Discovering novel enzymes in bacterial natural product biosynthesis

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The principles of construction of complex polyketides and nonribosomal peptides are now reasonably well understood, based on the unifying paradigm of assembly-line multimodular enzymes, in which each additional extension ketide or amino acid unit is processively introduced and shaped by a different set of enzymatic activities. However, the chemical diversity and biological activity of the end-products owe much to the action of further enzymes which either operate upon the full-length polyketide or polypeptide product, or interact directly with the growing chain on the modular synthase/synthetase. Among bacterial polyketides, one of the most intriguing of these transformations is the regio- and stereospecific cyclisation within the polyketide chain, required to form the multiple rings of polyethers. An extreme example of this is the biosynthesis of the polyether tetronate tetronasin from *Streptomyces longisporoflavus* which involves the formation of four different types of ring. For the central rings in particular, an unprecedented cyclisation mechanism seems required.

A different type of natural product polymer is represented by aminoglycosides such as the gentamicin C complex from *Micromonospora echinospora*, a globally important pseudotrisaccharide antibiotic still relied upon to combat serious Gram-negative bacterial infections. The pathway to gentamicins has also been largely worked out, except for the unusual removal of vicinal hydroxyl groups from one sugar ring of a late-stage biosynthetic intermediate. This modification is crucial because it makes the antibiotic less vulnerable to inactivation by resistance enzymes of the target pathogens. The identity, role and mechanism of the enzyme(s) that govern this double dehydroxylation are unknown.

We have set out to unravel the enzymology of all of these unusual biosynthetic transformations, using a combination of genetic and biochemical approaches, and culminating in stepwise in vitro reconstruction of the late steps of the biosynthesis. This has revealed several novel enzymes, and has given fresh insight into the mechanisms involved.