compounds.
***De novo* biosynthesis:** synthesis of complex molecules from simple precursors, such as sugars or amino acids, by a single microorganism equipped with the required metabolic pathways.
Isoprenoid: any organic hydrocarbon derived from one or more isoprene (C5) units.
Lead compounds: compounds with biological activity that serve as starting points in drug discovery for the development of pharmaceutical drugs. Lead compounds often require further structural optimization to improve characteristics like potency and pharmacokinetic properties.
Microbial cell factories: microbial cells that serve as production facilities for recombinant proteins and natural products, or as catalysts for biological transformations of industrial interest.
Natural preservatives: natural compounds that can be added to food to extend its shelf life. Typically, natural preservatives possess antimicrobial activity, whereby they contribute to microbial stability of food products, and can serve as natural alternatives to synthetic food preservatives.
Plastidial: localized to plastids, which are organelles found in various eukaryotic organisms. Plastids can differentiate into various forms, with the most well-known example being chloroplasts, green plastids that host chlorophyll for photosynthesis.
Prenyl: the shortest possible isoprenoid moiety, consisting of five carbon atoms, most commonly in a 3,3-dimethylallyl configuration.
Secondary or specialized metabolites: small molecules, which are not directly involved in growth, reproduction, and development of the plant, but contribute to, amongst others, defense against pathogens and chemical signaling.

To perform prenylation, plants use membrane-bound aromatic PTs that transfer isoprenoid moieties from pyrophosphate donor substrates to aromatic acceptor substrates. Until recently, few plant aromatic PTs had been identified. However, due to the increasing interest in bioactive plant metabolites, research in this field has been stimulated. In the past few years, this has led to the identification, gene cloning, and characterization of aromatic PTs from species such as soy bean (*Glycine max*) [9,10] peanut (*Arachis hypogaea*) [11] and hop (*Humulus lupulus*) [12].

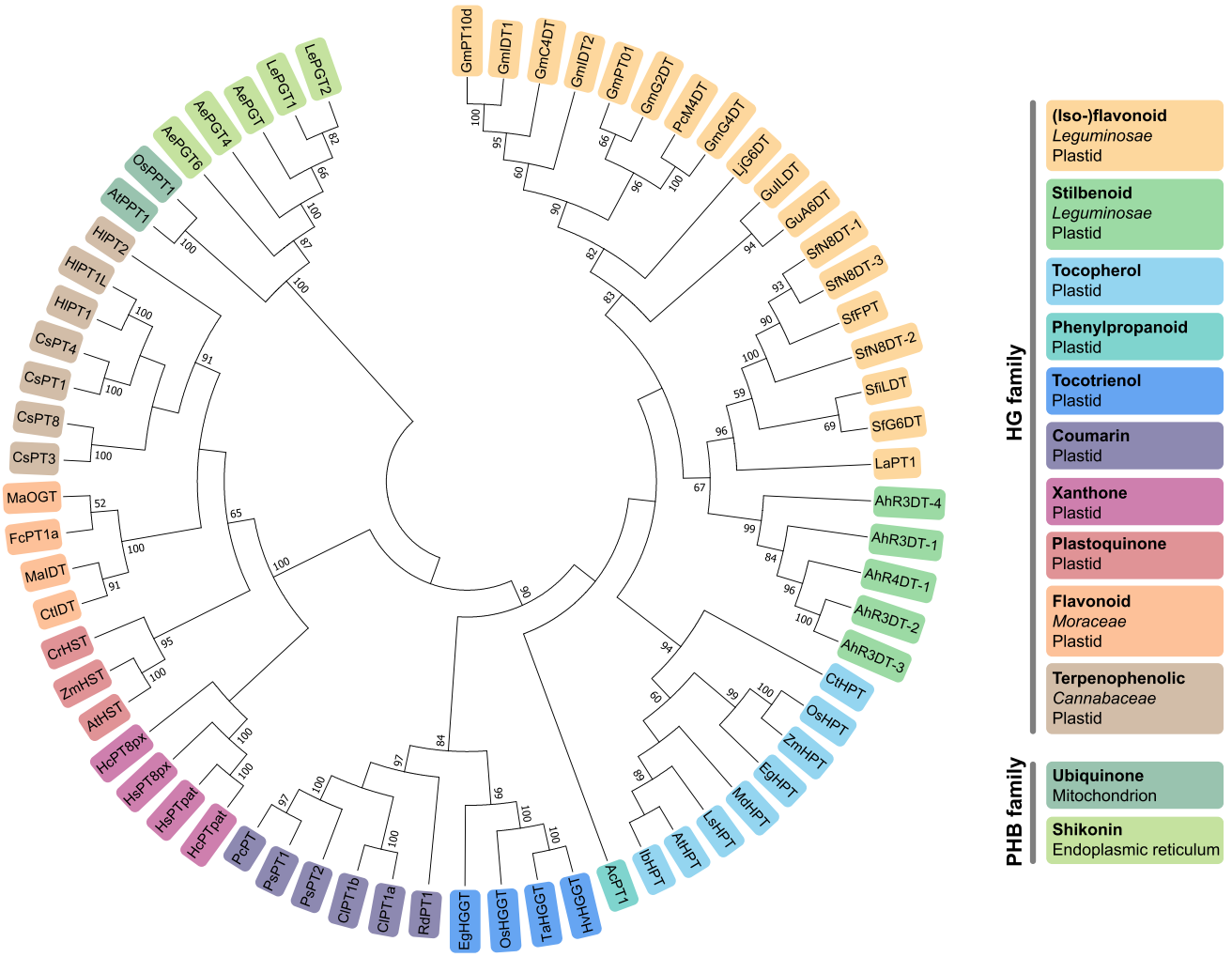

Figure 1. Phylogenetic Analysis of Plant Aromatic Prenyltransferases. Protein sequences of all plant prenyltransferases that have been reported to act on aromatic substrates were aligned using MUSCLE. A Neighbor-Joining tree (200 replicates) was constructed to illustrate their evolutionary relationships using the MEGA7 software [112]. The evolutionary distances were computed using the JTT matrix-based method [113]. Bootstrap values are indicated at the nodes of each branch. The abbreviations of the protein sequences and their corresponding accession numbers are listed in Table S1 (supplemental information online). Abbreviations: HG, homogentisate; PHB, *p*-hydroxybenzoic acid.

In this review, we aim to provide a comprehensive overview of the current state of the art on plant aromatic PTs and their exploitation in biotechnological platforms to produce prenylated (iso)flavonoids and stilbenoids.

Characterizing Plant Aromatic PTs

Plant aromatic PTs are members of the UbiA superfamily of intramembrane PTs. A recent review by Li gave a general overview of the entire superfamily [4]. Here, we focus on plant aromatic PTs, with special attention to those who catalyze the prenylation of (iso)flavonoids and stilbenoids.

Structure and Phylogeny

We compiled a list of known active, functionally characterized plant aromatic PTs (Table S1 in the supplemental information online). Based on phylogenetic analysis these enzymes can be divided into two distinct families: *p*-hydroxybenzoic acid (PHB) PTs and homogentisate (HG) PTs

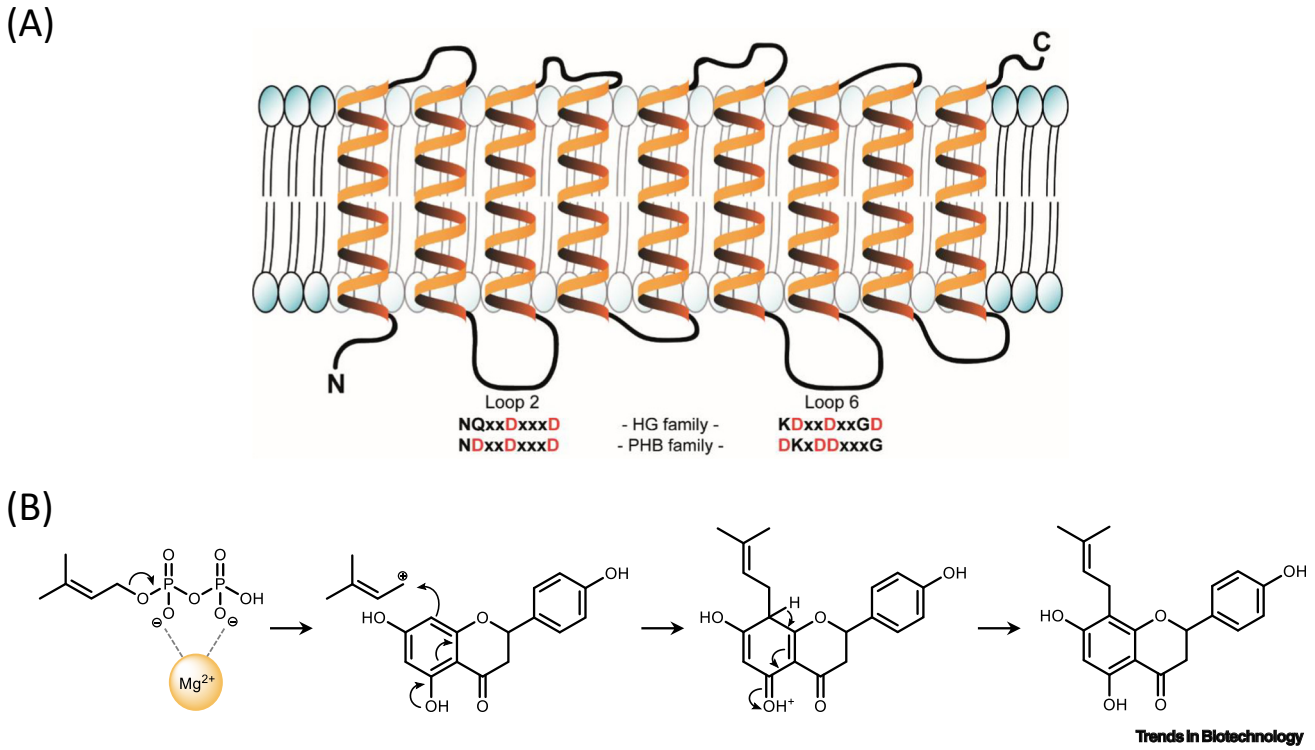

Figure 2. Canonical Fold of a Plant Aromatic PT and Mechanism of PT-Catalyzed Prenylation. (A) PTs are transmembrane proteins with 7–9 predicted transmembrane helices. The conserved aspartate-rich (D, in red) motifs in loops 2 and 6 coordinate Mg^{2+} ions, which stabilize the pyrophosphate moiety of the prenyl donor substrate. (B) Biosynthesis of 8-prenylnaringenin is used as an example. The pyrophosphate group of the donor substrate is bound and stabilized by magnesium (Mg^{2+}) ions facilitating prenyl carbocation formation and subsequent electrophilic substitution of the aromatic acceptor substrate. Abbreviations: HG, homogentisate; PHB, *p*-hydroxybenzoic acid; PT, prenyltransferase.

(Figure 1). All these PTs are transmembrane proteins with typically 7–9 predicted transmembrane helices. In addition, they possess two conserved aspartate-rich motifs in protein loops 2 and 6 (Figure 2A). Motifs for members of the HG PT family are NQxxDxxxD and KDxxDxxGD, whereas for members of the PHB PT family, the corresponding motifs are NDxxDxxxD and DKxDDxxxG (Figure 2A). Low resolution (>3 Å) crystal structures from two archaeal aromatic PTs (UbiA homologs) provide some insight in the structure of plant aromatic PTs, as most intramembrane PTs of the UbiA family seemingly share considerable structural similarity [13,14]. These crystal structures show that both aspartate-rich motifs coordinate Mg^{2+} ions, which stabilize the diphosphate moiety of the prenyl donor. Site-directed mutagenesis of a PHB PT from *Lithospermum erythrorhizon* confirms that residues in both motifs are critical for enzyme activity [15]. So far, no high-resolution structures of plant aromatic PTs have been reported.

Subcellular Localization

PHB PTs are located in the mitochondria or endoplasmic reticulum (ER) of the plant, whereas HG PTs are found in plastids (Figure 1). Most plant aromatic PTs reported today seem to have evolved from the HG PT family, and accordingly they are localized to the plastid compartment, as shown by GFP-fusion experiments [9,11,16–21]. **Plastidial** localization might be beneficial, and one of the reasons why most PTs have evolved from the HG rather than the PHB PT family, because this provides easy access to isoprenoid donor substrates. The plastid synthesizes large amounts of isoprenoids for the production of carotenoids and chlorophylls. Therefore, it is expected that the donor substrate flux through the plastidial 2-*C*-methylerythritol 4-

Key Table

Table 1. Overview of Functionally Characterized Aromatic PTs from Plants and Their Specificities

Species	Enzyme	Substrate specificity[a]		Regio-specificity	Refs
		Acceptor	Donor		
Arabidopsis thaliana	AtPPT1	PHB[b]	GPP[b]	*C*-3	[24]
	AtHPT (AtVTE2-1)	HG[b]	Phytyl-PP GGPP	*C*-3	[25–27]
	AtHST (AtVTE2-2)	HG HG γ-lactone Tyrosine PHB	Solanesyl-PP FPP GGPP Phytyl-PP	*C*-3[c]	[25,28]
Arachis hypogaea	AhR3′DT-1	Resveratrol Piceatannol Oxyresveratrol	DMAPP	*C*-3′	[11]
	AhR3′DT-2	Identical to AhR3′DT-1			[11]
	AhR3′DT-3	Identical to AhR3′DT-1			[11]
	AhR3′DT-4	Identical to AhR3′DT-1			[11]
	AhR4DT-1	Resveratrol Piceatannol Pinosylvin Oxyresveratrol	DMAPP	*C*-4	[11]
Arnebia euchroma	AePGT	PHB	GPP	*C*-3	[29]
	AePGT4	PHB	GPP	*C*-3	[29]
	AePGT6	PHB	GPP	*C*-3	[29]
Artemisia capillaris	AcPT1	*p*-Coumaric acid (*p*-Cou) *C*-3-Prenyl-*p*-coumaric acid Ferulic acid	DMAPP GPP, trace	*C*-3 *C*-5 (2nd prenyl on *p*-Cou)	[30]
Cannabis sativa	CsPT1	Olivetolic acid (OA) Phlorisovalerophenone Resveratrol Naringenin Olivetol	GPP	*C*-3 (OA) *C*-5 (OA)	[31,32]
	CsPT3	Apigenin Chrysoeriol	DMAPP[b] GPP	*C*-6	[33]
	CsPT4	OA + 5 OA derivatives	GPP[b]	*C*-3	[32]
	CsPT8	Apigenin	DMAPP	n.d.	[33]
Chlamydomonas reinhardtii	CrHST (CrVTE2-2)	HG HG γ-lactone	Solanesyl-PP FPP GGPP Phytyl-PP		[28]
Citrus limon	ClPT1a	Umbelliferone Esculetin 5,7-diOH-Coumarin 5-OCH_3-7-OH-Coumarin	GPP	*C*-8 *C*-6[d] *C*-5[d]	[34]
Citrus limon	ClPT1b	Identical to ClPT1a			[34]
Clitorea ternatea	CtHPT	HG[b]	Phytyl-PP[b]	*C*-3[c]	[35]
Cudriana tricuspidata	CtIDT	Isoliquiritigenin 2′,4′-diOH-Chalcone 2,4,2′,4′-tetraOH-Chalcone Butein Genistein 2′-OH-Genistein	DMAPP GPP	*C*-3′ (chalc.) *C*-6 (isoflav.)	[36]

Table 1. (continued)

Species	Enzyme	Substrate specificity[a]		Regio-specificity	Refs
		Acceptor	Donor		
Ficus carica	FcPT1a	Umbelliferone (Umb) 5-OCH_3-7-OH-Coumarin (5M7H)	DMAPP	*C*-6 (Umb) Diprenyl (5M7H)	[37]
Glycine max	GmC4DT	Coumestrol	DMAPP[b]	*C*-4	[10]
	GmG2DT	Glycinol	DMAPP[b]	*C*-2	[9,10]
	GmG4DT	Glycinol	DMAPP[b]	*C*-4	[9,17]
	GmIDT1	Genistein Daidzein	DMAPP[b]	B-ring[d]	[10]
	GmIDT2	Genistein Daidzein	DMAPP[b]	A-ring[d]	[10]
	GmPT01 (GmG2DT-2)	Glycinol	DMAPP[b]	*C*-2	[9]
	GmPT10d (GmIDT3)	Daidzein Genistein	DMAPP[b]	n.d.	[9]
Glycyrrhiza uralensis	GuA6DT	Apigenin Chrysin Luteolin + 3 other flavonoids	DMAPP GPP	*C*-6	[23]
	GuILDT	Isoliquiritigenin Naringenin chalcone 2,4′-diOH-Chalcone 2,4,2′,4′-tetraOH-Chalcone	DMAPP	*C*-3′	[38]
Hordeum vulgare	HvHGGT	HG[b]	GGPP[b]	*C*-3[c]	[39]
Humulus lupulus	HlPT-1	Phlorisovalerophenone Phlorisobutyrophenone Phlormethylbutanophenone Naringenin chalcone	DMAPP[b]	*C*-3 *C*-3′ (chalc.)	[21,40]
	HlPT1L[e]	Phlorisovalerophenone Phlorisobutyrophenone + prenylated derivatives Naringenin chalcone	DMAPP GPP	*C*-3 *C*-3′ (chalc.)	
	HlPT2[e]	Phlorisovalerophenone Phlorisobutyrophenone + prenylated derivatives	DMAPP GPP	*C*-3 *C*-5 (*gem*-diprenyl)	[12,19]
Hypericum calycinum	HcPT[f]	1,3,6,7-tetraOH-Xanthone[b]	DMAPP[b]	*C*-8	[41]
	HcPT8px	1,3,6,7-tetraOH-Xanthone[b] + prenylated derivative	DMAPP[b]	*C*-8 (mono- or *gem*-diprenyl)	[42]
	HcPTpat	1,3,6,7-tetraOH-Xanthone[b] + prenylated derivative	DMAPP[b]	*C*-8 (*gem*-diprenyl)	[42]
Hypericum sampsonii	HsPT8px	1,3,6,7-tetraOH-Xanthone[b] + prenylated derivative	DMAPP[b]	*C*-8 (mono- or *gem*-diprenyl)	[42]
	HsPTpat	1,3,6,7-tetraOH-Xanthone[b] + prenylated derivative	DMAPP[b]	*C*-8 (*gem*-diprenyl)	[42]
Ipomoea batatas	IbHPT	HG[b]	Phytyl-PP[b]	*C*-3[c]	[43]
Lactuca sativa	LsHPT	HG[b]	Phytyl-PP[b]	*C*-3[c]	[44,45]
Lithospermum erythrorhizon	LePGT1	PHB *o*-OH-Benzoic acid *m*-OH-Benzoic acid	GPP DMAPP (tr.) FPP (tr.) GGPP (tr.)	*C*-3	[15,46,47]
	LePGT2	PHB	GPP	*C*-3	[47]

(continued on next page)

Table 1. (continued)

Species	Enzyme	Substrate specificity[a]		Regio-specificity	Refs
		Acceptor	Donor		
Lotus japonicus	LjG6DT (LjPT1)	Genistein	DMAPP[b]	*C*-6	[20]
Lupinus albus	LaPT1	Genistein 2′-OH-Genistein	DMAPP	*C*-3′	[16]
Morus alba	MaIDT	Isoliquiritigenin 2′,4′-diOH-Chalcone 2,4,2′,4′-tetraOH-Chalcone Butein Genistein 2′-OH-Genistein Apigenin	DMAPP GPP	*C*-3′ (chalc.) *C*-6 (isoflav.)	[36]
	MaOGT	Oxyresveratrol Resveratrol	GPP	*C*-4	[22]
Oryza sativa	OsHGGT	HG[b]	GGPP[b]	*C*-3[c]	[39]
	OsHPT (RTD1)	HGe[b]	Phytyl-PP[b]	*C*-3[c]	[48]
	OsPPT1	PHB	GPP FPP GGPP	*C*-3[c]	[49]
Pastinaca sativa	PsPT1	Umb	DMAPP[b]	*C*-6[g] *C*-8	[50]
	PsPT2	Umb	DMAPP[b]	*C*-8[g] *C*-6	[50]
Petroselinum crispum	PcPT	Umb	DMAPP	*C*-6[g] *C*-8	[18]
Psoralea corylifolia	PcM4DT	Maackiain 3-OH-9-OCH_3-Pterocarpan	DMAPP	*C*-4	[51]
Rhododendron dauricum	RdPT1	Orsellinic acid	GPP FPP GGPP	*C*-3	[52]
Sophora flavescens	SfFPT	Naringenin Liquiritigenin Hesperetin + 8 other flavonoids	DMAPP GPP	*C*-8	[53]
	SfG6DT	Genistein Biochanin A	DMAPP GPP FPP (tr.)	*C*-6	[54]
	SfiLDT	Isoliquiritigenin	DMAPP	n.d.	[54]
	SfN8DT-1	Naringenin Liquiritigenin Hesperetin	DMAPP	*C*-8	[55]
	SfN8DT-2	Naringenin Liquiritigenin Hesperetin	DMAPP	*C*-8	[55]
	SfN8DT-3	Naringenin Liquiritigenin	DMAPP	*C*-8	[54]
Triticum aestivum	TaHGGT	HG[b]	GGPP[b]	*C*-3[c]	[39]
Zea mays	ZmHST (w3)	HG[b]	Solanesyl-PP[b]	*C*-3[c]	[56]
	ZmHPT	HG[b]	Phytyl-PP[b]	*C*-3[c]	[57]

phosphate (MEP) pathway is higher than through the cytosolic mevalonate (MVA) pathway (Box 1). On the other hand, mitochondria and ER, where PHB PTs are located, lack an endogenous isoprenoid pathway and need to acquire donor substrates from the cytosolic MVA pathway. As a result, PT activity may be impeded by limited availability of isoprenoid donor substrates.

Diversity in the HG PT Family

Within the HG PT family, several subgroups can be identified based on their aromatic substrate specificity, including flavonoid, xanthone, coumarin, stilbenoid, and terpenophenolic PTs (Figure 1). The majority of identified (iso)flavonoid and stilbenoid PTs have been isolated from legumes. Exceptions are MaIDT and CtIDT (Moraceae), and CsPT3 and CsPT8 (Cannabaceae), which are distantly related to their homologs in Leguminosae. This may indicate that flavonoid prenyltransferases in Leguminosae, Moraceae, and Cannabaceae have evolved independently.

Most plant aromatic PTs function independently as monomers. The hop PTs HlPT1L and HlPT2 occur in a heteromeric complex or metabolon, and the expression of HlPT1L was found to be essential for HlPT2 activity [19]. Moreover, the activity of these PTs could be enhanced when expressed together with chalcone isomerase-like enzymes [12]. There is currently no evidence of other plant aromatic PTs functioning as part of a complex or metabolon.

Mechanism of Aromatic Prenylation

The two conserved aspartate-rich motifs in protein loops 2 and 6 of the plant aromatic PTs coordinate divalent cations. Absence or chelation of these cations completely prevents PT activity [10,22]. The preferred cation is Mg^{2+}, although prenylation is possible with other cations such as Mn^{2+}, Co^{2+}, or Ca^{2+}, albeit at lower activity [10,17,20,22,23]. Their function is binding, stabilization, and orientation of the pyrophosphate group of the donor substrate, thereby facilitating the formation of a strongly electrophilic prenyl carbocation. Subsequently, the carbocation reacts with an aromatic acceptor substrate, leading to prenylation via a mechanism analogous to Friedel–Crafts aromatic electrophilic substitution (Figure 2B) [13,14].

Addition of an isoprenoid moiety increases the lipophilicity of the aromatic substrates and typically serves to enhance their potency. Prenylation can be the final step in a biosynthetic pathway, like with the production of 8-prenylnaringenin (Box 2), in which case the prenyl moiety remains unchanged. However, prenylation can also be an intermediate biosynthetic step, which is followed by further modification of the prenyl moiety or aromatic backbone, as in the case of THC (Box 2).

Most (iso)flavonoid and Stilbenoid PTs Are Highly Specific

The phylogenetic tree (Figure 1) shows that plant aromatic PTs catalyze prenylation of various classes of aromatic substrates. An overview of all functionally characterized plant aromatic PTs with their substrate- and regio-specificities is presented in Table 1 (Key Table), and the structures of

Notes to Table 1:
Abbreviations: chalc., chalcone; isoflav., isoflavonoid; n.d., not determined; tr., trace.
[a]Each paper only reports a subset of donor and acceptor substrates.
[b]Enzyme activity was only tested with the substrate(s) listed here.
[c]Position of prenylation inferred from the position in the expected product, but not explicitly determined in the cited study.
[d]Position of prenyl tentative.
[e]HlPT1L and HlPT2 form a metabolon which is essential for HlPT2 activity [19].
[f]Partial sequence in GenBank, not included in alignment and phylogenetic analysis.
[g]This position was strongly preferred.

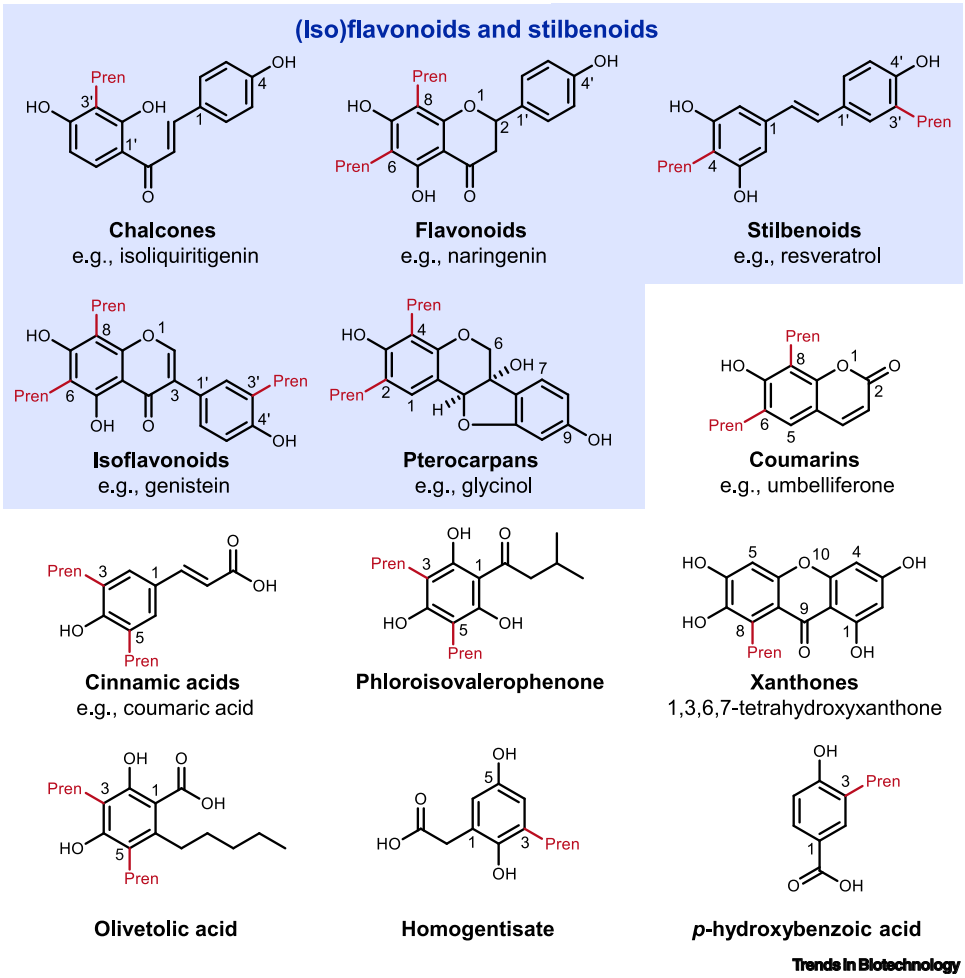

Figure 3. Overview of the Classes of Aromatic Acceptor Substrates Utilized by Plant Aromatic PTs. The group of (iso)flavonoids and stilbenoids is highlighted by blue shading. The positions of prenylation performed by known plant aromatic PTs (Table 1) are indicated in red. Abbreviation: PT, prenyltransferase.

the corresponding substrates are shown in Figure 3. With the exception of HG, PHB, and xanthones, all of these substrates can be prenylated at multiple positions by currently known PTs.

Acceptor Substrate Specificity

Most known plant aromatic PTs possess strict substrate specificity for the donor as well as the acceptor substrate. The prime example is the genistein- and dimethylallyl pyrophosphate (DMAPP)-specific PT from *Lotus japonicus* (LjG6DT), which solely produces 6-prenylgenistein (also known as wighteone) [20]. On the other hand, the flavonoid-specific PT from *Sophora flavescens* (SfFPT) is more promiscuous. This PT showed activity with all 12 tested flavonoids and accepted DMAPP and GPP as donor substrates [53]. SfFPT regio-specifically prenylated solely the *C*-8 position of the flavonoids in a stereospecific way; that is, it only accepted (–)-naringenin and not (+)-naringenin.

Overall, many (iso)flavonoid and stilbenoid PTs seem to recognize specific substructures in their aromatic acceptor substrates. For example, a common substructure used for recognition is resorcinol (*m*-dihydroxybenzene) as demonstrated by, for example, MaOGT, MaIDT, CtIDT, GuILDT, and SfG6DT [22,36,38,54]. Recognition of this substructure might explain the promiscuity of

some PTs, such as MaIDT, which is able to prenylate the resorcinol moiety of flavonoids as well as hydroxyxanthones [36,58]. Other examples are HlPT1 and HlPT1L, which are involved in the initial *C*-3 prenylation of phloroisovalerophenone (Table 1, Figure 3) in hop acid biosynthesis. Both PTs can also prenylate naringenin chalcone at *C*-3′ (Table 1) [19,40]. The phloroglucinol substructure recognized by these PTs in phloroisovalerophenone is also present in naringenin chalcone.

Donor Substrate Specificity

Regarding the donor substrate specificity, most of the (iso)flavonoid and stilbenoid PTs are specific for, or at least prefer, DMAPP. In some cases (e.g., SfG6DT), GPP or longer prenyl donors are accepted, but usually with much lower conversion rates [54]. Only two dedicated (iso)flavonoid or stilbenoid geranyltransferases have yet been described, namely CsPT3 from *Cannabis sativa* and MaOGT from *Morus alba* (Table 1). The sequences of these PTs are distant from the PT sequences within the legume family and more closely related to other geranyltransferases (e.g., xanthone geranyltransferases) (Figure 1). We speculate that a more spacious donor substrate binding site is required to accommodate longer chain prenyl donors (e.g., FPP), however, this can only be confirmed once high-resolution structures of several plant aromatic PTs become available.

Many Plant Aromatic PTs Are Still Unknown

As illustrated in Figure 1, 67 functional plant aromatic PTs have been identified and characterized thus far. However, there are still many prenylated (iso)flavonoids and stilbenoids found in plants for which the corresponding PTs have not yet been identified. An example is licorice (*Glycyrrhiza* spp.), which contains a wide variety of prenylated compounds, including amongst others *C*-6, *C*-8, and *C*-2′ (di)prenylated (iso)flavonoids, and *C*-2, *C*-3, and *C*-3′ prenylated chalcones [59] So far, however, only two licorice PTs have been identified, a flavonoid *C*-6 and a chalcone *C*-3′ PT (Table 1).

Plant Aromatic O-PTs

One of the major knowledge gaps in this field is that all characterized plant aromatic PTs reported thus far only perform *C*-prenylations. There has not been any report on the identification of an *O*-PT gene for aromatic substrates in plants, even though a growing number of natural *O*-prenylated (iso)flavonoids and stilbenoids have been identified. Examples include *C*,*O*-di-prenylated dihydrostilbene from *Glycyrrhiza glabra* [60], *C*,*O*-diprenylated chalcone from *Helichrysum teretifolium* [61], and several different *O*-prenylated (iso)flavonoids from *Amyris madrenis* and *Sophora interrupta* [62,63]. This is by no means a comprehensive list of naturally occurring *O*-prenylated aromatic compounds, but it does illustrate that *O*-prenylation is widespread in nature. It also indicates that *O*-PTs must be present in many different plant families including Leguminosae, Asteraceae, and Rutaceae. We presume that plant aromatic *O*-PTs make use of a similar catalytic mechanism and that they are related to *C*-PTs, but they may structurally differ and therefore belong to another subfamily of plant aromatic PTs.

Starting Points for Expanding the Variety of Characterized Plant Aromatic PTs

There are also cases in which (iso)flavonoid- or stilbenoid-specific PT activities have been detected in plant fractions but the corresponding PTs were not identified. Examples of PT activities described in plant fractions include chalcone *C*-3′ prenylation in *Morus nigra* [64], pterocarpan *C*-10 prenylation in *Phaseolus vulgaris* [65], and isoflavone *C*-6 prenylation in *Lupinus angustifolius* [66]. Similar experiments indicate the presence of *O*-PT activities in *Piper crassinervium* [67], *Ammi majus* [68], and *Citrus limon* [69]. An overview of plant tissues with PT activities can be found in Table S2 (see supplemental information online). In many cases, such as the examples mentioned earlier, the PT remains unidentified. The data presented in Table S2 (see supplemental

information online) might provide a starting point for identification of several yet uncharacterized PTs, including some *O*-PTs.

Plant Aromatic PTs as Tools for Microbial Cell Factories

Prenylated (iso)flavonoids and stilbenoids are of commercial interest because of their bioactivities (Box 2);however, currently their application is limited by their availability in nature. Plants produce these compounds in low quantities, thereby excluding plant-based production systems as a viable means of obtaining them. Alternatively, chemical synthesis has been successfully applied for the production of prenylated (iso)flavonoids and stilbenoids on laboratory scale [70,71]. Yet, chemical synthesis of these compounds at industrial scale is challenging due to harsh conditions, low yields, and production of undesirable byproducts. **Chemoenzymatic synthesis** of prenylated aromatic compounds using purified PTs is possible at laboratory scale [47,55]. but major drawbacks that restrict upscaling are the isolation of enzymes (or microsomal fractions) and the required addition of expensive isoprenoid pyrophosphates (DMAPP and GPP) as donor substrates. As an alternative, **microbial cell factories** may be used for environmentally friendly and efficient ***de novo* biosynthesis** of prenylated (iso)flavonoids and stilbenoids. Promiscuous microbial aromatic PTs have been investigated as tools for prenylation of (iso)flavonoids and stilbenoids [72–74]. However, substrate conversion by microbial PTs was often found to be low, and these enzymes lack substrate- and regio-specificity, limiting their suitability for targeted production of bioactive compounds. Recent advances with plant aromatic PTs could enable the use of these highly (regio-)specific enzymes in microbial cell factories for the biotechnological production of prenylated aromatic compounds.

Biotechnological Prenylation of Aromatic Compounds: Current Challenges

Several studies have shown the potential of yeast cell factories for the prenylation of (iso)flavonoids or stilbenoids. Yeasts possess similar cellular compartments as plant cells, including an ER, which is beneficial for the heterologous production of membrane-bound cytochrome P450 enzymes involved in (iso)flavonoid and stilbenoid biosynthesis [75]. *Saccharomyces cerevisiae*

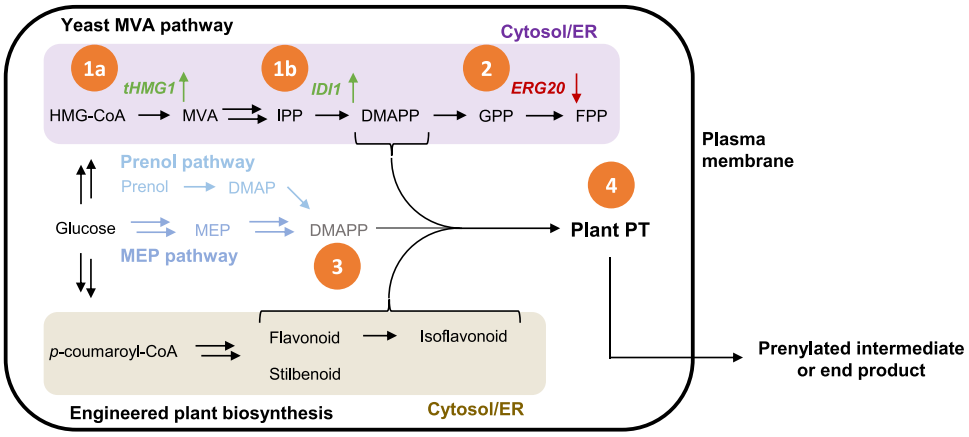

Figure 4. Schematic Representation of a Yeast Cell Factory for *de novo* Biosynthesis of Prenylated (Iso) flavonoids and Stilbenoids. Numbers indicate proposed strategies to remove bottlenecks and improve production efficiency. 1, Overcoming rate limiting steps of MVA pathway by overexpression of truncated HMG1 (1a) and increasing IDI1 activity (1b); 2, downregulation of FPP synthase (ERG20); 3, incorporation of an additional isoprenoid pathway to increase DMAPP production; 4, improve access of PT to substrates by targeting the plant PT to the ER. Abbreviations: DMAPP, dimethylallyl pyrophosphate; ER, endoplasmic reticulum; FPP, farnesyl pyrophosphate GPP, geranyl pyrophosphate; HMG-CoA, 3-hydroxy-methylglutaryl-CoA; IPP, isopentenyl pyrophosphate; MEP, 2-*C*-methylerythritol 4-phosphate; MVA, mevalonate; PT, prenyltransferase.

in particular has been proven to be a successful host for plant aromatic PTs [19,53,76,77]. This was first demonstrated for the bioconversion of naringenin by transgenic *S. cerevisiae* overexpressing SfN8DT-1 from *S. flavescens* [76]. This study showed that yeast could provide the prenyl donor (DMAPP) *in vivo*, and recombinant SfN8DT-1 converted supplemented naringenin to 8-prenylnaringenin. More recently, *de novo* biosynthesis of 8-prenylnaringenin was achieved by introduction of SfFPT from *S. flavescens* in a naringenin-producing yeast strain [77].

In both of these studies, however, prenylation yields were low; that is, less than 2% of the available naringenin was converted to 8-prenylnaringenin. Similarly, prenylation of phloroisovalerophenone to form lupulone (hop β-acids; Box 2) in yeast was only successful after chemical downregulation of ergosterol biosynthesis [78]. A likely limitation for a higher product yield in the aforementioned studies was the availability of the prenyl donor DMAPP, as supported by the findings of several other studies on the production of prenylated compounds in yeast [19,77–79].

Different subcellular locations of the donor and acceptor substrates, as well as of the PT, might also contribute to the low yields. Additionally, increasing the availability of acceptor substrate can boost titers of prenylated product, as was demonstrated for the prenylation of naringenin [77] and for the conversion of PHB to 3-geranyl-4-hydroxybenzoic acid (GBA) [29]. Advances in *de novo* biosynthesis of aromatic compounds, like naringenin, have enabled production of high titers of acceptor substrates from glucose or aromatic amino acids [77,80,81]. Thus, availability of basic acceptor substrates no longer seems to be one of the main limitations of microbial cell factories for the production of prenylated (iso)flavonoids and stilbenoids. Therefore, the main challenges are colocalization of substrates and PT, and donor substrate availability. These challenges are visualized in Figure 4 and the suggested optimizations to overcome them are discussed in the next paragraphs.

Subcellular Location of the Key Players in Aromatic Prenylation

The first key consideration is the subcellular compartments in which the aromatic PTs and their substrates are localized. The endogenous MVA pathway in yeast is mainly located on the ER and in the cytosol (Box 1). The same is believed to be true for the engineered aromatic acceptor substrate biosynthesis, like the plant flavonoid biosynthetic pathway. In the absence of a plastid organelle in yeast, the location of the heterologous membrane-bound PT is unknown. Current experimental evidence cannot be used to distinguish between different membrane locations. Specifically targeting the PT to the ER membrane (Figure 4, 4), to bring it closer to its substrates (Box 1), may contribute significantly to enhancing prenylation efficiency in yeast cell factories.

Improving Isoprenoid Donor Substrate Availability

A sufficient supply of isoprenoid donor substrate is required for the efficient biosynthesis of prenylated compounds. In *S. cerevisiae*, isoprenoids are produced via the MVA pathway (Box 1) and one of their main uses is as precursor for ergosterol biosynthesis. The supply of DMAPP, the prenyl donor utilized by most (iso)flavonoid or stilbenoid PTs (Table 1), can be manipulated in various ways.

A first strategy would be to overcome the rate-limiting steps of the MVA pathway for DMAPP biosynthesis (Box 1). 3-hydroxy-methylglutaryl-CoA (HMG-CoA) reductase (HMG1) is a key rate-limiting enzyme in the MVA pathway, and overexpression of a truncated HMG1 gene (tHMG1) is commonly used to remove negative feedback regulation, consequently increasing carbon flux through the MVA pathway [82]. This has been successfully applied to increase production of various terpenoids in yeast [83,84]. In addition, overexpression of isopentenyl pyrophosphate

(IPP) isomerase (IDI1), which catalyzes the conversion of IPP to DMAPP, contributed to significant enhancement of monoterpene titers [85]. Alternatively, DMAPP supply can be increased by overexpression of MAF1 [86], which is a negative regulator of another enzyme utilizing DMAPP, namely tRNA isopentenyltransferase (MOD5) [87]. Furthermore, DMAPP supply may be improved by deletion of ROX1, a transcription factor that represses many enzymes in the mevalonate and ergosterol biosynthesis pathway [88]. In addition, deleting the ubiquitin ligases UBC7 and SSM4/DOA10, and the ER resident protein PHO86, enhances stability of HMG1 and prevents its degradation [89]. Alternatively, overexpression of an IDI1-prenyltransferase fusion protein, mimicking natural biofunctional enzymes, may increase production of prenylated compounds [90]. It is expected that simultaneously overexpressing tHMG1 (Figure 4, 1a) and IDI1 (Figure 4, 1b) will be starting points to achieve improved prenylation of (iso)flavonoids and stilbenoids in microbial cell factories.

A second strategy to improve DMAPP availability could be downregulation of endogenous FPP synthase (ERG20) (Figure 4, 2). Mutation of ERG20 has been shown to increase GPP availability by decreasing FPP synthesis and consequently resulted in enhanced production of geraniol in yeast [91]. Although ERG20 mutations are targeted at increasing GPP availability for geranylation, the same mutation unexpectedly also enhances 8-prenylnaringenin yields from naringenin prenylation (44-fold increase) [19]. Various other mutations in ERG20 have been described that improve monoterpene production [91–93]. Whether these mutations also enhance DMAPP availability is not known, but based on previous success of ERG20 mutation for prenylation [19], this might be the case. Alternatively, the consumption of precursors of the ergosterol biosynthesis can be downregulated chemically using small molecules such as ketoconazole [78]. A drawback of the inhibition of ERG20, and thereby also ergosterol synthesis, is that it can result in major growth defects in *S. cerevisiae*, which can only be compensated for by ergosterol supplementation to the culture media. Alternatively, there are various other ways to control the flux through the ergosterol pathway without causing a detrimental shutdown. Manipulation of the ergosterol pathway with a pull approach has yielded very promising results, examples include promoter substitution, ERG3 mutations, and RNT1-based transcript destabilization to control gene expression levels in yeast [94–96].

A third strategy to improve the donor substrate availability is to implement alternative pathways to increase total DMAPP synthesis (Figure 4, 3), thereby providing enough isoprenoid precursors for prenylation as well as ergosterol biosynthesis. Evidently, the MEP pathway (Box 1) may serve this purpose. Synergy between MVA and MEP for isoprene production was demonstrated in *Escherichia coli* [97]. However, MEP incorporation in *S. cerevisiae* has proven challenging and has often been unsuccessful [98,99]. Nevertheless, several studies have shown that it is possible to functionalize MEP in *S. cerevisiae* cytosol [100,101]. The successful combination of MVA and MEP and its application to isoprenoid production has, however, not yet been demonstrated. As an alternative to the MEP pathway, a two-step pathway for IPP and DMAPP production in which isoprenol and prenol, respectively, were used as precursors was described [102,103]. This novel alcohol-dependent pathway is orthogonal to the endogenous IPP and DMAPP biosynthesis (i.e., the MVA pathway) and has potential for high metabolic flux because it is decoupled from the central carbon metabolism. Introduction of an additional pathway could, therefore, be an attractive approach to increase the cellular amount of DMAPP for improved prenylation in yeast.

Production of Structurally Diverse Aromatic Acceptor Substrates

The premise for efficient biotechnological production of prenylated (iso)flavonoids and stilbenoids is *de novo* biosynthesis of the acceptor substrate. The efficiency of producing basic (iso)flavonoids and stilbenoids, such as naringenin and resveratrol (Figure 3), has increased tremendously in

recent years [77,80,81]. Naringenin can be considered as the entry point to produce a large diversity of flavonoids. To access a wider variety of (iso)flavonoids and stilbenoids, both as acceptor substrates and for their own respective bioactivities, further developments in this field will be necessary. In particular, the functional expression of downstream biosynthetic enzymes from plants in microbes is still challenging. Production of highly functionalized flavonoids such as anthocyanins, which are produced from naringenin in several steps, results in low yields [104]. The rapidly growing amount of data from plant whole genome sequencing and omics techniques will play a key role in advancing our understanding of the plant metabolic pathways, and deliver candidate genes for efficient production of (iso)flavonoids and stilbenoids.

Above and Beyond: Diversifying Prenylation and Prenyl Modification

What has not yet been discussed so far is the activity and range of substrates utilized by PTs in biotechnological production platforms. Even though functionalization of various plant aromatic PTs in yeast has shown to be successful [19,53,76,77] only a limited number of aromatic substrates can be prenylated thus far. Extending prenylation to other acceptor substrates will require significant optimization of the microbial host for each combination of substrate and PT. Moreover, many of the required PTs, most evidently *O*-PTs, have not yet been identified.

In addition, to be able to produce the full range of plant prenylated compounds will also require expression of additional enzymes, such as cyclases, that act on the attached prenyl moiety. Successful application of these enzymes will require further optimization. To this end, yeasts possess various advantageous traits that support their industrial application as host, including their genetic accessibility, simple nutritional requirements, relatively high tolerance to substrate and product toxicity, and ability to express heterologous enzymes like cytochrome P450s [75]. The latter could be essential in the future to achieve prenyl modifications, such as cyclization, which is reportedly mediated by cytochrome P450 [105]. In addition to the aforementioned advantages, 8-prenylnaringenin produced in *S. cerevisiae* was primarily detected extracellularly [76,77], indicating that yeast possesses efflux pumps that can remove aromatic compounds from their cells, which facilitates recovery of the prenylated product.

Concluding Remarks

In the past 10 years, more than 60 plant aromatic PTs have been identified and functionally characterized. These enzymes play a key role in the biosynthesis of bioactive prenylated compounds in plants. Plant aromatic PTs often show remarkable substrate specificity, catalyzing the regio- and stereospecific prenylation of aromatic substrates. Unfortunately, the lack of high-resolution 3D structures currently limits mechanistic insight in the specificity of PTs. Besides this, a major gap in the current knowledge is the fact that no *O*-PTs have yet been identified (see Outstanding Questions). Discovery of the first aromatic *O*-PTs from plants will contribute to elucidation of the mechanisms underlying the specificity of plant PTs, and it will serve as a starting point for identifying additional aromatic *O*-PTs.

Exploitation of plant aromatic PTs in microbial cell factories could potentially enable the large scale production of valuable bioactive compounds, such as prenylated (iso)flavonoids and stilbenoids. However, so far *de novo* biosynthesis of these compounds in yeasts has generally resulted in low yields. The limiting factor, in most cases, was prenyl donor substrate (DMAPP) availability. To facilitate further developments in this field, we have described several approaches for optimization of prenyl donor substrate availability. Additionally, it will be essential to determine the subcellular location of plant aromatic PTs expressed in yeast (see Outstanding Questions). This information may contribute to enhanced prenylation efficiency by specifically colocalizing the aromatic PT and substrate biosynthesis in yeast cell factories.

Outstanding Questions

How can we enhance biotechnological efficiency of prenylation in microbial cell factories? Production of the isoprenoid donor substrate, in combination with localization of the PT and its substrates, currently seem to be the main bottlenecks for prenylation.

What is the subcellular location of PT in yeast? Expression of GFP fusions of PTs in yeasts could provide further information to aid in fine tuning the subcellular localization in yeast cell factories.

What determines the donor and acceptor substrate specificity of plant aromatic PTs?

Can the 3D structure of aromatic plant PTs be solved? A better understanding of the enzyme structure will facilitate identification of novel PTs, prediction of the function of uncharacterized PTs, and mechanistic insight in the specificity of these enzymes.

Do plant aromatic *O*-PTs belong to a separate subfamily of plant aromatic PTs?

Finally, expression of a plant aromatic PT alongside the plant flavonoid biosynthetic pathway has been demonstrated to yield *de novo* production of 8-prenylnaringenin, a natural compound with potent medicinal properties, from simple precursors. By overcoming the current bottlenecks for prenylation, as discussed in this review, microbial cell factories utilizing a similar approach could serve as efficient biotechnological production platforms for many other bioactive prenylated (iso)flavonoids and stilbenoids.

Supplemental Information

Supplemental information associated with this article can be found online at https://doi.org/10.1016/j.tibtech.2020.02.006.

References

1. Borghi, M. *et al.* (2017) The sexual advantage of looking, smelling, and tasting good: the metabolic network that produces signals for pollinators. *Trends Plant Sci.* 22, 338–350
2. Latif, S. *et al.* (2017) Allelopathy and the role of allelochemicals in plant defence. In *How Plants Communicate with Their Biotic Environment* (Becard, G., ed.), pp. 19–54, Academic Press
3. Winkelblech, J. *et al.* (2015) Prenyltransferases as key enzymes in primary and secondary metabolism. *Appl. Microbiol. Biotechnol.* 99, 7379–7397
4. Li, W.K. (2016) Bringing bioactive compounds into membranes: the UbiA superfamily of intramembrane aromatic prenyltransferases. *Trends Biochem. Sci.* 41, 356–370
5. Chen, X. *et al.* (2014) A systematic review on biological activities of prenylated flavonoids. *Pharm. Biol.* 52, 655–660
6. Yang, X.M. *et al.* (2015) Prenylated flavonoids, promising nutraceuticals with impressive biological activities. *Trends Food Sci. Technol.* 44, 93–104
7. Sanchez Maldonado, A.F. *et al.* (2015) Plant defence mechanisms and enzymatic transformation products and their potential applications in food preservation: advantages and limitations. *Trends Food Sci. Technol.* 46, 49–59
8. Venturelli, S. *et al.* (2016) Prenylated chalcones and flavonoids for the prevention and treatment of cancer. *Nutrition* 32, 1171–1178
9. Sukumaran, A. *et al.* (2018) Isoflavonoid-specific prenyltransferase gene family in soybean: GmPT01, a pterocarpan 2-dimethylallyltransferase involved in glyceollin biosynthesis. *Plant J.* 96, 966–981
10. Yoneyama, K. *et al.* (2016) Molecular characterization of soybean pterocarpan 2-dimethylallyltransferase in glyceollin biosynthesis: local gene and whole-genome duplications of prenyltransferase genes led to the structural diversity of soybean prenylated isoflavonoids. *Plant Cell Physiol.* 57, 2497–2509
11. Yang, T.H. *et al.* (2018) Stilbenoid prenyltransferases define key steps in the diversification of peanut phytoalexins. *J. Biol. Chem.* 293, 28–46
12. Ban, Z.N. *et al.* (2018) Noncatalytic chalcone isomerase-fold proteins in *Humulus lupulus* are auxiliary components in prenylated flavonoid biosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* 115, E5223–E5232
13. Cheng, W. and Li, W.K. (2014) Structural insights into ubiquinone biosynthesis in membranes. *Science* 343, 878–881
14. Huang, H. *et al.* (2014) Structure of a membrane-embedded prenyltransferase homologous to UBIAD1. *PLoS Biol.* 12, e1001911
15. Ohara, K. *et al.* (2009) Functional characterization of LePGT1, a membrane-bound prenyltransferase involved in the geranylation of *p*-hydroxybenzoic acid. *Biochem. J.* 421, 231–241
16. Shen, G.A. *et al.* (2012) Characterization of an isoflavonoid-specific prenyltransferase from *Lupinus albus*. *Plant Physiol.* 159, 70–80
17. Akashi, T. *et al.* (2009) Molecular cloning and characterization of a cDNA for pterocarpan 4-dimethylallyltransferase catalyzing the key prenylation step in the biosynthesis of glyceollin, a soybean phytoalexin. *Plant Physiol.* 149, 683–693
18. Karamat, F. *et al.* (2014) A coumarin-specific prenyltransferase catalyzes the crucial biosynthetic reaction for furanocoumarin formation in parsley. *Plant J.* 77, 627–638
19. Li, H.X. *et al.* (2015) A heteromeric membrane-bound prenyltransferase complex from hop catalyzes three sequential aromatic prenylations in the bitter acid pathway. *Plant Physiol.* 167, 650–659
20. Liu, J.Y. *et al.* (2018) Genistein-specific G6DT gene for the inducible production of wighteone in *Lotus japonicus*. *Plant Cell Physiol.* 59, 128–141
21. Tsurumaru, Y. *et al.* (2010) An aromatic prenyltransferase-like gene HlPT-1 preferentially expressed in lupulin glands of hop. *Plant Biotechnol.* 27, 199–204
22. Zhong, Z.H. *et al.* (2018) Molecular characterization of a geranyl diphosphate-specific prenyltransferase catalyzing stilbenoid prenylation from *Morus alba*. *Plant Cell Physiol.* 59, 2214–2227
23. Li, J.H. *et al.* (2014) GuA6DT, a regiospecific prenyltransferase from *Glycyrrhiza uralensis*, catalyzes the 6-prenylation of flavones. *ChemBioChem* 15, 1672–1680
24. Okada, K. *et al.* (2004) The AtPPT1 gene encoding 4-hydroxybenzoate polyprenyl diphosphate transferase in ubiquinone biosynthesis is required for embryo development in *Arabidopsis thaliana*. *Plant Mol. Biol.* 55, 567–577
25. Tian, L. *et al.* (2007) The *pds2* mutation is a lesion in the *Arabidopsis* homogentisate solanesyltransferase gene involved in plastoquinone biosynthesis. *Planta* 226, 1067–1073
26. Sadre, R. *et al.* (2010) Catalytic reactions of the homogentisate prenyl transferase involved in plastoquinone-9 biosynthesis. *J. Biol. Chem.* 285, 18191–18198
27. Savidge, B. *et al.* (2002) Isolation and characterization of homogentisate phytyltransferase genes from *Synechocystis* sp. PCC 6803 and *Arabidopsis*. *Plant Physiol.* 129, 321–332
28. Sadre, R. *et al.* (2006) Characterization of homogentisate prenyltransferases involved in plastoquinone-9 and tocochromanol biosynthesis. *FEBS Lett.* 580, 5357–5362
29. Wang, S. *et al.* (2017) Production of 3-geranyl-4-hydroxybenzoate acid in yeast, an important intermediate of shikonin biosynthesis pathway. *FEMS Yeast Res.* Published online November 1, 2017. https://doi.org/10.1093/femsyr/fox1065
30. Munakata, R. *et al.* (2019) Isolation of *Artemisia capillaris* membrane-bound di-prenyltransferase for phenylpropanoids and redesign of artepillin C in yeast. *Commun. Biol.* 2, 384
31. Page, J.E. and Boubakir, Z. National Research Council of Canada, Ottawa (CA); University of Saskatchewan, Saskatoon (CA). (2014) Aromatic prenyltransferase from *Cannabis*, *United States Patent* US8884100B2.
32. Luo, X.Z. *et al.* (2019) Complete biosynthesis of cannabinoids and their unnatural analogues in yeast. *Nature* 567, 123–126
33. Rea, K.A. *et al.* (2019) Biosynthesis of cannflavins A and B from *Cannabis sativa* L. *Phytochemistry* 164, 162–171
34. Munakata, R. *et al.* (2014) Molecular cloning and characterization of a geranyl diphosphate-specific aromatic prenyltransferase from lemon. *Plant Physiol.* 166, 80–90
35. Wunnakup, T. *et al.* (2018) Transient expression of the homogentisate phytyltransferase gene from *Clitoria ternatea* causes metabolic enhancement of α-tocopherol biosynthesis

and chlorophyll degradation in tomato leaves. *J. Plant Biochem. Biotechnol.* 27, 55–67
36. Wang, R.S. *et al.* (2014) Molecular characterization and phylogenetic analysis of two novel regio-specific flavonoid prenyltransferases from *Morus alba* and *Cudrania tricuspidata*. *J. Biol. Chem.* 289, 35815–35825
37. Munakata, R. *et al.* (2019) Convergent evolution of the UbiA prenyltransferase family underlies the independent acquisition of furanocoumarins in plants. *New Phytol.* 225, 2166–2182
38. Li, J.H. *et al.* (2018) Biocatalytic access to diverse prenylflavonoids by combining a regiospecific *C*-prenyltransferase and a stereospecific chalcone isomerase. *Acta Pharm. Sin. B* 8, 678–686
39. Cahoon, E.B. *et al.* (2003) Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. *Nat. Biotechnol.* 21, 1082–1087
40. Tsurumaru, Y. *et al.* (2012) HIPT-1, a membrane-bound prenyltransferase responsible for the biosynthesis of bitter acids in hops. *Biochem. Biophys. Res. Commun.* 417, 393–398
41. Fiesel, T. *et al.* (2015) Molecular cloning and characterization of a xanthone prenyltransferase from *Hypericum calycinum* cell cultures. *Molecules* 20, 15616–15630
42. Nagia, M. *et al.* (2019) Sequential regiospecific *gem*-diprenylation of tetrahydroxyxanthone by prenyltransferases from *Hypericum* sp. *New Phytol.* 222, 318–334
43. Ji, C.Y. *et al.* (2016) Molecular characterization of tocopherol biosynthetic genes in sweetpotato that respond to stress and activate the tocopherol production in tobacco. *Plant Physiol. Biochem.* 106, 118–128
44. Ren, W.W. *et al.* (2011) Molecular analysis of a homogentisate phytyltransferase gene from *Lactuca sativa* L. *Mol. Biol. Rep.* 38, 1813–1819
45. Ren, W.W. *et al.* (2011) Overexpression of homogentisate phytyltransferase in lettuce results in increased content of vitamin E. *Afr. J. Biotechnol.* 10, 14046–14051
46. Ohara, K. *et al.* (2013) Homogeneous purification and characterization of LePGT1 – a membrane-bound aromatic substrate prenyltransferase involved in secondary metabolism of *Lithospermum erythrorhizon*. *FEBS J.* 280, 2572–2580
47. Yazaki, K. *et al.* (2002) Geranyl diphosphate: 4-hydroxybenzoate geranyltransferase from *Lithospermum erythrorhizon*: cloning and characterization of a key enzyme in shikonin biosynthesis. *J. Biol. Chem.* 277, 6240–6246
48. Zhang, Y.H. *et al.* (2018) *Rice tocopherol deficiency 1* encodes a homogentisate phytyltransferase essential for tocopherol biosynthesis and plant development in rice. *Plant Cell Rep.* 37, 775–787
49. Ohara, K. *et al.* (2006) Functional characterization of OsPPT1, which encodes *p*-hydroxybenzoate polyprenyltransferase involved in ubiquinone biosynthesis in *Oryza sativa*. *Plant Cell Physiol.* 47, 581–590
50. Munakata, R. *et al.* (2016) Molecular evolution of parsnip (*Pastinaca sativa*) membrane-bound prenyltransferases for linear and/or angular furanocoumarin biosynthesis. *New Phytol.* 211, 332–344
51. He, J.B. *et al.* (2018) Regio-specific prenylation of pterocarpans by a membrane-bound prenyltransferase from *Psoralea corylifolia*. *Org. Biomol. Chem.* 16, 6760–6766
52. Saeki, H. *et al.* (2018) An aromatic farnesyltransferase functions in biosynthesis of the anti-HIV meroterpenoid daurichromenic acid. *Plant Physiol.* 178, 535–551
53. Chen, R.D. *et al.* (2013) Regio- and stereospecific prenylation of flavonoids by *Sophora flavescens* prenyltransferase. *Adv. Synth. Catal.* 355, 1817–1828
54. Sasaki, K. *et al.* (2011) Molecular characterization of a membrane-bound prenyltransferase specific for isoflavone from *Sophora flavescens*. *J. Biol. Chem.* 286, 24125–24134
55. Sasaki, K. *et al.* (2008) Cloning and characterization of naringenin 8-prenyltransferase, a flavonoid-specific prenyltransferase of *Sophora flavescens*. *Plant Physiol.* 146, 1075–1084
56. Hunter, C.T. *et al.* (2018) Maize *w3* disrupts homogentisate solanesyl transferase (*ZmHst*) and reveals a plastoquinone-9 independent path for phytoene desaturation and tocopherol accumulation in kernels. *Plant J.* 93, 799–813
57. Xie, L.H. *et al.* (2017) Evaluation of biosynthesis, accumulation and antioxidant activity of Vitamin E in sweet corn (*Zea mays* L.) during kernel development. *Int. J. Mol. Sci.* Published online December 20, 2017. https://doi.org/10.3390/ijms18122780
58. Wang, R.S. *et al.* (2016) Regiospecific prenylation of hydroxyxanthones by a plant flavonoid prenyltransferase. *J. Nat. Prod.* 79, 2143–2147
59. Song, W. *et al.* (2017) Biosynthesis-based quantitative analysis of 151 secondary metabolites of licorice to differentiate medicinal *Glycyrrhiza* species and their hybrids. *Anal. Chem.* 89, 3146–3153
60. Lim, T.K. (2016) Glycyrrhiza glabra. In *Edible Medicinal and Non-Medicinal Plants: Volume 10, Modified Stems, Roots, Bulbs*, pp. 354–457, Springer Netherlands
61. Popoola, O.K. *et al.* (2015) Inhibition of oxidative stress and skin aging-related enzymes by prenylated chalcones and other flavonoids from *Helichrysum teretifolium*. *Molecules* 20, 7143–7155
62. Peng, J.N. *et al.* (2012) Amyrisins A-C, *O*-prenylated flavonoids from *Amyris madrensis*. *J. Nat. Prod.* 75, 494–496
63. Munikishore, R. *et al.* (2013) A new *O*-prenylated flavonol from the roots of *Sophora interrupta*. *Nat. Prod. Res.* 27, 1823–1826
64. Vitali, A. *et al.* (2004) Chalcone dimethylallyltransferase from *Morus nigra* cell cultures. Substrate specificity studies. *FEBS Lett.* 557, 33–38
65. Biggs, D.R. *et al.* (1987) Dimethylallylpyrophosphate:3,9-dihydroxypterocarpan 10-dimethylallyl transferase from *Phaseolus vulgaris* – identification of the reaction-product and properties of the enzyme. *FEBS Lett.* 220, 223–226
66. Wojakowska, A. *et al.* (2015) Metabolic response of narrow leaf lupine (*Lupinus angustifolius*) plants to elicitation and infection with *Colletotrichum lupini* under field conditions. *Acta Physiol. Plant.* Published online 18 July 2015. https://doi.org/10.1007/s11738-11015-11896-11736
67. López, S.N. *et al.* (2010) Geranylation of benzoic acid derivatives by enzymatic extracts from *Piper crassinervium* (Piperaceae). *Bioresour. Technol.* 101, 4251–4260
68. Hamerski, D. *et al.* (1990) Induction of two prenyltransferases for the accumulation of coumarin phytoalexins in elicitor-treated *Ammi majus* cell suspension cultures. *Phytochemistry* 29, 1131–1135
69. Munakata, R. *et al.* (2012) Characterization of coumarin-specific prenyltransferase activities in *Citrus limon* peel. *Biosci. Biotechnol. Biochem.* 76, 1389–1393
70. Popłoński, J. *et al.* (2018) Synthesis and antiproliferative activity of minor hops prenylflavonoids and new insights on prenyl group cyclization. *Molecules* Published online March 28, 2018. https://doi.org/10.3390/molecules23040776
71. Li, W. *et al.* (2019) Regioselective synthesis of *C*-prenylated flavonoids via intramolecular [1,3] or [1,5] shift reaction catalyzed by acidic clays. *Tetrahedron Lett.* Published online October 10, 2019. https://doi.org/10.1016/j.tetlet.2019.151138
72. Araya-Cloutier, C. *et al.* (2017) Structural basis for non-genuine phenolic acceptor substrate specificity of *Streptomyces roseochromogenes* prenyltransferase CloQ from the ABBA/PT-barrel superfamily. *PLoS One* 12, e0174665
73. Yu, X. and Li, S.M. (2011) Prenylation of flavonoids by using a dimethylallyltryptophan synthase, 7-DMATS, from *Aspergillus fumigatus*. *ChemBioChem* 12, 2280–2283
74. Chen, R. *et al.* (2017) Molecular insights into the enzyme promiscuity of an aromatic prenyltransferase. *Nat. Chem. Biol.* 13, 226–234
75. Wang, Y.C. *et al.* (2011) Metabolic engineering of flavonoids in plants and microorganisms. *Appl. Microbiol. Biotechnol.* 91, 949–956
76. Sasaki, K. *et al.* (2009) Prenylation of flavonoids by biotransformation of yeast expressing plant membrane-bound prenyltransferase SfN8DT-1. *Biosci. Biotechnol. Biochem.* 73, 759–761
77. Levisson, M. *et al.* (2019) Toward developing a yeast cell factory for the production of prenylated flavonoids. *J. Agric. Food Chem.* 67, 13478–13486
78. Guo, X.J. *et al.* (2019) Enabling heterologous synthesis of lupulones in the yeast *Saccharomyces cerevisiae*. *Appl. Biochem. Biotechnol.* 188, 787–797

79. Backhaus, K. *et al.* (2017) Manipulation of the precursor supply in yeast significantly enhances the accumulation of prenylated β-carbolines. *ACS Synth. Biol.* 6, 1056–1064
80. Koopman, F. *et al.* (2012) *De novo* production of the flavonoid naringenin in engineered *Saccharomyces cerevisiae*. *Microb. Cell Factories* 11, 155
81. Lyu, X. *et al.* (2017) Enhancement of naringenin biosynthesis from tyrosine by metabolic engineering of *Saccharomyces cerevisiae*. *J. Agric. Food Chem.* 65, 6638–6646
82. Donald, K.A.G. *et al.* (1997) Effects of overproduction of the catalytic domain of 3-hydroxy-3-methylglutaryl coenzyme A reductase on squalene synthesis in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* 63, 3341–3344
83. Ro, D.K. *et al.* (2006) Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature* 440, 940–943
84. Dai, Z.B. *et al.* (2013) Metabolic engineering of *Saccharomyces cerevisiae* for production of ginsenosides. *Metab. Eng.* 20, 146–156
85. Ignea, C. *et al.* (2011) Improving yeast strains using recyclable integration cassettes, for the production of plant terpenoids. *Microb. Cell Factories* 10, 4
86. Liu, J.D. *et al.* (2013) Overproduction of geraniol by enhanced precursor supply in *Saccharomyces cerevisiae*. *J. Biotechnol.* 168, 446–451
87. Kamińska, J. *et al.* (2002) The isoprenoid biosynthetic pathway in *Saccharomyces cerevisiae* is affected in a *maf1-1* mutant with altered tRNA synthesis. *FEMS Yeast Res.* 2, 31–37
88. Özaydın, B. *et al.* (2013) Carotenoid-based phenotypic screen of the yeast deletion collection reveals new genes with roles in isoprenoid production. *Metab. Eng.* 15, 174–183
89. Ignea, C. *et al.* (2012) Positive genetic interactors of *HMG2* identify a new set of genetic perturbations for improving sesquiterpene production in *Saccharomyces cerevisiae*. *Microb. Cell Factories* 11, 162
90. George, K.W. *et al.* (2015) Metabolic engineering for the high-yield production of isoprenoid-based C_5 alcohols in *E. coli*. *Sci. Rep.* Published online June 8, 2015. https://doi.org/10.1038/srep11128
91. Fischer, M.J.C. *et al.* (2011) Metabolic engineering of monoterpene synthesis in yeast. *Biotechnol. Bioeng.* 108, 1883–1892
92. Fischer, M.J.C. *et al.* (2011) Identification of a lysine residue important for the catalytic activity of yeast farnesyl diphosphate synthase. *Protein J.* 30, 334–339
93. Ignea, C. *et al.* (2014) Engineering monoterpene production in yeast using a synthetic dominant negative geranyl diphosphate synthase. *ACS Synth. Biol.* 3, 298–306
94. Caspeta, L. *et al.* (2014) Altered sterol composition renders yeast thermotolerant. *Science* 346, 75–78
95. Yuan, J.F. and Ching, C.B. (2015) Dynamic control of *erg9* expression for improved amorpha-4,11-diene production in *Saccharomyces cerevisiae*. *Microb. Cell Factories* 14, 38
96. Babiskin, A.H. and Smolke, C.D. (2011) A synthetic library of RNA control modules for predictable tuning of gene expression in yeast. *Mol. Syst. Biol.* 7, 471
97. Yang, C. *et al.* (2016) Synergy between methylerythritol phosphate pathway and mevalonate pathway for isoprene production in *Escherichia coli*. *Metab. Eng.* 37, 79–91
98. Partow, S. *et al.* (2012) Reconstruction and evaluation of the synthetic bacterial MEP pathway in *Saccharomyces cerevisiae*. *PLoS One* 7, e52498
99. Carlsen, S. *et al.* (2013) Heterologous expression and characterization of bacterial 2-*C*-methyl-D-erythritol-4-phosphate pathway in *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* 97, 5753–5769
100. Maury, J. *et al.* (2008) Reconstruction of a bacterial isoprenoid biosynthetic pathway in *Saccharomyces cerevisiae*. *FEBS Lett.* 582, 4032–4038
101. Kirby, J. *et al.* (2016) Engineering a functional 1-deoxy-D-xylulose 5-phosphate (DXP) pathway in *Saccharomyces cerevisiae*. *Metab. Eng.* 38, 494–503
102. Lund, S. *et al.* (2019) Probing the substrate promiscuity of isopentenyl phosphate kinase as a platform for hemiterpene analogue production. *ChemBioChem* 20, 2217–2221
103. Chatzivasileiou, A.O. *et al.* (2019) Two-step pathway for isoprenoid synthesis. *Proc. Natl. Acad. Sci. U. S. A.* 116, 506–511
104. Levisson, M. *et al.* (2018) Engineering *de novo* anthocyanin production in *Saccharomyces cerevisiae*. *Microb. Cell Fact.* April 18, 2018. https://doi.org/10.1186/s12934-12018-10951-12936
105. Welle, R. and Grisebach, H. (1988) Induction of phytoalexin synthesis in soybean: enzymatic cyclization of prenylated pterocarpans to glyceollin isomers. *Arch. Biochem. Biophys.* 263, 191–198
106. Henry, L.K. *et al.* (2018) Contribution of isopentenyl phosphate to plant terpenoid metabolism. *Nat. Plants* 4, 721–729
107. Vranová, E. *et al.* (2013) Network analysis of the MVA and MEP pathways for isoprenoid synthesis. *Annu. Rev. Plant Biol.* 64, 665–700
108. Steenackers, B. *et al.* (2015) Chemical transformations of characteristic hop secondary metabolites in relation to beer properties and the brewing process: a review. *Food Chem.* 172, 742–756
109. Karabin, M. *et al.* (2015) Biotransformations and biological activities of hop flavonoids. *Biotechnol. Adv.* 33, 1063–1090
110. Shohami, E. and Horowitz, M. (2016) Cannabinoids in health and disease. *J. Basic Clin. Physiol. Pharmacol.* 27, 175–179
111. Galli, F. *et al.* (2017) Vitamin E: emerging aspects and new directions. *Free Radic. Biol. Med.* 102, 16–36
112. Kumar, S. *et al.* (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874
113. Jones, D.T. *et al.* (1992) The rapid generation of mutation data matrices from protein sequences. *Comput. Appl. Biosci.* 8, 275–282