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Enzymatic Aspects of Isoprenoid Chain Elongation

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I. Introduction

In the biosynthesis of isoprenoid compounds, which include numerous structurally different natural products, all of the carbon backbones are derived from linear prenyl diphosphates. These prenyl chains are constructed by the action of a group of enzymes commonly called "prenyltransferases". The reactions catalyzed by prenyltransferases are very unique and interesting from mechanistic viewpoints. The reaction starts with elimination of the diphosphate ion from an allylic diphosphate to form an allylic cation, which is attacked by the isopentenyl diphosphate (IPP) molecule, with stereospecific removal of a proton to form a new C−C bond and a new double bond in the product (Figure 1). By repeating this type of condensation between IPP and the allylic prenyl diphosphate product, prenyltransferase can synthesize a prenyl diphosphate with a certain length and stereochemistry fixed by its specificity. The chain length of prenyl diphosphates varies so widely that it ranges from geraniol (C_{10}) to natural rubber whose carbon chain length extends to several millions. To date 16 prenyltransferases with different catalytic functions have been characterized.

In a wide sense, prenyltransferase should involve any enzymes that catalyze the transfer of prenyl groups to acceptors that include not only IPP but also aromatic compounds and proteins etc. In this review, however, "prenyltransferase" is used in a narrow sense so that it means the prenyltransferase whose acceptor is IPP. In other words, "prenyltransferase" is equivalent to "prenyl diphosphate synthase".

II. Background

The study of isoprenoid biosynthesis was brought to an enzymological level by the discovery of IPP as the true biologically active isoprene unit, which was made independently by the groups of Lynen[1] and of Bloch[2] in 1958. The discovery[3] of IPP isomerase, which converts IPP to dimethylallyl diphosphate (DMAPP), as well as the prenyltransferase that catalyzes the head-to-tail condensations of IPP with

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Kyozo Ogura was born in 1933 in Mie, Japan, and graduated from Tohoku University in 1957, where he also obtained his doctorate in 1962. He became a research associate in 1962 at Tohoku University and was a postdoctoral fellow at Harvard University with Professor Konrad Bloch (1965–1967). He was promoted to Associate Professor in 1974 and to Professor in 1980 at the Chemical Research Institute of Non-Aqueous Solutions (renamed as Institute for Chemical Reaction Science in 1991), Tohoku University. He has been involved in enzymatic studies of isoprenoid biosynthesis. He received the Japanese Biochemical Society Award for Young Biochemists in 1972, Oence Lager Award of the Chemical Society of Japan in 1986, and Japan Synthetic Organic Chemistry Society Award in 1996. In 1997 he retired from Tohoku University and was given the title of Emeritus Professor of the same University.

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DMAPP and with geranyl diphosphate (GPP), to form (*E*,*E*)-farnesyl diphosphate (FPP) has led to the recognition that Ruzicka's hypothetical "active isoprene" [4] is the true biological building block for the tremendous number of isoprenoid compounds in nature.

In the following years, most of the stereochemical details of the biosynthetic pathway from mevalonic acid to squalene, including the FPP synthase reaction, have been established by Cornforth and Popják[5–7] in their beautifully designed experiments with supernatant fractions from mammalian liver or yeast. These works have been reviewed extensively.[8,9]

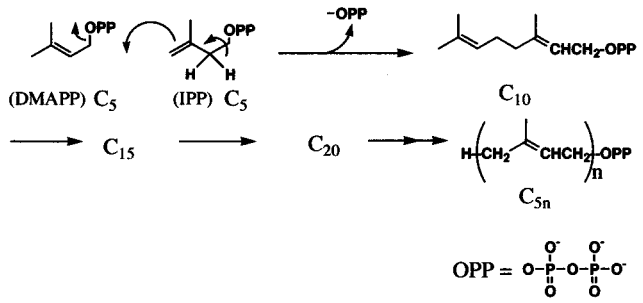

Figure 1. Reactions catalyzed by prenyltransferases.

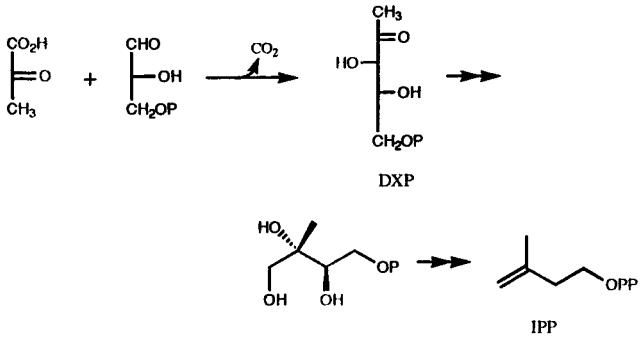

Figure 2. Hypothetical biogenetic scheme for the mevalonate-independent pathway for IPP synthesis.

The biosynthesis of IPP has long been assumed to proceed exclusively via the acetate/mevalonate pathway.[10–12] Recently, however, Rhomer et al.[13,14] have discovered a novel pathway to IPP in which mevalonate is not involved. On the basis of labeling patterns of bacterial hopanoids and ubiquinones derived from ^{13}C-labeled metabolites of glycolysis as well as [^{13}C]acetate, they have proved that glyceraldehyde 3-phosphate occupies the branch point of the glycolysis and the non-mevalonate pathway to IPP. They have also proposed a mechanism of the formation of IPP involving 1-deoxyxylulose 5-phosphate (DXP) yielded by the condensation between glyceraldehyde 3-phosphate and a C-2 unit resulted from thiamine diphosphate dependent decarboxylation of pyruvate (Figure 2). It has also been shown that the non-mevalonate pathway is operative in the biosynthesis of not only particular isoprenoids in bacteria but also of some terpenoids in plants[15] and algae.[16] Enzymatic and molecular biological elucidation of this pathway is expected.

From the mid 1970s the substrate specificities of prenyltransferases and IPP isomerase have been investigated extensively in our laboratory at Tohoku University and Popják's laboratory at UCLA. These studies indicated that FPP synthase utilizes a broad spectrum of artificial substrates to produce analogues of GPP or FPP. Prenyltransferase reactions with some of the artificial substrates have also been effectively applied for stereospecific syntheses of biologically active compounds.[17–19] Poulter and Rilling[20] at the University of Utah explored the reaction mechanisms of prenyltransferases by using fluorinated substrate analogues, and they have established the "ionization−condensation−elimination mechanism".

In 1987, a cDNA for rat liver FPP synthase was isolated by Clarke et al.[21] This was the first intro-

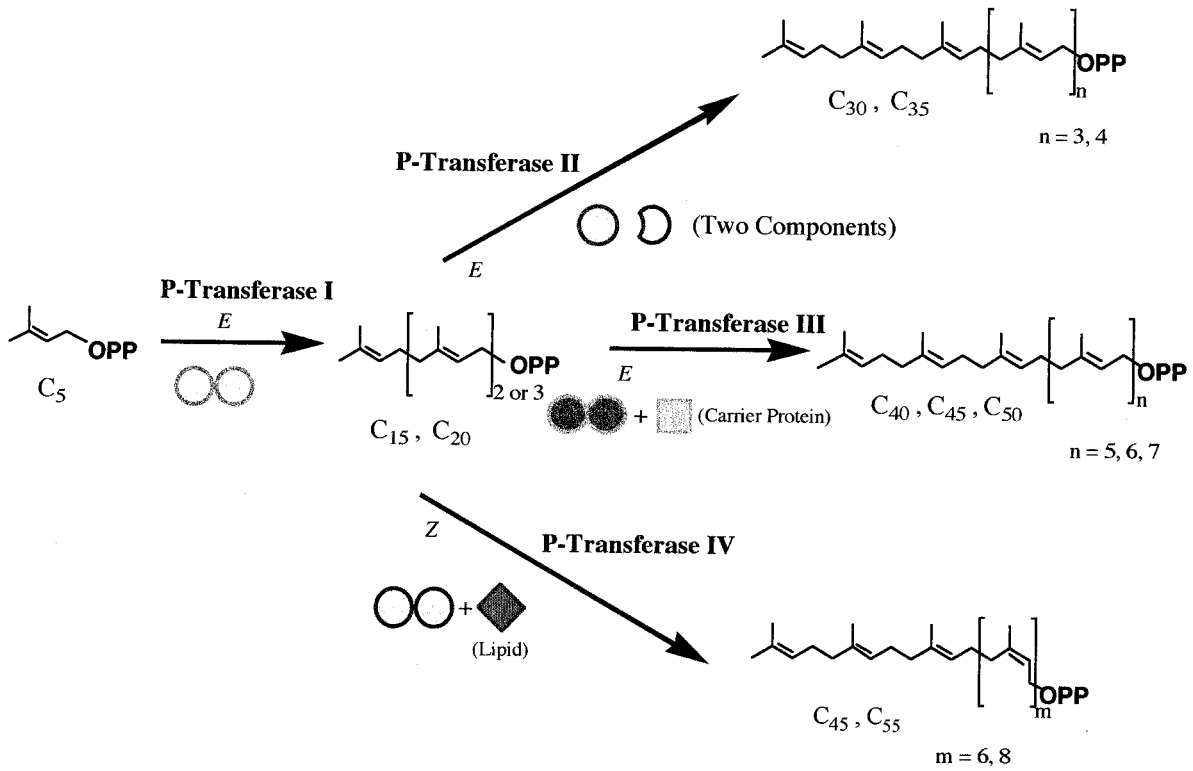

Figure 3. Classification of prenyltransferases.

duction of the molecular biological approach to the field of prenyltransferases and related enzymes. During the past 10 years, the genes or cDNAs for FPP- and geranylgeranyl diphosphate (GGPP) synthases of various organisms were identified and characterized. In the meantime, the hexaprenyl diphosphate synthase gene of *Saccharomyces cerevisiae*[22] and several bacterial genes encoding prenyltransferases that catalyze the synthesis of polyprenyl diphosphates were cloned. Two years later, after the cloning of rat liver FPP synthase gene,[21] the gene for IPP isomerase of *S. cerevisiae* was cloned by Anderson et al.[23] Six kinds of IPP isomerase genes or cDNAs have also been identified from human to bacteria.

In 1994, the three-dimensional structure of prenyltransferase was determined for the first time with FPP synthase of chicken liver by Poulter and Sacchettini.[24]

III. Occurrence and Classification of Prenyl Diphosphate Synthases

The prenyl chain elongation catalyzed by prenyltransferases is interesting in that the reaction proceeds consecutively and terminates precisely at discrete chain lengths according to the specificities of the enzymes. The chain length of products varies so widely that it ranges from geraniol (C_{10}) to natural rubber ($C_{>5000}$). Up to now 16 kinds of prenyltransferases with different catalytic functions have been characterized.

Despite the similarity of the reactions catalyzed by them, their properties are so different that they can be classified into four major groups according to the mode of subunit composition as well as the prenyl chain length and stereochemistry of the final product as depicted in Figure 3.

A. Short-Chain Prenyl Diphosphate Synthases (Prenyltransferase I)

Short-chain prenyl diphosphate synthases including FPP- (geranyl*trans*transferase, [EC 2.5.1.10]) and GGPP synthases (farnesyl*trans*transferase, [EC 2.5.1.30]) require no cofactor except divalent metal ions such as Mg^{2+} or Mn^{2+}, which are commonly required by all prenyltransferases.

Organisms constitutively contain at least one of these short-chain prenyl diphosphate synthases for the production of prenyl diphosphates which act as the priming substrates for the other groups of prenyltransferases. The short-chain prenyl diphosphates are also biosynthetic precursors of various isoprenoids including steroids, carotenoids, and prenylated proteins. It is noteworthy that GGPP synthase occurs not only in plants and bacteria but also in mammals.

The prenyltransferases in this group have been studied most extensively. Each enzyme of this group has a homodimeric structure in which the subunits are tightly bound to each other.

Geranyl diphosphate (GPP) synthase (dimethylallyl*trans*transferase, [EC 2.5.1.1.]) exists in higher plants producing monoterpenes. This synthase has been purified from *Lithospermum erythrorhizon* cell culture,[25] *Salvia officinalis* leaves,[26] and several higher dicotyledon plant leaves.[27] Farnesylgeranyl diphosphate (FGPP) synthase has also been purified from a haloalkaliphilic archaeon *Natronobacterium pharaonis*.[28]

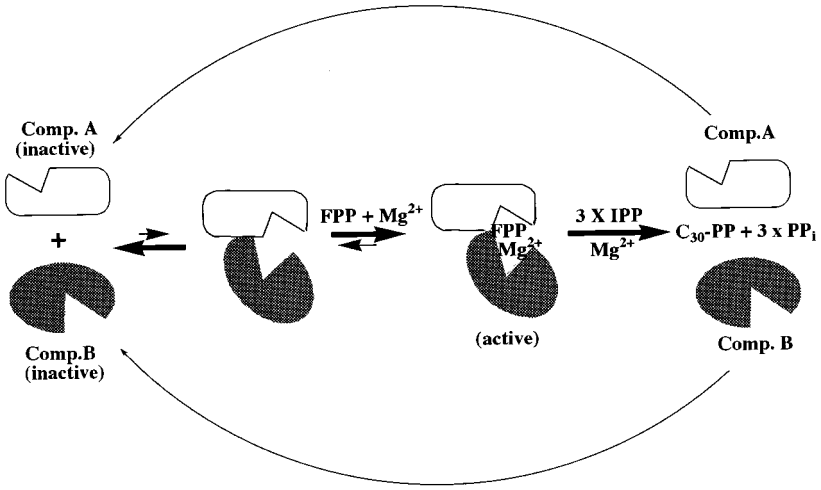

Figure 4. Mechanism of the dynamic formation and dissociation of the ternary complex of the heteromeric components of HexPP synthase.

B. Medium-Chain Prenyl Diphosphate Synthases (Prenyltransferase II)

Bacteria are good sources for prenyltransferases especially for the synthesis of long-chain prenyl diphosphates, because they produce menaquinones or ubiquinones having polyprenyl side chains whose chain lengths vary in a species-specific manner.[29] Thus, it was of interest to see whether there exists enzymes corresponding to the variety of the chain length of quinone side chains. It was also important to explore bacterial enzymes, because (*Z*,*E*)-undecaprenyl phosphate is known as a lipid carrier of glycosyl transfer in the biosynthesis of cell wall polysaccharide components in bacteria.

During the search for prenyltransferases in bacteria, we found that the C_{30} chain elongation is catalyzed by hexaprenyl diphosphate (HexPP) synthase (*trans*-pentaprenyl*trans*transferase, [EC 2.5.1.33]), which has a novel subunit system. It catalyzes the synthesis of (*all-E*)-HexPP by adding three molecules of IPP to FPP, but it cannot catalyze the steps of synthesis of GPP or FPP from DMAPP and IPP ($C_5 \rightarrow C_{10} \rightarrow C_{15}$).[30] This enzyme occurs in *Micrococcus luteus* B-P 26, which produces menaquinone-C_{30}. Similarly, heptaprenyl diphosphate (HepPP) synthase (*trans*-hexaprenyl*trans*transferase, [EC 2.5.1.30]), has been found in *Bacillus subtilis*, which produces exclusively menaquinone-C_{35}.[31] It catalyzes the synthesis of (*all-E*)-HepPP by adding four molecules of IPP to FPP, but it is not able to catalyze the $C_5 \rightarrow C_{10} \rightarrow C_{15}$ reactions either. FPP is supplied by FPP synthase, which occurs in all bacteria.

The purification of the HexPP synthase involved unexpected difficulties, due not only to the unexpected substrate specificities, but also to the fact that the synthesis of HexPP requires two separable proteins, neither of which is catalytically active alone.[30] Therefore, the HexPP synthase had been elusive before this fact was disclosed. Similarly two different proteins constituting HepPP synthase were separated and characterized from *B. subtilis*.[32] The components of HexPP synthase and HepPP synthase are so specific that neither of the two components of one enzyme is exchangeable with that of the other enzyme. Therefore, they appear to be novel heterodimeric enzymes with subunits easily dissociable under physiological conditions.

To obtain insight into the dynamic mechanism of the cooperative interaction between the components A and B of the HexPP synthase, we studied the properties of the heteromeric HexPP synthase using Superose 12 gel filtration.[33] Components A and B, when mixed without substrates, gave two elution peaks at 24 and 27 kDa positions, respectively. These eluates showed no enzyme activity. However, when a mixture of components A and B which had been incubated in the presence of Mg^{2+} and FPP, was filtered on Superose 12, a protein fraction which showed HexPP synthase activity was eluted at a position of approximately 50 kDa, suggesting that this fraction corresponds to a complex, A−B substrate, which probably represents a catalytically active state of the enzyme. Formation of a complex of components A and B was also observed in the presence of a relatively high concentrations of PPi or one of the substrates, IPP and FPP.[34] These results have led us to propose a mechanism of this unusual enzyme system as shown in Figure 4. In the presence of Mg^{2+} and FPP, components A and B exist as a ternary complex of the two components and FPP + Mg^{2+}, which is catalytically active as HexPP synthase. After three molecules of IPP is condensed to produce HexPP, which is water-insoluble, the complex dissociates into components A and B with a concomitant release of HexPP and PPi. This might account for the ability of these soluble proteins to exert an efficient turnover in the synthesis of amphipathic molecules from water-soluble substrate without association with membrane components. By inactivation studies using Cys- and Arg-specific reagents, we have shown that the catalytic site of the HexPP synthase is formed by cooperative interaction between the two components and that Cys and Arg residues on component B play important roles in the catalytic activity.[35]

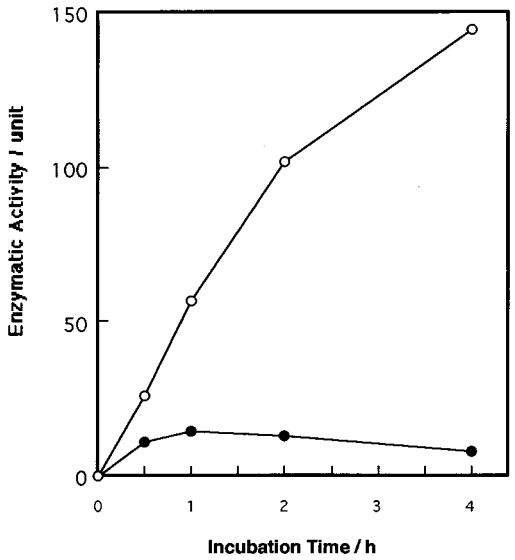

Figure 5. Time course of SPP synthase reaction. Reaction with purified *M. luteus* SPP synthase in the presence (○) or absence (●) of a high molecular component of a soluble fraction of the bacterium.

C. Long-Chain (*E*)-Prenyl Diphosphate Synthases (Prenyltransferase III)

By analogy with HexPP- and HepPP synthases, it was predicted that the enzymes for the synthesis of polyprenyl diphosphate with chain lengths longer than C_{35} might also be composed of two-component systems. However, this is not the case. (*E*)-Nonaprenyl (solanesyl) diphosphate (SPP) synthase (*trans*-octaprenyl*trans*transferase, [EC 2.5.1.11]) was isolated from *M. luteus*[36] as an enzyme catalyzing the chain elongation up to C_{45}-PP from GPP (C_{10}), and it was found that this enzyme is a homodimeric protein that is functionally active by itself.[37] In this respect, it resembles the enzymes in the group of prenyltransferase I (see section III.A), but it differs in that it requires a protein factor to maintain efficient catalytic turnover. This protein factor was separated as a high-molecular component of a soluble fraction of the same bacterium. It stimulates dramatically the enzymatic synthesis of SPP in a time-dependent manner as shown in Figure 5, but it does not affect the stability of the enzyme.[37] Bovine serum albumin or a detergent such as Tween 80 can substitute for this protein, showing a similar mode of stimulation. These results suggest that the protein facilitates catalytic turnover by removing from the active site hydrophobic products which otherwise would inhibit the reaction.

Bacterial (*all-E*)-octaprenyl PP (C_{40}) synthase and (*all-E*)-decaprenyl PP (C_{50}) synthase have been found and partially purified from *E. coli*[38] and *Paracoccus denitrificans*,[39] respectively. These polyprenyl diphosphate synthases have similar properties to those of the SPP synthase described above. The protein from *M. luteus* that stimulates the SPP synthase acts with these synthases as well.[37] However, the same protein shows no stimulation on *E. coli* undecaprenyl diphosphate synthase, which catalyzes the synthesis of (*Z*)-prenyl chains (see section III.D). Therefore, this protein is effective specifically on (*all-E*)-polyprenyl diphosphates with chain lengths of C_{40}, C_{45}, and C_{50}.

D. (*Z*)-Polyprenyl Diphosphate Synthases (Prenyltransferase IV)

Prenyltransferases that catalyze the synthesis of (*Z*)-prenyl chains occur more or less in association with the membrane fraction of bacterial cells and the microsomal fraction of eukaryotic cells. Their roles are to produce the precursors of polyprenyl lipids required as carbohydrate carriers in the biosynthesis of bacterial cell wall and of eukaryotic glycoproteins.

Undecaprenyl diphosphate (UPP) synthase (*di-trans,poly-cis*-decaprenyl*cis*transferase, [EC 2.5.1.31]) catalyzes the construction of a (*Z*)-prenyl chain onto the (*all-E*)-farnesyl moiety to yield a C_{55}-prenyl diphosphate with *E,Z* mixed stereochemistry. This enzyme has been partially purified and characterized from several bacteria including *M. luteus*,[40] *B. subtilis*,[41] *E. coli*,[38] and *Lactobacillus plantarum*.[42−44] The *L. plantarum* enzyme has been purified and studied most extensively by Muth and Allen.[45] It is associated with periplasmic membranes so loosely that it is easily solubilized, but it requires for activity a phospholipid or a detergent such as Triton X-100.[45] The enzyme was shown to be an acidic protein (pI = 5.1) with a molecular size of 56 kDa. SDS−PAGE analysis indicated that this enzyme is composed of two 30 kDa subunits.

It is interesting that *P. denitrificans*, which is assumed to be an ancestor of mitochondria,[46] has (*all-E*)-decaprenyl diphosphate (C_{55}) synthase and (*all-E*)-farnesyl-(*all-Z*)-hexaprenyl (C_{45}) diphosphate synthase[47] instead of UPP synthase, which is common in general bacteria.

Most plants contain large amounts of polyprenols with *E,Z* mixed stereochemistry, including betulaprenols and ficaprenols. From mulberry leaves, *Morus bombysis*, we partially purified a polyprenyl diphosphate synthase that catalyzes consecutive condensations of IPP with GGPP as an allylic primer to produce a series of ficaprenol-type *Z,E* mixed polyprenyl diphosphates with carbon chain length ranging from C_{40} to C_{60}.[48] FPP and GPP are also accepted as allylic primers, and Triton X-100 stimulates the enzymatic activity.

The stereochemistry of (*E*) medium- and (*Z*) long-chain prenyltransferase reactions has been demonstrated, using the *B. subtilis* enzymes, as shown in Figure 6.[49] The stereochemical courses of the *E* or *Z* chain elongations are similar to each other in that the C−C bond is formed on the same side as the C−H bond that is cleaved. The stereochemistry of HepPP synthase reaction is the same as demonstrated for mammalian FPP synthase by Cornforth et al.[5−7]

The (*Z*)-polyprenyl diphosphate synthase in eucaryotic cells is dehydrodolichyl diphosphate synthase, which catalyzes much longer chain elongations than do bacterial enzymes. This enzyme has been solubilized and characterized from the membrane fractions of Ehrlich ascites tumor cells,[50] rat tubulin,[51] *Saccharomyces carlsbergensis*,[52] and rat liver.[53] The chain length composition of the reaction products is the same as that of dolichols in each organism.

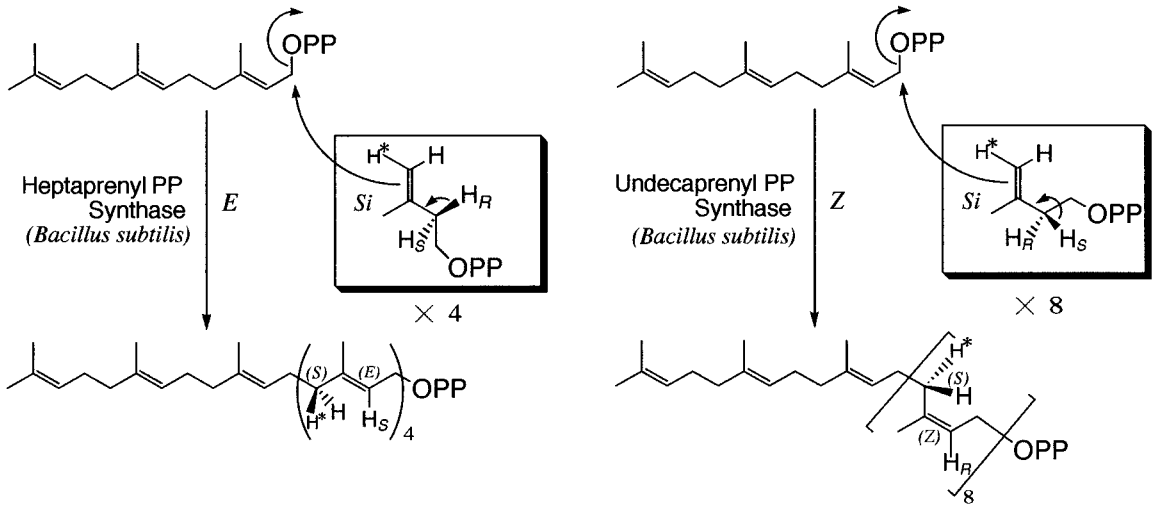

Figure 6. Absolute stereochemistry of (*E*) medium- (left) and (*Z*) long-chain (right) prenyl diphosphate synthase reactions.

Dolichols, which have saturated α-isoprene units, play important roles as the sugar carriers in cotranslational, N-linked glycoprotein biosynthesis.

Rubber transferase (rubber *cis*-polyprenyl*cis*transferase, [EC 2.5.1.20]) has been found in and characterized from *Hevea brasiliensis* by Archer et al.[54] and from Guayule, *Parthenium argentatum* by Cornish and Backhaus.[55] By analogy with the other prenyltransferases, it is believed that rubber transferase catalyzes the transfer of (*Z*)-polyprenyl diphosphates to IPP with elimination of PPi. However, the rubber transferase activity is formed only at the surface of the rubber particles. This activity is not diminished even after repeated washing, demonstrating a firm association of the enzyme with the particles.

Table 1 shows a comparative listing of typical prenyltransferases.

IV. Structures of (all-E)-Prenyl Diphosphate Synthases

A. Farnesyl Diphosphate Synthase

1. Gene Cloning and Overproduction

FPP synthase is one of the key enzymes responsible for cholesterol biosynthesis and exhibits changes in the level of its activity in response to the level of cholesterol in mammalian cells. Taking advantage of this fact, Clarke et al.[21] succeeded in isolating a cDNA for rat liver FPP synthase. It is noteworthy that the 30 amino acid "active site peptide" of chicken liver FPP synthase, which Rilling and co-workers[56,57] had identified by photoaffinity labeling with *o*-azidophenylethyl diphosphate, contributed to the identification of the gene. Since then, cDNAs or genomic clones encoding FPP synthase have been isolated from various organisms, including human,[58,59] yeast,[60] *E. coli*,[61] *Bacillus stearothermophilus*,[62] white lupin,[63] *Arabidopsis thaliana*,[64] and rubber tree.[65] All these FPP synthases are homodimeric enzymes of subunit sizes from 32 to 44 kDa. We have succeeded in overproduction of FPP synthase from *B. stearothermophilus* in *E. coli* cells and its purification.[62] Overproducing strains are able to produce thermostable FPP synthase in an amount equivalent to ~20% of the total soluble proteins. This recombinant enzyme is purified easily because of its thermostability and abundance. The enzyme is not inactivated even after treatment at 65 °C for 1 h. Song and Poulter[66] have succeeded in overproduction and purification of a recombinant yeast FPP synthase as a fusion protein having an α-tubulin epitope at the C-terminal, which enabled an efficient purification by immunoaffinity chromatography. Similarly, recombinant FPP synthases of rat liver,[67] human liver,[68] and avian liver[24] were overproduced and purified.

2. Crystallization and Three-Dimensional Structure of FPP Synthase

The first prenyltransferase that was purified to homogeneity was the FPP synthase from *S. cerevisiae*.[69] Crystallization of FPP synthase was first achieved for avian liver enzyme by Reed and Rilling in the same year of 1975.[70] After nearly 20 years, *B. stearothermophilus* FPP synthase was crystallized and a preliminary X-ray diffraction analysis was carried out to about 3 Å resolution.[71] Meanwhile, Sachettini and Poulter and their co-workers[24] made a great contribution to this field of study by succeeding in the crystal structure determination of avian FPP synthase to 2.6 Å resolution. This is the first three-dimensional structure for any prenyltransferases. Strikingly, the FPP synthase has a novel folding structure composed of all α-helices joined by connecting loops. They have shown that 10 core helices arrange around a large central cavity whose wall carries highly conserved amino acid residues which are assumed to be essential for catalytic function as described in later sections.

3. Comparative Primary Structures of FPP Synthases

Comparison of the amino acid sequences not only of FPP synthases from various organisms but also of GGPP synthase from *Neurospora crassa*[72] and HexPP synthase from *S. cerevisiae*[22] revealed the presence of seven conserved regions[62] as shown in Figure 7. The aspartate-rich motifs conserved in region II ($D^1D^2xxD^3xxxxR^1R^2G$) and in region VI

Table 1. Properties of Typical Prenyl Diphosphate Synthases[a]

prenyl-PP synthase	organism	class	enlongation range	subunit structure	size/monomer (kDa)	cation requirement	p*I*	activator	kinetic parameters	ref(s)
GPP synthase	*L. erythrorhizon*	I	$C_5 \rightarrow E \rightarrow C_{10}$	n.d.	73	Mg^{2+}, Mn^{2+}	4.95		K_m^{DMAPP} 83 μM, K_m^{IPP} 14 μM	25
FPP synthase	*S. cerevisiae*	I	$C_5 \rightarrow E \rightarrow C_{15}$	homodimer	40	Mg^{2+}	n.d.		K_m^{GPP} 6.7 μM, K_m^{IPP} 3.4 μM	66, 69
FPP synthase	rat liver	I	$C_5 \rightarrow E \rightarrow C_{15}$	homodimer	39	Mg^{2+}, Mn^{2+}	n.d.		K_m^{GPP} 1.0 μM, K_m^{IPP} 2.7 μM	67
FPP synthase	*B. stearothermophilus*	I	$C_5 \rightarrow E \rightarrow C_{15}$	homodimer	32	Mg^{2+}, Mn^{2+}	n.d.	K^+, NH_4^+	K_m^{DMAPP} 13.3 μM, K_m^{IPP} 16.2 μM	62, 97
GGPP synthase	*M. thermoautorophicum*	I	$C_5 \rightarrow E \rightarrow C_{15}$, C_{20}	homodimer	38	Mg^{2+}, Mn^{2+}	n.d.		K_m^{GPP} 105 μM, K_m^{IPP} 167 μM	79
GGPP synthase	bovine brain	I	$C_{15} \rightarrow E \rightarrow C_{20}$	homooligomer	37.5	Mg^{2+}, Mn^{2+}	6.2		K_m^{FPP} 0.74 μM, K_m^{IPP} 2 μM	111
GGPP synthase	*S. acidocaldarius*	I	$C_5 \rightarrow E \rightarrow C_{20}$	homodimer	37	Mg^{2+}	n.d.	K^+	K_m^{GPP} 6.7 μM, K_m^{IPP} 3.4 μM	80
FGPP synthase	*N. pharaonis*	I	$C_{20} \rightarrow E \rightarrow C_{25}$	n.d.	76	Mg^{2+}	n.d.		K_m^{FPP} 1.4 μM, K_m^{IPP} 25 μM	28
HexPP synthase	*M. luteus* B-P 26	II	$C_{15} \rightarrow E \rightarrow C_{30}$	heterodimer	17, 37	Mg^{2+}	n.d.		K_m^{FPP} 3.96 μM, K_m^{IPP} 3.50 μM	30, 35, 87
HepPP synthase	*B. stearothermophilus*	II	$C_{15} \rightarrow E \rightarrow C_{35}$	heterodimer	27, 36	Mg^{2+}	6.2	K^+, NH_4^+, TX-100	K_m^{FPP} 7.14 μM, K_m^{IPP} 16.7 μM	84, 89
HepPP synthase	*B. subtilis*	II	$C_{15} \rightarrow E \rightarrow C_{35}$	heterodimer	29, 36	Mg^{2+}, Mn^{2+}	5.1,5.2			31, 112
OctPP synthase	*E. coli*	III	$C_{15} \rightarrow E \rightarrow C_{40}$	homodimer	n.d.	Mg^{2+}	6.2	PCP?	n.d.	93
SPP synthase	*M. luteus*	III	$C_{15} \rightarrow E \rightarrow C_{45}$	homodimer	34	Mg^{2+}	n.d.	PCP	K_m^{FPP} 0.75 μM, K_m^{IPP} 17 μM	37
DecPP synthase	*P. denitrificans*	III	$C_{15} \rightarrow E \rightarrow C_{50}$	homodimer	36	Mg^{2+}	n.d.	K^+, NH_4^+, TX-100	K_m^{FPP} 0.06 μM	39, 95
NonaPP synthase	*P. denitrificans*	IV	$C_{15} \rightarrow Z \rightarrow C_{45}$	n.d.	n.d.	Mg^{2+}	n.d.	TX-100		47
UPP synthase	*L. plantarum*	IV	$C_{15} \rightarrow Z \rightarrow C_{55}$	homodimer	30	Mg^{2+}	5.1	TX-100	K_m^{FPP} 0.13 μM, K_m^{IPP} 1.92 μM	43−45
UPP synthase	*M. luteus* B-P 26	IV	$C_{15} \rightarrow Z \rightarrow C_{55}$	homodimer	29	Mg^{2+}	n.d.	TX-100	n.d.	110
DeDolPP synthase	*S. carlsbergensis*	IV	$C_{15} \rightarrow Z \rightarrow C_{80}$	n.d.	n.d.	Mg^{2+}	n.d.	TX-100	K_m^{FPP} 13.0 μM, K_m^{IPP} 0.54 μM	52

[a] Abbreviations: n.d., not determined; TX-100; Triton X-100; PCP, prenyl carrier protein; NonaPP, (*all-E*)-farnesyl-(*all-Z*)-hexaprenyl diphosphate; DeDolPP, dehydrodolichyl diphosphate.

(GxxFQxxD[4]D[5]xxD[6]) are the most characteristic for prenyltransferases.

B. Geranylgeranyl Diphosphate Synthase

In 1990, Armstrong et al.[73] determined the nucleotide sequence of three genes from the carotenoid biosynthesis gene cluster of *Erwinia herbicola*, a nonphotosynthetic bacterium, which encode homologues of the CrtB, CrtE, and CrtI proteins of *Rhodobacter capsulatus*, a purple nonsulfur photosynthetic bacterium.[74] These three proteins were thought to be involved in the early stage of carotenoid biosynthesis, because both organisms were expected to possess similar enzymes for synthesis and dehydrogenation of phytoene, the common precursor of all C_{40} carotenoids.

Meanwhile, Nelson et al.[75] isolated the gene encoding GGPP synthase from the filamentous fungus *N. crassa* by complementation of the Albino 3 (*al-3*) mutant, which was defective in GGPP synthase. By analyzing the nucleotide sequence of the *al-3* gene, Carattoli et al.[72] found that it showed significant homologies in three different regions including the Asp-rich motifs.

The *crtE* gene in *E. herbicola* was assigned for GGPP synthase by Math et al.[76] They isolated the *crtE* gene from *E. herbicola* cluster by PCR amplification and cloned the coding region into the *E. coli* expression vector pARC306N. The cell-free extracts from the *E. coli* transformants clearly showed GGPP synthase activity, indicating that *crtE* encodes GGPP synthase. Similarly the *crtE* gene of *E. uredovora* was assigned as the GGPP synthase gene by Sandman and Misawa.[77]

In this way, cDNAs or genomic clones encoding GGPP synthase have been isolated from various organisms including *Capsicum annuum*,[78] *M. thermoautotrophicum*,[79] *Sulfolobus acidocaldarius*,[80] *A. thaliana*,[81] white lupin,[82] and *S. cerevisiae*.[83]

C. Heptaprenyl Diphosphate Synthase

On the basis of the highly conserved amino acid sequences of prenyltransferases as well as the thermostability of HepPP synthase of a thermophilic bacterium *B. stearothermophilus*, we succeeded in identifying the genes encoding two protein components that constitute a medium-chain prenyl diphosphate synthase.[84] One of the two proteins constituting an enzyme system for the synthesis of HepPP synthase has 323 amino acid residues and shows a 32% sequence similarity to the FPP synthase of the same bacterium.[62] This protein designated as component II′ has seven highly conserved regions that have been shown typical in prenyltransferases. The other protein, designated as component I′, which is composed of 220 amino acids, has no such similarity, nor has any similar protein been found within the protein entries in databases. Therefore, it seems likely that the former carries the active sites for substrate binding and catalysis, while the latter plays an auxiliary but essential role in the catalytic function.

Protein database search for amino acid sequences similar to that of the component II′ of the HepPP

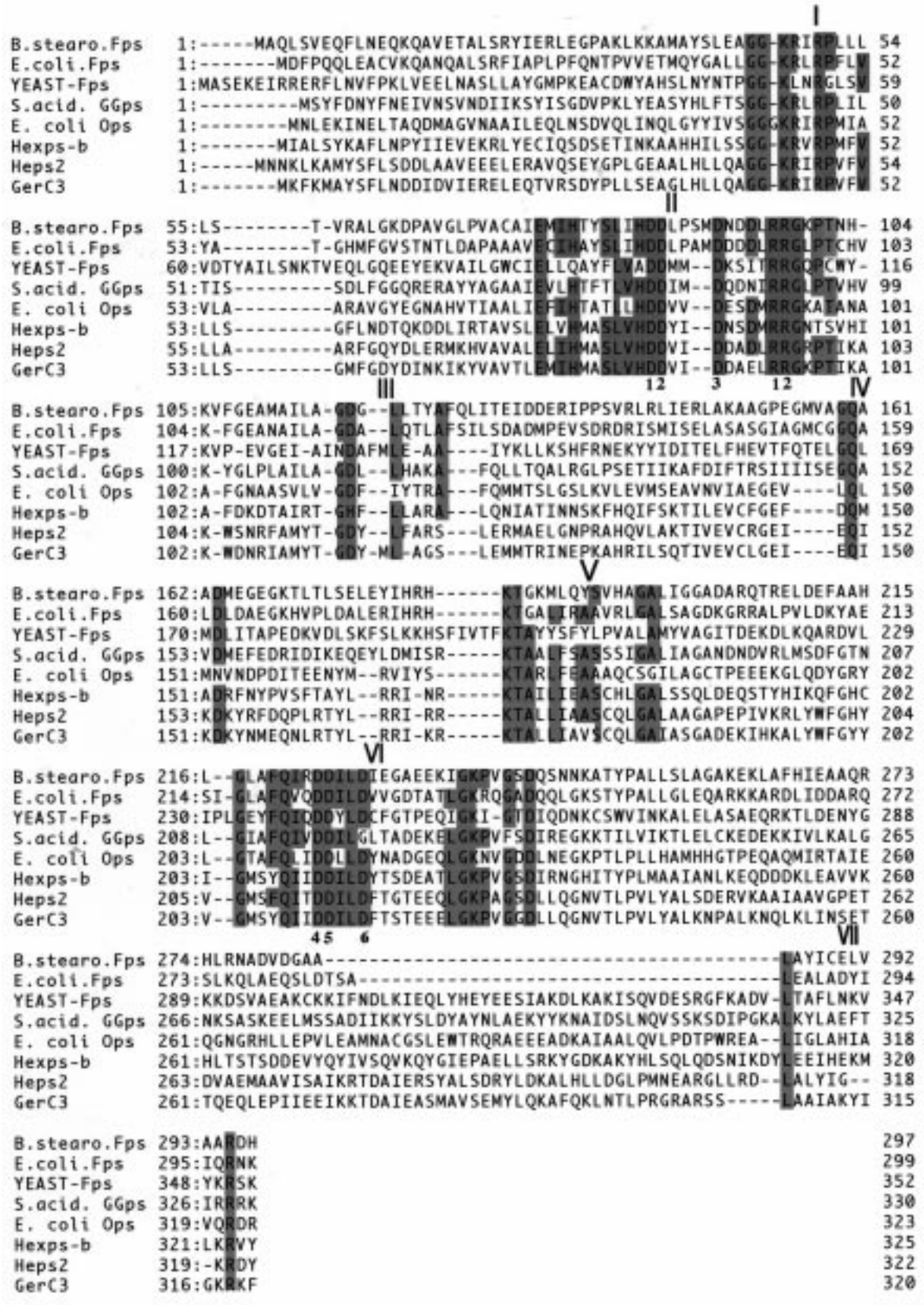

Figure 7. Amino acid sequence alignment of various prenyltransferases: B. stearo. Fps, FPP synthase of *B. stearothermophilus*;[62] E. coli Fps, FPP synthase of *E. coli*;[61] YEAST-Fps, FPP synthase of *S. cerevisiae*;[60] S. acid. GGps, GGPP synthase of *S. acidocaldarius*;[80] E. coli Ops, OctPP synthase of *E. coli*;[93] Hexps-b, component B of HexPP synthase of *M. luteus* B-P 26;[87] Heps2, component II′ of HepPP synthase of *B. stearothermophilus*;[84] GerC3, component II of HepPP synthase of *B. subtilis*.[86] The conserved amino acid residues in the seven conserved regions I to VII are shaded. The numbers at the bottom of the conserved Asp or Arg residues in regions II and VI correspond to the notation in the text (see section IV.A.3).

synthase from *B. stearothermophilus*[84] yielded the GerC3 protein of *B. subtilis*, which is encoded in a cluster of three open reading frames, *gerC1*, *gerC2*, and *gerC3*, and have been shown to be involved in vegetative cell growth and spore germination of this bacterium.[85] Two of the *gerC* products, GerC1 and GerC3 showed 38% and 65% identities with those of the component I′ and component II′, respectively, which constitute the HepPP synthase of *B. stearothermophilus*. To confirm that the *gerC* gene region encodes the HepPP synthase, we amplified the *gerC* gene by PCR and examined expression of prenyltransferase activities. When the two proteins were mixed, the HepPP synthase activity was detected, indicating that the HepPP synthase of *B. subtilis* is composed of the two dissociable components encoded by *gerC1* and *gerC3*.[86] Therefore, the proteins GerC1 and GerC3 exactly coincide with the two dissociable components I and II of *B. subtilis* HepPP synthase, respectively.

D. Hexaprenyl Diphosphate Synthase

The structural genes encoding the two essential components of HexPP synthase have been cloned recently from *M. luteus* B-P 26, and sequenced.[87] Within a 2130 bp DNA which expresses HexPP synthase in *E. coli* cells, there are three consecutive open reading frames (ORF1, ORF2, and ORF3) encoding proteins of 143, 246, and 325 amino acids, respectively. By constructing plasmids that contained each of the three ORFs, we found that the products of ORF1 and ORF3 were the two dissociable components A and B of HexPP synthase.[30] These genes were similarly located in a newly identified gene cluster participating in menaquinone biosynthesis.[84,88] The deduced amino acid sequence of the ORF3 product showed a 39% similarity to that of component II′ of the HepPP synthase (Heps-2, 320 a.a.) of *B. stearothermophilus*.[84] On the other hand, the sequence of the ORF1 product (143 aa) showed only an 8% similarity to that of component I′ (Heps-1, 220 aa).

We examined the possibility of forming a hybrid between two of the components of the three different medium-chain prenyl diphosphate synthases, components I and II of HepPP synthase from *B. subtilis*, components I′ and II′ of HepPP synthase from *B. stearothermophilus*, and components A and B of HexPP synthase from *M. luteus* B-P 26.[89] As a result, only the hybrid-type combination between component I and component II′ showed a distinct prenyltransferase activity. The hybrid-type enzyme catalyzed the synthesis of HepPP and showed a moderate heat stability ranking between those of the natural enzymes from *B. subtilis* and from *B. stearothermophilus*, which are mesophilic and thermophilic bacteria, respectively. The interchangeability of component I′ with component I but not with component A eliminates the involvement of a common factor in medium-chain prenyl diphosphate synthases of different specificity in the chain length of the product.

As shown in Figure 8, the larger components of the HepPP synthase from *B. stearothermophilus* or *B. subtilis* (component II′ or II) and the HexPP synthase

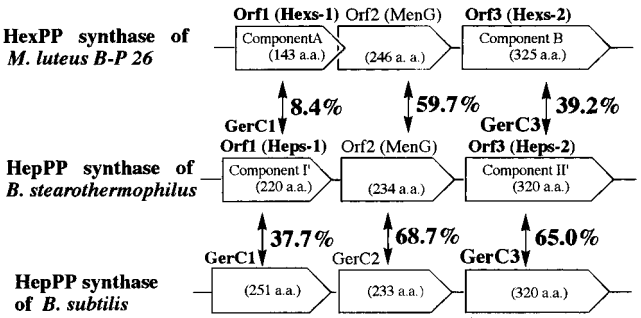

Figure 8. Comparison of the gene regions of HexPP- and HepPP synthases. Each box shows the open reading frames in the gene regions. Amino acid sequence similarities between the genes are described in percentages.

from *M. luteus* B-P 26 (component B) show high identities between the deduced amino acid sequences. In contrast, the identity between the smaller components of the HepPP synthase and the HexPP synthase is less than 10%.

In experiments with a yeast mutant[90] in coenzyme Q biosynthesis, Ashby and Edwards[22] have isolated a gene from a plasmid containing a wild-type genomic DNA fragment that is able to complement the mutant and restore HexPP synthase activity. They have also shown that this gene encodes a 473 amino acid protein having conserved regions characteristic of prenyltransferases. Therefore, this protein seems to correspond to the larger protein component of the two-heteromeric components of bacterial medium-chain prenyl diphosphate synthases as mentioned above. However, it is not known whether the yeast 473 amino acid protein acts as HexPP synthase by itself or in association with another gene product similar to the smaller protein component. If the latter is the case, the yeast mutant must be deficient in one of the two components of the HexPP synthase. It would be interesting to learn whether dissociable heterodimeric systems are common in medium-chain prenyl diphosphate synthases of both prokaryotic and eukaryotic cells.

E. Octaprenyl Diphosphate Synthase

On analysis of genes in *E. coli* chromosome, Choi et al.[91] found part of an open-reading frame that showed a significant similarity to the *ispA* gene, which encodes FPP synthase of *E. coli*.[61] Jeong et al.[92] also determined the whole sequence of this open-reading frame and indicated a high similarity of the gene product to the HexPP synthase of *S. cerevisiae*[22] and GGPP synthases of various organisms. Asai et al.[93] found that the activity of octaprenyl diphosphate (OctPP) synthase was increased in *E. coli* cells transformed with this gene. The deduced amino acid sequence of this enzyme also shows the presence of the seven conserved regions typical of prenyltransferases.[62]

F. Decaprenyl Diphosphate Synthase

In the study of ubiquinone biosynthesis, Suzuki et al.[94] have cloned the gene encoding decaprenyl diphosphate (DecPP) synthase of a fission yeast *Schizosaccharomyces pombe*. Takahashi et al.[95] have also

cloned the DecPP synthase gene of *P. denitrificans*, which produces ubiquinone-10. It is of interest that the genes encoding DecPP synthase (334 amino acid) and a methyltransferase are not located side by side, forming a cluster participating in prenylquinone biosynthesis in contrast to those of the bacteria synthesyzing menaquinones.[86−88]

V. Mutagenic Studies on FPP Synthase

A. Roles of Conserved Amino Acid Residues

To evaluate the roles of amino acids in the conserved regions in FPP synthases, a number of site-directed mutagenesis experiments have been carried out. Joly and Edwards[96] showed that the mutagenesis of Asp-104, Asp-107, Arg-112, Arg-113, and Asp-243 of rat liver FPP synthase, which correspond to D^2, D^3, R^1, R^2, and D^4 in regions II or VI, respectively (see Figure 7), resulted in a decreased V_{max} of approximately 1000-fold compared to the wild-type enzyme. However, no significant changes in K_m values for either IPP or GPP were observed in the conservative mutation in which Asp and Arg residues were replaced with Glu and Lys, respectively. These results suggest that these amino acid residues are involved in catalytic efficiency rather than binding affinity. On the other hand, Asp-103 (D^1) and Asp-247 (D^6) could be replaced with Glu without marked changes in kinetic properties. Using a recombinant form of yeast enzyme extended by a C-terminal -Glu-Glu-Phe α-tublin epitope, Song and Poulter[66] conducted mutagenesis studies in which the conserved Asp or Arg residue was replaced with Ala. They showed that the mutations of the Asp residues and nearby Arg residues in region II (D^1, D^2, R^1, and R^2) and the Asp residues in region VI (D^4 and D^5) drastically lowered the catalytic activity of the fused enzyme. However, the third Asp in region VI (D^6) and Lys-254, which is conserved downstream in region VI, were found to be replaced with Ala without marked changes in kinetic properties. We also showed that the mutagenesis of Asp-224 and Asp-225 (D^4 and D^5) of *B. stearothermophilus* FPP synthase resulted in approximately 10^4- to 10^5-fold decreases of k_{cat} values compared to the wild type.[97] On the other hand, replacement of Asp-228 (D^6) with Ala resulted in an ∼10-fold decrease of k_{cat} value and an 10-fold increase of the K_m value for IPP.

Taken altogether, these results indicate the importance of the six Asp residues in the consensus $D^1D^2xxD^3xxxxR^1R^2G$ (region II) and $GxxFQxxD^4D^5$-xxD^6 (region VI) motifs as follows: D^2, D^3, and D^4 are so critical that none of them can be replaced by even Glu without significant decrease in catalytic activity, whereas D^1 can be replaced by Glu but not by Ala. However, D^6 is so tolerant that even Ala can substitute for it.

Replacement of the first Asp residue (D^4, Asp-243) in the $GxxFQxxD^4DxxD$ motif in region VI of rat FPP synthase with Glu resulted in a 26-fold increase in the K_m value for IPP.[67] Replacement of the conserved Lys residues (Lys-47 and Lys-183) in regions I and V of *B. stearothermophilus* FPP synthase with aliphatic amino acids also resulted in marked depressions of the enzyme affinity for IPP, thereby causing a change in the distribution of products.[97] Both K47I and K183A have k_{cat} values ∼70-fold lower than that of the wild-type enzyme. Neither of them shows significantly changed K_m values for GPP, but they both show markedly increased K_m values for IPP. These facts indicate that both Lys residues conserved in regions I and V contribute to the binding affinity for IPP as well as D^6 (or D^4 in case of rat enzyme) in region VI.

Blanchard and Karst[98] isolated a mutant gene from a yeast strain that had the unusual property of excreting prenyl alcohols such as geraniol. By comparing the nucleotide sequence of the FPP synthase of the mutant, they have shown that the unusual property is attributed to a one-point mutation resulting in a substitution of Glu for Lys-197 of *S. cerevisiae* FPP synthase, which is the conserved Lys in region V and which corresponds to Lys-183 in the *B. stearothermophilus* enzyme. This is interesting in the light of the above-mentioned observation[97] that both K47I and K183A showed markedly increased K_m values for IPP, since a decrease of the affinity for IPP might also cause an accumulation of GPP. We examined this possibility by analyzing the relative accumulation of GPP in the FPP synthase reaction by the mutant enzymes, and obtained similar results[97] to those observed by Blanchard and Karst with the yeast mutant extracts.[98] It is noteworthy that K47I and K183A of *B. stearothermophilus* enzyme resemble each other with respect to both kinetic parameters and product distributions. These results raise the possibility that the release of GPP in the reaction catalyzed by the yeast mutant enzyme might also be due to depression of the binding affinity for IPP rather than for GPP.

The significance of the Phe-Gln motif (FQ) in region VI was examined by us.[99] The highly conserved Phe-220 and Gln-221 residues were replaced with Ala and Glu, respectively. These mutation resulted in 10^5 and 10^3 decreases in catalytic activity of the FPP synthase from *B. stearothermophilus*, respectively, indicating that the Phe residue has a crucial role in catalysis. Michaelis constants of the Q221E mutant for DMAPP and GPP increased ∼25- and 2-fold, respectively, compared to the wild type, whereas those for IPP were not altered much. These results suggest that the FQ motif is involved not only in substrate binding but also in catalysis. These facts suggest a hypothetical model for the binding of an allylic diphosphate to region VI. The dramatic effect on k_{cat} of the replacement of the Phe group by Ala suggests that Phe-220 might contribute to the acceleration of catalysis by stabilizing the prenyl cation formed at the beginning of the catalytic reaction through a cation-π interaction.[100]

Mammalian FPP synthase shares a C-terminal region (region VII) characterized as being so crowded with basic amino acids as shown in Figure 7. However, only the Arg residue at the third position from the C-terminus is completely conservative if bacterial FPP synthases are taken into account. To examine the significance of this residue, Arg-295 in the *B. stearothermophilus* enzyme was mutated to Val.[101]

This mutation, however, did not result in significant change in catalytic activity, indicating that the conserved Arg residue in region VII is not essential for catalysis. The R295V mutant showed a 3-fold increased K_m value for IPP but showed an unchanged K_m value for GPP as compared with the wild type. Song and Poulter[66] also examined the significance of the Arg-350 near the C-terminal of the yeast enzyme having an -EEF epitope. The R350A mutant showed only a slight additional increase in the K_m value for IPP. The addition of the -EEF epitope to the C-terminus of the wild-type enzyme resulted in a 14-fold increase of the K_m value for IPP and a 12-fold decrease of k_{cat}.

B. stearothermophilus FPP synthase is unique in that it possesses only two Cys residues in contrast to FPP synthases from other sources that have more than four Cys residues. To explore the significance of these Cys residues, we examined the effect of replacement of the Cys residues at 73 and 289 of this thermostable enzyme with Ser.[102] As a result, all of the mutant enzymes were active as FPP synthase, showing specific activities comparable to that of the wild-type enzyme. These results indicate that neither of the Cys residues is essential for catalytic function.

B. Random Chemical Mutagenesis

Random chemical mutagenesis often provides a powerful method for obtaining a desired mutation product if an effective procedure for screening positive clones is available. To clone the GGPP synthase gene of *S. acidocaldarius*, Ohnuma et al.[80] have developed an in vivo method for detecting the enzymatic activity by utilizing carotenoid biosynthesis genes of *Erwinia uredovora*[103] to produce a red colored clone expressing GGPP synthase activity.

The FPP synthase gene of *B. stearothermophilus* was subjected to random mutagenesis by $NaNO_2$ treatment to construct libraries of mutated FPP synthase genes. From the libraries mutants showing GGPP synthase activities were selected by the red−white screening method, and 11 red positive clones were obtained from 24 300 mutants.[104] Each mutant was found to contain a few amino acid substitutions in the FPP synthase, which resulted in acquisition of the catalytic activity of synthesizing GGPP as well as FPP. Mutants which had replacement of Tyr-81 by His showed the most efficient production of GGPP. From the analysis of the mutations it was possible to define three amino acids that determine the final chain length of the products. They were Leu-34, Tyr-81, and Val-157. In particular, the mutated enzyme that has a substitution of His for Tyr-81, which is situated at the fifth amino acid upstream to the DDxxD motif in region II, produced GGPP most effectively.

To investigate the role of Tyr-81 of *B. stearothermophilus* FPP synthase, Ohnuma et al. constructed 19 mutant enzymes each of which has a different amino acid at position 81.[105] All enzymes except Y81P showed prenyltransferase activities to catalyze condensation of IPP with an allylic diphosphate. When assayed with FPP as the allylic substrate, considerable activities were observed in almost all mutated FPP synthases. These results indicate that the mutated enzymes can catalyze the chain elongation beyond FPP. When DMAPP or GPP was employed as the allylic primer, mutated enzymes, Y81A, Y81G, and Y81S, produced HexPP as the longest product. These mutants produced geranylfarnesyl diphosphate (GFPP, C_{25}) as the main product. Y81A and Y81G gave larger amounts of HexPP and GFPP than did Y81S. These mutant enzymes, in which Tyr-81 was replaced with Cys, His, Ile, Leu, Gln, Thr, or Val, all produced GFPP as the longest product. These observations strongly indicate that the amino acid Tyr-81 directly contacts the ω-terminal of the final (longest) product during the catalytic isoprenoid chain elongation. This interaction in the catalytic site must be the critical step in determining the chain length of the product of prenyltransferase.

Assuming that the essential structure of the binding cavity of the *B. stearothermophilus* FPP synthase is similar to that of the avian FPP synthase in view of the sequence similarity between them, it is reasonable to predict, on the basis of the crystal structure of the avian enzyme,[24] that the Tyr-81 is situated on a point 11−12 Å apart from the first Asp-rich motif in the large central cavity of the FPP synthase. The distance is similar to the length of the hydrocarbon moiety of an allylic substrate bound by the diphosphate moiety at the Asp motif through a magnesium ion. As the distance is similar to the length of the hydrocarbon moiety of FPP, it is suggested that the aromatic ring of Tyr-81 might prevent further chain elongations longer than C_{15}.

VI. Mechanism of Chain-Length Determination

One of the most interesting subjects on the catalytic mechanism of prenyltransferases is to understand the mechanism by which individual enzymes determine product chain lengths.

Recently Poulter and his collaborators[106] have reported strong evidence on the mechanism of regulation of product chain length in the avian FPP synthase reaction by X-ray analyses of some mutated enzymes that acquired the catalytic capability of producing longer chain prenyl diphosphates. An analysis of the X-ray structure[24] of the avian FPP synthase, coupled with the information about conserved amino acids of many prenyltransferases so far cloned, led them to the idea that the phenyl groups of Phe-112 and Phe-113 in the avian enzyme, which are situated at the fifth and fourth amino acid upstream to the DDxxD motif in region II, are important for determining the ultimate length of the hydrocarbon chains. They then carried out several site-directed mutations in the avian enzyme with respect to these Phe residues. As a result, enzymes capable of producing GGPP (F112A), FGPP (F113S), and longer chain prenyl diphosphates (F112A/F113S) were obtained. X-ray analyses of the structure of the F112A/F113S mutant in the absence or presence of allylic substrates bound indicated an alteration of the size of the binding pocket for the growing isoprenoid chain in the active site of the enzyme. The proposed binding pocket in the mutant structure was increased

in depth by 5.8 Å as compared with that for the wild-type enzyme. Allylic diphosphates were observed in the holo structures, bound through Mg^{2+} to the first two Asp residues in the DDxxD motif (Asp-117 ≈ Asp-121), with the hydrocarbon tails of all the ligands growing down the hydrophobic pocket toward the mutation site.

From X-ray analyses of the wild-type avian FPP synthase, Tarshis et al.[106] have suggested that the ultimate length of the polyisoprenoid chain obtained during successive condensations of the growing allylic substrate with IPP is governed by the size of a hydrophobic pocket in the interior of the enzyme. They also showed that replacement of the benzyl groups in Phe-112 and Phe-113 with smaller side chains gave FPP synthase mutants that synthesized longer isoprenoid products than FPP.[106]

To obtain mutant prenyltransferases that have changes in the machinery for determination of the product chain length, Ohnuma et al.[107] carried out random mutation by $NaNO_2$ treatment on the GGPP synthase gene of an archaebacterium, *S. acidocaldarius*.[80] The library of mutated GGPP synthase genes was screened with a mutant deficient in HexPP synthase. As a result, three mutant enzymes showing catalytic activity to produce large amounts of FGPP with a concomitant formation of small amounts of HexPP were obtained. Sequence analysis revealed that the mutation of Phe-77, which is located at the fifth amino acid upstream from the first motif DDxxD in region II of prenyltransferases, is the most effective for elongating the ultimate product. This fact exactly coincides with the results on FPP synthase mutation[104,105] as described in section V.

By comparison of amino acid sequences of many kinds of FPP synthases with those of GGPP synthases, Ohnuma et al.[108] noticed that several homologous regions typical for FPP synthase are found around the first DDxxD motif in region II. They then introduced mutations into the region II of the archaeal GGPP synthase. In these mutants regions around the first DDxxD motif of GGPP synthase were replaced with the corresponding regions of FPP synthases from human,[59] rat,[21] *A. thaliana*,[64] *S. cerevisiae*,[60] *E. coli*,[61] and *B. stearothermophilus*.[62] By analyzing these mutated enzymes, they found that the region around the first DDxxD motif is essential for the product specificity of all FPP synthases and that the sequence in this region of FPP synthase differs between eukaryotic and prokaryotic enzymes. On the basis of these observations they have proposed that FPP synthases have evolved from a progenitor corresponding to the archaeal GGPP synthases in two ways.

VII. Gene Cloning of Undecaprenyl Diphosphate Synthase

During the past decade the structural genes for many kinds of prenyltransferases that catalyze *E*-type prenyl chain elongations have been identified and characterized as described in the previous section. Multiple sequence alignments have revealed the presence of more than five conserved regions in the primary structures of prenyltransferases identified to date, which contain two characteristic aspartate-rich DDxxD motifs (see sections IV.A.3 and V.A). One is followed by the RRG motif, and the other follows the FQ motif, which is also characteristic of the (*E*)-prenyl diphosphate synthases (Figure 7). However, one cannot define these highly conserved motifs as the essential features common to all prenyltransferases (prenyl diphosphate synthases) because no information has been available about the primary structures of the enzymes classified as Prenyltransferase IV, which catalyze (*Z*)-isoprene chain elongation.

MFPIKKRKAI KNNNINAAQI PKHIAIIMDG NGRWAKQKKM PRIKGHYEGM 50
QTVKKITRYA SDLGVKYLTL YAFSTENWSR PKDEVNYLMK LPGDFLNTFL 100
PELIKENVKV ETIGFIDDLP DHTKKAVLEA KEKTKHNTGL TLVFALNYGG 150
RKEIISAVQL IAERYKSGEI SLDEISETHF NEYLFTANMP DPELLIRTSG 200
EERLSNFLIW QCSYSEFVFI DEFWPDFNEE SLAQCISIYQ NRHRRFGGL* 249

Figure 9. Amino acid sequence of the UPP synthase of *M. luteus* B-P 26.

To isolate the gene for UPP synthase, we recently applied a method essentially similar to that developed by Raetz[109] for the rapid screening of *E. coli* colonies for mutants with defective enzymes of phospholipid metabolism. As a result, we have succeeded for the first time in the cloning of the gene for the prenyltransferase that catalyzes the (*Z*)-isoprene chain elongation.[110] The deduced amino acid sequence of the UPP synthase of *M. luteus* B-P 26 is shown in Figure 9. The primary structure of this enzyme is totally different from those of the prenyl diphosphate synthases such as FPP synthase and GGPP synthase catalyzing (*E*)-prenyl chain elongation, which have characteristic aspartate-rich motifs of DDxxD. Although there is a DDxxD motif-like structure in the region of residues 117−121 of the UPP synthase (Figure 9), it seems too premature to assign it as a critical region corresponding to the DDxxD motifs of the (*E*)-prenyl diphosphate synthases. It is very interesting to examine the similarity and difference in the three-dimensional structures of the prenyltransferases catalyzing (*Z*)-isoprene chain elongation and (*E*)-isoprene chain elongation.

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