A Molecular Ruler for Chain Elongation Catalyzed by Octaprenyl Pyrophosphate Synthase and Its Structure-Based Engineering To Produce Unprecedented Long Chain trans-Prenyl Products†,‡

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Taiwan International Graduate Program, Academia Sinica, Taipei 115, Taiwan. A.H.-J.W.: e-mail, ahjwang@gate.sinica.edu.tw; telephone, +886-2-2788-2043. P.-H.L.: e-mail, philiang@gate.sinica.edu.tw; telephone, +886-2-2785-5696 ext. 6070; fax, +886-2-2788-9759.

ABSTRACT: Octaprenyl pyrophosphate synthase (OPPs) catalyzes consecutive condensation reactions of farnesyl pyrophosphate (FPP) with five molecules of isopentenyl pyrophosphate (IPP) to generate C40 octaprenyl pyrophosphate (OPP) which constitutes the side chain of menaquinone. We have previously reported the X-ray structure of OPPs from Thermotoga maritima, which is composed entirely of α-helices joined by connecting loops and is arranged with nine core helices around a large central cavity [Guo, R. T., Kuo, C. J., Ko, T. P., Chou, C. C., Shr, R. L., Liang, P. H., and Wang, A. H.-J. (2004) J. Biol. Chem. 279, 4903–4912]. A76 and S77 are located on top of the active site close to where FPP is bound. A76Y and A76Y/S77F OPPs mutants produce C20, indicating that the substituted larger residues interfere with the substrate chain elongation. Surprisingly, the A76Y/S77F mutant synthesizes a larger amount of C20 than the A76Y mutant. In the crystal structure of the A76Y/S77F mutant, F77 is pushed away by Y76, thereby creating more space between those two large amino acids to accommodate the C20 product. A large F132 residue at the bottom of the tunnel-shaped active site serves as the “floor” and determines the final product chain length. The substitution of F132 with a small Ala, thereby removing the blockade, led to the synthesis of a C50 product larger than that produced by the wild-type enzyme. On the basis of the structure, we have sequentially mutated the large amino acids, including F132, L128, I123, and D62, to Ala underneath the tunnel. The products of the F132A/L128A/I123A/D62A mutant reach C95, beyond the largest chain length generated by all known trans-prenyltransferases. Further modifications of the enzyme reaction conditions, including new IPP derivatives, may allow the preparation of high-molecular weight polyisoprenyl products resembling the rubber molecule.
respectively, by these two enzymes (9–13). Despite the completely different structural folds, both enzymes contain a tunnel-shaped crevice as the active site to accommodate the long chain product. At the bottom of their active site, a large hydrophobic amino acid (L137 for UPPs and F132 for OPPs) serves as the “floor” to block further chain elongation of the correct product. By substitution of L137 in UPPs or F132 in OPPs with small Ala to remove the floor, the chain length is extended to mainly C75 in UPPs and C50 in OPPs (9, 13).

Compared to the large polymers, especially natural rubber, made by cis-prenyltransferases, trans-type enzymes generate shorter products (14). In the crystal structure of OPPs, several large amino acids, including Leu128, Ile123, and Asp62, were found underneath F132, which could provide additional blockage at the bottom of the active site. In this study, we have sequentially replaced these amino acids with smaller Ala residues and examined the product chain lengths made by the mutants. We have also determined the three-dimensional (3D) crystal structures of these mutants in an effort to provide the structural explanation for the unprecedented product chain length synthesized by the trans-prenyltransferases.

Wild-type OPPs from Thermotoga maritima catalyzes the chain elongation of FPP to C40 octaprenyl pyrophosphate that constitutes the side chain of menaquinone, an essential component involved in the electron transfer for oxidative phosphorylation (15). The 3D structure of this OPPs is composed entirely of α-helices joined by connecting loops and is arranged with nine core helices around a large central cavity (13). An elongated hydrophobic tunnel between α-helices D and F contains two DDxxD motifs on top for substrate binding and is sealed by F132 at the bottom. Two DDxxD motifs in the amino acid sequences were conserved in all trans-type prenyltransferases (see Figure 1) with the first motif responsible for FPP binding and the second motif for IPP binding (16–19). On the basis of the 3D structure and mutagenesis studies of farnesyl pyrophosphate synthase (another trans-prenyltransferase), a bulky amino acid residue located in the fifth position before the first DDxxD motif of FPPs appeared to block further elongation of the product FPP (20, 21). The corresponding amino acid in OPPs is a small amino acid, Ala (Figure 1), which may be required so that the steric obstacle preventing OPPs from synthesizing products larger than FPP could be avoided.

On the basis of the structure of OPPs, we identify several key amino acids which play critical roles in regulating the chain length of the final products. These include Ala76 and Ser77 for C20 production, F132 for the correct product of C40, and Asp62, Ile123, and L128, when mutated to a small Ala, to allow longer products to pass through the bottom of the tunnel.

EXPERIMENTAL PROCEDURES

Materials. Radiolabeled [14C]IPP (55 mCi/mmol) was purchased from Amersham Pharmacia Biotech, and FPP was obtained from Sigma. Reverse-phase thin-layer chromatography (TLC) plates were purchased from Merck. PfuTurbo DNA polymerase was obtained from Life Technologies, Inc. The plasmid mini-prep kit, DNA gel extraction kit, and NiNTA resin were purchased from Qiagen. Potato acid phosphatase (2 units/mg) was purchased from Roche Molecular Biochemicals. FXa and the protein expression kit (including the pET32Xa/LIC vector and competent JM109 and BL21 cells) were obtained from Novagen. The Quik-Change site-directed mutagenesis kit was obtained from Stratagene, Inc. All commercial buffers and reagents were of the highest grade.

Site-Directed Mutagenesis of OPPs. OPPs mutants were prepared by using the QuikChange site-directed mutagenesis kit in conjunction with the Thermotoga OPPs gene template in the pET32Xa/LIC vector. The mutagenic primers that were used were prepared by Biobasic Inc. The mutagenic oligonucleotides for performing site-directed mutagenesis are listed in Table 1. The basic procedure of mutagenesis utilizes a supercoiled double-stranded DNA (dsDNA) vector with an insert of interest and two synthetic oligonucleotide primers (forward and backward) containing the desired mutation. The mutation was confirmed by sequencing the entire OPPs mutant gene of the plasmid obtained from the overnight culture. The correct construct was subsequently transformed into Escherichia coli BL21(DE3) for protein expression. The procedure for protein purification followed our reported protocol (15, 22). Each purified mutant OPPs was verified by mass spectroscopic analysis, and its purity (>95%) was checked by SDS–PAGE.

Crystallization and Data Collection. Five mutants (A76Y, A76Y/S77F, F132A/L128A, F132A/L128A/I123A, and F132A/L128A/I123A/D62A) were crystallized using the hanging drop method from Hampton Research (Laguna Niguel, CA) by mixing 2 μL of the OPPs solution (10 mg/mL in 0.1% Triton X-100) with 2 μL of the mother liquor [0.1 M Na2Hepes (pH 7.5), 1.5–2.1 M LiSO4, and 4–10% PEG 900], equilibrating with 500 μL of the mother liquor. Within 4 days to 2 weeks, crystals had grown to dimensions of ~0.2 mm × ~0.2 mm × ~0.1 mm. The X-ray diffraction data sets for the A76Y, A76Y/S77F, F132A/L128A, F132A/L128A/I123A, and F132A/L128A/I123A/D62A were collected to 3.2, 2.7, 3.3, 3.35, and 3.4 Å resolution, respectively. The first data sets of the four mutants were collected in house using a Rigaku MicroMax002 X-ray generator equipped with an R-Axis IV++ image plate detector, whereas the F132A/L128A/I123A/D62A data set was collected at beamline BL17B2 in the National Synchrotron Radiation Research Center (NSSRC, Hsinchu, Taiwan).
The F132A/L128A mutant crystal belongs to tetragonal space group $P4_212$ with the following unit cell parameters: $a = b = 151.43$ Å and $c = 67.04$ Å. Crystals of A76Y, A76Y/S77F, F132A/L128A/I123A, and F132A/L128A/I123A/D62A belong to tetragonal space group $I422$ with the following unit cell parameters: $a = b = 152.94$ Å and $c = 65.02$ Å. All diffraction measurements were carried out on the crystals cryoprotected by adding 10% glycerol to the reservoir.

**Structure Determination and Refinement.** The wild-type OPPs structure was determined by the MIR (multiple isomorphous replacement) method using SOLVE (23) as described previously (13). The phases of all OPPs mutant structures were determined by the molecular replacement (MR) method using CNS (24).

**Kinetic Parameters for Mutant OPPs.** For enzyme activity measurements, each mutant OPPs enzyme was used at a concentration of 0.1 μM. The reaction was initiated in a 200 μL solution containing 100 mM Hepes (pH 7.5), 5 μM FPP, 50 μM [14C]IPP, 50 mM KCl, and 0.5 mM MgCl$_2$ at 25 °C. The enzyme concentration used in all experiments was determined from its absorbance at 280 nm for each mutant
OPPs. The reaction was terminated by adding 10 mM (final concentration) EDTA, and the product was extracted with 1-butanol. The product was quantitated by counting the concentration) EDTA, and the product was extracted with

<table>
<thead>
<tr>
<th>A76Y</th>
<th>A76Y/S77F</th>
<th>F132A/L128A</th>
<th>F132A/L128A/I123A</th>
<th>F132A/L128A/I123A/D62A</th>
</tr>
</thead>
<tbody>
<tr>
<td>space group</td>
<td>I422</td>
<td>I422</td>
<td>P42,2</td>
<td>I422</td>
</tr>
<tr>
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<td>2.7</td>
<td>3.3</td>
<td>3.35</td>
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<tr>
<td>no. of reflections unique</td>
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<td>9589</td>
<td>11660</td>
<td>5051</td>
</tr>
<tr>
<td>observed</td>
<td>71144</td>
<td>57001</td>
<td>85983</td>
<td>28516</td>
</tr>
<tr>
<td>completeness (%)</td>
<td>99.8 (100)</td>
<td>88.4 (80.5)</td>
<td>95.1 (91.3)</td>
<td>89.0 (87.7)</td>
</tr>
<tr>
<td>Rmerge (%)</td>
<td>7.1 (53.8)</td>
<td>4.7 (71.5)</td>
<td>10.0 (52.3)</td>
<td>10.0 (55.6)</td>
</tr>
<tr>
<td>I/σ(I)</td>
<td>34.89 (4.95)</td>
<td>30.31 (2.46)</td>
<td>15.05 (2.75)</td>
<td>15.51 (3.17)</td>
</tr>
<tr>
<td>refinement resolution limit (Å)</td>
<td>50–3.1</td>
<td>50–2.7</td>
<td>50–3.3</td>
<td>50–3.35</td>
</tr>
<tr>
<td>Rfactor (%)</td>
<td>24.43 (31.26)</td>
<td>21.98 (31.49)</td>
<td>27.70 (27.44)</td>
<td>24.07 (26.10)</td>
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<td>Rfree (%)</td>
<td>27.76 (32.52)</td>
<td>28.63 (41.21)</td>
<td>32.11 (32.57)</td>
<td>29.01 (35.59)</td>
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<td>deviations bond lengths (Å)</td>
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<td>0.0039</td>
<td>0.0041</td>
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<td>average B factors (Å²)</td>
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<td>73.5</td>
<td>58.4</td>
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<td>Ramachandran plot (%) favored</td>
<td>91.2</td>
<td>87.6</td>
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<td>7.6</td>
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<tr>
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<td>0.8</td>
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<tr>
<td>disallowed</td>
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a The unit cell dimensions for F132A/L128A (P42,2) are as follows: a = b = 151.43 Å and c = 67.04 Å (two molecules per asymmetric unit). For A76Y, A76Y/S77F, F132A/L128A/I123A, and F132A/L128A/I123A/D62A (I422) they are as follows: a = b = 152.94 Å and c = 65.02 Å (one molecule per asymmetric unit). b The values in parentheses are for highest-resolution shells.

RESULTS

Crystal Structures of the OPPs Mutants. The wild-type and F132A OPPs crystal structures have been previously reported (13). In the study presented here, we have generated three new mutants, F132A/L128A, F132A/L128A/I123A, and F132A/L128A/I123A/D62A, and determined their crystal structures. Moreover, we also determined the structures of S77F and F76Y/S77F which were prepared in our previous study (13). Among the five OPP mutants, F132A/L128A OPPs belongs to the P42,2 space group. Each asymmetric unit of the crystal unit cell contains one OPPs dimer, which is the active form of the enzyme. On the other hand, A76Y, A76Y/S77F, F132A/L128A/I123A, and F132A/L128A/I123A/D62A belong to the I422 space group. A crystallographic 2-fold axis is coincident with the dyad of the dimer, and each asymmetric unit of the crystal unit cell contains only one OPPs monomer. Data collection and refinement statistics for the structural analysis of A76Y, A76Y/S77F, F132A/L128A, F132A/L128A/I123A, and F132A/L128A/I123A/D62A OPPs are summarized in Table 2. Like wild-type OPPs, all mutant structures contain 12 α-helices, nine of them surrounding a large central cavity (helices A–I) and an elongated tunnel-shaped active site cavity surrounded by four α-helices (helices C, D, F, and H) (Figure 2A). Two DDxxD motifs for substrate binding are located in helices D and H near the top of the tunnel. At the bottom of helix F, F132 provides the floor to prevent extra chain elongation of the OPP product (13). Amino acids L128, I123, and D62 underneath F132 may form additional seals as shown by the side view and bottom-up stereoview of the tunnel in the wild-type OPPs crystal structure (Figure 2B,C). When those four amino acids were replaced with Ala, the F132A/L128A/I123A/D62A mutant shows a hollow tunnel with open space at the bottom of the tunnel as shown by the side view and the bottom-up stereoview (Figure 2B,D). The other mutants, F132A/L128A and F132A/L128A/I123A, exhibit a partially blocked tunnel (data not shown).
Products Synthesized by F132A/L128A, F132A/L128A/I123A, and F132A/L128A/I123A/D62A. By removing the blockade along the pathway of product chain elongation, we aimed to engineer mutant enzymes which can produce a product much larger than C40 OPP. We thus produced three OPP mutants (F132A/L128A, F132A/L128A/I123A, and F132A/L128A/I123A/D62A). These enzymes exhibit slightly lower activities than the wild type (Table 3), so the time for obtaining the final products was adjusted according to the relative activity of each mutant. Compared to the wild-type OPPs and the F132A mutant which synthesize C40 and C50 as the major products, respectively (lanes 1 and 2 of Figure 3), F132A/L128A (lane 3), F132A/L128A/I123A (lane 4), and F132A/L128A/I123A/D62A (lane 5) produce C60 as the major product (30, 38, and 21% for the latter three mutants, respectively). Finally, the quadruple mutant synthesizes an up to C95 product. Apparently, step-by-step removal of the large amino acids near the bottom of the tunnel gradually

**Figure 2:** Crystal structures of wild-type and F132A/L128A/I123A/D62A mutant OPPs. In panel A, the dimeric wild-type OPPs crystal structure is shown as a ribbon diagram. Two monomeric subunits are shown in blue and purple. Arrows indicate the active site locations where Asp-rich motifs are responsible for FPP and IPP binding. In panel B, the side views of the active site tunnels in wild-type and mutant OPPs are compared. The mutant apparently has an unblocked internal space that originated from the removal of the side chains. Panels C and D show the bottom-up stereoview structures of wild-type OPPs and the mutant, respectively. Four amino acids (F132, L128, I123, and D62) occupy the central area at the bottom of the tunnel. The internal part of tunnel becomes clear after these amino acids are replaced with the small Ala residue.
increases the chain lengths of the final products of the mutants. However, the $C_{60}$ major product shared by F132A/L128A, F132A/L128A/I123A, and F132A/L128A/I123A/D62A indicates that the chain of $C_{60}$ may reach the location of I123, and the I123A mutation is not able to create a sufficiently large opening to allow further chain elongation effectively. The replacement of I123 with an even smaller Gly residue in the mutant F132A/L128A/I123G/D62A still failed to provide sufficient space to efficiently “thread” the growing isoprenoid, and the major product remained $C_{60}$ (data not shown). Indeed, the diameter of the tunnel in the section of I123A is 5.2 Å, the smallest among the areas beside the four amino acids being mutated to Ala.

On the basis of these results, a molecular ruler for the chain elongation reactions catalyzed by OPPs is proposed, which shows that F132, L128, and I123 are located at the $C_{40}$, $C_{50}$, and $C_{60}$ key positions, respectively (Figure 4). The position of L128 is a bit lower, so a significant amount of $C_{55}$ was also produced along with $C_{50}$ (37:29 $C_{50}$:$C_{55}$ ratio, lane 2 of Figure 3).

Crystal Structures of A76Y and A76Y/S77F. Two sulfate ions (pyrophosphate-like) bound to the two DDxxD Asp-rich motifs via Mg$^{2+}$ represent the substrate binding sites. S77 and A76 are located in immediate neighborhood below FPP. Their substitution with large amino acids led to the formation of $C_{20}$ as a final product. F132, L128, and I123 are located on the bottom of helix D to block the chain elongation of $C_{60}$, $C_{50}$, and $C_{60}$, respectively.

L128A, F132A/L128A/I123A, and F132A/L128A/I123A/D62A indicates that the chain of $C_{60}$ may reach the location of I123, and the I123A mutation is not able to create a sufficiently large opening to allow further chain elongation effectively. The replacement of I123 with an even smaller Gly residue in the mutant F132A/L128A/I123G/D62A still failed to provide sufficient space to efficiently “thread” the growing isoprenoid, and the major product remained $C_{60}$ (data not shown). Indeed, the diameter of the tunnel in the section of I123A is $\sim 5.2$ Å, the smallest among the areas beside the four amino acids being mutated to Ala. Therefore, when F132 is converted to Ala, the product chain length is extended from the wild-type $C_{40}$ to $C_{60}$.

Surprisingly, the single mutant S77F generates $C_{20}$ in much smaller quantities than the double mutant A76Y/S77F (13). S77F has an activity similar to that of A76Y/S77F, although
their $k_{\text{cat}}$ values are significantly smaller than that of the wild type (Table 3). This seems contradictory since the replacement of two small amino acids, Ala and Ser, with large residues, Tyr and Phe, respectively, simultaneously should provide a larger seal for blocking the chain elongation than does the S77F single mutation. On the other hand, A76Y also produced a significant amount of C20 as the final product (13). This mutant has a $k_{\text{cat}}$ value similar to that of the wild type, but much larger than the $k_{\text{cat}}$ values of S77F and A76Y/S77F (Table 3).

The structures of S77F and A76Y/S77F determined in this study provide a possible answer to the above contradiction. The structure of A76Y/S77F shows that the large Y76 pushes F77 away and leaves a space between them, thereby allowing the FPP hydrocarbon terminus to wedge between the two aromatic side chains (see the green benzene rings for Y76 and F77 as shown in Figure 5). On the other hand, the F77 aromatic side chain (the orange benzene ring) of S77F is directly underneath the FPP hydrocarbon tail and could block the FPP chain elongation more effectively so that less C20 is produced. The previously determined structure of A76Y (13) shows that Y76 (the black benzene ring) alone could not completely stop the FPP elongation so a larger amount of C20 was obtained with this mutant. In the molecular ruler shown in Figure 4, we could add A76 and S77 at the stage of C20.

**DISCUSSION**

We previously hypothesized that the substitution of the large F132 with a smaller Ala removes the floor of the tunnel, thereby allowing the formation of products (C50) longer than the C40 produced by the wild type (13). From the side view of the tunnel (Figure 2B), F132 is well positioned to seal the bottom of the tunnel. Beneath F132, there are several additional amino acids (L128, I123, and D62) which may interfere with further chain elongation of the products. In this study, we have generated the F132A/L128A, F132A/L128A/I123A, and F132A/L128A/I123A/D62A mutants in addition to previously generated F132A to examine their products. The product can be extended from C40 to finally C95 for the quadruple mutant. The results have been rationalized by the crystal structures of these mutants showing that the mutants have a more open cavity at the bottom of the tunnel to allow the extra chain elongation to penetrate. By comparing the amino acid sequence of *T. maritima* OPPs with the sequences of other trans-prenyltransferases (Figure 1), we find that the amino acids corresponding to F132, L128, I123, and D62 of OPPs are Leu, Ala, Ser, and Gly, respectively, in short chain length FPPs. There are no particular requirements for these amino acids (large or small, hydrophobic or hydrophilic) since these residues are located out of the active site for the short chain length enzymes.
Structure and Mechanism of *T. maritima* OPPs

However, in the long chain length enzymes such as deca-
prenyl pyrophosphate synthase making the C30 product, the
respective amino acids are Leu, Val, Leu, and Ser, with
properties similar to those of F132, L128, I123, and D62 in
OPPs, respectively. Our results here thus provide a model
for other trans-prenyltransferases in understanding the me-
chanism of chain elongation. In OPPs, a molecular ruler is
defined as shown in Figure 4.

From the crystal structure of OPPs, A76 and S77 are right
below the first DDxxD motif where FPP is bound. A76 and
S77 are amino acids located at the fourth and fifth positions,
respectively, upstream from the first DDxxD motif in the
OPPs sequence (see Figure 1). When these small amino acids
were substituted with bulky Tyr and Phe, the final products
of the mutant OPPs became C20, suggesting the role of the
steric hindrance of the large amino acids at these positions for
substrate chain elongation. Moreover, it is notable that the
crystal structures of A76Y and A76Y/S77F remain

dimeric in contrast to the previous report that the A79Y
mutagenesis of conserved aspartate and arginine residues upon

proteins from *Thermotoga maritima* and *Escherichia coli*

mic reticulum protein localization mutations, encodes cis-prenyl-

Cornisk, K. (2001) Similarities and differences in rubber bio-

Asawatreratanakul, K., Zhang, Y.-W., Wittisuwannakul, D., Wittis-

suwannakul, R., Takahashi, S., Rattanapitayapornt, A., and

Koyama, T. (2003) Molecular cloning, expression and character-
ization of cDNA encoding cis-prenyltransferases from Hevea
brasilienesis. A key factor participating in natural rubber biosyn-

Ishii, K., Sagami, H., and Ogra, K. (1983) Decaprenyl pyro-
phosphate synthetase from mitochondria of pig liver, *Biochem.

Ko, T. P., Chen, Y. K., Robinson, H., Tsai, P. C., Gao, Y.-G.,

flexible loop in *Escherichia coli* undecaprenyl pyrophosphate

Chen, Y. H., Chen, A. P., Chen, T. C., Wang, A. H.-J., and Liang,
P. H. (2002) Probing the conformational change of *Escherichia coli*
undecaprenyl pyrophosphate synthase during catalysis using an
inhibitor and tryptophan mutants, *J. Biol. Chem.* 277, 7369–
7376.

Catalytic mechanism revealed by the crystal structure of undeca-
prenyl pyrophosphate synthase in complex with sulfate, magne-

Guo, R. T., Ko, T. P., Chou, C. C., Shr, H. L., Chu, H. M., Tsai,
diffraction analysis of octaprenyl pyrophosphate synthase crystals
from *Thermotoga maritima* and *Escherichia coli*, *Acta Crystallogr.*
D59, 2265–2268.

Guo, R. T., Kuo, C. J., Chou, C. C., Ko, T. P., Shr, H. L., Liang,
pyrophosphate synthase from hyperthermophia *Thermotoga maritima*
and mechanism of product chain length determination, *J. Biol.
Chem.* 279, 4903–4912.

genetic tree, and predictions of secondary structure, *Protein Sci.*
3, 600–607.

*Thermotoga maritima*: how is it different from that of the mesophilic

residues in domain II of farnesyl diphosphate synthase activity,
*J. Biol. Chem.* 267, 21873–21878.

farnesyl diphosphate synthase activity, *J. Biol. Chem.* 268, 26983–
26989.

Koyama, T., Tajima, M., Sano, H., Doi, T., Koike-Takeshita, A.,
Obata, S., Nishino, T., and Ogra, K. (1996) Identification of
significant residues in the substrate binding site of *Bacillus stea-

and arginine residues upon farnesyl diphosphate synthase activity, *J. Biol. Chem.* 268, 26983–
26989.

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References

269, 3339–3354.


substrate analogue to study ligand interactions for undecaprenyl

cloning, expression, and functional analysis of a cis-prenyltrans-
ferase from *Arabidopsis thaliana*, *J. Biol. Chem.* 275, 18482–
18488.

5. Sato, M., Sato, K., Nishikawa, S.-I., Hitara, A., Kato, J.-I., and
Nakano, A. (1999) The yeast RER2 gene, identified by endoplas-
mic reticulum protein localization mutations, encodes cis-prenyl-
transferase, a key enzyme in dolichol synthesis, *Mod. Cell. Biol.*
19, 471–483.


