Structure of Geranylgeranyl Pyrophosphate Synthase from *Saccharomyces cerevisiae* — Mechanism of Product Chain Length Determination of *trans*-prenyltransferases

The C_5-isopentenyl pyrophosphate (IPP) serves as the building block for the isoprenoid biosynthesis generating a very broad range of compounds used as hormones (sterol), pigments (carotenoid and chlorophyll), compositions of cell membranes (cholesterol and ergosterol) or cell walls (lipid I, II, and peptidoglycan), and components of signal transduction networks (Ras, Rho, Rap, and Rac) [1]. Condensation of IPP with dimethylallyl pyrophosphate (DMAPP) results in all isoprenoid diphosphates such as C_{15}-farnesyl pyrophosphate (FPP), C_{20}-geranylgeranyl pyrophosphate (GGPP), and longer species such as C_{40}-octaprenyl pyrophosphate (OPP) by *trans*-prenyltransferases forming the *trans* double bond during each IPP condensation reaction (Fig. 1(a)). At the active site, two conserved aspartate-rich motifs called DD*XX*D, where *X* encodes any amino acid, coordinate with Mg^{2+} for substrate binding and the subsequent reaction.

Based on previous study of C_{15}-FPP synthase (FPPs), two bulky amino acid residues at the 4th and 5th position before the first DD*XX*D motif in helix D form a blockage underneath the allylic substrate site to avoid the further elongation of FPP product [2]. However, C_{20}-GGPP synthase (GGPPs) of *S. cerevisiae*, *H. sapiens*, and *S. alba* (mustard) contain small residues at the 4th and 5th position prior the first DD*XX*D motif rather than bulky residues found in other GGPPs (Fig. 1(b)).

To rationalize the mechanism of product chain length determination, the first structure of GGPPs from *S. cerevisiae* has been determined at 1.98-Å resolution at beamline BL17B2 of the National Synchrotron Radiation Research Center (Taiwan) and the Taiwan Contract beamline **BL12B2** at SPring-8 (Japan) [3]. Each subunit is composed of 15 α-helices joined by connecting loops and a central crevice surrounded by α-helices A to I contains two conserved DD*XX*D motifs (helices D and I) at the top for substrate binding with one Mg^{2+} coordinated by Asp^{75}, Asp^{79}, and four water molecules. Helices F and G are involved in the dimer formation, with the major stabilization coming from the helices F (A chain)-F (B chain) and F (A chain)-G (B chain) intersubunit hydrophobic interactions and hydrogen bonding (Fig. 2).

Distinct from other known structures of *trans*-prenyltransferases, the N-termius of helix A and the following loop of *S. cerevisiae* GGPPs protrude from the helix core into the other subunit and contribute to the tight dimer formation proven by truncation mutants.

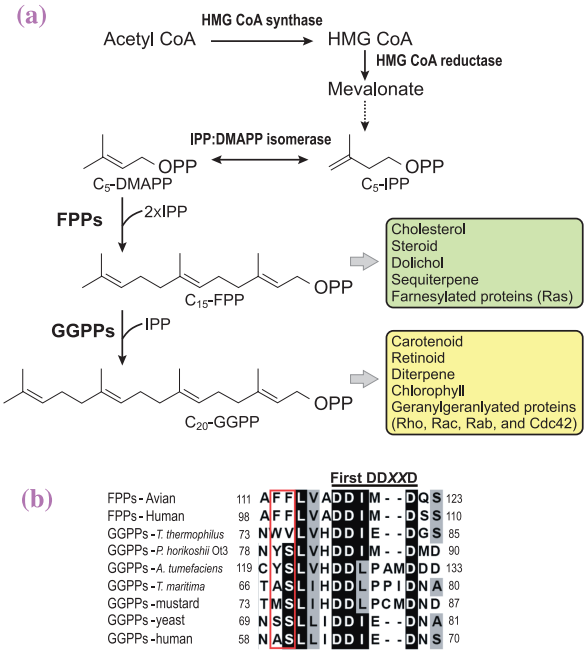

Fig. 1. **(a)** The mevalonate pathway of isoprenoid biosynthesis. **(b)** The partial amino acid sequence alignment of FPPs and GGPPs.

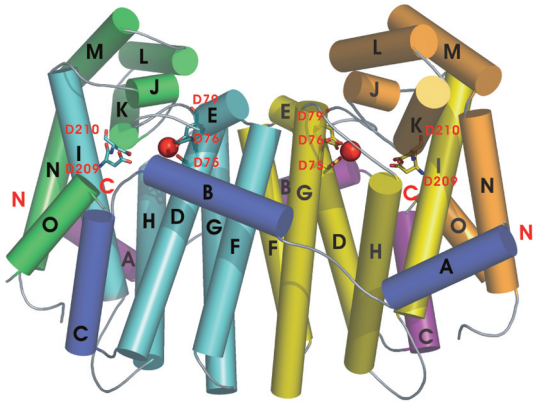

Fig. 2. Structure of *S. cerevisiae* GGPPs. The α-helices shown in cylinder diagrams and colored in blue, cyan, to green and purple, yellow, to orange from N- to C-terminus for the two individual subunits. Two DD*XX*D motifs locating at each active site are shown as sticks and one Mg^{2+} ion is colored in red ball.

Underneath the substrate-binding site, two bulky residues, Tyr^{107} (helix F) and His^{139} (helix G), occupy the bottom portion of the elongated crevice as a floor to block further chain length elongation of GGPP different from previous studying on FPPs [2] (Fig. 3(a)). Compared to the major product C_{30} synthesized by mutant H139A, the product generated by mutant Y107A is predominantly C_{40}, suggesting the most important role of Tyr^{107} in determining the product chain length (Fig. 3(b)).

Based on recently solved GGPPs crystal structures, these enzymes utilize large amino acids from different secondary structural elements to regulate the product chain length. GGPPs from *T. thermophilus*, *P. horikoshii* Ot3, and *A. tumefaciens* use a bulky residue at the 5th position before the first DD*XX*D motif and those from yeast, human, mustard, and *T. maritima* utilize two or three large residues in different helices.

In summary, a molecular ruler mechanism controls the chain length of *trans*-prenyltransferases shown in Fig. 4. The essential amino acids for product chain length determination of *trans*-prenyltransferases are Phe^{113} for C_{15}-avian FPPs, Trp^{74} for C_{20}-*T. thermophilus* GGPPs, Tyr^{107} and His^{139} for C_{20}-*S. cerevisiae* GGPPs, Leu^{164} for C_{30}-*S. solfataricus* HexPPs, and Phe^{132} for C_{40}-*T. maritima* OPPs [3-5].

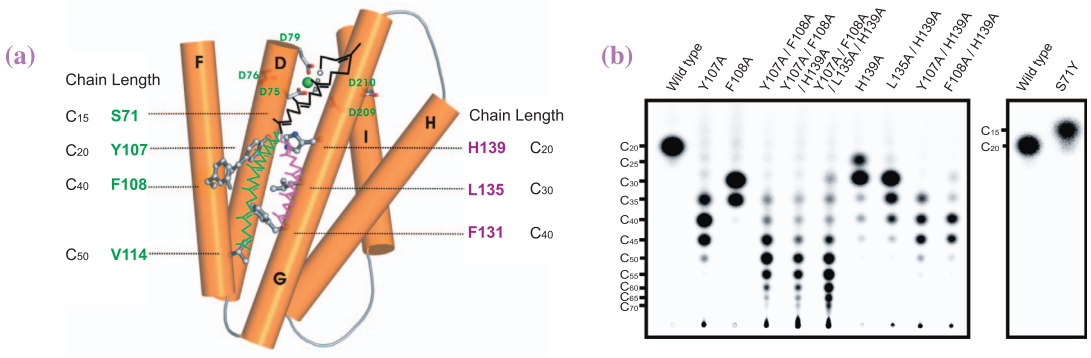

Fig. 3. **(a)** The diagram presents the mechanism of chain length determination of *S. cerevisiae* GGPPs. **(b)** The final products synthesized by wild-type and mutant GGPPs were analyzed through thin-layer chromatography.

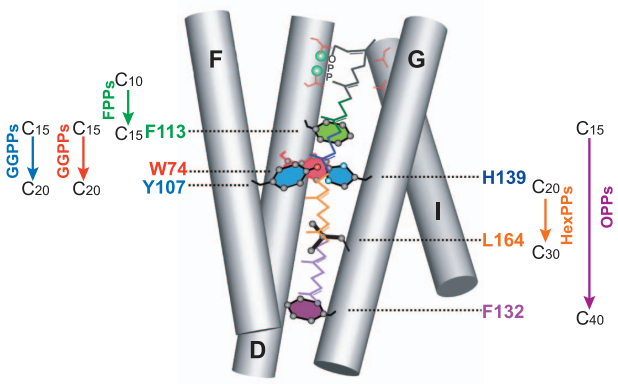

Fig. 4. The molecular ruler mechanism of *trans*-prenyltransferases. The key residues at the bottom of active site for chain length determination are marked.

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