Ronald Davis Joins Scripps Florida as Founding Chair of New Neuroscience Department

Ronald L. Davis, Ph.D., formerly the R.P. Doherty-Welch Professor of Science at Baylor College of Medicine, has joined The Scripps Research Institute as the founding chair of the Department of Neuroscience on the Scripps Florida campus. Davis is best known for his work in the areas of memory and learning, particularly on the molecular and cellular basis of memory formation and related brain disorders.

"It gives me great pleasure to announce Ron Davis's appointment," said Gerald Joyce, M.D., Ph.D., dean of the faculty at Scripps Research. "Ron will be a wonderful addition to our academic community and to the institute."

"I'm tremendously excited to be joining the Scripps Research faculty and to be building a new neuroscience department on the Scripps Florida campus," said Davis, who began the new position September 1, 2009. "There are so many great scientists at Scripps Florida. I'm proud to be associated with it." Davis, who is 57, visited the campus several times before making the decision to join.

"When I first visited Scripps Florida everyone was still in the temporary buildings," he said, "and I thought, well, this looks like it could be an intriguing idea. But after spending time here in these beautiful new buildings—which have been remarkably well designed—and with all the people here—whose enthusiasm and professionalism are unequal to any I've experienced in my career—I couldn't help but come away with an overall feeling of excitement for what's going on."

A Passion for the Field of Learning and Memory

While at Baylor College of Medicine, Davis and his colleagues used a variety of techniques and technologies to understand learning, memory, and other types of behavior in Drosophila (the common fruit fly) and other animal models. More recently, the research team extended those studies to humans by focusing on the genetics of mood disorders.

"Learning and memory have been my passion for many years," Davis said, "deciphering the molecular basis of how animals learn and then applying that knowledge to humans. There's so much we have yet to uncover about the underlying mechanisms. What we're trying to do is gain a fundamental understanding of the molecular architecture of learning and memory mechanisms, apply that information to understand the susceptibility factors for psychiatric and neurological diseases, and then use that to develop potential treatments."

For Davis, Scripps Florida's drug discovery program, with high-throughput screening and
drug development technologies, ranks high on the list of critically important resources that he plans to utilize.

"Trying to uncover potential agents for cognitive enhancement in various disease states, and in the normal state for that matter, is tremendously important for the work in my own lab," he said. "Given the levels of Alzheimer's disease diagnosed in the Baby Boom Generation, we hope these agents will be discovered over the next 10 years or so—and that Scripps Florida will play a role in those discoveries."

**Sparking Connections in Neuroscience**

The new Florida-based Department of Neuroscience will play an important role in the Program in Neurosciences, a recently created umbrella group that encompasses faculty in a number of Scripps Research departments, including the Molecular and Integrative Neurosciences Department (MIND), the Department of Neurobiology, and the Committee on the Neurobiology of Addictive Disorders (CNAD) on the La Jolla, California campus, as well as other investigators at Scripps Research with an interest in the field.

The program, which was created to foster a broad exchange of scientific ideas, is also designed to promote research collaborations, attract new sources of government and private funding, and facilitate the recruitment of new faculty to both campuses.

This fits in well with Davis's own research philosophy—that solving complex problems such as how memories are formed requires a multi-level, interdisciplinary approach. During his years at Baylor College of Medicine, this approach led to an affiliation with eight different departments, centers, and other programs at the Houston-based medical school, he said.

At Scripps Florida, Davis expects to hire about 10 new faculty members over the next several years, focusing on four main areas: learning and memory; diseases of learning and memory; sleep; and the mechanisms of anesthetics.

**Pioneering Scientist Donald Phinney Joins Molecular Therapeutics Faculty**

The Scripps Research Institute has appointed Donald G. Phinney, Ph.D., a nationally recognized expert in the study of adult bone marrow-derived stem cells, as a professor in the Department of Molecular Therapeutics.

Professor Phinney's laboratory will be located on the campus of Scripps Florida in Jupiter.

"We're pleased that Don chose to join us at Scripps Florida," said Patrick Griffin, Ph.D., chair of the Molecular Therapeutics Department at Scripps Florida. "His pioneering work in stem cells will add significantly to our current research capabilities in a number of areas, particularly in hormone receptors. This is a terrific match and we want to welcome
him to our department and our campus."

Before joining Scripps Florida, Phinney, 47, was a professor of microbiology and immunology and an associate director of research at the Center for Gene Therapy at Tulane University in New Orleans. During his last two years at the university, he was also director of the Good Manufacturing Procedure Facility at Tulane University Health Sciences Center.

Phinney, who has settled in Jupiter with his family, officially joined the Scripps Florida faculty on July 1, 2009.

"It's an honor to be part of Scripps Florida," Phinney said. "After many years of dividing my time between administrative work and science, I decided I wanted to focus on my own research full-time again. Scripps Florida is the kind of place that gives you the freedom to do your own work—plus it has tremendous resources and a supportive administration."

Phinney holds a B.A. in chemistry and mathematics from the University of Vermont and a Ph.D. in biochemistry from Temple University School of Medicine in Philadelphia. He did his postdoctoral work at the Fox Chase Cancer Center in Philadelphia.

From 2008 to 2009, Phinney was also co-editor of the journal *Stem Cells* and was a charter member of the International Society of Stem Cell Research.

Phinney's research interests include the basic biology and therapeutic applications of stem cells, specifically mesenchymal stem cells, which are derived from bone marrow and give rise to such structures as connective tissues, blood, lymphatics, bone, and cartilage. Since mesenchymal stem cells can develop into a number of differentiated cell types, they may have a number of potentially important therapeutic applications.

"Part of our work looks at the way these cells help promote wound healing," he said. "We're very interested in developing agonists or antagonists for these factors that could be turned into potential therapeutics. So, the drug discovery aspect of Scripps Florida fits in well with what we do."

Phinney is also looking forward to being part of the collaborative spirit of Scripps Florida.

"Researchers here—like Tom Burris and Pat Griffin in hormone receptors—are doing some important cell biology work," he said. "Because these types of receptors regulate the differentiation of the cells we work with, there's a great opportunity for collaboration."

### Noted Biochemist Paul Thompson Joins Department of Chemistry

Scripps Research Institute has appointed Paul R. Thompson, Ph.D., formerly on the faculty at the University of South Carolina, as an associate professor in the Department of Chemistry on the Scripps Florida campus.
Thompson's primary area of interest is the phenomenon of gene expression, particularly the study of histones—small, basic proteins that play a vital role in gene regulation."

We are thrilled with the appointment of Dr. Thompson to our Scripps Florida faculty," said K.C Nicolaou, Ph.D., chair of the Scripps Research Department of Chemistry. "He is one of the rising stars in the field of biochemistry as it relates to biology and medicine. His science provides a critical component to the drug discovery process and will enhance considerably the capabilities of the department in its mission to make biomedical breakthroughs."

William R. Roush, professor in the Department of Chemistry, executive director of Medicinal Chemistry, and associate dean of graduate studies at Scripps Florida, added, "We're delighted that Paul is joining us. He has a strong reputation, and his research will substantially broaden the chemistry group at Scripps Florida."

Thompson, who is 39 years old and lives in Jupiter, began his new position in May.

"I'm excited by the opportunity to join the Scripps Florida faculty," Thompson said. "The facilities and the faculty are second to none, and the focus on drug development is going to help push our research to the next level. To develop the best compounds, you have to look at everything—biology, chemistry, pharmacology—so the collaborative synergy at Scripps Florida is perfect for our work."

Thompson, who was born in Toronto, received his undergraduate and graduate degrees in biochemistry from McMaster University in Canada. He received a Canadian Institutes for Health Research postdoctoral fellowship for his work in pharmacology at Johns Hopkins University.

Thompson has been a member the American Chemical Society since 2003, and was elected a member of the society's Division of Biological Chemistry Nominating Committee in 2008. He received a New Investigator Award from the American Heart Association in 2005.

**Going After Gene Expression**

Currently, Thompson's research is focused on the mechanisms of two enzyme families that are involved in the modification of the amino acids (protein building blocks), specifically the enzymes arginine deiminase (PAD) and arginine methyltransferases (PRMT). His research is aimed at identifying the role of these compounds in cell signaling pathways and, ultimately, contributing to new therapies.

"We're particularly interested in developing inhibitors of the PAD enzyme family because two of them are overactive in cancer, multiple sclerosis, and rheumatoid arthritis," he said.

He added that members of the PRMT family also contribute to a number of disease pathologies, including cancer and heart disease.

In addition, Thompson and his colleagues are working to develop synthetic lectins,
natural proteins that bind to sugar structures and glycan structures on cells. Lectins are often overexpressed on virus and cancer cells, and play a role in metastatic cancers.

"Having the ability to bind to tumor cells, synthetic lectins could be potentially useful as diagnostics – attaching an imaging agent, for example," he said. "They could also work as a possible therapeutic."

Thompson has published more than 40 studies. His most recent, "Substrate Specificity and Kinetic Studies of Pads 1, 3, and 4 Identify Potent and Selective Inhibitors of Protein Arginine Deiminase 3," was published May 14, 2010, in an advance, online edition of the journal Biochemistry.

### Noted HIV Researcher Susana Valente Joins Scripps Florida Faculty

The Scripps Research Institute has appointed Susana T. Valente, Ph.D., as an assistant professor in the Department of Infectology.

Valente was an associate research scientist at Columbia University in New York before joining the Scripps Florida faculty on October 1.

"We are extremely pleased to have Susana join our department," said Charles Weissmann, M.D., Ph.D., chair of the Department of Infectology. "She has an innovative approach to HIV and brings a fresh perspective to the search for novel ways to interrupt viral replication. She is a significant addition to the Scripps Florida faculty and we're happy to have her with us."

Valente's research focuses on identifying the molecular interactions that occur within a host cell that are critical for viral replication, and on understanding mammalian genes that have evolved to block that replication. This research could lead to therapeutic targets for combating a number of viruses, including those that might be used as bioterrorism agents.

"It's a great honor to become part of the Scripps Florida faculty," Valente said. "The collaborative atmosphere here is unique and I'm looking forward to developing some strong working relationships with many of my colleagues. The fact that Scripps Florida is dedicated to finding potential therapeutics for many diseases, including HIV, is another reason I'm excited about joining Scripps Florida."

Valente, who was born in the United States but raised in Lisbon, Portugal, attended the New University of Lisbon, where she received her degree in Applied Chemistry/Biotechnology. She also earned a masters degree (Maitrise) in biochemistry from the University of Paris and a master's degree in biotechnology from Montfort University in England and the Hogeschool West-Brabant, the Netherlands. Valente was awarded a Ph.D. in microbiology and virology from the University of Paris VII in 2002. She conducted postdoctoral studies in the laboratory of Stephen Goff, Ph.D., at Columbia University, a pioneer in the use of genetic screens to identify factors in retroviral resistance.
Viruses often use the host cell's machinery in unusual ways during their replication. In a recently published study in the journal Molecular Cell, Valente and colleagues focused on screening expression libraries for genes or gene fragments to identify host factors that might interfere with HIV–1 replication.

What the scientists discovered was that overexpression of a host protein called eIF3f (eukaryotic initiation factor 3 subunit f), which is normally involved in the translation of the cellular gene's message into a protein, has the power to block HIV replication. It does so by reducing the processing of the viral messenger RNA; mRNA, which is synthesized from the viral DNA during transcription, carries the DNA code into the cytoplasm of the cell, where it becomes a template for protein synthesis. Without mRNA, the virus cannot replicate.

"Those results suggest an important role of eIF3f in the maturation of viral messenger RNA," Valente said, "and indicate that eIF3f can interfere with the processing of HIV–1 mRNAs. That means that the viral mRNA is open to manipulation by this and other host factors. This offers a potential target that may ultimately lead to a new means to suppress viral replication. That's one of the main areas we intend to focus on at Scripps Florida."

Nationally Known Memory Researcher Courtney Miller Appointed to Metabolism and Aging Faculty

The Scripps Research Institute has appointed Courtney Miller, Ph.D., as an assistant professor in the Department of Metabolism and Aging and the Department of Neuroscience on the Scripps Florida campus.

Miller, 31, is focused on research that seeks to understand the neurobiology of memory disorders, ranging from aberrations closely associated with drug addiction to age-related memory decline, with the goal of developing novel therapeutics. She was a scientific director and instructor in the Department of Neurobiology and McKnight Brain Institute at the University of Alabama before arriving at Scripps Florida.

"Courtney's breakthrough studies into the role of DNA in the formation of long-term memory opens new doors to understanding how aging affects cognition," said Roy Smith, Ph.D., chair of Metabolism and Aging at Scripps Florida. "Her research may also one day lead to solutions that will help keep our memories intact, even in the face of aging or such devastating diseases as Alzheimer's. We're extremely pleased to have her in our department and welcome her to Scripps Florida."

Miller said, "I'm very excited about joining Scripps Florida. This is a great opportunity to advance my work. I fell in love with Scripps Florida for a lot of reasons. The culture is very collaborative and the people are remarkably interesting. I am looking forward to developing some new collaborative studies, particularly in the area of addiction. The other draw was the drug discovery angle. My laboratory is interested in memory and we want to develop some novel targets for cognitive enhancers."

Earlier studies have shown that addiction and mild cognitive impairment represent
opposing disorders of the normal cognitive processes that Miller has been studying to date: the alterations produced by drugs like cocaine produced particularly strong memories, while aging weakens them. Now Miller is focused on solving both issues, possibly through a greater understanding of the same series of mechanisms, many of which remain unknown.

Miller and her husband plan to live in Jupiter. In addition to her work at Scripps Florida beginning October 5, Miller is a biopharmaceutical consultant, helping companies design studies to test lead compounds in models of memory, addiction, and mental illness.

*Early Memory Work*

After undergraduate work in biopsychology at the University of California, Santa Barbara, Miller received her Ph.D. in neuroscience from the University of California, Irvine, where she worked with Professor John Marshall, Ph.D., on the neural circuitry of drug addiction. She conducted postdoctoral studies in the laboratory of Professor David Sweatt, Ph.D., at the University of Alabama at Birmingham, investigating the role of epigenetics—changes in gene expression caused by something other than a change in the DNA sequence—in learning and memory.

In particular, Miller and Sweatt explored the role of DNA methylation in memory formation. In methylation, a specific section of DNA has a methyl group or molecule added to it. Once attached, the methylation process cuts the gene off from any possible transcription, repressing its function.

Traditionally, methylation has been studied in terms of development—the process helps during embryonic cell differentiation, allowing cells to pass on their phenotypes from one cell to another during early development. But Miller and Sweatt thought the process might have other roles.

"We believed that there could be environmental influences in an adult mammal's life that could have an epigenetic impact," Miller said. "We found that learning could actually alter methylation in the hippocampus. It goes back to the question 'how do you physically maintain a memory?' The proteins that encode these memories are being constantly turned over, so one way to preserve them could be epigenetics and methylation—because of the ability to permanently mark DNA during development and beyond."

Miller and Sweatt's study, which was published in 2007 by the journal Neuron, was the first to show that, in fact, methylation played an integral role in regulating gene activity involved in memory formation. At Scripps Florida, Miller looks forward to extending this work.

**Noted Learning and Memory Scientist Gavin Rumbaugh Joins Neuroscience Department**

The Scripps Research Institute has named Gavin Rumbaugh, Ph.D., as an assistant professor in the Department of Neuroscience at the institute's Florida campus.
Rumbaugh, who was an assistant professor at the University of Alabama, Birmingham, is known for his work on the brain mechanisms of information storage, learning, and memory, with a particular emphasis on the plasticity of neural circuits—the ability of neurons to adapt in the face of both developmental and environmental change.

"We're pleased to have Gavin join our department," said Ron Davis, Ph.D., chair of the Scripps Florida Department of Neuroscience. "He's a tremendously talented young scientist who is working in a field that holds the promise of new discoveries that could have an impact on the lives of millions of patients suffering from increasingly common neurodegenerative diseases like Alzheimer's. He's one of the people who can make a difference and we want to welcome him warmly to Scripps Florida."

Rumbaugh, 35, is married to Courtney Miller, Ph.D., who recently joined Scripps Florida as an assistant professor in the Department of Metabolism and Aging. The couple lives in Jupiter.

"Coming to Scripps Florida is a great opportunity for me," Rumbaugh said. "What has struck me the most is the incredible sense of collaboration among the scientists here. No matter what their area of research, if you need something—a piece of equipment or just a question answered—they're ready to help. Plus, the campus has advanced technological resources that are so critical to our research these days. Scripps Florida is a one-of-a-kind research facility and I'm extremely pleased to be here."

Rumbaugh received his bachelor's degree from Westminster College in Pennsylvania in 1996 and his Ph.D. from the Georgetown University School of Medicine in Washington, DC. He was a postdoctoral fellow in the Department of Neuroscience and the Howard Hughes Medical Institute at the Johns Hopkins University School of Medicine from 2000 to 2006 in the laboratory of Director of Neuroscience Richard L. Huganir, Ph.D.

"The goal of my lab is to discover how molecules store information, and how this process in the brain leads to what is commonly known as a 'memory,'" Rumbaugh said. "By understanding these processes in detail, we hope to develop new drugs that can restore memory function in persons with neurodegenerative disorders like Alzheimer's disease and dementia. Our work is also relevant to the development of intellectual abilities during childhood and adolescence, and may lead to treatments for conditions such as mental retardation, intellectual disabilities, autism, and schizophrenia."

Rumbaugh, who began at Scripps Florida in January 2010, is the first new faculty member to join the Department of Neuroscience. The department was created less than a year ago with the appointment of Davis as chair.

Rumbaugh's current research is examining the synaptic signaling pathways that influence the structure and function of neuronal circuits. In a study published in the December 9, 2009, edition of the journal Neuropsychopharmacology, Rumbaugh and his colleges showed that by inhibiting histone deacetylase, a family of signaling enzymes that play a role in cell growth, cell death, and cancers, the scientists could maintain stable memories in transgenic mice for more than two weeks, suggesting that inhibition of this class of enzymes might prove to be a promising avenue for treating the cognitive deficits.
associated with early-stage Alzheimer's disease. Rumbaugh received a Young Investigator Award in 2009 from National Alliance for Research on Schizophrenia and Depression (NARSAD), the world's leading charity dedicated to mental health research. He was also appointed as an investigator to the Evelyn F. & William L. McKnight Brain Institute at the University of Alabama in 2007.

Aging and Nutrition Investigator William Ja Joins Scrippps Florida

The Scripps Research Institute has named William Ja, Ph.D., as an assistant professor in the Department of Metabolism and Aging on the institute's Florida campus.

Ja, who was a National Institutes of Health (NIH) postdoctoral fellow in biology at the California Institute of Technology in Pasadena before joining Scripps Florida, is focused on researching various longevity-enhancing manipulations and their impact on aging and metabolism in Drosophila, the common fruit fly and one of the most widely used laboratory models. Among these manipulations are dietary restriction, and the effects on their hosts of certain types of bacteria that live in the gastrointestinal tract.

Ja, who is 32 and lives in Jupiter, officially joined the Scripps Florida faculty in January 2010.

"Bill Ja's recent studies of aging and nutrition in the fruit fly have been innovative and outstanding," said Roy Smith, Ph.D., chair of the Metabolism and Aging Department at Scripps Florida. "His most recent work has shown some novel ways to extend the lifespan of these organisms that could one day provide new tools to help us better understand the aging process and to control the diseases associated with it. We're glad he decided to join us and we want to extend him a warm welcome."

"I'm excited about joining Scripps Florida," Ja said. "It's one of the few places that combines basic and translational research with the advantages of advanced high-throughput screening technology. It's also one of the few places with serious and extensive research into the phenomenon of aging. The possibilities for collaboration with other faculty members are numerous, both in the Department of Metabolism and Aging and at Scripps Research as a whole, and I'm looking forward to exploring those opportunities."

In a 2009 study published in the journal Proceedings of the National Academy of Sciences, Ja and colleagues found that previous observations of a lifespan-increasing effect of dietary restriction turn out to have been a lifespan-reducing effect of dehydration on a diet of more concentrated food. Their results potentially compromise the findings from many previous studies. When dehydration stress is eliminated as a factor, the researchers suggest that the ratio of protein to carbohydrates in the diet of the fruit fly is the primary determinant of its lifespan—a possible model for future mammalian studies.

"Studies on the fruit fly have identified changes in metabolism and gene expression in response to dietary restriction," Ja said. "That's important because some of these changes also occur in humans. Now that we know what changes occur, we can use Scripps
Florida's high-throughput screening technology to look for potential drugs that cause the same changes as dietary restriction. Once you find these, you can you test for their potential effect on longevity in model organisms."

In 2008, Ja was awarded a National Institutes of Health Pathway to Independence grant to study the role of bacteria in the development and lifespan of the fruit fly.

"Gastrointestinal bacteria are critical to human health," he said. "In fact, there are ten times more bacterial cells found in our gut than human cells in our entire body. The trouble is, most cannot be grown and studied in the laboratory. We want to develop the fruit fly as a model to test some of these bacteria. Flies carry relatively few species of bacteria—around ten—and that should provide a simpler model for studying host-microbe interactions and lead to directions for future development. Think of all the probiotic microorganisms used in foods like yogurt, for example."

In addition to his scientific work at Caltech, Ja was also involved in sports, and was the assistant coach for the university's NCAA D-III women's volleyball team and head coach of Pasadena Polytechnic School's varsity boys' team.

**Innovative Molecular Biologist Min Guo Joins Cancer Biology Department**

The Scripps Research Institute has named Min Guo, Ph.D., as an assistant professor in the Department of Cancer Biology on the Scripps Florida campus.

Guo, who was a senior research associate in the Schimmel-Yang lab at Scripps California before joining Scripps Florida, researches cellular mechanisms involved directly in protein production, which could lead to novel treatments of melanoma, the most lethal form of skin cancer.

Guo, who is 31 and lives in Jupiter, officially joined Scripps Florida in April 2010.

"We're extremely pleased that Min Guo chose Scripps Florida and the Department of Cancer Biology," said John Cleveland, Ph.D., chair of the department. "His work in the intricacies of protein production and the role of enzymes in directing that process is both original and highly relevant to the development of possible cancer treatments. We fully expect that his contributions to science and to our department will be significant, and we want to offer our warmest welcome."

Guo received his bachelor's degree in Biology and his Ph.D. in Structural Biology from the University of Science and Technology of China (Hefei, People's Republic of China), before his postdoctoral work in the Schimmel-Yang lab.

"I'm honored to be joining the Cancer Biology faculty on the Scripps Florida campus," Guo said. "I appreciate the collaborative spirit at Scripps Research in both California and Florida and I'm looking forward to working with all of my new colleagues in Cancer Biology – and in other departments as well."
Going After Melanoma

For a number of years, Guo's research has focused on the functional interactions of aminoacyl-transfer RNA synthetases (tRNA synthetases), an ancient family of catalytic enzymes. tRNA synthetases transport specific amino acids to their tRNAs for the use of ribosomes, the protein-making machinery of the cell, so they can be added to the growing string of amino acids that eventually produce a specific protein.

In a pair of important studies published in the prestigious journals *Science* and *Nature* in August and December 2009, respectively, Guo and his colleagues showed the extraordinary lengths that the body goes to make certain that mistakes don't occur during protein building.

In the Science study, for example, the scientists found that the synthetase enzyme not only loads the tRNA with the correct amino acid, but adds one more function to double check it gets attached to the right spot. This editing function is a major mechanism to prevent mistranslation, where the wrong amino acid is inserted at a specific codon. For life to thrive, the challenge of preventing mistranslation through mischarging of tRNA had to be overcome. When the enzyme does make a mistake and it isn't corrected, that error leads to the accumulation of misfolded proteins, which in turn function incorrectly. In animal models, that has led to severe defects, including neurodegeneration.

For that reason, scientists believe that the addition of an editing function occurred prior to the divergence of life into the three kingdoms. The editing function was kept ever since, with strong selective pressure, throughout evolution.

But this addition was just a start. As the tree of life ascended, tRNA synthetases progressively added more functions beyond their original roles, such as being cytokines, or regulating gene transcription. Understanding how the functional switch redirects tRNA synthetases from protein synthesis to transcription regulation is the new focus of Guo at Scripps Florida.

"Cancer cells override the control processes that regulate cell growth and cell division," Guo said. "That ability to regulate gene transcription – the first step in producing a protein – clearly suggests a potential involvement of tRNA synthetases in cancer development." Guo plans to expand his research in this area, focusing on developing potential lead compounds to treat cancers, including melanoma, which accounts for more than 75 percent of all skin cancer deaths."

One of the most attractive resources of Scripps Florida is the small molecule screening center," he said. "We want to target tRNA synthetases’ gene transcriptional functions and develop screens to look for small molecules that can block this function."
Scripps Research Awarded $6 Million to Develop Potential Therapies to Fight Alcoholism and Autism-Linked Syndrome

Professor Claes Wahlestedt at Scripps Florida to Lead Work

The Scripps Research Institute has been awarded a pair of grants totaling $6 million by the National Institutes of Health (NIH) to develop new therapeutic approaches to alcohol addiction and Fragile X syndrome, a form of inherited mental retardation that has often been linked to autism.

Claes Wahlestedt, M.D., Ph.D., a professor in the Departments of Neuroscience and Molecular Therapeutics at Scripps Florida, is the principal investigator for both projects.

"The majority of the work in our laboratory is about drug discovery," Wahlestedt said. "So even though we have targeted two completely different disorders, both grants focus on expanding our drug discovery platforms significantly. The NIH has become very attuned to the drug discovery potential of places like Scripps Research. Since the pharmaceutical industry has shown little interest in disorders like alcoholism, even Fragile X syndrome, if we want better drugs to treat these conditions, they will have to come from academic institutes like ours."

The four-year grant of approximately $3.6 million to study alcohol addiction was awarded by the NIH's National Institute on Alcohol Abuse and Alcoholism (NIAAA). The five-year grant of approximately $2.4 million to study Fragile X syndrome was awarded by the NIH's National Institute of Mental Health.

Tackling Alcohol Dependence

With the NIAAA grant, the team of researchers will take a classic drug-discovery approach.

In this work, the researchers will focus on identifying, designing, and then synthesizing a number of potent and selective neuropeptide antagonists (neuropeptides serve as signaling molecules in the brain) as potential therapeutic agents of a specific molecular target, a neuropeptide receptor that is closely associated with alcohol dependence.

"The alcoholism study really is a team effort that builds on our academic and industry expertise in drug discovery," Wahlestedt said. "It is also a perfect example of the collaboration and cooperation between the two campuses of Scripps Research. Professor Edward Roberts of Scripps California is a phenomenally skilled medicinal chemist who will be in charge of developing potential therapeutic molecules, while another highly qualified colleague, Dr. Michael Cameron of Scripps Florida, will determine their drug-like properties. Dr. Shaun Brothers, a staff scientist in my laboratory, has also been
instrumental in the efforts to date. At the end of the four years we hope to have some vastly improved research tools as well as good drug candidates that we can move forward towards human trials.”

In addition to the work done at Scripps Research, a number of in vivo assessments of compound activity on alcohol dependence in animal models will be performed by Markus Heilig, MD, Ph.D., chief of the clinical studies laboratory and director of the division's clinical and biological research at NIAAA.

Unlocking the Mysteries of Fragile X

While the alcoholism study is focused on potential therapeutic agents, the Fragile X study's primary target is uncovering the mechanisms underpinning the disease, which is caused by the silencing of a specific gene, known as FMR1.

Several years ago, Wahlestedt and his colleagues discovered a second, so-called antisense gene, FMR4, which is also silenced in Fragile X patients. What the scientists intend to do over the next four years is to explore the genetic locus – the neighborhood surrounding gene FMR1 and gene FMR4 – to identify and characterize currently unknown genetic mechanisms that might help in the development of novel strategies to restore the FMR1 gene's expression.

FMR4 is not a conventional gene—it's a non-coding RNA transcript, which produces functional RNA molecules rather than encoding proteins. These non-coding RNAs are active in a number of different processes, including RNA modification and cell survival. Wahlestedt pointed out that several studies suggest that some non-coding RNA genes can be involved in various human diseases, including fragile X, and FMR4 clearly falls into that category.

"Like the alcoholism study, we think we can accomplish something significant in terms of a potential therapy for Fragile X," Wahlestedt said. "The long-term objective is to uncover the reasons behind the silencing of FMR1 – opening a window on this disorder – which would be the first step to reactivating it. No one has had any success at doing that and if we could, it would be a highly significant achievement."

Understanding how the gene has been shut off holds significant future promise for other disorders where non-coding genes like FMR4 may play a role, Wahlestedt noted.

Scripps Research Scientists Awarded $3.9 Million "Transformative" Federal Grant to Develop New Compound Screening Platform

Bicoastal Effort Could Help Revolutionize the Search for New Therapies

A pair of scientists from The Scripps Research Institute, one on each coast, has been awarded a five-year $3.9 million grant from the National Institutes of Health (NIH) to develop a new technology to accelerate the search for new protein ligands – compounds that bind to proteins and alter their function.
Current screening technology, which is slow and expensive, has caused what the NIH calls a "major bottleneck" in the search for these basic tools that are key for the broader study of biological processes and that lay the groundwork for development of most drugs.

The grant, awarded as part of the NIH's new Roadmap Transformative R01 Program, will be shared between the laboratories of Tom Kodadek, Ph.D., a professor in the Scripps Research Departments of Chemistry and Cancer Biology in Jupiter, Florida, and Benjamin Cravatt III, Ph.D., professor and chair of the Department of Chemical Physiology and member of The Skaggs Institute for Chemical Biology and Helen L. Dorris Child and Adolescent Neuro-Psychiatric Disorder Institute at Scripps Research in La Jolla, California.

"Ben and I are extremely pleased to win this highly competitive award and to be among the first selected for the new Transformative Grant program from the NIH," Kodadek said. "This is a perfect example of the tremendous collaborative possibilities available within Scripps Research. We worked on the proposal together and the fact that we're both part of the same national institution will make the work that much easier as we move ahead."

Cravatt added, "This project is a good reflection of what those of us at Scripps Research in La Jolla and in Florida are trying to accomplish – fostering collaborative interaction and working on complimentary research projects. This will help cement the strong working relationship between our two campuses."

The NIH Roadmap Transformative R01 (T-R01) Program awards were launched this year to support exceptionally innovative, high risk, original, and/or unconventional research projects that have the potential to create or overturn fundamental scientific paradigms.

"The appeal of the Pioneer, New Innovator, and now the T-R01 programs, is that investigators are encouraged to challenge the status quo with innovative ideas, while being given the necessary resources to test them," said NIH Director Francis S. Collins, M.D., Ph.D. "The fact that we continue to receive such strong proposals for funding through the programs reflects the wealth of creative ideas in science today."

*Two Innovative Methods and a Cab Ride*

The new Scripps Research project will combine two separate technologies from each laboratory – a peptoid library synthesis and screening platform developed in the Kodadek laboratory and an activity-based protein profiling system developed in the Cravatt laboratory.

Kodadek's screening platform involves the creation of vast libraries of peptoids (peptoids are synthetic molecules that are similar to peptides, compounds that when joined together make up proteins) displayed on microscopic beads that are screened against fluorescently tagged proteins that light up after binding with a high affinity, highly selective ligand.

"Our screening technology simulates the cellular environment," Kodadek said, "because the tagged proteins, which represent only a small fraction of the total, are mixed in with
un-tagged competitors. There is a specificity filter built into the process from the beginning."

The Cravatt Laboratory has pioneered the Activity-Based Protein Profiling technology, which allows scientists to identify protein classes based on their activity. The basic technology attaches a single label or probe to proteins from a particular subset of the proteome, which allows access to what are considered low abundance proteins and makes it ideal for massive parallel screening experiments. So far, Activity-Based Protein Profiling probes have been developed for more than a dozen distinct enzyme classes.

Cravatt's technology makes it possible to target what he calls "interesting classes of proteins" but in a highly parallel fashion – hundreds of screens at a time of those multi-million member peptoid libraries. Although both scientists have known one another for some time, many of the details of the collaboration were worked out on a cab ride from England's Heathrow airport to London last summer.

"Tom and I had an editorial board meeting in London, and shared a cab from the airport," Cravatt said. "The fact that Tom had recently joined Scripps Florida helped get us energized about the project."

"It's true," Kodadek added. "The ideas behind the grant proposal just popped out of that ride."

A Transformational Marriage

The combination of the Kodadek and Cravatt advanced technologies will allow the screening of massive peptoid libraries (1-10 million synthetic compounds) in parallel fashion, a novel strategy that the scientists predict will increase the rate of ligand discovery by several hundred times over current methods. "The gist of our proposal is quite simply marrying these two beautifully worked out technologies," Kodadek said. "We have a good track record on both sides, plus we're building off these innovative platforms, so if this works, and I'm certain it will, it will definitely be transformational."

That transformation, when it comes, should result in more lead drug candidates, Kodadek said, because while the scientists' success rate has been lower than those using current high throughput screening technology, the quality of the ligands identified has been significantly better. Some of this is due to the fact that simple synthetic compounds like peptoids have many advantages over other ligands such as antibodies. They can be modified easily for attachment to surfaces and can be produced in relatively large amounts at lower cost and rather quickly – a multi-million member peptoid library, for example, can be created in around three days.

"The way most science works today," Cravatt said, "is that researchers tend to huddle around those areas where there are tools available. By combining our technologies, we will have a streamlined, unbiased way to identify high quality protein ligands and that will give us access to a large part of the proteome that others can't study right now because the current technology is inadequate."
NIH Awards Scientists $2.3 Million Grant to Develop New Treatments for Drug Addiction

A pair of scientists on the Florida campus of The Scripps Research Institute has been awarded a $2.3 million grant by the National Institutes of Health (NIH) to conduct research relevant to developing new treatments for drug addiction.

Patricia McDonald, Ph.D., an associate scientific director in the Translational Research Institute at Scripps Florida and an assistant professor in the Department of Molecular Therapeutics, and Theodore Kamenecka, Ph.D., an associate scientific director in the Translational Research Institute, are co-principal investigators for the five-year project funded by the NIH's National Institute on Drug Abuse (NIDA).

The research will focus on identifying compounds that affect the Neurotensin receptor (NTSR1), a receptor that appears to play a significant role in drug addiction because of its ability to alter levels of the neurotransmitter dopamine in the brain.

"Despite almost three decades of work by the pharmaceutical industry and other researchers, there are still few compounds known to act on NTSR1," said McDonald. "This new funding will help us thoroughly explore the interactions between the receptor and its signaling pathways and its impact on dopamine, which helps drive addiction and relapse."

The neurotransmitter dopamine is released when addictive drugs stimulate a reward circuit in the brain. Drug-induced changes in the reward circuit then reinforce the link between the pleasurable experience and the drug, increasing the tendency towards addiction. Blocking the surge of dopamine could protect the brain from these addictive changes, while substantially reducing the risk of relapse.

Kamenecka noted, "Our grant was something special in the sense that the NIH was looking for something very specific – new ways to accelerate the search for potential treatments of central nervous system disorders, which is what we expect to deliver. It also provides a good opportunity for Patsy's and my laboratories to collaborate on an important therapeutic area – drug addiction is an area with a lot of unmet medical needs."

The scientists said they expect to use a multiple test or assay approach to identify compounds that act on NTSR1, a method both believe will be an improvement over the current single assay approach typically used in the pharmaceutical industry.

"We want to avoid missing any potentially valuable compounds," McDonald noted, "so we plan to cast as wide a net as possible to capture compounds that modulate the receptor through different mechanisms."

If the team identifies compounds of interest, the scientists plan to determine their "functional fingerprint," then work with addiction experts at Scripps Florida, such as Associate Professor Paul Kenny, to help validate the compounds as potential therapeutics for addiction. Their search may ultimately move beyond addiction, however, since NTSR1 and related receptors such as NTSR2 and NTSR3 are also involved in diseases such as Alzheimer's, Parkinson's, and even some cancers.
Scripps Research Scientists Share $2 Million in Florida State Research Grants

Multi-Year Funding Will Help Fuel Development of Advanced Cancer Therapies

The Florida Biomedical Research Program has awarded $2 million in biomedical research grants to three scientists from the Florida campus of The Scripps Research Institute.

This year’s awards went to Glenn Micalizio, Ph.D., an associate professor in the Scripps Research Department of Chemistry, who will receive $1,199,600 over five years; Thomas Bannister, assistant professor of medicinal chemistry and associate scientific director of Scripps Florida’s Translational Research Institute, who won a grant of $400,000 over three years; and Douglas Kojetin, an assistant professor in the Molecular Therapeutics Department, who also won $400,000 over three years.

The grants will begin on July 1, 2010.

The highly competitive grants from the Florida Biomedical Research Program support innovative research into the prevention, diagnosis, treatment, and/or cure of cancer and tobacco-related diseases. Funding comes primarily from taxes collected from the sale of tobacco products.

Exploring Innovative Cancer Treatments

Micalizio’s five-year grant will make it possible for him to study naturally occurring anticancer agents that could become potential chemotherapeutic agents.

"We’re looking at a protein called Hsp90, which is of considerable interest in cancer," Micalizio said, "because it plays a central role in controlling the function of a host of other proteins that are known to be oncogenic or cancer causing. Inhibiting Hsp90 results in the selective destruction of cancer cells. Unfortunately, the chemical structures of various natural products have proven difficult to optimize as therapeutic agents. Our aim is to develop ways to overcome those barriers. It’s an exciting opportunity for chemists to help drive the search for the next generation of anticancer chemotherapeutic agents."

For Bannister, the grant is an opportunity to pursue an equally novel form of potential cancer treatment as part of a collaborative research program in cancer therapy with William Roush, who is a professor in the Department of Chemistry, executive director of the Translational Research Institute Medical Chemistry Division, and associate dean of the Kellogg School of Science and Technology, and John Cleveland, chair of the Department of Cancer Biology.

"Cancer cells differ from most healthy cells in using one pathway, called glycolysis, to acquire nearly all of their energy from glucose," Bannister said. "The pathway makes lactic acid, a byproduct they must pump out in order to survive. Our research is aimed at
improving molecules we have discovered that block lactic acid export and acidify the tumor cells. Cancer cells also recognize our compounds as an amino acid that they need in abundance. This tricks tumor cells into taking in something that will kill them."

The major focus of Kojetin’s work is to understand how the structural dynamics of proteins contribute to their biological function. Modulation of a protein’s dynamic shape or conformation represents an avenue for drug discovery.

"For this grant, we’re looking at nuclear receptor transcription factor proteins, which are receptors for small molecules and important drug discovery targets for a variety of human diseases, including cancer and type II diabetes," he said. "When compounds bind to these receptors, other proteins called transcriptional co-regulator proteins also bind, all of which helps regulate expression of target genes. Hopefully, our work will help in the development of drugs targeting a specific receptor we’ve identified as promising."

The Florida Biomedical Research Programs are administered by the Florida Department of Health and Office of Public Health Research. In total, this year the Florida Department of Health awarded more than $45 million to more than 70 Florida scientists. Micalizio and Kojetin were funded through the James and Esther King Biomedical Research Program for tobacco-related projects, which awarded 42 grants out of 146 applications; Bannister was funded through the Bankhead-Coley Cancer Research Program, which awarded 35 grants from 186 applications.

Moffitt Cancer Center Receives NCI Grant to Create Bioengineered "Designer" Lymph Nodes with Scripps Florida

*Lymph Nodes Will Give Patients New Immune System to Fight Cancer and Increase Vaccine Potency*

Moffitt Cancer Center, in collaboration with researchers at Scripps Florida in Jupiter, has been awarded a five-year, nearly $2 million grant from the National Cancer Institute to design lymph nodes for cancer immunotherapy.

A patient diagnosed with cancer has a dysfunctional immune system either because of the tumor or the treatment being used to eradicate the tumor. These designer lymph nodes will help rebuild a patient's immune system in order to help fight disease. Researchers also hope to increase the potency of vaccines.

"We believe we will no longer be held hostage by what Mother Nature has given us with respect to an immune system," said James Mulé, Ph.D., executive vice president of Applied Research at Moffitt. "We anticipate we will be able to create fully-functioning, designer lymph nodes at will in the human body."

Mulé is partnering with John Cleveland, Ph.D., and Juliana Conkright, Ph.D., at Scripps Florida, who will be using high-throughput screening technologies to rapidly select the candidate genes to use in creating the human lymph nodes.
"Our collaborative efforts hold the real promise of restoring anti-tumor activity to the immune system of cancer patients, and could lead to cures for some cancer types," said Cleveland, Ph.D., chair of the Department of Cancer Biology at the Scripps Florida campus of The Scripps Research Institute. "It is also a perfect example of the creative, state-of-the-art science being driven by investigators at Moffitt and Scripps and the power of collaboration between the two institutes in moving biomedical science from the laboratory to the patient."

The creation of these designer lymph nodes is not limited to just cancer. Mulé plans to expand their use to other areas to boost immunity against a variety of infectious diseases and/or to improve the functions of the immune system during aging.

A clinical trial in melanoma is currently underway at Moffitt using one of the first candidate genes as a primitive lymph node. Twelve patients are presently enrolled.

The project described is supported by Award Number R01CA148995 from the National Cancer Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health.

Scripps Research Scientist Wins $1.75 Million Grant to Study Genome Hotspots

The Scripps Research Institute has been awarded a $1.75-million grant from the National Institutes of Health to study the phenomenon of recombination hotspots, small distinct regions of the genome where molecules of DNA are separated and then combined with different DNA molecules. Recombination is necessary for DNA repair and chromosome crossover, the process that produces the genetic difference between parents and children.

Philippe Bois, Ph.D., an assistant professor in the Department of Cancer Biology on the Scripps Florida campus, is the principal investigator. The grant is funded through the National Institute of General Medical Sciences.

The long-term goal of the five-year research project is to define the anatomy and control of these recombination regions in the mammalian genome, and to assess the impact of other factors known to play critical roles in recombination. These studies will lay the foundation for understanding how recombination is controlled in complex mammalian genomes.

Scripps Florida Scientist Awarded $1.4 Million in Federal Stimulus Funds to Study Drug Targets in Alzheimer’s, Alcoholism

Four Other Scripps Florida Scientists Receive Grants Totaling More Than $1 Million
A scientist from The Scripps Research Institute's Florida campus has been awarded a pair of research grants from the National Institutes of Health (NIH) totaling just over $1.4 million to pursue drug discovery work in both Alzheimer's disease and alcoholism.

The grants have gone to Claes Wahlestedt, M.D., Ph.D., who is professor and director of Neuroscience Discovery at Scripps Florida.

The first Wahlestedt grant, which will fund the study of non-coding RNA modulators in Alzheimer's disease, is for approximately $924,000 over two years; the other award, about $484,000 for one year, will be used to study and develop novel ligands for a specific receptor that plays a role in alcohol dependence.

The studies are being funded through the American Recovery and Reinvestment Act, the economic stimulus package passed by the Congress in February.

Other Scripps Florida scientists receiving funding through the Recovery and Reinvestment Act include John Cleveland, head of the Department of Cancer Biology, with approximately $368,000 for two years; Tom Kodadek, a professor in the Scripps Research Department of Chemistry and Department of Cancer Biology, with some $374,000 for two years; William Roush, a professor in the Department of Chemistry and executive director of the Scripps Florida Department of Medical Chemistry, with approximately $389,000 for two years; and Glenn Micalizio, an associate professor in the Department of Chemistry, with about $318,000 for two years.

To date about 100 Scripps Research Institute investigators in California and Florida have won supplemental grants from the NIH under the Recovery and Reinvestment Act.

**An Under-Studied Disease**

"These are two new applications that were awarded under the economic stimulus program from the NIH," Wahlestedt said, "and they will help move our research forward in two very important areas. The key to our ongoing research is that we shouldn't duplicate what's already being done but should seek out under-studied areas like drug discovery for alcohol abuse where any progress could truly have an impact on patient lives."

Alcohol dependence is not considered a major target by companies in the biotech-pharmaceutical industry, Wahlestedt said.

"In our study, we are searching for novel ligands for the nociceptin receptor," he said.

The nociceptin receptor may be involved in some brain disorders and could be a novel target in the development of new drugs to treat alcohol abuse; drug candidates bind to proteins like the nociceptin receptor and alter their function.

In the new study, Wahlestedt said he would work closely with Tom Bannister, associate scientific director of the Scripps Florida Translational Research Institute who will help design and synthesize novel receptor ligands through cheminformatics and various compound libraries; cheminformatics uses computers to store and retrieve vast amounts
of information about chemical compounds used in molecular design.

An Area of Interest

The two-year Alzheimer's disease study is an expansion of Wahlestedt's longtime interest in the disease. In research published in 2008, Wahlestedt and his colleagues showed that a noncoding form of RNA controlled the expression of β-secretase-1 (BACE1), an enzyme critical to Alzheimer's disease progression. Their work offered a rare positive glimpse of therapeutic potential in what has been a difficult research area.

There are several different types of small non-coding RNA, including microRNA and small interfering RNA (siRNA). The Wahlestedt laboratory also has a strong interest in long non-coding RNA, which regulate gene expression.

"Once again we're trying to gain a deeper understanding of the disease by creating a comprehensive inventory of non-coding RNA associated with Alzheimer's disease," Wahlestedt said. "We hope to develop biomarkers that can be used to determine the efficacy of potential treatments during clinical trials. While this is still very experimental, we think it could develop into something with a very practical use."

Currently, the most established source of biomarkers is cerebrospinal fluid (CSF), which can be obtained by lumbar puncture – sometimes known as a spinal tap.

Scripps Research Institute Scientists in Florida Awarded $1.3 Million NIH Grant to Develop New Tests for Potential Obesity and Diabetes Treatment

Expanded Funding Could Help Validate Novel Therapeutic Target

The Scripps Research Institute has been awarded a $1.3 million grant by the National Institutes of Health (NIH) to develop a series of tests at its Florida campus to help explore the potential of a protein that has emerged as a highly attractive target for the treatment of obesity and Type 2 diabetes.

Patricia McDonald, Ph.D., an associate scientific director in the Translational Research Institute at Scripps Florida and an assistant professor in the Department of Molecular Therapeutics, is the principal investigator for the three-year project funded by the NIH's National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

"Because obesity and diabetes are two of the most serious health problems facing us, the need for novel treatments has never been greater," McDonald said. "Some recent studies in animal models have shown that activating the G protein-coupled receptor GPR119 improves glucose homeostasis or balance, while positively affecting both food intake and weight gain. This funding will help us design new assays that will explore the overall potential of GPR119, and may one day lead to more effective treatments."

G protein-coupled receptors (GPCRs) are the largest and most diverse protein family in
the human genome. They transduce or convert extracellular stimuli including neurotransmitters, light, hormones, lipids, and peptides into intracellular signals through a number of signaling pathways. Approximately one third, and perhaps as many as half, of currently marketed drugs are designed to target these receptors.

GPR119 is expressed predominantly in the pancreas and gut of humans and rodents and in the rat brain. When activated, the receptor promotes secretion of a specific hormone, called Glucagon-Like Peptide-1 (GLP-1), in the intestines, which in turn increases insulin secretion from the pancreas; both are key components in regulating the balance of glucose in the body. Although some modulators of GPR119 have been discovered, they do not necessarily mimic the receptor's natural ligand and have thus turned out to be mostly unsuitable for use in studying the receptor's biology and function.

"In terms of treating metabolic disease through modulation of GPCRs," McDonald said, "an obvious candidate such as the GLP-1 receptor has been a historically difficult target to track with small molecules, but GPR119 is much more amenable to modulation, plus it also regulates the GLP-1 axis, which is what makes it such a potentially valuable target in diabetes and obesity. We chose this particular receptor for those reasons and the fact that it's being studied extensively by the pharmaceutical industry."

McDonald hopes that once the new assays are developed, and molecular probes created, the process will lead to the identification of small molecule compounds that can be used therapeutically. The probes themselves might even have potential in this regard.

"We'll be studying these probes to see if they have any drug-like properties, particularly if they show any significant activity against the GPR119 receptor," she said. "The obvious goal would be to improve a probe's therapeutic qualities, oral bioavailability, for example, while keeping its high level of activity, a process that can be a lot more difficult than it sounds."

*Expanding Knowledge in the Field*

With the human genome sequenced, science now has a good handle on just how many GPCRs exist at least 1,000 or more. Of those, McDonald said, scientists have a good understanding of what approximately 200 of them actually do and what activates them; another 600 or so are involved in taste and smell. The remaining receptors are known as orphan receptors, whose function and natural ligands have yet to be discovered (also a receptor class that the McDonald lab is actively pursuing).

"We want to look at developing assay environments that are more physiologically relevant to the disease state in question," she said, "to make them more akin to what's really going on in the whole animal. We hope that the in vitro pharmacology that we uncover in GPR119 will help bridge the gap between the limits of cell-based assays and in vivo studies. That's why this funding is so important to eventually find more effective treatments for diabetes and obesity."

In her work, McDonald collaborates with the medicinal chemists at Scripps Florida.

"When small molecule candidates demonstrate some sort of efficacy in our cell-based
assays, we work very closely with the chemists to improve their efficacy," she said. "The chemists modify these molecules and then they cycle back to the biologists and our assays for further evaluation. It's a very symbiotic relationship."

Scripps Research Scientist Awarded More than $1.2 Million to Identify Potential Treatments for Breast Cancer and Cardiovascular Disease

Award Will Be Used to Develop High-Throughput Screening Tests at Scripps Florida

The National Institutes of Health has awarded a three-year grant of more than $1.2 million to The Scripps Research Institute to develop a series of high-throughput screening tests that will help speed the discovery of potential small molecule therapies for breast cancer and cardiovascular disease.

Patrick Griffin, Ph.D., chair of the Scripps Research Department of Molecular Therapeutics and director of the Translational Research Institute at Scripps Florida, will lead the project as principal investigator. The grant will begin in January 2010.

The tests will focus on identifying ligands for the orphan nuclear receptor liver receptor homolog-1 or LRH-1, which plays a crucial role in hormone-driven breast cancer through its regulation of genes involved in hormone biosynthesis as well as fat and cholesterol metabolism – key risk factors in cardiovascular disease. Receptors like LRH-1 detect circulating signaling molecules (known as ligands) such as hormones or neurotransmitters; the ligand binds to the receptor, creating a biological response or blocking the receptor.

"Our goal is to uncover small molecules—either agonists or inverse agonists—that can be used to modulate or alter the activity of this important receptor," Griffin said. "Right now, there are no potent in vivo active LRH-1 agonists and no reports of any inverse agonists. Obviously, we need to better understand the function of this important receptor and its role in diseases like breast cancer if we’re going to development new treatments. To do that, first we need to accelerate the identification of chemical probes."

The functions of agonists and antagonists are well known—a compound that activates a receptor is an agonist and one that blocks it is an antagonist. But an inverse agonist, which binds to same site as an agonist, induces the opposite action of an agonist of that receptor.

Griffin has long maintained an interest in nuclear receptors.

"There has been a lot of interest in nuclear receptors like LRH-1 and the RORs [retinoic acid receptor-related orphan receptors] because they play separate but overlapping roles in many important biological processes which make them ideal targets for the development of therapies for a wide range of disease," Griffin said. "Even though researchers in the field have characterized all 48 known human nuclear receptors, we’re still looking for ligands for a number of them and, better still, more potent and more
selective ligands. That's what our work with LRH-1 will accomplish."

Eventually, Griffin said, the development of modulators for LRH-1 will fall under the Scripps Research Institute Molecular Screening Center (SRIMSC), part of a collaborative effort among teams of scientists at the Scripps Research California and Florida campuses, which includes Griffin as a co-principal investigator. The center employs assay development at both campuses, high-throughput robotics to screen large chemical libraries in Florida, and chemistry at both campuses to develop high quality chemical probes. The center is one of only four such large centers nationwide and was awarded more than $80 million by the National Institutes of Health in 2008 to expand its work.

**Kenan Charitable Trust Gives New $600,000 Grant to Continue Education Outreach Program at Scripps Florida**

The Scripps Research Institute has been awarded a three-year $600,000 grant from the William R. Kenan, Jr. Charitable Trust to continue its widely praised Education Outreach Program at Scripps Florida in Palm Beach County.

"I am extremely pleased that the William R. Kenan, Jr. Charitable Trust has renewed its significant support of our education outreach efforts," said Deborah Leach-Scampavia, who directs the program. "This is a great testament to the value of our program and to the hard work of everyone involved, particularly our main partner, the School District of Palm Beach County. Working together, we've been able to build our education outreach into something that is unique among biomedical research institutes."

To date, the trust has committed more than $1 million to support Scripps Florida's education outreach activities, which include a competitive summer intern program offering high school students and teachers from Palm Beach County a summer research experience in Scripps Florida laboratories. In addition, the program provides science seminars and presentations to district schools.

The new grant was approved after a day-long site visit by the Kenan Trust's board of trustees, Leach-Scampavia said. During that visit, the trustees received an overview of Scripps Florida's current programs and future plans, and spoke with a number of student, teacher, and faculty participants.

Volunteers from Scripps Florida's laboratories, including faculty, postdoctoral fellows, and Ph.D. students serve as mentors to the high school interns. One of the most effective components of the program has been the role of graduate student mentors. The original intent was to inspire in them a lifelong commitment to community service as part of their scientific careers. An unexpected benefit, however, has been to make it easier for the high school students to imagine themselves as budding scientists when they work with mentors who are almost as young.

"Our outreach program offers students and teachers the opportunity to work in some of the world's best scientific laboratories," Leach-Scampavia said. "That experience can mean opportunities beyond the classroom. Several of our summer intern students have
been offered part-time positions in our research laboratories."

Expanding Scripps Florida Outreach

Since the Scripps Florida Education Outreach Program began in 2006, nearly 60 teachers and students from 15 Palm Beach County public high schools have taken part in the summer intern program. Through the internships and a number of other education outreach programs, Scripps Florida scientists have reached thousands of students and community members throughout the region.

Scripps Florida education outreach programs include:

• The Summer Research Internship Program for Teachers. This program at Scripps Florida provides teachers the opportunity to study current laboratory techniques and procedures with working scientists. In addition to the intensive, hands-on six-week summer program, teachers are expected to use the laboratory experience to help create discovery-based learning for their students, and to serve as a resource for other educators.

• The Scripps Florida High School Student Summer Internship Program. This paid internship exposes students over six weeks to a variety of contemporary issues in basic biomedical research, provides hands-on laboratory experience, and helps motivate and prepare students for continuing education in the sciences. The program emphasizes the scientific process, research planning, bench experience, experimental design, and data analysis.

• The Scripps Florida Undergraduate Intern Program. This new offering was launched last year and is focused on providing additional opportunities to program alumni who have completed a summer high school internship at Scripps Florida and who wish to continue to develop their scientific careers at the college or university level.

• Scripps Florida Summer Teacher Institutes. This new professional development program directs greater efforts to the needs of Palm Beach County high school science teachers by providing basic science and advanced laboratory-based instruction.

For more information, visit: [www.scripps.edu/florida/edprograms/](http://www.scripps.edu/florida/edprograms/)

Scripps Research Scientists Receive Grant to Purchase Advanced Technology

The Scripps Research Institute has received a grant worth nearly half a million dollars to purchase advanced technology for the Scripps Florida campus that will allow scientists to do in-depth studies of various types of proteins.

The grant was awarded in early April by the National Center for Research Resources as
part of the Shared Instrumentation Grant Program.

The new technology is a LTQ Orbitrap XL mass spectrometer. The instrument, which was installed in June, is available for several national collaborations and to Scripps Research scientists for collaborative studies. In addition, it will provide training for research technicians, postdoctoral fellows, and graduate students, according to Patrick R. Griffin, Ph.D., chair of the Scripps Florida Department of Molecular Therapeutics.

Griffin said that the new instrument will used be to study protein dynamics through a technique known as hydrogen exchange (HDX), a process that maps protein kinetics through the rates of the hydrogen atom exchange between parts of the protein and a solvent.

“This high-resolution mass spectrometer is considered state-of-the-art in protein mass spectrometry, and has been interfaced with Scripps Florida’s HDX robotic system for automated experiments,” said Michael Chalmers, Ph.D., senior staff scientist and manager of the HDX laboratory.

Mass spectrometry calculates the mass or weight of molecules by measuring the ratio of mass-to-charge of a molecule that has been electrically charged. Mass spectrometry can be used to identify unknown molecules as well as revealing their structural and chemical properties.

The new spectrometer will also be used to study the transcriptional complexes that have been associated with diseases such as diabetes and cancer and, in collaboration with other labs, the dynamics of various protein receptors.

**Landenberger Foundation Awards Three New Grants to Scripps Florida**

Scripps Florida has received three new grants from the Philadelphia-based Margaret Q. Landenberger Research Foundation, totaling more than $400,000. The new funds will support scientific research by Professor Donny Strosberg and Assistant Professor Nagi Ayad, as well as a special conference for non-profit organizations hosted by Scripps Florida.

**Toward Treatments for Metabolic Disease**

The Landenberger Foundation awarded a two-year, $300,000 grant to Donny Strosberg, Ph.D., a professor in The Scripps Research Institute’s Department of Infectology, to support his lab’s studies on the possibility of developing novel treatments for metabolic diseases.

“I’m very pleased to receive this grant award from the Landenberger Foundation,” Strosberg said. “They have been very supportive of the work of Scripps Florida scientists, providing funding in areas that are not always able to attract significant funding from other sources. As such, they have been a terrific partner in helping get a number of
important projects off the ground and I’m grateful for their support.”

Specifically, the grant will support Strosberg’s research on the impact of agonists – compounds that bind to cell receptors and create a cellular response – on three receptors that play a critical role in the process of lipolysis, which is the breakdown of triglycerides within the cell to be used for fuel.

Strosberg is the fourth Scripps Florida scientist to receive an award from the foundation in the last four years. In 2008, Michael Conkright, an assistant professor in the Department of Cancer Biology, received a $150,000 grant. In 2007, Nagi Ayad, also an assistant professor in the Department of Cancer Biology, and Paul Kenney, an associate professor in the Department of Molecular Therapeutics, received grants of $125,000 and $65,000 respectively.

Research Relevant to Cancer

Ayad again received funding from the foundation this year, with a grant of $100,000 to continue his work in the field of cell cycle progression, which has serious implications for cancer research and treatment. Not a lot is known about the moment when cell division – proliferation – stops and when cell differentiation – when the cell decides what it wants to be when it matures – begins. If the cell goes into proliferative overdrive and fails to exit the cell cycle, these rapidly dividing cells can turn into cancer.

Ayad’s most recent study, Activation Domain Dependent Degradation of Somatic Wee1 Kinase, was published on December 29, 2009, in The Journal of Biological Chemistry.

Sharing Expertise

In addition to the grants for scientific research, the Landenberger Foundation approved a grant of $25,000 to Scripps Florida to host a special conference to help other small foundations that specialize in supporting biomedical research to develop greater expertise in their review and analysis of scientific grant requests.

The conference will took place on Tuesday, March 9, 2010 from 9 AM to 4 PM at the Scripps Florida campus.

Rendina Family Foundation Awards $150,000 Grant to Scripps Florida Scientist

The Rendina Family Foundation has awarded a $150,000 grant to Derek Duckett, Ph.D., an associate scientific director with the Translational Research Institute at Scripps Florida, a division of The Scripps Research Institute.

The two-year award will support the salary and training of a postdoctoral scientist working on potential treatments for glioblastoma, an aggressive form of brain cancer.

"I am delighted to receive this grant from the Rendina Foundation," Duckett said. "Our laboratory has recently developed several novel tools to help understand certain critical
aspects of this disease, so the help couldn't come at a more perfect time. This funding will enable us to generate some critically important proof-of-principle studies for potential new glioblastoma therapies."

Glioblastoma multiforme, the most malignant form of the disease and the most common, are tumors that form from glial cells, which support and protect neurons in the brain and spinal cord. Treatment options are extremely limited and patients with the disease usually die within a year of diagnosis. The primary reason for this grim prognosis is the complexity of the tumor itself, which is why the work of scientists like Duckett and the support of philanthropies like the Rendina Family Foundation are so important.

"We are pleased to support the work of scientists like Derek and his colleagues at Scripps Florida," said Michael Rendina, the foundation's president. "The death of my father and the recent death of Senator Kennedy from this cancer show just how far we have to go in finding new treatments. Derek's research represents the kind of innovative science that we need in the fight against cancers like glioblastoma, so that one day we can finally put an end to this terrible disease."

**Pointing the Way**

In his work, Duckett and his colleagues have focused on the Jun-N-terminal kinase or JNK as a potential treatment target for this virulent form of cancer.

The JNK kinases are enzymes involved in a range of cellular signaling pathways, and have been implicated in important processes including metabolic reactions, gene regulation, and cell proliferation—all areas where any significant disruption can lead to cancer, diabetes, or inflammatory disease.

For glioblastoma, the single most important factor in the virtually unstoppable progression of the disease is the ability of infiltrating tumor cells to disperse into distant brain tissue. Recently, *in vitro* experiments done by Duckett and his colleagues have shown that inhibiting JNK in glioblastoma cells, by either genetic modification or the use of small molecule inhibitors, dramatically inhibits both glioblastoma cell migration and invasion.

Duckett hopes his research program will lead to a better understanding of the role that uncontrolled JNK signaling plays in the migration and invasion of glioblastoma cells, as well as illuminating potential treatment options.

"We have reached the stage in our discovery efforts that our small molecule JNK inhibitors have desirable drug-like properties," Duckett said, "and we plan to study the efficacy of these molecules in glioblastoma tumor models. This generous grant from the Rendina Foundation will help us go one step further in terms of advancing novel treatments—to see how our small molecule inhibitors might enhance the action of other therapies such as chemotherapies and radiation."

Bruce Rendina and his wife Marji established the Rendina Family Foundation in 1997. Rendina was a recognized leader in the healthcare industry, founding the Rendina
Companies, a full-service medical real estate development firm. Since his death in 2006 from brain cancer, his family has led the foundation's work.
Scripps Research Scientists Identify Novel Hepatitis C Inhibitors

*Discovery Opens Door to Research on New Type of Therapeutic Compounds*

Scientists from the Scripps Florida campus of The Scripps Research Institute and their colleagues at Boston University have described their discovery of several novel drug-like inhibitors of the hepatitis C virus (HCV). These new inhibitors have the potential to substantially widen the current options to treat HCV infection.

The research, from the laboratory of Professor Donny Strosberg, Ph.D., of Scripps Florida, supported by members of the Scripps Florida Lead Discovery Division directed by Peter Hodder, Ph.D., and colleagues from Boston University, was published in the December 2009 edition of the journal *ASSAY and Drug Development Technologies* and appears in the December 15, 2009 print edition of the journal *Bioorganic & Medicinal Chemistry Letters*.

With more than 130 million people infected worldwide by HCV, new therapeutic strategies are urgently needed for this blood-borne disease, which is the main cause, with hepatitis B, of liver cancer, according to the National Cancer Institute.

Using a new fluorescence-based assay, the scientists were able to identify four small-molecule inhibitors of dimerization of the viral core protein. In this process, which is essential to the survival of the virus, the core protein binds to itself and related proteins to form the viral capsid, the outer lipid-encapsulated protein shell that protects the virus's genetic material like an eggshell protects its yolk sack.

"The fact that is so exciting is that no one has really considered the core protein as a viable target in HCV—in HIV, yes, but not HCV," said Strosberg. "With this study, there is now no good reason why researchers shouldn't go after the HCV core protein."

One of the problems in developing drugs for HCV is that it mutates at such prodigious rates; mutations in viral enzymes tend to lead to increased drug resistance.

By targeting the interactions of the core protein with itself and with other proteins, Strosberg and his colleagues have sought to reduce the problem of rapid mutation—because the core protein mutates much less than the other HCV proteins, and because mutations that affect the interface between core and itself or other proteins would be more likely to deactivate the virus anyway. Core proteins orchestrate the assembly and release of the infectious virus, as well as the disassembly of viral particles upon entering host cells.

Significantly, the new compounds not only inhibited dimerization of the core but also inhibited propagation of HCV in isolated hepatoma cells.
The New Assay

In a study that appeared in the *Journal of General Virology* earlier this year, Strosberg and his colleague described how peptides (molecules of two or more amino acids that are the building blocks of proteins) derived from the HCV core protein also inhibited its dimerization. Peptides however, are difficult to administer orally, unstable in the blood circulation, and are therefore difficult to use therapeutically.

The new assay goes one step further, allowing Strosberg and his colleagues to identify the three times smaller molecules with potential to interfere with the core protein function in the virus.

"While there is no similarity structurally between these new small molecule inhibitors and the peptides, functionally they behave precisely the same way," Strosberg said. "We developed an assay to screen small molecules that is robust and capable of revealing useful compounds that block protein-protein interactions and production of the virus."

Protein-protein interactions, which involve such key physiological actions as signal transduction and protein assembly, are highly desirable drug discovery targets, not only for HCV, but also for other viral infections because inhibitors of these protein associations have been shown to lack many clinical complications, such as the adverse side effects of recombinant therapeutic proteins. However, designing small molecules that inhibit protein-protein interaction remains problematic for a number of reasons, primarily because proteins are so large—interactions are thought to often take place over a wide area and conformation/site-selectivity is difficult to engineer.

"We always look for the simplest solution," Strosberg said. "We knew from our peptide study that we could split the core protein and use only one part that we knew still allowed the dimerization process. That simplified the process because the core protein is sometimes difficult to work with."

Next, Strosberg and his team uncovered a domain on the core protein—what they call "a hot spot"—that was essential for the interaction that creates the capsid and allows the virus to function.

"Since we had already established a proof-of-concept that certain peptides could disrupt capsid formation, we left the peptide world and moved into the small-molecule world," he said. "We developed the high-throughput version of the assay. That's what the industry always wants to know first—can you move from a peptide to a small-molecule and can you find inhibitors among screen large collections?"

From there, the team screened small-molecule compounds that could potentially disrupt the protein-protein gears that create the viral capsid, using the protein library and high-throughput screening technology available at Scripps Florida. For initial screening, Strosberg and his colleagues used a relatively small library containing nearly 2,250 indoline alkaloid-type compounds, produced by their colleagues at Boston University.

These studies revealed the four promising compounds described in the study.
"These new compounds definitely put us closer to the 'El Dorado' of finding viable protein-protein inhibitors for HCV," said Strosberg.

The small molecule inhibitor study made clear that three of the newly discovered inhibitors are relatively non-toxic compounds that could be the basis for the development of new anti-HCV drugs or could be used in combination with other compounds such as interferon on HCV targets other than the virus's core protein.

"These small-molecule candidates are quite promising," Strosberg said. "We continue to study the binding of these compounds with the HCV core protein and hope to design even more potent inhibitors based on their structures."

The first author of the ASSAY and Drug Development Technologies study, "A Time-Resolved Fluorescence-Resonance Energy Transfer Assay for Identifying Inhibitors of Hepatitis C Virus Core Dimerization," is Smitha Kota of The Scripps Research Institute. In addition to Strosberg, others authors include, Louis Scampavia, Timothy Spicer, Virginia Takahashi, and Peter Hodder of The Scripps Research Institute, and Aaron Beeler, John Snyder and John Porco of The Center for Chemical Methodology and Library Development, Boston University. See http://www.liebertonline.com/adt.

The first author of the Bioorganic & Medicinal Chemistry Letters study, "New Small Molecule Inhibitors of Hepatitis C Virus," is Wanguo Wei of The Center for Chemical Methodology and Library Development, Boston University; the corresponding author is John K. Snyder, also of Boston University. In addition to Strosberg, other authors include Smitha Kota and Virginia Takahashi of The Scripps Research Institute; and Cuifang Cai of Boston University. For more information, see Bioorganic & Medicinal Chemistry Letters at http://www.sciencedirect.com/science/journal/0960894X.

Both studies were supported by The Scripps Research Institute, the Factor Foundation of America, the National Institute of General Medical Sciences, and other funding from the National Institutes of Health.

Scripps Research Scientists Reveal Unique Cellular Architecture of the Thymus

Scientists from the Florida campus of The Scripps Research Institute have developed a technique to map the unique microenvironments in the thymus gland that produce several distinct types of mature lymphocytes or T-cells – a type of white blood cell that plays a central role in the human immune system.

The study was published in the December 18, 2009 edition of the journal Immunity.

In the study, the scientists used computers and laser dissection to identify gene expression of stromal cells (which form the structure of the thymus) in the various thymus regions. These genes induce progenitor cells to adopt the T-cell lineage, undergo multiple rounds of proliferation, and diverge into at least five major functionally distinct sub-lineages.
“Our approach provides an unprecedented opportunity to assess the relationship between the structural and functional microenvironments of the thymus in a panoramic representation of all stromal cells within any region of interest,” said Howard Petrie, Ph.D., a professor in the Department of Cancer Biology at Scripps Florida who led the study.

The first author of the study, "Spatial Mapping of Thymic Stromal Microenvironments Reveals Unique Features Influencing T Lymphoid Differentiation," is Ann V. Griffith of Scripps Research. Other authors include Mohammad Fallahi and Brandon Young of Scripps Research; Hiroshi Nakase of Yamaguchi University School of Medicine, Japan; and Mark Gosink of Pfizer, Inc.

The study was supported by the Department of Health and Human Services.

**Scientists Show "Lifeless" Prions Capable of Evolutionary Change and Adaptation**

Scientists from The Scripps Research Institute have determined for the first time that prions, bits of infectious protein devoid of DNA or RNA that can cause fatal neurodegenerative disease, are capable of Darwinian evolution.

The study from Scripps Florida in Jupiter shows that prions can develop large numbers of mutations at the protein level and, through natural selection, these mutations can eventually bring about such evolutionary adaptations as drug resistance, a phenomenon previously known to occur only in bacteria and viruses. These breakthrough findings also suggest that the normal prion protein—which occurs naturally in human cells—may prove to be a more effective therapeutic target than its abnormal toxic relation.

The study was published in the December 31, 2009 issue of the journal *Science Express*, an advance, online edition of the prestigious journal *Science*.

"On the face of it, you have exactly the same process of mutation and adaptive change in prions as you see in viruses," said Charles Weissmann, M.D., Ph.D., the head of Scripps Florida's Department of Infectology, who led the study. "This means that this pattern of Darwinian evolution appears to be universally active. In viruses, mutation is linked to changes in nucleic acid sequence that leads to resistance. Now, this adaptability has moved one level down—to prions and protein folding—and it's clear that you do not need nucleic acid for the process of evolution."

Infectious prions (short for *proteinaceous infectious particles*) are associated with some 20 different diseases in humans and animals, including mad cow disease and a rare human form, Creutzfeldt-Jakob disease. All these diseases are untreatable and eventually fatal.

Prions, which are composed solely of protein, are classified by distinct strains, originally characterized by their incubation time and the disease they cause. Prions have the ability to reproduce, despite the fact that they contain no nucleic acid genome.
Mammalian cells normally produce cellular prion protein or PrP\textsuperscript{C}. During infection, abnormal or misfolded protein—known as PrP\textsuperscript{Sc}—converts the normal host prion protein into its toxic form by changing its conformation or shape. The end-stage consists of large assemblies (polymers) of these misfolded proteins, which cause massive tissue and cell damage.

"It was generally thought that once cellular prion protein was converted into the abnormal form, there was no further change," Weissmann said. "But there have been hints that something was happening. When you transmit prions from sheep to mice, they become more virulent over time. Now we know that the abnormal prions replicate, and create variants, perhaps at a low level initially. But once they are transferred to a new host, natural selection will eventually choose the more virulent and aggressive variants."

\textit{Drug Resistance}

In the first part of the study, Weissmann and his colleagues transferred prion populations from infected brain cells to culture cells. When transplanted, cell-adapted prions developed and out-competed their brain-adapted counterparts, confirming prions' ability to adapt to new surroundings, a hallmark of Darwinian evolution. When returned to brain, brain-adapted prions again took over the population.

To confirm the findings and to explore the issue of evolution of drug resistance, Weissmann and his colleagues used the drug swainsonine or swa, which is found in plants and fungi, and has been shown to inhibit certain prion strains. In cultures where the drug was present, the team found that a drug-resistant sub-strain of prion evolved to become predominant. When the drug was withdrawn, the sub-strain that was susceptible to swainsonine again grew to become the major component of the population.

Weissmann notes that the findings have implications for the development of therapeutic targets for prion disease. Instead of developing drugs to target abnormal proteins, it could be more efficient to try to limit the supply of normally produced prions—in essence, reducing the amount of fuel being fed into the fire. Weissmann and his colleagues have shown some 15 years ago that genetically engineered mice devoid of the normal prion protein develop and function quite normally (and are resistant to prion disease!).

"It will likely be very difficult to inhibit the production of a specific natural protein pharmacologically," Weissmann said, "You may end up interfering with some other critical physiological process, but nonetheless, finding a way to inhibit the production of normal prion protein is a project currently being pursued in collaboration with Scripps Florida Professor Corinne Lasmezas in our department."

\textit{Quasi-Species}

Another implication of the findings, according to the study, is that drug-resistant variants either exist in the prion population at a low level prior to exposure or are generated during exposure to the drug. Indeed, the researchers found some prions secreted by infected cells were resistant to the drug before exposure, but only at levels less than one percent.
The scientists show that prion variants constantly arise in a particular population. These variants, or "mutants", are believed to differ in the way the prion protein is folded. As a consequence, prion populations are, in fact, comprised of multiple sub-strains.

This, Weissmann noted, is reminiscent of something he helped define some 30 years ago—the evolutionary concept of quasi-species.

The idea was first conceived by Manfred Eigen, a German biophysicist who won the Nobel Prize in Chemistry in 1967. Basically stated, a quasi-species is a complex, self-perpetuating population of diverse and related entities that act as a whole. It was Weissmann, however, who provided the first confirmation of the theory through the study of a particular bacteriophage—a virus that infects bacteria—while he was director of the Institut für Molekularbiologie in Zürich, Switzerland.

"The proof of the quasi-species concept is a discovery we made over 30 years ago," he said. "We found that an RNA virus population, which was thought to have only one sequence, was constantly creating mutations and eliminating the unfavorable ones. In these quasi-populations, much like we have now found in prions, you begin with a single particle, but it becomes very heterogeneous as it grows into a larger population."

There are some unknown dynamics at work in the prion population that leads to this increased heterogeneity, Weissmann added, that still need to be explored.

"It's amusing that something we did 30 years has come back to us," he said. "But we know that mutation and natural selection occur in living organisms and now we know that they also occur in a non-living organism. I suppose anything that can't do that wouldn't stand much of a chance of survival."

The joint first authors of the Science study, "Darwinian Evolution of Prions in Cell Culture," are Jiali Li and Shawn Browning of The Scripps Research Institute. Other authors include Sukhvir P. Mahal and Anja M. Oelschlegel also of The Scripps Research Institute. Weissmann notes that after the manuscript was accepted by Science, an article by Ghaemmaghami et al. appeared in PLoS Pathogens that described emergence of prions resistant to a completely different drug, quinacrine, providing additional support to the Scripps Research team's conclusions. For more information, see http://www.sciencemag.org/cgi/content/abstract/science.1183218.

The Scripps Research study was supported by a grant from the National Institutes of Health and by a generous donation to the Weissmann laboratory from the Alafi Family Foundation.

**Scripps Research Scientists Create Innovative New Way to Screen Libraries of 10 Million or More Compounds, Potentially Helping to Speed Drug Discovery**
The search for new drug compounds is probably worse than looking for a needle in a haystack because scientists are limited in the size of the haystacks they can rummage through—time and money make it virtually impossible to screen or search through super-large libraries of potential compounds. This is a serious problem, because there is enormous interest in identifying synthetic molecules that bind to proteins for applications in drug discovery, biology, and proteomics, and larger libraries should mean higher odds of success.

But large libraries come with large problems. Because even compounds with only modest affinity (binding to the target protein receptor with less force than those with high affinity) are usually marked as hits, researchers often end up with several hundred of them and, because of practical constraints involving time and money, no easy way to determine which might be the highest affinity or best compound to serve as a starting point to design a drug. These limitations and others have drastically blunted the use of very large libraries—monster libraries—in binding assays.

Now, in research published in the January 29, 2010 issue of the journal Chemistry & Biology, Tom Kodadek, Ph.D., a professor at The Scripps Research Institute's Florida campus, and his colleagues at Scripps Florida and the University of Texas Southwestern Medical Center have devised an innovative new way to solve this longstanding problem.

"Current methods severely limit the size of the libraries you can screen," said Kodadek. "If you get 20 hits out of a 100,000 compound library, it's feasible to re-synthesize each of those hits to test which are the most effective. But what if you want to screen 10 million compounds? It takes an impossible amount of time to re-synthesize promising compounds for further study. To find the most potent ligands, our new method stands head and shoulders over what is available to researchers today."

Ligands are compounds that attach to proteins and alter their expression, potentially affecting a particular biomolecular activity, say, a protein pathway involved in a disease.

The new method displays millions of compounds on the surface of resin-based beads, each type of compound on a different bead. The hits are culled from the beads using a unique magnetic signature and then transferred to a microarray format—glass slides or silicon chips that can hold large numbers of compounds on their surface. The microarray format allows quantitative comparison of binding affinity that can be carried out without the need for tedious re-synthesis of many different compounds.

In the study, the team used mixed peptide/peptoid libraries—peptides make up proteins; peptoids are molecules closely related to, but more stable than peptides, making them more convenient for testing—but the method could be applied to any class of compound, according to Kodadek.

* Changing the Paradigm

SF 2010 Scientific Report  -  Page 36 of 49
The Kodadek group's method combines several different technical advances to enable this convenient and efficient screening.

These days, most active molecules are discovered through screening of two basic types. There are functional screens, in which small molecules are introduced into the wells of microtiter plates—flat plates with multiple wells that can reach as high as 9,600—and tested individually for their ability to alter the activity of an enzyme. Alternatively, there are binding assays, an approach first developed for bead-displayed peptide libraries, where each bead displays many copies of a single molecule.

"Our new method for screening synthetic libraries and characterizing the resultant hits combines many of the features of bead library screening and microarray-based analysis in a seamless fashion," Kodadek said. "The new technique uses several million beads, each of which displays a unique ligand—theoretically as many as 64 million compounds. The target protein has an antibody attached to it that is covered with iron oxide particles—magnetic dust. If the peptoid ligand is a legitimate ligand, and attaches to the protein, we can pull it from the mass by using a magnetized centrifuge."

The selected compounds are then removed from the beads through a unique cleaving process and attached to glass microarray slides. These arrays are mixed with different concentrations of the target protein, allowing the affinity strength of each compound on the array to be determined quickly and efficiently.

"This technology is relevant to custom libraries that are produced on beads," Kodadek said. "Right now, that probably constitutes five percent of screening going on. My guess, however, is that ratio will change once researchers begin to adopt this new method."

Adoption of this new technique will take time and something of a paradigm shift, Kodadek notes. The new screening technology monitors binding of the bead-immobilized molecule to the target protein; currently, the most widely used high-throughput screens monitor function of the compound. In addition, not all laboratories currently have the equipment and expertise necessary to make microarrays of small molecules.

"I think our method can revolutionize medicinal chemistry," said Kodadek, "but this is only the first step."

The first author of the study, "Seamless Bead to Microarray Screening: Rapid Identification of the Highest Affinity Protein Ligands from Large Combinatorial Libraries," is John M. Astle of the University of Texas. In addition to Kodadek, other authors include Levi S. Simpson and Steven Connell of the University of Texas Southwestern Medical Center; and Yong Huang, M. Muralidhar Reddy, Rosemary Wilson, and Johnnie Wilson of The Scripps Research Institute. See http://www.cell.com/chemistry-biology/abstract/S1074-5521(10)00005-0

The study was supported by the National Institutes of Health Director's Pioneer Award.
Team Shows Therapy-Induced Inflammatory Response Speeds Up Development of Therapy-Resistant Prostate Cancer

In a study involving an international cast of researchers, including those from the Florida campus of The Scripps Research Institute, a team has solved the mystery of why prostate cancer almost always develops into dangerous hormone-refractory cancer after androgen-deprivation therapy—a standard treatment for advanced prostate cancer. Hormone-refractory prostate cancer is responsible for most deaths from the disease.

The study was published in the March 11, 2010 edition of the journal *Nature*.

The scientists found that the progression of hormone-refractory prostate cancer is associated with an inflammatory response triggered by the death of the hormone-deprived primary cancer, according to Jun-Li Luo, Ph.D., an assistant professor in the Department of Cancer Biology at Scripps Florida who is the co-first author of the study. In addition, other critical elements of this response are tumor-infiltrating B cells, part of the immune system, which help stimulate hormone-independent cancer growth.

According to the data collected from animal models, the interruption of this inflammatory response could delay the onset of hormone-refractory prostate cancer by up to three years, Luo said.

In addition to Luo, Massimo Ammirante of the University of California, San Diego, is a first author of the study, "B-Cell-Derived Lymphotoxin Promotes Castration-Resistant Prostate Cancer." Other authors include Sergei Grivennikov and Michael Karin the University of California, San Diego, and Sergei Nedospasov of the Engelhardt Institute of Molecular Biology, Moscow, Russia.

Light Activated "Warhead" Turns Modest Molecules into Super Protein Killers

Using a novel light activation technique, Scripps Research Institute scientists have been able to turn molecules with only a modest ability to fight specific proteins into virtual protein destroyers.

The new technique, which uses a "warhead" molecule capable of inactivating nearby proteins when triggered by light, could help to accelerate the development of new therapies by providing researchers with a new set of research tools and options.

The study was published March 14, 2010 in an advanced, online edition of the journal *Nature Chemical Biology*.

"High-throughput screening can produce a synthetic ligand [peptoid] capable of binding to just about any protein you want," said Thomas Kodadek, Ph.D., a professor in the Department of Chemistry at the Institute's Jupiter, Florida, campus, who led the study. "The problem is that they almost always have modest potency – which makes them less than ideal research tools. By attaching this 'warhead' molecule to a peptoid, we've shown
that we can increase that protein-killing potency by a thousand fold without going through an expensive and time-consuming optimization process."

The new technique offers researchers rapid access to some very potent, very selective light activated compounds that can knock out specific protein function, an important strategy in research into diseases such as cancer. Since light can be focused with high spatial resolution, this technology may open the door for knocking out proteins in only one region of a single cell, but not another, allowing, for example, the inactivation of a target protein in the nucleus, but not in the cytoplasm that surrounds it.

**A Choice of Warheads**

The technique is known as a CALI, which stands for chromophore-assisted light inactivation; chromophores are molecules that can absorb visible or ultraviolet light. While other researchers have made CALI reagents previously, they suffered from poor efficiency, largely due to self-inactivation. The new warhead used by the Scripps Florida team represents a significant advance.

They used a derivative of ruthenium, a metallic element that produces what is known as singlet oxygen, the well known oxygen molecule, $O_2$.

"When the ruthenium absorbs visible light," Kodadek said, "it has to dump that energy to return to a normal state. In the process, it produces an extremely reactive form of oxygen that rips apart whatever proteins it happens to encounter. Basically, it destroys those proteins forever."

While there have been reports of other "warhead"-carrying peptoids, the study said, the ruthenium derivative used by Kodadek and his colleagues is an important technical advance, one that allows scientists to target both extracellular and intracellular protein targets. Unlike organic singlet oxygen generators, the Ru complex is itself insensitive to singlet oxygen, greatly increasing the efficiency of CALI.

The other important point, the study noted, is that these new peptoids have no effect on any cellular components until they are activated by light.

Simple synthetic compounds like peptoids have many advantages over other ligands – molecules that bind to proteins and alter their function – such as antibodies, Kodadek pointed out. They can be modified easily for attachment to surfaces and can be produced relatively quickly in large amounts – a multi-million member peptoid library, for example, can be created in about three days.

This makes them ideal building tools for biomedical research, the study said.

Kodadek became interested in developing this new technique when he and Benjamin Cravatt, chair of the Scripps Research Department of Chemical Physiology, decided to combine separate technologies – a peptoid library synthesis and screening platform developed in the Kodadek laboratory in Florida and activity-based protein profiling (ABPP) developed in Cravatt's laboratory in California. The combination offered a powerful new method of screening and identifying more high quality lead drug
candidates.

"But when we first had this idea to collaborate to identify hundreds of protein ligands simultaneously, my enthusiasm was diminished by the fact that I knew they would all be modest potency compounds and the numbers would overwhelm our ability to optimize them all by traditional means," Kodadek said. "Our new 'warhead' technique solves that problem."

The first author of the study, "Potent and Selective Photo-inactivation of Proteins with Peptoid-Ruthenium Conjugates," is Jiyong Lee of the University of Texas Southwestern Medical Center and Scripps Research. In addition to Kodadek, other authors include D. Gomika Udugamasooriya of the University of Texas Southwestern Medical Center and Hyun-Suk Lim of University of Texas Southwestern Medical Center and the Indiana University School of Medicine. For more information, see http://www.nature.com/nchembio/journal/vaop/ncurrent/abs/nchembio.333.html

The study was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health.

Top Scientists Explore the Origin of Life in Lasker Lecture

A full house of faculty, staff, graduate students, and postdoctoral fellows filled Scripps Florida's Rodney B. Fink Education Pavilion on March 16, 2010, to hear Nobel laureate and Lasker Award winner Jack Szostak, Ph.D., and Scripps Research Assistant Professor Brian Paegel, Ph.D., speak. They addressed recent laboratory experiments that seek to recreate the formation of the first living cells from the basic chemical building blocks of nature.

"If it proves to be easy to build simple cells in the laboratory," Szostak said, "it might show that life could have formed quickly on earth and perhaps even other planets in similar ways."

Szostak's research at Harvard Medical School has focused on the use of fatty acids – the result of the breakdown of fats – to produce synthetic membranes or vesicles. Fatty acids, which are simple in their chemical structure, may have been part of the chemical soup found on early earth that gave rise to the first simple cellular life.

Szostak's research also addresses the rise of the first genetic system. "We are looking at the replication of primitive genetic materials, which is something of a challenge," Szostak said. "But if we can solve the problem of chemical replication of some genetic material, then we may be close to building simple living cells. Ultimately, we hope this will lead to a more complete explanation of how life itself began on the Earth."

Evolution in Real Time

Speaking after Szostak, Paegel joked that it was great having a Nobel Prize winner as an opening act, then set about describing his own work developing microchip technology for
driving evolutionary processes in the laboratory.

Working with Scripps Research Professor Gerald Joyce of the La Jolla, California campus, Paegel developed microfluidic processors for observing molecular evolution in real time. He also discussed a new circuit that generates millions of identical microscopic water droplets in oil. Chemistry conducted in these droplets simulated the compartmentalized evolution that is observed in cells.

"We are treating emulsions and vesicles – very complex systems composed of countless molecular components – like simple molecules that can be made by a series of chemical steps," he said. "We need to have complete control over droplet size and the loading of molecular cargo into these microscopic compartments. Microfluidic technology allows us to custom-tailor all of these characteristics by forming one droplet at a time."

Paegel's chemistry and engineering has a practical side. "These devices will serve as a starting point for constructing more sophisticated compartments, such phospholipid bilayer vesicles, the membrane material used in cells. We expect that there could be applications in drug delivery and aiding studies of proteins that must be housed in a membrane to be properly functional."

The Lasker Lecture is part of a series of forums presented by the Lasker Foundation designed to support and promote a public dialogue over critical issues involving the biological sciences. The inaugural Lasker Lecture, "Reading the Human Genome: Genes and Brains," by biologist and two-time Lasker laureate Sydney Brenner, took place at the Fred Hutchinson Cancer Research Center in Seattle in November 2009. Scripps Research is the second host institution of the program.

Questions from the Audience

For the evening portion of the program at Scripps Florida, the lectern was replaced by an intimate drawing room atmosphere, with comfortable chairs, floor lamps, and Bob Bazell, chief science and health correspondent for NBC News, sitting between the two scientists, taking questions from the audience and asking several of his own.

Before getting to those questions, Bazell complimented Scripps Research on having found two "beautiful research locations in the sun" for scientists to work. They're probably much more productive because of it, he said – drawing laughter from the Scripps Florida faculty in the audience.

Many of the questions came from the dozen or so Palm Beach County high school students and teachers who showed up for the evening with questions in hand.

When asked what it takes to become a successful scientist, Szostak and Paegel replied with similar answers – persistence, passion, and curiosity. Both, however, added their own caveats.

"Be sure that persistence doesn't turn into stubbornness," Szostak said, recalling a time when he spent a year on a laboratory problem that in the end wasn't worth the effort. Paegel cautioned against accepting conventional wisdom. "In biology there are certain
dogmas," he said, "but you shouldn't let them get in the way of thinking for yourself."

The best kind of student, Szostak noted, won't take "no" for answer: "They come to you with a new idea for an experiment and you tell them that it won't work. Then they go off and make it work and prove you wrong. That's who you want in your laboratory."

On the topic of Darwinian evolution – the subject of the day – one of the teachers asked Paegel how to convey the main message of evolution to his students and what precisely that main message ought to be.

"Evolution is the unifying theory of biology," Paegel told the audience. "Without evolution, biology makes no sense."

**Study Shows Compulsive Eating Shares Addictive Biochemical Mechanism with Cocaine, Heroin Abuse**

In a newly published study, scientists from The Scripps Research Institute have shown for the first time that the same molecular mechanisms that drive people into drug addiction are behind the compulsion to overeat, pushing people into obesity.

The new study, conducted by Scripps Research Associate Professor Paul J. Kenny, Ph.D., and graduate student Paul M. Johnson, was published March 28, 2010, in an advance online edition of the journal *Nature Neuroscience*.

The study's startling findings received widespread publicity after a preliminary abstract was presented at a Society for Neuroscience meeting in Chicago last October. Articles heralding the new discovery appeared in news publications around the world, focusing on the point obese patients have been making for years – that, like addiction to other substances, junk food binging is extremely difficult to stop.

The study goes significantly further than the abstract, however, demonstrating clearly that in rat models the development of obesity coincides with a progressively deteriorating chemical balance in reward brain circuitries. As these pleasure centers in the brain become less and less responsive, rats quickly develop compulsive overeating habits, consuming larger quantities of high-calorie, high-fat foods until they become obese. The very same changes occur in the brains of rats that overconsume cocaine or heroin, and are thought to play an important role in the development of compulsive drug use.

Kenny, a scientist at Scripps Research's Florida campus, said that the study, which took nearly three years to complete, confirms the "addictive" properties of junk food.

The new study, unlike our preliminary abstract, explains what happens in the brain of these animals when they have easy access to high-calorie, high-fat food," said Kenny. "It presents the most thorough and compelling evidence that drug addiction and obesity are based on the same underlying neurobiological mechanisms. In the study, the animals completely lost control over their eating behavior, the primary hallmark of addiction. They continued to overeat even when they anticipated receiving electric shocks,
highlighting just how motivated they were to consume the palatable food."

The scientists fed the rats a diet modeled after the type that contributes to human obesity—easy-to-obtain high-calorie, high-fat foods like sausage, bacon, and cheesecake. Soon after the experiments began, the animals began to bulk up dramatically. "

They always went for the worst types of food," Kenny said, "and as a result, they took in twice the calories as the control rats. When we removed the junk food and tried to put them on a nutritious diet – what we called the 'salad bar option' – they simply refused to eat. The change in their diet preference was so great that they basically starved themselves for two weeks after they were cut off from junk food. It was the animals that showed the "crash" in brain reward circuitries that had the most profound shift in food preference to the palatable, unhealthy diet. These same rats were also those that kept on eating even when they anticipated being shocked."

*Lethally Simple*

What happens in addiction is lethally simple, Kenny explained. The reward pathways in the brain have been so overstimulated that the system basically turns on itself, adapting to the new reality of addiction, whether its cocaine or cupcakes. "

The body adapts remarkably well to change—and that's the problem," said Kenny. "When the animal overstimulates its brain pleasure centers with highly palatable food, the systems adapt by decreasing their activity. However, now the animal requires constant stimulation from palatable food to avoid entering a persistent state of negative reward."

After showing that obese rats had clear addiction-like food seeking behaviors, Johnson and Kenny next investigated the underlying molecular mechanisms that may explain these changes. They focused on a particular receptor in the brain known to play an important role in vulnerability to drug addiction and obesity – the dopamine D2 receptor. The D2 receptor responds to dopamine, a neurotransmitter that is released in the brain by pleasurable experiences like food or sex or drugs like cocaine. In cocaine abuse, for example, the drug alter the flow of dopamine by blocking its retrieval, flooding the brain and overstimulating the receptors, something that eventually leads to physical changes in the way the brain responds to the drug.

The new study shows that the same thing happens in junk food addiction.

"These findings confirm what we and many others have suspected," Kenny said, "that overconsumption of highly pleasurable food triggers addiction-like neuroadaptive responses in brain reward circuitries, driving the development of compulsive eating. Common mechanisms may therefore underlie obesity and drug addiction."

Consistent with common mechanisms explaining addiction and obesity, levels of the D2 dopamine receptors were significantly reduced in the brains of the obese animals, similar to previous reports of what happens in human drug addicts, Kenny noted. Remarkably, when the scientists knocked down the receptor using a specialized virus, the development of addiction-like eating was dramatically accelerated.
"This addiction-like behavior happened almost from the moment we knocked down the dopamine receptors," Kenny noted. "The very next day after we provided access to the palatable food, their brains changed into a state that was consistent with an animal that had been overeating for several weeks. The animals also became compulsive in their eating behaviors almost immediately. These data are, as far as we know, the strongest support for the idea that overeating of palatable food can become habitual in the same manner and through the same mechanisms as consumption of drugs of abuse."

The study, "Addiction-Like Reward Dysfunction and Compulsive Eating in Obese Rats: Role for Dopamine D2 Receptors," was supported by a Bank of America Fellowship, The Margaret Q. Landenberger Research Foundation and the National Institutes of Health. See http://www.nature.com/neuro/journal/vaop/ncurrent/abs/nn.2519.html.

Scientists Break Barrier to Creating Potential Therapeutic Molecules

Scientists from The Scripps Research Institute have created a novel technique that for the first time will allow the efficient production of a molecular structure that is common to a vast array of natural molecules. This advance provides a means to explore the potential of this molecular substructure in the search for new therapies.

The study was published on May 23, 2010 in an advance online edition of the journal Nature Chemistry.

The structures in question, called "skipped polyenes," are shared by a large class of molecules that play a critical role in human health, including polyunsaturated fatty acids, which are vital to blood pressure regulation, inflammation, and immune response. The structures are also shared by a number of potent antibiotic, antifungal, and cytotoxic (toxic to living cells) compounds.

Simple and efficient methods for the preparation of skipped polyenes have generally been lacking, creating a significant barrier to exploring their potential as drugs. Currently, the production of molecules that contain simple variants of this substructure is quite labor intensive.

"Our study identifies a novel chemical reaction that will enable the accelerated production of this type of structural motif," said Associate Professor Glenn Micalizio, Ph.D., who authored the new study with a member of his Scripps Florida lab, Research Associate Todd K. Macklin, Ph.D. "This new reaction provides a means to explore the medicinal potential of molecules bearing complex skipped polyenes – something that we simply haven't been able to do until now."

Chemical Short Cuts

In essence, the new chemical method provides a means to replace long, step-by-step sequences of reactions that would have otherwise been required to prepare skipped polyenes. The new chemical process defines a fundamentally novel pathway (a new carbon-carbon bond forming process) to these complex structures that proceeds in just a
fraction of the number of chemical steps previously required.

As such, the new method not only saves time, but greatly increases efficiency for the production of molecules that house the skipped polyene core. In chemistry, each of the steps (or reactions) used to prepare a complex structure typically proceeds with less than 100 percent efficiency, notes Micalizio—maybe 80 to 90 percent of the initial material can successfully be advanced to the next chemical step. As a result, the requirement of long sequences of reactions, where yields per step are compounded mathematically through the sequence, typically result in poor overall efficiency.

"If one can invent reactions that decrease the length of sequences required to prepare complex structures, great enhancements of efficiency can result," said Micalizio. "A central focus of our laboratory is designing new chemical reactions that do just that. Since 2005, we have been advancing a large class of chemical transformations that can be seen as 'chemical short cuts' – so that ultimately scientists can better explore the therapeutic potential of molecules inspired by the vast and diverse structures that we see in nature."

The new technique described in the *Nature Chemistry* paper proceeds by bond formation between two specific classes of molecules, vinylcyclopropanes and alkynes (or vinylsilanes), using a metal-promoted cross-coupling reaction to assemble the key structural motif.

"That initial metal-promoted coupling leads to a very unstable intermediate molecule," Micalizio said. "Actually, the chemical intermediate spontaneously rearranges to stabilize the structure, through a process that establishes all of the complex architecture of the skipped polyene product."

The research for the paper, "Convergent and Stereoselective Synthesis of Complex Skipped Polyenes and Polyunsaturated Fatty Acids," was supported by the American Cancer Society, the Arnold and Mabel Beckman Foundation, Boehringer Ingelheim, Eli Lilly & Co., and the National Institutes of Health. For more information on the paper, see [http://www.nature.com/nchem/journal/vaop/ncurrent/abs/nchem.665.html](http://www.nature.com/nchem/journal/vaop/ncurrent/abs/nchem.665.html)

**Scientists Uncover Previously Unknown Natural Mechanism that Controls Cocaine Use**

Scientists from The Scripps Research Institute have found that a particular type of genetic material plays a key role in determining vulnerability to cocaine addiction and may offer an entirely new direction for the development of anti-addiction therapies. In animal studies, the scientists found that a molecule called microRNA-212 was increased in the brains of test animals that had extended access to cocaine. MicroRNA-212 controlled how much cocaine the animals consumed.

The study was published on July 8, 2010, in an advance, online edition of the prestigious journal *Nature*. 
"The key question that the study may answer is why one person is more vulnerable to the effects of cocaine than another," said team leader Paul Kenny, Ph.D., an associate professor in the Department of Molecular Therapeutics at Scripps Florida. "What we found is that a specific microRNA exerts enormous control over the response to the drug. When it is increased in the brain, it protects against addictive behavior, while a reduction raises vulnerability to addictive behaviors. The practical outcome of increased microRNA-212 expression is that it slams the brakes on any desire to take the drug."

MicroRNA-212 is a type of small non-protein coding RNA that can regulate the expression levels of hundreds or even thousands of genes. As such, microRNA-212 and other types of microRNAs are considered "master regulators" of gene expression. Because of their ability to coordinate the expression of related genes responsible for brain structure and function, it is thought that microRNAs might play important roles in complex psychiatric disorders, but little has been known about their involvement in addiction—until now.

What the new findings suggest, Kenny said, is that individuals with serious addiction problems may have damaged supplies of this particular non-coding RNA, or the microRNA may not function properly.

"Looking into the future," he said, "it might be possible to develop a small molecule therapeutic that mimics or stimulates the production of this particular microRNA. Once we understand the precise mechanism, we might uncover novel targets that would have a similar effect to acting on the microRNA directly."

A Molecule that Puts the Brakes on Cocaine Consumption

According to the 2007 National Survey on Drug Use and Health by the Department of Health and Human Services, nearly 1.6 million Americans' behavior met the criteria for cocaine abuse.

Cocaine triggers a constellation of brain reward systems, and cocaine addiction is commonly viewed as a disorder of neuroplasticity—the brain's ability to continually produce new neural connections in response to changes such as injury or disease. Long-lasting structural and functional modifications induced by cocaine are thought to increase sensitivity to the motivational effects of the drug, which in turn results in an eventual loss of control over intake.

In the study, the scientists set out to see how the brain changes in rats with extended or limited exposure to the drug using a model of compulsive drug-taking developed by Scripps Research scientist George Koob. The rats with extended access are those most likely to develop compulsive-like drug-seeking behaviors. The scientists allowed one group six hours of extended access to cocaine each day, compared to control groups, which were given limited exposure to the drug—one hour or none on a daily basis. In the extended-access group, microRNA-212 was increased nearly two fold in the dorsal striatum (a key brain region regulating the development of habit formation) compared to the brains of control rats.

The animals with increased microRNA-212 expression were progressively less motivated
to consume cocaine as their exposure to the drug increased.

"When we used a virus to drive a large over-expression of the microRNA, there was no effect on the behavior of the limited-access animals," Kenny said. "In the extended-access animals, however, their behavior changed abruptly – they took less and less cocaine. In fact, their intake became so low that it appeared they actively disliked the drug. Conversely, when we blocked the actions of the microRNA, the extended access animals began to consume the drug in a compulsive-like manner. MicroRNA-212 is therefore a protective factor helping to prevent the loss of control over drug-taking behavior. Individual differences in microRNA-212 signaling are therefore likely to play a key role in determining vulnerability to cocaine addiction."

In the study, the scientists took their investigation a step further to examine the molecular signaling mechanisms producing this effect. The scientists found that extended consumption of cocaine was associated with an overstimulation of various neurotransmitters in the brain, which increased the production of cAMP (a messenger associated with many biological processes), which in turn activated CREB signaling. (CREB is a transcription factor, a protein that turns on a gene by binding to it, known to regulate how much you like or dislike cocaine.) These changes led to the increase in expression of microRNA-212.

Surprisingly, the scientists found that the increases in microRNA-212 induced by CREB in turn dramatically amplified the actions of cocaine on CREB signaling, establishing a CREB-microRNA-212 "loop." In effect, the microRNA stimulated the pathway in the brain that increases aversive reactions to cocaine, thereby decreasing the motivation to consume the drug.

The first author of the study, "Striatal MicroRNA Controls Cocaine Intake through CREB Signaling," is Jonathan A. Hollander of The Scripps Research Institute. In addition to Kenny, other authors include Heh-In Im, Antonio L. Amelio, Jannet Kocerha, Purva Bali, Qun Lu, Claes Wahlestedt, and Michael D. Conkright of The Scripps Research Institute; and David Willoughby of Ocean Ridge Biosciences. See http://www.nature.com/nature/journal/v466/n7303/abs/nature09202.html.

The study was supported by the National Institute on Drug Abuse of the National Institutes of Health.

**Scripps Research Institute and Dana-Farber Scientists Uncover Novel Anti-Diabetes Mechanism**

*Findings Could Lead to Next Generation of Improved Therapies*

In a joint study, scientists from The Scripps Research Institute and the Dana-Farber Cancer Institute at Harvard University have uncovered a novel mechanism that dramatically increases insulin sensitivity and reduces the risk of developing type 2 diabetes and cardiovascular disease.
These findings offer a potent new target in the continuing search for new and improved anti-diabetic treatments. Currently, nearly 24 million children and adults in the United States have some form of the disease, according to the America Diabetes Association.

The new study, which focuses on controlling a fat-regulating protein known as PPARγ, was published July 22, 2010, in the journal *Nature* (Volume 466, Issue 7304).

"The field has become interested in finding drugs that can promote increased insulin sensitization but not activate the classical fat cell generating pathway of PPARγ," said Patrick R. Griffin, Ph.D., chair of the Department of Molecular Therapeutics at Scripps Florida who headed up the Scripps Research part of the study. "We examined the mechanism of action of compounds that bind to PPARγ that improve insulin sensitivity but have minimal induction of fat. It was clear from the studies that these compounds have a unique but overlapping mechanism with the class of drugs used clinically that target PPARγ."

Adipose or fat tissue lies at the center of the metabolic syndrome, a cluster of risk factors that increases the possibility of type 2 diabetes, as well as stroke, coronary artery disease, even certain cancers. Of those risk factors, excessive body fat is considered the most problematic. PPARγ can be considered the master gene of fat cell biology because it drives the conversion of cellular precursors into fat cells.

The collaborative studies showed obesity causes a modification on PPARγ that leads to alterations in the expression of a number of genes, including a reduction in the production of an insulin-sensitizing protein (adiponectin). This leads to an increase in insulin resistance. The reprogramming of genes controlled by PPARγ occurs when it undergoes phosphorylation (a phosphate group is added to a protein) by the cdk5 kinase, an enzyme that is involved in a number of important sensory pathways and that can be activated by pro-inflammatory proteins.

The scientists were able to use both full and partial agonists (compounds that activate a cellular response) to reverse these phosphorylation effects and improve the production of adiponectin. These results strongly suggest that cdk5-mediated phosphorylation is involved in the development of insulin-resistance and open the door to a novel opportunity for creating an improved generation of anti-diabetic drugs.

*Pointing the Way*

In 2007, Griffin and his colleagues published a study in the journal *Structure* (October 16, 2007, Volume 15, Number 10, pp.1258-1271) that explained the difference between how full and partial agonists interacted with PPARγ. Full agonists interacted strongly with a region of the receptor known to be important for the classical fat generation program. On the other hand, partial agonists, which are poor agonists of the receptor, did not interact with this region at all but interacted more strongly with a potentially critical region of the receptor. From a drug development point of view, these results offered a new area of the protein to focus on to optimize therapeutic molecules that would be potent insulin sensitizers without driving fat generation.

"Bruce Spiegelman at Dana-Farber was starting to uncover the fact that the
phosphorylation of PPARγ takes place in the very region where MRL-24, one of the partial agonists interacted," Griffin said. "I suggested that compounds like MRL24 might be better at antagonizing the cdk5 site given their strong interaction in this region of the receptor. For the new study, we provided significant amounts of compound to support the animal studies and provided an plausible mechanism for how partial agonists might recruit co-activator proteins to the cdk5 surface of PPARγ."

While the team found that PPARγ phosphorylation effects were reversed by both full and partial agonists, partial agonists indeed accomplished this as well or better than the full agonists. Mimicking the effects of just blocking the phosphorylation event by mutation of the site on the receptor showed improvements in the production of adiponectin.

The new study also suggests a unified framework for understanding the relationship between fat cell dysfunction in obesity and anti-diabetic therapies based on PPARγ. In animal studies, high fat diets activate the cdk5 kinase, initiating phosphorylation, disrupting a number of key metabolic regulators including adiponectin and adipisin, a fat cell-selective gene whose expression is altered in obesity.

"The great paradox of this whole effort is we're targeting a receptor critical for fat production to offset the problem of fat overproduction," Griffin said. "Unfortunately, current drugs that target PPARγ increase fat as one of their unwanted long-term side effects."

While the study is a big step forward, important questions still remain such as does a high fat diet and obesity lead to activation of cdk5 in non-fat tissues, Griffin said, since the negative effects of obesity extend far beyond metabolic syndrome to diseases like cancer and neurodegeneration.

The first authors of the study, "Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARγ by Cdk5," are Jang Hyun Choi, Alexander S. Banks Jennifer L. Estall, and Shingo Kajimura of the Dana-Farber Cancer Institute and Department of Cell Biology, Harvard Medical School. Other authors include Pontus Bostrom, Dina Laznik, Bruce M. Spiegelman and Jorge L. Ruas of the Dana-Farber Cancer Institute and Department of Cell Biology, Harvard Medical School; Michael J. Chalmers, Theodore M. Kamenecka of The Scripps Research Institute; and Matthias Bluher of the University of Leipzig, Germany.

The study was supported by the National Institutes of Health and the Deutsche Forschungsgemeinschaft (DFG).