The C5-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 C15-farnesyl pyrophosphate (FPP), C20-geranylgeranyl pyrophosphate (GGPP), and longer species such as C40-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 DDXXD, where X encodes any amino acid, coordinate with Mg2+ for substrate binding and the subsequent reaction.

Based on previous study of C15-FPP synthase (FPPs), two bulky amino acid residues at the 4th and 5th position before the first DDXXD motif in helix D form a blockage underneath the allylic substrate site to avoid the further elongation of FPP product [2]. However, C20-GGPP synthase (GGPPs) of S. cerevisiae, H. sapiens, and S. alba (mustard) contain small residues at the 4th and 5th position prior the first DDXXD 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 DDXXD motifs (helices D and I) for substrate binding with one Mg2+ coordinated by Asp75, Asp79, 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-terminus 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.
Underneath the substrate-binding site, two bulky residues, Tyr\textsuperscript{107} (helix F) and His\textsuperscript{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\textsubscript{30} synthesized by mutant H139A, the product generated by mutant Y107A is predominantly C\textsubscript{40}, suggesting the most important role of Tyr\textsuperscript{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 \textit{T. thermophilus}, \textit{P. horikoshii} Ot3, and \textit{A. tumefaciens} use a bulky residue at the 5\textsuperscript{th} position before the first DDXXD motif and those from yeast, human, mustard, and \textit{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\textsuperscript{113} for C\textsubscript{15}-avian FPPs, Trp\textsuperscript{74} for C\textsubscript{20}-\textit{T. thermophilus} GGPPs, Tyr\textsuperscript{107} and His\textsuperscript{139} for C\textsubscript{20}-\textit{S. cerevisiae} GGPPs, Leu\textsuperscript{164} for C\textsubscript{30}-\textit{S. solfataricus} HexPPs, and Phe\textsuperscript{120} for C\textsubscript{40}-\textit{T. maritima} OPPs [3-5].

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{(a) The diagram presents the mechanism of chain length determination of \textit{S. cerevisiae} GGPPs. (b) The final products synthesized by wild-type and mutant GGPPs were analyzed through thin-layer chromatography.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{The molecular ruler mechanism of trans-prenyltransferases. The key residues at the bottom of active site for chain length determination are marked.}
\end{figure}

References