SPRING 2006 VOLUME NINE / NUMBER ONE

THE SCRIPPS RESEARCH INSTITUTE

ENDEAVOR

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VOLUME **NINE** / NUMBER **ONE** **SPRING 2006**

FEATURES:

ALSO:

ENDEAVOR IS A PUBLICATION OF *THE SCRIPPS RESEARCH INSTITUTE*

This issue of *Endeavor* magazine features investigators working at the forefront of the field of RNA research. Once thought to be a simple stepping stone in the process of translating genetic information from DNA into protein, RNA has turned out to be a far more important player than previously imagined.

At the Forefront

Scientists Discover "Unprecedented" Functional Amyloid Plays Beneficial Role In Human Cells

A group of scientists at The Scripps Research Institute have shown that the amyloid protein structure, which has been linked to diseases of the brain including Alzheimer's, Parkinson's, and Huntington's, carries out an important functional role in human physiology. The discovery raises the possibility that current research into curtailing amylolid formation to treat these diseases might ultimately do as much harm as good.

"The fact that we have found a structure in humans that is usually only associated with pathology is a critical new finding," said Jeffery W. Kelly, Ph.D., whose positions at Scripps Research include Lita Annenberg Hazen Professor of Chemistry, member of The Skaggs Institute for Chemical Biology, and vice president of academic affairs. Kelly led the study with Scripps Research Professor William E. Balch, Ph.D.

REFERENCE: PLoS *Biology*, 4(1), 10.1371 (2006).

Researchers Find Protein Controls Tumor Growth in Certain Breast Cancers

Scientists from Scripps Research and the Xiamen University School of Life Sciences, Fujian, The People's Republic of China, have uncovered a new and potentially important function for a protein previously thought to play a role solely in the innate immune system's response to bacterial infection. In the study, researchers showed that the presence of the protein Nod1 strongly inhibits the growth of estrogen-sensitive human breast cancer cells.

Richard Ulevitch, Ph.D., chair of the Scripps Research Department of Immunology, said, "These unexpected findings offer the first real evidence that this pathway may regulate tumor growth and suggest a potentially new mechanism for controlling this type of breast cancer."

REFERENCE: *PNAS*, 103(6), 1840-1845 (2006).

World Community Grid Targets AIDS in Giant Research Effort

IBM and Scripps Research have launched a new effort to help battle AIDS using the massive computational power of the World Community Grid, a global community of Internet users who donate unused time on their personal computers. With computational power already placing it among the top 10 supercomputers in the world, the World Community Grid is the first "virtual supercomputer" devoted specifically to AIDS research. Donating unused computer time is fast, easy, and secure. For more information on how to participate, go to www.worldcommunitygrid.org.

Scripps Florida Opens Cutting-Edge Screening Technology to Florida Scientists

Scripps Florida has launched its "Access to Technologies" program, which invites scientists from Florida universities and other academic research institutions to use state-of-the-art screening technologies at the institute's Jupiter facilities for qualifying projects. Access to Scripps Florida's new High Throughput Screening operation, similar to that used widely by the pharmaceutical industry, should speed up the process of discovering new drugs to treat a variety of human illnesses.

For more information, go to the Scripps Florida Access to Technologies website at www.scripps.edu/florida/technologies/hts.

Small Molecule Generates Neurons from Adult Stem Cells

A group of scientists from Scripps Research and the Salk Institute for Biological Studies have uncovered a synthetic small molecule that generates functional neurons from adult neural stem cells. The molecule, named neuropathiazol, selectively and potently induces neuronal differentiation of neural stem or progenitor cells. The results of this study, led by Sheng Ding, Ph.D., a Scripps Research investigator, may ultimately help in the development of future small molecule therapeutics. These could stimulate the regeneration of neurons in patients suffering from neurodegenerative disorders, such as Alzheimer's and Parkinson's disease, or from brain injuries.

REFERENCE: *Angewandte Chemie*, 45(4), 591-593 (2006).

"Scientists have been looking for a long time at known disease-causing genes and not finding answers. At least some of the answers may be contained in this new universe of non-coding RNA…"

CLAES WAHLESTEDT, M.D., PH.D.

Exploring a New Genetic World

CLAES WAHLESTEDT VENTURES BEYOND THE CENTRAL DOGMA

It is the nature of scientific inquiry that solving one puzzle often leads to many others. Such is the case with the Human Genome Project, which deciphered all three billion nucleotides, the basic building blocks of DNA that comprise the totality of human genes. The massive international research effort, begun in 1990 and completed in 2003, was one of the great feats of scientific exploration and provided the ability, for the first time, to read nature's complete genetic blueprint for humans. To scientists like Claes Wahlestedt, M.D., Ph.D., professor and director of Central Nervous System Disorders at The Scripps Research Institute's Florida campus, this achievement, as astounding as it was, was just the starting point.

"We now have an enormous list of genes, but this is almost equivalent to having a phone book with a list of names—it doesn't tell us how the information contained in genes is expressed."

And therein lies one of the puzzles that Wahlestedt and his research collaborators have in the past few years set out to solve: how does gene expression—the process whereby different cells in our bodies use genetic information—actually work? Their focus, however, is less on DNA than on its little-recognized cousin, ribonucleic acid, or RNA. Recent discoveries about the function of RNA in gene expression made by Wahlestedt, his collaborators, and others in the field have shaken up a central dogma of biology that holds that the main function of RNA is to translate DNA codes into proteins, which are needed to sustain life.

While it has been known for some time that not all of a cell's RNA is involved in protein synthesis, the importance of so-called "non-coding" RNA has not been well understood or even thought significant. Wahlestedt maintains, however, that "non-coding" RNA plays a crucial role—one that will become increasingly valued as it is better understood—in the processes of expression and repression of hereditary information.

MAPPING THE "TRANSCRIPTOME"

For the past several years, Wahlestedt's research has concentrated on non-coding RNA. This focus led to his participation in an international consortium involved in an effort akin to the Human Genome Project—sequencing the human "transcriptome"—defined as all the RNA transcribed from genes within a given genome. The consortium announced results of its work in the September 2, 2005 issue of the journal *Science*.

"The results of the study give scientists unprecedented insights into both the nature of the genome

"We want to find out why most drugs are really only effective for a small number of people. The holy grail is to really be able to practice preventive, personalized medicine, to assess an individual's risk and then treat that person before symptoms appear, because once symptoms show up it's often too late."

CLAES WAHLESTEDT, M.D., PH.D.

and the importance of genome organization to its function. The data will serve as a basis for discovery for many years," Wahlestedt says.

One of the startling findings of this massive sequencing project is that more than half of the 40,000 RNA transcripts—the "messages" translated from DNA codes—were found by Wahlestedt and his consortium colleagues to be non-coding. The amount of non-coding RNA is vast, far more than most scientists would have imagined even a few months earlier and even greater than the amount of protein-encoding RNA.

"Even we were surprised to discover how much of the RNA doesn't fit the one gene/one protein paradigm," Wahlestedt says. "These results indicate that while proteins make up the essential components of our cells, the development of mammals may also be controlled by non-coding RNA."

The premise put forth by Wahlestedt and his collaborators is that non-coding RNA somehow regulates which genes are expressed and which are not. Now that the sequences of these atypical non-coding genes are known, scientists can begin to look at how they function. (See Hogenesch article.)

"This is arguably one of the next major steps after the Human Genome Project and it raises many interesting questions," Wahlestedt says. "For example, why would the cell expend so much energy making RNA transcripts that do not code for proteins? It seems unlikely that it's just sitting there in the cell without a purpose."

THE TRANSCRIPTOME REVEALS ANOTHER MYSTERY

Wahlestedt's work with the transcriptome project has led to another significant finding—this one having to do with "antisense" genes. DNA is double-stranded and only one of its strands, the so-called "sense" strand, encodes for proteins. In normal DNA transcription, the two strands split apart, and only the sense strand is copied. The other DNA strand, the "antisense" strand, can also be transcribed into RNA, but the transcript has the reverse sequence.

Wahlestedt and his group analyzed the transcriptome and found evidence that antisense transcription is extremely common and contributes to gene regulation by affecting the corresponding sense transcripts. The results of this work appear in a separate paper in the September 2, 2005 issue of *Science*.

This groundbreaking discovery has significant implications for the future of biological research, medicine, and biotechnology because antisense genes are numerous and they are likely to participate in the control of many, perhaps all, cell and body functions. If correct, these findings may fundamentally change our understanding of genetics and how information is stored in the genome.

SEARCHING FOR ANSWERS TO DISEASE

The goal of much genetic research is to find genes that contribute to disease. Finding these genes should allow an understanding of the disease process, so that drugs might be developed to prevent and treat the disease. For diseases with a relatively straightforward genetic basis, the single-gene disorders such as cystic fibrosis or Huntington's disease, current methods are usually sufficient to find the genes involved.

Most people, however, do not have single-gene disorders, but develop more common, "complex" diseases—heart disease, stroke, diabetes, cancers, or psychiatric disorders—which are affected by many genes and environmental factors. Why?

"We know that the DNA of all human beings on earth is 99.9 percent identical and that individuals of all races and genders are almost carbon copies of each other," Wahlestedt says. "Still, some people are tall, others short, some are heavy, some are thin,

some people get terrible diseases, others don't. These characteristics must be the result of differences in just the .1 percent of DNA that is variable—in combination with environmental factors."

Wahlestedt would like to pinpoint the basis for these DNA differences to learn why one person is more susceptible to disease than another. "Of course, the reasons for susceptibility may reside in the world of genes already known, but I believe non-coding RNA may eventually be found to be involved in the cause of many of the complex diseases."

As an expert in pharmacogenomics—the science of understanding the correlation between an individual patient's genetic make-up and his or her response to drug treatment—Wahlestedt says, "We want to find out why most drugs are really only effective for a small number of people. The holy grail is to really be able to practice preventive, personalized medicine, to assess an individual's risk and then treat that person before symptoms appear, because once symptoms show up it's often too late.

Wahlestedt now is applying the insight gained from studying the transcriptome to understanding more about the genetic variations behind diseases. He is closely following the work of another massive mapping project—the International Haplotype Map Project—HapMap, for short—a partnership of scientists and funding agencies from around the world that is helping researchers find genes associated with human disease and that will eventually describe the common patterns of human genetic variation.

Through the use of "association studies"—those that look for differences among many individuals—Wahlestedt is looking at a large number of people with complex diseases and matching them to a large number of unaffected people of the same ethnic background to try to find out whether differences in their genomes—including non-coding RNA—may be behind their diseases. If this turns out to be the case, drugs may be found that can target the causative RNA. This is a different approach to drug development from what is mostly used today where most drugs treat symptoms, at best, but don't cure.

"Scientists have been looking for a long time at known disease-causing genes and not finding answers," Wahlestedt says. "At least some of the answers may be contained in this new universe of non-coding RNA, much of which acts as regulatory guardians of the cell. It's likely that it is part of a higher-level control system—if something goes wrong with a cell and disease occurs, it's possible that these control systems are involved or even the reason why things go wrong in the first place."

AT WORK ACROSS THE GLOBE

A native of Sweden, Wahlestedt received both his medical degree and Ph.D. from Lund University. His collaborative work currently spans three continents—the United States, where he has been based at Scripps Florida since early 2005 and has a growing lab, the Karolinska Insitute in Sweden where he has a sizable but shrinking lab at the Center for Genomics and Bioinformatics, and Japan, where he has long-standing ties at the Riken Institutes in Tokyo and Yokohama.

"The work we are doing is very exciting," says Wahlestedt, who now lives in Palm Beach with his wife Lisa, a medical doctor, and daughter Ella, 7 and son Thor, 5. "It's not that often in scientific research that you get to feel you're venturing into uncharted territory. We feel that with our work, we have a good chance to change the scientific outlook dramatically. That doesn't happen every day. Right now, we feel a bit the way Christopher Columbus may have felt. There's sometimes skepticism when an explorer describes what he has seen, but if people keep an open mind, they may realize that a world of possibility is opening up where it did not exist before."

ANNA SOBKOWSKI


Science-Sentinel.
Classifieds
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The Scripps Research Institute
Science-Sentinel.
'That's the genius of evolution'
Technique lauded for ingenuity,
mencement honors graduates

"Our method gives a systematic, high-throughput way to find those noncoding RNAs that do have functions. We can now identify the ones we should be focusing on."

JOHN HOGENESCH, PH.D.

Looking for a Job Description for RNA

JOHN HOGENESCH BRINGS NEW TOOLS TO THE TASK

The most puzzling result of the Human Genome Project, announced in 2001, was that humans had no more genes than mice or, worse yet, the little weed *Arabidopsis*. It was a source of humility, and led some scientists to comment that the mechanisms that generate the complexity of creatures must derive from something other than sheer numbers of genes or base pairs.

Biochemistry professor and neurobiologist John Hogenesch, Ph.D., and his colleagues at The Scripps Research Institute in Florida and California are currently exploring one likely source of that complexity: RNA.

For nearly 50 years, molecular biology has taken Francis Crick's idea that "DNA makes RNA makes protein" as its paradigm or, as it came to be known, the "central dogma." RNA was simply an intervening stage between the gene and the protein that did the work; genes were discovered and characterized by studying their protein products.

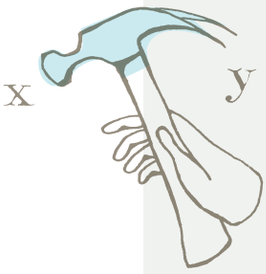
By the early 1990s, however, work on the worm *C. elegans* had revealed a flaw in the central dogma. Small bits of RNA, called microRNA, controlled the timing of the worm's development without ever being translated into proteins. Moreover, scientists using cloning and computational methods to compare the genomes of mice and humankind have recently been surprised to find that more than half of the RNA transcripts they observe do not code for proteins (see Wahlestedt story).

Why, then, are these noncoding RNA in the cell? Hogenesch is looking for answers.

QUESTIONING THE PARADIGM

In a study supported by the Novartis Research Foundation and an NIH Kirschstein National Research Service Award, Hogenesch, postdoc Aaron Willingham, Ph.D., Professor Peter G. Schultz, Ph.D., and their colleagues entered what the journal *Science* has called the "underworld of RNA." Adapting a common method of screening pharmaceuticals, the team proved that at least some of these noncoding RNA transcripts play crucial roles in cell functions.

The study's results, published in the September 2, 2005 issue of *Science* alongside several other landmark papers on RNA, caused some scientists to rethink their assumptions. "We may have seriously misunderstood the nature of genetic programming in the higher organisms by assuming that most genetic information is expressed as and transacted by proteins," says John S. Mattick of the University of Queensland, who wrote an accompanying commentary. Noncoding RNA could, he continued, "constitute a critical hidden layer of gene regulation." →



"The system doesn't have to work perfectly, it just has to work. That's the genius of evolution."

JOHN HOGENESCH, PH.D.

TOOL BUILDING FOR BIOLOGY

Hogenesch himself is not convinced that molecular biology has been, as he puts it, "looking under the wrong lamppost all these years." Of the thousands of noncoding RNA transcripts, he suspects only a subset, perhaps a few hundred, are important for cell function.

"There is much evidence to suggest that many noncoding RNAs are not functional," he says. Noncoding RNAs don't turn up, for example, in large-scale detection schemes for the causes of disease. Nor can their effects be seen in knock-out experiments: A mouse missing an entire megabase of noncoding genome looks and acts no differently than a normal mouse.

Could some noncoding RNA simply be, as Hogenesch puts it, "translational fluff"? Hogenesch believes this is one possibility: "Nonfunctional RNA wouldn't really cost the cell much", according to Hogenesch, as "the real energy costs are in translation [making proteins from RNA], not transcription [making RNA from DNA] and the cost for making the transcription process 100 percent leakproof is likely to be quite high … The system doesn't have to work perfectly, it just has to work. That's the genius of evolution."

And yet Hogenesch's research shows there's a component of the genome that the central dogma overlooks. "So we know the function of a subset of this noncoding RNA. It could, after all, be a real paradigm shift," Hogenesch concedes. "The hard part is to systematically link these noncoding RNAs to biology."

That part—the hard part of finding an efficient way to test whether or not noncoding RNAs really do have a function in the cell—was what attracted Hogenesch to the topic in the first place.

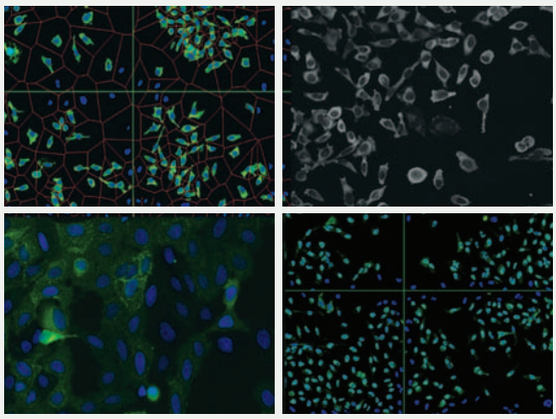
A self-described "gearhead" who is the son of a chemist and a nursing professor, Hogenesch had developed a cell-based screening method to study circadian rhythms, the so-called "body clock." Another project that involved a similar sort of screening method was the Gene Atlas that Hogenesch created with colleagues at the Genomics Institute of the Novartis Research Foundation. The resulting website cross-indexes 36,000 genes and 80 tissues, revealing which genes are expressed in what tissues. (See http://symatlas.gnf.org)

"So I was very comfortable with large-scale science and with cell-based assays," he explains. "The pharmaceutical industry has used these for many years to screen drugs. We took a page from their book."

Having set up a new robotic cell-based screening lab at Scripps Florida to look at protein-coding DNA, Hogenesch began to wonder if it could be refined to study noncoding RNA. "First I ask, 'What are the techniques I need to approach a problem I'm interested in?' And once I have them working, I look around to see what other questions those techniques can be applied to. Sure, it's tool building. But it's tool building to answer a biological question. The beauty is that once you have a tool to do x, then you can use that tool to do y."

He also credits the influence of Schultz, who holds the Scripps Family Chair and is a member of

Professor John Hogenesch, Ph.D., is deeply involved in the Cell-Based Screening Program at Scripps Florida, which can be used to assign novel functions to genes. (Images from Beckman Coulter IC100 Image Cytometer, analyzed using Cytoshop.)



The Skaggs Institute of Chemical Biology at Scripps Research in California. "Basically, this entire project has been due to Pete and his vision," Hogenesch says. "Pete is one of few scientists with truly penetrating insights into many different scientific areas. He encourages us to look for the important problems in biology and to ask, 'Can I contribute?'"

CELL-BASED SCREENING

To screen noncoding RNAs in a cell-based array, Hogenesch and his colleagues took advantage of the phenomenon called RNA interference. RNA interference is a process whereby cells identify and destroy double-stranded RNA—usually the mark of an RNA virus, as the cell's own RNA is single-stranded. Hogenesch's team made part of the targeted noncoding RNA into a double strand, thereby tricking the cell into destroying its own noncoding RNA and silencing or "knocking down" the team's target.

The researchers first chose 512 noncoding RNA sequences, all relatively large (not microRNAs) present in both mice and humans. They then constructed a library of short-hairpin RNAs that would anneal to—and interfere with—the target RNAs, and tested the effects of the knockdown on the cell's signaling pathways and other vital processes.

Only eight of the 512 cells showed a reproducible and measurable effect when a specific noncoding RNA sequence was silenced. This "hit rate," Hogenesch notes, is low—an order of magnitude lower than the results he sees from cell-based screening of protein-coding genes.

Of the eight affected cells, six had their viability compromised in ways not yet determined. One interrupted Hedgehog signaling, a well-studied pathway essential for cell development, maintenance, and repair.

The researchers chose to investigate the remaining functional noncoding RNA further. This one repressed the transcription factor NFAT (nuclear factor of activated T-cells), which plays a vital role in the immune response by interacting with T-cells. A remarkably sensitive transcription factor, it is also necessary for the proper development of the heart, blood vessels, muscles, and nerves. The noncoding RNA that the researchers dubbed NRON (for noncoding repressor of NFAT) was found to interact with 11 proteins, of which three were members of the "importin" family. Importins regulate transport of molecules into and out of the nucleus. NRON was found to most closely associate with importin-beta 1, suggesting that NRON modulates NFAT by controlling its location in the cell, perhaps by providing a scaffold for the making of protein complexes that regulate traffic into and out of the nucleus.

Hogenesh is reserving judgment for now on whether these results help explain how so few genes can result in such complex organisms.

"The jury's still out," Hogenesch says, "but our method gives a systematic, high-throughput way to find those noncoding RNAs that do have functions. We can now identify the ones we should be focusing on. Studying them will determine the contribution of noncoding RNA to the diversity and complexity of higher organisms."

NANCY MARIE BROWN

"This has been the case all along. Just when you think there are no more remarkable RNA discoveries, another one pops up."

MARTHA FEDOR, PH.D.

The Remaking of RNA

MARTHA FEDOR CONNECTS FORM AND FUNCTION

Welcome to RNA World, a combination of primordial theme park and research fraternity, where this rather simple string of nucleotides is now taking star billing. After what many now think of as years of unwarranted neglect relegated to a handoff role between genes and proteins, RNA has become the Tom Hanks of molecules—it writes, acts, produces, and directs, and it looks darn good doing it. In fact, it may even have had a hand in founding the theater.

A lot of the credit for this newfound spotlight on RNA goes to the work of Martha Fedor, an associate professor at The Scripps Research Institute, and her laboratory colleagues at The Skaggs Institute for Chemical Biology. What her research has shown is that RNA, once thought of as kind of a genetic nonsequitor, can do more—in fact, a whole lot more.

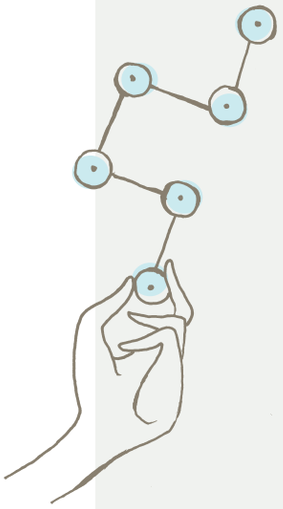
PRIMORDIAL SOUP

RNA's makeover began in the 1980s, when a researcher named Tom Cech at the University of Colorado, Boulder made the astonishing discovery that RNA enzymes could perform catalytic functions, quickly gaining them the nickname *ribozymes*. It was something no one had ever thought of before—so astonishingly new, in fact, that Cech won the Nobel Prize in Chemistry for the discovery in 1989.

It was also Fedor's first exposure to the endless possibilities posed by this new understanding of RNA—her husband, Professor Jamie Williamson, also at Scripps Research, was working in Cech's lab when the prize was announced. Fedor herself was at the university doing postdoctoral work after receiving her doctorate from the University of California, Berkley and spending time at Stanford, "looking at yeast," as she describes it.

Cech's discovery led a number of scientists to reconsider RNA and the whole primordial soup theory of life. Suddenly, here was a likely candidate for life's early beginnings. In a world of pre-biological evolution, RNA could have provided not only the basic genetic material of life but also the enzyme engine that created more progeny. Support for the idea was bolstered by the fact that retroviruses like HIV use only RNA as their genetic material, simultaneously storing that information and replicating it. If the RNA theory of early life is correct, then retroviruses and RNA are living reminders of how we all got started, a reverse molecular telescope that looks backward into genetic time.

Welcome to RNA World. →



"The concept that gave rise to RNA world," Fedor says, "is the notion that RNA can do many different kinds of things. For a long time, everyone thought that proteins were the end-all of genetic expression. After Tom Cech's discovery, it became clear that RNA was much more than a transmitter—it was a repository of genomic information, a catalyst to accelerate biological functions. This is what intrigues me the most, that RNA can do so many things—and to do all these things, there has to be a functional structure."

A PRACTICAL BENT

Fedor was born in Michigan, where a certain practicality is infused into everybody's sensibility almost from birth, especially in an oldest daughter with three siblings. Fedor says she admires the idea of what might have arisen out of that bowl of primordial RNA stew, but what she really likes is doing experiments.

"While it's interesting to think about how things might have arisen eons ago, I'm much more of an experimental biologist," she says. "I like imposing order on the universe. I like making sense out of complicated scenarios. I'm the reductionist thinker in our family." (Her brothers and sisters are also in the science business. One is a forensic chemist and works with DNA fingerprinting. Another has a degree in biochemistry. Another translates the language of high technology into everyday English for the layperson.)

The initial discovery that RNA could catalyze a biochemical reaction was made when she was a graduate student at Berkeley. Fedor, along with everyone else, thought that they were living in the *fin de siecle* of RNA discovery. Now, Fedor says, new RNA functions are being uncovered on a regular basis. In the last few years, discoveries have been made about a form of the molecule called small noncoding RNAs and the fact that they play key roles in gene expression.

Once the notion that RNA was as formless as a bowl of wet pasta was cast aside, Fedor wanted to know more about it—especially about the *how* of this intriguingly functional structure. Because proteins have more chemistry to play with than RNA, it makes sense that proteins have a far easier time creating a precise structure. But RNA's simple chemical structure doesn't seem to hinder it much.

"The thing that's really intriguing about RNA is that it's a very simple chemical molecule—just four building blocks—that can do a variety of complicated things," she says. "You can think of it like Tinkertoys with different colors that come in pairs. There are only so many pair combinations, but you can build anything from a car to a factory with them. What's more, they assemble themselves."

What is most amazing about all this, Fedor says, is that at first glance RNAs seem unlikely to catalyze biological reactions because they're almost inactive, virtually inert. Which is a very good thing in terms of genetic material because you don't really want it

"The thing that's really intriguing about RNA is that it's a very simple chemical molecule—just four building blocks—that can do a variety of complicated things. You can think of it like Tinkertoys with different colors that come in pairs. There are only so many pair combinations, but you can build anything from a car to a factory with them. What's more, they assemble themselves."

MARTHA FEDOR, PH.D.

to be wildly active; you want it to stay the same from transference to transference. And yet here was RNA, catalyzing biological reactions, an ability seemingly incompatible with its ability to serve as a stable repository of genetic information.

WHEN EVERYTHING YOU KNOW TURNS OUT TO BE WRONG

Fedor's initial research, which began before she arrived at Scripps Research in 1997, looked at RNA's alleged need to bind to metals to perform certain functions, gaining them another nickname, *metalloenzymes*. The positive charges of metals supposedly helped RNA balance the highly negative charges that accumulate during the most difficult steps in the reactions they catalyze. What Fedor did was show just how wrong this theory about RNA was.

"I found that not all RNAs use metals to catalyze reactions," she says. "The ribozyme we studied didn't need metals to function because it worked perfectly well without them. It surprised a lot of people who thought the RNA story was over. This has been the case all along. Just when you think there are no more remarkable RNA discoveries, another one pops up. My boss at the time said it's a wonderful feeling when everything you know turns out to be wrong."

Fedor used her work debunking the *metalloenzymes* theory (R.I.P.) in her job interview at Scripps Research.

But that still left the exact how of RNA catalysis to probe. Like proteins, RNA needs to fold into a precise three-dimensional structure to perform. But while proteins generally have only one stable structure, RNA can fold itself into any number of stable structures, with the catch that only one of them is a properly assembled molecular machine; the rest are virtually worthless. If it folds correctly, it works; incorrectly and it gets stuck in a malformed dead end and remains woefully inert.

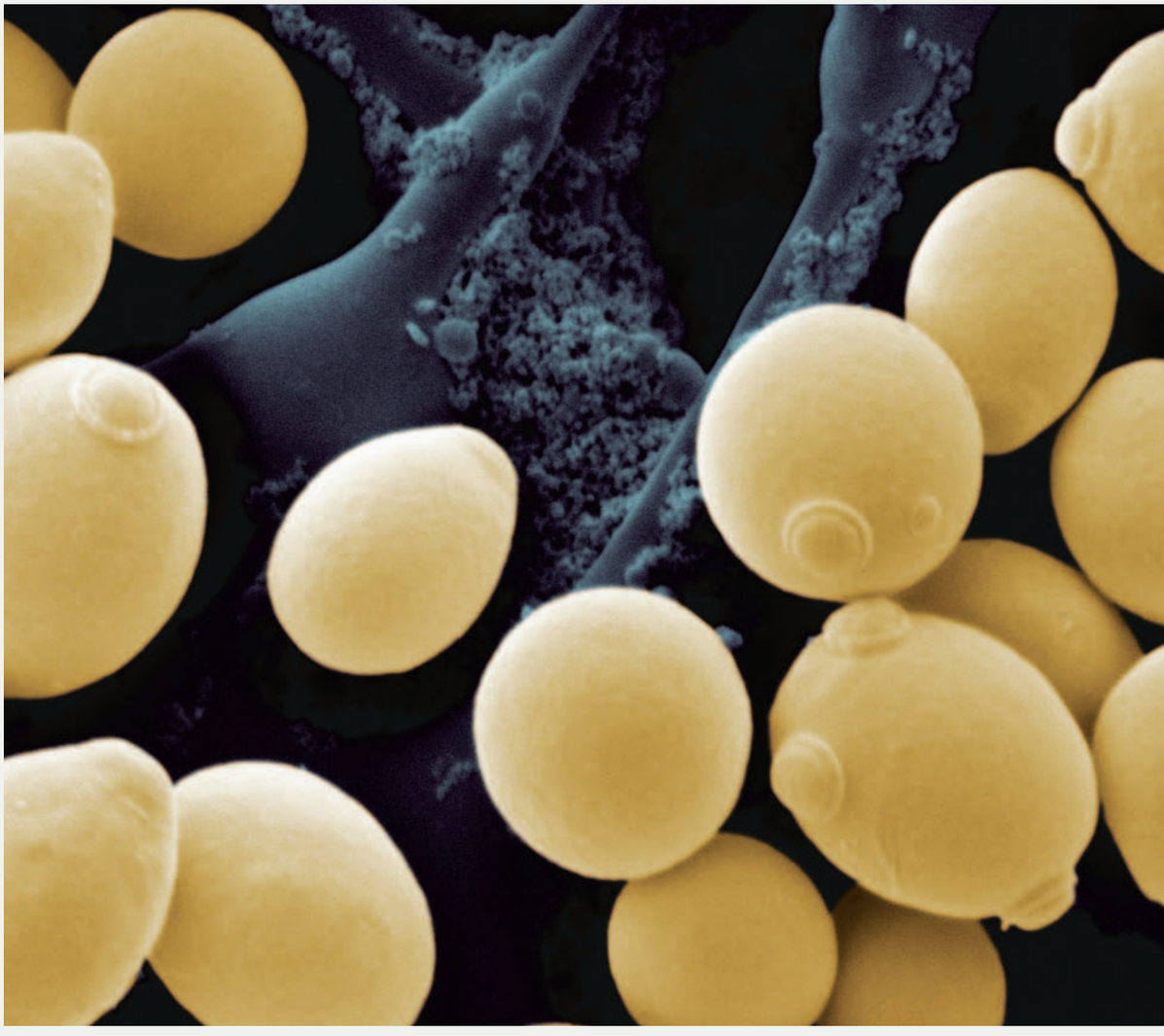
In the lab, Fedor and her colleagues chose the hairpin ribozyme, a good choice because folding into the correct *hairpin* structure (which looks, you guessed it, just like a hairpin) makes this RNA cut itself in two, allowing easy detection of proper assembly. They manipulated the folding process by inserting inhibitory bits into the ribozyme at different points to see if the folding occurred sequentially, from one end to the other as the RNA is made, or if all the parts interacted simultaneously, regardless of their position in the RNA.

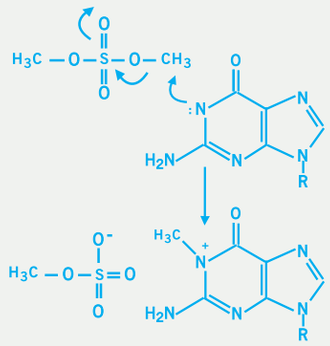
Sequential folding is precisely what they found in simple test tube folding reactions. If they put an inhibiting sequence at the end of the RNA, the folding process produced normal ribozymes, and was more or less oblivious to the inhibitory part at the end; if they put it closer to the beginning, the inhibitory bit folded back onto the ribozyme part and trapped the RNA in a functionless form. All of which Fedor expected to find again when they tried the same thing with living yeast. →

"There are errors in RNA processing that are associated with certain diseases. If we know how the RNA processing goes wrong, and we can find a way to correct those errors, then we may be able to develop something that could alter the disease pathway."

MARTHA FEDOR, PH.D.

When Martha Fedor, Ph.D., moved her research on RNA folding from test tube to yeast (*Saccharomyces cerevisiae* shown here), the results were unexpected.





Chemical structure mapping of RNA using a dimethylsulfate modification reaction.

Except that they didn't. No matter where they stuck the inhibitory structures in the yeast ribozyme, the creature simply folded itself into a corner. So, either sequential folding was something that just didn't happen in the real world or something else entirely was going on.

Fedor has a couple of ideas.

"I don't think this is a case of nature being sloppy," she says, "even though some argue that nature might keep at it until it gets it right. To me, taking apart misfolded RNAs to recycle their building blocks just seems so wasteful. We can watch RNAs getting stuck in the wrong structures in the test tube, but that's not what happens inside a cell. Maybe nature has ways to orchestrate the folding process to make it more efficient that we don't know anything about."

"A POTENTIAL TOOL AND A POTENTIAL TARGET"

This is where the idea of the RNA chaperone comes in. Fedor theorizes that these chaperones are proteins that help RNA during the folding process. For example, when RNA folds itself into an inert structure, the chaperones come along and destabilize the inert structure, essentially giving RNA the chance of a do-over folding. Although some chaperone behavior has been seen in the lab, no one has been able to prove conclusively that they actually help assemble RNA machines in cells. But the whole idea adds another layer of potentiality to RNA.

"One thing we say is that RNA is a potential tool and potential target," Fedor says. "There are errors in RNA processing that are associated with certain diseases. If we know how the RNA processing goes wrong, and we can find a way to correct those errors, then we may be able to develop something that could alter the disease pathway. With rapidly dividing cells, like those in cancer, much of the cells' energy goes into making ribosomes that generate proteins. And they do so in a radical way. It's as if all the parts of a car were laid out on a tarp and suddenly the car builds itself. Interfering with RNA self-assembly could lead to shutting the process down, so one of the things we're interested in is small molecules that block the RNA assembly process."

Fedor and her colleagues are testing two *in vivo* models to see what cells contribute to the RNA folding process so far missing from simple laboratory reactions. One line of research searches for chaperones that work inside cells. The other investigates whether the three types of RNA synthesis machinery produce three different patterns of assembly, clarifying whether the machinery that makes RNA also helps it fold properly.

"Characterizing how RNA folds and assembles offers a very basic insight into the nature of life and biology," Fedor says. "It shows how macromolecules achieve the functions that are unique to life and make a living organism different than a collection of inert chemicals. That's what I say to my students: 'I'm going to tell you all about the difference between something that's alive and a pot of chemical goo.'"

ERIC SAUTER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4

Behind the Scenes

New Events Bring Together Scientists, Donors, and Business Executives

1

Over 30 South Florida business executives who support Scripps Florida attended the late 2005 kick-off lunch for the Scripps Florida Corporate Club at the Raymond F. Kravis Center for Performing Arts in West Palm Beach. Deborah A. Mosca, Ph.D., senior director, business development, spoke and introduced Polly A. Murphy, V.M.D., Ph.D., Scripps Research's new senior vice president for business and scientific services. Pictured here are Mike Dyer, regional managing director for Wachovia Wealth Management; Murphy and Mosca; and Tommy Mayes, regional managing director, Wachovia-Calibre Family Office, Palm Beach.

2, 3

Approximately 75 guests attended Scripps Research's first annual donor appreciation dinner in the fall at the Beckman Center for Chemical Sciences. At the dinner, held for annual donors of $1,000 or more, Scripps Research President Richard A. Lerner, M.D., spoke about the institute's extraordinary history, culture, and future. Following Lerner's remarks, Scripps Research department heads described the breakthrough work of their scientists. Pictured here are Ernest Beutler, M.D., chair of molecular and experimental medicine with donors Izetta and Sheldon Magazine. Also pictured is Donald Haake, president of the Donald E. and Delia B. Baxter Foundation, and Steve A. Kay, Ph.D., chair of biochemistry, professor of cell biology, and director of the Institute for Childhood and Neglected Diseases.

4

Scripps Florida is collaborating with the South Florida Science Museum (SFSM) to create a permanent bioscience exhibit at its planned new museum facility in West Palm Beach. Scripps Florida's Science Saturday program for high school students, funded by the William R. Kenan, Jr. Charitable Trust, will also expand to include the SFSM facility. Pictured at the SFSM's recent Diamond Constellation Ball at the Mar-a-Lago Club in Palm Beach are Harry Orf, Ph.D., vice president of scientific operations for Scripps Florida; Jennifer Busby, Ph.D., associate scientific director of proteomics; Donald Trump; Teresa Reyes, Ph.D., associate professor of biomedical sciences; and Gala Chair Kathryn Vecellio, wife of Leo A. Vecellio, Jr. The Vecellio Group is a member of the Scripps Florida Corporate Club.

"While the last century has been the century of physical sciences, I believe the next century will be the century of biological sciences."

ANDREW VITERBI, PH.D.

Scripps Research Supporter Andrew J. Viterbi Looks to the Future

Inventor, entrepreneur, and Scripps Research trustee Andrew J. Viterbi, Ph.D., has spent half a century capturing and capitalizing on the physical sciences. Best known for the Viterbi Algorithm used in digital communications and other fields and a co-founder of QUALCOMM, Inc., a leading developer and manufacturer of mobile satellite communications and digital wireless telephony, Viterbi nonetheless sees biological sciences as the wave of the future.

"While the last century has been the century of physical sciences, I believe the next century will be the century of biological sciences," says Viterbi.

Viterbi's interest in the biological sciences first led him to Scripps Research as a member of the Scripps Cancer Center Advisory Board. The center is dedicated to quickly bringing advanced cancer treatments from the laboratory bench to the patient's bedside through breakthrough translational research. Demonstrating his commitment, he and his wife, Erna, made a $2 million gift for state-of-the-art research led by Jorge Nieva, M.D., assistant professor of chemistry at Scripps Research.

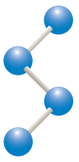
"While it's difficult to bridge the gap between the research laboratory and the clinical environment, translational medicine is critical to effective drug discovery," says Viterbi.

A doctoral graduate of the University of Southern California, where the school of engineering was recently named in his honor, Viterbi's decision to contribute to Scripps Research was influenced by the freedom afforded to scientists to take their laboratory work in the direction that they think is most promising.

"I'm very oriented to independent research institutions such as those in private universities," he said. "Private institutions have more flexibility and control over their destiny, particularly in the recruitment of scientists—they're in a better position to control quality and limit bureaucracy."

As a scientist, businessman, and philanthropist, Viterbi believes in basic research. "Basic research will yield tremendous dividends towards the fight against a variety of diseases. The key is getting extremely intelligent people involved and giving them free reign," he says. But he is equally devoted to advanced technologies, noting, "More and more, the computer is becoming an indispensable tool in medical research."

A Scripps Research trustee since 2004, Viterbi is a life fellow of the Institute of Electrical and Electronic Engineers and a fellow of the American Academy of Arts and Sciences. Among his many honors, he was inducted into the National Academy of Engineering in 1978 and the National Academy of Sciences in 1996.



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A PUBLICATION OF
THE SCRIPPS RESEARCH INSTITUTE

Office of Communications—TPC30
10550 North Torrey Pines Road
La Jolla, California 92037
www.scripps.edu

PUBLISHER:
Keith McKeown

EDITOR:
Mika Ono Benedyk

DESIGN:
Miriello Grafico

PRODUCTION:
Miriello Grafico
Kevin Fung

COVER ILLUSTRATION:
Dennis Clouse

PORTRAIT PHOTOGRAPHY:
Martin Trailer
Bruce Hibbs
Jamey Stillings

PRINTING:
Precision Litho