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Profiling Natural Product Biosynthesis

Natural products are a rich source of therapeutics; however, artificially reengineering the biosynthetic pathways that generate these compounds could potentially generate "designer" drugs. Last month in *Chemistry & Biology*, Burkart and coworkers reported their technique to track and better understand the components of these pathways [1].

Many pharmacologically active natural products are of polyketide or polypeptide origin and are synthesized via secondary metabolism processes [2]. Although the structures of polyketides are myriad and diverse, natural products from this class are biosynthesized by a general mechanism involving the construction of poly-β-keto repeating chains in an "assembly line" fashion from the condensation of carboxylic acid (C_2) precursors by polyketide synthetases (PKS) [3–7]. Similarly, nonribosomally encoded peptide-derived natural products are assembled from amino acid precursors by the action of nonribosomal peptide synthetases (NRPS) [8–10].

Both PKSs and NRPSs are modular in construction, with separate enzymatic subdomains responsible for precursor recognition, activation, condensation, and postassembly modification. In preparation for polyketide synthesis and concomitant chain elongation, a serine residue of a carrier protein (CP) domain is phosphopantetheinylated by a 4′-phosphopantetheinyl transferase (PPTase) [11]. The terminal thiol of the phosphopantetheinyl arm is then acylated by an adjacent acyltransferase (AT) domain that utilizes acyl-Coenzyme A (acyl-CoA) as a substrate. The ketosynthase (KS) domain catalyzes the subsequent addition of the β-keto acid on a downstream CP domain to the monomer unit of an upstream CP domain. In any given module the resultant ketone may be functionalized by any combination of ketoreductase, dehydratase, and enoylreductase domains to yield the nascent linear product. Subsequent tailoring enzyme activities such as cyclization, epimerization, methylation, glycosylation, or hydroxylation are often employed during conversion of a linear precursor to the mature natural product.

In a similar fashion, NRPS enzymes consist of basic modules containing adenylation (A) domains, carrier protein (CP) domains, and condensation (C) domains. The NRPS CP domain is also 4′-phosphopantethienylated by a PPTase prior to loading with the activated amino acid (as an adenylate, the product of the A domain). The C domain then catalyzes transfer of the growing peptide chain of a downstream CP domain to the amino acid of an upstream CP domain via amide bond formation. Like PKS systems, NRPS modules may also contain any of a number of modification domains that contribute to the chemical diversity of the resultant product. In addition to isolated PK and NRP synthetases, hybrid PKS/NRPS systems have been observed for natural products such as bleomycin, epothilone, and others [12–16].

However, despite our current growing body of knowledge of the organization, mechanisms, and substrate specificity of NRPS and PKS assemblies, several formidable obstacles still preclude harnessing this machinery for metabolic engineering purposes. For example, there are significant challenges associated with performing genetic manipulations in many host producer microbial strains. There are also marked difficulties associated with expressing soluble active synthetases or subdomains in heterologous hosts. In part this is due to both the inherently large size of PK and NRP megasynthetases, impaired promotion of gene expression, codon usage differences between native and heterologous hosts, and improper folding of the recombinant proteins. In light of these issues, there is a pressing need for the development of alternative methods to identify, quantify, purify, and dissect the function of fully folded, active NRPS and PKS assemblies, both from host-producing organisms as well as heterologous hosts.

In last month's issue of *Chemistry & Biology,* University of California at San Diego assistant professor Michael Burkart, visiting scientist James La Clair, and coworkers described the development of novel activity-based proteomic tools for profiling PKS and NRPS activation [1]. This is the first example of activity-based protein profiling applied to natural product biosynthesis machinery. Burkart, La Clair, and colleagues described a method to covalently label CP domains of PK and

NRP synthetases with analogs of coenzyme A containing fluorescent or affinity probes. Their method of natural product profiling takes advantage of the broad substrate tolerance of the PPTase Sfp to transfer the analogs enzymatically to carrier proteins [11, 17, 18]. When combined with techniques such as affinity purification, this approach will allow the functional characterization of active domains within PK and NRP synthetases in host-producing organisms, a goal not fully achievable through conventional molecular biology approaches.

Recently, Walsh and coworkers utilized an enzymatic strategy to load aminoacyl-CoAs onto CP domains in order to probe C domain specificity in NRP synthetases [19]. This study expands upon this important precedent by developing facile synthetic methodology for the construction and biosynthetic incorporation of labeled reporter CoA derivatives that are not thioester linked. The creation of a nonhydrolyzable linker between reporter and CoA eliminates subsequent scattering of the probe by condensation or hydrolyzing enzymes and allows for the visualization, quantification, and purification of PKS and NRPS CP domains from crude cell lysates. Burkart, La Clair, and colleagues carefully illustrated this technique by incorporating labeled CoA analogs into recombinantly expressed VibB, the vibriobactin modular NRP synthetase of *Vibrio cholera*. Similar success was illustrated for recombinantly expressed CP domains from three iterative type II PK producer strains, frenolicin from *Streptomyces roseofulvus*, oxytetracycline from *S. rimosus*, and tracenomycin from *S. glaucescens*. It is clear that this technique may find universal applicability for natural product biosynthesis activity-based profiling.

While fluorescent derivatives proved useful for visualization of CP domain loading and PPTase activity, a biotinylated-CoA derivative provided a more sensitive (5 ng/ml) means of activity profiling. When combined with Western blot detection, this reagent promoted visualization of CP domain 4′-phosphopantetheine loading of the 6-deoxyerythronolide synthetase (DEBS) type I modular PKS from a crude lysate of the host-producing organism *Saccharopolyspora erythraea*. Although, as the authors point out, the efficiency of NRPS and PKS labeling of CP domains in lysates is potentially limited by the degree of intrinsic 4′-phosphopantethienylation, the technique appears to be remarkably effective as an activity-based profiling tool. In addition to this application, the authors presented both biotinylated and mannosylated CoA derivatives that were shown to enable affinity purification of synthetases.

On a broader level, this report represents a way to characterize, purify, and identify NRPS and PKS modules on a whole proteome scale, bypassing any difficulties associated with heterologous expression and purification of these systems. These tools allow the researcher to track and quantify metabolically engineered pathways by monitoring protein expression, solubility, activity, and native posttranslational modification events. Although CP domains derivatized with the described affinity CoA analogs were not functional in this example, isolation may allow the identification of other functional subunits within a biosynthetic complex. In the future, we envision that labeled CoA analogs such as those developed by Burkart and La Clair may be used alone or in combination with other domain specific probes for functional dissection of NRP and PK synthetases. This study will provide much-needed tools for the functional analysis of these intriguing biosynthetic machines.

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