JMU, dubbed the National Schools Observatory, 5% of the telescope's observing time will be allocated to schools. Registered groups can request observations from the telescope using a comprehensive Web site stuffed with astronomical information and activities. And thanks to the virtual astronomer, the students won't get pushed aside by "proper" researchers. "We'll get equal footing," says astronomer Andy Newsam, who is developing the school's Web site at JMU.

Faulkes is also planning an ambitious educational project with his two telescopes. The sites were chosen so that U.K. students would have dark sky when they are in the classroom. His plan is to offer 700 schools a chance to control the telescopes themselves, often collaborating with researchers on real science projects. "It's all about understanding science as it happens," he says.

Although juggling all these projects is creating anxious moments for the TTL staff, they track precisely with what the company's founders hoped to achieve, namely, to serve a universe of customers. "This is not just a university design exercise, it's a business," says Daly. "We succeed or fail as a business. If we fail, we close." Henry Ford would be proud.

–DANIEL CLERY

With reporting by Ding Yimin in Beijing.

BIOENGINEERING

Working Outside the Protein-Synthesis Rules

Proteins built in the ribosome are subject to certain restrictions, so researchers are harnessing a nonribosomal system that might one day make new drugs

Most proteins are built according to a set of rules as strict as any list of "don'ts" posted at a public swimming pool. Only 21 types of amino acids are permitted. Proteins must not loop. Non–amino acids may not be included in the protein.

Life has gotten by pretty well with these restrictions. They are imposed on all proteins built by a cell's ribosomes, and for higher organisms, that means all proteins. But many bacteria and fungi can turn to an alternative system that allows them to toss aside the standard rules of molecular biology. Bypassing the ribosome, they manufacture some of their most important short proteins using giant enzymes that recognize amino acids and link them directly into chains.

Because the so-called nonribosomal peptide synthetases (NRPSs) are not bound by the ribosome's rulebook, they are able to produce an array of peptides with unusual properties. Peptides manufactured by NRPSs include some of the most potent pharmaceuticals known, from penicillin to the immunosuppressant cyclosporin. Researchers have known about this alternative protein-construction system for decades, but only recently have they begun to build a solid molecular understanding of how the system works. With this knowledge, they hope to alter the NRPS machinery to make even more effective variants of powerful existing drugs as well as novel drugs built along the same lines. Although the goal of designing drug-factory enzymes is still a long way off, recently researchers have begun to engineer new nonribosomal proteins.

An alternative system

Bacteria and fungi use nonribosomal peptides for critical tasks such as killing parasites, communicating with members of their own species, and regulating the movements of ions. The peptides are ideal for these delicate tasks because they resist degradation and are unlikely to be mistaken for other compounds. These properties are a result of their unique composition: Scientists have spotted several hundred different molecular ingredients in various nonribosomally manufactured peptides, most of which the ribosomes have never heard of. These compounds include so-called right-handed amino acids, the rare twins of the standard left-handed model, as well as molecular relatives of amino acids such as acyl acids. But it's not just strange starting materials that make these peptides unconventional. They frequently contain loops, which are almost never found in standard proteins. The eccentric structures of these proteins foil protein-eating enzymes called proteases, which prefer to digest conventional strings of amino acids.

Who needs the ribosome? This mold uses nonribosomal peptide synthetase to make penicillin.

CREDIT: ANDREW SYRED, SPL/PHOTO RESEARCHERS INC.

The NRPSs that build these odd peptides are the largest enzymes known in nature. They're made up of a series of modules, each of which is responsible for adding one particular unit—be it a specific left- or right-handed amino acid or some other compound—to a growing peptide chain.

Each module in the NRPS consists of three subunits. The first is an adenylation (A) element that recognizes a free-floating amino acid, say, and prepares it for incorporation into the chain. Next are two subunits that attach the new amino acid to its neighbors in the chain, known as the condensation (C) and thiolation (T or PCP) domains. The sequence of modules in an enzyme constitutes a unique blueprint designed to produce a particular protein. The final module of the chain has a special termination (Te) domain that releases the protein, sometimes drawing it into a loop.

Most NRPSs consist of four to 10 modules, but the enzymes—and therefore the peptides they produce—can reach up to 50 units in length. NRPSs can be enormous, for an enzyme. The 11-module cyclosporin NRPS, for example, weighs in at a whopping 1700 kilodaltons (kD), compared to about 20 kD for myoglobin, a ribosomally produced protein that helps the body store oxygen.

Each species of bacterium or fungus that relies on the enzymes carries just one or two different NRPSs, in part because they are so unwieldy. But given the diversity of species that use the alternative protein-building system, researchers expect that a wealth of NRPS diversity must be out there. Figuring out how the modules recognize their target molecules and string them together might allow researchers to build on one of the existing NRPS plans.

Breaking down the problem

The immense size of NRPSs has prevented scientists from deducing their structure—a key to understanding how the enzymes work—using the standard tricks. "Some people think they are impossible to crystallize," says biochemist Hans von Dohren of the

Technical University of Berlin in Germany. To get around this problem, researchers have broken the enzymes apart and studied the structures of the subunits. The publication of the Te subunit structure in the March issue of *Structure* marks the completion of sample structures for all of the subunits except the condensation domain.

The structure of the A subunit, responsible for the core task of recruiting new amino acids for the growing protein, has been determined in the greatest detail. In 1997, Mohamed Marahiel and his colleagues at the Philipps University of Marburg in Germany reported the structure of an adenylation subunit from *Bacillus brevis*, the source of the antibiotic gramicidin; this subunit recognizes the amino acid phenylalanine. They found that this subunit has an active site flanked by two domains, each resembling the well-studied luciferase protein that makes fireflies glow. More recently, in work submitted for publication, Marahiel's group has found that a second adenylation subunit from *Bacillus subtilis* (this one recognizes a carboxyl acid) has a similar structure, suggesting that all A subunits are built according to the same general plan.

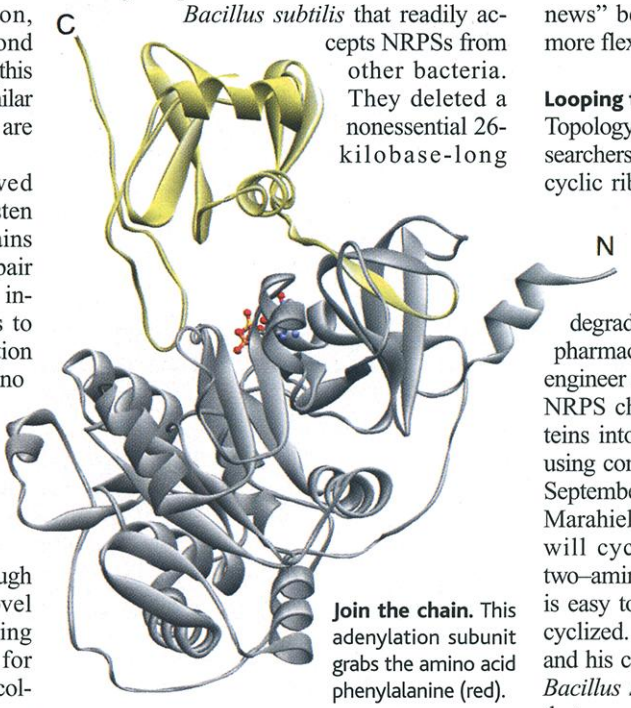
The structural information allowed Marahiel and Marburg biochemist Torsten Stachelhaus to figure out how A domains recognize their substrates. In 1999 the pair combined structural data with sequence information for 160 different A domains to identify 10 critical residues that adenylation subunits use to discriminate among amino acids. By modifying these residues, they were able to predictably alter which compounds an adenylation subunit will recognize, for example, coaxing a phenylalanine subunit into recognizing leucine.

But modifying NRPS subunits is a tough way to customize NRPSs to make novel products. Far easier is cutting and pasting complete modules to make blueprints for new protein sequences. Marahiel and his colleagues first used such a strategy to alter an NRPS in 1995, replacing the A subunit that recognizes the amino acid leucine, along with the T subunit that stitches it into the growing peptide chain, with A and T subunits dedicated to other amino acids. By swapping these subunits, they switched which amino acid was incorporated into the chain.

This was a major advance, but researchers were stymied when they tried to tailor other enzymes using the same strategy. It turned out that switching A and T subunits wasn't enough. As biochemist Christopher T. Walsh of Harvard Medical School in Boston discovered, in most cases, the A needs to be accompanied by the appropriate C domain, in addition to T. Armed with this information and a sharper understanding of subunit boundaries gleaned from the structural data, Marahiel and colleagues were able to modify more NRPSs, adding various third modules onto several two-part NRPSs, they reported in the 23 May 2000 *Proceedings of the National Academy of Sciences*. The study was the "first clear indication that you can swap modules and get them to function," says Walsh.

On the assembly line

There are many additional obstacles to altering NRPSs, however. Many bacteria and fungi, some familiar and others exotic, use the enzymes. But scientists do not know how to genetically manipulate most of these organisms—most lab strains stick to ribosomes when they're building proteins. This has held back progress on altering NRPSs, despite the recent biochemical advances. To address the problem, Marahiel and his colleagues produced a modified strain of *Bacillus subtilis* that readily accepts NRPSs from other bacteria. They deleted a nonessential 26-kilobase-long gene cluster in the bacterium, replacing it, as proof of principle, with a 49-kilobase NRPS gene cluster that produces the antibiotic bacitracin. The gene was taken from the bacterium *Bacillus licheniformis*, which cannot be genetically manipulated. In September 2001, the team reported that the enzyme was produced correctly, despite their concerns that inserting a large foreign gene would cause genomic instability.



Join the chain. This adenylation subunit grabs the amino acid phenylalanine (red).

CREDIT: M. MARAHIEL/PHILIPPS UNIVERSITY OF MARBURG

Other major obstacles remain to be overcome before researchers can engineer novel NRPSs in vivo. For one, many of the unusual amino acid variants in these peptides are made by enzymes not normally present in the cell. Ultimately, researchers will have to insert genes for the enzymes that manufacture these oddball amino acids into the engineered host systems, a challenge that no one has yet reported attempting.

Yet more complications arise from the variations in structure between NRPSs. In 1998, Guido Grandi at Chiron S.p.A. in Siena, Italy, discovered that a *Pseudomonas syringae* NRPS that manufactures an antibiotic called syringomycin has an unusual building plan. Modules encoding eight of its nine amino acids are arranged in a line, as is standard, but the ninth amino acid is added by an A subunit located on a different protein. This protein then intermingles with the eighth residue's C and T domains to help them hitch up the ninth amino acid.

Since then, many other variations on the standard NRPS theme have been discovered. Although these oddities mean that it may be harder for researchers to alter some NRPSs, Walsh says that the variations on the assembly-line structure may still turn out to be "good news" because they mean that NRPSs are more flexible than previously thought.

Looping the loops

Topology provides another opportunity for researchers to cook up useful proteins. Whereas cyclic ribosomal peptides are rare, proteins manufactured by NRPSs are commonly looped, a feature that helps them bind their targets securely and decreases their vulnerability to degradation—both desirable properties for pharmaceuticals. Researchers would like to engineer novel looped peptides as well as use NRPS chemistry to turn known linear proteins into loops, which is nearly impossible using conventional chemical tricks. In the 14 September 2000 issue of *Nature*, Walsh and Marahiel showed that termination domains will cyclize any protein with a simple two–amino acid signature, suggesting that it is easy to manipulate whether a protein gets cyclized. Following up on this study, Walsh and his colleagues successfully used purified *Bacillus subtilis* Te to cyclize novel peptides that were variations on the theme of the 10-unit peptide that the Te normally loops.

Eventually researchers hope to create large pools of looped and unlooped peptides as part of their long-sought goal: screening many variants of potential and successful drugs to look for even more potent medications, a powerful drug-discovery technique. Walsh suspects that such screens may be possible within perhaps 5 years. "It's harder than one might have expected at the beginning, but enough of the rules will be deciphered and controllable" that researchers will be able to borrow techniques from NRPSs to build libraries of candidate drugs, he says. Ultimately, researchers hope that these law-breaking enzymes will rewrite the rules on what proteins can do.

–JOSH GEWOLB

Josh Gewolb is a writer in Boston.