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Crystal Structure of a Bifunctional Heterotetrameric Terpene Synthase: Functional Switch Via Protein-Protein Interaction.
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Terpene synthases (TPSs) are involved in the biosynthesis of terpenes (isoprenoids), a versatile class of natural compounds that can function as critical mediators in metabolism, as plant volatiles, and in ecological communication. To generate these structurally complicated and functionally diverse compounds, divergent evolution has allowed TPSs to optimize the contours of their individual catalytic pockets to achieve catalytic fidelity and diversity. A family of heteromeric TPSs, distinct from homomeric TPSs, has recently been discovered. Although the role of the heteromeric structure may be regulatory, its mechanism has remained obscure. Here, we report the crystal structure of a bifunctional heterotetrameric geranyl pyrophosphate/ geranylgeranyl pyrophosphate synthase (GPP/GGPPS) from *Mentha piperita* (mint) that is composed of two small subunits (SSU) and two large subunits (LSU). *M. piperita* GPP/GGPPS catalyzes the condensation of C_5-dimethylallyl pyrophosphate with C_5-isopentenyl pyrophosphate to generate C_{10}-GPP and C_{20}-GGPP. Monoterpenes derived from C_{10}-GPP are plant volatiles with important bioactivity. C_{20}-GGPP is a precursor in synthesizing electron carriers (carotene, chlorophyll) and is involved in intercellular signaling (Rho, Rap, Rac). The LSU possesses catalytic function while the SSU serves as a regulatory unit acting with LSU to control the ligand-binding pocket of the LSU. Our structural study sheds light on a functional enzyme switch that acts via protein-protein interaction, and may have future application in the biosynthesis of novel terpenoids.

Keywords: crystallography of complex structures; protein interactions; terpenes

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Crystal Structure of a Replicative Helicase DnaC and Its Complex With ssDNA.
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DNA helicases are motor proteins that play essential roles in DNA replication, repair, and recombination. In the replicative hexameric helicase, the fundamental reaction is the unwinding of duplex DNA; however, our understanding of this function remains vague due to insufficient structural information. Here, we report two crystal structures of the DnaB-family replicative helicase from *Geobacillus kaustophilus HTA426* (*Gk*DnaC) in the apo-form and bound to single-stranded DNA (ssDNA) [1]. The *Gk*DnaC-ssDNA complex structure reveals that three symmetrical basic grooves on the interior surface of the hexamer individually encircle ssDNA. The ssDNA-binding pockets in this structure are directed toward the N-terminal domain collar of the hexameric ring, thus orienting the ssDNA towards the DnaG primase to facilitate the synthesis of short RNA primers. These findings provide insight into the mechanism of ssDNA binding and provide a working model to establish a novel mechanism for DNA translocation at the replication fork.

[1] Lo, Y.-H.; Tsai, K.-L.; Sun, Y.-J.; Chen, W.-T.; Huang, C.-Y.; Hsiao, C.-D. *Nucleic Acids Res.* **2009**, 37, 804.

Keywords: DNA replication; replicative helicase; ATPase

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Structures and Substrate Transfer Mechanisms of Eukaryotic Fatty Acid Synthases.
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Fatty acids are basic building blocks of cells and their biosynthesis is an essential process in all kingdoms of life. In bacteria, fatty acid synthesis is carried out by sets of individual mono-functional enzymes. In contrast, eukaryotes harbor giant multifunctional fatty acid synthases (FASs), that catalyze all steps of cyclic fatty acid synthesis from malonyl- and acetyl-CoA precursors. We have recently succeeded in crystallographic structure determination of the two distinct types of eukaryotic FAS, the 2.6 MDa heterododecameric fungal [1] and the 540 kDa dimeric animal FAS [2]. Although both enzymes catalyze identical overall reactions with closely related chemistries, they have completely dissimilar architectures. Animal FAS is X-shaped and consists of a lower condensing part and an upper part containing the β-carbon modification domains. Two previously uncharacterized structured non-catalytic domains provide a clear evolutionary link to polyketide synthases. Apart from these domains, only 9% of the total sequence is invested in domain linking, and the condensing and modifying parts of animal FAS are only loosely connected. While fungal FAS is a barrel shaped, rigid structure with two tightly defined reaction chambers, animal FAS is not constrained by scaffolding and displays a remarkable degree of flexibility, which is required for efficient substrate shuttling.

[1] Jenni S., Leibundgut M., Boehringer D., Frick C., Mikolasek B., Ban N., *Science*, **2007**, 316, 254. [2] Maier, T., Leibundgut, M., Ban, N., *Science,* **2008**, 321,1315.

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