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Prenyl transfer to aromatic substrates: genetics and enzymology

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Aromatic prenyltransferases catalyze the transfer of prenyl moieties to aromatic acceptor molecules and give rise to an astounding diversity of primary and secondary metabolites in plants, fungi and bacteria. Significant progress has been made in the biochemistry and genetics of this heterogeneous group of enzymes in the past years. After 30 years of extensive research on plant prenylflavonoid biosynthesis, finally the first aromatic prenyltransferases involved in the formation of these compounds have been cloned. In bacteria, investigations of the newly discovered family of ABBA prenyltransferases revealed a novel type of protein fold, the PT barrel. In fungi, a group of closely related indole prenyltransferase was found to carry out aromatic prenylations with different substrate specificity and regiospecificity, and to catalyze both regular and reverse prenylations.

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Introduction

Prenyltransferases attach isoprenoid moieties, derived from allylic isoprenyl diphosphates like dimethylallyl diphosphate (DMAPP; C_5), geranyl diphosphate (GPP; C_{10}) or farnesyl diphosphate (FPP; C_{15}), to an acceptor molecule [1]. The archetypal prenyltransferase of primary metabolism, FPP synthase [2] utilizes the homoallylic substrate isopentenyl diphosphate (IPP) as acceptor molecule in two successive 1′-4 linkages, yielding the all-*trans* C_{15} compound FPP, the precursor of membrane sterols in all living organisms (Table 1). The reaction proceeds via formation of a carbocation, resulting from dissection of the pyrophosphate group from C-1 of the allylic substrate, followed by electrophilic attack on the 3,4-double bond of IPP. A tertiary carbocation is formed at C-3 of the added IPP molecule, and finally hydrogen is eliminated from C-2 of this IPP forming a *trans* 2,3 double bond. Other *trans* polyprenyl diphosphate synthases carry out similar reactions, leading, for example, to geranylgeranyl diphosphate (GGPP; C_{20}) as the precursor of carotenoids in plants, or to the all-*trans* polyprenyl diphosphates, comprising usually 30–50 carbons, which give rise to the side chains of ubiquinones, menaquinones and plastoquinones. All these polyprenyl diphosphate synthases show two conserved DDxxD motifs that represent the binding sites for the allylic and the homoallylic substrate, respectively. The substrates bind in form of Mg^{2+} complexes, and the aspartate residues in the conserved motifs serve to chelate the Mg^{2+} ion, facilitating both substrate binding and catalysis. The three-dimensional structure of *trans* polyprenyl diphosphate synthases is an all α-helical fold. By contrast, *cis* (*Z*) polyprenyl diphosphate synthases, like the undecaprenyl diphosphate synthase of *E. coli* that generates undecaprenol (bactoprenol) as lipid carrier for peptidoglycan biosynthesis, have a completely different amino acid sequence and three-dimensional structure [3]. They do not contain DDxxD motifs, but binding of IPP and catalytic activity still depends on the presence of Mg^{2+} [4].

Protein prenyltransferases are important for the post-translational modification of proteins in eukaryotes and represent important drug targets. Ras farnesyltransferase [5] utilizes a Zn^{2+}-activated thiolate as acceptor substrate for the allylic isoprenyl moiety that is formed from a cysteine residue in a CAAX-motif. Protein prenyltransferases have a different primary sequence and structure than the polyprenyl diphosphate synthases, and they do not contain DDxxD motifs.

Other prenyltransferases utilize amino, carboxyl or hydroxy groups as acceptors for the allylic isoprenyl moiety. Examples are adenosine phosphate isopentenyl transferases and tRNA isopentenyl transferases [6] as well as chlorophyll synthase [7] in plants, and glycerol-1-phosphate prenyltransferases in archeal lipid biosynthesis [8].

In secondary metabolism, prenyltransferases can catalyze the linkage between allylic and homoallylic isoprenyl diphosphates in four different patterns of C–C coupling [9]. Subsequent rearrangements, and highly divergent cyclization mechanisms, lead to ten thousands of natural terpenoids [10].

Finally, prenyltransferases can catalyze the transfer of allylic isoprenyl moieties to aromatic acceptor molecules, forming C–C bonds between C-1 or C-3 of the isoprenoid substrate and one of the aromatic carbons of the acceptor substrate. These enzymes give rise to a huge diversity of secondary metabolites in plants, fungi and bacteria.

Table 1

Families of prenyltransferases.

Type	Example	Prenyl donor	Prenyl acceptor	Soluble or membrane-bound	Asp-rich motifs for substrate binding	Magnesium-dependent	Protein structure	Occurrence
trans polyprenyl diphosphate synthases	Farnesyl diphosphate synthase	DMAPP, GPP	IPP	Soluble	DDxxD DDxxDxxxD	Yes	All alpha-helical fold	All living organisms
cis polyprenyl diphosphate synthases	Undecaprenyl diphosphate synthase	FPP	IPP	Soluble	Absent	Yes	Alpha and beta protein	Archaea, bacteria and eukaryotes
Protein prenyltransferases	Protein farnesyl-transferase	FPP (GGPP)	Proteins (Cys residue in CAAX motif)	Soluble	Absent	No	α Subunit: alpha-alpha superhelix β Subunit: alpha/alpha toroid	Eukaryotes
Aromatic prenyltransferases of lipoquinone biosynthesis	4-hydroxybenzoate octaprenyl-transferase (UbiA)	All-*trans* prenyl diphosphates	Phenols	Integral membrane protein	NDxxDxxxD	Yes	Structural model: all alpha-helical fold	All living organisms
Aromatic prenyltransferases of prenylflavonoid biosynthesis	Naringenin 8-dimethylallyl-transferase (N8DT)	DMAPP	Phenols	Integral membrane protein	NQxxDxxxD	Yes	Unknown	Plants
ABBA aromatic prenyltransferases	NphB	GPP	Phenols	Soluble	Absent	NphB: yes	Antiparallel β/α barrel ('PT barrel')	Bacteria (*Streptomyces*), fungi
	CloQ	DMAPP				CloQ: no		
Fungal indole prenyltransferases	Dimethylallyl tryphtophan synthase (DMATS)	DMAPP	Indoles	Soluble	Absent	No	Unknown	Fungi

Often, they catalyze the rate-limiting step in the respective pathway. Until recently, biochemical and especially genetic knowledge on this group of enzymes was very limited, but substantial progress has been made in the past years. The present review will focus on the recent advances in the genetics and biochemistry of these enzymes, hereafter called 'aromatic prenyltransferases'. Special emphasis is given to the time period since 2006.

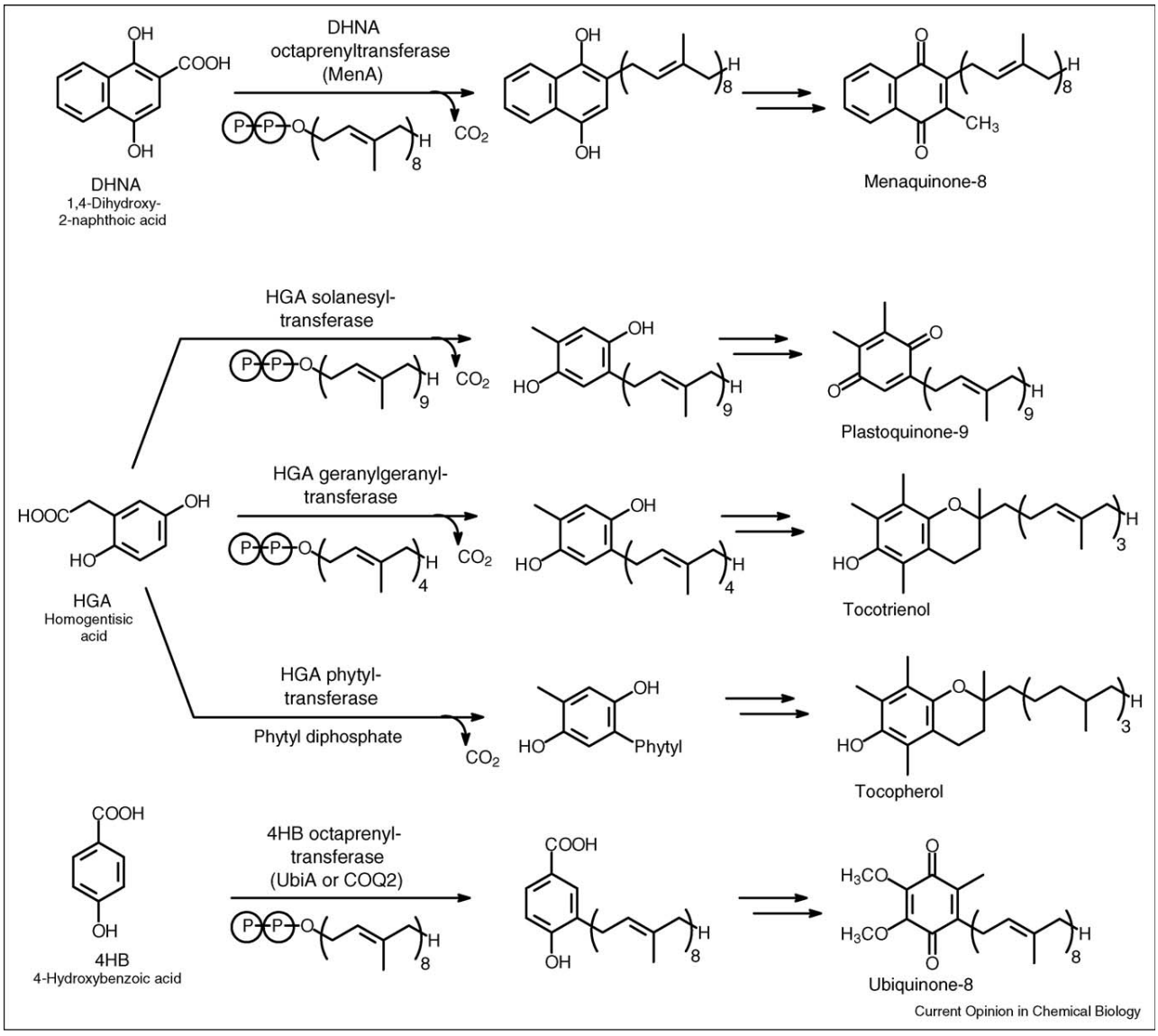
Aromatic prenyltransferases of lipoquinone biosynthesis

In the biosynthesis of ubiquinones, menaquinones and plastoquinones, aromatic prenyltransferases catalyze the transfer of all-*trans* prenyl moieties to the acceptor molecules 4-hydroxybenzoate (4HB), 1,4-dihydroxy-2-naphthoate (DHNA) or homogentisate (HGA), respectively (Figure 1).

MenA, the DHNA octaprenyltransferase of *E. coli*, catalyzes a simultaneous prenylation and decarboxylation at C-2 of DHNA. Surprisingly, little biochemical data is available on this prenyltransferase [11], which has been suggested as a potential target of antibacterial therapy [12].

In plants, plastidically localized HGA prenyltransferases of different substrate specificity exist for the transfer of solanesyl (C_{45}), geranylgeranyl (C_{20}) or phytyl

Figure 1



Aromatic prenyltransferase reactions in lipoquinone biosynthesis.

(C_{20}, partially saturated) moieties for the biosynthesis of plastoquinones, tocotrienols and tocopherols (Figure 1) [13,14]. Tocopherols and tocotrienols are also known as vitamin E. In all these cases, the prenylation of the aromatic ring of HGA proceeds with simultaneous decarboxylation of the side chain.

The best-studied prenyltransferase of lipoquinone biosynthesis is 4HB polyprenyltransferase (also called 4HB oligoprenyltransferase). Dependent on the predominant isoprenoid chain length of ubiquinones in the respective organism, it transfers prenyl moieties of different size (usually 30–50 carbons) to position 3 of 4HB. The subsequent decarboxylation is catalyzed by a different enzyme (UbiD in *E. coli*). After initial discovery of the 4HB polyprenyltransferase gene in yeast (COQ2) and *E. coli* (UbiA) [15], further orthologues have been investigated, for example, from humans [16] and plants [17•]. The 4HB polyprenyltransferases usually show a broad substrate specificity for the isoprenoid substrate, accepting also short chain isoprenoids like FPP and GPP, but not DMAPP and not *cis*-prenyl diphosphates.

All prenyltransferases of lipoquinone biosynthesis comprise an aspartate-rich motif (e.g. NDxxDxxxD), similar to the corresponding motif of FPP synthase, for binding of the prenyl diphosphate via a Mg^{2+} ion. Their activity is absolutely dependent on the presence of Mg^{2+} or similar cations like Mn^{2+}, Co^{2+} or Ni^{2+}. They are integral membrane proteins, and no experimentally determined structure is available yet. However, a structural model of UbiA of *E. coli* has been developed recently [18••]. In this model, a deep lipophilic pocket between the transmembrane helices allows to accommodate prenyl diphosphates of different chain length. The pyrophosphate group is bound to an arginine residue (R137) and, via a Mg^{2+} ion, to the latter two aspartate residues of the NDxxDxxxD motif. A second aspartate-rich motif fixes the conformation of R137 and forms a hydrogen bond to the 4-OH group of the aromatic substrate 4HB.

UbiA has been successfully used for the chemoenzymatic synthesis of prenylated aromatic compounds [19].

Aromatic prenyltransferases of plant secondary metabolism

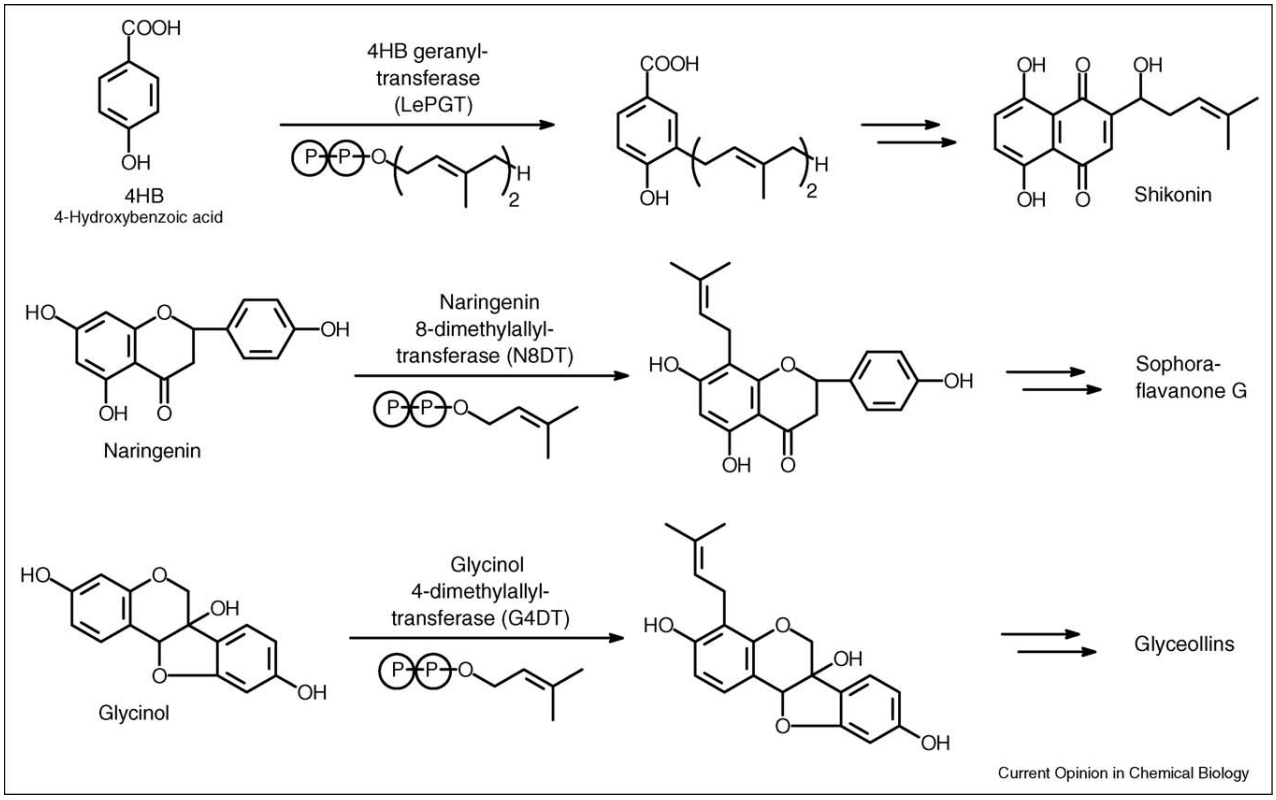
Aromatic prenylation reactions contribute significantly to the diversity secondary metabolites in plants. Particularly, prenylated flavonoids and isoflavonoids are important in plant defence mechanisms and may be of considerable interest for human health [20]. The first prenyltransferase genes have recently been identified by the pioneering work of the group of K Yazaki. This group first cloned a 4HB geranyltransferase involved in the biosynthesis of the plant naphthoquinone shikonin, the first plant secondary metabolite to be industrially produced by plant cell cultures [21]. The reaction is identical to that of COQ2 of ubiquinone biosynthesis (Figures 1 and 2), except that the enzyme is specific for GPP (C_{10}) as isoprenoid substrate, and is localized at the ER rather than in mitochondria. Exploiting sequence similarity to known 4HB prenyltransferases of ubiquinone biosynthesis, the enzyme was cloned by PCR from a cDNA library of a shikonin producing cell culture. Two isoenzymes of very similar sequence and catalytic activity were found and termed LePGT-1 and LePGT-2. Both contained a signal peptide for targeting to the endoplasmatic reticulum and a NDxxDxxxD motif, and both catalyzed a Mg^{2+}-dependent prenylation of 4HB with strict substrate specificity for GPP, in clear contrast to the 4HB prenyltransferases of ubiquinone biosynthesis [21].

In prenylflavonoid and prenylisoflavonoid biosynthesis, prenylation is carried out by membrane-bound prenyltransferases localized in plastids. In an attempt to clone the naringenin 8-dimethylallyltransferase (N8DT, Figure 2) from *Sophora flavescens* (Fabaceae), Yazaki and co-workers [22•] generated a cDNA library from a methyl jasmonate induced cell culture. They sequenced approximately 12 000 EST clones, searching for sequences that comprised a plastid transit peptide, transmembrane domains and an aspartate-rich motif as a potential binding site for the prenyl diphosphate substrate. This led to the identification of the naringenin 8-dimethylallyltransferase gene SfN8DT-1. The predicted polypeptide showed clear similarity to the HGA prenyltransferases of vitamin E biosynthesis. It comprised nine transmembrane α-helices and an NQxxDxxxD motif for prenyl diphosphate binding. The enzymatic reaction was specific for DMAPP and strictly dependent on Mg^{2+}. Using internal sequences of SfN8DT-1 as primers, RT-PCR resulted in the discovery of an isogene, SfN8DT-2, coding for a very similar protein with the same enzymatic activity, and two genes with somewhat lower similarity and yet unknown function.

Exploiting the knowledge generated from the study on *Sophora flavescens*, Yazaki and co-workers [23••] subsequently searched the publicly available EST database of soybean for sequences with similarity to *Arabidopsis* HGA phytyltransferase. They identified, besides the putative soybean HGA phytyltransferase, an aromatic prenyltransferase gene involved in the biosynthesis of gyceollins, the principal phytoalexins of soybean. These compounds derive from the prenylation of the pterocarpan glycinol, an isoflavonoid derivative. The discovered gene coded for a glycinol 4-dimethylallyltransferase (G4DT) and showed close sequence similarity to N8DT of *Sophora*.

It appears likely that the plastidic secondary metabolic enzymes N8DT and G4DT evolved from the plastidically localized HGA prenyltransferases of primary metabolism. On the contrary, 4HB geranyltransferase of

Figure 2



Membrane-bound aromatic prenyltransferases of plant secondary metabolism.

shikonin biosynthesis may have evolved from a 4HB polyprenyltransferase of ubiquinone biosynthesis, adopting a signal peptide for ER rather than mitochondrial targeting.

Aromatic prenylations of substrates with phloroglucinol or resorcinol structures occur in the biosynthesis of cannabinoids, lupulone and hyperforin [24–26]. These reactions are apparently catalyzed by soluble rather than membrane-bound enzymes, and none of the responsible genes has been cloned yet.

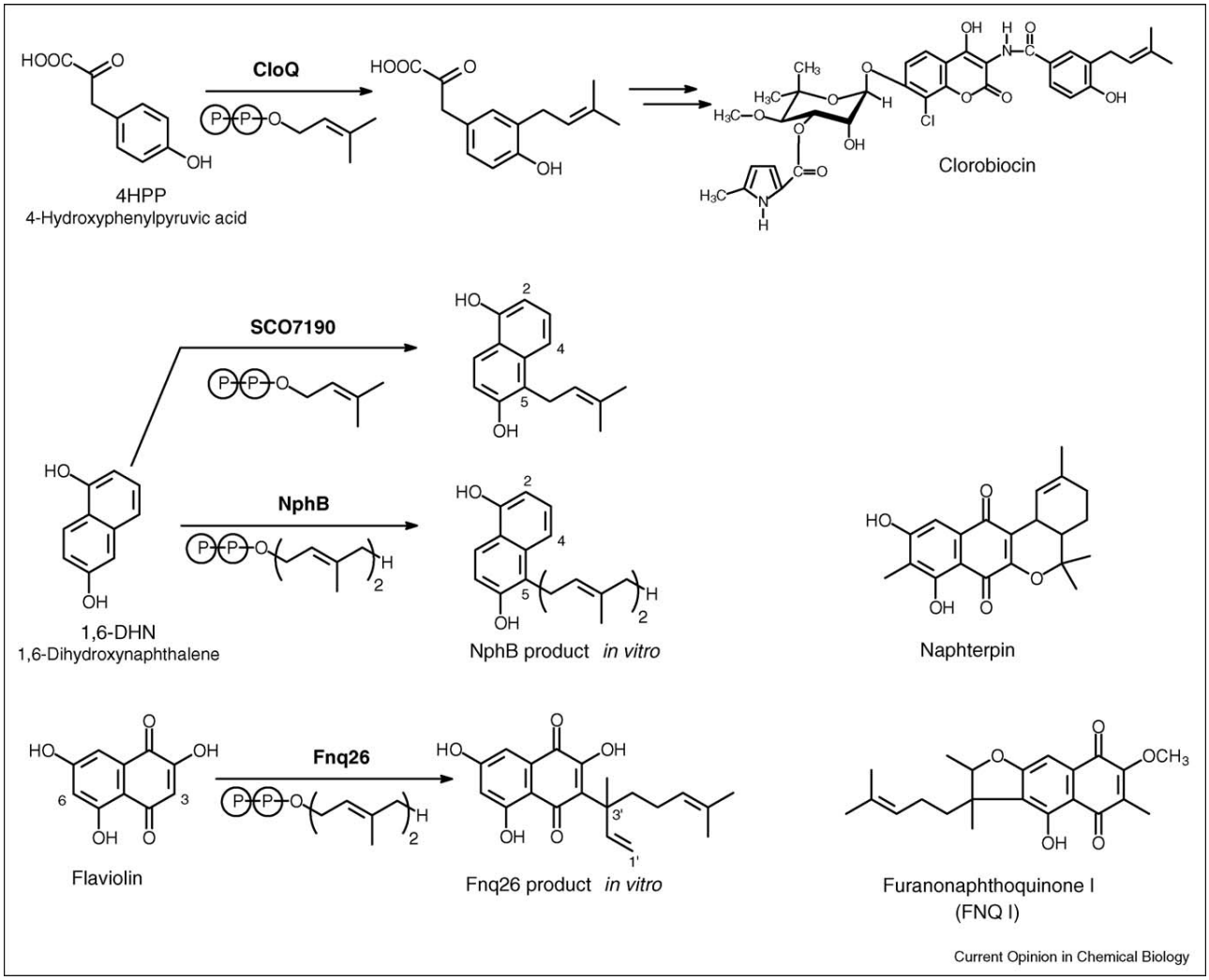
The ABBA family of aromatic prenyltransferases

Prenylations at C-3 of 4-hydroxybenzoate (4HB) occur in the biosynthesis of ubiquinones (Figure 1) and shikonin (Figure 2). A 3-prenylated 4HB moiety is also found in the structure of the antibiotic clorobiocin (Figure 3), produced by a Gram-positive bacterium of the genus *Streptomyces*. Surprisingly, however, the biosynthetic gene cluster of this antibiotic did not contain genes with similarity to known prenyltransferases. Eventually, it was shown that the prenyltransferase of clorobiocin biosynthesis was encoded by *cloQ*, coding for a 324 amino acid protein with no sequence similarity to known proteins in the database [27]. Heterologous expression of CloQ yielded a soluble, monomeric 35 kDa protein that catalyzed a C-prenylation at position 3 of 4-hydroxyphenylpyruvate (4HPP), using DMAPP as prenyl donor (Figure 3). The reaction product is subsequently converted to 3-dimethylallyl-4HB by the bifunctional dioxygenase CloR [28].

CloQ did not contain a DDxxD motif and was active in the absence of Mg^{2+} or other divalent cations. In contrast to protein prenyltransferases, CloQ did not contain Zn^{2+}. In the first report on CloQ [27], it was therefore speculated that it may belong to a previously unrecognized group of prenyltransferases.

This was confirmed by Kuzuyama *et al.* [29••]. On the basis of sequence similarity with CloQ, they identified NphB (initially termed Orf2) from the biosynthetic gene cluster of the meroterpenoid (prenylated polyketide) naphterpin, produced by a *Streptomyces* strain, as an aromatic prenyltransferase. The heterologously expressed protein (307 amino acids) catalyzed a C-geranylation of the artificial substrate 1,6-dihydroxynaphthalene (1,6-DHN) and of other phenolic substrates (Figure 3). The X-ray structure of the crystallized NphB showed new type

Figure 3



Reactions catalyzed by ABBA prenyltransferases.

of β/α fold with antiparallel strands. Owing to the α-β-β-α architecture of this fold, this group of enzymes was later designated as ABBA prenyltransferases [30••].

A gene with sequence similarity to *cloQ* and *nphB*, SCO7190, is found in the genome of *Streptomyces coelicolor* A3(2). Heterologous expression of the 296 aa protein yielded a soluble enzyme that catalyzed, like NphB, the C-prenylation of 1,6-DHN, but with specificity for DMAPP rather than GPP [29••].

Two further putative ABBA prenyltransferase genes, *fnq26* and *fnq28*, were discovered in the gene cluster of the meroterpenoid furanonapthoquinone I (FNQ I) [31]. Gene inactivation experiments revealed that *fnq26*, but not *fnq28*, was required for FNQ I biosynthesis. Heterologous expression of Fnq26 (300 aa) yielded a soluble monomeric protein that preferentially utilized flaviolin (Figure 3) and GPP as substrates [32•]. C-3 rather than C-1 of GPP is connected to the aromatic moiety, a reaction referred to as 'reverse' prenylation.

While the catalytic activities of CloQ, SCO7190 and Fnq26 are independent of the presence of Mg^{2+}, NphB requires Mg^{2+} for catalysis. X-ray crystallography showed that Mg^{2+} is coordinated by four water molecules, arranged in a plane, by one of the oxygens of the α-phosphate group of GPP, and by an aspartate residue of NphB [29••]. The structures of CloQ, SCO7190 and Fnq26 were modelled after the experimentally determined NphB structure [30••], and it was suggested that positively charged Lys or Arg residues in these proteins

may functionally substitute for the divalent cation in the binding of the α-phosphate group of DMAPP or GPP. No experimentally determined structure is available yet for these three proteins, but the successful crystallization of CloQ has been reported [33].

CloQ, NphB, SCO7190 and Fnq26 are the only ABBA prenyltransferases that have been biochemically characterized. However, five other putative ABBA prenyltransferase genes from *Streptomyces* strains are found in the database, including genes from the biosynthetic gene clusters of the meroterpenoids furaquinocin [34] and napyradiomycin [35]. Interestingly, four further genes with similarity to the ABBA family have been identified in fungal genomes [30••]. Like SCO7190, these genes appear not to reside in secondary metabolic gene clusters, and it remains to be shown whether they do have a metabolic function in their respective organisms.

The genuine substrates of Fnq26 and NphB are yet unknown. The naphthoquinone nucleus of meroterpenoids like FNQ I and naphterpin is formed by a type III polyketide synthase [36,37], which yields 1,3,6,8-tetrahydroxynaphthalene (THN) as immediate product. *In vitro* and *in vivo* THN is rapidly oxidized to flaviolin, the preferred substrate of Fnq26. However, *in vitro* the prenylation catalyzed by Fnq26 occurred at C-3 of flaviolin [32•], rather than at C-6 as required for FNQ I biosynthesis (Figure 3). Therefore, not flaviolin but an earlier or later intermediate of the biosynthetic pathway may be the genuine substrate of Fnq26. Fnq26 accepts also 1,3-dihydroxynaphthalene (1,3-DHN) and 4HB, catalyzing *O*-prenylations rather than *C*-prenylations with these substrates. By contrast, Fnq26 does not accept 1,6-DHN that is the preferred substrate of NphB.

Recently, Kuzuyama and co-workers [38•] showed that NphB does not prenylate flaviolin or 1,3-DHN to a significant extent, indicating principal differences in the prenylation reactions of naphterpin and FNQ I biosynthesis. 1,6-DHN is prenylated by NphB preferentially at C-5, to a lesser extent at C-2, and a very minor product is prenylated at C-4. In a computational study, Cui *et al.* [39•] showed that 1,6-DHN can be expected to bind in three different modes to the active centre of NphB, which may account for the three different products observed.

ABBA prenyltransferases are soluble biocatalysts that can easily be obtained as homogeneous proteins in significant amounts. Their substrates are accommodated in a surprisingly spacious central cavity that explains their promiscuity for different aromatic substrates. For example, NphB can catalyze *C*-prenylations and *O*-prenylations of flavonoids, isoflavonoids, resveratrol and olivetol [38•]. Therefore, the enzymes of this family represent attractive tools for the chemoenzymatic synthesis of bioactive molecules [40].

Fungal indole prenyltransferases

In fungi, the prenylation of indole moieties, derived from tryptophan, leads to a large structural diversity of alkaloids [41]. The gene for 4-dimethylallyl tryptophan synthase (DMATS), a key enzyme of ergot alkaloid biosynthesis, was first discovered in *Claviceps purpurea* [42]. DMATS orthologues are encoded in many fungal genomes [43,44]. In pioneering work, Shu-Ming Li and co-workers cloned, overexpressed and characterized several enzymes with sequence similarity to DMATS, catalyzing indole prenylations with different substrate specificity and regiospecificity [45••,46••]. Similar fungal indole prenyltransferases include TidB of terrequinone A biosynthesis [47,48] and the enzymes involved in the biosynthesis of indole diterpenes [49,50]. Genetic and biochemical data on the fungal prenyltransferases have been reviewed elsewhere [46••]. These enzymes carry out regular and reverse *C*-prenylations and *N*-prenylations, are soluble enzymes, do not show (N/D)DxxD motifs and are active in the absence of Mg^{2+}. Therefore, they resemble the biochemical properties of the ABBA prenyltransferases, but the primary sequences of these enzyme families do not show similarities. No X-ray structure of an indole prenyltransferase has been reported yet.

Also the cyanobacterial indole prenyltransferase LtxC from lyngbyatoxin biosynthesis [51] is not related to any of the prenyltransferases described above, and may indicate the existence of a further family of aromatic prenyltransferases.

Conclusions

Aromatic prenyltransferases are key enzymes in the generation of metabolic diversity in primary and especially in secondary metabolism of plants, fungi and bacteria. Different types of these enzymes have been identified in the past years (Table 1), and a simple division in 'membrane-bound' and 'soluble' aromatic prenyltransferases does not reflect the state of our knowledge any longer. Structural investigations on the ABBA prenyltransferases in bacteria led to the identification of a new, unique protein fold. However, the structure of the fungal indole prenyltransferases remains to be elucidated. While evolutionary relationships can be shown in each of the families of aromatic prenyltransferases, the possible existence of such relationships between these families still need to be examined. The ABBA prenyltransferases show an unusual large central cavity that allows for a promiscous prenylation of different aromatic substrates. Their substrate range may, in future, be further expanded by site-directed mutagenesis, guided by mechanistic insights from structural biology. Thereby, aromatic prenyltransferases may become an important tool for the chemoenzymatic synthesis of bioactive compounds.

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