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The ABBA family of aromatic prenyltransferases: broadening natural product diversity

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Aromatic prenyltransferases (PTases) catalyze the transfer of a C5 (dimethylallyl), C10 (geranyl) or C15 (farnesyl) prenyl group derived from the corresponding isoprenyl diphosphate metabolites onto a variety of electron-rich aromatic acceptors. Prenyl groups appear in a wide variety of bioactive natural products of microbial and plant origin, including amino acids, stilbenes, alkaloids, polyketides and phenylpropanoids such as flavonoids, creating natural product hybrids with altered or enhanced bioactivities. Prenylation of flavonoids enhances some of the desirable pharmacological properties of these plant compounds [1], as demonstrated for apigenin and liquiritigenin [2]. Prenylation appears in many cases to provide a higher level of bioactivity compared to the non-prenylated precursor, often by increasing affinity for biological membranes and interactions with cellular targets [3]. With the recent identification of these enzymes there is increased interest in the role of these regiospecific catalysts in expanding the diversity and bioactivities of several important classes of natural products *in vivo* and *in vitro*.

One way in which PTases can be categorized depends on whether they catalyze the synthesis of isoprenyl diphosphates, the prenylation of a protein or the prenylation of an aromatic substrate. Isoprenyl diphosphate synthases catalyze the chain elongation of an allylic isoprenyl diphosphate substrate by reaction with isopentenyl diphosphate [4]. Protein PTases transfer a geranyl-geranyl or farnesyl group to the Cys residue on a CaaX motif at the C-terminus of several proteins to facilitate membrane anchoring in eukaryotes and possibly archaea [5]. Small-molecule aromatic PTases constituting the third category can be subdivided into membrane-associated and functionally soluble PTases. Membrane-associated PTases contain a characteristic (N/D)DXXD Mg²⁺-diphosphate binding motif which is also found in the isoprenyl diphosphate synthases and are involved e.g. in the biosynthesis of ubiquinones and

menaquinones [6], in the biosynthesis of membrane lipids in archaea [7] and in the formation of plant secondary metabolites [8].

The functionally soluble PTases do not possess an obvious Mg^{2+} -diphosphate binding motif. Moreover, most of the currently known soluble aromatic PTases segregate into two subgroups based on their primary sequence similarity and on the small-molecule substrates they accept for prenylation. The first subgroup commonly prenylates indole-containing ring systems. Examples of these PTases include the fungal enzymes FgaPT1 and FgaPT2 involved in the biosynthesis of fumigaclavin C [9], as well as the newly discovered N-reverse PTase CdpNPT [10] and the 7-dimethylallyl-tryptophan synthase Afu3 g12930 [11]. LtxC, which is involved in the biosynthesis of lyngbyatoxins obtained from the cyanobacteria *Lyngbya majuscula* [12], also prenylates an indole-containing ring system but shares no sequence similarity with the fungal enzymes named above.

The second subgroup of functionally soluble aromatic PTases encompasses the most recently discovered ABBA family of enzymes, for most of which the genuine substrates have yet to be identified (Fig. 1). This subgroup possesses a unique three-dimensional antiparallel β/α -barrel structure referred to as the PT-barrel fold and is the subject of this review.

Discovery of a new subgroup of aromatic PTases

cloQ was the first gene identified to encode a completely new subgroup of aromatic PTases. It was identified in the gene cluster involved in the biosynthesis of Ring A (3-dimethylallyl-4-hydroxybenzoic acid) of clorobiocin, an aminocoumarin antibiotic produced by *Streptomyces roseochromogenes* [13]. *In vitro* biochemical characterization of the encoded gene product demonstrated that 4-hydroxyphenylpyruvate (4-HPP) served as the aromatic acceptor with the C5 isoprenoid diphosphate, DMAPP, serving as the prenyl donor in a divalent cation-independent reaction [14]. Moreover, the closely related NovQ protein found in *S. spheroides* [15] (84% sequence identity to CloQ) was speculated to participate in the dimethylallylation of Ring A of the structurally related aminocoumarin novobiocin [14].

Two years after these initial discoveries, an open reading frame designated *orf2* (now renamed *nphB*) was identified based on its similarity to *cloQ/novQ* in a gene cluster associated with the biosynthesis of the geranylated natural product naphterpin in *Streptomyces* sp. strain CL190 [16]. Biochemical and structural characterization of the encoded enzyme NphB (~22% sequence identity with CloQ and NovQ) showed preferential specificity for GPP as the prenyl donor, Mg^{2+} dependency and impressive promiscuity with respect to aromatic acceptors [16]. Diverse phenolic compounds, including the anti-aging stilbene resveratrol [17], many (iso)flavonoids and the CloQ substrate 4-HPP, were among the variety of substrates prenylated by NphB [16]. Recently, we observed that resveratrol geranylated at C4 of the dihydroxylated ring possesses anti-bacterial activity against *Staphylococcus aureus*, while the non-prenylated starting material shows no such activity [unpublished data]. In addition to the novelty of the NphB and CloQ/NovQ reactions, the catalytic promiscuity of NphB opens the door to the structure-guided exploitation of this newly discovered family of aromatic PTases as biosynthetic tools for the chemoenzymatic diversification of small molecules of natural and synthetic origin.

The PT-barrel fold

The x-ray crystallographic determination of the NphB structure in complex with substrates and substrate analogues at high resolution revealed a novel antiparallel β/α barrel, termed the PT-barrel [16] (Pre-SCOP entry number 135530 [18]). The high resolution of the small-molecule complexes with NphB (PDB codes: 1ZDY, 1ZCW, 1ZB6 and 1ZDW) afforded insights into the molecular basis of substrate binding. Moreover, experimental NphB structures combined

with computer-assisted modelling and mutagenesis provided a starting point to understand the enzymatic mechanism accompanying both Mg^{2+} -dependent and -independent prenyl group transfer.

Recently, the crystallization and preliminary characterization of CloQ crystals was reported [19] with structural elucidation underway [D. M. Lawson, personal communication]. The discovery of the novel PT-barrel fold, and in particular, the characteristic five α - β - β - α secondary structure repeat elements of the overall fold topology led to the ABBA designation of this subgroup of aromatic PTases (see Fig. 2, central panel).

A growing family of ABBA PTases

Following the initial identification and characterization of CloQ/NovQ and NphB, a number of related genes and encoded proteins were identified in *Streptomyces* species. SCO7190, initially designated HypSc, is a PTase from *S. coelicolor* that was shown to encode *in vitro* PTase activity against aromatic substrates [16]. Moreover, modelling the SCO7190 structure based on homology to NphB accurately predicted SCO7190's specificity for DMAPP and Mg^{2+} independence when using 1,6-dihydroxy-naphthalene as an aromatic acceptor. To date, it is not clear what *in vivo* substrate SCO7190 prenylates.

More recently, two newly discovered PTases, Fnq26 and Fnq28, were identified as being encoded in the gene cluster responsible for the biosynthesis of furanonaphthoquinone I in *S. cinnamonensis* [20]. Recombinant Fnq26 was biochemically characterized and exhibited specificity for GPP with limited substrate promiscuity and Mg^{2+} independency [21]. Notably, Fnq26 catalyzes a C3-reverse geranyl chain transfer in addition to the regular C1 directed transfer. However, as for NphB and SCO7190, the precise *in vivo* substrate of Fnq26 is still unknown.

Several additional PTases have been tentatively classified as ABBA PTases based on sequence similarities. Fur7, encoded in the furaquinocin A gene cluster of *Streptomyces* sp. strain KO-3988 [22], and NapT8 and NapT9, encoded in the napyradiomycin gene cluster in *S. aculeolatus* and *Streptomyces* sp. strain CNQ-525 [23], possess significant sequence similarity to NphB, Fnq26 and Fnq28 (see Fig. 1). Fur7 and NapT8 are more closely related to Fnq26, possessing 66 and 46% sequence identity, respectively. NapT9 is more closely related to Fnq28, with 42% sequence identity. By analyzing various fungal databases, we uncovered a number of sequences with a high degree of certainty as members of the ABBA family, based on sequence identity of important active-site residues. These newly identified ABBA family members outside of the bacterial kingdom include hypothetical protein BC1G_01295 from *Botryotinia fuckeliana* B05.10, hypothetical protein SS1G_09465 from *Sclerotinia sclerotiorum* 1980, a predicted protein from *Aspergillus terreus* NIH2624 as well as a region of encoded similarity in the draft genome of *Penicillium marneffei*, all within the *Pezizomycotina* subphylum. Interestingly, the encoded protein sequences are more similar to CloQ/NovQ than to other identified members of the ABBA subgroup of PTases (see Fig. 1).

Phylogenetic analysis of the ABBA subgroup of PTases divides them into two distinct clades, as shown in Figure 1. The first clade includes NphB, Fnq26, Fnq28, Fur7, NapT8 and NapT9. The second clade encompasses CloQ, NovQ, SCO7190 and the hypothetical fungal proteins. Interestingly, all the enzymes belonging to the NphB clade appear to be associated with biosynthetic pathways putatively having 1,3,6,8-tetrahydroxynaphthalene (THN) as an intermediate (see Fig. 1). For the second clade, only the CloQ substrate has been defined. It remains to be seen whether the phylogenetic groupings for the CloQ clade will be associated with similar aromatic acceptors as for the NphB clade.

Structure-based engineering of ABBA PTases

To facilitate our mechanistic understanding of ABBA PTases and their development as enzymatic tools for small-molecule diversification, additional structure determinations of subgroup members are under way. Currently available structures have proven to be a reliable starting point to understand divalent cation dependencies and prenyl chain length selectivity.

In analogy to other prenyl transfer and cyclization reactions, the Mg^{2+} ion was proposed to facilitate isoprenoid diphosphate ionization through pyrophosphate loss and prenyl cation formation to initiate the assumed electrophilic prenylation reaction [16]. Asp62 (numbering refers to NphB unless otherwise stated) was demonstrated to be responsible for Mg^{2+} coordination in NphB [16]. In Mg^{2+} -independent PTases, modelling studies suggested that positively charged residues (replacing Ser51 in NphB) spatially and functionally substitute the divalent cation for diphosphate binding (see Fig. 2): Lys54 in CloQ, Arg47 in SCO7190 and Arg46 in Fnq26. Therefore, Asp62 is not strictly conserved among Mg^{2+} -independent PTases.

The structural features governing the prenyl chain length specificity appear to be more variable. Comparison of the NphB experimental structure with computational models of several of the other ABBA PTases suggest that three amino acid residues play a key role in chain length specificity. These residues include Ser64, Ser66 and Gly286 (see Fig. 2). In CloQ and SCO7190, Ser64 is replaced by Arg66 and Arg61, respectively, and the side chains directly clash with the location of the GPP chain in the NphB structure (see Fig. 2). Glu281 in CloQ and Glu274 in SCO7190 appear to be forming a salt bridge with the Arg residue, blocking the end of the prenyl binding site and preventing the binding of any isoprenoid chain length longer than C5. Additionally, a Phe residue in CloQ (Phe68) sits at the end of the prenyl binding 'tunnel' in substitution of Ser66 in NphB and is located very close to Arg64, therefore providing additional bulkiness. This is in agreement with the observed DMAPP specificity for both enzymes. However, in the Fnq26 model Arg60 replaces Ser64, and Gln276 replaces Gly286, and both side chains are close enough to suspect a direct interaction, although the enzyme has demonstrated to be GPP-specific. Consequently, although the diphosphate would probably be positioned at the same place compared to NphB, the side chain of GPP may display a different orientation.

However, further structural and biochemical characterization of additional members of the ABBA family of aromatic PTases, such as the novel fungal members, will afford a more complete understanding of the key features involved in substrate selection and specificity/promiscuity. Rational engineering of these regiospecific catalysts would then provide a powerful tool to expand the diversity and bioactivities of many synthetic and natural compounds through enzyme-directed site-specific prenylation.

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References

1. Botta B, Vitali A, Menendez P, Misiti D, Delle Monache G. Prenylated flavonoids: pharmacology and biotechnology. *Curr. Med. Chem* 2005;12:717–739. [PubMed: 15790308]
2. Watjen W, Weber N, Lou YJ, Wang ZQ, Chovolou Y, Kampkotter A, Kahl R, Proksch P. Prenylation enhances cytotoxicity of apigenin and liquiritigenin in rat H4IIE hepatoma and C6 glioma cells. *Food Chem. Toxicol* 2007;45:119–124. [PubMed: 17045382]
3. Botta B, Delle Monache G, Menendez P, Boffi A. Novel prenyltransferase enzymes as a tool for flavonoid prenylation. *Trends Pharmacol Sci* 2005;26:606–608. [PubMed: 16229901]

4. Liang PH, Ko TP, Wang AH. Structure, mechanism and function of prenyltransferases. *Eur. J. Biochem* 2002;269:3339–3354.
5. Benetka W, Koranda M, Eisenhaber F. Protein prenylation: An (almost) comprehensive overview on discovery history, enzymology, and significance in physiology and disease. *Monatshefte für Chemie* 2006;137:1241–1281.
6. Meganathan R. Biosynthesis of menaquinone (vitamin K2) and ubiquinone (coenzyme Q): a perspective on enzymatic mechanisms. *Vitam. Horm* 2001;6:173–218. [PubMed: 11153266]
7. Hemmi H, Shibuya K, Takahashi Y, Nakayama T, Nishino T. (S)-2,3-Di-O-geranylgeranylglycerol phosphate synthase from the thermoacidophilic archaeon *Sulfolobus solfataricus*. Molecular cloning and characterization of a membrane-intrinsic prenyltransferase involved in the biosynthesis of archaeal ether-linked membrane lipids. *J. Biol. Chem* 2004;279:50197–50203. [PubMed: 15356000]
8. Yazaki K, Kuniyoshi M, Fujisaki T, Sato F. Geranyl diphosphate :4-hydroxybenzoate geranyltransferase from *Lithospermum erythrorhizon*. Cloning and characterization of a ket enzyme in shikonin biosynthesis. *J. Biol. Chem* 2002;277:6240–6246. [PubMed: 11744717]
9. Unsold IA, Li SM. Reverse prenyltransferase in the biosynthesis of fumigaclavine C in *Aspergillus fumigatus*: gene expression, purification, and characterization of fumigaclavine C synthase FGAPT1. *Chembiochem* 2006;7:158–164. [PubMed: 16397874]
10. Yin WB, Ruan HL, Westrich L, Grundmann A, Li SM. CdpNPT, an N-prenyltransferase from *Aspergillus fumigatus*: overproduction, purification and biochemical characterisation. *Chembiochem* 2007;8:1154–1161. [PubMed: 17525915]
11. Kremer A, Westrich L, Li SM. A 7-dimethylallyltryptophan synthase from *Aspergillus fumigatus*: overproduction, purification and biochemical characterization. *Microbiology* 2007;153:3409–3416. [PubMed: 17906140]
12. Edwards DJ, Gerwick WH. Lyngbyatoxin biosynthesis: sequence of biosynthetic gene cluster and identification of a novel aromatic prenyltransferase. *J. Am. Chem. Soc* 2004;126:11432–11433. [PubMed: 15366877]
13. Pojer F, Li SM, Heide L. Molecular cloning and sequence analysis of the clorobiocin biosynthetic gene cluster: new insights into the biosynthesis of aminocoumarin antibiotics. *Microbiology* 2002;148:3901–3911. [PubMed: 12480894]
14. Pojer F, Wemakor E, Kammerer B, Chen H, Walsh CT, Li SM, Heide L. CloQ, a prenyltransferase involved in clorobiocin biosynthesis. *Proc. Natl. Acad. Sci. USA* 2003;100:2316–2321. [PubMed: 12618544]
15. Steffensky M, Muhlenweg A, Wang ZX, Li SM, Heide L. Identification of the novobiocin biosynthetic gene cluster of *Streptomyces spheroides* NCIB 11891. *Anti-microb. Agents Chemother* 2000;44:1214–1222.
16. Kuzuyama T, Noel JP, Richard SB. Structural basis for the promiscuous biosynthetic prenylation of aromatic natural products. *Nature* 2005;435:983–987. [PubMed: 15959519]
17. Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the *in vivo* evidence. *Nat. Rev. Drug. Discov* 2006;5:493–506. [PubMed: 16732220]
18. [accessed 12 February 2008]. <http://www.mrc-lmb.cam.ac.uk/agm/pre-scop/135530.html>.
19. Keller S, Pojer F, Heide L, Lawson DM. Crystallization and preliminary X-ray analysis of the aromatic prenyltransferase CloQ from the clorobiocin biosynthetic cluster of *Streptomyces roseochromogenes*. *Acta Crystallograph. Sect. F. Struct. Biol. Cryst. Commun* 2006;62:1153–1155.
20. Haagen Y, Gluck K, Fay K, Kammerer B, Gust B, Heide L. A gene cluster for prenylated naphthoquinone and prenylated phenazine biosynthesis in *Streptomyces cinnamonensis* DSM 1042. *Chembiochem* 2006;7:2016–2027. [PubMed: 17103476]
21. Haagen Y, Unsold I, Westrich L, Gust B, Richard SB, Noel JP, Heide L. A soluble, magnesium-independent prenyltransferase catalyzes reverse and regular C-prenylations and O-prenylations of aromatic substrates. *FEBS Lett* 2007;581:2889–2893. [PubMed: 17543953]
22. Kawasaki T, Hayashi Y, Kuzuyama T, Furihata K, Itoh N, Seto H, Dairi T. Biosynthesis of a natural polyketide-isoprenoid hybrid compound, furaquinocin A: identification and heterologous expression of the gene cluster. *J. Bacteriol* 2006;188:1236–1244. [PubMed: 16452404]
23. Winter JM, Moffitt MC, Zazopoulos E, McAlpine JB, Dorrestein PC, Moore BS. Molecular basis for chloronium-mediated meroterpene cyclization: cloning, sequencing, and heterologous expression of

the napyradiomycin biosynthetic gene cluster. J. Biol. Chem 2007;282:16362–16368. [PubMed: 17392281]

Figure 1.

Phylogenetic analysis of the members of the ABBA PTase family and structure of the final compounds of the biosynthetic pathways in which the enzymes participate. The phylogenetic tree was generated using the AlignX component of Vector NTI Advance 10.3.0, Invitrogen.

Figure 2.

Details of the structural differences of NphB with respect to the models of CloQ, Fnq26 and SCO7190. In the centre, the structure of NphB (1ZB6) is shown with GPP, Mg²⁺ and 1,6-DHN bound in the active site. Details of the GPP and Mg²⁺ binding site are shown in NphB and have been modeled for CloQ, Fnq26 and SCO7190 using the package Jackal (http://wiki.c2b2.colum-bia.edu/honiglab_public/index.php/Software:Jackal). In the models, GPP, Mg²⁺ and the relevant side chains of NphB have been overlaid and the C-terminal helix has been removed for clarity.