the recombinant enzyme was characterized biochemically. Although the 5α-hydroxylase displayed a narrow substrate specificity, interestingly it also converted the isomeric taxa-4(20),11(12)-diene to the same 5α-hydroxyl product **4** with a V_{rel}/K_m that was twice that of the presumed natural substrate **3**. Further screening for P450 enzymes catalyzing downstream oxidations was complicated by the absence of suitable natural substrates. Croteau chose instead to incubate microsomes of transformed yeast with a surrogate substrate, taxusin (**5**), a presumed analog of the natural but as yet unidentified taxoid intermediate (Figure 1) [17]. The resulting product, shown to be the corresponding 7β-hydroxyl derivative **6**, was rigorously identified by NMR spectroscopic methods. The 7β-hydoxylase itself was then expressed in insect cells and characterized biochemically. Notably, the 7β-hydoxylase displayed a relatively broad substrate specificity for polyoxygenated and acylated taxoid substrates. Thus, although the *site* of hydroxylation is beyond doubt, the natural substrate for this P450-catalyzed 7β-hydroxylation remains to be determined. Combined with other studies of taxoid-oxidizing P450s, the Croteau group continues to unmask the remaining secrets of this intricate and important biosynthetic pathway.

David E. Cane
Department of Chemistry, Box H
Brown University
Providence, Rhode Island 02912

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Plumbing New Depths in Drug Discovery

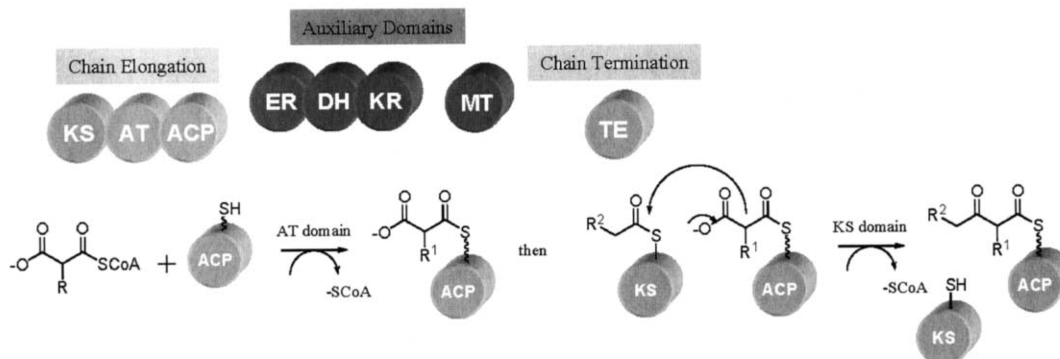
Marine natural products are an untapped source for drug development. In this month's *Chemistry & Biology*, Gerwick and coworkers [1] describe the biosynthetic genes for novel neurotoxins, jamaicamides A–C from *Lyngbya majuscula*, bringing closer the goal of producing marine natural products in terrestrial hosts.

Following on the failure of combinatorial chemistry to deliver the anticipated wealth of new drug candidates, interest in natural products as pharmaceutical leads has been significantly rekindled [2]. One of the least characterized but most promising sources for new drug candidates is the ocean: to survive in this environment, it seems that microorganisms must produce a tremendous diversity of toxic metabolites [3]. Not only are these compounds cytotoxic, but many exhibit important medicinal activities including anticancer, antifungal, antimalarial, antiviral, immunosuppressive, and antimicrobial properties. As an illustration of their potential utility, approximately half of all current anticancer discovery efforts are focused on marine organisms [3].

Exploiting the chemical diversity of marine metabolites, however, has been greatly hampered by their extremely low natural abundance. In addition, all but a small fraction of marine microbes are difficult to culture using conventional methods, particularly those that are symbionts of aquatic invertebrates [4]. One promising alternative is to clone the biosynthetic genes and transfer them wholesale from the natural hosts to more productive alternatives. This strategy not only offers a more secure source of these compounds, but also paves the way for the genetic engineering of unnatural variants of these molecules for use in drug discovery [5, 6]. However, research in this area is relatively new, with only three complete gene clusters sequenced to date [7–9].

From a "retrobiosynthetic" analysis, it appears that many marine metabolites are of polyketide or nonribosomal polypeptide origin, and a significant number incorporate features of both [4]. Assembly of such structures in terrestrial microorganisms occurs on gigantic multienzymes called polyketide synthases (PKSs) and nonribosomal polypeptide synthases (NRPSs), respec-

A PKS systems



B NRPS systems

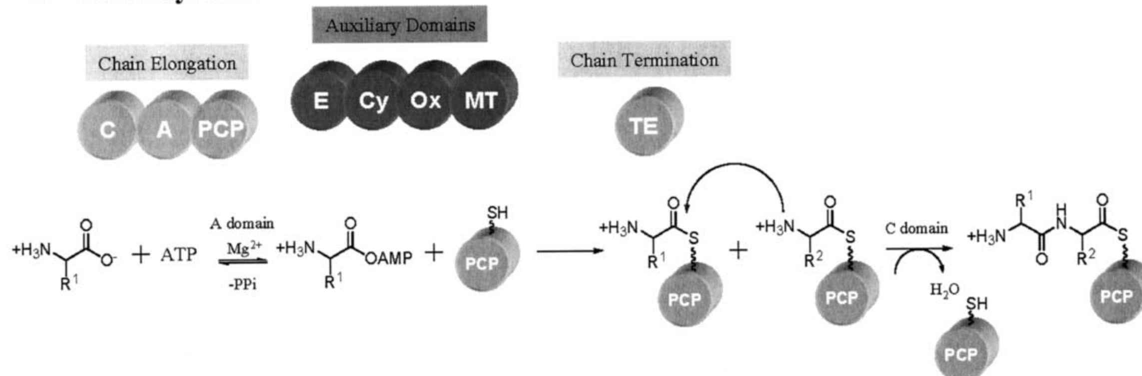


Figure 1. Organization of Modular Biosynthetic Systems

(A) Complement of domains in polyketide synthases (PKSs). Each PKS module minimally incorporates ketosynthase (KS), acyl transferase (AT), and acyl carrier protein (ACP) domains, which cooperate in chain extension. Modules can also include optional reductive activities, ketoreductase (KR), dehydratase (DH), and enoyl reductase, as well as methyl transferases (MT). Chain extension is typically terminated by a thioesterase (TE) domain.

(B) Complement of domains in nonribosomal polypeptide synthetases (NRPSs). Each NRPS module minimally incorporates condensation (C), adenylation (A), and peptidyl carrier protein (PCP) domains, which cooperate in chain extension. Modules can also include optional activities: epimerization (E), cyclization (C), oxidoreductase (Ox), and methyl transferase (MT) domains. Chain extension is often terminated by a thioesterase (TE) domain (figure adapted from [3]).

tively, which catalyze the sequential condensation of simple carboxylic acid (PKS) or amino acid (NRPS) building blocks into a growing chain [10]. The individual catalytic domains within these enzymes are organized into modules, where each module is typically responsible for a single round of chain extension and any necessary modification reactions. Each module of a PKS minimally contains a ketosynthase (KS) domain responsible for carbon-carbon bond formation, an acyltransferase (AT) domain for building block selection, and an acyl carrier protein (ACP) to which the growing chain is tethered (see Figure 1). The corresponding activities within the NRPSs are the condensation (C), adenylation (A), and peptidyl carrier protein (PCP) domains. In addition, modules in both pathways contain a variety of auxiliary domains, including reductive activities in the case of PKSs and epimerases and cyclases in the case of NRPSs. In most systems, there is a dedicated domain for each step in the biosynthesis, with the consequence that it is often possible to deduce the complement of domains in a PKS or NRPS from its product and visa versa.

The underlying similarity between the marine and terrestrial pathways means that biosynthetic genes from terrestrial microorganisms can be used to locate analogous genes in marine strains; this is particularly the case for terrestrial microbes that make compounds similar or identical to those from marine sources [4]. However, many marine natural products also possess functional groups without terrestrial counterparts, and these structural differences will undoubtedly be reflected in their biosynthetic pathways. Therefore, identifying marine PKS and NRPS pathways could significantly expand the "molecular toolbox" of genes used in the genetic engineering of new drugs.

Among marine microbes, cyanobacteria (blue-green algae) have a proven track record as producers of novel, biologically active compounds. The clear metabolic "superstar" is the tropical, filamentous cyanobacterium *Lyngbya majuscula*, which biosynthesizes fully 30% of all natural products so far isolated from cyanobacteria [3]. Analysis of the over 110 *L. majuscula* metabolites characterized to date shows that an impressive 75% have some sort of biological activity. While 58% of these are lipopeptides of mixed polyketide-polypeptide origin, many other structural classes are also represented, including fatty acids, alkaloids, and pyrroles [3]. The meta-

bolic richness of *L. majuscula* and its relative amenability to culturing in the laboratory makes it a model organism for developing genetic techniques applicable to cyanobacteria and other marine microbes.

In this issue of *Chemistry & Biology*, Gerwick and coworkers report the isolation of yet more bioactive metabolites from *L. majuscula*—the novel lipopeptides jamaicamides A–C, which exhibit cytotoxicity toward cancer cells as well as sodium channel-blocking activity [1]. The jamaicamides have several notable structural features, including a rare alkynyl bromide, an unusually located vinyl chloride, and a terminal pyrrolinone functionality; the genes responsible for these functionalities are therefore very attractive targets for inclusion in an engineering toolbox. Using feeding studies with isotopically labeled precursors, the authors were able to establish conclusively that the jamaicamides are of mixed polyketide-polypeptide origin. These experiments also suggested the involvement of a 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS) in forming the vinyl chloride function [11].

Based on this information, Gerwick and his colleagues were able to design specific probes for the PKS genes of the jamaicamide pathway using conserved KS sequences from terrestrial PKSs, and then to refine their search among four PKS hits in *L. majuscula* by probing for sequences with similarity to terrestrial HMGCS genes. In this way, they identified the putative jamaicamide biosynthetic cluster spanning 17 open reading frames (*jamA–Q*) over 58 kbp; this represents only the second natural product cluster to be described from a marine cyanobacterium. For technical reasons, the authors were unable to demonstrate conclusively the involvement of the cluster in jamaicamide biosynthesis, but the complement of domains is at least consistent with the observed structures, and several other strong lines of evidence support this assignment. This experiment serves as further proof of principle [7–9] that knowledge of terrestrial biosynthetic pathways can be used to locate analogous genes in marine microorganisms, even when the structure of the marine metabolite has no terrestrial counterpart [4].

Pleasingly, the deduced gene sequence suggests plausible mechanisms by which the alkynyl, vinyl, and pyrrolinone features are formed, although the timing and mechanism of the critical halogenation reactions remain uncertain. For example, the pathway contains a candidate "β-modifying gene cassette" for vinyl group formation similar to that observed in other PKS pathways, such as mupirocin [11], and an NRPS condensation domain homolog that may be involved in pyrrolinone ring formation. Although the goal of expressing the entire *jam* cluster in a heterologous host remains distant, specific genes from the pathway could have more immediate value in attempts to create further structural diversity among polyketides and peptide natural products through genetic engineering.

Kira Weissman
Department of Biochemistry
University of Cambridge
80 Tennis Court Road
Cambridge CB2 1GA
United Kingdom

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Bending at Microtubule Interfaces

Microtubule-destabilizing drugs alter the interfaces between subunits so that they cannot assemble into straight filaments [1]. Most target β-tubulin, but pironetin binds to the supposedly inactive α subunit [2]. This unusual drug binds covalently to lysine but is nevertheless specific for α-tubulin.

The central role of microtubules in the process of segregating duplicated chromosomes before cell division makes them an important target for antimitotic drugs. Microtubules that make up the mitotic spindle are in a delicate state of balance between assembly and disassembly. This is important because both the formation of the spindle and the movement of chromosomes to opposite spindle poles depend on carefully coordinated extension or shrinkage at both ends of the microtubules in the spindle. The protein subunits, αβ-tubulin heterodimers, together with two bound molecules of guanosine triphosphate (GTP), assemble head to tail in a polar