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

Selected Reading

1. Edwards, D.J., Marquez, B.L., Nogle, L.M., McPhail, K., Goeger, D.E., Roberts, M.A., and Gerwick, W.H. (2004). Chem. Biol. *11*, this issue, 817–833.
2. Rouhi, A.M. (2003). Chem. Eng. News *81*, 77–91.
3. Burja, A.M., Banaigs, B., Abou-Mansour, E., Burgess, J.G., and Wright, P.C. (2001). Tetrahedron *57*, 9347–9377.
4. Salomon, C.E., Magarvey, N.A., and Sherman, D.H. (2004). Nat. Prod. Rep. *21*, 105–121.
5. Walsh, C.T. (2004). Science *303*, 1805–1810.
6. Donadio, S., and Sosio, M. (2003). Comb. Chem. High Throughput Screen. *6*, 489–500.
7. Piel, J., Hertweck, C., Shipley, P.R., Hunt, D.M., Newman, M.S., and Moore, B.S. (2000). Chem. Biol. *7*, 943–955.
8. Li, A.Y., and Piel, J. (2002). Chem. Biol. *9*, 1017–1026.
9. Chang, Z.X., Flatt, P., Gerwick, W.H., Nguyen, V.A., Willis, C.L., and Sherman, D.H. (2002). Gene *296*, 235–247.
10. Cane, D., and Walsh, C. (1999). Chem. Biol. *6*, R319–R325.
11. El-Sayed, A.K., Hothersall, J., Cooper, S.M., Stephens, E., Simpson, T.J., and Thomas, C.M. (2003). Chem. Biol. *10*, 419–430.

Chemistry & Biology, Vol. 11, June, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.chembiol.2004.06.004

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

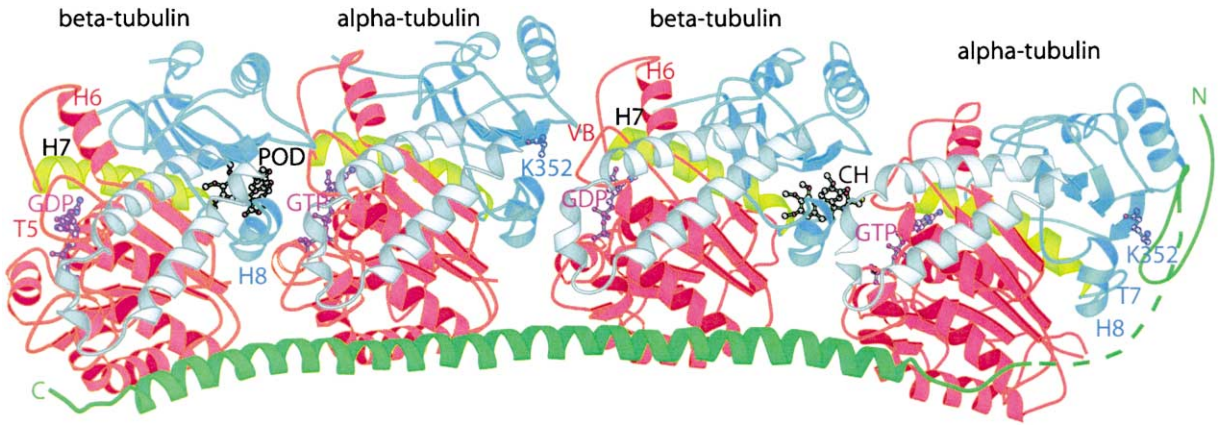

Figure 1. Ribbon Diagram of the Crystal Structure of the Tubulin-Stathmin Complex Showing the Binding Sites for Various Microtubule Depolymerizing Drugs

Tubulin GTPase domains are colored pink, the activation domains are blue, and the central helices (H7) are yellow; the C-terminal helices are in gray. Stathmin, shown in green, induces curvature of this short segment of tubulin protofilament, and its N-terminal domain caps one end [1]. GTP is sandwiched between α- and β-tubulin subunits of each heterodimer; that bound to each β-tubulin has been hydrolyzed to GDP through contact with helix H8 and loop T7 of another α-tubulin subunit. The depolymerizing drugs colchicine (CH) and podophyllotoxin (POD) bind to similar sites on β-tubulin. Pironetin binds to a lysine (K352, shown as a ball and stick model) on α-tubulin [2]. Vinblastine (VB), with which pironetin competes, binds to the GTPase domain of β-tubulin, probably to loop T5 or the loop between H6 and H7. (Figure prepared with Molscript [13].)

fashion as linear protofilaments, and these protofilaments combine to make up a microtubule. Their atomic structure is known from 3.5 Å resolution maps, the first of which came from electron crystallography of zinc-induced two-dimensional sheets, subsequently refined [3]. Recent data, including important information about conformational change, have been obtained by X-ray crystallography [1]. Each monomer has two main globular domains, on each side of a central helix. The larger domain, comprising the N-terminal half of the polypeptide, contains a binding site for guanosine nucleotide on its surface, and this region also contacts the smaller globular domain of the next subunit in the protofilament (Figure 1).

GTP is in direct contact with loops T1 to T6 of the GTPase domain, and, during assembly into protofilaments, loop T7 and helix H8, in the smaller domain of the next subunit, are brought close to the phosphates of the nucleotide [4]. The idea that the smaller globular domain (of tubulin or of FtsZ, the bacterial monomeric homolog of tubulin) acts as a GAP (GTP hydrolysis-activating protein) [4, 5] to the GTPase domain has been confirmed experimentally by mutagenesis of FtsZ [6, 7]. Also, in work that is soon to be published (M. Oliva, S.C. Cordell, and J. Löwe, submitted), a new crystal structure of a mutant FtsZ is revealed whose subunits form a dimer by interchanging their smaller globular domains, showing that each domain can fold up independently. These researchers have suggested that the GTPase domain and the "activation" domain were once separate proteins that became fused. The protofilament thus consists of alternating GTPase domains and activation domains (Figure 1). After assembly and hydrolysis, the location of the nucleotide at the center of the interface prevents its exchange from β-tubulin until the subunit disassembles. GTP bound to α-tubulin is nonexchangeable, being permanently trapped between the two monomers of the heterodimer and is never hydrolyzed because β-tubulin has lysine at the lower end of helix H8, in place of glutamic acid (as in α-tubulin E254) or aspartic acid (as in FtsZ), amino acids that are required for hydrolysis [4].

Although tubulin protofilaments with bound GDP are curved [8, 9], after GTP hydrolysis contacts between neighboring subunits in a microtubule constrain the protofilaments to remain associated in a straight form. The resulting tension is proposed to store conformational energy that is released during depolymerisation. The straight structure solved to near-atomic resolution by Nogales and colleagues [3] corresponds to the "strained" state. When a microtubule disassembles, its protofilaments roll up to form rings. The curved state seen in cocrystals of tubulin and the tubulin-sequestering protein stathmin [1] shows ~12° bends between the tubulin subunits (see Figure 1). Each stathmin molecule interacts alongside a pair of tubulin heterodimers, while its amino-terminal domain caps one of the α-tubulin subunits, preventing any further interactions. Changes within the subunit, compared with the straight conformation, include a relative rotation between the GTPase and activation domains. Contacts between subunits are preserved by local movements of helices H6, H7 and H8, and loop T5.

The bending at all interfaces between monomers, both between and within heterodimers, was first seen by electron microscopy [8, 9]. Bending in both places is unexpected because there is still GTP bound in the intradimer interface, and it occurs even in the absence of destabilizing agents such as stathmin or colchicine. These observations suggest some sort of cooperation between tubulin monomers, involving a signal between the nucleotide binding site and the bottom of the activation domain. The central helix H7, which is able to slide like a piston [1], seems the most likely means of communicating between these two surfaces.

A variety of drugs appear to inhibit microtubule assembly by favoring the curved conformation of tubulin and thus a distorted protofilament structure that cannot support microtubule polymerization [10]. They can affect the amount of curvature: heterodimers in rings induced by cryptophycin-1 have 13° bends between them and 32° bends within [8]. Although this drug binds only to the β subunit, it protects both α- and β-tubulin against proteolysis by trypsin, suggesting specific conformational changes in both subunits. The new crystal structure [1] confirms earlier predictions that colchicine binds to β-tubulin near to the interface between monomers. Its binding location necessarily requires a distortion *within* the dimer structure that would inhibit its polymerization into straight protofilaments. In contrast, vinblastine, which turns protofilaments into tightly wound helices, binds to β-tubulin somewhere on residues 175–213 [11]. This peptide includes regions that are involved in contacts *between* dimers (Figure 1).

Most antimicrotubule drugs exert their effects by binding to β-tubulin, but Osada and colleagues [12, 2] have discovered a natural compound, pironetin, that strongly inhibits microtubule assembly by binding to α-tubulin. A surprising feature of pironetin is that, when bound to α-tubulin, it inhibits the binding of vinblastine to β-tubulin. This competition suggests that the two drugs occupy overlapping sites in the interface between tubulin heterodimers (see Figure 1), where they must bind without actually disassembling the protofilaments. Also, colchicine binds more readily to the intradimer site if either vinblastine or pironetin is bound to the interdimer interface [12]. This suggests that the binding of either drug produces curved protofilaments, with the intradimer interfaces partially opened up. In an impressive series of new experiments detailed in this issue of *Chemistry & Biology* [2], the group demonstrate that pironetin binds covalently to a specific lysine residue. Crosslinking experiments revealed that it binds to a peptide of α-tubulin. The precise site was then located by alanine scanning all cysteine and lysine residues within that peptide and measuring the binding of pironetin to the mutated proteins, expressed in an in vitro translation system. Only the removal of lysine 352 completely inhibited pironetin interaction. The high specificity of the drug for α-tubulin, among all of the lysine-containing proteins of a cell, is predicted to be due to a precise match between the whole molecule and its binding site, as shown by computational modeling in the paper. This property and the relatively small size of the molecule make pironetin a very promising candidate for chemotherapy.

Linda A. Amos
MRC Laboratory of Molecular Biology
Hills Road
Cambridge CB2 2QH
United Kingdom

Selected Reading

1. Ravelli, R.B.G., Gigant, B., Curmi, P.A., Jourdain, I., Lachkar, S., Sobel, A., and Knossow, M. (2004). Nature *428*, 198–202.
2. Usui, T., Watanabe, H., Nakayama, H., Tada, Y., Kanoh, N., Kondoh, M., Asao, T., Watanabe, H., Nishikawa, K., Kitahara, T., and Osada, H. (2004). Chem. Biol. *11*, this issue, 799–806.
3. Löwe, J., Li, H., Downing, K.H., and Nogales, E. (2001). J. Mol. Biol. *313*, 1045–1057.
4. Nogales, E., Downing, K.H., Amos, L.A., and Löwe, J. (1998). Nat. Struct. Biol. *5*, 451–458.
5. Erickson, H.P. (1998). Trends Cell Biol. *8*, 133–137.
6. Mukherjee, A., Saez, C., and Lutkenhaus, J. (2001). J. Bacteriol. *183*, 6190–6197.
7. Scheffers, D.J., de Wit, J.G., den Blaauwen, T., and Driessen, A.J. (2002). Biochemistry *41*, 521–529.
8. Watts, N.R., Cheng, N., West, W., Steven, A.C., and Sackett, D.L. (2002). Biochemistry *41*, 12662–12669.
9. Nogales, E., Wang, H.-W., and Niederstrasser, H. (2003). Curr. Opin. Struct. Biol. *13*, 256–261.
10. Checchi, P.M., Nettles, J.H., Zhou, J., Snyder, J.P., and Joshi, H.C. (2003). Trends Pharmacol. Sci. *24*, 361–365.
11. Rai, S.S., and Wolff, J. (1996). J. Biol. Chem. *271*, 14707–14711.
12. Kondoh, M., Usui, T., Nishikiori, T., Mayumi, T., and Osada, H. (1999). Biochem. J. *340*, 411–416.
13. Kraulis, P.J. (1991). J. Appl. Crystallogr. *24*, 946–950.