RNA Editing: What's in a Mechanism?

A new model of RNA editing brings it out of the radical fringe and back in line with mainstream molecular dogma

EVERY SO OFTEN, PERHAPS WHEN SCIENTISTS get complacent and begin thinking they understand how Mother Nature works, she manages to throw up one of those little surprises that challenge their most cherished dogmas. Then the fun begins as researchers struggle to explain what has at first seemed impossible. Take the discovery of the phenomenon known as RNA editing. In the mid-1980s, when editing was first noticed in a variety of one-celled parasites, including *Trypanosoma brucei*, which causes African sleeping sickness, it appeared as if new information—not encoded in the DNA, the cell's master repository of information—was being added to certain RNA molecules. Molecular biologists struggled for years to explain how that might happen, but only in the past year have they begun to crack the problem.

While what takes place during editing was deceptively simple to describe, it was potentially revolutionary in its implications. All biologists know that during protein synthesis the information encoded in the DNA of a cell's genes is first transcribed into messenger RNA, which in turn directs the assembly of amino acids into proteins. But in the trypanosome parasites, nucleotides were being mysteriously added and deleted at specific sites in certain messenger RNAs, thereby altering—or editing—the sequence information they carried. And for several years researchers couldn't find any source for that new information in the parasites' genes.

"When you see it for the first time, you don't believe it," recalls Rob Benne, a molecular biologist at the University of Amsterdam in The Netherlands, of RNA editing. He ought to know better than most since he experienced the initial surprise firsthand, as the leader of one of three independent teams that stumbled onto the phenomenon while investigating gene expression in the mitochondria of the trypanosome parasites. The discovery so disconcerted molecular biologists that some were even willing to entertain the thought that information might flow from protein to nucleic acid, rather than the other way around.

The recent work suggests that such radicalism isn't necessary, however. RNA editing, which occurs in plants as well as in the parasites, seems to be the work of a newly discovered form of RNA, called guide RNA, that directs the insertion and deletion of nucleotides at "correct" sites on the unedited messenger RNAs.

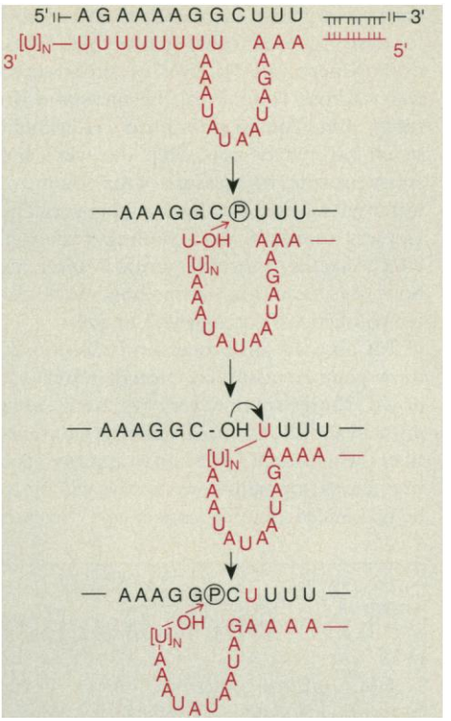
And the new RNA may be a guide to more than just editing. Although there's still disagreement about how the RNA editing machinery operates, some scientists are already speculating that it may be a key to understanding the evolution of life—what Thomas Cech of the University of Colorado in Boulder calls a "molecular fossil," a living relic that provides a look backward to life's origins. What's more, since RNA editing occurs primarily in trypanosomes and related parasites, it may be a weak link in the organisms' armor that can be exploited as a potential target for therapeutic drugs.

All this has the discoverers of RNA editing breathing a sigh of relief. While the phenomenon may now have achieved a firm molecular footing, there were lots of doubters when the first signs of editing turned up in independent studies done first by Benne's group, and then by those of Larry Simpson at the University of California, Los Angeles, and Kenneth Stuart at the Seattle Biomedical Research Institute.

In the cells of higher organisms, genes can be found not just in the nucleus, but also in the small particles called mitochondria, which produce most of the cell's energy. While studying the mitochondrial genes in the parasites *T. brucei* and *Leishmania tarentolae*, the three groups kept running into a puzzling observation: In several instances, functional proteins seemed to be made from genes that had mutations that should have prevented them from being active. Some genes contained premature "stop" signals, for example, while other didn't have the appropriate "start" signals.

Benne first thought, he says, that the genes were the mitochondrial equivalent of the inactive "pseudogenes" known to dot the nuclear genome. He expected that his group would turn up the authentic full-length genes, but despite an exhaustive search, they couldn't come up with any.

So Benne and his colleagues tried a different approach. They compared the DNA sequence of one of the presumed mutant



Guiding the message. *In the transesterification model of RNA editing, the guide RNA* (red) *splits the messenger RNA molecule* (black) *to insert uridine nucleotides.*

genes with the sequence of the messenger RNA that actually directs the synthesis of the protein encoded by the gene. And that's when the researchers found an astonishing result: The sequence of the messenger RNA should have corresponded to that of the gene, but it didn't. The RNA contained information that couldn't be found within the DNA. Somehow the gene was being corrected—but at the level of the RNA. The sequence analysis also showed that corrections were accomplished by inserting uridine nucleotides in some places and deleting them in others. Those changes took place at very specific sites, too. They didn't just happen randomly.

The first thing Benne did when he saw these unprecedented findings was to send Just Brakenhoff, the undergraduate student who had done the work, back to the bench to repeat the experiment. But, Benne says, the results were always the same for that gene and for others as well.

About a year later, Stuart and his colleagues followed up the Benne group's work with new data, which Stuart says, "alerted everyone to the wonders of RNA editing." The Seattle workers showed that in some cases more than half of the nucleotides in a messenger RNA could be determined at the editing stage. They found one RNA, for example, in which 550 uridines had been added and 41 removed to make the mature

message. In that case, 60% of the message was determined during editing, a troubling result in view of the fact that no one could find a gene template dictating where the uridines should be added and deleted.

"It was hard to take in the beginning," Simpson says. "We didn't want to discard genetic dogma, but if there was a hidden gene somewhere with the complete sequence, we just couldn't find it." Yet he and the other researchers ultimately remained faithful to the notion that the information had to be encoded by a nucleic acid. "The editing process is really very precise," Benne explains, and that made it very difficult to come up with a mechanism other than the standard base-pairing between nucleic acids to explain how editing occurred.

But adhering to the idea of a nucleic acid template got harder and harder as the years rolled by, and no evidence for one could be found. Then in 1989, Simpson came up with an idea that would ultimately crack the problem. What if these puzzling mitochondrial genes weren't encoded in single long DNA segments, Simpson asked, but in many small segments?

Mitochondrial DNA comes in two forms, large circles known as maxicircles and the smaller minicircles. In the organism Simpson was studying, *L. tarentolae*, all of the known mitochondrial genes were on the maxicircles. So that's where he focused his attention, betting that the additional information needed for messenger RNA editing would be on the maxicircles, too. But the original search for maxicircle sequences that correspond exactly to the edited RNA came up empty.

Only when Simpson and his colleagues allowed for the possibility that the match might not be perfect did they hit paydirt. They found short sequences of maxicircle DNA that encode small RNA molecules that contain fewer than 40 nucleotides and apparently carry the instructions for uridine insertions and deletions. Because of their proposed function, Simpson called those RNAs "guide RNAs." They had been missed before, he says, because of their diminutive size. Researchers simply dismissed them as bits of RNA contaminating their nucleic acid preparations.

Soon afterwards, the Simpson group found that minicircle DNA in *Leishmania* also encodes guide RNAs. Indeed, work by Stephen Hajduk and his colleagues at the University of Alabama in Birmingham, as well as by the Benne and Simpson groups, showed that minicircle-encoded guides are the rule, rather than the exception in African trypanosomes, thereby solving a long-standing mystery. "People had wondered," Simpson says, "why the mitochondria for these organisms contain so much DNA. The maxicircles contain the mitochondrial genes, but we hadn't understood the need for minicircles."

But while the guide RNAs may carry the information needed to edit messenger RNAs, their discovery doesn't explain how the editing actually takes place. A more recent result from the Simpson group may help there, however. The researchers found that tails consisting of strings of uridine nucleotides are added to the guides, and suggested that these were the source of the uridines that are inserted into messenger RNAs.

Meanwhile, the Simpson group's identification of guide RNAs, which they described in *Cell* in January of last year, had piqued the curiosity of Boulder's Cech, whose own work had previously led to another surprising finding about RNA—the discovery that some RNAs have catalytic activity (which won Cech a share of the 1989 Nobel Prize). Many messenger RNAs contain stretches of noncoding sequences, called introns, that have to be spliced out of the molecule to form the mature message, and the catalytic RNAs Cech discovered catalyze that splicing reaction. In any event, when Cech learned about the guide sequences, he says, "I suddenly saw a parallel between editing and splicing."

He proposed that the uridines added to or removed from messenger RNAs during editing might just be very small introns. They might then be cut out the same way that more typical introns are, and inserted by the reverse of that reaction. Chemical reactions generally are reversible, Cech points out.

As Cech originally described his editing scheme, the guide RNAs can direct both the uridine insertions and deletions. In the first step, the guide aligns itself with the unedited RNA. Then the uridine tail of the guide invades the unedited RNA, splitting it in two and forging a new bond between one of the ends and the uridine at the tip of the tail (see diagram on p. 136). In the next phase, the unattached end of the RNA undergoing editing attacks at the newly attached uridine, forming a bond with it, thereby rejoining the RNA and releasing the tail of the guide so that it can initiate another round of reactions. The simplicity of this reaction, called transesterification, is attractive to Cech, but, he adds, "that doesn't mean it is correct."

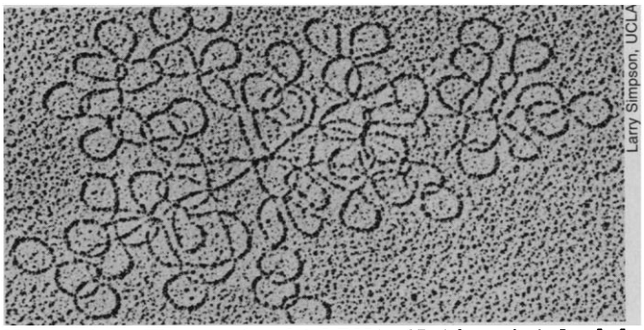
Earlier this year, however, Simpson and his colleagues provided evidence for Cech's proposed mechanism. The UCLA workers essentially caught guide and unedited RNAs in the act. They found four different chimeric molecules in which guide RNA is joined by a length of uridine nucleotides to messenger RNA—just the sort of intermediate that Cech's model predicts.

But not everyone finds the chimeric molecules to be convincing evidence for the transesterification mechanism. Molecular biologist Barbara Sollner-Webb of Johns Hopkins University School of Medicine has proposed an entirely different role for the guide RNAs in editing, and she says, chimeras would be consistent with her proposal as well.

In her view, the sites at which uridines are added and deleted are not determined by the guides. To bring about those changes, the RNA being edited has to be cut first, and with Hajduk's group, Sollner-Webb has found that some sites on the messenger are inherently more susceptible to being cut by cellular enzymes than others. "Maybe that means," she says, "that the most delectable, most favorable cutting site is a function of the secondary structure of the RNA."

But if guide RNAs aren't responsible for telling the cellular machinery where to put the uridines, what are they doing? According to Sollner-Webb, their main job is to join to the correctly edited portion of the messenger RNA and protect it from being cut again. Despite these differences with the Cech-Simpson model, the uridine tail of the guide RNA might still serve as the source of uridines being added during editing, Sollner-Webb says, and in that event chimeric molecules would be formed. Finding chimeric molecules is, she maintains, "a far distance from proving transesterification."

But if RNA editing does proceed by transesterification, it might provide a glimpse back to the origins of life. Although Cech initially viewed editing as a form of RNA splicing, in the past few months he has begun considering another possibility as the result of a suggestion made by one of his



Larry Simpson, UCLA

Many minis. *The mitochondrial DNA of* Leishmania *is loaded with minicircles, which have a key function in RNA editing.*

graduate students, John Grey.

The first nucleic acids to have evolved are thought to be RNAs that have both the information and the catalytic abilities to replicate themselves. Grey pointed out a chemical similarity between that reaction and the transesterification reaction postulated to occur during editing. "The idea in both cases is that you have RNA-directed RNA replication," Cech says. Viewed in this way, he adds, editing may not be a variant of splicing that was invented by the trypanosomes, but something much older. "I'm not saying that the mechanism today is exactly like what was there in the prebiotic world. I'm sure it, too, evolved," Cech explains. "But editing has the right feel to be a direct descendant from RNA replication."

Whatever the origins of RNA editing, Stuart has new evidence suggesting what it's modern function might be. His lab has shown that edited messages are present in the trypanosomes that cause African sleeping sickness only when the proteins they synthesize are needed. Editing may therefore be a way of regulating which proteins are synthesized when. If so, and if editing is found just in the parasite and not in the host, then the editing machinery may be a possible target for drugs that control trypanosome infections in humans.

Such drugs are not likely to come any time soon, however. "Right now the best [therapeutic] targets are still the surface antigens," Sollner-Webb says. "Guides are not only not on the surface, but are hidden away in mitochondria, and not enough is known about the whole editing process for them to be a good target."

The next goal, the researchers all say, is to develop good test tube systems for studying editing so that they can work out the exact functions of all the components of the machinery. When that's been done, they may or may not have their therapeutic target, but they will for sure have removed the last veil of mystery from another of Mother Nature's surprises. ■ MICHELLE HOFFMAN

ADDITIONAL READING

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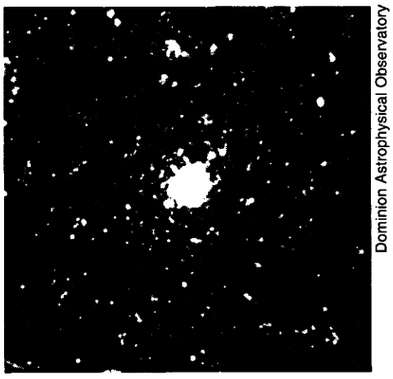
A Long, Hard Look at the Virgo Cluster

New images reveal the stars in a distant galaxy—and heat up a long-simmering debate about the universe's age and size

MIT ASTRONOMER JOHN TONRY THOUGHT he might see something new last February, when he aimed the powerful Canada-Hawaii-France telescope at the distant collection of galaxies called the Virgo Cluster. A visiting astronomer at the Dominion Astrophysical Observatory's facility on Mauna Kea, Tonry had just finished his own observing run and planned to use his remaining minutes to photograph the Virgo Cluster as a favor to Dominion colleagues Robert McClure and Michael Pierce. Working in Tonry's favor were crystal-clear weather, the thin air on the 14,000-foot peak, and a prototype adaptive-optics system, which eliminates atmospheric turbulence by monitoring the twinkling of a star and adjusting the telescope's optics to compensate (see *Science*, 28 June, p. 1786).



Dominion Astrophysical Observatory

Seeing stars. *NGC 4571, one of the galaxies in the Virgo Cluster.*

Yet the quality—and the unsettling cosmological implications—of the resulting images startled Tonry. In every earlier photograph of the Virgo Cluster, which lies at a distance of tens of millions of light-years, entire galaxies had been smudges, their individual stars indistinguishable. Astronomers hadn't expected to make out individual stars in such distant galaxies until the Hubble Space Telescope is cured of its blurry vision. But now, when McClure and Pierce analyzed Tonry's images, they could pick out individual stars within one of those galaxies—the most distant stars ever seen. The achievement is testimony to the rapid progress being made in ground-based astronomy. "Every day observations from the ground get better," says Tonry.

At the same time, the images have sent a ripple of uncertainty through the cosmological community. The reason? Analysis of Tonry's sharp images suggests that the universe is smaller and younger than most cosmologists have assumed.

The grand cosmological implications of these images flow from the fact that McClure and Pierce were able to use them as cosmic distance measurements. The individual stars seen in the galaxy images gave McClure and Pierce the astronomical equivalent of scale bars. By assuming that the brightest of those stars are giving off as much light as the brightest stars in our own galaxy, the astronomers were able to estimate how far away the Virgo Cluster has to be to explain the stars' apparent brightness. The result—50 million light-years—is tens of millions of light-years closer than had been assumed.

"This is extremely important for estimating the distance scale of the universe," says McClure. That's because the Virgo Cluster's distance gives astronomers a new yardstick for measuring the distances to other galaxies, even ones whose individual stars still can't be seen. Ordinarily, the distance to another galaxy has to be estimated based on its red shift —the degree to which the galaxy's light is dragged toward the red end of the spectrum by its velocity away from our own galaxy.

Because of the expansion of the universe, the red shift increases with distance. But astronomers can't translate a specific red shift into an exact distance. The translation involves a number called the Hubble constant, which describes how the recession rate increases with distance. The trouble is that the Hubble constant is far from certain—something Simon Lilly, an astronomer at the University of Toronto, calls "pretty much a disgrace." "All astronomers should be working on that problem," he says. Among those who are, the favored value is 50—the units are kilometers per second per megaparsec—but a few cosmologists have held out for a value approaching 100.

Calibrating the Hubble constant more precisely has required two things: the red shift of a sample galaxy and an independent measure of its distance. But the sample galaxy should be a distant one, with a high red shift that is unlikely to reflect local motions