Spying on nature's drug factories

Martina Habeck, freelance writer

Microorganisms have their own special machinery to assemble compounds with antibiotic, immunosuppressive or cytostatic activity. New revelations about the architecture of these drug factories should enhance our ability to manipulate these assembly-line processes to discover new drugs.

Bacteria and fungi are a rich source of natural products that are of therapeutic interest. Many of these are used in the clinic, including penicillin, vancomycin, cyclosporin and bleomycin. The building blocks of these compounds are amino acids and carboxylic acids that are

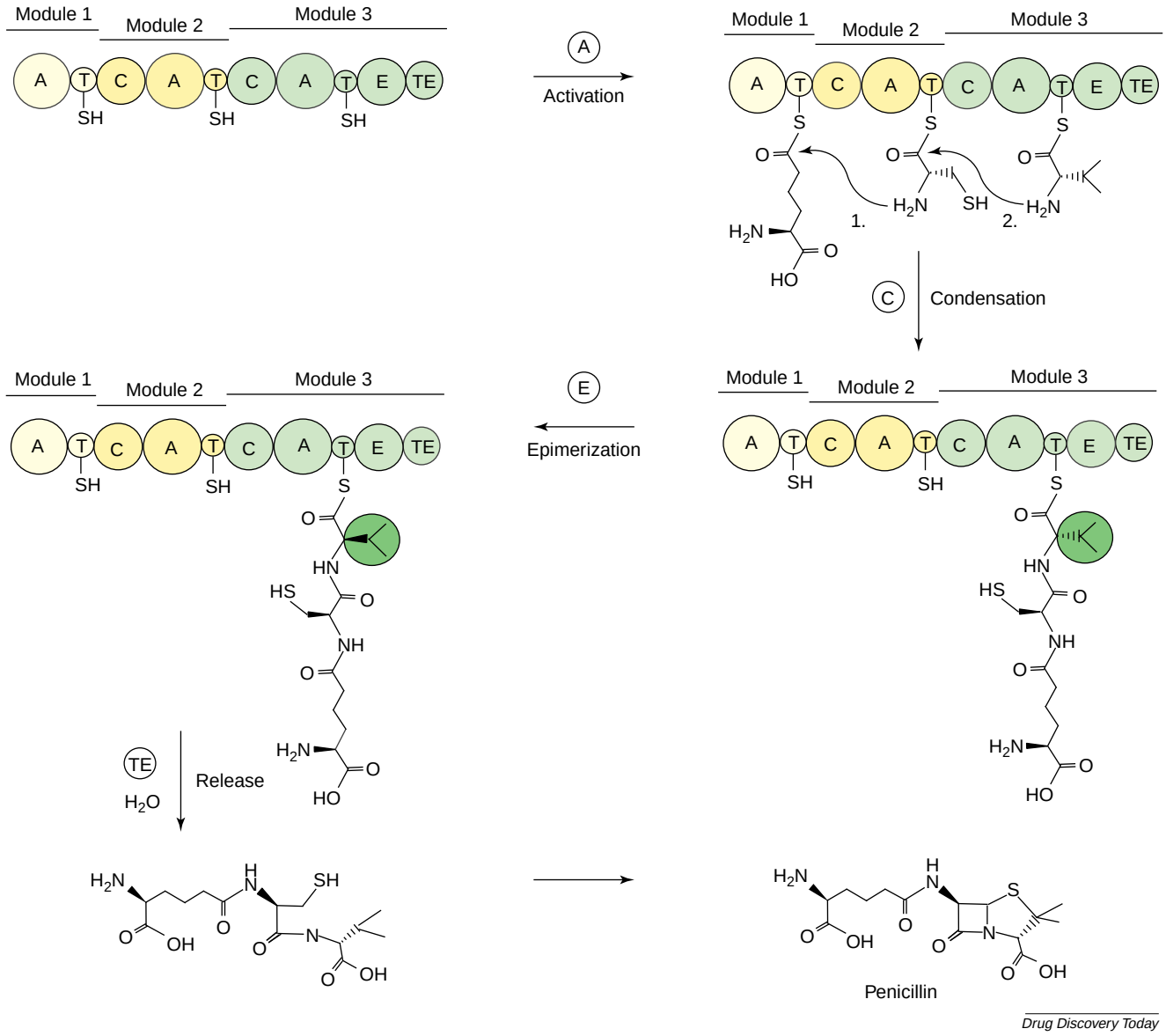

Figure 1. The biosynthesis of penicillin. Penicillin is produced by a modular non-ribosomal peptide synthetase (NRPS). Each module has various domains. The adenylation domain (A domain) recognizes and activates an amino-acid monomer by ATP hydrolysis. The thiolation domain (T domain) then tethers the amino acid by forming a thioester bond, and the condensation domain (C domain) adds the amino acid to the growing peptide chain. An amino acid can also be modified by epimerization (change from the L- to D-configuration; E domain). Finally, the full-length chain is released through hydrolysis by a C-terminal thioesterase domain (Te domain). Figure kindly provided by the Marahiel group at the University of Marburg, Germany (http://www.uni-marburg.de).

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assembled by giant proteins called nonribosomal peptide synthetases (NRPSs) and polyketide synthetases (PKSs). These proteins have a modular structure, and each module acts as an independent multifunctional enzyme that joins one amino acid or carboxylic acid to the growing polypeptide or polyketide chain and makes modifications possible (Fig. 1). The specific order of the modules defines the sequence of the incorporated building blocks.

Combinatorial biosynthesis

Many scientists have dreamt of redesigning this assembly line to create new products with therapeutic activity. The idea is to recombine the modules and thus arrive at a novel compound – a process known as combinatorial biosynthesis.

'In principle, you could do this combination at the DNA level,' said Christopher Walsh of Harvard Medical School (http://www.hms.harvard.edu). He points out that scientists can already tell from looking at the DNA sequence whether something will be a PKS or an NRPS assembly line, and even which particular acyl or aminoacyl monomer each module will select.

However, to engineer new PKS and NRPS proteins successfully, the 3D structure of these drug factories also needs to be understood. It is well established that PKSs are only active as homodimers [1,2]. The same holds true for fatty acid synthases [3], closely related megasynthases that use an assembly-line strategy equivalent to that of the PKSs. Therefore, people assumed that NRPSs would also be oligomeric – but a new study says otherwise [4].

Teams led by Walsh, and Mohamed Marahiel at the University of Marburg in Germany (http://www.uni-marburg.de), looked at modules from the gramicidin S, tyrocidine and enterobactin biosynthetic systems and investigated their architecture with biophysical and biochemical techniques, such as cross-linking, gel filtration, analytical ultracentrifugation and mutant complementation experiments. Independent of the method used, the answer was the same: 'We always find they are monomers,' said Walsh.

Ben Shen at the University of Wisconsin-Madison (http://www.pharmacy.wisc.edu) is impressed with the thoroughness of their approach: 'Many of the conclusions people draw in the field [are based on] only one set of experiments, and sometimes [the results] are over-interpreted. But this group of scientists used both biochemical and biophysical methods, and even within each category, they have done every single experiment you can think of, and they come to the same conclusion. I think their result is very sound.' Because they studied three different NRPS systems, Shen believes their finding is generally applicable.

Implications for drug discovery

Where does this finding leave scientists who wish to engineer new 'natural' products? According to Shen, PKS engineering gained much momentum with the discovery of the dimeric structure of these proteins. He predicts that the discovery of Walsh and Marahiel 'should have the same effect and help us engineer peptide synthetases for new structures.'

The finding is important for another reason: some natural products of therapeutic interest are polyketide and polypeptide hybrids. Among those hybrids are the anticancer drugs bleomycin and also epothilone, which is currently in clinical trials. This suggests that the polyketide and NRPS assembly lines have to be able to mix and match. But how exactly does that work? How does a dimer interact with a monomer at the molecular level?

That is what the Walsh and Marahiel teams are now investigating, for example, by studying the epothilone biosynthetic pathway in more detail. The epothilone assembly line consists of a PKS subunit (epoA), followed by an NRPS subunit (epoB), followed by another PKS subunit (epoC). In other words, there are two PKS–NRPS switch points. 'We are trying to understand how A and B interact and how B and C interact,' said Walsh. 'If we understand the rules of compatibility between the module boundaries, maybe we could swap in different modules and make new versions of the natural product.'

References

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