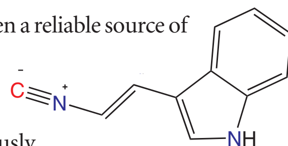
Uncultured antibiotic biosynthesis



Although cultured soil bacteria have been a reliable source of many current antibiotics, an untapped source of new antibiotics may be found in bacteria that have been difficult or impossible to culture. Previously, Brady and Clardy had devised a method to screen DNA extracted from uncultured bacteria in environmental samples (eDNA) for antibiotic activity by identifying eDNA clones that, when expressed, could inhibit bacterial growth. In a recent publication, Brady and Clardy uncovered an antibiotic from uncultured soil bacteria that inhibited *Bacillus subtilis* growth. After isolation of the active compound, structural analysis revealed that it was a tryptophan derivative containing an unusual isocyanide group. To study its biosynthesis, the authors expressed the *isnA* and *isnB* genes from the eDNA clone. Coexpression of these two genes was sufficient to produce the antibiotic in *E. coli*. The authors further explored the biochemical pathway and found that both nitrogens (shown in blue) originated from tryptophan. Despite the identification of the first isocyanide-containing natural product approximately 50 years ago, the origin of the carbon atom (shown in red) was still unknown. In a follow-up paper, the authors used an elegant combination of isotope labeling and auxotrophic strains of *E. coli* to identify the origin of this carbon, which was shown to be the C2 carbon of ribulose-5-phosphate. In addition to identifying a new compound with antibacterial activity, the characterization of the biosynthetic pathway reported in these papers will likely be generalizable to other microbially produced isocyanides. (*Angew. Chem. Int. Ed.* **44**, 7045–7048 and 7063–7065, 2005) *GW*

If the nucleotide fits...

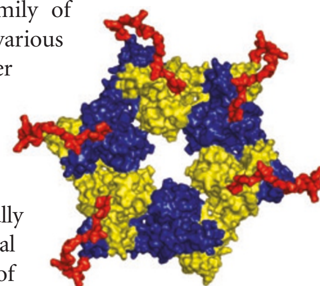
Watson-Crick base pairing is an essential element of DNA structure and function. By extension, it had been assumed that complementary hydrogen bonding between an incoming deoxynucleotide triphosphate (dNTP) and a DNA template base guarantees specificity during DNA replication. However, recent studies have suggested that DNA polymerases may use other mechanisms to select the correct nucleotide during DNA replication. For example, DNA polymerases accept nucleotides with aromatic bases that retain the shape of a natural base, but lack hydrogen bonding donors or acceptors. A new study by Kool, Essigmann and coworkers suggests that replication efficiency and fidelity may be driven by optimal steric fit of nucleotide bases in DNA polymerase active sites. Starting with a nonnatural nucleotide based on toluene, the authors inserted halogen atoms of increasing size (F, Cl, Br, I) to expand the size of the nucleotide base in sub-angstrom increments. *In vitro* kinetic studies with *E. coli* DNA polymerase I showed that nucleotide incorporation efficiency and fidelity increased markedly with increasing base size, up to the dichlorinated analog. Greater expansion of base size (Br, I substitutions) led to sharply lower incorporation efficiencies. These results were confirmed *in vivo* using an *E. coli* replication and mutagenesis assay, suggesting that *E. coli* polymerases can tolerate substrates somewhat larger than a natural base pair. The authors hypothesized that the tightness of the DNA polymerase active site may control replication fidelity, and suggest that subtle structural and dynamic control of active site architectures may explain the mutation rates across different classes of DNA polymerases. *(Proc. Natl. Acad. Sci. USA 102, 15803–15808, 2005) TLS*

Research Highlights written by Greg Watt, Mirella Bucci and Terry L. Sheppard.

Substrate-guided protease inhibition

Proteases, which cleave peptide bonds in proteins, are central to normal and aberrant cellular processes. As a result, selective protease inhibition offers a tool for understanding biological processes and therapeutic intervention. Because protease inhibitors have traditionally been modeled on peptide substrates, there are few drug-like protease inhibitors. Furthermore, weak inhibition by fragment-based compounds to proteases is difficult to detect and can produce false hits during high-throughput screening. In a recent publication, Wood, Ellman and coworkers report a new strategy to obtain small-molecule protease inhibitors, termed substrate activity screening (SAS). SAS eliminates the generation of false hits by screening substrate fragment libraries directly for protease activity. Using the cysteine protease cathepsin S, the authors screened a library of 105 fluorescently labeled acyl aminocoumarin fragments. Protease cleavage revealed two potential substrate scaffolds, which were then modified to generate two focused analog libraries. A second round of screening produced a number of highly potent substrates. Structurally related analogs containing an aldehyde in place of the cleavable fluorescent group were potent cathepsin S inhibitors in the low nanomolar range, in which substrate activity increased proportionally with inhibitor potency. Thus, SAS provides a simple means to identify and convert novel, nonpeptidic substrates into inhibitors and offers a therapeutic lead for treating cathepsin S–related diseases such as arthritis. (*J. Am. Chem. Soc.*, published online 13 October 2005, doi: 10.1021/ja547230) *GW*

AAAsymmetry in symmetry



Some enzymes of the AAA+ family of ATPases ('ATPases associated with various cellular activities') use ATP to power conformational changes in target proteins or complexes for their ultimate degradation, disaggregation or disassembly. These enzymes assemble into oligomeric rings, usually consisting of six subunits. The bacterial ClpXP proteolytic system consists of ring-shaped hexamers of ClpX bound to ClpP, a barrel-shaped protease. Substrates are threaded through the ClpX hexamer's central pore and unfolded before translocation into ClpP. The unfolding of substrates may come from ATP-driven pulling through the central pore. To determine how ATP hydrolysis is coupled to the mechanical tasks of ClpX, Martin, Sauer and coworkers studied the functional roles of individual ClpX subunits. By linking ClpX subunits together and then introducing mutations into specific subunits, they were able to look at various combinations and subunit symmetries. In variants consisting of trimers of dimers, the ClpP-dependent degradation was proportional to the number of active ClpX subunits. The fact that some subunits could be inactive ruled out the idea that all six subunits must bind and hydrolyze ATP concertedly. Other hexameric variants suggested that the active ClpX subunits do not need to be symmetrically arranged, and one remarkable variant had only one active subunit but could still drive unfolding and translocation. Thermodynamic analysis suggested that many diverse arrangements of active and inactive subunits had comparable efficiencies and that translocation efficiencies render the variants more or less active. A mechanism in which individual ClpX subunits hydrolyze ATP in a probabilistic and asymmetric fashion, rather than in strict sequence, could help explain how asymmetric AAA+ ATPase complexes like the 19S proteasome can operate. (*Nature* **437**, 1115–1120, 2005) *MB*

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