

MODERN RELIQS

by Lisa Seachrist Chiu

Bits of possibly ancient RNA are turning up in bacteria and other modern organisms. Their capacity to monitor the environment and regulate gene expression makes them a welcome target for new antibiotics that could confuse bacteria into starving to death.

illustration by Christian Northeast



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onald R. Breaker is a man comfortably caught between two worlds. His training as both a biologist and a chemist enables him to apply those disciplines to the search for relics of an ancient world within modern organisms.

In this so-called RNA World—where RNA reigned supreme—the molecule known for its role as a messenger and intermediary would have needed to accomplish a much broader array of activities. Breaker has zeroed in on RNA’s need to monitor the available nutrients in its environment. Survival for all organisms requires them to conserve nutrients and shut down cellular activities that are not needed—otherwise they starve, says Breaker, an HHMI investigator at Yale University. “To do that,” he adds, “RNA would have had to sense its own surroundings.”

Research spearheaded by Breaker has opened a window back in time, offering a tantalizing glimpse at just how RNA organisms may have achieved such complex environmental surveillance. He has found sequences of RNA that control gene expression by binding vital nutrients and switching on or off genes involved in producing or transporting those nutrients.

Following a tradition that molecules involving RNA begin with the word *ribo*—as in *ribosome*—Breaker dubbed these RNA sequences *riboswitches*. “The name didn’t impress one of the reviewers of our first paper,” Breaker laughs. “He

said it sounded like something that came from the back of a cereal box.” But the name stuck and these snippets of antiquity have proven useful to scientists exploring evolution.

Breaker, however, is most recently interested in targeting certain *riboswitches* in the modern world to develop new types of antibiotics against bacterial scourges like *salmonella* and *anthrax*.

A Technology That Time Did Not Forget

To hear Breaker tell it, the entire field of *riboswitches* began with an intellectual exercise. He decided to engineer RNA sensors in the lab to demonstrate that components of the RNA World could monitor RNA’s environment. As a chemist, he wanted to understand the chemical limitations of RNA, figuring it must be capable of doing more than simply folding and catalyzing the cleavage reactions that Yale University’s Sidney Altman and University of Colorado researcher (and now HHMI president) Thomas R. Cech described in winning the 1989 Nobel Prize in Chemistry.

“I wanted to test the RNA World hypothesis in a small way,” Breaker says. “If we had failed to create RNA sensors, that would have struck a significant blow against the RNA World hypothesis.”

He built on the work of HHMI investigator Jack W. Szostak, at the Massachusetts General Hospital, and Larry Gold, at the University of Colorado, who independently discovered that short RNAs could form structures capable of binding molecules such as vitamins and amino acids. Szostak dubbed these RNAs “*aptamers*,” and he and others subjected them to evolution in a test tube by selecting only those RNAs that were exceptionally good at binding a target molecule.

Breaker engineered his RNA sensors to include an *aptamer* that bound a target molecule, like a vitamin or drug, followed by sequences of RNA that folded into a structure capable of cleaving the RNA strand at a specific location. The cleavage structure formed only if the *aptamer* section bound the target molecule. Once the metabolite was bound, Breaker could watch the RNA cut itself in two: the RNA truly sensed its environment and took action as a result.

“We were engineering *riboswitches* long before we discovered them in nature,” Breaker says. “There was no evidence at the time that these switches were still in existence. But, it was so easy to build them that we thought there was no way that nature had forgotten this technology.”

With that thought, Breaker decided to search for ancient RNA switches in modern bacteria.

An Early Glimpse with Riboflavin

While Breaker’s group was building RNA sensors, Mikhail S. Gelfand, then at the Research Institute of Genetics and Selection of Industrial Microorganisms in Moscow, his student Alexey Vitreschak, and biologist Yuri Kozlov were probing how bacteria regulate production of *riboflavin*, one of the B vitamins the body uses to metabolize fats, carbohydrates, and proteins.

They knew the vitamin derivative flavin mononucleotide (FMN) stopped expression of certain genes involved in riboflavin biosynthesis. “But, the biologists were saying that there wasn’t a protein involved. It was about that same time that people started writing about RNA

messenger RNA (mRNA) called the 5' prime untranslated region (5' UTR). “These very different species had a region of similarity in the 5' UTR that was completely unexpected,” Gelfand says.

Their computational work revealed that the 5' UTR of the RNA could fold into a

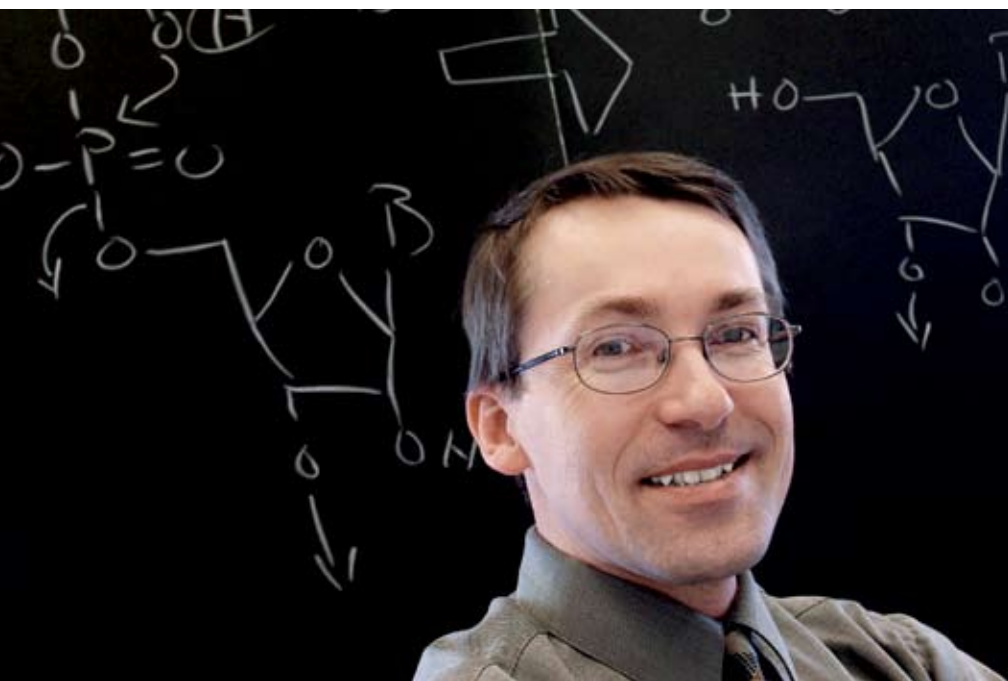
Back in Connecticut, Breaker began looking for examples of genes that are sensitive to the amount of a vital nutrient, but for which scientists had been unable to find a regulatory protein that orchestrates response to the level of that nutrient. If there was no protein, maybe there was a riboswitch.

To Modern Cells

Breaker’s biologist side was taking over. “As a biologist, I want to study something that is actually in a cell,” Breaker says. “Evolution isn’t kind to inefficient molecules, and the biologist in me argued that if RNA was so good at sensing metabolites, there were probably still riboswitches in modern cells.” His group was studying a gene whose product helps transport vitamin B12 into the cell. By identifying parts of the mRNA that formed new structures in the presence of B12, Breaker’s group discovered a structure that bound a B12 derivative and prevented ribosomes from reading the mRNA.

Nature hadn’t forgotten the RNA sensor technology at all, as Breaker and his team detailed in the September 2002 issue of *Chemistry & Biology*. Soon thereafter, Breaker’s team documented riboswitches at work in the biosynthesis of riboflavin and another B vitamin, thiamine.

Since their discovery in 2002, dozens of natural riboswitches, which bind vitamin derivatives, amino acids, nucleic acids, and other metabolites, have been discovered. The constant among them is that they consist of an aptamer sequence that senses



Ronald Breaker says riboswitches are strong proof of the RNA World hypothesis—and they make great targets for new antibiotics.

aptamers,” says Gelfand, now an HHMI international research scholar at the A.A. Kharkevich Institute for Information Transmission Problems in Moscow.

By examining the genomes of diverse bacterial species, the group found an unusual similarity in a section of the

cloverleaf structure that was highly conserved among species. He speculated that this structure bound FMN. What’s more, Vitreschak and Gelfand discovered that the RNA could form another structure that would stop the transcription of mRNA dead in its tracks and suggested that the RNA was regulating gene expression.

Normally, when a cell needs to shut down production of a protein from an mRNA, regulatory proteins jump onto the 5' UTR—the real estate on the mRNA in front of the section coding for protein. That gums up the works, stopping the ribosome from making the protein.

▶ RNA ON PATROL FOR MAGNESIUM

Eduardo A. Groisman knows the frustration of searching for a protein that isn't there. Ever since he was a post-doctoral fellow, Groisman, an HHMI investigator at Washington University School of Medicine in St. Louis, has been studying how *Salmonella typhimurium* bacteria regulate magnesium via the PhoP/PhoQ system—a two-component regulatory system that controls two of three magnesium-transporter genes. ▶ **MAGNESIUM REGULATION IS VITAL** for cell survival. The metal exists as a positively charged ion in biological systems, where it stabilizes DNA and RNA and is critical to certain enzymatic reactions as well as membrane and ribosome stability. In *Salmonella*, the PhoQ protein monitors magnesium and responds to changes in concentration by riding herd on the PhoP protein. When magnesium concentrations are low, PhoQ keeps PhoP in a form that turns on expression of the genes encoding magnesium transporters, which pump the metal ion into the cell. When magnesium is abundant, PhoQ changes PhoP into a form that shuts down expression of the three genes. ▶ **"AT ONE POINT,** we decided to make a PhoP mutant that works independently of PhoQ," Groisman says.

Presumably, genes regulated by PhoP should then be blind to the magnesium concentration. And that was true for all the genes they looked at except *mgtA*. This gene still turned on when magnesium was scarce and turned off when it was plentiful. ▶ **UNABLE TO IDENTIFY THE PROTEIN** regulating *mgtA*, Groisman's team examined the unusually large section of the mRNA transcript preceding the sequence that actually encodes the protein. When they mutated the 5' UTR, the gene wasn't repressed in the presence of high levels of magnesium. ▶ **COMPUTATIONAL STRUCTURAL ANALYSIS** suggested that a riboswitch may be involved in the gene's expression, and the group then proved that the 5' UTR could bind magnesium and alter the RNA's structure to halt transcription. In work published in the April 7, 2006, issue of *Cell*, Groisman and his colleagues announced that they had found a magnesium-binding riboswitch, the first example of a riboswitch that responds to a metal. ▶ **HE BELIEVES THE 5' UTR** of *mgtA* may offer some surprises—the RNA fragment can be detected in the cell after it is cleaved, indicating it may still have some unidentified function. "My fantasy," says Groisman, "is that the 5' untranslated region has a life of its own in addition to that of a riboswitch." – L.C.

metabolites and a sequence that turns gene expression on or off in response to that environmental monitoring. It's an elegant model that allows the riboswitch to control its gene expression by binding a metabolite and either preventing or inducing termination of mRNA synthesis, protein synthesis, or RNA self-destruction through self-cleavage.

Riboswitches demonstrate striking complexity. For example, the glycine riboswitch in *Bacillus subtilis* employs two glycine-binding domains to control a set of genes whose products allow the bacteria to live off of glycine, an essential amino acid. The two-aptamer regions cooperate with each other to allow the

bacteria to sense small changes in available glycine, says Breaker.

Another riboswitch has proven itself as sophisticated as certain proteins, findings that Breaker's team reported in the October 13, 2006, issue of *Science*. The bacteria *Escherichia coli* and *Bacillus clausii* both have two different enzymatic proteins that turn the amino acid homocysteine into methionine. One pathway is more efficient but needs vitamin B12 to help. The other is less efficient, and bacteria use it only when B12 and another nutrient, S-adenosylmethionine (SAM), are in short supply. *E. coli* uses proteins to monitor the concentrations of these nutrients and regulate the expression of these enzymes. *B. clausii*, on the other hand, uses a riboswitch to control expression of the less efficient enzyme. What's interesting is that the riboswitch harbors two different aptamer binding sites—one for SAM and one for B12. If either nutrient

binds the riboswitch, the gene for the less efficient enzyme is switched off.

Although metabolite-binding riboswitches are relatively common among bacteria, only one riboswitch has been found in a higher organism: the TPP riboswitch, which exists in bacteria, is also found in fungi and the flowering plant *Arabidopsis*. In bacteria, this riboswitch regulates thiamine production and transport by binding thiamine pyrophosphate. In fungi, it's involved in a process called RNA splicing, which removes nonsensical bits from mRNA transcripts.

“As bioinformatics search strategies improve, we might still find some riboswitches in common between bacteria and humans,” Breaker suggests. “If humans do have riboswitches, they likely form different shapes and sense different compounds than those found in bacteria.”

For Humanity’s Benefit

The application of riboswitches to human health does not necessarily depend on whether humans have them. More than a dozen bacteria that are dangerous to humans rely on riboswitches, which offers “a real opportunity to specifically target bacterial processes,” Breaker says.

He believes the capacity of riboswitches to control gene expression by monitoring nutrient availability could provide desperately needed targets for developing antibiotics. Although bacteria have a prodigious talent for replicating themselves, they still struggle to survive in a nutrient-poor environment. Drugs that mimic vital metabolites could take advantage of that fact. Breaker has cofounded a company called BioRelix to pursue this technology.

“Drugs targeted at unique and essential riboswitches could trick microorganisms into believing that they are swimming in nutrients when they are actually starving for them,” says Breaker, who described in the January 2007 issue of *Nature Chemical Biology* that several antibacterial compounds, which never became drugs for humans, appear to target lysine riboswitches in anthrax and other bacteria. He also believes that designing metabolite mimics that target only riboswitches presents an opportunity to ameliorate adverse drug interactions.

Breaker speculates that, if bacteria were to develop resistance to the metabolite mimics, they wouldn’t have much of a survival advantage because they would

continue to produce unnecessary proteins, exhausting resources. For example, Tina Henkin at Ohio State University discovered a mutation that deregulates the riboswitch-regulated SAM synthetase gene, leaving *Bacillus subtilis* struggling to grow in laboratory growth media.

Still unclear is whether riboswitches are actual relics of the RNA World. To date, the closest living descendant of that world is the ribosome—a protein-enrobed RNA machine that translates mRNA into

Gelfand, who is using riboswitches to study evolution, agrees noting that “the riboswitches that are present in diverse bacteria are likely very old.” That’s not to say that all riboswitches are ancient. Gelfand and Breaker posit that a number of riboswitches may have emerged much later than the RNA World. “This doesn’t contradict the hypothesis of ancient origin of some riboswitches,” Gelfand says. “Rather it demonstrates that the process of their creation continues.”



After his computational studies suggested that RNA regulates gene expression in riboflavin biosynthesis, Mikhail Gelfand began using riboswitches to study evolution.

protein. Scientists speculate that the ribosome marks the place where RNA ceded its supremacy to more stable and efficient molecules. Riboswitches could be one step further back in evolution.

“It is very tempting to speculate that at least some riboswitches are ancient relics from the RNA World,” Breaker says. “Some riboswitch classes sense metabolites that certainly were present in the last common ancestor of all modern cells, and these riboswitches are preserved with very little variation in most bacteria.”

From his vantage point in the modern world, Breaker sees riboswitches as good proof of the RNA World hypothesis. Even though a self-replicating RNA would offer ultimate proof of the RNA World, Breaker notes, “All you have to do is crack open a modern cell and see how common these RNA elements are and everything screams RNA World.” ■