

Gene-silencing Breakthrough Heard Loud and Clear

By Alison M. Duffy

The discovery of RNA interference ushers in an explosive new field of research—and brings remarkable acclaim to UMMS scientists.

Perhaps fortunately for UMass Medical School and the field of basic research as a whole, a young Craig Mello never found a dinosaur fossil. If he had, he might have followed in his paleontologist father's footsteps, pursuing the dusty remnants of the largest creatures to roam the Earth instead of the smallest of molecular particles within a microscopic worm. His work in picking apart the genetic screen of *C. elegans* has led to one of the most pivotal discoveries in biological research, upending previously held tenets about the role of a basic cellular material, ribonucleic acid (RNA).

Scientists had long generally believed RNA to be a simple messenger, shuttling the genetic code contained in DNA from the cell's nucleus to its ribosomes where proteins are made, and "proofreading" the proteins to ensure that they are correctly constructed to perform their function in living organisms. Although vital to the functioning of the cell, RNA was given little credit beyond its messenger duties. Over the last decade, however, unexpected discoveries have thrust the humble RNA into the center of an explosive new field of research.

In the early 1990s, puzzled researchers scratched their heads over a white petunia, the unanticipated result of a botanical experiment in which extra-pigment genes inserted into petunias were expected to produce a vibrant purple bloom. Instead, both the extra genes and the plant's own copies of the same pigment gene were silenced. Although plant literature described various genetic mechanisms, it wasn't clear exactly what was shutting down the pigment genes—scientists believed it might have something to do with a developmental mechanism involving RNAs, but that wasn't well understood.

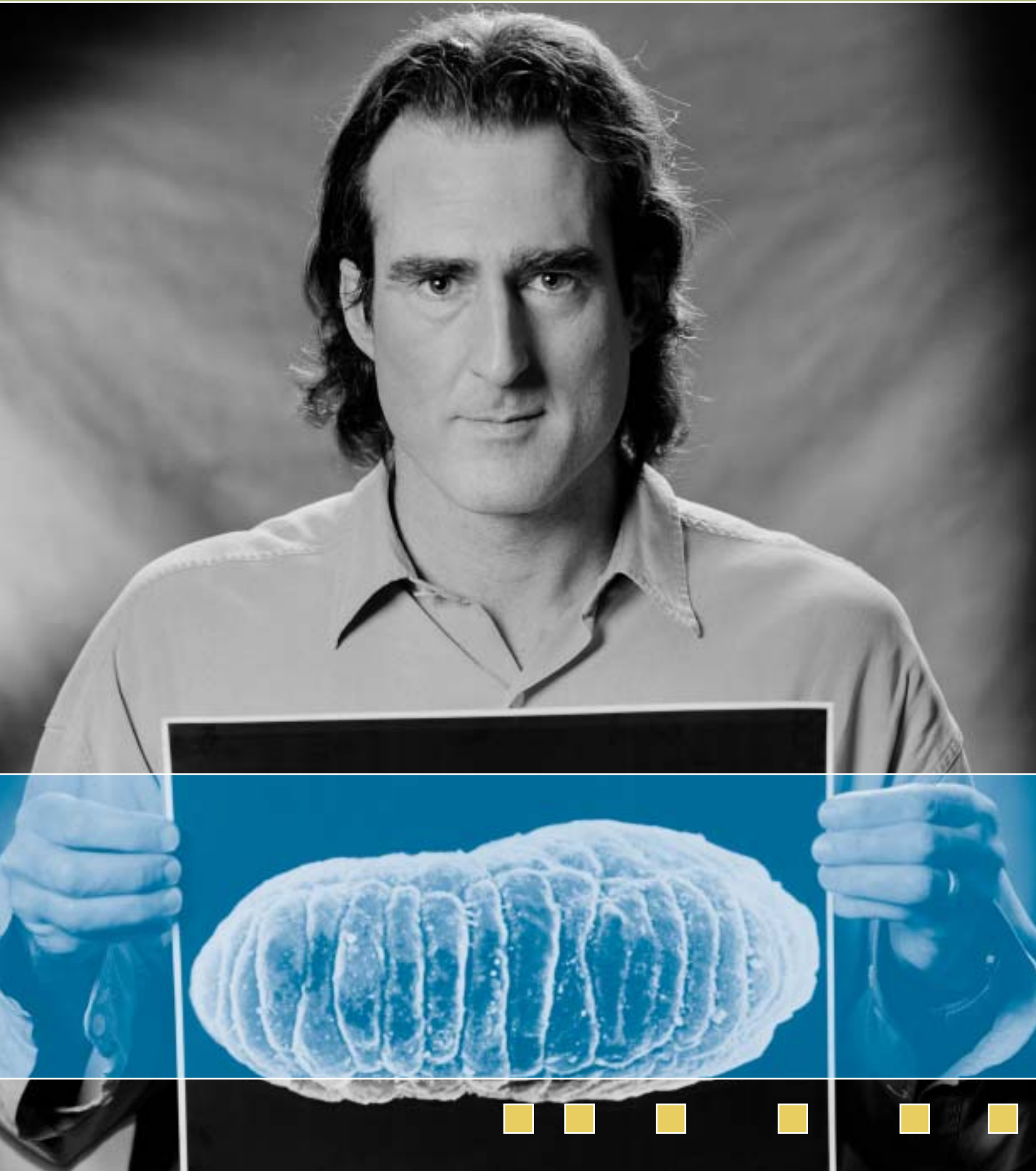
Some years later, Dr. Mello's work in *C. elegans* led him to investigate a similar gene-silencing phenomenon. An investigator of the prestigious Howard Hughes Medical Institute and a UMMS Distinguished Professor of Molecular Medicine, Mello and colleagues in his laboratory found that upon injection into *C. elegans*, RNA could cause an interference effect that could spread from the site of injection throughout the body of the worm and could even be transmitted via the sperm or egg for at least two generations. Andrew Fire, PhD, of the Carnegie Institution of Washington, a respected colleague with whom Mello had collaborated in the

past, suggested that double-stranded RNAs that were unintentionally present in Mello's RNA preparation might be causing the potent interference effect.

Double-stranded RNA is a form of RNA that is structured much like the classical DNA double helix, with two complementary strands wound around each other. Mello and Dr. Fire found that purified double-stranded RNA was at least ten to 100 times more potent than single-stranded RNA at inducing genetic interference. This astounding breakthrough, published in 1998 in *Nature*, revealed double-stranded RNA as the molecule responsible for the gene-silencing mechanism.

The reaction among the scientific community to RNA interference (RNAi), as it was called, was one of dumbfounded excitement at the magnitude of the discovery, particularly because it completely rearranged the understanding of the fundamental role of RNA within the cell. "Organisms have faced challenges from viruses for billions of years," said Mello, "and RNAi appears to be an ancient protective mechanism that's been in place all along. We just never noticed it."

The birth of a new field brings with it remarkable acclaim: *Science* magazine called RNAi a 2002 "Breakthrough of the Year;" the National Academy of Science tapped Mello and Fire for its Award for Molecular Biology, and the two shared the Wiley Prize in Biomedical Sciences with a handful of other RNA researchers. "Our work was just one piece of a puzzle, but I think it was an important piece because it brought several fields of work together," Mello said. "Now it feels like we are standing on the very edge of a new frontier: RNAi has opened a new door through which we can go to learn so much more."



*Craig Mello, PhD, holds an electron micrograph of a *C. elegans* embryo, six hours after fertilization. Approximately one-tenth the diameter of the period at the end of this sentence, the embryo consists of just over 500 cells. The resulting adult worm has all of the major cell types found in humans, including skin, neurons, muscle and intestine, but consists of only 1,000 cells.*



These three images show top, side and bottom views of the *C. elegans* embryo from page 7. Rows of skin cells are visible on each side of the animal. The oblong rectangular shapes are individual skin cells, which spread over and surround the embryo at mid-embryogenesis and then contract to elongate the body until it is threefold the length of the egg. Adult animals are self-fertile hermaphrodites and can produce 300 progeny in three days. The simple anatomy and rapid generation time of *C. elegans* makes it an excellent system in which to study a variety of questions relevant to basic research. The electron micrographs shown here and on page 7 were taken by James R. Priess of the Fred Hutchinson Cancer Research Center in Seattle and provided courtesy of the Craig Mello laboratory.

Indeed, Mello and Fire opened this astounding door and suddenly scientists everywhere, with a new appreciation for these tiny, previously unnoticed RNA molecules, fumbled to turn on the light: how does RNAi work? One of those poised to flip the switch was Phillip D. Zamore, PhD, UMMS associate professor of biochemistry & molecular pharmacology, who remembers clearly his reaction upon reading Mello and Fire's *Nature* article, which he calls simply "the paper."

"Virtually every project in my lab has utilized RNAi. It has had an enormous impact on biological research. [Mello and Fire's] initial work made the field, and they continue to make extraordinarily interesting contributions to this exciting [and] useful phenomenon."

—Iva Greenwald, PhD, professor of biochemistry and molecular biophysics, Columbia University

"You had to have been lobotomized, I think, to not understand that it was a paper on the magnitude of Watson and Crick—it was inescapable," said Dr. Zamore, who had devoted his postdoctoral work at MIT's Whitehead Institute to the developmental mechanisms in the very early embryo *Drosophila* (fruit flies). He was immediately intrigued and realized that, with double-stranded RNA identified as the key to the interference mechanism in *C. elegans*, the next important step would be to replicate the process not only in other organisms but also in the test tube, in order to pick it apart and examine it step by step.

Zamore, a 2002 W. M. Keck Foundation Distinguished Young Scholar, and Whitehead colleague Thomas Tuschl, PhD, now of the Max Planck Institute for Biophysical Chemistry in Germany, both of whom were between their postdoctoral work and their faculty appointments, quickly set up an *in vitro* biochemical system for RNAi using extracts from Zamore's microscopic

Drosophila embryos. The key experiment was to see if double-stranded RNA for the luciferase gene could turn off the production of luciferase enzyme. "Just the top two numbers coming out of the thermal printer made it perfectly clear that the experiment had worked beautifully—that we had RNAi in the test tube."

Zamore's experiment shed the light on how RNAi worked by indicating that it was the small double-stranded RNA, the result of an enzymatic chopper called "dicer," that precisely guided the silencing reaction Mello had identified. Capturing the RNAi process in an *in vitro* system was a defining moment for Zamore. "It completely changed the path of my research. Everything I'd planned to do for 20 years was put on hold." Zamore now focuses his work on the biochemical analysis of *in vitro* extracts to study the machinery of the RNAi. "The RNAi pathway is a wonderful thing to tease apart because we're learning three very different kinds of things: fundamental biochemistry, developmental biology and technology."

The development of RNAi technology as a research tool has been key for UMMS, and the school, with the Carnegie Institution, has been awarded a broad patent for the application of that technology. (See brief story, page 12.) Numerous companies have sprung up to capitalize on the process and further the field, many seeking to use RNAi to develop therapeutic drugs. The effect of RNAi has been felt in the scientific community as laboratories here at UMMS (see story, page 10) and around the world have adopted the technology: RNAi is simply a better tool for researchers, allowing them to silence a specific gene to understand its function, instead of laboring through the inaccurate mutation process. "Think of the Human Genome Project, which identified some 30,000 genes," said Mello. "It created some 30,000 questions: 'What does this one do? What about that one?' RNAi lets us answer those questions directly by asking the organism what's important."

It's fantastic to see RNAi become such a powerful tool for researchers around the world and it's understandable that the RNAi work being done here is getting a lot of attention," Mello continued, "but our strength in RNAi is just one example of the high quality of research going on at UMass Medical School. Again and again we're showing it's not a fluke. When people look at us what they are really seeing is the depth and strength of our research programs in numerous fields."

“There is incredible excitement about RNAi, not only in academia, but in biotech and pharmaceutical companies. It is a powerful tool, and I think may become a new kind of drug itself. With Craig Mello and [fellow UMMS investigator] Phil Zamore, your institution is a powerhouse. You have two of the leaders in the field of RNAi.”

—Judy Lieberman, MD, PhD, senior investigator, Center for Blood Research, Harvard Medical School

Milestones like the discovery of RNAi illustrate that there are still breathtaking surprises out there. No one knows whether RNAi’s ultimate legacy will be as a powerful tool that enables researchers to understand the full function of every gene, or whether it will itself prove to be the genetic silver bullet able to directly combat human disease.

Both Mello and Zamore believe that the critical mass of creative and energetic people in the labs, the brilliant colleagues down the hall including the “phenomenal junior faculty we’ve recruited,” and an enthusiastically supportive research administration fuel the very best work at UMMS. As Zamore said, “Breakthroughs don’t happen in obscure places to solitary scientists. The insight may suddenly come while you’re in the shower or driving to work, but the seed was planted during a conversation with your colleagues.”

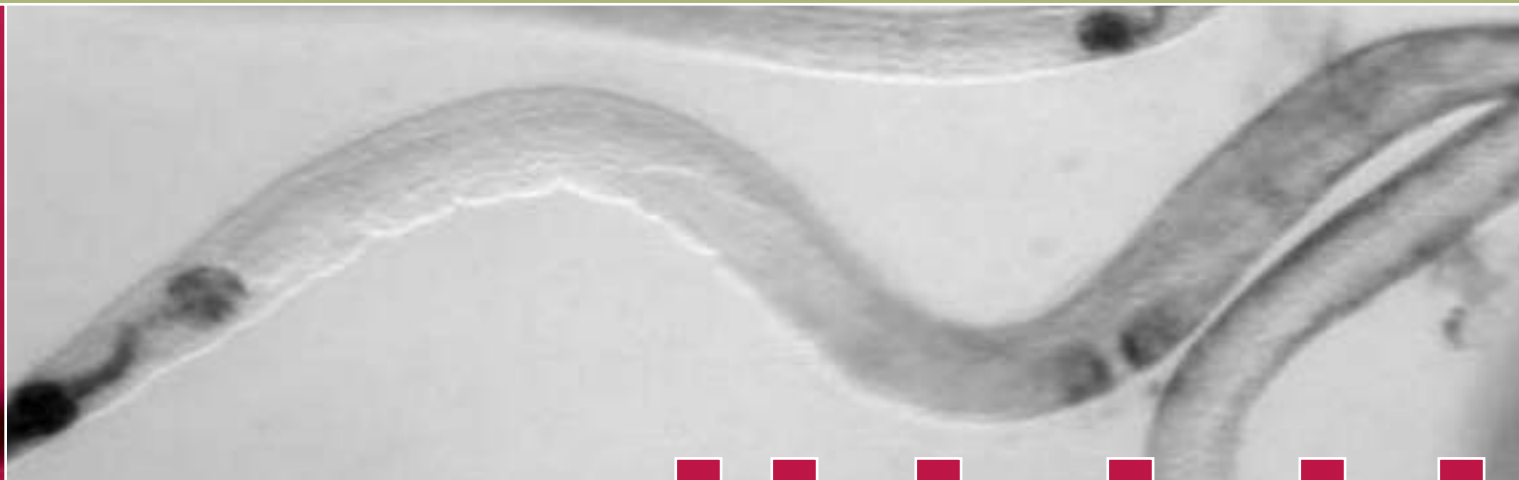


Phillip Zamore, PhD

A Real Knockout

By Michael I. Cohen

RNA interference accelerates biomedical research at UMMS, and around the world.



Staining appears around the mouth area (left) of *C. elegans*, where muscle RNA was not targeted by RNA interference, while reduced or nonexistent staining along the body wall shows the inactivation of muscle genes through RNAi.

What began as a curiosity in petunias and worms has emerged as a breakthrough technology taking aim at the causes and potential cures of major diseases afflicting humanity.

The process is called RNA interference, or RNAi, and was discovered by UMass Medical School researcher Craig C. Mello, PhD, and his colleague Andrew Fire, PhD, of the Carnegie Institution of Washington. (See story page 6.) Since their findings were published in *Nature* in 1998, the technology of RNAi has swept through laboratories like a virus, changing the way biomedical researchers work, giving them a long-sought-after tool for rapid analysis of the genes and proteins that underlie biological processes.

“This is a revelation we’ve been waiting on for years,” said Michael P. Czech, PhD, chair of the Program in Molecular Medicine at UMMS. “It’s really a breath-taking advance.”

Dr. Czech’s lab is at the forefront of research in type 2 diabetes and the tools of RNAi are now central to much of his work. In recent years, Czech’s lab applied human genome data to identify hundreds of genes and proteins that appear to be involved in the biological processes that lead to type

2 diabetes. But identifying those genes and proteins was only a first step, Czech said. “It’s been very exciting, discovering all these novel genes. But it’s also been very frustrating because you get much more data than you bargained for.”

Before RNAi technology, researchers worked one gene at a time. It was a laborious process that used chemical methods to “knock out” a single gene and study the impact of its removal. The “conundrum,” as Czech describes it, is that not every gene or protein identified has a vital role in the processes of diabetes. His lab had a mountain of genetic data, but no quick, reliable way to test that data to see which genes and proteins were truly important for the progression of the disease.

RNAi changes all that. “The absolute beauty of RNAi is that it allows us to begin sorting out and prioritizing the genes we discover,” Czech said. “With RNAi, we can knock out genes individually, and in combinations, in culture and see if they really function in the process

we’re examining. What used to take us months, for one gene, now takes a week.”

Czech’s team recently applied RNAi technology to a “molecular motor” they discovered in a previous genomic screen. The experiments found that the motor positions a “pipe” in the wall of the fat cell that allows the cell to take up glucose. Cellular uptake of glucose doesn’t work properly in people with type 2 diabetes. “If we could control that process, determine which genes are defective, and provide an intervention that would bring it back to normal, I think we’d have a major therapeutic advance in the field,” Czech said.

The discovery and refinement of RNAi as a biological technique is so important that nearly every biological laboratory in the world will be using it, predicted Czech. “And for those of us at UMass Medical School, it is exceedingly exciting to have one of our colleagues making a breakthrough of this magnitude.”

Background image: *C. elegans* that have not been treated with RNAi; expression of muscle RNA in the mouths and body walls is indicated by staining. Images courtesy of the Craig Mello laboratory.

“This was a great discovery. It provides a great tool to study gene function. The high praise [for Mello and Fire] is fully justified.”

—Ronald Plasterk, PhD, professor of developmental genetics and director of the Hubrecht Laboratory, the Netherlands Institute for Developmental Biology

Mario Stevenson, PhD, the David J. Frelander Professor of AIDS Research at UMMS and professor of molecular medicine, agrees. “I think Craig Mello deserves immense credit. He’s provided an approach which will have an impact on research in so many areas, not just HIV,” Dr. Stevenson said. “In our work, we can now ask questions about HIV that would have been difficult, if not impossible, to ask prior to the advent of RNAi.”

Stevenson’s team is using RNAi to focus on the processes that enable HIV to penetrate a cell’s nucleus. HIV can’t replicate on its own—it needs to co-opt the DNA of another cell to reproduce. So if the virus can be kept out of the nucleus and away from the DNA, then it can be stopped. “We demonstrated that if you introduce those small interfering RNAs into cells that are infected by HIV, they

has been hampered by a number of potential genetic targets and no “blueprint” illustrating the genes and proteins that actually play a role in the HIV life cycle, according to Stevenson. He likened the problem to trying to figure out how a car runs, without knowing which parts are more important than others. “You end up letting air out of the tires to see if that makes the engine stop,” Stevenson said. “With RNAi, we can quickly validate what cellular proteins are important for HIV, and that’s a first step toward drug discovery. I can’t overestimate the impact RNAi is going to have on the field, as it focuses our energies on the information that’s really important. Instead of letting air out of the tires, we’ll be able to go right for the fuel line to stop the engine.”

Cancer research at UMMS also uses RNAi technology. Stephen J. Doxsey, PhD,

Before the advent of RNAi technology, Doxsey’s team used chemical means to work with a single protein that they knew was important in centrosome function. Then, RNAi came along. “The life of the cell biologist has changed dramatically by having the ability to use RNAi,” Doxsey said. “Rather than working on one molecule at a time with less precise means, the ability to work on all the molecules we’re interested in simultaneously, and conducting the definitive experiment that provides results in two days, is simply unprecedented.”

When it was clear that RNAi could silence genes “as clean as a knife cutting them out,” Doxsey refocused some of the work of his lab. He launched an effort to study every protein that was part of, or interacts with, the centrosome. In just the past few months, that work has produced some tantalizing clues. “Data now suggest that centrosomes are important for moving through the cell cycle; functional disruption of many individual centrosome proteins arrests the cell cycle and prevents cells from dividing,” Doxsey said. “Now, we may be able to take advantage of that ability of centrosome proteins to arrest cells, to attack cancer cells. We just received a grant to study that.”

“Just by allowing us to figure out what specific proteins do in cells, the discovery of RNAi is worthy of a Nobel Prize. Now, if it turns out to be therapeutically useful as well, and there are indications that it may, then this is truly an unbelievable advance.”

—John Sullivan, MD, director, UMMS Office of Research

dramatically shut down virus replication,” Stevenson said. “But what we really want to do is catch it earlier, immediately after it infects the cell, before it enters the nucleus and has a chance to replicate. And I believe it’s just a matter of time before we figure out a way to attack that stage more efficiently.”

Like the work in diabetes, HIV research

associate professor of molecular medicine, biochemistry & molecular pharmacology and cell biology, studies the role of the centrosome in cancer cells. The centrosome is a small part of every human cell that directs the chromosomes to line up during cell division. Dr. Doxsey’s lab has shown that in nearly all carcinomas, including breast, prostate, lung, brain and cervical cancers, the centrosomes are defective.

Beyond its use in research, the potential for RNAi to become a therapy for diseases is being studied. To facilitate therapeutic uses, Tariq M. Rana, PhD, professor of biochemistry & molecular pharmacology and director of the Program in Chemical Biology at UMMS, is working to enhance the effectiveness of the RNAi technology. For example, early research showed the



RNA Interference Will Have Broad Application

As described in the preceding pages, RNAi is now the state-of-the-art method by which scientists can knock down the expression of specific genes in cells to thus define the biological functions of those genes.

"It has been extremely exciting and rewarding to see RNA interference grow as a research tool and field of study—from a strange phenomenon in a simple microscopic worm into so many exciting new discoveries and whole new research fields," said UMMS researcher Craig Mello, PhD, who discovered RNAi with colleague Andrew Fire, PhD, of the Carnegie Institution of Washington.

"Given the fundamental and broad-based impact of RNAi, I am extremely pleased that we can make this widely available to researchers at universities and other institutions seeking answers to genetic puzzles," said Dr. Fire. *"I look forward to what I believe will be astonishing discoveries that will result from the use of RNAi."*

A patent, "Genetic Inhibition by Double-Stranded RNA," (US Patent 6,506,559 B1) issued to UMMS and Carnegie, is expected to have far-reaching licensing potential both in the lab and in drug development. Because both institutions were eager to bring RNAi to bear as broadly as possible to hasten genetic research, they developed a licensing policy by which companies can readily obtain, for a basic fee, a wide-ranging and non-exclusive license for scientists to use the technology for research. A significant number of companies have already licensed the invention and additional companies have expressed interest.

"RNAi is an amazing contribution to molecular biology."

—Cori Bargmann, PhD, professor of anatomy and Howard Hughes Medical Institute Investigator, University of California at San Francisco

RNAi effect in human cells lasted only about 66 hours. To improve the potential for a therapeutic effect, Dr. Rana's lab set out to extend the duration of RNAi in human cells. "In the last year, our research has taken a huge leap," Rana said. "We developed a way to modify short RNA strands needed for RNAi that enhance their lifetime *in vivo* for up to six days."

Rana's lab is also working to chemically modify snippets of these short RNAs so they can specifically suppress the activity of a mutant gene, even, potentially, if it is only one nucleotide (base) different than the normal gene related to it. Single-base mutations have been linked to Alzheimer's and Parkinson's diseases and Amyotrophic Lateral Sclerosis (Lou Gehrig's disease), among others. "We have shown that we can increase the specificity for a gene targeted for RNAi in human cells in culture using these modified RNAs," Rana said. "Now we're ready to use this knowledge to target mutant genes linked to diseases that have representative mouse models and see if there is a positive effect on that disease in the mouse."

The technology and application of RNAi in many areas has grown so fast at UMMS not only because some of the world's leaders in basic research are here, but also because the model used by the Medical School's research enterprise fosters intellectual cooperation among the various disciplines, said John L. Sullivan, MD, director of the UMMS Office of Research. "We try to identify outstanding young investigators or mid-career investigators and bring them here to conduct their science," Dr. Sullivan said. "There is support at the leadership level for interaction and collaboration that is then encouraged for all. We all get together, talk about our science and ideas flow, making us unique among research institutions."