

Natural product biosynthesis moves *in vitro*

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Heterologous production of natural products in non-native bacteria can be used to increase yields of certain bioactive compounds; however, producing small molecules inside bacteria has numerous limitations. Two reports of the *in vitro* reconstruction of entire biosynthetic pathways highlight the advantages and challenges of this approach for pathway engineering.

Many drugs of clinical significance are natural products produced by bacteria and fungi; examples include antibiotics (erythromycin), anticancer agents (mitomycin), immunosuppressants (tacrolimus) and cholesterol-lowering agents (lovastatin). Two major natural product classes of interest as drugs are aromatic polyketides and nonribosomal peptides. Well-known examples of aromatic polyketides are tetracycline and doxorubicin, and vancomycin and cyclosporin are representative nonribosomal peptides. Tremendous advances in our understanding of the genetics and biosynthesis of natural products in microorganisms during the last 15 years have reinvigorated the study of natural products for drug discovery¹. However, the metabolic engineering of biosynthetic pathways and *in vivo* combinatorial biosynthesis of natural product libraries has had limited success. In this issue of *Nature Chemical Biology*, Cheng *et al.*² and Balibar *et al.*³ independently report, respectively, the *in vitro*, enzymatic total synthesis of aromatic polyketide (wailupemycins and enterocin) and mixed nonribosomal peptide–isoprenoid (terrequinone A) natural products. These studies suggest new ways to explore natural product biosynthesis for drug discovery.

Polyketides and nonribosomal peptides are biosynthesized in a manner similar to fatty acids by large, multifunctional enzymes that typically carry out condensation, reduction and dehydration reactions. A key feature

of these polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) systems is their modularity. Genetic manipulation of individual modules, or the exchange of a module for one from a different pathway, have been the most widely studied methods for pathway engineering. The slow growth and genetic intractability of certain natural product-producing bacteria present significant challenges for metabolic engineering. Heterologous expression of biosynthetic pathways in *Escherichia coli* is playing an increasingly important role in natural product production, particularly for unculturable organisms⁴. Total chemical synthesis can also be a viable route for drug discovery, and the recent total synthesis of ambiguine H by Baran and co-workers is an elegant example of a biomimetic strategy that rivals the efficiency of the producing cyanobacteria⁵. However, chemical synthesis is not often practical for producing libraries of complex natural products. The approach of Cheng *et al.* and Balibar *et al.*, which takes the entire biosynthetic machinery out of the cell, represents a new strategy for producing complex natural products of biomedical interest.

Enterocin (**Fig. 1a**) is a bacteriostatic polyketide produced by a type II PKS in the marine bacterium *Streptomyces maritimus*. Its unique non-aromatic caged core is the result of an enzymatic Favorskii rearrangement by the flavoprotein EncM. Although all of the proteins for enterocin biosynthesis have previously been expressed and studied individually *in vitro*, Moore and co-workers have now taken biosynthetic pathway reconstruction to a new level. Combination in a single flask of the first eight recombinant proteins from the enterocin pathway (EncA/B, -C, -D, -N, -M, -K), the primary

metabolite precursors (benzoic acid and malonyl-CoA) and the necessary cofactors (*S*-adenosyl-L-methionine (SAM), NADPH, ATP and Mg²⁺) afforded 5-deoxyenterocin and the shunt natural products wailupemycins F and G. Extraction of the reaction mixture and addition of EncR, three commercial enzymes and NADPH converted 5-deoxyenterocin to enterocin. (Inhibition of EncR by SAM prevented reconstruction of the entire pathway in one pot.) This remarkable two-step enzymatic cascade forms ten C–C bonds, five C–O bonds and seven stereogenic centers in approximately 25% overall yield.

Whereas the work of Moore and colleagues demonstrates the sheer power of enzymatic synthesis, Walsh and co-workers elegantly show the utility of biosynthetic pathway reconstruction for revealing unusual mechanisms in natural product biosynthesis. Terrequinone A (**Fig. 1b**), a cytotoxic fungal metabolite, is a representative member of the bisindoylbenzoquinone family of natural products. Its biosynthetic pathway contains a unique single-module NRPS, TdiA, that lacks a traditional condensation domain. TdiA also contains a thioesterase (TE) domain, but the normal functional groups resulting from a TE domain (carboxylic acids, cyclic amides and esters) are not present in terrequinone A. Walsh *et al.* have now cloned and expressed all five enzymes responsible for terrequinone biosynthesis. Reconstruction of the entire pathway constitutes the *in vitro* total synthesis of terrequinone A in three enzymatic steps. Significantly, this work reveals the mechanisms of the unique TE-catalyzed C–C bond cyclization of TdiA and the prenyltransferase TdiB. Also of interest is the still-unclarified role of TdiE. TdiE was predicted to be a methyltransferase;

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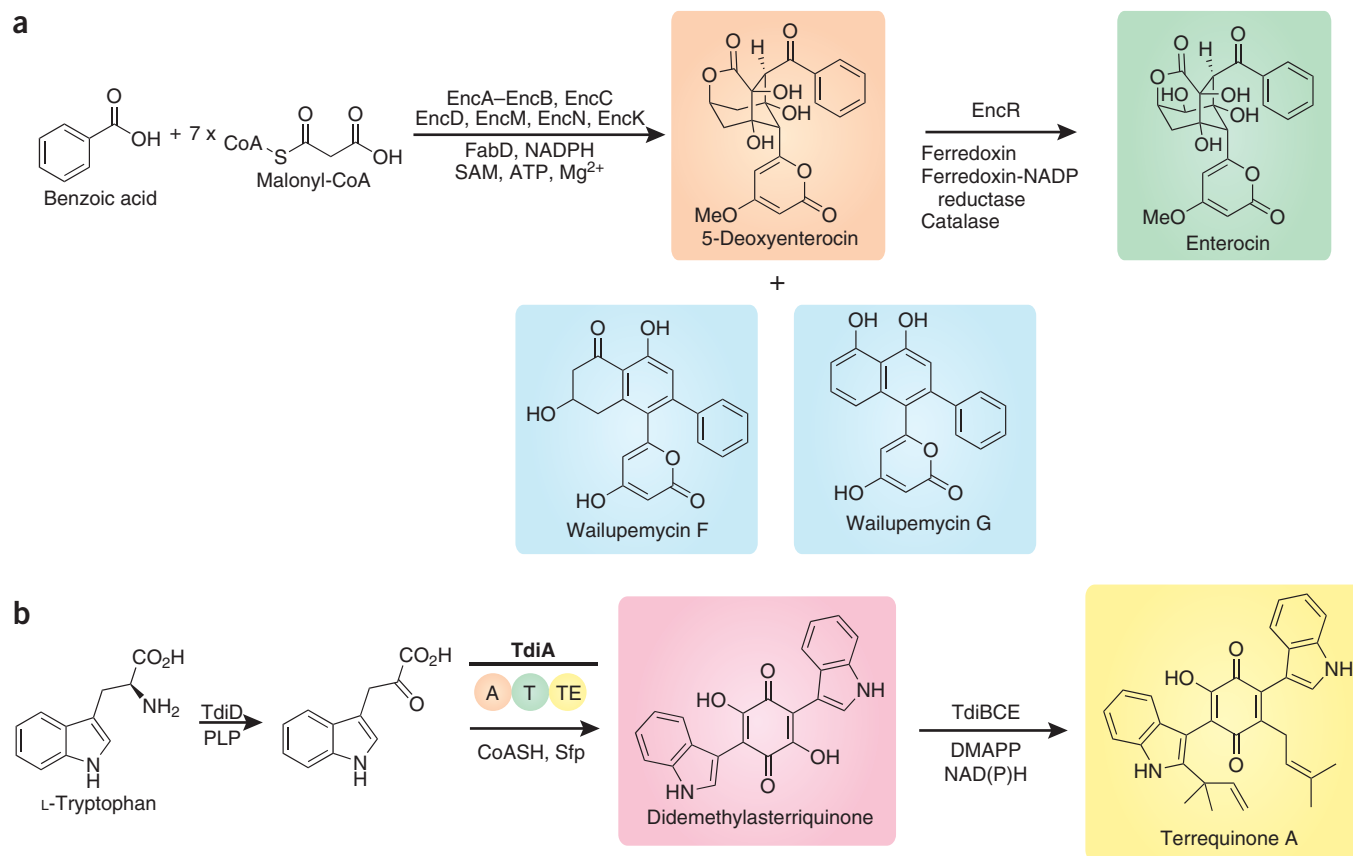


Figure 1 Biosynthetic pathways. (a,b) The *in vitro* total biosynthesis of enterocin and wailupemycins F and G (a) and of terrequinone A (b). A, adenylation domain; ATP, adenosine 5'-triphosphate; CoASH, coenzyme A; DMAPP, dimethylallyl diphosphate; NADPH, nicotinamide adenine dinucleotide phosphate; PLP, pyridoxal 5-phosphate; SAM, S-adenosyl methionine; T, thiolation domain; TE, thioesterase.

however, it seems to have a gatekeeper function in preventing the formation of a dead-end side product.

Each of the two papers highlights different advantages to the study of entire biosynthetic pathways *in vitro*. Reaction of benzoic acid and malonyl-CoA with the minimal enterocin PKS system (EncA/B, -C, FabD) resulted in nonaketide products that have not been observed *in vivo*, revealing aspects that regulate polyketide chain length². In the terrequinone pathway, the study of multienzyme combinations *in vitro* enabled the function of TdiE to be partially understood when gene sequence predicted a different role³. Perhaps the most exciting aspect of these studies is

the number of unanswered questions that are now before us. It remains to be seen at what scale natural products from reconstructed pathways can be produced and how amenable to protein engineering these systems are when removed from a microorganism. The *in vitro* enzymatic total synthesis of natural products suggests new avenues to study these pathways and provides additional opportunities for metabolic engineering. For instance, immobilization of biosynthetic enzymes may further increase their activity and stability, and successive combinations of enzymes may quickly reveal those with relaxed substrate specificity suitable for combinatorial biosynthesis. Despite the challenges ahead

in reconstructing biosynthetic pathways, the power of multifunctional enzymes to conduct complex chemistry will continue to inspire scientists to harness them for practical purposes.

COMPETING INTERESTS STATEMENT

The author declares no competing financial interests.

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