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Review

Farnesyl diphosphate synthase; regulation of product specificity✪

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Farnesyl diphosphate synthase (FPPS) is a key enzyme in isoprenoid biosynthesis which supplies sesquiterpene precursors for several classes of essential metabolites including sterols, dolichols, ubiquinones and carotenoids as well as substrates for farnesylation and geranylgeranylation of proteins. It catalyzes the sequential head-to-tail condensation of two molecules of isopentenyl diphosphate with dimethylallyl diphosphate. The enzyme is a homodimer of subunits, typically having two aspartate-rich motifs with two sets of substrate binding sites for an allylic diphosphate and isopentenyl diphosphate per homodimer. The synthase amino-acid residues at the 4th and 5th positions before the first aspartate rich motif mainly determine product specificity. Hypothetically, type I (eukaryotic) and type II (eubacterial) FPPSs evolved from archeal geranylgeranyl diphosphate synthase by substitutions in the chain length determination region. FPPS belongs to enzymes encoded by gene families. In plants this offers the possibility of differential regulation in response to environmental changes or to herbivore or pathogen attack.

Since the isolation of farnesyl diphosphate (FPP) synthase (FPPS) by Lynen *et al.* (1959) our knowledge regarding this enzyme has advanced tremendously and new data appear in-

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Abbreviations: CLD, chain length determination; DMAPP, dimethylallyl diphosphate; FARM, first aspartic rich motif; FPP, farnesyl diphosphate; FPPS, farnesyl diphosphate synthase; GFPP, geranylfarnesyl diphosphate; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate; IPP isopentenyl diphosphate; SARM, second aspartic rich motif.

dicating the important role it plays in all cells (Fig. 1). FPPS catalyzes consecutive *E*-condensations of two isopentenyl diphosphates

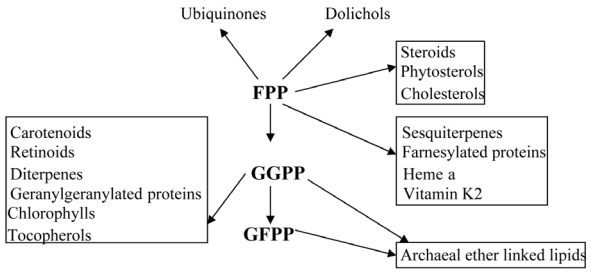

Figure 1. FPP participation in biosynthetic pathways.

(IPPs) with dimethylallyl diphosphate (DMAPP) to form the intermediate geranyl diphosphate (GPP) and the product FPP (Fig. 2). FPP is used as substrate for squalene synthase that will ultimately be con-

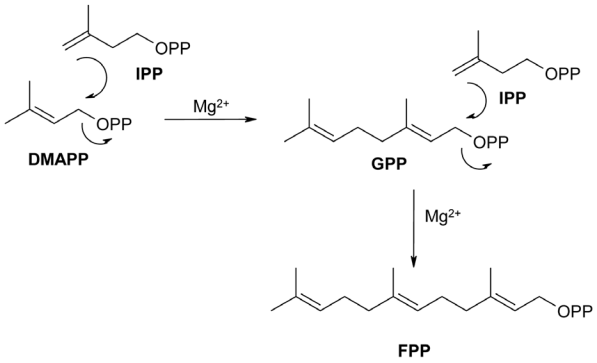

Figure 2. Condensation of two IPP molecules with DMAPP to form an intermediate GPP and the product FPP.

verted to sterols. Some bacteria use FPP as a precursor of bacterial hopane. FPP is also used as a primary substrate for the synthesis of geranylgeranyl diphosphate (GGPP) which similarly to FPP is acted on by the protein geranylgeranyl or farnesyl transferase that catalyze lipid modification of proteins with a CAAX box at their C-terminus, such as Ras and lamin A. Plant carotenoids, retinoids and chlorophyll originate from GGPP. Archea use GGPP to form their membranous ether lipids (Tachibana *et al.*, 2000). Long-chain *E*-isoprenyl synthases use FPP as primer for the formation of the side chain of ubiquinones. FPP also serves as a substrate for dehydrodolichyl diphosphate synthases (*Z*-isoprenyl diphosphate synthases) whose products function as sugar carriers in the formation of glycoproteins and glycolipids. It also is a side chain of heme a. Farnesol, the dephosphorylated form of FPP acts as a regulator of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (Correll *et al.*, 1994; Shearer & Hampton, 2005) and is a signal molecule that interacts with an orphan receptor (Shivdasani *et al.*, 1995). Reilly *et al.* (2002) suggest a novel role of FPPS in fibroblast growth factor-mediated signal transduction.

FPPS has been localized in the cytosol (Grunler *et al.*, 1994), mitochondria (Runquist *et al.*, 1994; Cunillera *et al.*, 1997), peroxisomes (Oliver & Krisans, 2000) and chloroplasts (Sanmiya *et al.*, 1999). However, the plastidial localization is questioned (Hemmerlin *et al.*, 2003).

The excellent studies of the group of Cornforth (1966) described the stereochemistry of C–C bond formation in FPPS. The major research of Poulter and Rilling using biochemical methods led to the establishment in the 70s and 80s of the reaction kinetics (Poulter & Rilling, 1976; Laskovics & Poulter, 1981). The enzyme binds both substrates IPP and DMAPP and an allylic cation is formed at the C′ position of the allylic substrate. Mg^{2+} is the activator of the reaction. The carbocation electrophylically attacks the fourth carbon of the C-4 position of IPP resulting in the formation of a C–C bond between IPP and the allylic substrate DMAPP, and subsequently GPP. The product is then released from the active site. However, earlier (Roos *et al.*, 1998) and recent findings show that GPP but not DMAPP may serve as primary substrate. FPPS from a rare rubber-producing mushroom, *Lactarius chrysorrheus*, uses geranyl

diphosphate as the primary substrate, with a 2.4-fold higher k_{cat}/K_m value for GPP than for dimethylallyl diphosphate (Mekkriengkrai *et al.,* 2004).

PURIFICATION OF FPPSs AND CLONING OF THEIR GENES

The first purifications of the enzyme to homogeneity from several eukaryotic sources, including *Saccharomyces cerevisiae* (Eberhardt & Rilling, 1975), and avian (Reed & Rilling, 1975), porcine (Yeh & Rilling, 1977) and human (Barnard & Popjak, 1981) livers allowed to establish that in all these organisms FPPS is a dimer of 80–84 kDa.

IMPORTANCE OF ASPARTIC ACID RESIDUES IN THE II AND VI CONSERVED REGIONS OF FPPS

Sequence alignment of FPPSs from many different organisms revealed I to VII conserved regions (Koyama *et al.,* 1993) (Fig. 3) with two characteristic Asp-rich motifs, one in region II with DDXX(XX)D (D being Asp and X any amino acid) called FARM (first Asp-rich motif). This motif is highly conserved in all known *E*–prenyltransferases and has been designated as the chain length determination (CLD) region. The second motif is in region VI with the sequence DDXXD called SARM (second Asp-rich motif) (Ashby & Edwards, 1990). It became obvious that the

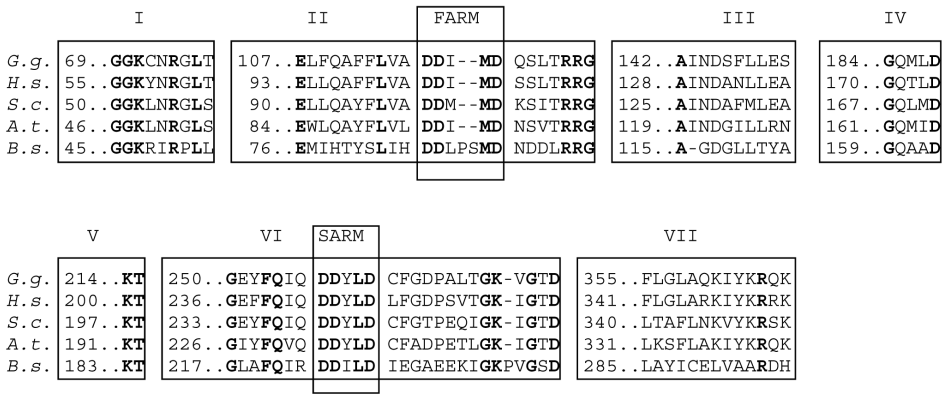

Figure 3. Amino-acid sequence alignment of conserved regions I–VII of various FPPSs.

Numbers to the left of the sequences indicate the positions of the first amino acid displayed. Typical conserved amino acids are in boldface. Boxed FARM and SARM denote the first and the second Asp-rich motif, respectively. *G.g., Gallus gallus; H.s., Homo sapiens; S.c., Saccharomyces cerevisiae; A.t., Arabidopsis thaliana; B.st., Bacillus stearothermophilus.*

The development of molecular biology at the end of the 80s provided further insight into the nature of the enzyme. Clarke *et al.* (1987) cloned the gene encoding rat liver FPPS. Two years later Poulter and his group isolated the gene for FPPS from yeast genomic DNA by hybridization with a synthetic probe based on the N-terminal amino-acid sequence of the enzyme from *S. cerevisiae* (Anderson *et al.,* 1989). The human FPPS gene was cloned in 1990 (Wilkin *et al.,* 1990). At present about twenty FPPS genes have been cloned and new sequences appear.

amino acids in these regions must participate in the formation of the C–C bond between IPP and the allylic substrate, and in the determination of the chain length of the product. To evaluate the roles of individual amino acids in these regions a number of site-directed mutagenesis experiments were performed. Joly and Edwards (1993) reported in their work on the liver enzyme that the conserved Asp and Arg residues in FARM (region II) are critical for efficient catalysis, since their substitutions resulted in significant decreases in V_{max} without significant changes in the K_m

for IPP and GPP. Marrero *et al.* (1992) reported that mutation in first Asp residue in SARM (region VI) of the rat liver enzyme drastically reduced the catalytic activity and increased the K_m value for IPP. Song and Poulter (1994) also found that mutation in SARM at the first and second Asp residues of *S. cerevisiae* FPPS severely reduced the catalytic activity. They investigated a series of site-directed mutants of the conserved Asp and Arg residues in region II of yeast recombinant FPPS and documented that substitution of charged by noncharged residues, including Asp to Ala and Arg to Gln, dramatically reduced the catalytic activity of the enzyme. The results of these experiments indicated that Asp in the FARM and SARM motifs are crucial for the activity of the enzyme. The studies of Koyama *et al.* (1995) documented that another conserved sequence designated FQ in region VI is crucial for enzyme activity. Replacements Phe220Ala and Gln221Glu in *Bacillus stearothermophilus* FPPS resulted in a 10^5 and 10^3 decreases in the catalytic activity, respectively. The evolutionarily conserved FQ amino acids upstream SARM motif are located in the substrate binding cavity and are responsible for holding of the DMAPP or GPP in a proper conformation necessary for the condensation with IPP.

ROLE OF CONSERVED ARGININES AND LYSINES IN OTHER CONSERVED REGIONS OF FPPS

The influence of amino-acid substitutions in motifs other than FARM and SARM on FPPS activity has also been investigated. The Arg residue in the third position from the C-terminus of the enzyme (region VII) is highly conserved. Koyama *et al.* (1994) examined the significance of this amino acid by replacing Arg295 in *B. stearotermophilus* with Val. This mutation, however, did not result in a marked change in catalytic activity, indicating that the Arg residue in region VII is not essential for catalysis (Koyama *et al.*, 1994). Lys is conserved in regions I, V and VI. Blanchard and Karst (1993) substituted Lys197 conserved in region V with Glu in *S. cerevisiae* FPPS. The substitution resulted in a decrease in enzyme activity and excretion of geraniol. Koyama and his group substituted the conserved Lys in regions I, V and VI in *B. stearothermophilus;* their results suggested that Lys in regions I and V contribute to the binding of IPP (Koyama *et al.*, 1996). These results show that substitution of Lys in region V decreases the affinity for IPP rather than for GPP which in turn leads to GPP release and a decrease in enzyme activity.

THREE-DIMENSIONAL STRUCTURE AND DIMER FORMATION

Crystallization of FPPS was first achieved with the avian liver enzyme by Reed and Rilling (1975). A preliminary X-ray diffraction analysis to about 3 Å resolution of *B. stearothermophilus* FPPS was performed by Nakane *et al.* (1993). But the first three-dimensional structure of chicken liver FPPS to 2.6 Å resolution was determined by Tarshis *et al.* (1994). This dramatically advanced the knowledge about isoprenyl diphosphate synthases. The enzyme exists as a homodimer and exhibits a novel fold called "terpenoid synthase fold", composed entirely of α-helices joined by loops. The most prominent structural feature of the enzyme is the arrangement of ten core helices around a large central cavity. The two highly conserved Asp-rich sequences FARM and SARM were found on opposite walls of this cavity approximately 12 Å apart and facing each other. The dimer configuration places the single active site of each subunit at the top of the dimer and facing away from each other so that they each open outwards to the solvent. Substrate binding experiments revealed that each subunit of the avian liver enzyme has a single allylic

binding site in FARM (region II) accommodating DMAPP, GPP or FPP and one putative homoallylic binding site in SARM (region VI) for IPP (Tarshis *et al.,* 1996). Hence, there are two sets of binding sites in one molecule of FPPS with a tightly coupled homodimer structure. But it is still difficult to elucidate the dynamic interaction of the catalytic site(s) that directly participate in the reaction performed by the homodimeric enzyme.

To date all purified FPPSs are homodimeric with tightly coupled subunits ranging from 32 to 44 kDa. Koyama *et al.* (2000) constructed hybrid-type heteromeric dimers of site-directed mutants of *B. stearothermophilus* FPPSs. They showed that heterodimers combining wild type subunits with subunits carrying a mutation in region II exhibited 78% of the activity of the wild type homodimer. Moreover, the heterodimer formed with a subunit mutated in region II with a subunit mutated in region VI was moderately active. These results suggest 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. An interesting result was obtained by Burke and Croteau (2002); they demonstrated that the small subunit of the heterodimeric *Mentha piperita* GPP synthase and the subunit of the homodimeric GGPP synthase of *Taxuus canadiensis* or *Abies grandis* yielded a functional hybrid heterodimer that synthesized GPP. However, there were no interactions between the GPP synthase small subunit and the FPPS subunit originating from the same peppermint.

PRODUCT CHAIN-LENGTH DETERMINATION

An X-ray analysis of a double mutant showed that substitution of the aromatic rings of Phe112 and Phe113 (fourth and fifth position before FARM) by the hydrogen of Ala and the hydroxyl of Ser, respectively, removes the "floor" of the binding pocket and creates a channel that extends from the top of the binding pocket through the center of the protein, into the dimer interface, and out to the solvent allowing synthesis of longer products, up to C_{70} with C_{35} products dominating. On the other hand, amino-acid substitutions Ala116Trp and Asn144Trp that reduced the size of the binding pocket for the hydrocarbon residue of the allylic substrate altered product selectivity of avian FPPS to favour synthesis of GPP (Stanley Fernandez *et al.,* 2000). In *B. stearothermophilus* FPPS substitution of Ser by Phe at the fourth position before FARM resulted in exclusive synthesis of GPP. However, substitution of the same amino acid by Tyr with an accessible surface bigger than that of Phe did not change product specificity (Narita *et al.,* 1999). Similar results were obtained in the yeast system. Based on the 3-D model of *S. cerevisiae,* FPPS (Plochocka *et al.,* 2000), substitution of chosen Phe96 (Fig. 4) in the CLD region by the bulkier Trp did not change product specificity (Karst *et al.,* 2004).

Ohnuma and his group constructed libraries of mutated FPPS genes in *B. stearothermo-*

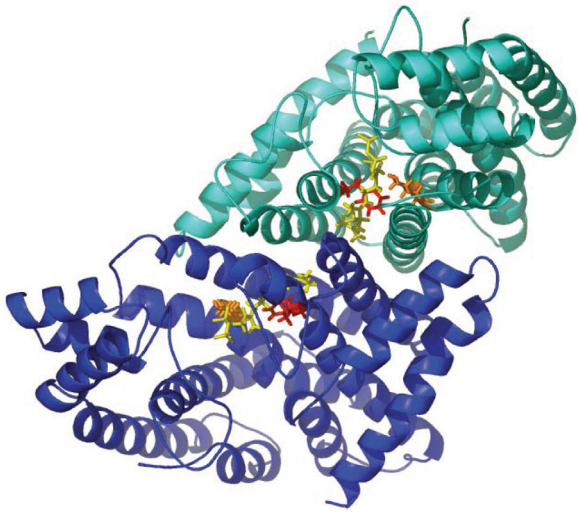

Figure 4. Ribbon diagram of the yeast FPPS homodimer model.

Subunits are dark and light blue. Farnesyl diphosphate is in yellow, Phe96 in red and Lys197 in orange.

philus by random mutagenesis with $NaNO_2$ (Ohnuma *et al.*, 1996a). They found that the mutated enzyme with the substitution Tyr81His in the CLD region at the fifth amino acid upstream of the FARM motif was active and produced GGPP instead of FPP most effectively. In another set of experiments Tyr81 was replaced by small amino acids such as Ala, Gly or Ser and such mutants produced HexPP as the longest product, whereas substitution with Cys, Ile, Leu, Gln, Thr or Val resulted in the synthesis of geranylfarnesyl diphosphate as the longest product (Ohnuma *et al.*, 1996b).

Both lines of evidence originating from the 3-D structure of FPPS presented by the group of Poulter (Tarshis *et al.*, 1994; 1996) and the molecular approach of Ohnuma *et al.* (1996a; 1996b; 1996c) clearly demonstrated that the product chain lengths of natural FPPS and GGPPS are mainly regulated by the amino--acid residues located in the fourth and fifth position before FARM at the bottom of the active pocket through direct interaction with the ω-terminal region of the allylic products. Some reports indicate that amino acids located in regions other than that defined previously as CLD play an important role in product chain-length determination (Hemmi *et al.*, 2003; Kawasaki *et al.*, 2003). These residues are placed before the evolutionarily conserved G(Q/E) motif. They are suggested to be in spatial proximity to the 4th and 5th positions before the FARM so they determine product cleft size and through their bulky functional groups regulate the chain termination.

The product specificity of isoprenyl diphosphate synthases may also be changed by modulating the reaction constants of catalysis. Koyama *et al.* (1996) showed that at equal concentrations of IPP and DMAPP, wild type FPPS synthesized practically only FPP. When the concentration of IPP was reduced the amount of GPP synthesized significantly increased. Similar results were obtained by Ohnuma *et al.* (1997). Yeast and bacterial FPPS mutants with markedly increased K_m values for IPP synthesized more GPP than FPP (Koyama *et al.,* 1996; Blanchard & Karst, 1993; Karst *et al.,* 2004). These data suggest that an allylic substrate competes with allylic intermediates for binding to the active site, and that an intermediate with a chain length near that of the substrate is easily expelled by the allylic substrate resulting in the synthesis of shorter products. Likewise, accelerating the rate of allylic carbocation formation by Co^{2+} or Mn^{2+} (Ohnuma *et al.,* 1993) or by the increase of IPP concentration over the allylic substrate or intermediate causes a shift of the chain length towards longer products (Matsuoka *et al.,* 1991; Plochocka *et al.,* 2000).

EVOLUTION OF *E*-ISOPRENYL DIPHOSPHATE SYNTHASES

Chen and Poulter (1993) and Chen *et al.* (1994) suggested divergent evolutionary pathways for *E*-isoprenyl diphosphate synthases. In their model, a bifunctional FPP/GGPP archaebacterial synthase which they purified from *Methanobacterium thermoautotrophicum* acts as a "pre-enzyme" from which long and short isoprenyl diphosphate synthases diverged. Subsequent chain length control evolved independently after this archeal GGPPS segregated into Archea, Eubacteria and Eucarya. Experiments carried out by Ohnuma *et al.* (1997) and Narita *et al.* (1999) showed that FPPSs can be classified into types I and II depending on their sequences in the CLD region. If GGPP is the "pre-enzyme", type I FPPS (eukaryote) activity should have been conferred by a substitution of the amino acid at the fourth position before FARM. Regarding type II FPPS (eubacteria), replacement of the amino acid at the fifth position before FARM, insertion of two amino acids in FARM and other modifications probably occurred to change the product specificity. Moreover, Narita *et al.* (1999) suggested

that the structure of Phe in the fourth position before FARM is just as important as its size. None of the currently known natural FPPSs have Tyr or Trp at this position, and all type I FPPSs have Phe (Fig. 5). Tachibana *et al.* (2000) question the assumption that

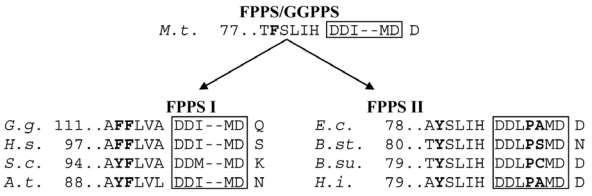

Figure 5. Hypothetical evolution from archeal FPP/GGPP synthase to the two types of FPP synthases.

FPPS I and FPPS II are respectively eukaryotic and prokaryotic FPPSs. Numbers represent position of the first amino acid indicated. Amino acids in the fourth and fifth positions before FARM (boxed) and the insertion of two amino acids in FARM are in boldface. *M.t., Methanobacterium thermoauthotrophicum; G.g., Gallus gallus; H.s., Homo sapiens; S.c., Saccharomyces cerevisiae; A.t., Arabidopsis thaliana; E.c., Escherichia coli; B.st., Bacillus stearothermophilus; B.su., Bacillus subtilis; H.i., Haemophilus influenzae.*

archeal GGPP synthases are the ancestral types for eukaryotic and bacterial FPP/GPP synthases. Recently Dhiman *et al.* (2004) identified an *ω,E,E*-farnesyl diphosphate synthase from *Mycobacterium tuberculosis* that is unique among FPPSs in that it does not contain the type I (eukaryotic) or the type II (eubacterial) *ω,E,E*-FPPS signature. It does not have four amino acids between the Asp residues of FARM. Instead, it has a structural motif similar to that of the type I GGPP synthases found in *Archaea*. Thus, the enzyme represents a novel class of *ω,E,E*-FPPS. An interesting novel molecular evolutionary approach applying genome organization analysis (intron number, size, placement and phase, and exon size) of plant terpenoid synthases was proposed by Trapp and Croteau (2001). They suggest that the ancestral terpenoid synthase gene resembled a contemporary conifer GGPPS gene.

FPPS BELONGS TO ENZYMES ENCODED BY GENE FAMILIES

FPPS is an enzyme encoded by a gene family. At least two different FPPS isoforms are found in white lupin (Attucci *et al.,* 1995), *Arabidopsis thaliana* (Cunillera *et al.,* 1996), guayule (Pan *et al.,* 1996), rice (Sanmiya *et al.,* 1999) or *Artemisia tridentata* (Hemmerlin *et al.,* 2003). Cunillera *et al.* (1997) demonstrated that one of the *A. thaliana* FPPS-encoding genes is controlled at the transcriptional level. It can be transcribed and translated into an isoform that bears a mitochondrial targeting peptide or a protein without the signal sequence. Hemmerlin *et al.* (2003) isolated from *A. tridentata* cDNA library three full-length cDNAs encoding putative isoprenoid synthases, FDS-1, FDS-2 and FDS-5 with greater than 89% similarity. One of the open reading frames, FDS-5, encodes a protein with an N-terminal amino-acid extension that was identified as a plastidial targeting peptide. FDS-1 and FDS-2 synthesize FPP as the final chain elongation product but their kinetic behaviour varies. FDS-1 prefers GPP as allylic substrate and is active at the acidic pH found in the plant cytosol dur-

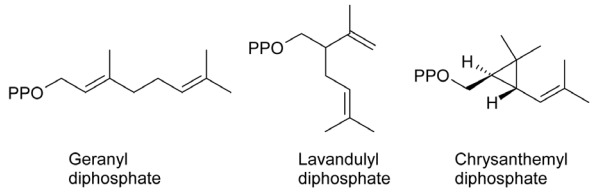

Figure 6. Monoterpene alcohols synthesized by farnesyl diphosphate synthase from *Artemisia tridentata.*

ing elicitation (Roos *et al.,*1998), whereas FDS-2 prefers DMAPP and the pH of non-elicited cells. The level of *FDS-1* transcripts was 20-fold higher than of *FDS-2* in a cDNA library from fresh shoots; thus FDS-1 might provide a high level of FPP for the biosyn-

thesis of isoprenoids needed in rapidly growing shoots, while FDS-2 might play a housekeeping function for the biosynthesis of metabolites such as phytosterols or prenylated proteins. In contrast, FDS-5 synthesizes two irregular monoterpenoids, chrysanthemyl and lavandulyl diphosphates when incubated with DMAPP and an additional product, the regular monoterpene GPP, when incubated with IPP and DMAPP (Fig. 6).

FINAL REMARKS

Even though data on FPP synthase has been accumulating for over forty years with tremendous acceleration in the last decade, many unanswered questions remain. We do not know whether the activity of FPPS is regulated at the transcriptional, translational or perhaps post-translational level. What is the interplay between hydroxy-methylglutaryl coenzyme A reductase, squalene synthase and FPPS? The cytosolic, peroxisomal and mitochondrial localization of FPPS is well established but plastidial localization is controversial. Are substrates for FPPS synthesized in the same cellular compartment or are they imported? Why is it that even with about 80% identity with two other FPP synthases from A. *tridentata,* FDS-5 synthesizes the cyclic chrysanthemyl, the branched lavandulyl and a regular monoterpene alcohols? Why does the FPPS from *M. tuberculosis* with significantly different regions from all known CLD regions still synthesize FPP? These and other questions await an answer.

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REFERENCES

Anderson MS, Yarger JG, Burck CL, Poulter CD. (1989) Farnesyl diphosphate synthetase. Molecular cloning, sequence, and expression of an essential gene from *Saccharomyces cerevisiae*. *J Biol Chem.*; **264**: 19176–84.

Ashby MN, Edwards PA. (1990) Elucidation of the deficiency in two yeast coenzyme Q mutants. Characterization of the structural gene encoding hexaprenyl pyrophosphate synthetase. *J Biol Chem.*; **265**: 13157–64.

Attucci S, Aitken SM, Gulick PJ, Ibrahim RK. (1995) Farnesyl pyrophosphate synthase from white lupin: molecular cloning, expression, and purification of the expressed protein. *Arch Biochem Biophys.*; **321**: 493–500.

Barnard GF, Popjak G. (1981) Human liver prenyltransferase and its characterization. *Biochim Biophys Acta.*; **661**: 87–99.

Blanchard L, Karst F. (1993) Characterization of a lysine-to-glutamic acid mutation in a conservative sequence of farnesyl diphosphate synthase from *Saccharomyces cerevisiae*. *Gene.*; **125**: 185–9.

Burke C, Croteau R. (2002) Interaction with the small subunit of geranyl diphosphate synthase modifies the chain length specificity of geranylgeranyl diphosphate synthase to produce geranyl diphosphate. *J Biol Chem.*; **277**: 3141–9.

Chen A, Poulter CD. (1993) Purification and characterization of farnesyl diphosphate/geranylgeranyl diphosphate synthase. A thermostable bifunctional enzyme from *Methanobacterium thermoautotrophicum. J Biol Chem.*; **268**: 11002–7.

Chen A, Kroon PA, Poulter CD. (1994) Isoprenyl diphosphate synthases: protein sequence comparisons of a phylogenetic tree, and prediction of secondary structure. *Protein Sci.*; **3**: 600–7.

Clarke CF, Tanaka RD, Svenson K, Wamsley M, Fogelman AM, Edwards PA. (1987) Molecular cloning and sequence of a cholesterol-re-

pressible enzyme related to prenyltransferase in the isoprene biosynthetic pathway. *Mol Cell Biol.*; **7**: 3138–46.

Cornforth JW, Cornforth RH, Popjak G, Yengoyan L. (1966) Studies on the biosynthesis of cholesterol. XX. Steric course of decarboxylation of 5-pyrophosphomevalonate and of the carbon to carbon bond formation in the biosynthesis of farnesyl pyrophosphate. *J Biol Chem.*; **241**: 3970–87.

Correll CC, Ng L, Edwards PA. (1994) Identification of farnesol as the non-sterol derivative of mevalonic acid required for the accelerated degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J Biol Chem.*; **269**: 17390–3.

Cunillera N, Arro M, Delourme D, Karst F, Boronat A, Ferrer A. (1996) *Arabidopsis thaliana* contains two differentially expressed farnesyl-diphosphate synthase genes. *J Biol Chem.*; **271**: 7774–80.

Cunillera N, Boronat A, Ferrer A. (1997) The *Arabidopsis thaliana* FPS1 gene generates a novel mRNA that encodes a mitochondrial farnesyl-diphosphate synthase isoform. *J Biol Chem.*; **272**: 15381–8.

Dhiman RK, Schulbach MC, Mahapatra S, Baulard AR, Vissa V, Brennan PJ, Crick DC. (2004) Identification of a novel class of omega,E,E-farnesyl diphosphate synthase from *Mycobacterium tuberculosis*. *J Lipid Res.*; **45**: 1140–7.

Eberhardt NL, Rilling HC. (1975) Prenyltransferase from *Saccharomyces cerevisiae*. Purification to homogeneity and molecular properties. *J Biol Chem.*; **250**: 863–866.

Grunler J, Ericsson J, Dallner G. (1994) Branch-point reactions in the biosynthesis of cholesterol, dolichol, ubiquinone and prenylated proteins. *Biochim Biophys Acta.*; **1212**: 259–77.

Hemmerlin A, Rivera SB, Erickson HK, Poulter CD. (2003) Enzymes encoded by the farnesyl diphosphate synthase gene family in the Big Sagebrush *Artemisia tridentata* ssp. *spiciformis*. *J Biol Chem.*; **278**: 32132–40.

Hemmi H, Noike M, Nakayama T, Nishino T. (2003) An alternative mechanism of product chain-length determination in type III geranylgeranyl diphosphate synthase. *Eur J Biochem.*; **270:** 2186–94.

Joly A, Edwards PA. (1993) Effect of site-directed mutagenesis of conserved aspartate and arginine residues upon farnesyl diphosphate synthase activity. *J Biol Chem.*; **268**: 26983–9.

Kawasaki T, Hamano Y, Kuzuyama T, Itoh N, Seto H, Dairi T. (2003) Interconversion of the product specificity of type I eubacterial farnesyl diphosphate synthase and geranylgeranyl diphosphate synthase through one amino acid substitution. *J Biochem (Tokyo).*; **133**: 83–91.

Karst F, Plochocka D, Meyer S, Szkopinska A. (2004) Farnesyl diphosphate synthase activity affects ergosterol level and proliferation of yeast *Saccharomyces cerevisiae*. *Cell Biol Int.*; **28**: 193–7.

Koyama T, Obata S, Osabe M, Takeshita A, Yokoyama K, Uchida M, Nishino T, Ogura K. (1993) Thermostable farnesyl diphosphate synthase of *Bacillus stearothermophilus*: molecular cloning, sequence determination, overproduction, and purification. *J Biochem* (*Tokyo*).; **113**: 355–63.

Koyama T, Obata S, Osabe M, Saito K, Takeshita A, Nishino T, Ogura K. (1994) Thermostable farnesyl diphosphate synthase of *Bacillus stearothermophilus*: crystallization and site-directed mutagenesis. *Acta Biochim Polon.*; **41**: 281–92.

Koyama T, Tajima M, Nishino T, Ogura K. (1995) Significance of Phe-220 and Gln-221 in the catalytic mechanism of farnesyl diphosphate synthase of *Bacillus stearothermophilus. Biochem Biophys Res Commun.*; **212**: 681–6.

Koyama T, Tajima M, Sano H, Doi T, Koike-Takeshita A, Obata S, Nishino T, Ogura K. (1996) Identification of significant residues in the substrate binding site of *Bacillus stearothermophilus* farnesyl diphosphate synthase. *Biochemistry.*; **35**: 9533–8.

Koyama T, Gotoh Y, Nishino T. (2000) Intersubunit location of the active site of farnesyl diphosphate synthase: reconstruction of active enzymes by hybrid-type heteromeric dimers of site-directed mutants. *Biochemistry.*; **39**: 463–9.

Laskovics FM, Poulter CD. (1981) Prenyltransferase; determination of the binding mechanism and individual kinetic constants for farnesylpyrophosphate synthetase by rapid quench and isotope partitioning experiments. *Biochemistry.*; **20**: 1893–901.

Lynen F, Agranoff BW, Eggerer H, Hennig U, Moslein EM. (1959) $\gamma\gamma$-Dimethyl-allyl-pyrophosphat und Geranyl-pyrophosphat, biologische Vorstufen des Squalens. *Angew. Chem.*; **21**: 657–84.

Marrero PF, Poulter CD, Edwards PA. (1992) Effects of site-directed mutagenesis of the highly conserved aspartate residues in domain II of farnesyl diphosphate synthase activity. *J Biol Chem.*; **267**: 21873–8.

Matsuoka S, Sagami H, Kurisaki A, Ogura K. (1991) Variable product specificity of microsomal dehydrodolichyl diphosphate synthase from rat liver. *J Biol Chem.*; **266**: 3464–8.

Mekkriengkrai D, Sando T, Hirooka K, Sakdapipanich J, Tanaka Y, Fukusaki E, Kobayashi A. (2004) Cloning and characterization of farnesyl diphosphate synthase from the rubber-producing mushroom *Lactarius chrysorrheus*. *Biosci Biotechnol Biochem.*; **68**: 2360–8.

Nakane H, Koyama T, Obata S, Osabe M, Takeshita A, Nishino T, Ogura K, Miki K. (1993) Crystallization and preliminary X-ray diffraction studies of *Bacillus stearothermophilus* farnesyl diphosphate synthase expressed in *Escherichia coli*. *J Mol Biol.*; **233**: 787–8.

Narita K, Ohnuma S, Nishino T. (1999) Protein design of geranyl diphosphate synthase. Structural features that define the product specificities of prenyltransferases. *J Biochem (Tokyo).*; **126**: 566–71.

Ohnuma S, Koyama T, Ogura K. (1993) Alteration of the product specificities of prenyltransferases by metal ions. *Biochem Biophys Res Commun.*; **192**: 407–12.

Ohnuma S, Nakazawa T, Hemmi H, Hallberg AM, Koyama T, Ogura K, Nishino T. (1996a) Conversion from farnesyl diphosphate synthase to geranylgeranyl diphosphate synthase by random chemical mutagenesis. *J Biol Chem.*; **271**: 10087–95.

Ohnuma S, Narita K, Nakazawa T, Ishida C, Takeuchi Y, Ohto C, Nishino T. (1996b) A role of the amino acid residue located on the fifth position before the first aspartate-rich motif of farnesyl diphosphate synthase on determination of the final product. *J Biol Chem.*; **271**: 30748–54.

Ohnuma S, Hirooka K, Hemmi H, Ishida C, Ohto C, Nishino T. (1996c) Conversion of product specificity of archaebacterial geranylgeranyl-diphosphate synthase. Identification of essential amino acid residues for chain length determination of prenyltransferase reaction. *J Biol Chem.*; **271**: 18831–7.

Ohnuma S, Hirooka K, Ohto C, Nishino T. (1997) Conversion from archaeal geranylgeranyl diphosphate synthase to farnesyl diphosphate synthase. Two amino acids before the first aspartate-rich motif solely determine eukaryotic farnesyl diphosphate synthase activity. *J Biol Chem.*; **272**: 5192–8.

Olivier LM, Krisans SK. (2000) Peroxisomal protein targeting and identification of peroxisomal targeting signals in cholesterol biosynthetic enzymes. *Biochim Biophys Acta.*; **1529**: 89–102.

Pan Z, Herickhoff L, Backhaus RA. (1996) Cloning, characterization, and heterologous expression of cDNAs for farnesyl diphosphate synthase from the guayule rubber plant reveals that this prenyltransferase occurs in rubber particles. *Arch Biochem Biophys.*; **332**: 196–204.

Plochocka D, Karst F, Swiezewska E, Szkopinska A. (2000) The role of *ERG20* gene (encoding yeast farnesyl diphosphate synthase) mutation in long dolichol forma-

tion. Molecular modeling of FPP synthase. *Biochimie.*; **82**: 733–8.

Poulter CD, Rilling HC. (1976) Prenyltransferase: the mechanism of the reaction. *Biochemistry.*; **15**: 1079–83.

Reed BC, Rilling HC. (1975) Crystallization and partial characterization of prenyltransferase from avian liver. *Biochemistry.*; **14**: 50–4.

Reilly JF, Martinez SD, Mickey G, Maher PA. (2002) A novel role of farnesyl pyrophosphate synthase in fibroblast growth factor-mediated signal transduction. *Biochem J.*; **366**: 501–10.

Roos W, Evers S, Hieke M, Tschope M, Schumann B. (1998) Shifts of intracellular pH distribution as a part of the signal mechanism leading to the elicitation of benzophenanthridine alkaloids. Phytoalexin biosynthesis in cultured cells of *Eschscholtzia californica Plant Physiol.*; **118**: 349–64.

Runquist M, Ericsson J, Thelin A, Chojnacki T, Dallner G. (1994) Isoprenoid biosynthesis in rat liver mitochondria. Studies on farnesyl pyrophosphate synthase and *trans*-prenyltransferase. *J Biol Chem.*; **269**: 5804–9.

Sanmiya K, Ueno O, Matsuoka M, Yamamoto N. (1999) Localization of farnesyl diphosphate synthase in chloroplasts. *Plant Cell Physiol.*; **40**: 348–54.

Shearer A, Hampton RY. (2005) Lipid mediated, reversible misfolding of sterol-sensing domain protein. *EMBO J.*; **24**: 149–59.

Shivdasani RA, Rosenblatt MF, Zucker-Franklin D, Jackson CW, Hunt P, Saris CJ, Orkin SH. (1995) Transcription factor NF-E2 is required for platelet formation independent of the actions of thrombopoietin/MGDF in megakaryocyte development. *Cell.*; **81**: 695–704.

Song L, Poulter CD. (1994) Yeast farnesyl-diphosphate synthase: site-directed mutagenesis of residues in highly conserved prenyltransferase domains I and II. *Proc Natl Acad Sci USA.*; **91**: 3044–8.

Stanley Fernandez SM, Kellogg BA, Poulter CD. (2000) Farnesyl diphosphate synthase. Altering the catalytic site to select for geranyl diphosphate activity. *Biochemistry.*; **39**: 15316–21.

Tachibana A, Yano Y, Otani S, Nomura N, Sako Y, Taniguchi M. (2000) Novel prenyltransferase gene encoding farnesyl-geranyl diphosphate synthase from a hyperthermophilic archaeon, *Aeropyrum pernix.* Molecular evolution with alteration in product specificity. *Eur J Biochem.*; **267**: 321–8.

Tarshis LC, Yan M, Poulter CD, Sacchettini JC. (1994) Crystal structure of recombinant farnesyl diphosphate synthase at 2.6-Å resolution. *Biochemistry.*; **33**: 10871–7.

Tarshis LC, Proteau PJ, Kellogg BA, Sacchettini JC, Poulter CD. (1996) Regulation of product chain length by isoprenyl diphosphate synthases. *Proc Natl Acad Sci USA.*; **93**: 15018–23.

Trapp SC, Croteau RB. (2001) Genomic organization of plant terpene synthases and molecular evolutionary implications. *Genetics.*; **158**: 811–32.

Wilkin DJ, Kutsunai SY, Edwards PA. (1990) Isolation and sequence of the human farnesyl pyrophosphate synthetase cDNA. Coordinate regulation of the mRNAs for farnesyl pyrophosphate synthetase, 3-hydroxy-3-methylglutaryl coenzyme A reductase, and 3-hydroxy-3-methylglutaryl coenzyme A synthase by phorbol ester. *J Biol Chem.*; **265**: 4607–14.

Yeh LS, Rilling HC. (1977) Purification and properties of pig liver prenyltransferase: interconvertible forms of the enzyme. *Arch Biochem Biophys.*; **183**: 718–25.