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FIRST REACTIONS

Traffic Control in Modular Polyketide Synthases

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Polyketides and their derivatives offer a wide range of therapeutics in today's pharmaceutical market, with annual sales reaching over 20 billion dollars.[1] Key examples include antibiotics (erythromycin A), antifungals (amphotericin B), immunosuppressants (rapamycin), and anticancer agents (epothilone B). These chemically diverse and important products are made in biological systems by modular polyketide synthase (PKS) pathways. These megasynthases act as assembly lines, building the polyketide chain through consecutive condensation of coenzyme A-derived subunits.[2] This month in *ACS Central Science*, Chaitan Khosla and co-workers provide insight on the mechanisms behind the impressive processivity demonstrated in the large multifunctional type 1 PKS assemblies.[3]

So what are the precise mechanisms that guide biosynthetic intermediates from one active site to the next? And what is the relative importance of protein−protein and protein−substrate interactions to the vectorial nature of these pathways? Finding the answers to these questions is essential to the success of future engineering.

Beginning in the 1990s, the emergence of whole genome sequencing methods resulted in the discovery of numerous PKS gene clusters at a dizzying pace.[4] At around the same time, Katz and co-workers provided the first evidence that PKS modules function in the linear order indicated by their sequence.[5] This co-linearity of gene products, along with the predictability of the corresponding polyketide natural product, put modular PKS pathways in the spotlight for rational engineering, and an army of aspiring synthetic biologists were born. Combinatorial biosynthesis was hailed as an approach to engineer new "unnatural" natural products with potentially novel or increased bioactivity, ushering in a new world of molecular design and production by "simple" DNA shuffling and fermentation.[2] Numerous academic groups and independent companies threw their hats into the ring and attempted to restructure these pathways. Yet after more than a decade and millions of dollars spent, these efforts were met with very limited success, and researchers were left scratching their heads about what they were missing.

Lowry et al. greatly enhance the mechanistic understanding of the vectorial nature of PKS machines utilizing ACP cross-linking probes.

It turns out that we were missing quite a lot. We lacked 3D structures of complete modular PKSs, and while the basic mechanisms of these enzyme domains could be rationalized, we had little molecular understanding of the protein−protein interactions and processivity rules inherent to these unique assembly line machines. Leaders in this research field realized that we had to go back and study the fundamentals of these pathways before we could proceed to manipulate them. Recent years have seen the emergence of new molecular tools,[6] structural biology techniques,[7] and kinetic activity assays[8] designed to investigate the details of modular biosynthetic enzymes.

"A Turnstile Mechanism for the Controlled Growth of Biosynthetic Intermediates on Assembly Line Polyketide Synthases" by Lowry et al. introduces a new concept to our current understanding of modular PKS mechanisms (Figure 1).[3] One of the largest intellectual challenges in type I PKS enzymology is understanding how polyketide intermediates are linearly directed through the synthase modules.[9] The vectorial nature of these assembly lines is truly remarkable considering that they do not utilize a template like DNA and RNA polymerization, nor coded monomer delivery in protein synthesis. It was first thought that substrate recognition might dictate the order of

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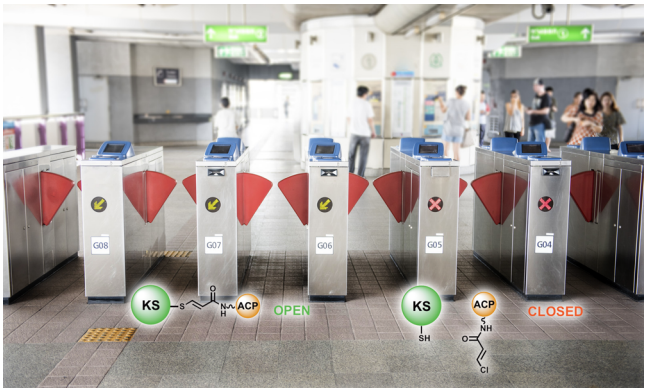

Figure 1. Lowry et. al demonstrate that the open and closed states of the KS complex act as a turnstile controlling the linear growth of the polyketide chain.

modification, but inherent substrate promiscuity has since been shown numerous times in these pathways.[10] So what are the precise mechanisms that guide biosynthetic intermediates from one active site to the next? And what is the relative importance of protein−protein and protein−substrate interactions to the vectorial nature of these pathways? Finding the answers to these questions is essential to the success of future engineering.

A minimal PKS module necessary for one round of chain elongation is composed of ketosynthase (KS), acyl transferase (AT), and acyl carrier protein (ACP) domains. The KS domain accepts a polyketide chain from the upstream ACP and then catalyzes a Claisen-like condensation between this chain and an ACP-bound α-carboxyacyl-CoA extender unit.[2] Briefly, Lowry et al. studied 6-deoxyerythronolide B synthase (DEBS), a prototypical example of a multimodular PKS that synthesizes the precursor to erythromycin. Their research uncovered a turnstile mechanism, where, after the Claisen condensation between the KS and ACP occurs, the KS is not immediately loaded by the upstream ACP (closed conformation). Only once the ACP's cargo gets offloaded by a downstream KS or TE domain does the module move to an open conformation, allowing the KS to be reprimed with a growing polyketide chain. This mechanism, with a minimal three-state catalytic model, was demonstrated through the application of synthetic intermediates and subsequent MS analysis, as well as the application of cross-linking probes designed to tether ACPs to KS domains, monitored by gel shift analysis.[3]

The concept that the KS of each module does not become acylated by the upstream ACP until the acylated product from the prior catalytic cycle has been passed on to the downstream module is a new and crucial detail in megasynthase processivity (Figure 2A). This work suggests mono-occupancy of a PKS subunit, whereby modular reactions are energetically coupled to the translocation of the growing polyketide chain to the next module, facilitated

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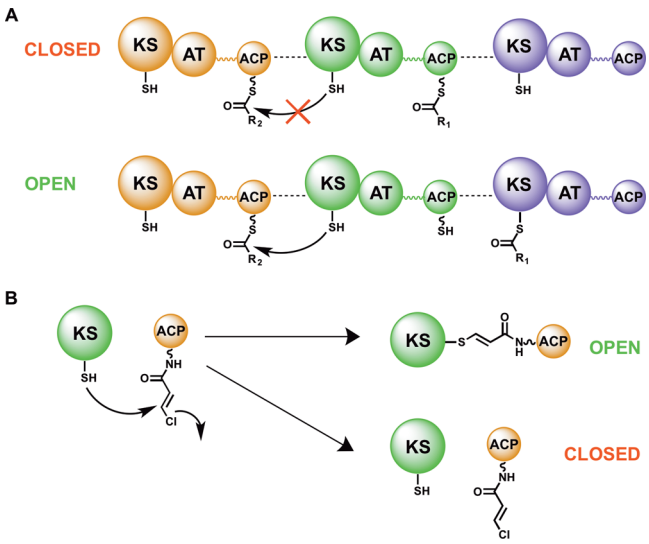

Figure 2. Open and closed representations of the PKS turnstile mechanism. (A) Three modules of a PKS, each composed of a ketosynthase (KS), acyl transferase (AT), and acyl carrier protein (ACP), are depicted as orange, green, and purple ball models. The KS of the green module cannot trans-acylate its polyketide substrate while the ACP in the same module still holds its elongated product (CLOSED conformation). Once the polyketide intermediate is transferred to the downstream module (purple KS), the green KS is in an OPEN conformation and available for acylation. (B) CLOSED and OPEN conformations of the KS are determined by Lowrey et al.[8] using a tethered ACP-KS cross-linker.[11] When the KS is OPEN, it can attack the ACP choloroacrylamide probe to covalently cross-link the two proteins for in vitro SDS PAGE analysis. When the KS is in the CLOSED conformation, the KS does not attack the ACP-bound probe, and no cross-linking is seen.

by finely choreographed protein−protein interactions. This work was made possible by the elegant application of ACP cross-linking probes (Figure 2B), which to date have been applied primarily to understanding protein−protein interactions and their structural implications.[6,11] As shown here, an advanced mechanistic understanding of megasynthases will not be achieved without the application of carefully designed small molecule probes, an emerging chemical biology technique coming into the spotlight within the past decade.

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In their conclusion, Lowry et al. propose a few theories for the chemical basis of the open and closed KS conformations that require further studies and analysis to confirm. Two theories include stable conformational changes or KS capture of the CO_2 leaving group from the Claisen condensation that alters the protein active site until subsequent release.[3] Future work must include structural characterization of modules with bound intermediates or small molecule probes that help explain such hypotheses. AT domains, which select the starting substrate, are another major target for engineering applications, and advanced tools for studying these domains will also be needed to probe catalytic mechanisms and protein−protein interactions.[2] In order to engineer chimeric assembly lines we must assume that the mechanisms underlying these synthases are structured and conserved, and the present work is a significant step toward illuminating the fundamental mechanisms of these elegant molecular machines.

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