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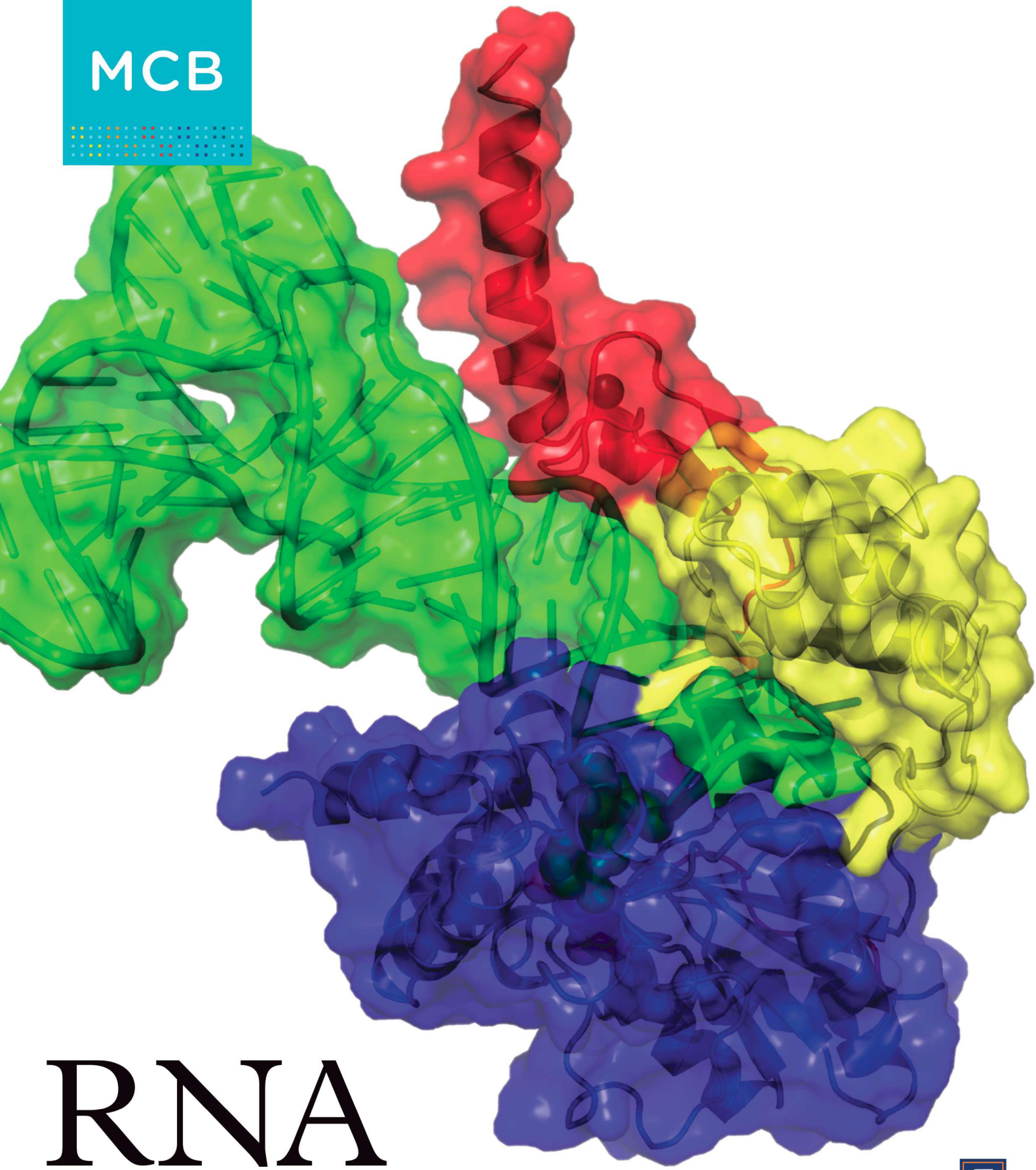

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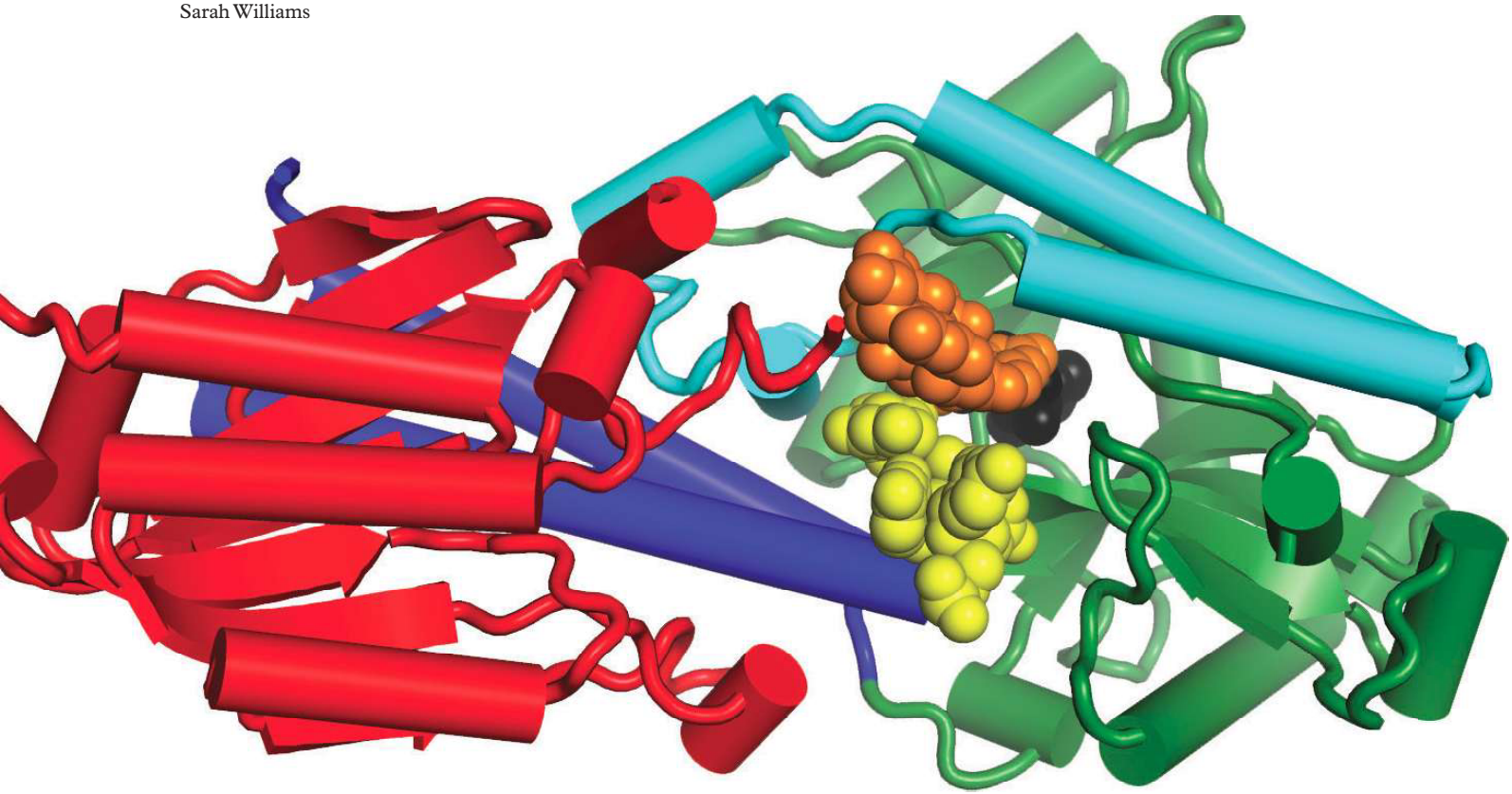


Once shrugged off as DNA's lesser sibling, RNA is now emerging in its own right as a vital mediator of dozens of processes in the cell. RNA molecules can be classified based on their size and function.

The central dogma of biology, set forth by Francis Crick in 1958, states that information in any living cell moves in one direction—from DNA to RNA to protein. Information is stored in molecules of DNA, transcribed to RNA, and then translated into proteins that carry out physical and chemical tasks for the cell. For years, this role of RNA as the intermediary between DNA and protein was seen as its primary function in the cell.

In the past decade, however, scientists have begun to uncover many more jobs of RNA. RNA molecules have their own functions beyond coding for proteins. In MCB and around the globe, researchers are now probing these functions. Some RNA molecules may play a role in human diseases; others could mediate processes of learning and memory in the brain. While DNA most often weaves two strands together to form a double helix, RNA molecules are more often single-stranded, allowing them to form more varied and complex structures. As these diverse roles and structures of the molecules have been discovered, clear categories of RNA have emerged.

Types of RNA

Messenger RNA (mRNA)

This classic RNA molecule carries information between DNA and proteins. Like all RNA, it is composed of chains of four nucleotides: uracil, adenine, guanine, and cytosine (non-mRNAs have more than 100 other nucleotides that are created by chemical modifications after they are synthesized). During the process of transcription, RNA polymerases use DNA as a template to assemble mRNA molecules. The mRNA strands then exit the nucleus of the cell, where DNA is generally confined, to reach a ribosome. Complexes of RNA and proteins, ribosomes translate mRNA sequences to proteins. Research by Associate Professor of Cell and Developmental Biology Stephanie Ceman is showing that which ribosome an mRNA strand is translated by isn't random. In the brain, she's found, one protein helps shepherd some mRNA molecules to locations where they're most needed (see page 4).

Non-coding RNA (ncRNA)

RNA that isn't translated into proteins is now dubbed ncRNA. In humans, some ncRNA is made by the same transcription process as mRNA, some is bits of RNA removed from mRNA strands, and some ncRNA arises as a response to outside sources, like viruses and bacteria. Biologists use size and function to categorize ncRNA molecules like those listed below.

Transfer RNA (tRNA)

The first ncRNAs that scientists knew about were those involved in translating mRNA to protein. tRNAs are molecules that contain the code to translate a codon of mRNA—three nucleotides—into a single amino acid of protein. All tRNA molecules are about 80 nucleotides long and share a T-shaped structure. One section of a tRNA molecule has three nucleotides that bind to its cognate codon in the mRNA; another section has a binding site that links to a specific amino acid. Professor and Head of Biochemistry Susan Martinis studies how cells ensure that mistakes in translation are minimized and corrected. By blocking the tRNA editing capabilities of cells, Martinis has found ways to alter the genetic code and attach an incorrect amino acid to a tRNA molecule (see page 6).

Ribosomal RNA (rRNA)

Like tRNA, rRNA plays an integral role in mRNA-to-protein translation. rRNA is the major component of the ribosomal complex, along with proteins, and is the active agent of translation. Ribosomes coordinate the binding of tRNA to an mRNA strand and direct the subsequent joining of an amino acid to the growing protein chain. Professor of Microbiology Carl Woese discovered in the 1970s that the sequence of an organism's rRNA can be used to classify it in the phylogenetic tree of life. His technique led to the discovery of a new class of organism, Archaea (see page 10).

MicroRNA (miRNA) and small interfering RNA (siRNA)

Researchers have recently discovered that ncRNAs do more than help carry out the process of translation. miRNA and siRNA molecules both have the capability to bind directly to mRNA or DNA and cause it to be cut up, block it from being translated, or regulate how much of a gene is transcribed. Associate Professor of Molecular and Integrative Physiology Jongsook Kim Kemper has discovered that the pathway the human body uses to regulate glucose and lipid levels is controlled in part by miRNA. Obese mice, she's found, have different levels of these miRNA molecules than thinner mice (see page 12). miRNA also may aid in the process of learning in brain cells, according to work by Professor of Cell and Developmental Biology David Clayton. Clayton's lab recently learned that levels of miRNA molecules in a songbird's brain change after it hears a new song for the first time (see page 8). Other miRNA and siRNA molecules have been implicated in cancers and other diseases.

Bacterial small non-coding RNA (sRNA)

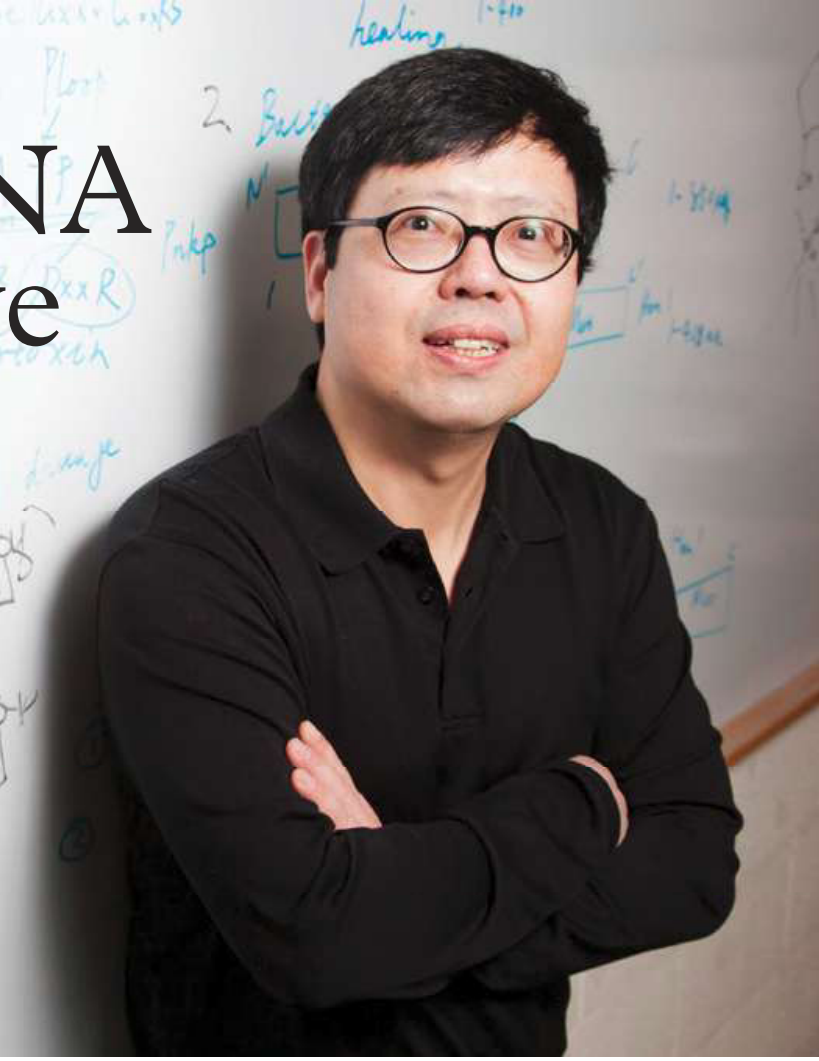
It's not just in human cells that RNA molecules have diverse sets of functions. In bacteria, sRNA molecules have been implicated in the regulation of numerous cellular pathways. Assistant Professor of Microbiology Cari Vanderpool studies how bacteria use these sRNAs to control their responses to external stresses, such as lack of nutrients. sRNA, she's found, binds to different regions of mRNA to effect its translation during times of stress. Her next task: determine what these mRNA targets are and how the regulation occurs (see page 14). Associate Professor of Biochemistry Raven Huang is also studying the importance of RNA to bacteria. He probes how bacteria exposed to harsh environments keep RNA molecules intact through constant editing (see page 16).

Long non-coding RNA (lncRNA)

Most ncRNA molecules are short—a tRNA is around 80 nucleotides long, siRNAs span 19 to 23 nucleotides, and miRNAs contain 21 to 25 nucleotides. But some RNA molecules are dramatically longer. A lncRNA is any non-coding RNA molecule that has more than 200 nucleotides. lncRNA is only beginning to be understood, but scientists already know that many long RNA molecules have similar functions as miRNA and siRNA in controlling gene expression.

Repairing RNA to Stay Alive

Sarah Williams



"It's basic offense and defense," says Raven Huang. "Somebody tries to kill you by cleaving your RNA molecules, and so you survive by fixing them."

For a cell to stay alive is a constant battle to keep its molecular parts intact. The effects of harsh environments, pathogens, and toxins perpetually strike every part of a cell, from its outer membrane to its nucleic acids and proteins. When some parts stop working, the cell can survive for long enough to rebuild them. But other parts are more vital—they must be fixed immediately.

For example, when a toxin leaves its mark on an RNA molecule, cutting or degrading the essential molecule, fix-it proteins jump to action.

Associate Professor of Biochemistry Raven Huang studies how these RNA repair programs work. He's discovered that RNA repair enzymes not only fix breaks in an RNA molecule, but add protection to keep that RNA safer in the future.

The chemicals that target RNA molecules are called ribotoxins. The most famous example is ricin, a potent poison that comes from castor plant seeds. Ricin can quickly kill a human by cleaving a section of ribosomal RNA necessary for a cell to produce new proteins. Other ribotoxins target essential mRNA and tRNA molecules.

"Ribotoxins directly target RNA molecules involved in protein synthesis in order to kill cells," explains Huang. "If a cell can't make proteins, it dies."

But bacteria, which often live in rough conditions and are exposed to constant bombardment by toxins, have evolved a way to stay alive when they're exposed to ribotoxins. The mechanism revolves around the protein Hen1.

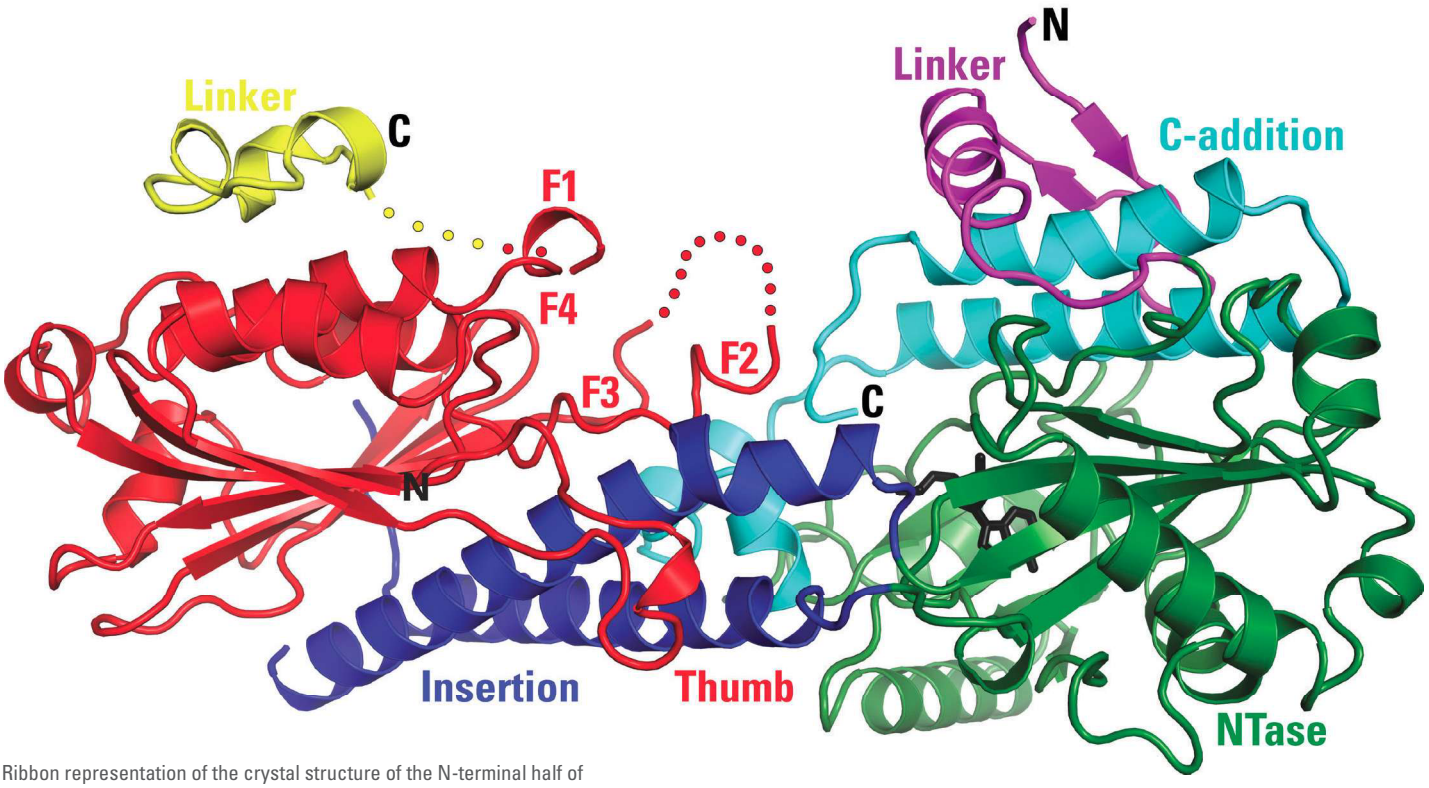
In human cells, Hen1 is involved with modifying RNA molecules participating in RNA interference, a form of gene regulation. Huang wanted to find out what the protein's function in bacterial cells was. So he started studying bacteria that contained Hen1. All bacteria with a gene for Hen1, he discovered, also had a gene vital to RNA repair. He started to suspect that in bacteria, Hen1 was linked to RNA repair.

In a set of papers published in 2009, Huang revealed that Hen1 doesn't just repair RNA—when the protein links back together a broken section of RNA, it adds a methyl group—a small chemical moiety that sticks off the repaired RNA molecule.

"Hen1 helps to repair the RNA to better than new," says Huang. "Ribotoxins can no longer cleave it because this methyl group is sitting in the way."

Repairing RNA to "better than new" is how bacteria can avoid expending all their energy on RNA repairs after damage from ribotoxins.

"Bacteria in these harsh environments don't necessarily have just a one-time attack by a ribotoxin they have to fend off," explains Huang. "They have constant bombardment by harsh temperatures and chemicals. A normal repair system would be working perpetually, carrying out repairs 24 hours a day. So this system has a very elegant solution."



Ribbon representation of the crystal structure of the N-terminal half of bacterial Hen1 (Hen1-N, colored red and yellow) in complex with the C-terminal half of bacterial Pnkp (Pnkp-C, colored magenta, green, blue, and cyan). The ligase-activating domain of Hen1-N (red), shaped like a left hand (with the Thumb and four fingers labeled), activates the RNA ligase activity of Pnkp-C for RNA repair by grabbing the flexible insertion module of Pnkp-C (blue) and fixing it in a correct orientation.

Eventually, all RNA molecules wear out and need to be replaced. But the longer the cell can reuse an RNA molecule, the less energy it expends on producing new ones.

"Biology is all about dosage. If an RNA can last twice as long, it can do twice as much," says Huang.

The lessons from how Hen1 works don't just have implications for bacteria and for understanding how RNA repair works. In humans, Hen1 adds methyl groups not to repaired RNA, but to the ends of microRNA molecules (miRNA). miRNA is increasingly thought to be important to controlling the expression of genes in the cell—it can bind to a gene and turn it on or off. Huang thinks that the methyl groups Hen1 adds to miRNA molecules help protect their ends from degradation. While his lab doesn't study Hen1 in human cells, what he learns could help scientists understand miRNA and its regulation better.

Huang has no shortage of questions about how proteins modify RNA molecules, or how ribotoxin damage is fended off by bacteria. Attacking a cell's RNA is an incredibly effective way of killing it, he explains, and likely a mechanism used by a plethora of pathogens and toxins.

"We probably know about a dozen ribotoxins right now," says Huang. "If I had to estimate how many are out there, I would say a million."

And it's not just methyl groups that can be added to RNA. The four basic RNA building blocks—the nucleobases of adenine, guanine, uracil, and cytosine—can be modified by bonding with additional chemicals in 106 different ways. Huang wants to know how each variation is composed, and whether it serves an important cellular function.

In his most recent work, Huang focused back on Hen1's role in bacterial RNA repair. He studied how Hen1 interacts with Pnkp, the protein that actually seals two strands of RNA back together, a process called ligation. Through studying the RNA repair in the test tube, Huang found that in RNA ligation Pnkp is inactive by itself. It requires Hen1 to bind to it to activate it. This helps the cell ensure that any RNA damage isn't just ligated back together, but is first methylated by Hen1.

"To our knowledge, this is the first time in the field of DNA/RNA repair that ligation is turned on by another protein," says Huang. "Normally you don't need that level of control, you just want ligation to happen."

For Huang, the possible implications of his work are a side effect. What drives him forward is his inquisitiveness about the basic biology.

"Maybe in the future this will have applications. It could help scientists engineer repair systems to treat illness from ribotoxins, or help us understand parasites better," says Huang. "But for now, my main purpose is to satisfy my curiosity. How does this work?" ■

FURTHER READING

Chan, CM, Zhou, C, and Huang, RH. 2009. "Reconstituting bacterial RNA repair & modification system in vitro." *Science.*

Chan, CM, Zhou, C, Brunzelle, JS, and Huang, RH. 2009. "Structural and biochemical insights into 2'-O-methylation at the 3'-terminal nucleotide of RNA by Hen1." *Proceedings of the National Acadamy of Sciences.*