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these fundamental questions can begin to be addressed. ■

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BIOCHEMISTRY

Enzyme's black box cracked open

David H. Sherman

Polyketide synthase enzymes make compounds from molecules that synthetic chemists can't easily control. The basis of the enzymes' ability to use such unstable precursors has been laid bare.

Polyketides form a structurally diverse — and often medicinally useful — family of compounds derived from bacteria, fungi and plants. These compounds include the antibiotic erythromycin, the anticancer agent epothilone and the cholesterol-lowering drug lovastatin. But it's not all good news. Some polyketides are dangerous, such as aflatoxin B_1, which causes liver cancer in humans. The biological activities of polyketides are prescribed by their molecular shapes and sizes. These, in turn, are ultimately determined by the biosynthetic machinery that makes the molecules — polyketide synthase (PKS) enzymes, along with other auxiliary enzymes that tailor polyketide structures to provide additional chemical diversity[1].

Among PKS enzymes, some of the most confounding and mysterious are the fungal non-reducing, multi-domain iterative PKSs (NR IPKSs). These enzymes perform a truly remarkable 'ring-closing' transformation: they convert linear polyketide fragments into a series of connected aromatic rings, known as polycycles. On page 1139 of this issue, Crawford *et al.*[2] provide stunning insight into how NR IPKSs bind and fold polyketides to control the structural outcome of these transformations. They have obtained a crystal structure of a domain of PksA — an NR IPKS found in certain species of *Aspergillus* moulds that is involved in the assembly of a polycyclic precursor to aflatoxin B_1, known as noranthrone.

The stage was set for Crawford and colleagues' work when a region of PksA known as the product-template (PT) domain was reported last year to be responsible for catalysing polyketide ring-closing reactions[3]. This unique functional domain was shown to be evolutionarily conserved in NR IPKSs from diverse fungi. But how does it work? The mechanism might reasonably be expected to be similar to that of fatty acid synthase (FAS) enzymes, which are phylogenetically and biochemically related to NR IPKSs. FAS enzymes make the long hydrocarbon chains of saturated fatty acids by repeating a cycle of reactions, in which the chain is first elongated by the addition of a small molecular building block and then chemically reduced[4].

In fact, although PksA makes molecular chains using elongation steps similar to those of FAS enzymes, it lacks the processing machinery necessary to perform reduction reactions. This results in the formation of a polyketide chain that, in isolation, would be so chemically unstable as to be practically useless. But in NR IPKS enzymes, the polyketide is covalently tethered to an arm (known as the phosphopantetheine arm) of one of the protein domains that makes up the enzyme. The combination of the covalent linkage and the NR IPKS protein scaffold provides a protective environment that stabilizes the polyketide.

The importance of chain stabilization in polyketide biosynthesis was suggested several decades ago when a series of linear polyketides was prepared by chemical synthesis[5]. The compounds were made to see how they might be involved in the biosynthesis of natural products that are polycyclic and aromatic, many of which are derived from fungi. These studies showed that, in the absence of external influences, polyketides undergo several different uncontrolled cyclization reactions (rather than just one), yielding a mixture of products (Fig. 1a). This ultimately suggested that NR IPKS enzymes are responsible for much more than just chemical catalysis — they must also direct the polyketide chain toward a pre-cyclization configuration, something that was unimaginable at the time. Such molecular control would enable faithful generation of a single reaction product out of a myriad of possibilities.

The 'black box' of fungal NR IPKS has now

50 & 100 YEARS AGO

50 YEARS AGO

The Scientific Principles of Crop Protection. By Hubert Martin — To say that, nuclear wars apart, the greatest problem of the future will be to feed the rapidly growing human population is none the less true for being trite. Happily there is no need to think it is insoluble. Average crop yields are so low that the scope for improvement is enormous, and starvation can be avoided for a long time simply by improving the health of crops. Over much of the world most crops are left to fend for themselves, unaided in their struggle with pests and diseases. What annual toll these predators take cannot be estimated at all accurately, but there is little doubt that human beings will have at least twice as much to eat when they stop sharing their crops with pests and diseases.

ALSO:

Prof. Jaroslav Heyrovsky ... has been awarded the Nobel Prize for Chemistry for 1959, for his discovery and development of polarography ... The number of papers dealing with polarography now approaches the 10,000 mark, and the technique finds application in many fields of chemistry and biochemistry ... For example, the kinetics of electrode reactions and of chemical reactions associated with redox processes have been studied, redox potentials have been determined and the energetics of the reduction of organic compounds have been elucidated.
From *Nature* 24 October 1959.

100 YEARS AGO

Considerable interest attaches to the discovery of large quantities of shells of the pearl-mussel (*Unio margaritifer*) in gravel of apparently Pleistocene age in the Thames near Mortlake ... The cause of the extinction of the species is explained by the fact that as the land sank the river became more sluggish, and silt and mud commenced to accumulate. Such conditions would prove highly detrimental to its welfare, and the species soon ceased to exist.
From *Nature* 21 October 1909.

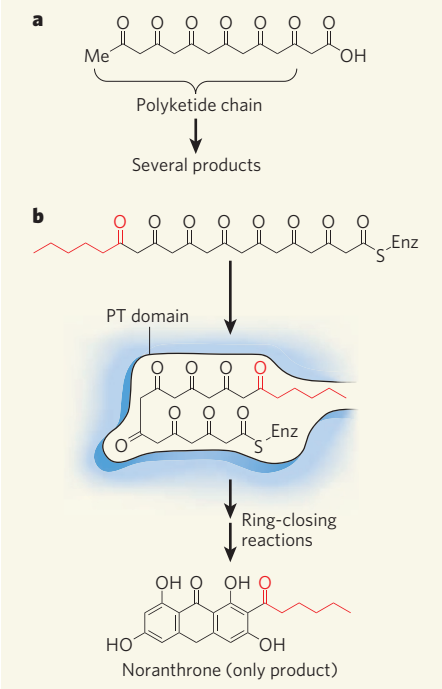

Figure 1 | Controlling the reactivity of polyketide chains. a, Molecules containing polyketide chains are highly unstable. In the absence of external control, they undergo several reactions, yielding a mixture of products. **b**, Fungal non-reducing, multi-domain iterative polyketide synthase (NR IPKS) enzymes make polyketide-containing compounds as precursors of natural products. The polyketide chains are covalently linked to the enzyme (Enz) and may undergo further modification — in this case, a hexanoyl group (red) is attached at one end of the chain. The resulting product is then guided into the product-template (PT) domain of the enzyme, which folds the polyketide chain into the optimal conformation for subsequent ring-closing reactions. Crawford *et al.*[2] have obtained crystal structures of the PT domain of a fungal NR IPKS, revealing the mechanisms used by the enzyme to control its substrate, which allow it to faithfully form a single compound, noranthrone, rather then a mixture of products. Me, methyl group.

been cracked wide open in Crawford and colleagues' penetrating account[2] of the pivotal role of the PT domain. First, the authors find that, although the amino-acid sequence of PksA bears little resemblance to those of FAS enzymes, the crystal structure of the PT domain of PksA has a similar 'double hotdog fold'[6] to that of certain domains of FAS enzymes — specifically, the dehydrase domains that form part of the FAS machinery responsible for reductive processing reactions. This indicates that the PT domain has evolved from a FAS dehydrase domain, or from its counterpart in bacterial modular PKSs.

The authors also observe a clear binding region for the phosphopantetheine arm of PksA that guides polyketide substrates into the PT domain's binding pocket (Fig. 1b). Furthermore, by obtaining the crystal structure of PksA in which a model substrate is bound, Crawford *et al.* reveal the presence of a spacious cyclization chamber that can harbour two aromatic rings. Herein lie the two amino-acid residues that initiate the key ring-closing reactions catalysed by the PT domain. Finally, the authors' crystal structures strongly suggest that the binding pocket orientates the polyketide chain ready for catalysis by anchoring the chain's two termini at opposite ends of the pocket.

Crawford and colleagues' analysis[2] of PT domains from other NR IPKS systems reveals that, within an otherwise highly conserved amino-acid sequence, the most significant level of divergence occurs within the cyclization chamber. This is consistent with the idea that the chambers of different enzymes control the overall configurations of specific substrates prior to reaction, and that they have each evolved to accommodate the correct number of ring-forming reactions necessary to generate a specific product. Taken together, the authors' findings show that the PT domain is the main determinant of chemical architecture produced from NR-IPKS biosynthetic pathways. After the cyclization reaction, the final release of the polyketide product is catalysed by the terminal domain of NR IPKS enzymes, a process that often results in the formation of another ring in the natural product.

The data presented by Crawford *et al.*[2], along with those from a recent study of another previously elusive enzyme from a bacterial PKS[7], bring new clarity to the once murky chemical world of polyketide ring cyclization in natural-product assembly. This fresh insight will certainly provide exciting opportunities for engineering metabolic systems for the preparation of biologically active natural products that have great potential as medicinal agents. ■

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MICROSCOPY

Light from the dark

Stefan W. Hell and Eva Rittweger

Fluorescence microscopy is the most popular way to image biomolecules, but it leaves many of them in the dark. Non-fluorescent, light-absorbing molecules can now be viewed by a method that turns them into mini-lasers.

What happens when a molecule absorbs a photon from a beam of light? It moves from the ground state to an excited, higher-energy state and then quickly relaxes, giving off the absorbed energy as heat. There are, however, notable exceptions — molecules called fluorophores, which, after some 'wiggling', relax by emitting a lower-energy photon. Employed as molecular tags, fluorophores are invaluable in biomedical microscopy and diagnostics because they render dark molecules visible with high specificity. But what if fluorescent tagging is not an option, as in applications such as endoscopy, and the molecules under investigation stay hidden in the dark? As Min *et al.*[1] report on page 1105, it is still possible to squeeze photons out of such molecules to produce three-dimensional images of biological systems, such as living cells and tissues.

How is this possible? Well, another mechanism for molecular relaxation exists that can be induced by a beam of light. In this process, called stimulated emission, a photon encountering an excited molecule produces a copy of itself, thus adding another photon of the same colour and propagation direction to the beam. To be effective, the energy of the stimulating photon must match the gap between the excited and the ground state. In fact, the stimulating photons need to be slightly lower in energy than their excitation counterparts, because some of the excited molecule's energy is usually lost as vibrational motion (wiggling) before the photon arrives[2]. Stimulated emission is used to amplify light in lasers[3], and to overcome the resolution barrier in fluorescence microscopy[4]. As a molecular process it is almost as effective as light absorption, because both processes depend on the molecule's photon-capture area of about 0.2×0.2 nanometres, which is roughly the area of the molecule itself.

The role of photon-capture area in light absorption and stimulated emission can be thought of as follows. Imagine two synchronized trains of laser pulses directly focused on a molecule: pulses containing excitation photons are followed by pulses of photons for stimulated emission (Fig. 1), with each pulse containing N photons. If one could produce focal light spots that are the size of the molecule, then every photon in the pulses would interact with the molecule — a single photon