Using Surrogates to Bypass Missing Catalytic Components

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Bryostatin is a natural product that has many medically promising biological activities. Understanding how bryostatin is assembled by the producing symbiotic bacterium has been hampered by the limited availability of genetic information. In the new report, Buchholz et al. (2010) circumvented this issue by using surrogates to replace missing catalytic components.

The study of the biosynthesis of natural products has benefited enormously from the technological advances in DNA sequencing and bioinformatics. It is now routine to associate a cluster of genes with the production of a particular natural product and propose a biosynthetic scheme based on the bioinformatic analysis of the encoded proteins. These hypothetic biosynthesis schemes are subsequently tested by biochemical and genetic studies. While routine for many systems, there are unique challenges when studying the biosynthesis of natural products produced by microorganisms that cannot be cultured from their natural environment. The challenge comes from the fact that in many cases the completeness of the genetic information is in question because the structure of the associated natural product calls for catalytic components that are not encoded by the available DNA sequence. In these circumstances, testing hypotheses for even early biosynthesis steps may be inhibited by the apparent absence of key catalytic components. In this issue, Buchholz et al. (Buchholz et al., 2010) demonstrate a valuable way to circumvent this problem in the analysis of bryostain biosynthesis. Using surrogate acetyl-acyl carrier protein (ACP) donors in place of the cognate counterpart that has yet to be identified, they were able to biochemically validate the proposed function of BryR, a 3-hydroxy-3-methylglutaryl-CoA synthase (HMGS) homolog. Furthermore, replacing missing components by surrogates is a step toward the heterologous production of the bryostatins in a culturable host even without identifying all the cognate genes/enzymes.

Polyketides are a diverse group of natural products characterized by the polymerization of short carboxylic acids by large enzyme complexes called polyketide synthases (PKSs). PKSs, particularly type I PKSs, are capable of using a diversity of starter units and extender units. Substituents at the α-carbons of these precursors is one mechanism for branching away from the core of a polyketide, and much work has gone into understanding how these precursors are biosynthesized (Chan et al., 2009; Moore and Hertweck, 2002). Less common are polyketides that have branching at the β-carbons. The mechanism for how this type of branching is biosynthesized remained elusive until recently when a number of groups began to decipher its mechanism (Calderone, 2008). The enzymology involved in β-branching typically involves the following set of proteins, referred to as the HMGS cassette: a free-standing donor ACP (ACP_D), a HMGS homolog, a decarboxylating ketosynthase (KS_{DC}) domain, and one or two enoyl-CoA hydratase (ECH) homologs (Figure 1). In the canonical β-branching pathway, an acyltransferase domain loads a malonyl unit onto ACP_D. The KS_{DC} domain catalyzes the decarboxylation of the malonyl unit on ACP_D, generating acetyl-*S*-ACP_D. The HMGS homolog subsequently catalyzes the aldol attack by the acetyl-*S*-ACP_D on a β-ketothioester intermediate on an acceptor ACP (ACP_A) within the PKS. This generates an HMG-*S*-ACP_A intermediate that can be dehydrated and decarboxylated by the ECH homologs to generate the β-branched product (Figure 1). While this is the canonical pathway, diversion from this pathway extends the β-branching structural possibilities, but it is hypothesized that they will all involve an HMGS-like homolog, with the exception of rhizoxin biosynthesis (Partida-Martinez and Hertweck, 2007).

Bryostatin is a polyketide natural product with a number of promising biological activities including anticancer, neuroprotective, and reduction in the levels of a toxic protein implicated in Alzheimer's disease (Banerjee et al., 2008; Khan et al., 2009). The excitement for using bryostatin for these medical purposes is tempered by the fact that it is produced in small quantities by an unculturable bacterium that is a symbiont of a marine bryozoan (Sudek et al., 2007). Additionally, its total chemical synthesis is difficult; thus, eliminating the possibility of using total synthesis as a means for obtaining more material (Singh et al., 2008). To circumvent these issues, Sherman and Haygood have collaborated to access the genetic information that codes for the biosynthesis enzymes with the ultimate goal of moving these to a heterologous host for production (Sudek et al., 2007). To this end, they have identified candidate gene clusters encoding bryostatin biosynthesis enzymes, but the gene clusters appear incomplete and the "missing" genetic information has yet to be identified. This initially appeared to limit the analysis of the pathway. This problem was sidestepped by the use of surrogate catalytic components from related enzymology.

A modified HMGS cassette is hypothesized to be involved in the generation of the two β-branching points of bryostatin (Figure 1). It is not clear, however, how the β-branching initiates because the gene clusters encode for HMGS (BryR)

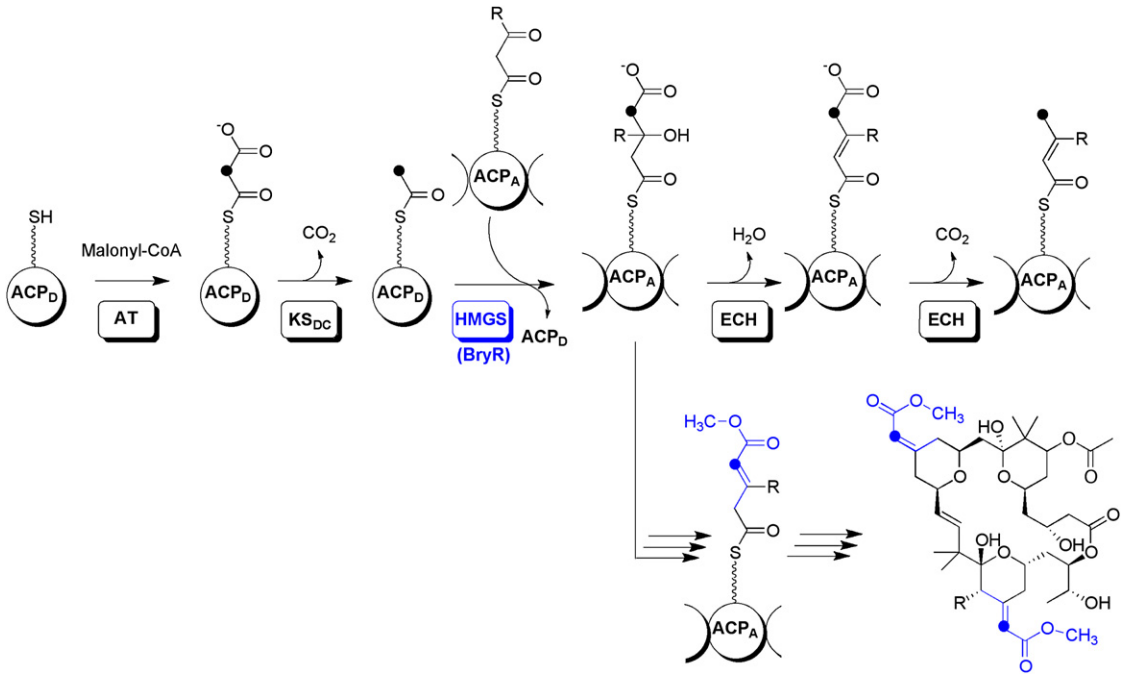

Figure 1. Schematic of the Canonical β-Branching Pathway and the Relationship of This Pathway to Bryostatin Production
The enzyme characterized in this study is an HMGS homolog BryR.

and KS_{DC} (BryQ) homologs, but not for an ACP_D homolog. To investigate whether BryR functions as an HMGS, Buchholz et al. searched for surrogate ACPs to function in place of the cognate ACP_D. They tested a variety of ACPs from type I and II PKSs, ACPs involved in bacterial fatty acid biosynthesis, and ACP_Ds from other HMGS cassettes. Each of these ACPs was modified to form acetyl-*S*-ACPs, and these were tested for whether they were competent as substrates for BryR. BryR was shown to only recognize the acetyl group when tethered to ACP_Ds from other HMGS cassettes. This strongly suggests BryR specifically recognizes an ACP_D-linked substrate in vivo. Furthermore, the failure of the authors to detect interactions between BryR and other ACPs, even though these ACPs carried the same substrate as the ACP_Ds, suggests protein-protein interactions between BryR and the ACP_D are essential for substrate recognition. A model acetoacetyl-*S*-ACP_A, from Module 3 of the bryostatin PKS, was used to confirm that BryR was able to catalyze the complete HMGS reaction.

The use of surrogate proteins by Buchholz et al. enabled them to biochemically verify the function of BryR and address how β-branching initiates during bryostatin biosynthesis. The applications for this approach are two fold: first, this or similar methods can be used to test the catalytic functions of domains in otherwise "incomplete" biosynthetic pathways; second, the method carries with it important implications on metabolic engineering. Marine-derived natural products showing interesting biological activities are often produced by unculturable bacterial symbionts of eukaryotic hosts. Thus far the only way to obtain large quantities of such compounds has been to purify them from enormous quantities of the host organisms. It is of interest to produce these molecules from a heterologous host so that appropriate quantities can be acquired. When dealing with missing biosynthetic components, using surrogates is a promising solution, as well as a step toward heterologous production.

REFERENCES

Banerjee, S., Wang, Z., Mohammad, M., Sarkar, F.H., and Mohammad, R.M. (2008). J. Nat. Prod. *71*, 492–496.

Buchholz, T.J., Rath, C.M., Lopanik, N.B., Bardner, N.P., Häkansson, K., and Sherman, D.H. (2010). Chem. Biol. *17*, this issue, 1092–1100.

Calderone, C.T. (2008). Nat. Prod. Rep. *25*, 845–853.

Chan, Y.A., Podevels, A.M., Kevany, B.M., and Thomas, M.G. (2009). Nat. Prod. Rep. *26*, 90–114.

Khan, T.K., Nelson, T.J., Verma, V.A., Wender, P.A., and Alkon, D.L. (2009). Neurobiol. Dis. *34*, 332–339.

Moore, B.S., and Hertweck, C. (2002). Nat. Prod. Rep. *19*, 70–99.

Partida-Martinez, L.P., and Hertweck, C. (2007). ChemBioChem. *8*, 41–45.

Singh, R., Sharma, M., Joshi, P., and Rawat, D.S. (2008). Anticancer Agents Med. Chem. *8*, 603–617.

Sudek, S., Lopanik, N.B., Waggoner, L.E., Hildebrand, M., Anderson, C., Liu, H., Patel, A., Sherman, D.H., and Haygood, M.G. (2007). J. Nat. Prod. *70*, 67–74.

