

Relaxed specificity in aromatic prenyltransferases

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Prenylation represents a critical step in the biosynthesis of many natural products. A new study reveals how aromatic prenyltransferase enzymes tolerate diverse aromatic polyketides while still controlling the length of prenyl side chains.

Natural products remain a valuable source of chemical diversity and provide tools for probing biological mechanisms and developing therapeutic lead compounds¹. Secondary metabolites of plants and bacteria are among the most intensively investigated natural products. Although some natural products are easily classified as terpenes, alkaloids or polyketides, many are structural hybrids of these broader classes. Naphterpin², for example, is a natural product that contains a polyketide aromatic core that is fused to a terpenoid framework by prenyl transfer. But how are hybrid natural products, such as naphterpin, synthesized in cells, and how do the enzymes involved in their biosynthesis adapt to the array of related metabolites involved in the pathway? In a recent Letter to *Nature*, Kuzuyama *et al.*³ provided biochemical and structural insight into these questions. Their studies show how a prenyltransferase enzyme may tolerate diverse aromatic compounds as substrates, while regulating the size of the prenyl side chain that is attached during the biosynthetic reaction.

'Prenylation' is a general term for the enzymatic addition of a hydrophobic side chain to a biomolecule. Prenyl groups can be relatively small, five-carbon (C₅) units, including isopentenyl or dimethylallyl groups, or they can be larger side chains comprising multiple five-carbon units, such as geranyl (C₁₀) or farnesyl (C₁₅) moieties. *In vivo*, prenylation is catalyzed by prenyltransferase enzymes, in which terpene diphosphates are used as prenyl donors. The structures of several protein farnesyltransferases and geranyltransferases have been described, providing new insights into the chemistry and function of this important post-translational modification⁴.

Prenylation represents a critical step in the biosynthesis of natural products of mixed origin, such as naphterpin. However, in contrast to protein prenyltransferases, very little is known about small aromatic molecule prenyltransferases. For example, Pojer *et al.*⁵ recently identified a new class of aromatic prenyltransferases

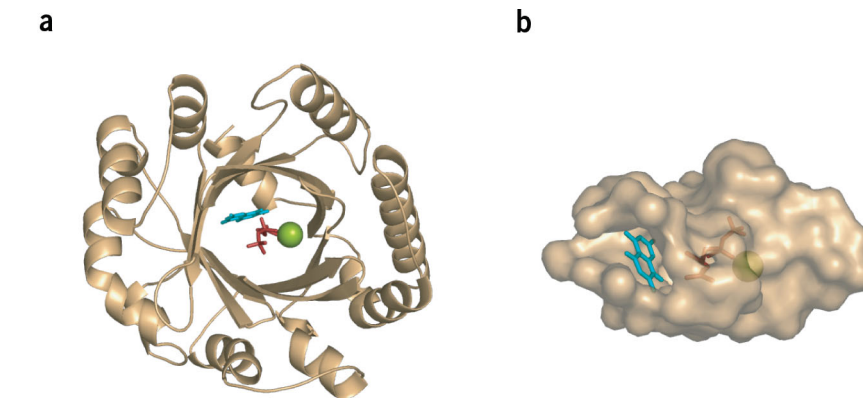


Figure 1 Structure of the aromatic prenyltransferase Orf2, from *Streptomyces*, complexed with aromatic and prenyl substrates. **(a)** A representation of the prenyltransferase barrel structure of Orf2, with the central barrel axis oriented vertically. The bound prenyl group, geranyl-S-thiolodiphosphate (GSPP), and the aromatic substrate, flaviolin, are shown in red and blue, respectively. Orf2 is magnesium-dependent, and the bound Mg²⁺ is shown as a green sphere. **(b)** A side view of the barrel reveals a large binding pocket for the aromatic substrate. The envelope of atoms lining the cavity inside the prenyltransferase barrel is shown as transparent to allow visualization of the bound substrates. Both panels were rendered using Pymol (<http://www.pymol.org>). The pocket was identified with software available at: <http://www.cs.ucdavis.edu/~koehl/ProShape>. PDB coordinates (1ZDW) were kindly provided by T. Kuzuyama, J.P. Noel and S.B. Richard prior to publication.

whose genes show no sequence similarity to those of protein prenyltransferases. They studied two of these enzymes from *Streptomyces* strains, CloQ and NovQ, which are products of the gene clusters for the biosynthesis of novobiocin and clorobiocin, respectively. Neither CloQ nor NovQ contain the typical substrate binding site for prenyl diphosphate, nor do they require magnesium for their enzymatic activity, in contrast to most known prenyltransferases.

By studying the gene cluster responsible for naphterpin production in *Streptomyces*, Kuzuyama *et al.*³ found the gene *orf2*, which showed strong sequence similarity to *cloQ* and *novQ*. The corresponding protein, Orf2, is monomeric and soluble and shows promiscuous prenyltransferase activity *in vitro* on a large variety of flavonoids and related plant polyketides, including the prenylation substrate of CloQ. Orf2 shows intriguing specificity for prenyl diphosphate substrates. Unlike CloQ and NovQ, which accept only C₅ prenyl units, Orf2 selects against these shorter chains and prefers to transfer geranyl and farnesyl side chains to the aromatic substrates.

The characterization of the high-resolution structure of Orf2 in both the absence and the presence of aromatic substrates by Kuzuyama *et al.*³ revealed several features of the enzyme's substrate promiscuity and selectivity. Although it was expected that the three-dimensional structure of Orf2 would show no similarity to structures of protein prenyltransferases, it was surprising that Orf2 adopted a new α/β barrel fold (**Fig. 1a**), in which 10 antiparallel β -strands form a barrel filled with solvent molecules. Such α/β barrel folds are common among enzymes, and usually include a core of 4–20 strands arranged into a closed barrel, surrounded by a ring of solvent-exposed helices⁶. Orf2 adopts an unusual β -barrel that resembles the TIM fold⁷, but with antiparallel β -strands. Two additional Orf2 ternary complex structures revealed that the geranyl diphosphate analog and the aromatic substrate bind in the barrel (**Fig. 1a**). Most notably, the Orf2 structure creates a large pocket at the site of aromatic substrate binding, which may explain its ability to accept a variety of aromatic prenylation substrates (**Fig. 1b**).

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To gain further insight into how aromatic prenyltransferases select the appropriate prenyl donor, Kuzuyama *et al.*³ used the three-dimensional structure of Orf2 to model the active sites of other known aromatic prenyltransferases, including CloQ, NovQ and another prenyltransferase from *Streptomyces*, HypSc. In the crystal structures of Orf2–prenyl complexes, the bound prenyl group is sequestered inside the barrel. The structural models for CloQ, NovQ and HypSc, based on the Orf2 structure, each contained a salt bridge between arginine and glutamine residues inside the barrel that prevents longer prenyl groups from entering. Based

on the structure of Orf2 bound to its substrates, the authors suggested electrophilic aromatic substitution as the likely mechanism for prenyl transfer. However, their models for aromatic substrate versatility, prenyl group specificity and the reaction mechanism invite additional mutagenesis and biochemical experiments.

Kuzuyama *et al.*³ provide a glimpse into the structure and biochemistry of the prenylation of aromatic natural products by prenyltransferases. Their observations provide a testable mechanistic hypothesis for aromatic prenyl transfer reactions in secondary metabolism. They also suggest that these enzymes may be

used to create libraries of prenylated aromatic analogs for use in probing biological systems and in drug development.

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