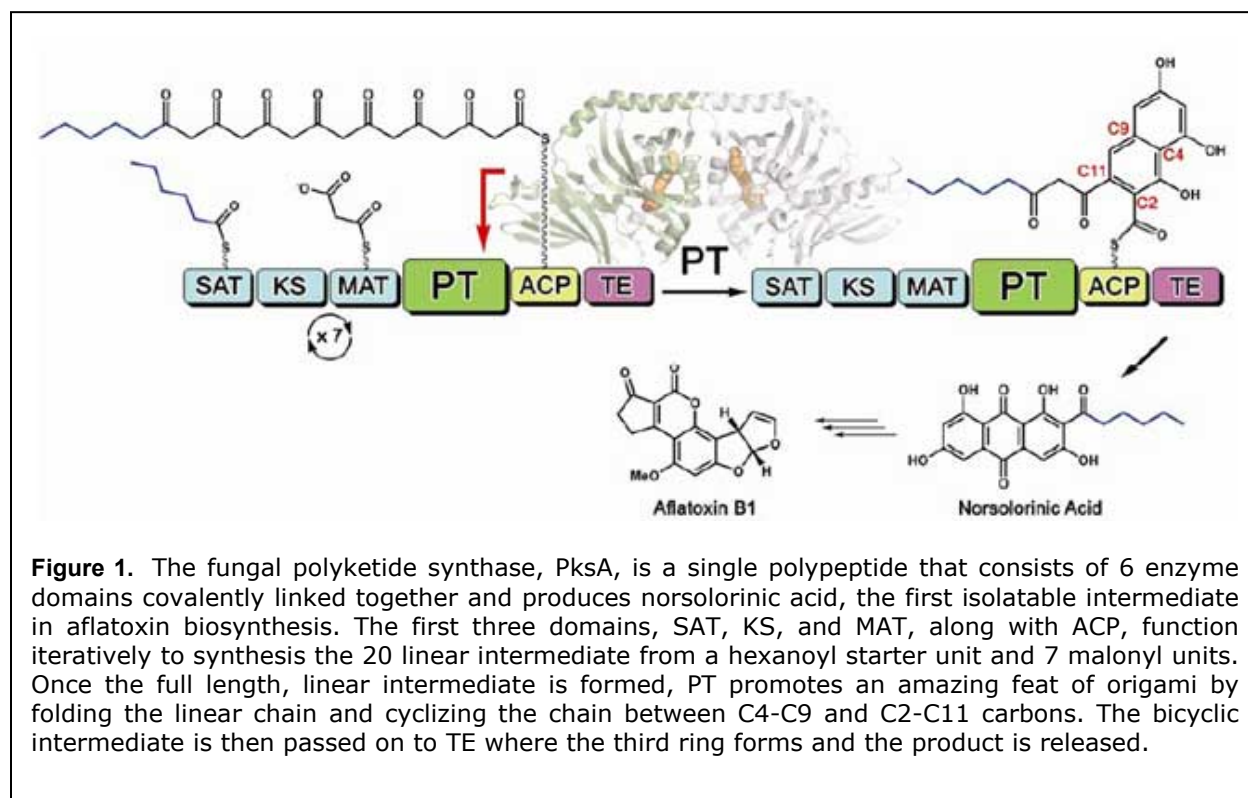


Deadly Carcinogen Unraveled: The Molecular Origami of Fungal Polyketides

Aflatoxin is an unavoidable food contaminant in grains and nuts produced in developing countries. Chronic ingestion of nuts and grains contaminated with aflatoxin-producing molds such as *Aspergillus parasiticus* leads to a high rate of liver cancer, and is a large problem in developing countries. Aflatoxins belong to a class of natural products called polyketides, which are biosynthesized in many bacteria and fungi (1,2).

Polyketide natural products produced by bacteria and fungi are often characterized by the presence of multiple aromatic rings that are responsible for the activity of polyketides as antibiotic, anticancer, and toxic compounds (2). Polyketide ring formation by fungal PKSs results from regiospecific cyclizations of reactive poly- β -keto intermediates of specific length that is mediated by the product template (PT) domain (Fig. 1). The mechanism for aromatic ring formation, where a linear intermediate is transformed into a multicyclic product with high fidelity, has remained unclear. To reveal the cyclization mechanism, the Tsai lab at the University of California, Irvine, in collaboration with the Townsend lab at The Johns Hopkins University, solved the structure of the isolated PT domain of PksA to 1.8 Å using data from the SSRL beamline 9.1 and ALS beamline 8.2.2. The work was reported in the Oct 22 issue of *Nature*.



The PksA megasynthase, containing six enzyme domains covalently linked together, catalyzes the iterative reactions that couple one hexanoyl group with 7 malonyl-CoAs to produce a 20-carbon linear poly β -keto intermediate (Fig. 1). At this point, if left unprotected, the highly reactive poly β -keto moieties would undergo random cyclization to produce dead-end products. Instead, the linear intermediate enters PT, where it is stabilized and transformed into a specific bicyclic intermediate with a highly specific cyclization pattern exclusively observed in fungi. How PT accomplishes this amazing feat of origami remains a mystery.

In contrast to PT in fungi, bacteria would fold the linear chain into a completely different pattern, as promoted by an enzyme called aromatase/cyclase (ARO/CYC) that share no sequence homology with PT. Therefore, although starting with the same linear chain, fungi and bacteria would fold the chain differently, resulting in completely different natural products. How bacteria and fungi conduct such highly-specific cyclizations has fascinated researchers in the natural product field for the past decade.

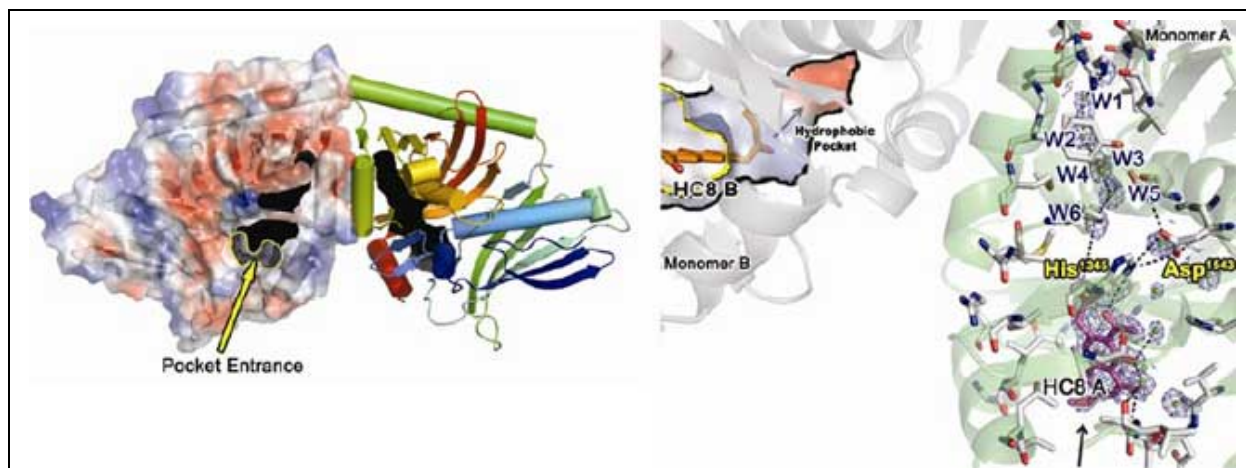
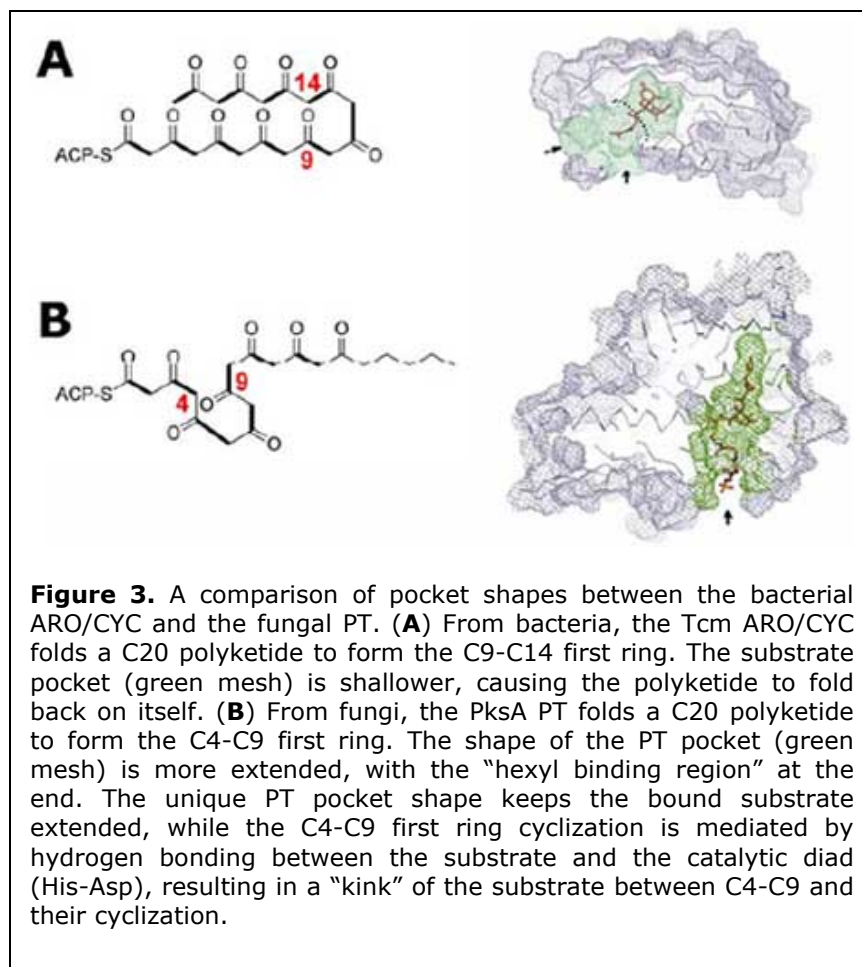


Figure 2. The PT dimer crystal structure. **(A)** The monomer shown in cartoon (right) is colored from the N-terminal (blue) to the C-terminus (red). The long hydrophobic pocket (left, in black shadow) is colored black. The surface representation highlights the single opening to the PT active site on each face of the dimer. **(B)** A closeup view of the PT active site with the bicyclic substrate analog HC8 bound. The hydrophobic hexyl binding region is filled with 6 crystallographic waters. HC8 binds just below the catalytic diad of His1345 and Asp1543 in a region called the cyclization chamber that is big enough to accommodate a bicyclic intermediate.

The crystal structure of the fungal PT domain contains a dimer of double hot-dog folds reminiscent of ARO/CYC. Each monomer has an interior pocket (Figure 2), and the finite PT pocket length limits the possible substrate chain length while also orienting the linear polyketide via the presence of a hydrophilic “wet side” and a hydrophobic “dry side” in the pocket. Two cocrystal structures were solved. In the first structure, a fortuitously bound palmitic acid molecule (from *E. coli* purification) established the long, hydrophobic pocket and suggested that the 20-carbon intermediate enters the narrow PT pocket in a linear conformation. The second structure, soaked with a bicyclic substrate analog, showed that the interior chamber can accommodate two aromatic rings in a region in the middle of the chamber near the catalytic diad (Figure 3). Together with biochemical data, the structures confirm the catalytic capability of PT to catalyze first and second ring cyclization/aromatization, and established a general mechanism for fungal polyketide cyclization.

The fungal PT crystal structure, together with the bacterial ARO/CYC structure published in 2008, have changed the way we visualize polyketide cyclization. Although researchers had known for half a century that polyketides adopt special “Streptomyces Fold” (the S-fold, C7-C12 or C9-C14 cyclized) for bacterial-generated polyketides, or “Fungal Fold” (the F-fold, C7-C2, C9-C4 or C11-C6 cyclized) for fungal polyketides, no information is available about how Nature folds and cyclizes a linear polyketide chain in such a highly specific manner. The ARO/CYC and PT structures revealed that despite of low sequence homology, both the bacterial ARO/CYC and fungal PT have similar topology that look very similar to their cousin,

the fatty acid synthase dehydratase (DH), and both have an interior pocket, whose size and shape directs the distinct ring patterns observed in bacteria and fungi.



Primary Citation

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References

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