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New Start and Finish for Complex Polyketide Biosynthesis

The polyketide vicenistatin has significant anticancer activity. In the January issue of *Chemistry & Biology*, Kakinuma and coworkers [20] reported on the vicenistatin biosynthetic gene cluster and demonstrated in vitro glycosylation of its aglycone core, raising the possibility of producing analogs with altered sugar residues.

The polyketide group of natural products includes numerous antibiotics, anticancer drugs, and immunosuppressants. Biosynthesis of these important compounds involves assembly of carbon chains from small acyl precursors. In general, chain elongation is catalyzed by a set of enzymes that together make up a polyketide synthase (PKS). A starter or growing acyl chain migrates onto the active site cysteine of a ketosynthase (KS), and an acyltransferase (AT) loads a dicarboxylic acyl extender unit onto the phosphopantetheine thiol of an acyl carrier protein (ACP). A decarboxylative Claisen condensation leaves an extended β-ketoacyl chain attached to the ACP [1]. A complete chain extension cycle can involve three more steps: conversion of the β-ketone group to an alcohol by a ketoreductase (KR), dehydration by a dehydratase (DH) to form a *trans* α-β unsaturated acyl intermediate, and finally reduction by an enoyl reductase (ER) to give a saturated chain. The condensations between acyl thioesters may be repeated many times, but PKSs rarely take β-ketone processing all the way to the third stage. As a result, ketones, hydroxyl groups, and double bonds appear at defined positions within polyketide chains.

Chemical and genetic studies have now uncovered many different types of PKS [2]. Of these, the modular PKSs are the most amenable to redesign for production of novel compounds. These systems contain a set of enzymes or modules for every cycle of chain extension, as well as a loading module for transferring the starter acyl group onto the first KS domain. This modular organization allows programmed assembly of a defined sequence of starter and extender units, together with controlled processing of each β-ketone group. The final product may be cyclized by a thioesterase (TE) to give a macrolactone.

Starter units may derive from acyl CoAs that are recruited from primary metabolism and used directly. Acetyl and propionyl starters can also be generated by decarboxylation of malonyl and methylmalonyl groups attached to loading module ACPs. Other more unusual organic acids are activated as acyl adenylates before transfer to CoA or an ACP [3]. Modular PKSs generally use malonyl and methylmalonyl-CoA as extender units. Ethylmalonyl and methoxymalonyl units are used less frequently [4]. The sequences of malonyl and methylmalonyl-specific AT domains can be distinguished by characteristic motifs. A separate region of the AT domain contains critical residues that appear to determine substrate specificity. Methylmalonate-specific AT domains use only the (*2S*) isomer of methylmalonyl CoA [5]. When this branched extender is used, condensation occurs with inversion of stereochemistry at C-2 so that a (*2R*) 2-methyl-3-ketoacyl chain is generated initially [6]. With some modules epimerization must occur to give the final alkyl stereochemistry. Exactly how this epimerization is achieved is still unclear. The epimerase activity seems to reside within the KS domain [7], but there are no obvious differences between the primary sequences of epimerizing and nonepimerizing KSs.

KR domains are responsible for determining the final stereochemistry at chiral centers derived from reduction of β-ketones to alcohols [8]. Conserved amino acid residues at a few key positions appear to be useful for predicting KR stereospecificity [9, 10]. Predictive methods suggest that the DH domains of modular PKSs normally act on (*3R*)-hydroxyacyl chains to give *trans* double bonds. Few complex polyketides have *cis* double

bonds, and it is still unclear how these are introduced. ER domains occur relatively infrequently in PKS modules and are so far the least studied of all of the constituent domains. These domains must determine alkyl stereochemistry when incorporation of a branched extender is followed by complete reduction of the β-ketone.

Numerous modular PKSs have now been investigated and considerable progress has been made in engineering these systems to make new macrolactones [1, 11]. Polyketide structures can be modified further, notably by glycosylation with various deoxyhexoses and aminodeoxyhexoses [11–13]. Alteration of these sugar moieties can dramatically alter biological activity [13, 14]. Glycosylation engineering is therefore an area of considerable interest for developing novel therapeutics.

Studies on the biosynthesis of other complex polyketides could allow novel modifications of macrolactone rings, and further diversification of carbohydrate moieties. The group led by Kakinuma has investigated the biosynthesis of vicenistatin, an antitumor compound produced by *Streptomyces halstedii* HC34 [15–18]. Vicenistatin is a 20-membered macrolactam ring to which a novel aminodeoxysugar, vicenisamine, is attached. A minor cometabolite, vicenistatin M, contains the neutral sugar D-mycarose in place of vicenisamine [15]. Vicenistatin M is not cytotoxic and has no antitumor activity.

Chemical feeding with labeled precursors indicates that the vicenistatin carbon chain is assembled from acetate and propionate extender units [16]. The starter is a 3-amino-2-methylpropionyl unit that is generated by decarboxylation of glutamate-derived 3-methylaspartate. Surprisingly, however, labeled 3-amino-2-methylpropionate is not incorporated as a starter. This suggests that 3-methylaspartate must be activated as an ACP or CoA thioester prior to decarboxylation and transfer to the PKS. More detailed feeding studies revealed that (*2S, 3S*)-3-methylaspartate is converted to a starter and incorporated whereas the (*2S, 3R*)-isomer is not [17]. This indicates that after conversion to a thioester, the acyl chain undergoes epimerization, as well as decarboxylation, to generate the (*2R*)-3-amino-2-methylpropionyl group that would give the final stereochemistry observed in vicenistatin [18].

In the January issue of *Chemistry & Biology*, Kakinuma and colleagues published an extension of their chemical studies by analyzing the vicenistatin biosynthetic genes. The cluster encodes a B12-dependent mutase that rearranges glutamate to (*2S*, *3S*)-3-methylaspartate. Two enzymes appear to be capable of activating this starter amino acid to an acyl adenylate. One of these has a CoA ligase domain and may attach the starter to CoA or to a discrete ACP as a thioester. A pyridoxal phosphate (PLP)-dependent amino acid decarboxylase is likely to generate the 3-amino-2-methylpropionyl group. There is no candidate epimerase gene within the cluster. The decarboxylation reaction is likely to involve condensation of the PLP aldehyde group with the amino group of the activated 3-methylaspartyl thioester. Decarboxylation would position the methyl-branched carbon atom, now designated C-2, between the carbon atom of a protonated imine and the thioester carbonyl carbon. This could well favor an epimerization reaction by increasing the acidity of the C-2 proton. Biochemical studies will be required to investigate further the origin of this intriguing starter unit. Whereas many PKSs epimerize chiral centers derived from C-2 of a branched extender unit, the vicenistatin-synthesizing system is unusual in epimerizing the primer.

The vicenistatin PKS contains eight extension modules contained within four multienzyme polypeptides. The first extension module is preceded by an unusual loading module that contains only an ACP domain. It is unclear exactly how the final starter unit is transferred from a discrete ACP to the loading module. Vicenistatin also has a double bond that cannot result from straightforward extension of an α-β unsaturated intermediate. The position of this bond could be accounted for by an isomerization to give a *trans* β-γ double bond prior to further chain elongation. Other possible explanations include double bond migration after closure of the macrolactam ring. An understanding of this process could lead to a new means of diversifying engineered polyketide structures.

The cluster also encodes the five enzymes necessary for formation of dTDP-D-vicenisamine from glucose-1-phosphate. The previous identification of vicenistatin M [15] indicates that the VinC glycosyltransferase shows some sugar flexibility and can transfer D-mycarose as well as vicenisamine onto the aglycone core. Initial hybridization experiments detected two sets of genes for NDP-hexose 4,6 DH and NDP-4-keto-6-deoxyhexose 2,3 DH enzymes in the genome of *S. halstedii*. This suggests that an entirely separate cluster of genes is involved in biosynthesis of dTDP-D-mycarose. Alternatively, D-mycarose could result if a vicenisamine pathway intermediate were intercepted by a C-methyl transferase and a reductase that are not encoded within the vicenistatin cluster.

No attempts to genetically manipulate the vicenistatin producer have yet been reported. However, the vicenistatin glycosyltransferase was overproduced in *Escherichia coli* and was shown to modify the purified aglycone with vicenisamine. It should therefore be possible to carry out in vitro glycosylation with alternative aminodeoxyhexoses so as to produce new vicenistatin analogs. This would reduce the need to carry out numerous gene replacements. In addition, there has been significant recent progress in expanding the repertoire of dTDP-deoxysugars that can be generated by enzymatic synthesis in vitro [19]. These advances could lead to the development of improved vicenistatin-based anticancer agents.

Patrick Caffrey
Centre for Synthesis and Chemical Biology
Department of Industrial Microbiology
Conway Institute of Biomolecular and
Biomedical Research
University College Dublin
Dublin 4
Ireland

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Understanding Diseases via Receptor Regulation

In this issue of *Chemistry & Biology*, Guy and coworkers [11] demonstrate that they can selectively recruit individual nuclear receptors by using small molecules (proteomimetics) in combination with specific agonists. This may ultimately lead to a link between the receptor's signaling pathway and its role in individual diseases.

A number of diseases, including cancer and metabolic disorders, are known to be associated with the inappropriate regulation of the nuclear hormone receptor transcription factors (NRs). [1] NRs regulate transcription based on hormone levels. The complex mechanisms involved in these signaling pathways are poorly understood. Selectively examining each nuclear receptor's signaling pathway will provide insight into their connection to specific diseases.

Uncoupling the signaling pathways of these NRs is challenging. The ability to selectively activate one NR in the presence of other NRs using small molecules has been limited by the similarity of one NR isoform over another and the challenge of replacing a protein-protein interaction with a small molecule-protein interaction. Upon discovery of lead small molecules, compound libraries targeted to individual NRs could be screened for their specificity for each NR. The discovery of unique small molecule leads for each NR would offer selective control of the signaling pathways for the individual nuclear receptor, illuminating the connection between the specific signals regulating the pathways and the disease state.

NRs bind to small molecule agonists: hormones. These hormones activate the NRs, leading the NR•agonist complex to recruit a specific steroid receptor coactivator (SRC)[1, 2] (Figure 1). This NR•agonist•SRC complex then initiates transcription. The ability to selectively bind one NR isoform to a SRC would allow the unraveling of individual signaling pathways for that NR and SRC complex. There are three known SRCs: SRC1, SRC2, and SRC3 [3–5]. They appear to play distinct but, perhaps, partially overlapping functions [4, 6]. The NRs bind to an area of a SRC protein known as the nuclear receptor-interacting domain (NID). This NID area contains multiple, conserved interfaces that have identical sequences specific for each SRC, which are known as NR boxes. The NR boxes contain sequences of the motif $L_1XXL_2L_3$, [1, 2], where L depicts a position of diversity, and X is an amino acid specific to that SRC. Thus, for example, the SRC2 NR box contains multiple, conserved interfaces within the NID that are sequence LERLL.

One series of structurally similar NRs are two isoforms of estrogen receptors: ERα and ERβ. Both ERα and ERβ bind to SRC2 to activate transcription. However, they regulate entirely different gene transcription pathways [7]. Both ERα and ERβ bind to the second box of SRC2 (SRC2-2) in the presence of a number of agonists, including estradiol, diethyl stilbesterol, or genistein.

In earlier work using a small molecule library of compounds that mimicked the second SRC2 NR box (SRC2-2), Guy and coworkers discovered a small molecule proteomimetic that selectively blocked binding of ERα in the presence of ERβ to SRC2 [8]. Thus transcription regulation by ERα was inhibited in the presence of ERβ, while transcription regulated by ERβ was unaffected. In addition, they found a proteomimetic that could preferentially inhibit the binding of ERβ to SRC2 in the presence of ERα. This new tool for selectively inhibiting individual NRs using a small molecule for regulating nuclear receptors is an excellent lead for the development of small, drug-like compounds that will ultimately illuminate the function of these individual receptors.

This issue of *Chemistry & Biology* includes an article by Guy and coworkers that demonstrates how small molecule proteomimetics can be used to selectively recruit individual NRs that are specific to the hormone