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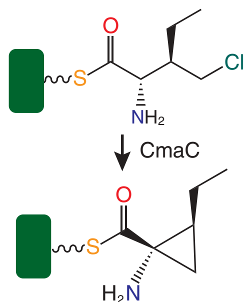
Chlorine closes the deal

Although many natural products are made by standard anabolic pathways, certain compounds are assembled from building blocks derived from multiple biosynthetic routes. Coronatine, for example, is a compound produced by *Pseudomonas syringae* that is composed of two biosynthetic units: an unusual cyclopropyl amino acid (CMA), from nonribosomal peptide synthesis (NRPS), and coronafacic acid, from a nonstandard polyketide pathway. In a recent issue of *Nature*, Walsh and co-workers deduced the biosynthetic pathway of CMA and revealed the remarkable chlorination and cyclization sequence that produces its cyclopropane ring. The authors previously identified an NRPS gene cluster from *P. syringae* and showed that the first protein in the cluster, CmaA, became charged with L-*allo*-isoleucine, the known precursor to CMA. In the current work, the team demonstrated the functions of the four remaining enzymes of the *cma* cluster by reconstituting the pathway with purified proteins. Once CmaA is loaded with L-*allo*-isoleucine, a second protein, CmaE, shuttles the amino acid, through thioester exchange reactions, to a single-domain protein called CmaD. Subsequently, a nonheme iron protein, CmaB, chlorinates the γ-position of L-*allo*-isoleucine while it is bound to CmaD. Finally, CmaC catalyzes the formation of the cyclopropane moiety by an intramolecular displacement reaction. Although further studies will be required to characterize the mechanisms of these Cma proteins, this pathway shows how organisms generate new chemical structures through biosynthesis. (*Nature* **436**, 1191–1194, 2005) *TLS*



VIRAL TAKE ON TAKING OFF UBIQUITIN

Post-translational addition of (poly) ubiquitin (Ub) chains targets proteins to the proteasome for degradation. Ub modification of proteins controls diverse processes including cell-cycle progression and membrane trafficking. As expected for a fundamental biological process, ubiquitination is tightly regulated. In mammalian genomes there are hundreds of Ub ligases—enzymes that attach Ub to substrates—and nearly a hundred deubiquitinating enzymes (DUBs), which reverse this process. Viral proteins and host-cell ubiquitination machinery are known to interact during many stages of the viral life cycle. In a recent issue of *Molecular Cell*, Ploegh and co-workers identified the first virally encoded DUB. The authors took advantage of a previously developed activity-based probe composed of a hemagglutinin epitope tag and an electrophilic ubiquitin derivative. This probe covalently modifies the active site cysteine in cysteine protease DUBs. During herpes simplex virus 1 (HSV-1) infection, this probe allowed identification of a fragment of the HSV-1 UL36 protein. The N-terminal fragment of UL36 was shown to have DUB activity. UL36 does not have sequence similarity to known host DUBs. However, all herpesviruses possess a UL36 homolog, which suggests that UL36 defines a new family of DUBs. Deletion of UL36 is lethal to the virus, indicating that this newly identified DUB performs a critical function during viral infectivity. However, characterization of the precise role of UL36 and related DUBs will require future studies. (*Mol. Cell* 19, 547–557, 2005) *JK*

Research Highlights written by Greg Watt, Joanne Kotz and Terry L. Sheppard.

Dynamic DNA duplex binding

In addition to the double helix, DNA may adopt other secondary structures, which are both recognized and stabilized by small molecules. The possible involvement of DNA secondary structures—in particular, quadruplexes—in the regulation of gene transcription has generated interest in targeting them for tumor-therapy development. Distamycin is an *N*-methylpyrrole-containing polyamide that has been shown to bind to DNA duplexes and more recently to G-quadruplex structures in a dimeric manner. Ladame *et al.* recently reported the generation of a dynamic combinatorial library of distamycin analogs, which they screened using duplex and quadruplex DNA to identify the strongest binding components. The distamycin analogs contained one, two or three *N*-methylpyrrole residues with a terminal sulfide group to allow dynamic coupling with a second distamycin analog under equilibrium conditions. The authors found that pairing of analogs containing two and three pyrrole residues was significantly enhanced in the presence of a DNA quadruplex. In agreement with previously reported findings, DNA duplexes had an even more pronounced effect on distamycin-analog coupling. The authors then showed that the thermal stability of DNA duplexes was higher in the presence of the larger distamycin-analog dimers. The amplification of stronger binding ligands in combinatorial mixtures under dynamic equilibrium offers a potentially useful tool for the discovery of new DNA-binding ligands. (*Angew. Chem. Int. Ed.*, 44, S736–S739, 2005) *GW*

TB drug-resistance regulon

The increasing appearance of drug-resistant tuberculosis (TB) strains is a serious health concern. *Mycobacterium tuberculosis* evades many commonly used antibiotics because of its impermeable cell envelope. However, it presents stubborn resistance even after antibiotics have penetrated its armor. Several genes are known to enhance drug resistance in *Mycobacteria*, whose products include membrane pumps, compound modifying enzymes and protective thiol-containing compounds. In a recent article, Morris *et al.* identified *whiB7*, an *M. tuberculosis* gene that activates an entire complementary drug-resistance system upon exposure to a broad range of antibiotics. Genetic analysis of a drug-sensitive mutant of *M. tuberculosis* identified *whiB7* as the gene responsible for coordinating multidrug resistance. A *whiB7 M. tuberculosis* knockout had reduced resistance against several antibiotics, whereas a strain overexpressing *whiB7* had increased antibiotic resistance. The authors demonstrated that sublethal concentrations of several antibiotics induced the expression of *whiB7* and of other genes within the *whiB7* regulon, including several known antibiotic-resistance genes. In a more physiological setting, they showed that a *Mycobacterium whiB7* mutant within monocyte-like cells was more susceptible to spectinomycin antibiotic compared to the wild-type strain. The authors argue that the stubborn antibiotic resistance of *M. tuberculosis* could be a legacy of its distant, soil-dwelling ancestors, the Actinomycetes, which possess a large repertoire of defensive antibiotic-synthesis genes, including the corresponding antibiotic-resistance genes homologous to the *whiB7* regulon. Further studies will be needed to understand the precise mechanism of *whiB7* activation and to explore whether compromising this system could lead to new therapies against TB. (*Proc. Natl. Acad. Sci. USA* **102**, 12200–12205, 2005) *GW*