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**New insights into short-chain prenyltransferases:
structural features, evolutionary history and potential for selective inhibition**

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Abstract

Isoprenoids form an extensive group of natural products involved in a number of important biological processes. Their biosynthesis proceeds through sequential 1'-4 condensations of isopentenyl diphosphate (C₅) with an allylic acceptor, the first of which is dimethylallyl diphosphate (C₅). The reactions leading to the production of geranyl diphosphate (C₁₀), farnesyl diphosphate (C₁₅) and geranylgeranyl diphosphate (C₂₀), which are the precursors of mono-, sesqui- and diterpenes, respectively, are catalyzed by a group of highly conserved enzymes known as short-chain isoprenyl diphosphate synthases or prenyltransferases. In recent years, the sequences of many new prenyltransferases have become available, including those of several plant and animal geranyl diphosphate synthases, revealing novel mechanisms of product chain-length selectivity and an intricate evolutionary path from a putative, common ancestor. Finally, there is considerable interest in designing inhibitors specific to short-chain prenyltransferases, for the purpose of developing new drugs or pesticides that target the isoprenoid biosynthetic pathway.

Introduction

With more than 55,000 compounds identified in bacteria, archaea and eukaryotes, isoprenoids are the most abundant and structurally diverse natural products. They play essential roles in numerous biochemical pathways: as quinones in electron transport chains, as components of membranes (prenylipids in archaeobacteria and sterols in eubacteria and eukaryotes), in subcellular targeting and regulation (prenylation of proteins), as photosynthetic pigments (carotenoids, side chain of chlorophyll), as hormones (gibberellins, brassinosteroids, abscisic acid, retinoic acid, juvenile hormone), and as semiochemical compounds in plants and insects (monoterpenes, sesquiterpenes, diterpenes) [1,2].

The universal precursors of isoprenoids generated by the mevalonate pathway are synthesized by sequential 1'-4 condensations of isopentenyl diphosphate (IPP, C₅) with an allylic diphosphate, in reactions catalyzed by enzymes commonly referred to as prenyltransferases or isoprenyl diphosphate synthases (IPPS). Each member of this enzyme family is classified according to the stereochemistry of the double bonds formed during product elongation and the length of the final product [3]. In general, *trans*-prenyltransferases synthesize products up to C₅₀ in length, with *trans* (*E*) double bonds, while most *cis*-prenyltransferases characterized to date generate longer products featuring *cis* (*Z*) double bonds. Although *trans*- and *cis*-prenyltransferases catalyze similar reactions, they are evolutionary and structurally distinct [4]. *Trans*-prenyltransferases can be further divided into short-chain (SCIPPS; C₁₀–C₂₅), medium-chain (MCIPPS; C₃₀–C₃₅), and long-chain (LCIPPS; C₄₀–C₅₀) prenyltransferases [3]. Whereas most short-chain prenyltransferases are active as homodimers, both homo- and heteromers have been observed among the medium- and long-chain enzymes.

Enzymes of the short-chain prenyltransferase subfamily

Geranyl diphosphate synthase (GPP synthase or GPPS): GPPS catalyzes the condensation of two C₅ co-substrates, IPP and dimethylallyl diphosphate (DMAPP), to produce GPP, the precursor of all monoterpenes (Fig. 1). Despite the multiple functions of monoterpenes, which are found most commonly in plants and insects where they act as essential oils and pheromones, respectively, our understanding of their biosynthesis remains limited. GPPS genes have been characterized from only a handful of plant species [5-13] and, so far, only one study has reported on the existence of a GPPS in an insect [14]. However, a second putative GPPS has recently been cloned by our group from a longhorn beetle (unpublished data; see *Evolution of short-chain prenyltransferases* for more information), and an enzyme producing both GPP and FPP has been cloned from aphids [15,16]. Based on sequence comparisons, three different classes of plant GPPSs may be distinguished, one of which contains heterodimeric proteins, while the other two are active as homodimers [13]. In the first heterodimeric GPPS to have been isolated (from *Mentha piperita*), the two subunits were shown to display very limited similarity to one another, with only one subunit recognized as a typical prenyltransferase [6]. Among the homodimeric plant GPPSs, one class has so far been found only in conifers [13] while the other class has members among diverse plant species, including *Arabidopsis thaliana* [8]. These two homodimeric GPPS types display only 20% amino acid sequence identity to each other. The first GPPS to be characterized from an animal was isolated from the bark beetle *Ips pini*, in which it plays a role in aggregation pheromone biosynthesis [14]. Plant and insect GPPSs appear to have no common proximate ancestor (see *Evolution of short-chain prenyltransferases*).

Farnesyl diphosphate synthase (FPP synthase or FPPS): FPPS is the most extensively studied short-chain prenyltransferase due to its central position in the mevalonate pathway.

FPPS catalyzes the condensation of IPP and DMAPP to form the intermediate GPP, which then undergoes a second condensation step to generate FPP (Fig. 1). The FPP product serves as precursor to sesquiterpenes, sterols, dolichols, and some mitochondrial ubiquinones, and is used for protein farnesylation. FPPSs were purified to homogeneity from a number of organisms, including *Saccharomyces cerevisiae* [17], chicken [18], pig [19], human [20], and green pepper, *Capsicum annuum* [21]. All of these purified enzymes were found to form homodimers, with tightly coupled subunits ranging from 32 to 44 kDa in size. In addition, FPPS cDNAs were cloned from various organisms [22-31]. FPPSs may be divided into two broad categories: type I (eukaryotic) and type II (eubacterial) FPPSs [32,33]. Recent work on insect FPPSs suggests that the type-I enzymes include various subtypes displaying features that are distinct from those of vertebrate, plant and yeast FPPSs [27,34].

In addition to the *trans*-FPPSs described above, some *cis*-FPPSs have recently been cloned and characterized from bacteria [35-37] and plants [38]. Whereas the bacterial enzymes use GPP as allylic co-substrate and produce *E,Z*-FPP, the plant enzyme uses DMAPP and generates *Z,Z*-FPP. Little is known about the mechanism of product chain length regulation in these enzymes [37,38].

Geranylgeranyl diphosphate synthase (GGPP synthase or GGPPS): GGPPS supplies the essential acyclic precursor GGPP for the biosynthesis of a structurally diverse group of compounds that includes diterpenes, carotenoids, chlorophylls, geranylgeranylated proteins and archaeal ether-linked lipids. In plants and bacteria, GGPP is synthesized by a GGPPS that catalyzes successive additions of IPP to DMAPP, GPP, and FPP (Fig. 1), while yeast and mammalian GGPPSs can only accept FPP as the allylic co-substrate [39]. To date, cDNAs encoding GGPPS have been cloned from various organisms ranging from bacteria to higher eukaryotes [40-46]. GGPPSs have been divided into three categories [32,33]: type I (archaea

and some bacteria), type II (plants and bacteria), and type III (eukaryotes other than plants) (see *Evolution of short-chain prenyltransferases* for further details).

Farnesylgeranyl diphosphate synthase (FGPP synthase or FGPPS): Tashibana [47] reported on the presence of farnesylgeranyl diphosphate (FGPP) synthase activity, generating C₂₅ isoprenyl diphosphates, in *Natronobacterium pharaonis*, a haloalkaliphilic archaeon that has C₂₀-C₂₅ diether lipids in its membranes, in addition to the C₂₀-C₂₀ diether lipids commonly found in archaea. The highest activity of this FGPPS was observed when GGPP was used as the allylic substrate. Tashibana et al. [48] observed the same behavior for an FGPPS from the aerobic hyperthermophilic archaeon *Aeropyrum pernix*, the membrane of which contains only C₂₅-C₂₅ diether lipids. These archeal FGPPSs are very closely related to archeal GGPPSs [48], thus justifying their inclusion here in the group of short-chain prenyltransferases (see also *Evolution of short-chain prenyltransferases*).

Short-chain prenyltransferases displaying “catalytic promiscuity”: short-chain prenyltransferases displaying dual FPP/GGPP synthase activity have been reported for a protozoan [49], a hyperthermophilic archaea [50], and maize [51]. In the reactions catalyzed by these enzymes, the use of either DMAPP or GPP as allylic co-substrate leads to the formation of both FPP and GGPP, whereas the use of FPP generates GGPP as the sole product. This is in contrast with results obtained for conventional GGPPSs, which tend to generate only GGPP, irrespective of the allylic co-substrate used.

In aphids, Vandermoten et al. [15] identified the first animal short-chain prenyltransferase that can generate both GPP and FPP. Although the recombinant enzyme yielded more GPP than FPP, and the proportion of GPP increased with a rise in the concentration of DMAPP, it generated only FPP if supplied with GPP as the sole allylic co-

substrate [15]. In addition, the recombinant aphid enzyme could generate both monoterpenes and sesquiterpenes in linked assays where appropriate terpene synthases were added to the assay buffer [16]. In contrast, the recombinant *Drosophila melanogaster* FPPS generated almost exclusively FPP in the presence of IPP and DMAPP [15].

In Norway spruce, *Picea abies*, Shimdt et al. [13] reported on the presence of two GPPSs belonging to two separate groups of homodimeric proteins. While one enzyme produced GPP as its sole *in vitro* product, the second enzyme produced substantial amounts of FPP and GGPP, in addition to GPP (42% GPP, 33% FPP, and 25% GGPP). In parallel assays performed on a related enzyme from the angiosperm *Quercus robur*, GPP and FPP were produced in proportions of 55 to 45, while no GGPP was detected [13].

These dual (or multiple) activities observed for some short-chain prenyltransferases may be examples of “catalytic promiscuity”, an expression that was coined to describe the ability of some enzymes to display an adventitious secondary activity at the active site responsible for the primary activity [52]. Catalytic promiscuity is of interest because an adventitious secondary activity may become useful to the organism at some point, and the enzyme may be recruited to provide the secondary product. For example, the existence of a GPP/FPP synthase in aphids is perhaps not too surprising, given that these insects require both monoterpenes and sesquiterpenes as precursors for sex pheromone, alarm pheromone and juvenile hormone biosynthesis [15].

Catalytic mechanism

Short-chain prenyltransferases catalyse chain elongation reactions, where the growing chain of an allylic isoprenoid diphosphate (DMAPP, GPP or FPP) undergoes coupling with IPP through electrophilic alkylation of its carbon-carbon double bond. In all organisms, this reaction occurs following a sequential ionization-condensation-elimination mechanism

referred to as head-to-tail condensation. After binding of both co-substrates to the enzyme, a carbocation is formed at the C1' position of the allylic substrate; this step is activated by divalent cations such as Mg^{2+} or Mn^{2+} . The carbocation electrophilically attacks the C4 position of IPP resulting in formation of a C-C bond between IPP and the allylic substrate. The product is then released from the active site [53]. The stereochemistry of the 1'-4 condensation was elucidated by Cornforth's group [54], who established that the allylic carbocation attacks the double bond of IPP from the *Si*-face. The *proR*-H at the C2 position of IPP is then removed to form a new allylic *trans*-double bond

Structure and active site

Current knowledge about short-chain prenyltransferase 3D-structures is based in large part on the work of Tarshis et al., who reported the crystal structure of unliganded avian FPPS at 2.6-Å resolution [55], as well as that of the corresponding double F112A/F113S mutant, liganded with DMAPP, GPP, and FPP [56]. More recently, Hosfield et al. [57] reported the crystal structures of two prokaryotic FPPSs at 2.4-Å resolution: the unliganded *Staphylococcus aureus* FPPS and the *Escherichia coli* enzyme bound to both IPP and a noncleavable DMAPP analogue. In addition, the structure of the *Trypanosoma cruzi* FPPS, both unliganded and complexed with substrates and inhibitors, has been elucidated [58]. Furthermore, Kavanaght et al. [59] reported the high-resolution X-ray structures of the human FPPS in complex with two bisphosphonate inhibitors, risedronate and zoledronate, respectively. Finally, Chang et al. reported the first GGPPS structure, which was determined for the yeast *Saccharomyces cerevisiae* at 1.98-Å resolution [60].

Altogether, these studies have indicated that short-chain prenyltransferases are typically active as homodimers and exhibit a fold composed of 13 α -helices joined by loops. Ten of these helices form a helical bundle that surrounds a central cavity where the active site

is located. Two highly conserved aspartate-rich motifs face each other on opposite walls of this cavity. Using hybrid-type heterodimers of *Bacillus stearotherophilus* FPPSs, constituting different types of mutated monomers in regions II and IV (see below), Koyama et al. suggested that FPPS subunits interact with each other to form a shared active site in the homodimer structure, rather than an independent active site in each subunit [61]. More recently, the determination of the first mammalian GGPPS crystal structure revealed a novel hexameric quaternary structure [62]. The regions involved in hexamer formation (from three homodimers) are largely conserved for mammalian and insect GGPPSs, but not for plant, bacterial, fungal, or archaeal GGPPSs, suggesting that insect GGPPS may be hexameric as well. Unlike the homodimeric FPP or GGPP synthases purified to date, both homo- and heteromeric forms of plant GPPSs have been identified [6,7,9].

The deduced amino acid sequences of all short-chain prenyltransferases tend to display a high level of similarity to one another, and multiple sequence alignments have revealed the presence of seven conserved regions [63]. Regions II and VI contain aspartate-rich motifs (DDx(xx)xD; where x represents any amino acid) referred to as the first (FARM) and second (SARM) aspartate-rich motifs, respectively. **Site-directed mutagenesis studies targeting the aspartate residues within the FARM have pointed to their involvement in catalysis and binding of the allylic substrate [63,64]. Binding of IPP, however, involves the C-terminus of the enzyme, which interacts with Lys, Arg and His residues contacting the IPP pyrophosphate [57]. Among the five residues located upstream from the FARM, some have been shown to be involved in product chain-length determination; this portion of the sequence is therefore referred to as the chain-length determination (CLD) region [33], although additional residues, located on distinct α -helices, can play a role in defining product chain-length [60].**

Mechanisms of product chain-length regulation

Although they share the same catalytic mechanism, short-chain prenyltransferases rarely generate a product whose chain length differs from that which is pre-determined for the enzyme. Over the past several years, there has been considerable interest in identifying the mechanisms responsible for product chain-length regulation by scIPPSs. In this context, it has been observed that factors such as salt and metal ion concentrations [65,66] and the ratio between allylic and IPP co-substrates [15,67,68] can, to a limited extent, modulate the product chain-length selectivity of some prenyltransferases. Under certain circumstances, temperature can also have an impact on this variable, as shown by Fujiwara et al. [69] for the dual FPP/GGPP synthase isolated from the hyperthermophilic archaea *Thermococcus kodakaraensis*.

However, the primary molecular mechanism regulating product chain length is dependent upon structural features specific to each subgroup of prenyltransferases. Our current understanding of the mechanisms of product chain-length determination by these enzymes is derived primarily from the elucidation of their 3D structures, combined with several site-directed and random mutagenesis studies. The analysis of the crystal structures of wild-type and mutated avian FPPS suggested that the ultimate product length was dictated by the size of the active site hydrophobic pocket [56]. More specifically, the two aromatic residues Phe112 and Phe113, located at the fourth and fifth positions upstream from the FARM, were shown to form the “floor” of the catalytic pocket, and replacement of these two amino acids with smaller residues directly altered the size of the binding pocket, resulting in the formation of longer products. Additional studies further demonstrated that the product chain-length of wild-type FPP and GGPP synthases was in large part determined by the nature of the amino-acid residues located at both the fourth and fifth positions upstream from the FARM [11,56,70,71]. The side-chains of these amino-acid residues can either prevent or

allow additional condensations to take place, with smaller side-chains typically permitting further product elongation. In bacterial FPPSs, which typically have a two-amino-acid insertion within the FARM (i.e., DDxxxxD instead of DDxxD), only the residue at position -5 relative to the FARM seems critical to defining product chain length selectivity [70,71], possibly because of the structural changes brought about by the two-amino-acid insertion [33].

Residues other than those at positions -4 and -5 relative to the FARM can also play a role in the chain-length selectivity of short-chain prenyltransferases. For example, the Ala116Trp (-1 relative to the FARM) and Asn144Trp (+23 relative to the FARM) avian FPPS mutants displayed a smaller binding pocket for the hydrocarbon chain of the allylic substrate, with altered product selectivity favoring synthesis of shorter products such as GPP [72]. In type-III GGPPSs, in which the fourth and fifth residues upstream from the FARM are either Ser or Ala (Fig. 2), other residues located deeper in the catalytic cavity prevent chain elongation beyond C20. In yeast GGPPS, for instance, Tyr107, Phe108 and His139 seal the bottom of the active site cavity [60].

Several insect GPP and FPP synthases do not display an aromatic residue at either or both the fourth and fifth positions upstream from the FARM [27]. For example, the aphid GPP/FPP synthase displays a Gln residue at positions -4 relative to the FARM (YQxxxDDxxD), a feature shared with lepidopteran type-1 FPPSs [27], for which the exact product(s) remain(s) to be identified [73]. Site-directed mutagenesis and molecular dynamics simulations indicated that the dual GPP/FPP synthase activity of the aphid enzyme was largely defined by Gln107 and Leu110, at positions -4 and -1 relative to the FARM, respectively. Although the mechanism responsible for this dual activity involves steric constraints on the ligand, as shown for other short-chain prenyltransferases, two additional factors have been identified, namely ligand stabilization and the disruption of H-bonds formed

between Gln107 and residues across the catalytic cavity, which constitutes a novel chain-length determination mechanism for sCIPPSs [Vandermoten et al., unpublished data].

Although the mechanism of product chain-length determination of homodimeric plant GPPSs has not been elucidated, that of the heterodimeric GPPS from mint [6] has been shown to be dependent upon the interaction of the small subunit with the large, GGPPS-like subunit [74]. However, this interaction does not appear to result strictly from constraints imposed by the small subunit on the size of the active site cavity in the large subunit [10]. Thus, additional geometric features of the catalytic cavity apparently contribute to product chain-length determination in these GPPSs.

Evolution of short-chain prenyltransferases

Phylogenetic trees of *trans*-isoprenyl diphosphate synthases were published earlier by other groups [32,33,48,75], but many prenyltransferases have since been cloned and sequenced, including several plant and animal GPPSs, insect FPPSs displaying unusual features and various medium- and long-chain polyprenyl diphosphate synthases, to which short-chain prenyltransferases are related. For this reason, it seemed appropriate to re-examine the phylogeny of this group of enzymes.

All *trans*-isoprenyl diphosphate synthases are believed to be derived from a common ancestor, and some have argued that archeal GGPPSs are the closest relatives to this common ancestor [33,75], although this hypothesis has been challenged [48]. Given the high incidence of horizontal gene transfer that has occurred among procaryotes [76], it will remain difficult to identify the prenyltransferase that is closest to the root of the tree. For this reason, the present tree (Fig. 2) was left unrooted; as a result, its branching order may not always reflect the evolutionary course that these enzymes have taken. Nevertheless, the analysis clearly indicates that *trans*-isoprenyl diphosphate synthases form two large and distinct clusters that

have presumably originated from a common procaryotic ancestor: animal GPPSs and eukaryotic FPPSs, on the one hand, and all other *trans*-prenyltransferases, on the other. Within the first cluster, the residues at positions –4 and –5 relative to the FARM tend to be aromatic amino acids (F or Y), although this is not always the case, particularly among the FPPSs of unicellular organisms such as *Plasmodium* and *Trypanosoma*, and the enzymes of insects, where substitutions at these positions can lead to the formation of GPP, instead of FPP, as the principal product [15]. The level of amino acid sequence similarity among these enzymes displays a wide range of variation in pairwise comparisons, even within a species (e.g., spruce budworm FPPS1 and FPPS2). Bark beetle GPPS and FPPS are very divergent and display only a limited degree of similarity to one another, despite a nearly identical CLD region (the GPPS has an uncommon cysteine residue at position –3 relative to the FARM). The nature of the residue at the first position upstream from the FARM, which can have an impact on product chain length [72], also tends to vary considerably within this group (L, M, I, V, A, S or E). In contrast, all enzymes in the other cluster, with the exception of animal and type-II bacterial GGPPSs, have a histidine residue at the equivalent position. In fact, many of these enzymes, including all eukaryotic **polyprenyl diphosphate synthases [PPPSs; i.e., all MCIPPSs and LCIPPSs, including those whose exact product chain-length (C30-C50) is unknown]**, a sub-group of plant homodimeric GPPSs, archeal PPPSs, and some bacterial medium-chain and long-chain IPPSs share the same ASxxHDDxxD “FARM signature”. The ubiquity of this histidine residue suggests that it plays an important structural role, although its presence in enzymes displaying vastly different product chain-length selectivities (e.g., plant GPPSs and animal LCIPPSs) points to a limited role in defining product chain length.

Interestingly, animal GGPPSs are closely related to the yeast enzyme (both are type-III GGPPSs), while the latter is clearly distinct from plant GGPPSs (Fig. 2). Type-III GGPPSs are highly divergent from their intra-specific FPPS counterparts. In fact, inter-taxonomic

GGPPS-GGPPS comparisons reveal a much higher degree of similarity than that measured for intra-specific FPPS-GGPPS comparisons (e.g., Blastp expect values for human versus *Drosophila* GGPPS: $1e^{-103}$; for human FPPS versus human GGPPS: $1e^{-007}$). These observations strongly suggest that the two enzymes have not evolved from one another, but have been acquired independently, possibly through horizontal gene transfer in a common ancestor. A more obvious case of the latter phenomenon is seen with plant GGPPSs and a sub-group of plant GPPSs, which display a CLD region almost identical to that of some bacterial FPPSs and GGPPSs (including the insertion of two extra residues within the FARM), from which they appear to have originated (Fig. 2).

Whereas FPP and GGPP synthases occur nearly ubiquitously in plants, animals, fungi and bacteria, GPPSs appear to have a more limited distribution in nature, having been identified almost exclusively in plants and insects. The present analysis indicates that insect GPPSs have evolved from insect FPPSs while those of plants have evolved once from eukaryotic PPPSs (most homodimeric plant GPPSs) and once from plant GGPPSs (heterodimeric plant GPPSs). The homodimeric grand fir GPPS stands out from the other homodimeric plant GPPSs included in the present analysis, with its distinctive bacterial “FARM signature”. Clearly, *trans*-prenyltransferases from all organisms share many features that are indicative of common ancestry, but they also seem to have followed a convoluted evolutionary path that blurs some of the branching points in the tree shown here.

Inhibitors of short-chain prenyltransferases and perspectives for applied research

Over the past several years, there has been considerable interest in developing inhibitors that are specific to certain enzymes of the mevalonate pathway. This concept has found widespread clinical use with statins, which are drugs that inhibit hydroxymethylglutaryl-CoA (HMG CoA) reductase and, as a result, reduce cholesterol biosynthesis. Because short-chain

prenyltransferases play a pivotal role in the biosynthesis of numerous mevalonate pathway end-products, the inhibition of their activity is also of great interest in applied research. Among this enzyme family, FPPS is a key target, and several groups have investigated the possibility of inhibiting FPPS with nitrogen-containing bisphosphonates (N-BPs) [77-80]. N-BPs are pyrophosphate analogs in which the oxygen bridge between the two phosphorus atoms has been replaced with a carbon atom bearing two side chains, the nature of which affects the compound's chemical properties, mode of action and potency.

In mammals, the inhibition of FPPS by N-BPs blocks the formation of FPP, which is required for protein prenylation. A deficit in protein prenylation results in osteoclast apoptosis and inhibition of the bone-destroying action of these cells [81]. Therefore, N-BPs are used to treat disorders characterized by bone resorption such as osteoporosis, Paget disease, hypercalcemia caused by malignancy, tumour metastases in bone, and other ailments [82-85].

A number of N-BPs have recently been shown to have curative effects in *in vivo* models of visceral [86] and cutaneous [87] leishmaniasis. Their significant activity was also demonstrated against the proliferation of *T. brucei* and *T. cruzi*, the causative agents of African trypanosomiasis (sleeping sickness) and Chagas' disease, respectively. Other N-BPs also show potential as anti-cancer drugs as they can inhibit human GGPPS, which is required for the prenylation of proteins such as Ras involved in cancer development [88].

In the field of agrochemical research, the replacement of the amino group of N-BPs by hydroxyl, ureido, thioureido, or amino moieties leads to compounds inhibiting FPPS and having significant herbicidal properties [89]. Some of the N-BPs that are effective in inhibiting FPP and GGPP synthases also inhibit plant heterodimeric GPPSs [10]; the herbicidal action of N-BPs could therefore result from their inhibitory activity on more than one prenyltransferase. It has also been suggested that the uncommon structural features of some insect GPP and FPP synthases, including those of caterpillars [27] and aphids [15],

could be exploited for the design of pest-specific inhibitors displaying insecticidal activity. Since FPPSs are required for the production of juvenile hormone in insects, FPPS inhibition is expected to cause a decrease in the production of this hormone, which would result in a precocious and lethal metamorphosis [90].

Conclusion

Although considerable new information has accumulated on short-chain prenyltransferases during the past decade, with important advances in their cloning and characterization, these enzymes have not yet revealed all their secrets. Indeed, the recent discovery of new types of short-chain prenyltransferases that produce a mixture of products with different chain-lengths points to a new level of complexity in the mechanism regulating product size, which is not always dictated only by steric constraints, as originally suggested. Many of the recently cloned enzymes have yet to be characterized, including several putative insect FPPSs, which could turn out to have properties that differ from those initially inferred from blastp searches, given the sometimes unorthodox nature of their CLD regions. In addition, the mechanism of product chain-length selectivity of homodimeric plant GPPSs, which are in many respects very similar to eukaryotic long-chain prenyltransferases, needs to be examined so as to identify the substitutions that have led the presumed conversion of enzymes that generate long polyprenyl chains into GPPSs; this would help elucidate their exact evolutionary history.

Finally, there are still exciting prospects with respect to the development of inhibitors specific to short-chain prenyltransferases, both in the field of drug discovery and in crop/forest protection. Progress in this area will undoubtedly continue to benefit from sustained efforts in the structural and enzymatic characterization of novel prenyltransferases displaying features that suggest that they might be inhibited in a taxon-selective manner.

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Figure Legends

Figure 1: Elongation reactions catalyzed by short-chain prenyltransferases, along with examples of final products generated by the geranyl diphosphate (GPP), farnesyl diphosphate (FPP), geranylgeranyl diphosphate (GGPP), and farnesylgeranyl diphosphate (FGPP) precursors.

Figure 2: Phylogenetic tree (unrooted) of *trans*-isoprenyl diphosphate synthases from diverse taxa. Nucleotide sequences were retrieved from Genbank and imported into MEGA4 [91], where they were conceptually translated into proteins. Alignments were performed on the amino acid sequences using ClustalW, as implemented by MEGA4, with default settings except for penalties relating to multiple alignment gap openings and extensions, which were set to 3.0 and 1.8, respectively [92]. The back-translated nucleotide alignment was then used to construct a Neighbor-Joining tree using MEGA4’s default settings, except for gap deletions, where the pairwise deletion option was selected. Bootstrapping was performed on 2000 pseudoreplicates, and all branches with less than 50% bootstrap support were collapsed. For each enzyme or enzyme lineage, residues within the chain-length determination (LCD) region [i.e., the first aspartate-rich motif (“FARM” = DDxxD or DDxooxD) and the five residues upstream from it] are shown as a color-coded sequence, where x = L, I, V or M, and o = any amino acid. Residues at position –4 and –5 relative to the FARM are shown in green, while

the residue at position –1 is shown in red. Aspartate residues within the FARM are shown in blue. Accession numbers: GPPS: algae, XM_001691017; Arabidopsis, Y17376; bark beetle, AY953508; grand fir, AF513112; longhorn beetle, not deposited yet; Mentha, AF182828; spruce; EU432048; tomato, DQ286930; GPPS/FPPS: aphid, AY968586; FPPS: Arabidopsis, L46350; bark beetle, AY953507; Drosophila, NM_058032; E. coli, D00694; human, NM_002004; Micrococcus, AB003187; mosquito, XM_308653; nematode, NM_060626; Plasmodium-AY219707; spruce budworm-1, AY954920; spruce budworm-2, AY954919; Trypanosoma, AF386102; yeast, NC_001142; FPPS/GGPPS: Methanothermobacter, S75695; Mycobacterium, NC_000962; GGPPS: Arabidopsis, NM_127943; Drosophila, AF049659; human, NM_004837; Pantoea, D90087; Sulfolobus, NC_007181; **Yeast, AY692852**; FGPPS: Aeropyrum, AB025791; **hexaprenyl diphosphate synthase (HPPS; C30)**: Micrococcus, AB003188; **heptaprenyl diphosphate synthase (HepPPS; C35)**: Bacillus, EF191544; **octaprenyl diphosphate synthase (OPPS; C40)**: Archeoglobus, AE000782; E. coli, U18997; Trypanosoma, XM_799480; **solanesyl diphosphate synthase (SPPS)**: Arabidopsis, NM_001084371; mouse, NM_019501; Mucor mold, AJ496300; Rhodobacter, AB001997; **decaprenyl diphosphate synthase (DPPS; C50)**: fission yeast, NM_001021183; human, NM_014317; **PPPS**: aphid, XM_001947162; body louse, DS235241; chicken, XM_418592; Drosophila, NM_170546; Methanosarcina, NC_007355; mosquito, XM_565746; Plasmodium, XM_001349505; Tribolium, XM_968226; wasp, XM_001602302.