

# Breakthrough

# #1

## The Winner

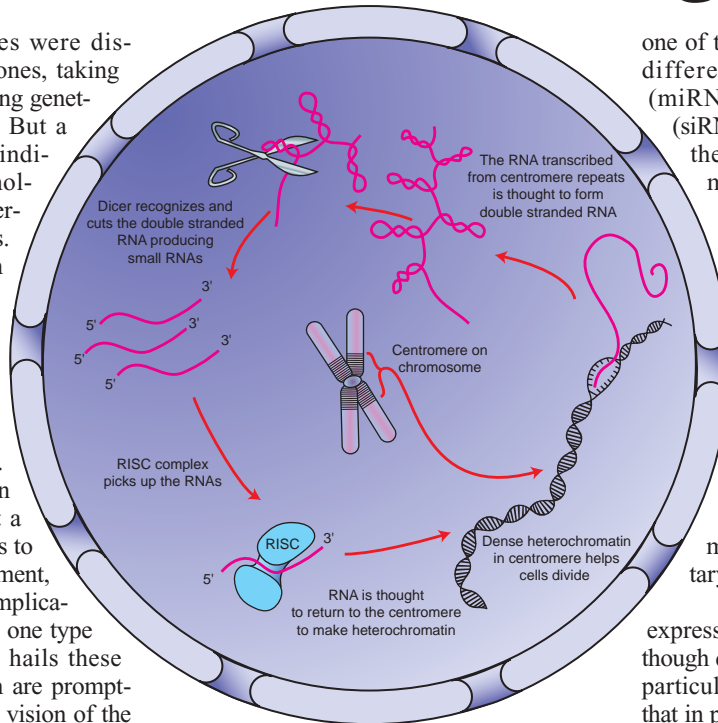
Just when scientists thought they had deciphered the roles played by the cell's leading actors, a familiar performer has turned up in a stunning variety of guises. RNA, long upstaged by its more glamorous sibling, DNA, is turning out to have star qualities of its own.

## Small RNAs Make Big Splash

For decades, RNA molecules were dismissed as little more than drones, taking orders from DNA and converting genetic information into proteins. But a string of recent discoveries indicates that a class of RNA molecules called small RNAs operate many of the cell's controls. They can turn the tables on DNA, shutting down genes or altering their levels of expression. Remarkably, in some species, truncated RNA molecules literally shape genomes, carving out chunks to keep and discarding others. There are even hints that certain small RNAs might help chart a cell's destiny by directing genes to turn on or off during development, which could have profound implications for coaxing cells to form one type of tissue or another. *Science* hails these electrifying discoveries, which are prompting biologists to overhaul their vision of the cell and its evolution, as 2002's Breakthrough of the Year.

These astonishing feats are performed by short stretches of RNA ranging in length from 21 to 28 nucleotides. Their role had gone unnoticed until recently, in part because researchers, focused on the familiar larger RNA molecules, tossed out the crucial small ones during experiments. As a result, RNA has long been viewed primarily as an essential but rather dull molecule that ferries the genetic code from the nucleus to the ribosomes, the cell's protein factories, and helps assemble amino acids in the correct order during protein synthesis.

Signs that RNA might be more versatile came in the early 1990s, when biologists determined that some small RNAs could quash the expression of various genes in plant and, later, animal cells. But they didn't appreciate the molecules' true powers until 1998. That's when Andrew Fire of the Carnegie Institution of Washington in Baltimore, Maryland, Craig Mello of the University of Massachusetts Medical School in Worcester, and



**Life cycle.** With a helping hand from proteins RISC and Dicer, small RNAs are born. We now know that these molecules keep DNA in line and ensure a cell's good health.

their colleagues injected stretches of double-stranded RNA into worms. Double-stranded RNA forms when a familiar single strand kinks back in a hairpin bend, putting two complementary sequences alongside each other. To the researchers' surprise, double-stranded RNA dramatically inhibited genes that had helped generate the RNA in the first place. This inhibition, which was later seen in flies and other organisms, came to be known as RNA interference (RNAi). It helped prove that RNA molecules were behind some gene silencing.

Another crucial step came last year, when Gregory Hannon of Cold Spring Harbor Laboratory in New York and his colleagues identified an enzyme, appropriately dubbed Dicer, that generates the small RNA molecules by chopping double-stranded RNA into little pieces. These bits belong to

one of two small RNA classes produced by different types of genes: microRNAs (miRNAs) and small interfering RNAs (siRNAs). siRNAs are considered to be the main players in RNAi, although miRNAs, which inhibit translation of RNA into protein, were recently implicated in this machinery as well.

To bring about RNAi, small RNAs degrade the messenger RNA that transports a DNA sequence to the ribosome. Exactly how this degradation occurs isn't known, but scientists believe that Dicer delivers small RNAs to an enzyme complex called RISC, which uses the sequence in the small RNAs to identify and degrade messenger RNAs with a complementary sequence.

Such degradation ratchets down the expression of the gene into a protein. Although quashing expression might not sound particularly useful, biologists now believe that in plants, RNAi acts like a genome "immune system," protecting against harmful DNA or viruses that could disrupt the genome. Similar hints were unearthed in animals this year. In labs studying gene function, RNAi is now commonly used in place of gene "knockouts": Rather than delete a gene, a laborious process, double-stranded RNA is applied to ramp down its expression.

The year's most stunning revelations emerged in the fall, in four papers examining how RNA interference helps pilot a peculiar—and pervasive—genetic phenomenon known as epigenetics. Epigenetics refers to changes in gene expression that persist across at least one generation but are not caused by changes in the DNA code.

In recent years, researchers have found that one type of epigenetic regulation is caused by adjustments in the shape of complexes known as chromatin, the bundles of DNA and certain fundamental proteins that make up the chromosomes. By changing shape—becoming either more or less compact—chromatin can alter which genes are expressed. But what prompts this shape-

ILLUSTRATION: C. SLANTEN/G. RIDDHOUGH

# of the Year

shifting remained mysterious.

This year, scientists peering closely at RNAi in two different organisms were startled to find that small RNAs responsible for RNAi wield tremendous control over chromatin's form. In so doing, they can permanently shut down or delete sections of DNA by mechanisms not well understood, rather than just silencing them temporarily.

That news came from several independent groups. In one case, Shiv Grewal, Robert Martienssen, and their colleagues at Cold Spring Harbor Laboratory compared fission yeast cells lacking RNAi machinery with normal cells. When yeast cells divide, their chromosomes untangle and migrate to opposite sides of the cell. The researchers already knew, broadly, that this chapter of cell division is governed by a tightly wrapped bundle of chromatin, called heterochromatin, around the centromere—the DNA region at the chromosome's "waist." The biologists found that their mutant cells, which were missing the usual small RNAs, couldn't properly form heterochromatin at their centromeres and at another DNA region in yeast that controls mating. This suggests that without small RNAs, cell division goes awry. The scientists theorized that in healthy yeast cells, small RNAs elbow their way into cell division, somehow nudging heterochromatin into position to do the job. That exposes DNA to different proteins and dampens gene expression.

Meanwhile, David Allis and his colleagues at the University of Virginia Health System in Charlottesville, along with Martin Gorovsky of the University of Rochester in New York and others, were focusing on a different organism, a single-celled ciliate called *Tetrahymena*. Biologists treasure *Tetrahymena* because it stores the DNA passed to offspring in a different nucleus from the one containing DNA expressed during its lifetime, making it easy to distinguish one gene set from the other. The researchers found that in *Tetrahymena*, small RNAs trigger deletion or reshuffling of some DNA sequences as a cell divides. RNAi appeared to be targeting structures analogous to heterochromatin, only this time strips of DNA were discarded or moved elsewhere. The mechanism remains unclear, however.

The two sets of experiments might help explain why small RNAs exist in the first place. In both the yeast and *Tetrahymena*, small RNAs' frenetic activity is focused on genome regions, such as centromeres, that contain repetitive DNA resulting from trans-

posons. Transposons are bits of DNA that can jump around the genome and insert themselves at different locales; at times, they jam transcription machinery and cause disease. It appears possible—although still largely hypothetical—that small RNAs evolved very early in life's history to help protect the genome against instability.

This is just one of many areas that remain to be explored. Researchers are still trying to sort out how the well over 100 different miRNAs function and which species contain which ones. There are hints that they behave differently in plants and animals. And some recent work suggests that miRNAs exert more control over gene expression than pre-

viously believed. Also a focus of research are the proteins, such as Dicer, that are critical cogs in the RNAi machinery.

Researchers are also probing RNAi's possible role in development and disease. RNAi has been implicated in guiding meristems, the plant version of stem cells, so some biologists believe that it might help establish the path taken by human and other mammalian stem cells as they differentiate into certain tissues. If so, RNAi could prove an essential tool in manipulating stem cells. And if small RNAs influence cell division in humans as they do in yeast and *Tetrahymena*, minor disruptions in the machinery could lead to cancer.

The extraordinary, although still unfulfilled, promise of small RNAs and RNAi has split the field wide open and put RNA at center stage. Having exposed RNAs' hidden talents, scientists now hope to put them to work. —JENNIFER COUZIN

## THE RUNNERS-UP

Science applauds discoveries ranging from the dawn of time to the dawn of our species.

**#2 Neutrinos nailed.** Neutrinos, mysterious and misunderstood, are finally getting the respect they deserve. For years, neutrinos were the terra incognita on the particle chart. Electrons, muons, taus, and quarks had all been analyzed for years, their properties measured and dissected. But neutrinos? Nobody knew even whether they had mass until a few years ago. They were essential-

tario, put the final nail in the coffin of the solar neutrino paradox. The nuclear reactions in the sun should produce a large number of electron neutrinos, but all observations had shown that only about one-third of the expected number were actually reaching Earth.

If neutrinos have mass, they can change flavors—from electron neutrinos into tau or mu neutrinos, for example—and that could explain the missing electron neutrinos. SNO showed, once and for all, that this is the case. In April, scientists at SNO announced that they had measured the abundances of all three types of neutrinos—electron, mu, and tau—by detecting when they split apart atoms of deuterium. When they added up the solar electron, mu, and tau neutrinos streaming through the detector, the total matched the number that should be created by nuclear reactions. Electron neutrinos change flavor during their journey to Earth.

As a bonus, the SNO measurements allowed scientists to drastically limit the "mixing angles" that define the neutrinos' flavor-changing abilities and, in December, the KamLAND experiment in Japan restricted the limits even further—with nuclear reactor-created antineutrinos instead of solar neutrinos. Although physicists still don't know how much neutrinos weigh, the evanescent



**Positive ID.** A huge sphere of heavy water caught fugitive neutrinos as they changed from one flavor to another.

ly unknowns.

No longer. In the last decade, physicists finally proved that neutrinos have mass, and since then, a flurry of experiments has begun to flesh out the elusive neutrinos' properties.

This year, the Sudbury Neutrino Observatory (SNO), a 1000-ton sphere of heavy water deep inside a nickel mine in Sudbury, On-