Scripps Research Florida 2011 Scientific Report
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Part 1: New Faculty

Scripps Research Appoints Noted Chemist to Florida Faculty

The Scripps Research Institute has appointed Ben Shen as a professor on its Jupiter, Florida campus.

Prior to his appointment to the Scripps Research Department of Chemistry and Department of Molecular Therapeutics, Shen was the Charles M. Johnson Distinguished Chair and Professor of Pharmaceutical Sciences and Chemistry at the University of Wisconsin, Madison.

"Ben is one of the finest chemists in the country and will be a formidable member of the Scripps Research faculty," said K.C. Nicolaou, who chairs the Scripps Research Department of Chemistry. "His work in natural product drug development, particularly creating potential anti-cancer and antibiotic compounds, is outstanding and at the cutting edge of science. We want to extend our warmest welcome to Ben and his laboratory colleagues."

"Ben's multidisciplinary approach is a perfect fit for Scripps Florida," said Patrick R. Griffin, chair of the Scripps Research Department of Molecular Therapeutics and director of the Translational Research Institute at Scripps Florida. "We look forward to his contributions, and to working with him to exploit the enormous potential of the natural products he is interested in."

Shen received a bachelor's degree in chemistry from Hangzhou University in 1982, and a master's degree in chemistry from the Chinese Academy of Sciences in 1984. He received his doctorate in organic chemistry/biochemistry from Oregon State University 1991 and held a postdoctoral appointment at the University of Wisconsin, Madison from 1991 to 1995.

Before joining the University of Wisconsin, Madison faculty in 2001, he was a member of the faculty at the University of California, Davis. He arrived at Scripps Research this month and is now living with his family in Jupiter.

The focus of Shen's research is to identify and produce natural products, especially those produced by bacteria, and to genetically engineer these bacteria to produce novel natural product analogues for use in anti-cancer and antibiotic drug discovery. Some recent highlights from his work include manipulating a South African soil microbe to overproduce two novel antibacterial antibiotics, setting the stage for the manufacture of large amounts of drugs for future clinical studies, and engineering a Chinese soil microbe to synthesize designer enediynes, a bacterial product that could potentially treat tumors.

Shen and his colleagues use a wide variety of disciplines, including organic chemistry, biochemistry, enzymology, microbiology, structural biology, and genomics, to delve more deeply into these natural products, defining their structures and biological profiles as well as the biosynthetic machineries that control their production. By understanding, and thereby rationally manipulating these biosynthetic machineries, the Shen group looks for new ways to redesign these existing natural products to create something new and more effective in terms of therapeutic potential.

"In some ways, it's like creating something from Lego® parts," he said. "Once you learn the parts and their connections to constitute functional biosynthetic machineries, you can take them apart and put them back together to make something completely new and different."

Shen points out that natural products remain the best sources of drugs and drug leads.

"Natural products have a fantastic track record," Shen said. "In the anti-cancer area, almost three-quarters of anti-cancer drugs in current clinical use are from natural products or inspired by them. The same is true for antibiotics. To speed up the discovery process, you need libraries of natural products and their analogs, but to produce them is a daunting challenge. It is sobering to note that only one percent of the 2010 Molecular Libraries Small Molecule Repository collection at the National Institutes of Health is annotated as natural products and bioactives, highlighting the urgent need to discover, produce, and diversify natural products to enrich the chemical space available for drug discovery."

Shen will also launch the Natural Products Library initiative at Scripps Research to expand the natural products in the institute's current small molecule libraries. "Highly collaborative translational research is very much a way of life at Scripps Florida," he said, "and I look forward to becoming part of it."

Scripps Research Appoints Innovative Biochemist to Florida Faculty

The Scripps Research Institute has appointed Matthew Gill as an assistant professor in the Florida campus's Department of Metabolism and Aging.

Prior to his appointment, Gill, 38, was an assistant research professor at the Buck Institute for Research on Aging in Novato, California, a research facility focused on understanding the connection between aging and chronic disease.

"Matt is one of the most innovative scientists working in the field of aging today," said Roy Smith, chair of the Department of Metabolism and Aging. "His recent study of the hormonal aspects of lifespan extension in the nematode worm *Caenorhabditis elegans* is impressive and he has been instrumental in developing automated screening processes involving florescent signaling, to identify drugs that influence aging. We offer him a warm welcome."

"I'm honored to be part of the Scripps Research faculty," said Gill, who lives in Jupiter with his family. "I've been impressed with the breadth of talent and resources in the Metabolism and Aging Department and with the resources of Scripps Florida in general, particularly the high-throughput screening facilities. Having access to chemical libraries with hundreds of thousands of compounds is going to open up many opportunities for my work."

Gill received a degree in Biochemistry from the University of Newcastle-upon-Tyne, United Kingdom, followed by a PhD in Endocrinology from the University of Manchester, UK. His PhD thesis focused on the development of tests of various growth factors that could be used in the assessment of normal and disordered growth in children.

In 1998, he was awarded a Medical Research Council Research Fellowship to examine growth in C. elegans, a simple organism that is widely studied in biomedical research. In recent years the nematode has emerged as an important model in which to study the aging process, as well as age-related diseases such as Alzheimer's disease. In 2002, Gill received a Brookdale National Fellowship to examine the hormonal control of the roundworm's lifespan. He has published more than 30 papers.

At Scripps Florida, Gill will focus on the nematode endocannabinoid system, a signaling pathway that affects nematode lifespan, and that holds the potential one day to be manipulated to minimize the effects of aging. Gill noted the close collaboration between biologists and chemists that has been a hallmark of Scripps Research, and he looks forward to working with chemists at Scripps Florida.

"We simply couldn't have done our recent work by focusing solely on biology," he said. "We need the help of chemists. The strong emphasis on chemistry and the whole drug development area excites me about Scripps Florida."

Scripps Research Appoints Noted Neuroscientist to Faculty

The Scripps Research Institute has appointed Kirill Martemyanov as associate professor in the Department of Neuroscience.

Martemyanov, who is 36, will work on the Scripps Florida campus in Jupiter. Prior to his appointment, he was assistant professor in the Department of Pharmacology at the University of Minnesota.

"Kirill's work has already uncovered some novel mechanisms of G proteins and has clearly indicated their potential as targets for drug development," said Ron L. Davis, chair of the Scripps Research Department of Neuroscience. "We want to offer Kirill a warm welcome to Scripps Florida and the department."

Martemyanov's laboratory is investigating the fundamental principles governing signal transduction in G protein-coupled receptor pathways, which mediate a vast number of biological processes such as perception of light and odor, as well as responses to hormones and neurotransmitters. Dysfunction in G protein pathways is associated with a range of the disorders, including mental, neurological, visual, and cardiovascular diseases.

"I'm extremely pleased to join the Scripps Florida," said Martemyanov, who lives in Jupiter with his family. "This is a remarkable environment because the people here are focused on doing great research, not to mention working in amazing facilities. I have been hearing about The Scripps Research Institute since I started in science, so joining its faculty is a tremendous honor for me."

Martemyanov received a degree in biochemistry from Samara State University, Russia, graduating *summa cum laude* in 1996. He received his Ph.D. degree from Institute of Protein Research, Russian Academy of Sciences, in 2000.

Martemyanov received a 1998 European Academy Prize in Biology and the 1999 Russian Biochemical Society Award. He was awarded a Postdoctoral Fellowship in Molecular Mechanisms of Ocular Diseases from Harvard University for 2000-2002. He received an American Heart Association Fellowship in 2002. Two years later, he received the Knights Templar Foundation Award and, in 2008, was awarded a McKnight Land-Grant Professorship.

In some respects, Martemyanov said, Scripps Research already feels familiar.

"I received my doctoral training at the Institute of Protein Research," he said, "and the head of the institute really tried to fashion it after Scripps Research. So, I find I'm very much at home with the way things are done here."

At Scripps Florida, Martemyanov will focus on studying the role of powerful regulators of G protein pathways called RGS proteins in controlling signaling pathways in the nervous system. "RGS proteins act as a braking mechanism in controlling the extent of G protein signaling," said Martemyanov. Specifically, his laboratory will study how dysfunction in RGS proteins leads to the development of neuropsychiatric diseases, drug addiction, and blindness. A major effort will be to assess the potential of pharmacologically targeting these proteins with the goal of developing novel treatments.

"Everything that we do is medically driven," Martemyanov said. "We are trying to understand the mechanisms behind devastating diseases with the hopes of finding ways that will ultimately cure them."

For more information on Martemyanov's work, see his website at www.scripps.edu/martemyanov.

Scripps Research Appoints Noted Autism Researcher to Neuroscience Faculty

Scripps Research Institute has appointed Damon Page as assistant professor in the Department of Neuroscience.

Page, 36, will work on the Scripps Florida campus in Jupiter. Prior to his appointment, he was a senior analyst at the Allen Institute for Brain Science in Seattle, Washington.

"Damon's research on autism makes a valuable addition to our department," said Ron L. Davis, chair of the Department of Neuroscience. "His discovery of genes that can cause autism-like symptoms is a breakthrough in the complex origins of the disease and offers new potential therapeutic targets to investigate. We're delighted he is joining us."

"This is a wonderful opportunity to be part of a dynamic, highly collaborative organization, with a breadth of basic and translational research that meshes perfectly with my research," Page said. "Scripps Florida is a unique place to explore the basic science of how the brain develops and then to use that knowledge to develop potential new treatments for autism."

Page, who lives in Jupiter, received his bachelor's degree in biology from Eastern Oregon University in 1999 and his PhD from the University of Cambridge in 2002. He was a postdoctoral fellow at the MRC Laboratory of Molecular Biology from 2002 to 2004 and the Massachusetts Institute of Technology (MIT) from 2004 to 2009; he worked as a research scientist at MIT from 2009 to 2010.

It was during his stint at MIT that Page led a groundbreaking study that resulted in the discovery of a novel mechanism whereby two autism risk factors interact to shape autism-like symptoms in an animal model. That discovery showed for the first time that genes acting in two distinct molecular pathways implicated in autism can interact to significantly influence the severity of symptoms. The study pointed to the intersection of these pathways as a potential new target for therapeutic development.

Autism is a complex neurodevelopmental disorder that impairs the normal development of social and communication skills, among other facilities. Autism is the most severe form of autism spectrum disorders; milder forms include Asperger syndrome. According to the National Institutes of Health, six children out of every 1,000 have autism spectrum disorder, with males four times more likely to be afflicted than females.

"There are a number of risk factors for autism," Page said, "but at present we don't understand how these interact in the developing brain to cause the disorder. My aim is to shed light on this problem, but, more importantly, to apply what we learn in the laboratory to help individuals and families affected by the disorder."

For more information, see Page's faculty web page at http://www.scripps.edu/research/faculty/page .

Part 2: Grant Awards

Scripps Research and University of Pennsylvania Win $8 Million Grant to Develop Addiction Treatment

The Scripps Research Institute and the University of Pennsylvania School of Medicine have been awarded approximately $8.2 million over five years to develop novel compounds that could eventually become drug candidates for the treatment of tobacco addiction. Of the funds awarded, $5.7 million will go to Scripps Research, and $2.5 million to the University of Pennsylvania.

The grant from the National Institutes of Health's (NIH) National Institute on Drug Abuse (NIDA) will fund research focused on potential new treatments for tobacco addiction, specifically the development of novel kinds of potent and selective compounds that affect nicotinic receptors in the brain.

Consortium principal investigators are Paul J. Kenny, an associate professor in the Department of Molecular Therapeutics on the Jupiter, Florida campus of Scripps Research, and Jon Martin Lindstrom, professor of Neuroscience at the University of Pennsylvania School of Medicine.

"This project capitalizes on the unique drug discovery capabilities at Scripps Florida, and combines that with the work Jon Lindstrom has been doing on defining the structure and function of nicotinic receptors," Kenny said. "We have exceptional collaborators here at Scripps Florida, including Ted Kamenecka and Michael Cameron. In combination with Jon's expertise, we believe that the collaboration funded by this generous grant has the potential to produce effective therapeutic compounds to help people stop smoking."

Lindstrom added, "We have been studying the properties of cloned human nicotinic receptor subtypes *in vitro* for years with the hope of using the methods and cell lines we have developed to discover new drugs for these receptors. This collaboration with Paul, and the support of NIDA, provides critical elements to turn these hopes into reality."

Recent studies have shown that genetic variations in several nicotinic receptor subunits (building blocks of the "receptor" proteins that respond to nicotine) actually increase vulnerability to tobacco addiction, Kenny said, although initially little was actually known about these variations or their function.

"We got interested in one of these lesser-studied subunits that influences smoking and have developed a good understanding of how it works in the brain," he said. "It seems that this subunit is part of a distinct subtype of nicotinic receptor that actually protects against tobacco addiction. Now we want to find compounds that boost the activity of this receptor subtype in the hopes that they will be effective in reducing tobacco smoking. This approach contrasts with most anti-

smoking treatments today, which are aimed at reducing the positive impact of nicotine on the brain's reward centers. Instead, we aim to enhance the inhibitory effects of the drug on these reward centers."

Kenny said he met Lindstrom a year ago and their common interests led to the collaboration.

"Jon is one of the leaders in the molecular pharmacology of nicotinic receptors, and has been for many years," Kenny said. "Our work is complementary – he approaches the problem from the bottom up, starting at the receptor. We approach the problem from the top down, starting from a behavioral perspective."

Kenny said that he plans to use Scripps Florida's compound screening resources to look for compounds that Lindstrom can then test to provide a clearer understanding of how they work on the receptor subunits. The researchers then hope to conduct further studies on the effectiveness of the most promising compounds.

Bi-Coastal Team Awarded $7.5 Million to Identify Potential Drug Candidates to Treat Nicotine Addiction

The Scripps Research Institute and the University of California, San Diego, (UCSD) School of Medicine have been awarded approximately $7.5 million over five years to develop novel compounds that could eventually become drug candidates for the treatment of nicotine dependence, and possibly other drug addictions. Of the funds awarded, $5 million will go to Scripps Research, $2.5 million to UCSD.

The grant from the National Institutes of Health (NIH) will fund research focused on finding novel positive modulators for $GABA_B$ receptors that have the potential to become treatments for nicotine addiction. $GABA_B$ receptors, found in the central nervous system, mediate some of the actions of GABA, the major inhibitory neurotransmitter in the brain involved in regulating several brain functions, including reward signals that play a role in drug addiction.

Consortium principal investigators are Patrick R. Griffin, chair of the Department of Molecular Therapeutics and director of the Translational Research Institute on the Jupiter, Florida campus of Scripps Research; M.G. Finn, a professor in the Department of Chemistry and the Skaggs Institute for Chemical Biology on the La Jolla, California campus of Scripps Research; and consortium director Athina Markou, a professor in the Department of Psychiatry at UCSD and adjunct professor in the Scripps Research Molecular and Integrative Neurosciences Department (MIND).

"We're looking for small molecules that will affect very complex feedback mechanisms in the brain, but in a subtle way," Finn said of the new project. "While the field has focused on some obvious receptor pathways in the brain, these are involved in many different functions and side

effects are impossible to avoid. Positive modulation of $GABA_B$ receptors – if we can find the right agent – has strong potential to help people resist the addiction impulse without messing with the main circuitry of the brain."

This consortium grant is a competitive renewal of a previous award to Markou, in collaboration with Novartis, when her laboratory was located at Scripps Research. Chemists at Novartis involved in the previous phase of the program discovered a number of small-molecule modulators of the $GABA_B$ receptor. Further study resulted in the development of the first highly selective positive modulators for $GABA_B$ receptors. Subsequently, work in the Markou laboratory showed that these compounds had desirable effects on nicotine dependence in animal models, while offering a better side-effect profile than other alternatives under study (full agonists at the same receptors).

"As a result, a strong preclinical proof-of-concept has already been established for this novel approach to the treatment of nicotine addiction that drives the harmful tobacco smoking habit," Markou said. "The preclinical results in our animal models are really exciting and have provided the momentum for me to continue on this project, even after Novartis indicated that it no longer wished to maintain this collaboration."

Markou reached out to her Scripps Research colleagues and formed a new research team to continue work on the initiative. "I am very fortunate to have such outstanding collaborators who are cutting-edge chemists and have extensive experience in drug discovery," she said. "After the neurobiological studies pointed out the important role of the $GABA_B$ receptor in nicotine reward, and we had positive data in a variety of animal models of nicotine dependence, it was time to focus our efforts on discovery of new molecules that could become therapeutics to assist people to quit smoking."

The new funding will advance this effort.

Griffin noted, "We want to expand the pipeline of possible compounds that could be developed into potential therapies. Once we have a better mechanistic understanding of the factors that drive selective modulation of $GABA_B$ receptors, we can assess how these new compounds affect various behaviors in animal models. In the end, this approach has the best potential to provide potent innovative therapies for human nicotine addiction."

Scientists on the two Scripps Research campuses will collaborate in the design of new $GABA_B$ receptor modulators. The compounds will then be synthesized in California and tested in Jupiter. Key personnel from the Florida campus include Patricia McDonald and Michael Cameron, both assistant professors in the Department of Molecular Therapeutics. Compounds that successfully make it past several rounds of evaluation will be tested in animal models at UCSD.

"In many ways, this is the kind of collaboration that [Scripps Research President] Richard Lerner had in mind when he founded Scripps Florida," Finn said. "Jupiter is the state-of-the-art for assay development, chemical screening, and compound evaluation to support biomedical research in

the country, possibly the world. And in La Jolla, we are developing some unique approaches to synthesis. It's a great match. This really is what everybody hoped would happen."

"This is not a typical individual investigator-driven science grant," Griffin said. "It's really a highly integrated and collaborative research program to discover innovative drug candidates. In this particular program to discover novel modulators of $GABA_B$, the Scripps Florida team took on the translational role of a large pharmaceutical company. We are providing the framework and support to discover potent and functionally selective $GABA_B$ modulators that are efficacious in animal models of tobacco addiction. This project is very exciting and very energizing."

Scripps Florida Scientist Awarded $4.2 Million for Type 1 Diabetes Research

A scientist at The Scripps Research Institute has been awarded $4.2 million from the National Institutes of Health in a program to advance what the agency calls “bold and creative research” into Type I diabetes.

Thomas Kodadek, a professor in the Department of Chemistry on the Scripps Florida campus, is the principal investigator on the study. The award will be shared with researchers at the University of Miami and Opko, a Florida-based biotechnology company.

The new four-year grant from the NIH National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) is a special Type I Diabetes Impact Award (DP3). Type I diabetes is an autoimmune disease, in which the immune system attacks the body’s own tissues. In Type 1 diabetes, the immune system attacks cells in the pancreas that produce insulin, which leads to insulin deficiency; the condition is treated with regular insulin injections.

The new grant to Kodadek and his colleagues will fund research to determine early autoimmune reactions that drive the development of Type I diabetes, as well as to look for ways to selectively block such autoimmune diseases without shutting down or damaging the entire immune response.

“Once the earliest autoimmune reactions have been identified,” Kodadek said, “we can develop compounds that specifically target the autoimmune cells to see if we can block the disease in mice without affecting the normal function of the ‘good’ parts of the immune system. This would set the stage for similar studies in human diabetic patients. Obviously, if we succeed in developing a therapy for humans, it would have a radical impact on the detection and treatment of diabetes—and other autoimmune conditions as well.”

The research funded by the new grant may also lead to new ways to detect Type 1 diabetes. Currently, immunoassays, a technique that detects auto-antibodies for human insulin, are used as early diagnostic markers for Type I diabetes, and for screening and risk assessment in clinical trials. Because progression of diabetes is often haphazard, additional markers are needed to improve overall risk assessment.

The novel approach Kodadek uses in his research involves peptoids, synthetic molecules similar to peptides that make up proteins when joined together. His lab uses these peptoids to screen or search for molecules that bind to and affect the action of a type of immune system molecule called an antibody.

Like the handmade flies used by fishermen, the synthetic peptoids are a lure to capture disease-specific antibodies—in this case, for diabetes—well enough to pull them from blood samples. It's a novel way to short-circuit the discovery process that has been used successfully in the lab for Alzheimer's disease. In the new project, once novel autoimmune cells for diabetes have been identified, Kodadek said, the scientists will begin to determine whether they can be turned off selectively, proof-of-principle for what could be a powerful therapeutic strategy.

Scripps Research Awarded More than $3 Million to Develop Therapies for Biological Clock Disorders

The Scripps Research Institute has been awarded $3.17 million over four years to develop compounds that will counteract disruptions of the human biological clock – the circadian rhythm that regulates our patterns of activity and rest over a 24-hour daily cycle. Circadian rhythm disruptions have been associated with sleep disorders, as well as bipolar disease and schizophrenia.

The grant, from the National Institutes of Health (NIH), was awarded to Thomas Burris, a professor in the Department of Molecular Therapeutics at Scripps Florida.

The NIH also selected the Burris lab to receive a one-year award of $580,000 to develop compounds that might act against metabolic diseases such as diabetes and obesity and a one-year award of $243,000 to investigate a method of finding compounds that might lead to new treatments for diseases including cancer, inflammation, and diabetes.

"These grants will help move our research forward," Burris said. "For the four-year grant to study circadian rhythm disorders, we put together a group of four investigators at Scripps Florida – Pat Griffin, Ted Kamenecka, Andrew Butler, and myself – to investigate the widely accepted idea that circadian rhythms are involved in various disorders such as depression and schizophrenia. We have an initial lead that we believe will result in several new and more effective compounds."

Pat Griffin is chair of the Department of Molecular Therapeutics and director of the Translational Research Institute at Scripps Florida; Ted Kamenecka is associate scientific director of Scripps Florida's Translational Research Institute; and Andrew Butler is an associate professor in the Scripps Florida Department of Metabolism and Aging.

The one-year award to develop compounds for metabolic diseases, Burris said, is a seed grant that could grow into a new and much larger five-year grant involving the same group of Scripps Research scientists.

The Role of Nuclear Receptors

The work for all three grants is related to the Burris lab's work on specific "nuclear receptors," which might be modified with small molecules to counteract a number of disorders. The nuclear hormone receptor family is a large a group of protein molecules that recognize and regulate hormones as well as other natural substances in our body. As a result, these receptors control an organism's metabolism by activating gene expression.

Nuclear receptors make tempting drug targets because they can bind directly to DNA and activate genes through specific ligands—molecules that affect receptor behavior—such as the sex hormones, vitamins A and D, and glucocorticoids, which affect the body's response to stress. Nuclear receptors have been implicated in a number of cancers, including prostate, breast, and colon cancers, and other diseases as well, including type 2 diabetes, atherosclerosis, and metabolic syndrome.

Burris's research involves what are known as orphan nuclear receptors called RORs (retinoic acid receptor-related orphan receptors), a subgroup that plays a role in the expression of genes involved in the regulation of carbohydrate and fat metabolism, as well as circadian rhythm.

"The caveat is that since no one has ever developed ROR-related drugs, we don't know what the side effects might be," Burris said. "On the positive side, however, there is compelling evidence that these receptors are associated with these diseases, particularly the circadian rhythm disorders, and we have several compounds that can target these mechanisms and control them. We're on the cutting edge of this research."

In November of last year, Burris published a study in the Journal of Biological Chemistry that identified for the first time a novel mechanism that regulates circadian rhythm. The mechanism involved a specific ROR as well as another member of the nuclear receptor family.

Another Burris study, published around the same time in the journal *ACS Chemical Biology*, identified a novel compound acting on a pair of nuclear receptors that could provide new and potentially more effective therapeutic approaches to a range of metabolic diseases.

Scripps Florida Scientists Awarded $3 Million NIH Grant to Accelerate Identification of Learning and Memory Genes

The Scripps Research Institute has been awarded a three-year, $3.2 million grant by the National Institutes of Health (NIH) to identify the full spectrum of genes involved in learning and memory

in *Drosophila*, the common fruit fly. The research could lead to a number of new therapeutic targets for several major cognitive and neurological disorders, including Alzheimer's disease.

Ronald Davis, chair of the Scripps Research Department of Neuroscience on the Florida campus, is the principal investigator for the project.

Past research has shown that genes involved in *Drosophila* olfactory learning are remarkably similar in both structure and function in mammalian organisms.

"A large number of genes we expect to identify in *Drosophila* over the next several years should, in fact, play analogous roles in human learning and memory," Davis said. "As a consequence, our discoveries could provide several new candidate genes that could become potential targets for the development of drugs to treat a range of cognitive disorders."

Because *Drosophila* learning genes underlie specific behavior, they have long been considered test genes for understanding human brain disorders. For example, the Drosophila gene known as *dunce* helped define the human form of the gene as a serious risk factor for the devastating psychiatric condition of schizophrenia; and the Nf1 gene, which underlies neurofibromatosis type 1, a human genetic disorder, is important for cognition in both humans and flies.

"We're still early in the process of making connections between *Drosophila* memory and learning genes and the pathology of human disease," Davis said, "but it's already clear that many of these genes will provide potentially important insight into human brain disorders."

Screening the Fruit Fly Genome

A major objective in the field of learning and memory is to identify all gene products that have essential roles in this complex neurobiological process. Employing some recently developed, genome-wide RNAi transgene libraries of *Drosophila* genes, Davis said they expect to screen more than 90 percent of the genome for genetic functions involved in acquisition, short-term memory stability, long-term memory, and retrieval.

Davis said they would screen approximately 15,000 RNAi transgenes to cover most of the *Drosophila* genome; at its most basic, a transgene is a DNA sequence that has been implanted into a new organism. *Drosophila* RNAi transgenes can inactivate gene function, which allows scientists to systemically analyze specific gene function in a range of tissues.

"Our approach will also utilize techniques recently developed in our laboratory to induce transgene expression in neurons of the adult brain," he said. "This approach will allow us to identify the spectrum of genes across the Drosophila genome involved in learning, stabilizing, and retrieving information about odors – the most commonly studied learning process in fruit flies."

RNA interference (RNAi), which consists of microRNA and small interfering RNA, helps control the selection and activity of genes. These small RNAs can increase or decrease the

activity of other RNAs by binding to them, helping direct gene expression, and protecting cells against viral mutation.

These properties make RNAi valuable for the kind of advanced large-scale screens developed by Davis and his colleagues. Because RNAi can shut down genes in the cell systematically, it can help identify various components involved in cell processes or a specific event such as cell division.

As the research progresses, Davis said he plans to construct a website to make the results of these new screens more widely available.

Scripps Florida Scientists Awarded $2.35 Million to Study New Obesity Treatment

The Scripps Research Institute has been awarded a $2.35 million grant from the National Institutes of Health to study a new way of treating obesity as part of a national consortium with the Dana-Farber Cancer Institute.

The long-term aim of the research is to develop compounds that can control the formation and function of a particular type of fat cell that burns calories and reduces weight.

Patrick R. Griffin, chair of the Department of Molecular Therapeutics and director of the Translational Research Institute at Scripps Florida, and Theodore Kamenecka, associate scientific director of the Translational Research Institute, are consortium principal investigators, along with Bruce M. Spiegelman, of the Boston-based Dana-Farber Cancer Institute and Harvard Medical School.

The total for the three-year award, a highly competitive National Institutes of Health Director's Opportunity Grant, is $4.2 million with $2.35 million going to Scripps Research in Florida.

"This grant speaks to the critical research components we have developed at Scripps Florida," Griffin said, "plus the fact that we have a host of resources that aren't found anywhere else in academia. Bruce Speigelman is expert in the understanding of fat and energy utilization, and we're experts in understanding how certain receptors are activated and modulated by small molecule drugs, which is central to the success of the research. Our collaboration offers the best team possible to exploit this potentially life-changing biology."

The new research is aimed at creating new therapeutic approaches to the national problem of obesity based on regulating brown fat, a type of cell that plays a critical role in controlling metabolic rates and fighting obesity. Western countries - the U.S. in particular - are in the midst of a growing epidemic of obesity and its associated problems, including high blood pressure, type 2 diabetes, and certain cancers. Currently, there are no generally effective medical therapies

to treat obesity, and efforts so far have been limited to education extolling diet and exercise and in extreme cases surgery.

"This type of grant, the Director's Opportunity grant, is aimed at large and critically important projects that have the potential to impact clinical care and public health," Griffin said. "Our joint project will take the discoveries and insight in Bruce's laboratory, develop assays for these novel targets, and look to develop compounds that stimulate white fat to burn calories in the intense way that brown fat does."

Brown fat, which has been identified in healthy humans, serves as a kind of molecular mammalian furnace, generating heat by burning large numbers of calories in creatures like newborn babies (and rodents) that lack the ability to shiver effectively to keep warm. The fat gets its name because it is loaded with mitochondria, the cell's energy plant, which contains iron and gives the fat its red-brown tint. Mitochondria use nutrients to produce energy for the cell.

The grant, Griffin said, is built on several key discoveries the team has made over the last few years. One is the initial discovery of PRDM16, a protein capable of determining whether certain types of immature cells will develop into brown fat cells. The protein works in tandem with another protein and together they act as the catalyst to the development of brown fat in different cell types. One aim of the funded research program will be to investigate regulation of whole body energy homeostasis and to isolate and characterize a type of brown fat cell in white fat tissues with substantial heat-producing capacity. This will be combined with the synthesis and evaluation of small molecules that can regulate PRDM16's activity.

A second discovery by the research team, which was published earlier this year in the journal *Nature*, was a novel signaling pathway trigged by phosphorylation of PPARg, the molecular target of the antidiabetic drugs called thiazolidenediones or TZDs. The research team is building on this discovery and is in the process of generating proof-of-concept compounds that modulate only this pathway. These compounds, in addition to being antidiabetic, have the potential to enhance brown fat leading to weight loss.

Scripps Florida Scientist Awarded $2.2 Million Grant to Study Hepatitis C

The Scripps Research Institute has been awarded a $2.2 million grant by the National Institutes of Health (NIH) to determine how the hepatitis C virus (HCV) induces liver cancer. The research could lead to potentially new therapeutic targets for treating those chronically infected with the virus.

Timothy Tellinghuisen, an assistant professor on the Florida campus of Scripps Research, is the principal investigator for the project.

Hepatitis C virus infection is a major public health problem worldwide. Estimates place the number of HCV infected individuals at approximately 170 to 200 million, representing nearly

three percent of the world's population, according to the World Health Organization. HCV infection and its assorted pathologies are responsible for an estimated 250,000 deaths a year worldwide.

A majority of patients remain chronically infected, which can lead to progressive liver damage, cirrhosis, and often the development of hepatocellular carcinoma—liver cancer. An estimated 60 to 70 percent of all those infected develop chronic infections and most progress to major liver damage. Each year, as many as five percent of these chronically infected patients will develop liver cancer.

While the mechanisms by which HCV induces liver cancer are largely unknown, Tellinghuisen's ongoing research points to host cell signaling pathways that are likely altered by the virus, creating a replication niche for the virus that avoids the body's innate immune system.

"We have identified a host protein—called CARD14—as an important factor for HCV RNA replication," he said. "We believe that a pathway regulated by this protein gets manipulated by the virus to maintain chronic infections and that this contributes, in part, to liver cancer development. The new grant will help us explore the extensive role of CARD14 in HCV replication and, quite possibly, identify new ways to attack chronic HCV infection."

Overall, the new grant will enable Tellinghuisen and his colleagues to characterize how the virus manipulates this host cell pathway, identify the genes regulated by this pathway and determine their effect on viral infection and persistence, and define the function of this protein in normal liver physiology.

Scripps Research Scientists Awarded $2.2 Million to Develop Treatment for Multi-Drug Addiction

The Scripps Research Institute has been awarded a $2.2 million grant from the National Institutes of Health (NIH) to develop novel therapeutics for the treatment of addiction to multiple substances in a national effort with the University of Kansas.

The total award is for $3,698,130, with the Florida campus of Scripps Research receiving $2.19 million over five years and the University of Kansas receiving approximately $1.5 million.

According to the National Survey on Drug Use and Health, in 2009 an estimated 21.8 million Americans aged 12 or older had used illicit drugs within the last month, approximately 8.7 percent of the population aged 12 or older, an increase over 2008. Illicit drugs include marijuana/hashish, cocaine (including crack), heroin, hallucinogens, inhalants, and prescription-type psychotherapeutics used nonmedically. Multiple drug use is common among substance abusers, according to the National Institute on Drug Abuse (NIDA).

The grant funds research directed by Scripps Research Associate Professor Laura Bohn to develop new compounds targeting a specific receptor playing a critical role in the persistence of compulsive drug seeking and taking. (Receptors bind substances, triggering certain biological effects.)

“All addictive drugs share certain reinforcing properties that make it difficult to quit and remain abstinent,” said Bohn. “There is significant evidence spanning decades that the kappa opioid receptor is a good candidate in terms of disrupting this addictive cycle. Right now we’re in the early stages of R&D but we have a lot of confidence in what we’ve studied so far.”

The kappa opioid receptor, which is located on neurons, helps regulate the release of dopamine – a neurotransmitter that plays a key role in drug addiction. Drugs of abuse often cause the brain to release large amounts of dopamine, flooding the brain’s reward system and reinforcing the addictive cycle.

The basis of the studies proposed in the current award results from years of screening by the Molecular Libraries Probe Production Centers Network supported by the NIH. After screening more than 300,000 small molecule compounds, the group, along with the specialized chemistry center at the University of Kansas, identified five novel chemical candidates for clinical development.

For the next five years, the collaborative research team at Scripps Research and the University of Kansas will work to improve selectivity, potency, efficacy, and bioavailability of these compounds to provide a strong preclinical framework for clinical utilization of new drugs targeting the kappa opioid receptor in the hopes of finding a way to help improve recovery from a variety of addictive drugs.

Scripps Research Wins $2 Million to Study Prostate Cancer

The Scripps Research Institute and Tampa's Moffitt Cancer Center have been awarded more than $2 million to study the formation and progression of prostate cancer. Of the funds awarded, approximately $1.9 million will go to Scripps Research, with the remaining $138,380 supporting Moffitt Cancer Center work.

The five-year grant from the National Institutes of Health (NIH) will fund research to advance the development of novel therapeutic strategies for prostate cancer treatment and prevention.

"This new funding will help us continue our work into the origins of prostate cancer and the role that inflammation plays in its development," said Jun-Li Luo, an assistant professor on the Florida campus of Scripps Research and principal investigator for the new study. "We are pleased that Moffitt, one of the country's leading treatment and research centers, will be our

partner in this research. Gaining a better understanding of the inflammatory process should help lay the foundation for developing novel therapeutic strategies for this disease."

"This collaboration with Scripps Florida is a great opportunity to help uncover the underlying mechanisms of prostate cancer," said Shohreh Dickinson, an assistant professor at Moffitt, where scientists will study and interpret pathology slides of human cells as part of the new study. "It's also a great opportunity for two Florida research centers to advance the science that, hopefully, will one day help put an end to this terrible disease."

Prostate cancer—which, according to the American Cancer Society, will affect one in six American men in their lifetime—is the second-leading cause of death after lung cancer in American men. Prostate cancer is driven by androgen, the male sex hormone, and androgen deprivation is considered a first-line treatment of the disease once it spreads beyond the prostate gland.

Eventually, all prostate cancer becomes resistant to the treatment, and the disease grows independently of androgen. This can occur almost anytime during treatment. Currently there are no effective treatments for what is known as hormone-refractory prostate cancer.

Luo's work has long been focused on the role of inflammation in cancer and the body's innate inflammatory response, which encourages tumor growth. In earlier studies, he found that blocking one of the factors involved in inflammation—the nuclear factor-kappa B (NF-κB)—dramatically impaired development of the disease. In addition, Luo has identified tumor-infiltrating B cells as another critical component of the inflammatory response that enhances androgen-independent tumor growth.

The new study will further define how B cells control the spread of hormone refractory cancer.

Scripps Florida Scientist Wins Prestigious NIH Innovator Award

Assistant Professor Brian Paegel of the Florida campus of The Scripps Research Institute has won a prestigious National Institutes of Health (NIH) Director’s New Innovator Award.

The award, announced by NIH Director Francis S. Collins at the Seventh Annual NIH Director’s Pioneer Award Symposium September 20, will provide Paegel with $1.5 million in research funding over five years.

“The NIH Director’s Award programs reinvigorate the biomedical work force by providing unique opportunities to conduct research that is neither incremental nor conventional,” said James M. Anderson, director of the Division of Program Coordination, Planning and Strategic Initiatives, who guides the NIH Common Fund’s High-Risk Research program. “The awards are intended to catalyze giant leaps forward for any area of biomedical research, allowing investigators to go in entirely new directions.”

Paegel, a member of the Scripps Research Department of Chemistry, will use his award to evolve new molecular tools for protein sequencing.

“Imagine being asked to take apart a sophisticated race car with a single Phillips-head screwdriver,” said Paegel. “This is basically where we are today with protein sequencing technology. We will evolve a suite of custom-tailored molecular tools that will allow us to identify all sites of protein modification, and to correlate those changes with normal cellular function and disease. Our approach integrates the institute’s strengths in chemistry and high-throughput screening with my laboratory's expertise in microfluidic technology development and evolution.”

Winners of the NIH Director's New Innovator Award are selected on the basis of individual creativity, the innovativeness of his or her research approaches, and the potential of the proposed project, if successful, to have a significant impact on an important biomedical or behavioral research problem. More information on the New Innovator Award is at http://commonfund.nih.gov/newinnovator, including information on this year's awardees.

Another Scripps Research scientist, Associate Professor Michael Petrascheck, based at the institute’s La Jolla, California campus, also received an NIH Director’s New Innovator Award this year. Petrascheck, a member of the Department of Chemical Physiology, the Department of Molecular and Experimental Medicine, and the Dorris Neuroscience Center at Scripps Research, will use the award to conduct research on aging and lifespan in C. elegans, a flatworm widely used in aging research. The project will test strategies that might be used in human therapies.

“The innovator award will allow me to focus more of my attention on science,” said Petrascheck. “We now have the means necessary to develop the tools that will allow us to determine how sensory perception influences aging and how sensory perception could be targeted by small molecules to treat age-related disease.”

Scripps Florida is Awarded Grant to Create National Anti-Addiction Network

The Florida campus of The Scripps Research Institute has received a multistage cooperative grant to create a national public-private network that will work to combat the nation’s lingering addiction to tobacco.

The new National Institutes of Health (NIH) program will eventually become a broad collaborative effort between academia, the pharmaceutical industry, and charitable organizations to deliver new anti-smoking medicines—in essence the first large-scale federally sponsored tobacco addiction research and drug development center in the United States.

Scripps Florida was awarded $125,000 to complete the first stage of the multistage cooperative NIH grant. The first stage is a planning stage, which kicks off this month. The leadership team is

well into developing several projects that could influence its chances of next year being chosen as the national center's managing partner.

"We have a number of important objectives for the coming year, including a major international scientific symposium with tobacco addiction experts from academia, the Food and Drug Administration, the NIH, and the pharmaceutical industry," said Patrick R. Griffin, chairman of the Department of Molecular Therapeutics and director of the Translational Research Institute at Scripps Florida, and program director of the new project.

Griffin will collaborate with Scripps Florida Associate Professor Paul J. Kenny, a noted addiction expert and the grant's principal investigator, to host this symposium and to create a Web portal that will include a vast range of tobacco addiction data—basically, everything there is to know scientifically about the issue will be available on the site. This all-encompassing resource will be available to public, providing information about the addiction, which kills approximately 440,000 Americans each year, according to the National Institute on Drug Abuse, and costs the nation $160 billion annually. One in every five American deaths is the direct result of smoking.

Griffin and Kenny will also conduct an extensive review of the science of tobacco addiction, which will summarize the data from the new website, outcomes from the symposium, and other findings by the close of the planning year.

"We intend this review to be the most focused and comprehensive on tobacco addiction to date," Griffin said.

Currently, there are six active drug discovery research programs at Scripps Florida, all supported by the NIH, aimed at developing novel compounds with the potential to help smokers quit.

In January of this year, for example, Kenny identified a novel pathway in the brain that regulates an individual's vulnerability to the addictive properties of nicotine. Kenny's laboratory is already working on research in collaboration with scientists at the University of Pennsylvania to develop new drugs that could decrease the addictive properties of nicotine.

The Griffin lab has recently developed novel modulators of the nuclear receptor PPARG, a target currently being investigated in clinical trials for smoking cessation. "Our compounds may offer a significant advantage in terms exposure to the target in the brain, as well as a much-improved side effect profile compared with the drug currently being evaluated in the clinic," noted Griffin.

Scripps Florida Grant Supports Advanced Technology

Scripps Research 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 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 on protein and receptor dynamics and on transcriptional complexes that have been associated with diseases such as diabetes and cancer. In addition, it will provide training for research technicians, postdoctoral fellows, and graduate students, according to Patrick R. Griffin, chair of the Scripps Florida Department of Molecular Therapeutics.

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

Scripps Florida Scientist Awarded Pair of Unconventional Grants

While scientists depend on grants for their research, applying for them is a long and frequently frustrating process. For one Scripps Research Institute scientist, however, the process was shorter.

William Ja, an assistant professor in the Department of Metabolism and Aging who joined the institute's Florida campus in January 2010, was recently awarded an unsolicited grant of $60,000 by the Glenn Foundation, which supports an array of biomedical research with a strong emphasis on aging studies.

Ja said the award was totally unexpected when he was informed of it at a Glenn Foundation meeting for former scholarship winners.

"I had received a small scholarship for summer work in 2004 when I was a graduate student," he said. "As small as it was, it was very important because it gave me the encouragement to make the move from chemistry to biology. You could say it was the seed that started the early stage of my academic career. Still, I was surprised by the invitation [to the foundation's meeting]."

The real surprise came at the end of the meeting when Ja was told he was to receive a $60,000 grant to continue his work in aging. Ja's award, along with several others, was formally announced by the Glenn Foundation on November 2, 2010.

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.

The Glenn Awards were initiated in 2007 to provide unsolicited funds to researchers investigating the biology of aging. The grants are to assist scientists where funding shortages threaten to impede scientific progress. Award recipients are selected from nominees provided by an anonymous scientific advisory committee. Applications are not accepted.

A Second Unconventional Grant

Ja has also recently received a two-year grant of approximately $200,000 for the study of non-surgical sterilization methods for dogs and cats. The award, named the Michelson Prize, came from the newly created Found Animals Foundation, a privately funded, non-profit organization dedicated to minimizing shelter euthanasia. Ja was one of the first to apply for it, even though it was outside his previous field of research.

"I saw an advertisement for it while I was still at Caltech and thought it would be an interesting project," he said. "A few of us threw out a bunch of ideas, most of which had been attempted 20 years ago. But I came up with something different, sent it in, and it got favorably reviewed. With a few modifications, we're already working on it."

The project involves using a cytotoxin – a cell killer – to attack the cells critical to reproduction in dogs and cats by targeting the follicle stimulating hormone receptor (FSHR), which is found in certain cells of the sexual organs. Blocking or destroying these cells can lead to infertility.

"What makes this study so interesting," Ja said, "is that we're going to use some of the tools we develop to ask some basic scientific questions, so what we're doing is a nice combination of basic and applied science." The development of the tools, he said, is just as important as finding the right cytotoxin.

"Even if the toxin or the target isn't the right one," he said, "we'll be able to share these tools with other labs and quickly mix-and-match other molecules for testing. The foundation has been very understanding about our approach. They said, 'Go for it.'"

Susana Valente Awarded Grant from Landenberger Foundation to Study HIV

Susana T. Valente, an assistant professor in the Department of Infectology, has received a two-year $240,000 grant from the Philadelphia-based Margaret Q. Landenberger Research Foundation. The grant will fund the expansion of her study of the many genes and protein products that HIV, the virus that causes AIDS, uses to live inside human cells. These unique

molecular interactions between retrovirus and host cell are potential therapeutic targets that might be exploited to disarm the virus without endangering the viability of the cell.

"I'm honored to receive this award from the Landenberger Foundation," Valente said. "This award will help me continue to focus on understanding how viruses like HIV use the machinery of host cells to replicate, as well as to explore some novel ways to prevent that from happening. The foundation has been very supportive of the work of Scripps Florida scientists, and I'm pleased that they selected our laboratory for this latest award."

Valente is the fifth Scripps Florida scientist to receive an award from the foundation in the last four years, an exceptional showing in a highly competitive environment. In the future, all awards will likely be restricted to a single grant per year per institution, according to a Landenberger spokesman. Previous grantees include Assistant Professor Nagi Ayad, Associate Professor Paul Kenney, Assistant Professor Michael Conkright, and Professor Donny Strosberg.

Part 3: Scientific Accomplishments

Researchers Shed Light on How Serotonin Works

Scripps Research Institute scientists have shown for the first time that the neurotransmitter serotonin uses a specialized signaling pathway to mediate biological functions that are distinct from the signaling pathways used by hallucinogenic substances. The new findings could have a profound effect on the development of new therapies for a number of disorders, including schizophrenia and depression.

The study was published in the October 6, 2010 issue of the *Journal of Neuroscience*.

Serotonin has tremendous influence over several brain functions, including the control of perception, cognition, sleep, appetite, pain, and mood and mediates these effects through interactions with receptors located throughout the central and peripheral nervous systems.

"Our study shows that while both serotonin and hallucinogens act at the serotonin 2A receptor, serotonin utilizes a very specific pathway and its actions are independent of those produced by hallucinogens," said Laura Bohn, an associate professor on the Florida campus of The Scripps Research Institute. "Future drug discovery efforts to identify lead compounds for treatment of depression may consider focusing upon those that only engage that pathway. This work may also lend insight into the mechanisms that underlie the hallucinations that occur in schizophrenia."

This may be particularly important, Bohn said, for the treatment of depression because traditional therapies, which focus on elevating serotonin levels, can sometimes produce serious side effects such as a serotonin syndrome. This syndrome is often accompanied by

hallucinations, and is especially serious when antidepressant treatments such as selective serotonin reuptake inhibitors (SSRIs) are mixed with monoamine oxidase inhibitors (MAOIs).

The scientists' current study supports a long-standing hypothesis that hallucinations may arise from the metabolites formed from elevated serotonin levels. Since there is a difference in the way the two neurotransmitters signal, this may represent a means to preserve the effects of serotonin while preventing the adverse side effects caused by the metabolites.

Serotonin Versus Hallucinogens

The study, coauthored by Cullen Schmid, a graduate student in the lab, showed that serotonin signals through the serotonin 2A receptor by recruiting a regulatory protein called βarrestin2, and that the actions of serotonin at the receptor are far different than those produced by hallucinogenic N-methyltryptamines, a class of naturally occurring substances found in several plants and in minute amounts in the human body and which includes the abused drug, DMT. The study found that the N-methyltryptamines activate the serotonin 2A receptor independently of βarrestin2.

Both serotonin and the N-methyltryptamines produce what is known as a head twitch response in animal models, which indicates that the serotonin 2A receptor has been activated. Any interruption in the exclusive serotonin pathway prevents that behavioral response to serotonin, but has no effect on N-methyltryptamine-induced head twitches, indicating a distinct divergence in the signaling pathways utilized by these two neurotransmitters.

"Despite the fact that they activate the same receptor, serotonin leads to the assembly of a number of proteins associated with the receptor that the metabolites of serotonin do not produce," Bohn said. "But whether the lack of this complex formation is why compounds like DMT lead to hallucinations is not clear."

Bohn continues to investigate these and other questions.

In addition to Bohn, the study, "Serotonin, But Not N-Methyltryptamines, Activates the Serotonin 2A Receptor via an βArrestin2/Src/Akt Signaling Complex *in Vivo*," was authored by Cullen L. Schmid of The Ohio State University Neuroscience Graduate Studies Program and Scripps Research. See http://www.jneurosci.org/cgi/content/abstract/30/40/13513

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

Study Challenges Conventional Theory of Modern Drug Design

Scientists from The Scripps Research Institute have uncovered new evidence that challenges the current theory about a process key to the way modern drugs are designed and how they work in the human body.

The new study was published October 10, 2010 in an advance, online edition of the journal *Nature Chemical Biology*.

Currently, the theory about ligands – compounds that bind to proteins and trigger a specific biological action – and how they bind to proteins runs along the lines of a one person-one vote paradigm. Ligands are considered to be the relatively static partner in the process, and easily rejected if the protein dramatically changes shape.

In contrast, working with the molecular systems that recognize the hormone estrogen, the new Scripps Research study found that as protein receptors change shape ligands can adapt to that change, binding productively to both active and inactive structures.

"To our great surprise, the ligand bound differently to the active and inactive conformations of the receptor," said Kendall Nettles, an associate professor in the Department of Cancer Biology at Scripps Florida. "This strongly suggests a novel mechanism for managing [cell] signaling activity. The implications of this are profound, both for our understanding of how ligands regulate protein activity, and as a novel approach in drug discovery."

Changing the Drug Discovery Model

In the current study, the scientists worked with a receptor (which binds substances triggering certain biological effects) for the hormone estrogen and a well known estrogen receptor antagonist (which blocks the receptor). Estrogen receptors are activated by the hormone estrogen, which is one of two primary female sex hormones (the other is progesterone). Disturbances in estrogen levels play a role in number of disorders including cancers, heart disease, and stroke in women.

When ligands bind to a specific subset of receptors, the ligands stabilize specific protein conformations, turning on (or off) molecular switches that control diverse cellular functions. For example, the binding of the breast cancer treatment tamoxifen is specific for the inactive conformation of the estrogen receptor – this locks the receptor in place, blocks the active conformation and prevents tumor growth.

"Our new findings suggest that we need to think not only about an ensemble of protein conformations, but also an ensemble of ligand binding orientations when we think about therapeutic compounds," Nettles said. "As the protein and ligand move together, each can have a unique affinity, and activity profile, which working *together* defines the signaling output."

Nettles is excited by the possibility the new study suggests of working with an ensemble of ligand conformations, perhaps combining one with anti-inflammatory properties – which play a role in cancer – with another that blocks tumor growth. "This would give you dual therapeutic activity, potentially doubling the effectiveness of the treatment," he said.

Nettles is also eager to find out whether the new study's findings apply to other ligand-protein pairs. "If ligand dynamics turn out to be a general feature of small molecule signaling," he said, "then our findings have the potential to transform how we think about chemical biology."

The first authors of the study, "Coupling of receptor conformation and ligand orientation determine graded activity," are John Bruning of The Scripps Research Institute and Alex A. Parent of the University of Illinois. In addition to Nettles, Bruning, and Parent, other authors include German Gil, Min Zhao and Jason Nowak of The Scripps Research Institute; Margaret C. Pace and Carolyn L. Smith of Baylor College of Medicine; Pavel V. Afonine and Paul D. Adams of the Lawrence Berkeley National Laboratory; and John A. Katzenellenbogen of the University of Illinois. See http://www.nature.com/nchembio/journal/vaop/ncurrent/abs/nchembio.451.html

The study was supported by The National Institutes of Health.

Scientists Identify New Mechanism Regulating Daily Biological Rhythms

Scientists from the Florida campus of The Scripps Research Institute have identified for the first time a novel mechanism that regulates circadian rhythm, the master clock that controls the body's natural 24-hour physiological cycle. These new findings could provide a new target not only for jet lag, shift work, and sleep disturbances, but also for disorders that result from circadian rhythm disruption, including diabetes and obesity as well as some types of cancer.

The study is published in the November 10, 2010 edition (Volume 285, Number 45) of the *Journal of Biological Chemistry*.

"It's well known that the nuclear receptors RORα and REV-ERBα regulate expression of the gene BMAL1, which is vital to virtually every aspect of human physiology and a core component of the circadian clock," said Tom Burris, a professor in the Department of Molecular Therapeutics at Scripps Florida who led the study. "BMAL1 functions as an obligate heterodimer (only working as a dimer with a partner) with either CLOCK or NPAS2 so it was unclear how RORα and REV-ERBα could control this complex. In this study, we show that both partners are targets. As we understand more about the relationship between these receptors and their gene targets, we can consider the possibility of modulating the body's core clock, especially as we continue to develop synthetic ligands targeting these two nuclear receptors."

Circadian rhythms are conserved across a wide variety of organisms, from *Drosophila* (fruit flies) to humans. In mammals, these rhythms respond to light signals and are controlled by the

"master clock" in the brain. In the periphery, semi-autonomous clocks can respond to signals from the brain and from other cues including nutrient status

Disorders linked to dysfunctional circadian rhythms can be severe and potentially deadly, Burris said.

"When you're dealing with circadian rhythm, the most obvious disease target is sleep – for people who do shift work, critical jobs like police work, fire fighting, and medicine," he said. "If circadian rhythm is disrupted, you're prone to metabolic disorders like diabetes and obesity and even breast cancer – because the core clock is closely linked to the cell cycle. If your clock goes awry, you run the risk of your cell cycle going awry as well."

The Role of Nuclear Receptors

Nuclear receptors are proteins that recognize and regulate hormones as well as other molecules. As a result, they control an organism's metabolism by activating gene expression.

The study found that oscillations in the expression of RORα and REV-ERBα not only influence the pattern of circadian expression of BMAL1, but also of NPAS2, a protein that is part of the circadian clock. The fact that NPAS2 is a target of both receptors suggests that there is a specific mechanism that coordinates the relative levels of each receptor to maintain correct circadian function.

"Based on the fact that BMAL1 and NPAS2 work together within the circadian clock, it seems highly unlikely that these two nuclear receptors would only regulate one of them," Burris said. "Our study shows for the first time that, like BMAL1, NPAS2 is also a direct target for RORα and REV-ERBα. This discovery makes this complex a very good therapeutic target." The expression of RORα and REV-ERBα follows a 24-hour circadian pattern (with opposing phases) leading to the correct circadian pattern of gene expression of BMAL1 and NPAS2.

"We think it's something of a competition between these two receptors for binding to promoters of these genes that triggers either the activation (RORα) or repression (REV-ERBα) of the gene," Burris said.

Nuclear receptors make tempting drug targets because they can bind directly to DNA and activate genes through specific ligands—molecules that affect receptor behavior—such as the sex hormones, vitamins A and D, and glucocorticoids, which modulate the body's response to stress. Nuclear receptors have been implicated in a number of cancers, including prostate, breast, and colon cancers, and other diseases as well, including type 2 diabetes, atherosclerosis, and metabolic syndrome.

The other important aspect of nuclear receptors is their practicality. Scientists can design small molecule therapeutics to force them to change their ways. Burris said that he has already identified several new synthetic ligands (drug like molecules) for both receptors.

The first author of the study, "Characterization of the Core Mammalian Clock Component, NPAS2, as a REV—ERBα/RORα Target Gene," is Christine Crumbley of The Scripps Research Institute. Others authors include Yongjun Wang and Douglas J. Kojetin, also of Scripps Research. For more information, see http://www.jbc.org/content/285/46/35386.abstract

This work was funded by the National Institutes of Health.

Scientists Identity First Synthetic Activator of Two Critical Proteins

Scientists from the Florida campus of The Scripps Research Institute have identified a novel synthetic activator of a pair of proteins that belong to a protein family playing key roles in human metabolism and immune function. The discovery could provide new and potentially more effective therapeutic approaches to diseases ranging from diabetes to osteoporosis.

The study was published in the November issue (Volume 5, Issue 11) of the journal *ACS Chemical Biology*.

"This new compound is particularly important because it works *in vivo*, and it is selective for certain receptors," said Tom Burris, a professor in the Department of Molecular Therapeutics at Scripps Florida who led the study. "These two properties give it significant potential as a possible therapeutic compound."

The new discovery represents the very first synthetic ligand (binding partner) that functions as an agonist (activator) of retinoid-related orphan (ROR) nuclear receptor. Nuclear receptors are protein molecules that mediate hormone activity inside the cell; they have been implicated in the progress of a number of cancers, and have also become drug development targets for diseases including type 2 diabetes, atherosclerosis, and metabolic syndrome.

Although scientists don't know the full therapeutic significance of the new synthetic ligand, its potential usefulness is clear, Burris noted.

"For example, loss of RORα in animal models renders them resistant to weight gain," he said, "while RORγ has been shown to be involved in development of cells that are implicated in autoimmune diseases – and loss of RORγ results in animals that are resistant to these types of disease."

RORα has also been shown to be required for normal bone development; animal models lacking this receptor develop osteoporosis, strongly suggesting that RORα agonists may have potential as a treatment of this disease. Osteoporosis affects as many as 44 million Americans, according to the National Institutes of Health. Burris and his colleagues also discovered a pathway stimulating liver secretion of FGF21—which has been shown to treat diabetic animals—via activation of

ROR. Diabetes is estimated to affect 23.6 million Americans, according to the National Institutes of Health.

Second Major Discovery

This new agonist is the second that Burris and his Scripps Florida colleagues have identified.

In 2009, Burris and Patrick R. Griffin, chair of the Department of Molecular Therapeutics and director of the Translational Research Institute at Scripps Florida, identified a high affinity synthetic *inverse* agonist of this same pair of nuclear receptors. An inverse agonist, which binds to the same site as an agonist, induces the *opposite* action of an agonist of that receptor. For this new study, Burris said they used that first discovery, a compound known as T1317, as a molecular scaffold to synthesize an array of compounds and assess their activity against a number of receptors, including RORα and RORγ.

The one compound that stood out was SR1078, which displayed a unique pharmacological profile that indicated it had a high potential for use as a chemical probe for assessing ROR receptor function in general.

"Unexpectedly, we found that SR1078 functioned as a ROR agonist," Burris said. "When we treated cells with SR1078 we got a significant increase in RORα transcription. Similarly, with RORγ, SR1078 treatment resulted in a stimulation of RORγ dependent transcription activity. Basically, it produced more of these receptor proteins, significantly so."

The first author of the study, "Identification of SR1078, a Synthetic Agonist for the Orphan Nuclear Receptors RORα and RORγ," is Yongjun Wang of Scripps Research. Others authors include Naresh Kumar, Philippe Nuhant, Michael D. Cameron, Monica A. Istrate, William R. Roush, and Patrick R. Griffin, also of Scripps Research. For more information on the study, see http://pubs.acs.org/doi/abs/10.1021/cb1002575

The study was supported by the National Institutes of Health.

Team Develops Groundbreaking Technology to Detect Alzheimer's Disease

Scientists from the Florida campus of The Scripps Research Institute, have developed a novel technology that is able to detect the presence of immune molecules specific to Alzheimer's disease in patients' blood samples. While still preliminary, the findings offer clear proof that this breakthrough technology could be used in the development of biomarkers for a range of human diseases.

The study, led by Scripps Research Professor Thomas Kodadek, was published in the January 7, 2011 edition of the journal *Cell*.

Traditionally, antigens—a substance such as a protein from a virus or bacteria that stimulates an immune response—have been necessary for the discovery of antibody biomarkers. There has previously been no way to identify an antibody (a type of targeted immune molecule) without first knowing the antigen that triggers its production. The new study, however, challenges conventional wisdom and uses synthetic molecules rather than antigens to successfully detect signs of disease in patients' blood samples.

These synthetic compounds have many advantages – they can be modified easily and can be produced quickly in relatively large amounts at lower cost.

"Dr. Kodadek has conceived of a new approach for identifying antibody biomarkers of human disease that bypasses the conventional, but difficult, step of identifying the natural antigens or antigen mimics," said James M. Anderson, director of the National Institutes of Health (NIH) Division of Program Coordination, Planning, and Strategic Initiatives, who helps oversee the NIH Common Fund's Pioneer Award Program. "The results in the paper suggest great potential for using this approach to rapidly develop diagnostic biomarkers for a variety of significant human diseases. Such boldness to challenge conventional paradigms to achieve important scientific advances is a hallmark of the NIH Director's Pioneer Award Program, which supported much of this research."

"This study essentially puts an end to the notion that the only way to pull a potentially useful antibody from blood samples is with a specific antigen," said Kodadek. "Because the antigen identification problem has proven to be so difficult, we decided to take it out of the equation."

A Focus on the Immune System

To test the concept, Kodadek and his colleagues used comparative screening of combinatorial libraries of synthetic molecules – peptoids – against serum samples obtained from mice with a multiple-sclerosis-like condition or healthy controls. Those synthetic molecules that retained more immunoglobulin (IgG), a major type of antibody, from the blood samples of the diseased animals were identified as potential agents for capturing diagnostically useful molecules. This worked well.

The team next turned to serum samples from six Alzheimer's patients, six healthy individuals, and six Parkinson's disease patients. Three peptoids were identified that captured at least three-fold higher levels of IgG antibodies from all six of the Alzheimer's patients than any of the control or Parkinson's patients. The results showed that two of the peptoids bind the same IgG antibodies; a third binds different antibodies, resulting in at least two candidate biomarkers for the disease.

"We use these peptoids as a lure to capture the lgG antibodies," Kodadek said. "Some of these synthetic molecules recognize the antigen-binding sites of disease-specific antibodies well enough to pull them from blood samples, although they almost certainly don't bind as well as the native antigens. This ability should make it possible to short circuit the discovery of the natural antigens."

The first author of the study, "Identification of Candidate IgG Antibody Biomarkers for Alzheimer's Disease through Screening of Synthetic Combinatorial Libraries," is M. Muralidhar Reddy of Opko Health Laboratories and Scripps Research.Other authors include Rosemary Wilson and Johnnie Wilson of Opko Health Laboratories; and Steven Connell, Anne Gocke, Linda Hynan, and Dwight German of The University of Texas Southwestern Medical Center. For more information, see http://www.cell.com/abstract/S0092-8674(10)01376-0.

Scientists Show Prions Mutate and Adapt to Host Environment

Scientists from the Florida campus of The Scripps Research Institute have shown that prions, bits of infectious protein that can cause fatal neurodegenerative disease such as bovine spongiform encephalopathy (BSE) or "mad cow disease," have the ability to adapt to survive in a new host environment.

In this regard, although they lack DNA and RNA, they behave much like viruses, producing distinct self-perpetuating structural mutations that provide a clear evolutionary advantage.

The study was published December 14, 2010, in the online Early Edition the journal *Proceedings of the National Academy of Sciences*.

"We found that when a particular prion strain is transferred from brain cells to a different cell line, its properties gradually change, giving rise to a variant strain that is better adapted to this new cellular environment," said Charles Weissmann, the head of Scripps Florida's Department of Infectology, who led the study. "If those same prions are subsequently transferred to another cell line, they change again, adapting to these new host cells. And if returned to the brain, the prions gradually regain their original properties. We found physical evidence that, at least in one case, the fold of the prion changed when its properties changed."

Darwinian Evolution Without DNA

These new findings come approximately one year after Weissmann and colleagues published a study in the January 1, 2010 edition of the journal *Science* that showed that prions were capable of Darwinian evolution.

That earlier study also showed that prions can develop large numbers of mutations and that these mutations can bring about such evolutionary adaptations as drug resistance, a phenomenon previously known to occur only in bacteria and viruses. This study also suggested that the normal prion protein - which occurs naturally in mammalian cells - may prove to be a more effective therapeutic target than its abnormal toxic relation.

"Because prions can adapt to changing environments, it now becomes clear that it will be more difficult than originally thought to find drugs that will work against them," Weissmann said. "But

if you could develop a drug that inhibits formation of the normal prion protein, you could, in essence, starve the infectious prions and prevent them from reproducing. This approach to treatment, although technically demanding, can be envisaged because, as we have shown earlier, deprivation of PrP is not detrimental to health - at least to the health of mice."

Folding and Misfolding

Prions, which are composed solely of protein, are classified by distinct strains, characterized by their incubation time and the disease they cause. In addition to BSE/mad cow disease in cattle, diseases caused by prions include scrapie in sheep, chronic wasting disease in deer, and variant Creutzfeldt-Jakob disease in humans. 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^{C}. During infection, abnormal or misfolded protein - known as PrP^{Sc} - converts the normal host prion protein into its toxic form by changing its conformation or shape. The end-stage consists of large sheets (polymers) of these misfolded proteins, which causes massive tissue and cell damage.

"The infectious prion protein can fold in different ways, and depending on the fold, a different prion strain results," Weissmann said. "As long as prions are maintained in the same host, they retain their characteristic fold, so that strains breed true."

When prions multiply, however, that fold is not always reproduced correctly, so a prion population contains many variants, albeit at low levels.

The new study found that when a prion population is transferred to a different host, one of the variants may replicate faster - an evolutionary advantage - and become the dominant strain. This new population also contains variants, one of which may be selected over others when transferred to a different host.

"The result is that prions, although devoid of genetic material, behave similarly to viruses and other pathogens, in that they can mutate and undergo evolutionary selection," Weissmann said. "They do it by changing their fold, while viruses incur changes in their nucleic acid sequence."

Diverse Yet Related

The new study suggests that prion populations constitute a "quasi-species" similar in nature to RNA viruses and retroviruses, such as flu viruses and HIV.

The idea of a quasi-species was first conceived by Manfred Eigen, a German biophysicist who won the Nobel Prize in Chemistry in 1967. Basically, a quasi-species is a complex, self-perpetuating population of diverse and related entities that act as a whole. It was Weissmann, however, who in 1978 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.

But that's where the comparison ends, Weissmann said.

"The fact that they behave like viruses doesn't mean they're anything like a virus," he said. "A bicycle is like a car in that it gets you from one place to the other, but they're not the same. The end *effect* is the same, however. Prions and viruses are both able to change their structure to survive."

The first author of the study, "Transfer of a Prion Strain to Different Hosts Leads to Emergence of Strain Variants," is Sukhvir P. Mahal of Scripps Research. Other authors include Shawn Browning, Jiali Li, and Irena Suponitsky-Kroyter, also of Scripps Research. For more information, see http://www.pnas.org/content/early/2010/12/13/1013014108.abstract .

The study was supported by the National Institutes of Health and the Alafi Family Foundation.

Scientists Identify Key Interaction in Hepatitis C Virus

Scientists from the Florida campus of The Scripps Research Institute have identified a molecular interaction between a structural hepatitis C virus protein (HCV) and a protein critical to viral replication. This new finding strongly suggests a novel method of inhibiting the production of the virus and a potential new therapeutic target for hepatitis C drug development.

The study was published in the January 2010 issue (Volume 92, Part 1) of the *Journal of General Virology.*

These new data underline the essential role of the viral protein known as "core" as a primary organizer of the infectious HCV particle assembly and support a new molecular understanding of the formation of the viral particle itself.

"While our finding that the HCV core interacts with the non-structural helicase protein was not totally unexpected, this had not really been confirmed until this study," said Scripps Florida Professor Donny Strosberg, who led the study. "But the most exciting part is that small molecule inhibitors of dimerization [the joining of two identical subunits] of core actually inhibit interaction between core and helicase, thus possibly preventing production of an infectious viral particle."

A Viral Plague

Hepatitis C virus infects between 130 and 170 million people worldwide and is the cause of an epidemic of liver cirrhosis and cancer. Because current HCV treatments are only partially effective, a number of alternative molecular mechanisms are actively being pursued as possible drug targets.

One of the critical problems of finding inhibitors for the hepatitis C virus is that it mutates at such prodigious rates. An RNA virus such as hepatitis C can mutate at a rate estimated as high as one million times that of DNA viruses such as the herpes virus.

With this in mind, Strosberg has been examining the core protein, the most conserved protein among all HCV genotypes. Core plays several essential roles in the viral cycle in the host cell. It is particularly important in the assembly of the hepatitis C nucleocapsid or capsid, an essential step in the formation of infectious viral particles; the nucleocapsid is the virus genome protected by a protein coat. By interacting with various structural and non-structural viral proteins, core plays an essential role in the HCV cycle during assembly and release of the infectious virus as well as disassembly of viral particles upon entering host cells. Core also interacts with a number of cellular proteins, possibly contributing to the disarmament of several host defense mechanisms and to the activation of oncogenic pathways.

Last year, Strosberg developed a novel quantitative test for monitoring these protein-protein interactions with the specific goal of identifying inhibitors of the core dimerization, which would block virus production. Strosberg and his colleagues uncovered peptides derived from the core protein of hepatitis C that inhibit not only dimerization of the core protein, but also production of the actual virus.

That earlier study led to the discovery of non-peptidic small organic molecules that strongly inhibited HCV production, one of which, SL201, was used in the new study.

In the new study, Strosberg and his colleagues focused on non-structural proteins that provide functions relating to HCV production, in particular NS3 helicase. The scientists' findings support a growing body of evidence that this protein participates in the assembly and production of infectious viral particles. The interaction of the core protein with this non-structural protein also confirms core as a key organizer of virus assembly and suggests it acts to facilitate the packaging and integration of the newly synthesized viral RNA.

The first author of the study, "Dimerization-Driven Interaction of Hepatitis C Virus Core Protein with Ns3 Helicase," is Guillaume Mousseau of Scripps Research. Additional authors include Smitha Kota and Virginia Takahashi of Scripps Research, and David Frick of the University of Wisconsin, Milwaukee. For more information, see http://vir.sgmjournals.org/cgi/content/abstract/92/1/101 .

The study was supported by the state of Florida, The Factor Foundation, and the National Institutes of Health.

Study Uncovers Switch Controlling Protein Production

A scientist from the Florida campus of The Scripps Research Institute has discovered a molecular switch that controls the synthesis of ribosomes. Ribosomes are the large machineries inside all living cells that produce proteins, the basic working units of any cell. These new findings offer a novel target for potential treatments for a range of diseases, including cancer.

The study is published in the December 24, 2010 edition of the *Journal of Molecular Biology*.

The study identified the molecular switch, essentially formed by a small sequence of RNA, that controls a critical part of ribosome synthesis to allow for strict, albeit temporary, regulation of the process.

"These kinds of switches in RNA are thought to be slow acting," said Katrin Karbstein, an assistant professor in the Department of Cancer Biology at Scripps Florida who helped lead the study. "That suggests a point where we might intervene to modify the process – then you could potentially shut down the pathway, because if you don't produce ribosomes, you cannot make proteins. Thus, cells can't grow. That would be a desirable outcome in cancer, for example."

This slowness may be there precisely so these regulatory points can be introduced for cells to downregulate growth when nutrition is scarce.

"Perhaps, nature has found a way to exploit RNA's Achilles' heel – its propensity to form alternative structures that can lead to protein misfolding, which, in turn, can cause diseases ranging from Alzheimer's to diabetes," Karbstein said. "Nature might be using this to stall important biological processes and allow for quality control and regulation." The synthesis of proteins involves ribosomes, large macromolecular machines required for cell growth in all organisms. Ribosomes read the genetic code carried by messenger RNA and then catalyze or translate that RNA code into proteins within cells, assembling them from amino acids.

To produce mature ribosomal RNAs (rRNAs), the catalysts that control protein synthesis in all cells, the body first needs perfectly formed intermediate or pre rRNAs, which can be further processed into fully functioning ones. The intermediate form is produced as an RNA transcript that is cleaved or cut in multiple steps to produce mature rRNA.

"While we believe that this switch is essential for ribosome assembly, it seems unlikely that this is the only event that regulates cleavage," Karbstein said. "However, tight regulation of ribosome synthesis is essential to ensure the structural integrity of mature ribosomes."

Cutting Extra Material

The ribosomal RNA that is transcribed has extra material in it, Karbstein said, so it is necessary to cut it down – that's why these cuts or cleavages are so essential to the process of producing the final rRNA product.

The study also suggests RNA itself exploits its own natural ability to form these stable structural switches to order and regulate various RNA-dependent biological processes.

"What is interesting," Karbstein said, "is that as the organism becomes more complex, the number of cleavages needed increases. This may make the process more accurate and that may be an evolutionary advantage, but even in bacteria this cutting is not done in a simple way. We still don't know exactly why that is."

Perhaps these strictly ordered cleavage steps are introduced to produce singularly perfect intermediates, she added. This is important because cleavage is an irreversible energy-releasing process with the potential to shift the landscape of assembly towards the final product. As a result, cleavage steps should be carefully controlled and should only occur if the assembly intermediate is correct.

"Ribosomes make mistakes rarely, on the order of one in 10,000 amino acid changes," Karbstein said. "A lot of this accuracy depends on conversations between different parts of the ribosomes, so if the structure of the RNA isn't correct, these conversations can't happen. And that means more mistakes, and that's not good because it can lead to any number of disease states."

For now, Karbstein said she's interested in looking at small molecules that perturb the switch, and finding out if this affects the quality of the ribosomes produced.

"Certain kinds of antibiotics work by making the ribosomes produce more mistakes – it's not a huge increase but it's enough to make these cells die," she said. "Maybe we can find molecules that similarly lead to the production of 'worse' ribosomes."

In addition to Karbstein, Allison C. Lamanna is an author of the study, "An RNA Conformational Switch Regulates Pre-18S rRNA Cleavage" (doi:10.1016/j.jmb.2010.09.064).

The study was supported by the National Institutes of Health.

Chemists Devise New Method to Quantify Protein Changes

A scientist from the Florida campus of The Scripps Research Institute has devised a new method of analyzing and quantifying changes in proteins that result from a common chemical process. The new findings could provide new insights into the effects of a highly destructive form of stress on proteins in various disease models, particularly cancer.

The study, published January 5, 2011, in the online Early View of the journal *Angewandte Chemie*, was designated by the journal as a “very important paper,” a distinction bestowed on less than five percent of its publications.

"This new technique allows us to home in on which proteins are modified to a significant extent during periods of stress and how that changes during disease progression," said Kate Carroll, an associate professor in the Scripps Research Department of Chemistry who conducted the study with Young Ho Seo, a research fellow at The University of Michigan. "It gives us the chance to look more closely at targets for possible therapeutic intervention. From a practical standpoint, the technique is simple and will be accessible to biologists and chemists alike."

The new technique focuses on the process of cysteine S-hydroxylation, which plays a significant role in a number of events related to physiology in both health and disease, including the regulation of signaling proteins in various disease states.

The ability of the new technique to focus on signaling pathways, particularly in diseases such as cancer, is critical.

"Chronic disease states such as cancer can involve the modification of signaling proteins through S-hydroxylation, but other housekeeping proteins may also be targets," she said. "Key to distinguishing which of these proteins may be involved in pathogenesis is the ability to measure the amount of S-hydroxylation at specific sites within a protein. Now you'll be able to tell. This should help accelerate target identification in these disease-related signaling pathways and allow us to focus on proteins that are important to the process."

During periods of cellular stress, caused by factors such as exposure to UV radiation or many disease states, the level of highly reactive oxygen-containing molecules can increase, resulting in inappropriate modification of proteins and cell damage.

One oxidant, hydrogen peroxide, functions as a messenger that can activate cell proliferation through oxidation of cysteine residues in signaling proteins, producing sulfenic acid (i.e., S-hydroxylation); cysteine is an amino acid is synthesized in the body.

Extending the Gains of an Earlier Study

In a 2009 study, Carroll found that sulfenic acid served as an early warning biomarker of the reaction between hydrogen peroxide and cysteine. Carroll tagged the miniscule reaction target with a fluorescent dye antibody. With it, Carroll was able to read the levels of sulfenic acid levels in various cell lines, including breast cancer cells.

The new technique takes those findings several steps further by allowing scientists not only to quantify the modifications to various proteins, but also to monitor these changes at the level of individual cysteines within a single protein.

Carroll used a class of reagents called isotope-coded dimedone and iododimedone, which traps and tags sulfenic acids, allowing the cysteine sites and modified proteins to be easily identified. These probes, which are highly selective for sulfenic acid, allow the S-hydroxylation process to be monitored at the exact site of the modification.

The tagged proteins can be then be analyzed by mass spectrometry, a standard technology used to determine the precise make-up of proteins and other molecules.

“This technique should be widely accessible to the scientific community because it’s so simple,” Carroll said. “It should allow researchers to identify proteins with altered S-hydroxylation profiles whose function may lend insight into events in disease progression and have utility as potential markers for disease detection.”

The study, “Quantification of Protein Sulfenic Acid Modifications Using Isotope-Coded Dimedone and Iododimedone (ICDID*),*” was funded by the Camile Henry Dreyfus Teacher Scholar Award and the American Heart Association Scientist Development Award. For more information on the paper, see
http://onlinelibrary.wiley.com/doi/10.1002/anie.201007175/abstract

Scientists Reveal Key Mechanism Governing Nicotine Addiction

Scientists from the Florida campus of The Scripps Research Institute have identified a pathway in the brain that regulates an individual's vulnerability to the addictive properties of nicotine. The findings suggest a new target for anti-smoking therapies.

The study appeared January 30, 2011, in an advance, online issue of the journal *Nature*.

In the study, the scientists examined the effects of a part of a receptor (a protein molecule to which specific signaling molecules attach) that responds to nicotine in the brain. The scientists found that animal models with a genetic mutation inhibiting this receptor subunit consumed far more nicotine than normal. This effect could be reversed by boosting the subunit's expression.

"We believe that these new data establish a new framework for understanding the motivational drives in nicotine consumption and also the brain pathways that regulate vulnerability to tobacco addiction," said Scripps Research Associate Professor Paul Kenny, who led the study. "These findings also point to a promising target for the development of potential anti-smoking therapies."

Specifically, the new study focused on the nicotinic receptor subunit α5, in a discrete pathway of the brain called the habenulo-interpeduncular tract. The new findings suggest that nicotine activates nicotinic receptors containing this subunit in the habenula, triggering a response that acts to dampen the urge to consume more of the drug.

"It was unexpected that the habenula, and brain structures into which it projects, play such a profound role in controlling the desire to consume nicotine," said Christie Fowler, the first author of the study and research associate in the Kenny laboratory. "The habenula appears to be activated by nicotine when consumption of the drug has reached an adverse level. But if the

pathway isn't functioning properly, you simply take more. Our data may explain recent human data showing that individuals with genetic variation in the α5 nicotinic receptor subunit are far more vulnerable to the addictive properties of nicotine, and far more likely to develop smoking-associated diseases such as lung cancer and chronic obstructive pulmonary disease."

A Previously Unknown Pathway Inhibits Motivation

Tobacco smoking is one of the leading causes of death worldwide, with more than five million people dying each year as a result of it, according to statistics cited in the study. Smoking is considered the cause of more than 90 percent of lung cancer deaths. Scientists have established that a tendency towards smoking can be inherited – more than 60 percent of the risk of becoming addicted to nicotine can be laid at the door of genetic factors.

Nicotine is the major addictive component of tobacco smoke, and nicotine acts in the brain by stimulating proteins called nicotinic acetylcholine receptors (nAChRs). These nAChRs are made up of different types of subunits, one of which is the α5 subunit—the focus of the new study.

In their experiments, the Scripps Research scientists set out to determine the role of nAChRs-containing α5 subunits (α5* nAChRs) in regulating nicotine consumption.

First, the team assessed the addictive properties of nicotine in genetically altered mice lacking α5* nAChRs. The results showed that when these "knockout" mice were given access to high doses of nicotine, they consumed much larger quantities than normal mice. Next, to determine if the subunit was responsible for the sudden shift in appetite for nicotine, the scientists used a virus that "rescued" the expression of α5* nAChRs only in the medial habenula and areas of the brain into which it projects. The results showed the nicotine consumption patterns of the knockout mice returned to a normal range.

The scientists repeated the experiments with rats and produced similar results. In this case, the scientists used a virus to "knock out" α5 nAChR subunits in the medial habenula. When the α5* nAChRs were decreased, the animals were more aggressive in seeking higher doses of nicotine. When the subunit remained unaltered, the animals showed more restraint.

The scientists then worked out the biochemical mechanisms through which α5* nAChRs operate in the medial habenula to control the addictive properties of nicotine. They found that α5* nAChRs regulate just how responsive the habenula is to nicotine, and that the habenula is involved in some of the negative responses to nicotine consumption. So when α5* nAChRs do not function properly, the habenula is less responsive to nicotine and much more of the drug can be consumed without negative feedback from the brain.

The scientists are optimistic that their findings may one day lead to help for smokers who want to kick the habit. Based on the new findings, the Scripps Florida scientists have started a new program of research in collaboration with scientists at the University of Pennsylvania to develop new drugs to boost α5* nAChR signaling and decrease the addictive properties of nicotine.

In addition to Kenny and Fowler, authors of the paper, "Habenular α5* Nicotinic Receptor Signaling Regulates Nicotine Intake," include Qun Lu and Paul M. Johnson of Scripps Research and Michael J. Marks of the University of Colorado, Boulder. For more information, see http://www.nature.com/nature/journal/vaop/ncurrent/abs/nature09797.html.

The study was funded by the National Institutes of Health and The James and Esther King Biomedical Research Program, Florida Department of Health.

Scripps Research Molecular Screening Center Work Leads to First Clinical Trial

The Scripps Research Institute Molecular Screening Center has become the first of such National Institutes of Health (NIH) centers to yield a drug candidate tested in humans. The compound is a potential treatment for multiple sclerosis.

"Although many MLP [Molecular Library Probe] probes have been tested in *in vivo* studies (e.g., rat models), this is the first example of an MLP probe to have been further developed far enough along the drug discovery pipeline to be tested in humans, a landmark achievement for the program," notes the NIH Molecular Libraries Program website.

Compound Blocks Brain Cell Destruction in Parkinson's Disease

Scientists from the Florida campus of The Scripps Research Institute have produced the first known compound to show significant effectiveness in protecting brain cells directly affected by Parkinson's disease, a progressive and fatal neurodegenerative disorder.

Although the findings were in animal models of the disease, the effectiveness of the compound, combined with its potential to be taken orally, offers the tantalizing possibility of a potentially useful future therapy for Parkinson's disease patients.

The results were published in two separate studies in the journal *ACS Chemical Neuroscience*.

"These studies present compelling data on the first oral, brain-penetrating inhibitor to show significant efficacy in preventing neurodegeneration in both mouse and rat models of Parkinson's disease," said team leader Philip LoGrasso, a professor in the Department of Molecular Therapeutics and senior director for drug discovery at Scripps Florida. "The compound offers one of the best opportunities we have for the development of an effective neuroprotective treatment."

The new small molecule—labeled SR-3306—is aimed at inhibiting a class of enzymes called c-jun-N-terminal kinases (JNK). Pronounced "junk," these enzymes have been shown to play an important role in neuron (nerve cell) survival. As such, they have become a highly viable target for drugs to treat neurodegenerative disorders such as Parkinson's disease.

"A drug like SR-3306 that prevents neurodegeneration would be a quantum leap in the clinical treatment of Parkinson's because all current therapies treat only the symptoms of the disease, not the underlying pathologies," LoGrasso said.

Patients with Parkinson's disease suffer from the loss of a group of neurons in the substantia nigra pars compacta (SNpc), part of the midbrain involved in motor control. These cells produce dopamine, a neurotransmitter that plays a key role in motor reflexes and cognition. The disease also affects projecting nerve fibers in the striatum, a part of the forebrain filled with cells that interact with dopamine.

Stopping the Progression of Neuron Destruction in Animal Models

The SR-3306 compound, which has been in development at Scripps Florida for several years, performed well in both cell culture and animal models. In cell culture, the compound showed greater than 90 percent protection against induced cell death of primary dopaminergic neurons, while in mouse models of induced neuron death, the compound showed protective levels of approximately 72 percent.

The scientists went one step further, testing the new compound in a rat model, which duplicates the physical symptoms often seen with the human disease—a pronounced and progressive loss of motor skills. The results showed SR-3306 provided a protection level of approximately 30 percent in the brain, a level that reduced the dysfunctional motor responses by nearly 90 percent.

"It was a surprise that level of neuroprotection reduced the behavioral impact so strongly," LoGrasso said, "but it's indicative of how it might perform in human patients. While SR-3306 doesn't represent a cure, it does appear to have the potential of stopping the progression of the disease."

The new studies are part of a $7.6 million multiyear grant awarded to LoGrasso in 2008 by the National Institutes of Neurological Disorders and Stroke (NINDS). The grant will enable Scripps Research and potential partners to file an application for an investigational new drug (IND)—the first step in the lengthy clinical trials process required by the U.S. Food and Drug Administration before a new drug can be brought to market.

The first authors of the study, "Small Molecule c-jun-N-terminal Kinase (JNK) Inhibitors Protect Dopaminergic Neurons in a Model of Parkinson's Disease," are Jeremy W. Chambers and Alok Pachori of Scripps Research. Other authors include Shannon Howard, Michelle Ganno, Donald Hansen Jr., Ted Kamenecka, Xinyi Song, Derek Duckett, Weimin Chen, Yuan Yuan Ling, Lisa Cherry, Michael D. Cameron, Li Lin, and Claudia H. Ruiz, also of Scripps Research. See http://pubs.acs.org/doi/abs/10.1021/cn100109k .

The first author of the study, "JNK Inhibition Protects Dopamine Neurons and Provides Behavioral Improvement in a Rat 6-hydroxydopamine Model of Parkinson's Disease," is Candice E. Crocker of Dalhousie University, Halifax, Nova Scotia, Canada. Other authors include Susan Khan and Michael D. Cameron of Scripps Research, and Harold A. Robertson and George S. Robertson of Dalhousie. See http://pubs.acs.org/doi/abs/10.1021/cn1001107 .

Both studies were supported by the National Institutes of Health. Harold A. Robertson and George S. Robertson were supported by funding from the Atlantic Innovation Fund.

Scripps Research Scientists Create Cell Assembly Line

Borrowing a page from modern manufacturing, scientists from the Florida campus of The Scripps Research Institute have built a microscopic assembly line that mass produces synthetic cell-like compartments.

The new computer-controlled system represents a technological leap forward in the race to create the complex membrane structures of biological cells from simple chemical starting materials.

"Biology is full of synthetic targets that have inspired chemists for more than a century," said Brian Paegel, Scripps Research assistant professor and lead author of a new study published in the *Journal of the American Chemical Society*. "The lipid membrane assemblies of cells and their organelles pose a daunting challenge to the chemist who wants to synthesize these structures with the same rational approaches used in the preparation of small molecules."

While most cellular components such as genes or proteins are easily prepared in the laboratory, little has been done to develop a method of synthesizing cell membranes in a uniform, automated way. Current approaches are capricious in nature, yielding complex mixtures of products and inefficient cargo loading into the resultant cell-like structures.

The new technology transforms the previously difficult synthesis of cell membranes into a controlled process, customizable over a range of cell sizes, and highly efficient in terms of cargo encapsulation.

The membrane that surrounds all cells, organelles and vesicles—small subcellular compartments—consists of a phospholipid bilayer that serves as a barrier, separating an internal space from the external medium.

The new process creates a laboratory version of this bilayer that is formed into small, cell-sized compartments.

How It Works

"The assembly-line process is simple and, from a chemistry standpoint, mechanistically clear," said Sandro Matosevic, research associate and co-author of the study.

A microfluidic circuit generates water droplets in lipid-containing oil. The lipid-coated droplets travel down one branch of a Y-shaped circuit and merge with a second water stream at the Y-junction. The combined flows of droplets in oil and water travel in parallel streams toward a triangular guidepost.

Then, the triangular guide diverts the lipid-coated droplets into the parallel water stream as a wing dam might divert a line of small boats into another part of a river. As the droplets cross the oil-water interface, a second layer of lipids deposits on the droplet, forming a bilayer.

The end result is a continuous stream of uniformly shaped cell-like compartments.

The newly created vesicles range from 20 to 70 micrometers in diameter—from about the size of a skin cell to that of a human hair. The entire circuit fits on a glass chip roughly the size of a poker chip.

The researchers also tested the synthetic bilayers for their ability to house a prototypical membrane protein. The proteins correctly inserted into the synthetic membrane, proving that they resemble membranes found in biological cells.

"Membranes and compartmentalization are ubiquitous themes in biology," noted Paegel. "We are constructing these synthetic systems to understand why compartmentalized chemistry is a hallmark of life, and how it might be leveraged in therapeutic delivery."

"Stepwise Synthesis of Giant Unilamellar Vesicles on a Microfluidic Assembly Line," was published February 10, 2011. The research was supported by the National Institutes of Health. For more information, see http://pubs.acs.org/doi/abs/10.1021/ja109137s.

Scientists Develop Compound that Effectively Halts Progression of Multiple Sclerosis

Scientists from the Florida campus of The Scripps Research Institute have developed the first of a new class of highly selective compounds that effectively suppresses the severity of multiple sclerosis in animal models. The new compound could provide new and potentially more effective therapeutic approaches to multiple sclerosis and other autoimmune diseases that affect patients worldwide.

The study appeared April 17, 2011, in an advance online edition of the journal *Nature*.

Current treatments for autoimmunity suppress the patient's entire immune system, leaving patients vulnerable to a range of adverse side effects. Because the new compound, known as SR1001, only blocks the actions of a specific cell type playing a significant role in autoimmunity, it appears to avoid many of the widespread side effects of current therapies.

"This is a novel drug that works effectively in animal models with few side effects," said Tom Burris, a professor in the Department of Molecular Therapeutics at Scripps Florida who led the study, which was a multidisciplinary collaboration with scientists including Patrick Griffin, William Roush, and Ted Kamenecka of Scripps Research, and Paul Drew of the University of Arkansas for Medical Sciences. "We have been involved in several discussions with both pharmaceutical and biotechnology firms who are very interested in developing it further."

A lengthy process of drug development and review is required to ensure a new drug's safety and efficacy before it can be brought to market.

"This impressive multidisciplinary team has used a combined structural and functional approach to describe a class of molecules that could lead to new medicines for treating autoimmune diseases," said Charles Edmonds, who oversees structural biology grants at the National Institutes of Health. "Breakthroughs such as this highlight the value of scientists with diverse expertise joining forces to solve important biological problems that have the potential to benefit human health."

Targeting Specific Receptors

For the past several years, Burris and his colleagues have been investigating small-molecule compounds that affect particular disease-related receptors (structures that bind other molecules, triggering some effect on the cell). In particular, the scientists have been interested in a pair of "orphan nuclear receptors" (receptors with no known natural binding partner) called RORα and RORβ involved in both autoimmune and metabolic diseases.

These particular receptors play a critical role in the development of TH17 cells, a form of T helper cells that make up part of the immune system. A relatively new discovery, TH17 cells have been implicated in the pathology of numerous autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and lupus. TH17 cells produce Interleukin-17, a natural molecule that can induce inflammation, a characteristic of autoimmunity.

"If you eliminate TH17 cell signals, you basically eliminate the disease in animal models," Burris said. "Our compound is the first small-molecule orally active drug that targets this specific cell type and shuts it down. Once SR1001 is optimized, chances are it will be far more potent and effective."

The compound works without affecting other types of T helper cells and without any significant metabolic impact, Burris added.

The first author of the study, "Inhibition of TH17 Differentiation and Suppression of Autoimmunity by a Selective Synthetic ROR Ligand," is Laura A. Solt of Scripps Research. In addition to Burris, Griffin, Roush, Kamenecka, Drew, and Solt, other authors include Naresh Kumar, Philippe Nuhant, Yongjun Wang, Janelle L. Lauer, Jin Liu, and Monica Istrate of Scripps Research; Dušica Vidović, Stephan C. Schŭrer of Scripps Research and the Center for Computational Science, University of Miami; and Jihong Xu and Gail Wagoner of the University of Arkansas for Medical Sciences. See http://www.nature.com/nature/journal/vaop/ncurrent/abs/nature10075.html .

The study was supported by the National Institutes of Health's National Institute of General Medical Sciences, National Institute of Diabetes and Digestive and Kidney Diseases, and National Institute of Mental Health as well as the NIH's Molecular Libraries Program (part of the NIH Cmmon Fund).

Team Uncovers New DNA Role in Modifying Gene Function

For years, scientists have thought of DNA as a passive blueprint capable only of producing specific proteins through RNA transcription. Now, research led by scientists from the Florida campus of The Scripps Research Institute has shown DNA can also act to fine-tune the activity of certain proteins known as nuclear receptors.

These new findings may make it possible to design therapies that could activate specific genes in a highly targeted manner in a number of important diseases including osteoporosis, obesity, autoimmune disease, and cancer.

The study was published April 10, 2011, in the journal *Nature Structural & Molecular Biology*.

"This study offers the first direct evidence of what we now recognize as critically important interactions," said team leader Patrick R. Griffin, chair of the Department of Molecular Therapeutics and director of the Translational Research Institute at Scripps Florida. "This new understanding could lead to the development of ways to promote highly targeted activity, which is exactly what you need in order to produce safe and effective therapies."

The new study focuses on the interactions between a protein complex comprising the vitamin D receptor and the retinoic X receptor and their ligands, vitamin D and 9-cis-retinoic acid (a metabolite of vitamin A), respectively, as well as DNA, and a coregulatory protein. Receptors are proteins to which one or more specific kinds of signaling molecules bind.

Scientists at Eli Lilly and Company collaborated on this study to better understand how vitamin D works at its most basic level, given that vitamin D plays a major role in bone health and is thus linked to the company's research platform in osteoporosis.

"These findings will potentially enable us to design safer medicines that work via the vitamin D pathway to help the osteoporotic patient," said Jeffrey A. Dodge, senior research fellow at Lilly. "Specifically, the technology developed at Scripps to understand these receptor ligand interactions has been critical and is a great example of industry-academia collaboration to solve important scientific questions."

Dynamic Interactions

In the new study, the scientists used a technology known as hydrogen-deuterium exchange (HDX) mass spectrometry to measure the interaction of various ligands with the vitamin D receptor complex. Ligands can be small synthetic compounds, hormones, other proteins, or DNA and they bind to large molecules such as proteins and change these molecules' behavior. In this study, the ligands were vitamin D, a metabolite of vitamin A, DNA, and a coactivator protein known as SRC1 (steroid receptor coactivator 1).

"HDX mass spectrometry is a high-precision, high-sensitivity mapping technique," Griffin explained. "With it, we can find the specific regions of the protein complex that are altered upon interaction with ligand. This information can be used to infer structural changes that are the result of a specific interaction."

In their research, the scientists found that DNA can actually alter the structure and function of the receptor complex through a wide variety of long-range structural effects. These long-range effects had been hypothesized, but this study is the first to actually detect them directly with high spatial resolution. The changes wrought by these dynamic interactions were impressive.

The study shows these binding events have substantial consequences; for example, that the DNA binding at one site alters the stability of both the ligand binding site as well as coactivator interaction surface at opposite ends of the complex and vice versa. Those alterations influence a number of key processes, from coactivator-mediated interactions with various cofactors to the modification of the DNA binding domain so that the receptor can recognize specific DNA sequences. "

There is an elaborate biochemical dialogue going on between the receptor, ligand, coregulatory proteins, and the specific DNA sequence that the nuclear receptor complex is bound to," said Griffin. "But until this study, it was not completely clear what the structural basis for this crosstalk was."

The first author of the study, "DNA Binding Alters Coactivator Interaction Surfaces of the Intact VDR/RXR Complex," is Jun Zhang of Scripps Research. In addition to Griffin, other authors include Michael J. Chalmers, Yongjun Wang, Scott A. Busby, Bruce D. Pascal, Ruben D Garcia-Ordonez, and Thomas P. Burris of Scripps Research; Keith R. Stayrook, Lorri L. Burris, and Jeffrey A. Dodge of Lilly Research Laboratories; and John B. Bruning of Texas A&M University. See http://www.nature.com/nsmb/journal/vaop/ncurrent/abs/nsmb.2046.html

The study was supported by the National Institutes of Health.

Scientists Identify Mechanism of Long-Term Memory

Using advanced imaging technology, scientists from the Florida campus of The Scripps Research Institute have identified a change in chemical influx into a specific set of neurons in the common fruit fly that is fundamental to long-term memory.

The study was published in the April 13, 2011 issue of *The Journal of Neuroscience*.

"In studying fruit flies' learning and long-term memory storage, we observed an increase in calcium influx into a specific set of brain neurons in normal fruit flies that was absent in 26 different mutants known to impair long-term memory," said Ron Davis, chair of the Scripps Research Department of Neuroscience, who led the study. "The logical conclusion is that this increase, which we call a memory trace, is a signature component of long-term memory."

The memory trace in question is an increased influx of calcium into a set of neurons after long-term memory forms in a part of the insect brain known as mushroom bodies, a pair of oversized lobes known to mediate learning and memory, particularly the memories of smell. They have been compared to the hippocampus, a site of memory formation in humans.

Increases in calcium influx also occur with learning in other animal models, Davis said, and it seems highly likely a similar correlation exists in humans.

Measuring Memory Traces

To measure the changes in the *Drosophila* neurons, Davis and his colleagues used functional optical imaging, an advanced technology that his laboratory helped pioneer for the study of learning and memory. Using protein sensors that become fluorescent when calcium levels are increased, the team was able to highlight changes in the levels of calcium influx into the mushroom body neurons in response to odor learning. These observed memory traces occur in parallel with behavioral changes.

Interestingly, these memory traces occur only with spaced conditioning – where the insects receive multiple episodes of learning but with periods of rest between each episode. Spaced conditioning is required for long-term memories to form.

In an earlier study last December, also published in *The Journal of Neuroscience*, Davis found not only that fruit flies receiving spaced conditioning exhibited a long-term memory trace, but also that their memories lasted between four and seven days. In flies that were given a single episode of learning, memory formation lasted only a day and the long-term memory trace failed to form. These two studies are the newest in a series of six studies on the topic, including those published in the journal *Neuron* in 2004 and 2006, *Cell* in 2005, and *Nature Neuroscience* in 2008. Davis reviewed all of his studies of memory traces in the most recent issue of *Neuron*.

"The phenomenon of spaced conditioning is conserved across all species," Davis said. "No one really knows why it's important to long-term memory formation but there appears to be something magical about that rest period during learning."

The co-authors of the most recent study, "The Long-Term Memory Trace Formed in the *Drosophila* α/β Mushroom Body Neurons Is Abolished in Long-Term Memory Mutants," are David-Benjamin G. Akalal and Dinghui Yu of the Baylor College of Medicine. See http://www.jneurosci.org/content/31/15/5643.abstract.

The study was supported by the National Institutes of Health.

Research Identifies New Pathway Affecting Lifespan

A team led by a scientist from the Florida campus of The Scripps Research Institute has identified a new role for a biological pathway that not only signals the body's metabolic response to nutritional changes, but also affects lifespan.

The study, published in the May 12, 2011 issue of the journal *Nature*, was conducted on *Caenorhabditis elegans* (nematodes or roundworms), which are a widely accepted model for human aging research.

"We been able not only to identify some of these molecules for the first time in the worm, but also to show they act as a signal of nutrient availability and ultimately influence the worm's lifespan," said Gill. Gill, an assistant professor in the Scripps Research Department of Metabolism and Aging, conducted the research while at The Buck Institute for Research on Aging in Novato, California. "What makes this important is that the same molecules are present in both humans and *C. elegans*, so these molecules may play similar roles in both organisms."

Dietary restriction is a well-known means of extending lifespan and postponing age-related disease in many species, including yeast, worms, flies, and rodents. However, until this study, little was known about the molecular signals involved.

The molecules identified in the new study are N-acylethanolamines (NAEs), a group of signaling molecules derived from lipids that help indicate nutrient availability in the environment and maintain an animal's internal energy balance. In the study, Gill and his colleagues showed that NAE abundance in the worm is reduced during periods of dietary restriction, and that NAE deficiency in the presence of abundant food is sufficient to extend the animal's lifespan.

"It is well known that if you put *C. elegans* on a restricted diet, you can extend its lifespan by 40 to 50 percent," Gill said. "However, we were amazed to see that if you add back just one of these NAE molecules, eicosapentaenoyl ethanolamide, it completely abrogates the lifespan extension."

Importantly, this particular NAE is similar to endocannabinoids in mammals, which regulate many different physiological processes including nutrient intake and energy balance, as well as inflammation and neuronal function. "The identification of other components of a novel endocannabinoid system in the worm now brings a new model system to the many researchers studying NAE and endocannabinoid physiology," said Gill.

Intriguingly, the study also established a link among fat, NAE levels, and longevity. Other studies in rodents have shown that the availability of fatty acids can influence NAE levels. However, Gill and his colleagues found that in a genetically altered strain of *C. elegans* the inability to produce certain polyunsaturated fatty acids was not only associated with a reduction in levels of specific NAEs but also with lifespan extension. He added that the study's findings could shape future drug development efforts to influence aging and age-related disease.

The first author of the study, "N-Acylethanolamine Signaling Mediates the Effect of Diet on Lifespan in *C. elegans*," is Mark Lucanic, a postdoctoral fellow at the Buck Institute for Research on Aging. Other authors include Jason M. Held, Maithili C Vantipalli, Jill B. Graham, Bradford W. Gibson, and Gordon J. Lithgow of the Buck Institute for Research on Aging; and Ida M. Klang of the Buck Institute for Research on Aging and the Karolinska Institute. For more information, see http://www.nature.com/nature/journal/v473/n7346/abs/nature10007.html.

The study was supported by the Larry L. Hillblom Foundation and the National Institutes of Health.

Scientists Create New Genetic Model of Premature Aging Diseases

Working with a group of national and international researchers, scientists from the Florida campus of The Scripps Research Institute have developed a new genetic model of premature aging disorders that could shed light on these rare conditions in humans and provide a novel platform for large-scale screening of compounds to combat these and other age-related diseases.

In the new study, which published in May 2011 in the open-access publication *PLoS ONE*, the scientists found a way to use zebrafish (*Danio rerio*) to model two rare human genetic disorders: Hutchinson-Gilford Progeria Syndrome and laminopathies.

"This is a robust model system of human aging that corresponds directly to the human genes involved in these diseases," said Scripps Florida Assistant Professor Shuji Kishi, who led the study. "This model is ready now and can be used to screen and develop chemical compounds to treat these and other age-related diseases."

Kishi noted that zebrafish, which display an array of signs of aging resembling those in humans, have emerged over the past decade as a powerful system to study diseases associated with aging and development.

Hutchinson-Gilford Progeria Syndrome is a rare disease that causes symptoms of advanced aging such as cardiovascular problems, hair loss, and distressed skin in young children. The laminopathies are a cluster of at least 13 different genetic disorders, whose symptoms range from muscular dystrophy to premature aging. They are grouped together because they are all caused by mutations in the genes that encode proteins of the nuclear membrane, the double-hulled envelope that surrounds the cell nucleus.

The gene associated with both progeria and laminopathies is the lamin A gene (LMNA), which presumably is also involved in the normal process of human aging, although the underlying mechanisms of the process are still relatively unknown.

In the new research, scientists set out to block the protein production of the LMNA gene in zebrafish. This resulted in apoptosis or programmed cell death, as well as interruption of the normal cell cycle. Deletion of some specific amino acid residues in the lamin A protein also produced aging in embryonic zebrafish.

Intriguingly, the study also found that farnestyl transferase inhibitor (FTI), a new class of anti-cancer drugs, reduced abnormalities in the nuclear membrane and prevented significant aging in the embryonic zebrafish models, which survived to adulthood but with a shortened lifespan.

"Utilizing our 'embryonic senescence' zebrafish model, our next goal will be to find modifier genes as well as chemical compounds to reverse accelerated aging and restore the normal aging process," Kishi said. "These findings could contribute to healthy aging in normal individuals, because the moderate defects of lamin A are also associated with the normal aging process."

The first author of the study, "Embryonic Senescence and Laminopathies in a Progeroid Zebrafish Model," is Eriko Koshimizu of Harvard University and the Tokyo University of Marine Science and Technology. Other authors include Shintaro Imamura, Jamal Toure and Jun-ichi Hanai of Harvard University; Christopher E. Carr of the Massachusetts Institute of Technology; Jie Qi of Harvard University and The Scripps Research Institute; and Delgado M. Valdez Jr. of The Scripps Research Institute. See http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0017688.

The work was supported by The Ellison Medical Foundation and The Glenn Foundation for Medical Research.

Led by Advances in Chemical Synthesis, Team Discovers that a Rare Natural Product Has Potent Pain-Killing Properties

Scientists from the Florida campus of The Scripps Research Institute have for the first time accomplished a laboratory synthesis of a rare natural product isolated from the bark of a plant

widely employed in traditional medicine. This advance may provide the scientific foundation to develop an effective alternative to commonly prescribed narcotic pain treatments.

The study, published May 23, 2011, in an advanced online edition of the journal *Nature Chemistry*, defines a chemical means to access meaningful quantities of the rare natural product conolidine. Based on data from mouse models, the study also suggests that synthetic conolidine is a potent analgesic as effective as morphine in alleviating inflammatory and acute pain, with few, if any, side effects.

In recent years, there has been significant interest in developing alternatives to opiate-based pain medications such as morphine. While widely prescribed for pain, morphine has a number of adverse side effects that range from the unpleasant to the lethal, including nausea, chronic constipation, addiction, and breathing depression.

The rare natural product central to the study is derived from the bark of a widely grown tropical flowering plant *Tabernaemontana divaricata* (also known as crepe jasmine). Long part of traditional medicine in China, Thailand, and India, extract from the leaves has been used as an anti-inflammatory applied to wounds, while the root has been chewed to fight the pain of toothache. Other parts of the plant have been used to treat skin diseases and cancer.

Conolidine belongs to a larger class of natural products, called C5-nor stemmadenines, members of which have been described as opioid analgesics, despite a substantial discrepancy between potent *in vivo* analgesic properties and low affinity to opiate receptors. Conolidine is an exceptionally rare member of this family for which no therapeutically relevant properties had ever been described. Despite the potential value of conolidine and related C5-nor stemmadenines as leads for therapeutics, efficient methods to prepare these molecules were lacking.

"This was a classic problem in chemical synthesis," said Glenn Micalizio, an associate professor in the Department of Chemistry, who initiated and directed the study, "which we were able to solve effectively and efficiently—an achievement that made subsequent assessment of the potential therapeutic properties of this rare natural product possible."

Micalizio and his colleagues began working on the synthesis of the molecule after they arrived at Scripps Florida in 2008.

Testing For Potency

Once the synthesis was complete, research shifted to pharmacology for evaluation. The pharmacological assessment, performed in the laboratory of Scripps Florida Associate Professor Laura Bohn, showed that the new synthetic compound has surprisingly potent analgesic properties.

"Her pharmacological studies confirmed that while it's not an opiate, it's nearly as potent as morphine," Micalizio said.

In various models of pain, the new synthetic compound performed spectacularly, suppressing acute pain and inflammatory-derived pain, two key measures of efficacy. Not only that, but the new compound passed easily through the blood-brain barrier, and was present in the brain and blood at relatively high concentrations up to four hours after injection.

Bohn herself was surprised by the compound's potency and by the fact it so readily enters the brain.

"While the pain-relieving properties are encouraging, we are still challenged with elucidating the mechanism of action," she said. "After pursuing more than 50 probable cellular targets, we are still left without a primary mechanism."

So far, the compound has shown remarkably few, if any, side effects, but that is something of a double-edged sword.

"The lack of side effects makes it a very good candidate for development," Bohn said. "On the other hand, if there were side effects, they might provide additional clues as to how the compound works at the molecular level."

That remains a mystery. While the synthetic compound might be as effective as morphine, it doesn't act at any of the receptors associated with opiates. In fact, it misses most of the major neurotransmitter receptors completely, suggesting it may be highly tuned towards relieving pain while not producing multiple side effects. While still in the early stages of development, further characterizations of conolidine may suggest further development as a human therapeutic for the treatment of pain.

The first author of the study, "Synthesis of Conolidine, a Potent Non-Opioid Analgesic for Tonic and Persistent Pain," is Michael A. Tarselli of Scripps Research. Other authors include Kirsten M. Raehal, Alex K. Brasher, John M. Streicher, Chad Groer, and Michael D. Cameron, also of Scripps Research. For more information, see: http://www.nature.com/nchem/journal/v3/n6/abs/nchem.1050.html.

This research was made possible by Scripps Florida start-up funds, resulting from a one-time appropriation of federal economic development funds by the State of Florida, as well as support from Palm Beach County.

Scientists Find Way to Block Stress-Related Cell Death

Scientists from the Florida campus of The Scripps Research Institute have uncovered a potentially important new therapeutic target that could prevent stress-related cell death, a characteristic of neurodegenerative diseases such as Parkinson's, as well as heart attack and stroke.

In the study, published in May 2011 in the journal *ACS Chemical Biology*, the scientists showed they could disrupt a specific interaction of a critical enzyme that would prevent cell death without harming other important enzyme functions.

The enzyme in question is c-jun-N-terminal kinase (JNK), pronounced "junk," which has been implicated in many processes in the body's response to stresses, such as oxidative stress, protein misfolding, and metabolic disorder. JNK also plays an important role in nerve cell survival and has become a target for drugs to treat neurodegenerative disorders such as Parkinson's disease.

In recent studies, JNK has been found to migrate to the mitochondria—the part of the cell that generates chemical energy and that is involved in cell growth and death. That migration, coupled with JNK activation, is associated with a number of serious health issues, including apoptosis or programmed cell death, liver damage, neuronal cell death, stroke and heart attack.

"Activated JNK migrates to the mitochondria in reaction to a stress signal," said Philip LoGrasso, professor in the Department of Molecular Therapeutics and senior director for drug discovery at Scripps Florida who led the study. "Once there, it amplifies the effects of reactive oxygen species that cause significant damage to the cell. We developed a small peptide that intervenes in JNK migration and blocks those harmful effects—specifically cell death."

LoGrasso noted that the team was able to block JNK mitochondrial interaction without harming any other important enzyme processes, such as JNK's impact on gene expression. These findings, LoGrasso said, suggest that this interaction could be exploited in the development of a new drug.

"The peptide we developed will never be a drug, but it is an important new investigative tool that we can use to selectively probe mitochondrial biology," he said. "Our hope is to produce a small molecule that can mimic the inhibitory effect of this peptide. If we can do that, we might be able to selectively inhibit JNK mitochondrial interaction and use it to treat a number of diseases."

The first author of the study, "Selective Inhibition of Mitochondrial JNK Signaling Achieved Using Peptide Mimicry of the Sab Kinase Interacting Motif-1 (KIM1)," is Jeremy W. Chambers of Scripps Research. Other authors include Lisa Cherry, John D. Laughlin, and Mariana Figuera-Losada, also of Scripps Research. For more information, see http://pubs.acs.org/doi/abs/10.1021/cb200062a.

The study was supported by National Institutes of Health and the Saul and Theresa Esman Foundation.

Study Shows Component of Common Supplement May Alleviate Fatty Liver Disease, Improve Insulin Sensitivity

Researchers from Baylor College of Medicine, the Emery School of Medicine, and The Scripps Research Institute have found that a natural product increases sensitivity to insulin and reduces fatty liver in mice, suggesting it may be able to provide a treatment for prediabetic patients.

The study, which appeared in the June 23, 2011 print edition of the journal *Nature*, focuses on a trace component of lecithin called DLPC (dilauroyl phosphatidylcholine). Lecithin, a major constituent of cell membranes, is found in foods including egg yolk, soybeans, grains, wheat germ, fish, legumes, yeast, and peanuts; it is also available in supplement form.

The new study found that, in mice, DLPC induced the production of bile acid enzymes, lowered fat in the liver, and dramatically improved insulin sensitivity.

"Their overall body weight was not changed," said David D. Moore, professor of molecular and cellular biology at Baylor College of Medicine. "But they had improved sensitivity to insulin (which helps keep glucose levels in check) and less fatty livers."

Fatty liver, which is associated with diabetes, high blood pressure, and cardiovascular disease, can lead to scarring of the liver and poor liver function. Insulin sensitivity is important for maintaining healthy levels of blood sugar, which helps prevent type 2 diabetes.

Clinical Trial Planned

The researchers first became interested in DLPC as a tool for studying the function of a receptor protein—liver receptor homolog-1 or LRH-1—that regulates the production of bile acids in the liver. The team then screened compounds to identify candidates that activated LRH-1 function, finding that DLPC enhanced LRH-1 activity in cells.

"Our lab at Scripps Florida has been developing assays to support discovery of synthetic inverse agonists of LRH1 as potential treatments as anti-cancer agents," said Patrick Griffin, chair of the Department of Molecular Therapeutics and director of the Translational Research Institute at Scripps Florida. "These same assays were used in our collaboration with David Moore's efforts demonstrating the natural product DLPC bound specifically to LRH1 and modulated the interaction with coactivator proteins."

While DLPC decreased fatty liver and lowered glucose levels in the blood in two kinds of mice that had resistance to insulin, DLPC had no effect in mice lacking LRH-1 in the liver, underlining the dependence on LRH-1 for these biological effects.

A pilot clinical study of DLPC for patients with prediabetes has been launched at Baylor in Waco, Texas; those who wish to enroll should contact Dr. Kerem Ozer at 713-798-7684.

In addition to Moore and Griffin, authors of the Nature article, "A nuclear-receptor-dependent phosphatidylcholine pathway with antidiabetic effects," include Jae Man Lee, Yoon Kwang Lee, and Jennifer L. Mamrosh of Baylor; Scott A. Busby of Scripps Florida; and Manish C. Pathak

and Eric A. Ortlund of Emory. For more information, see
http://www.nature.com/nature/journal/vaop/ncurrent/abs/nature10111.html

Funding for this research was provided by the National Institutes of Health, the Alkek Foundation, the National Institute of Diabetes and Digestive and Kidney Diseases, and the Robert R.P. Doherty Jr. - Welch Chair in Science.

Scripps Research Scientists Identify Critical Role for Night Blindness Gene

Scientists from the Florida campus of The Scripps Research Institute have determined how a particular gene makes night vision possible.

The study, which was published in the August 10, 2011 edition of *The Journal of Neuroscience*, focuses on a gene called nyctalopin. Mutations in the gene result in inherited "night blindness," a loss of vision in low-light environments.

"Until now, our understanding of the role of this gene in the visual signaling pathway has been very limited," said Kirill Martemyanov, an associate professor on the Florida campus of The Scripps Research Institute and senior author of the study. "This is the first time we have uncovered a functional role for it—and we linked that function to a much larger molecular complex that's needed for low-light vision."

Quick as a Flash

Our vision begins when photons hit light-sensitive photoreceptor cells in the retina. When excited by light, photoreceptors generate a response that needs to be rapidly transmitted to the downstream neurons (nerve cells) for the signal to be processed and sent to the brain, which then interprets the visual picture. The hand off of the information occurs at the specialized contact points called synapses.

"The proper function of a particular type of synapse between rod photoreceptors and bipolar cells is absolutely critical for the transduction of the visual signal," Martemyanov explained. "Even if rods generate response to light but are unable to properly transmit the signal, this results in an inability to see in the dark. Without this signaling, we'd have a tough time surviving in the world where it is dark half of the time."

In addition, the transmission across the synapse must occur rapidly. "The quickness of our signaling response to light creates a clear temporal resolution of what we see," he said. "For example, when you turn your head suddenly, you see different objects clearly, not just a blur. We couldn't drive a car without it."

In the new research, the scientists searched for proteins associated with nyctalopin in the mouse retina. Scientists had known for a decade that the gene encoding nyctalopin is one of the most

frequent culprits of night blindness, but its function had remained a mystery. The results showed that the protein expressed by the gene serves as a kind of molecular glue that holds together key elements of the signal transduction machinery at the synapse, allowing for the rapid and intact transmission of these sensory signals.

In molecular terms, the study strongly suggests that nyctalopin coordinates the assembly and precise delivery to the synapse of the macromolecular complex consisting of mGluR6, a neurotransmitter receptor protein, which directly communicates with rod photoreceptors and TRPM1, a protein channel that generates the response, making vision possible.

While the new findings are relevant to the processing of low-light vision, Martemyanov said, the role of nyctalopin might go far beyond the eye. Proteins similar to nyctalopin exist in the central nervous system, and it is possible that they coordinate synaptic signaling in a manner similar to the retina. Indeed, communication between neurons across synapses is fundamental to the nervous system function and disruption of this process is thought to be the main factor contributing to a range of the neuropsychiatric diseases.

The first author of the study, "TRPM1 Forms Complexes with Nyctalopin In Vivo and Accumulates in Postsynaptic Compartment of ON-Bipolar Neurons in mGluR6-dependent Manner," is Yan Cao. In addition to Martemyanov and Cao, Ekaterina Posokhova is an author of the paper. All of the authors work at Scripps Research. See http://www.jneurosci.org/content/31/32/11521.abstract

The study was supported by the National Institutes of Health.

Scripps Research Scientists Expand Knowledge of Cell Process Involved in Many Diseases

As part of a joint research effort with the University of Michigan, scientists from the Florida campus of The Scripps Research Institute have for the first time defined the structure of one of the cell's most basic engines, which is required for cell growth, as it assembles from its components.

The study reveals a series of redundant mechanisms that assure production of these critical structures while avoiding any missteps that could lead to their destruction or to the production of incorrect cellular building blocks. These findings throw new light on a process that is integrally involved in a number of disease states, including cancer and Alzheimer's disease.

The study, published on August 11, 2011, in the advance online edition of the prestigious journal *Science*, reveals the structure of an assembly intermediate of the small ribosomal subunit.

Ribosomes, which are large macromolecular machines required for cell growth in all organisms, catalyze the production of proteins in all cells. They read the genetic code carried by messenger

RNA, and then catalyze or translate that code into proteins within cells, assembling them from amino acids.

Understanding the process of ribosome assembly—which involves almost 200 essential proteins known as "assembly factors" in addition to the four RNA molecules and 78 ribosomal proteins that are part of the mature ribosome—is a potentially fruitful area of research because of the importance of ribosome assembly for cell growth. The link between defects in ribosome assembly and cancer clearly points to this pathway as a new target for anti-cancer drugs.

In the current study, the scientists used cryo-electron microscopy (where samples are studied at temperatures of –150 °C) to image the 40S ribosome structure.

"This is the best-defined ribosomal assembly intermediate we have ever had with true structural information on the location of each assembly factor," said Katrin Karbstein, an associate professor at Scripps Florida and one of the senior authors of the study. "It will be helpful in determining what's going on in what is still a relatively unknown process."

While most ribosome assembly takes place in the nucleolus, a protein-nucleic acid structure inside the nucleus, the final maturation process occurs in the cytoplasm, the "general" cellular compartment where protein translation occurs. In the cytoplasm, these pre-mature ribosomal subunits encounter large pools of mature subunits, messenger RNA, and various translation factors

This cellular stew presents a unique challenge, especially keeping the translation process from acting on the subunits prematurely, which would result in their rapid degradation or in the production of incorrectly assembled proteins, both processes with potentially lethal outcomes for the cell.

The new study shows that that the bound assembly factors cooperate with one another in a highly redundant and multi-pronged approach to prevent such occurrences, chaperoning the pre-40S subunits to keep them from falling victim to the translational apparatus.

"We had thought the role of assembly factors was to help mature this intermediate form of the ribosome," said Karbstein. "But our new research has shown that these assembly factors also prevent a number of unwanted things from happening. If one of these intermediate forms were to bind prematurely to a messenger RNA, there could be no protein produced, or worse, a wrong protein might be produced and that could lead to early cell death."

It's important to note that this is a single snapshot of the late-stage assembly process, Karbstein added. "We know better how the process works but this is by no means a final statement," she said.

The first authors of the study, "Ribosome Assembly Factors Prevent Premature Translation Initiation by 40S Assembly Intermediates," are Bethany S. Strunk of Scripps Research, and Cherisse R. Loucks and Min Su of The University of Michigan. Other authors include Harish

Vashisth, Shanshan Cheng, Justin Schilling, Charles L. Brooks III, and Georgios Skiniotis of The University of Michigan. For more information, see http://www.sciencemag.org/content/early/2011/08/10/science.1208245.abstract?sid=adbd44ad-f124-492f-8d73-ebb6dc4761aa

The study was supported by the National Institutes of Health and the National Science Foundation.

Scripps Research Scientists Help Pinpoint Cause of Stress-Related DNA Damage

Working closely with a team of researchers from Duke University, scientists from the Florida campus of The Scripps Research Institute have helped identify a molecular pathway that plays a key role in stress-related damage to the genome, the entirety of an organism's hereditary information.

The new findings, published in the journal *Nature* on August 21, 2011, could not only explain the development of certain human disorders, they could also offer a potential model for prevention and therapy.

While the human mind and body are built to respond to stress—the well-known "fight or flight" response, which lasts only a few minutes and raises heart rate and blood glucose levels—the response itself can cause significant damage if maintained over long periods of time.

When stress becomes chronic, this natural response can lead to a number of disease-related symptoms including peptic ulcers and cardiovascular disorders. To make matters worse, evidence indicates that chronic stress eventually leads to DNA damage, which in turn can result in various neuropsychiatric conditions, miscarriages, cancer, and even aging itself.

Until the new study, however, exactly how chronic stress wreaks havoc on DNA was basically unknown.

"Precisely how chronic stress leads to DNA damage is not fully understood," said Derek Duckett, associate scientific director of the Translational Research Institute at Scripps Florida. "Our research now outlines a novel mechanism highlighting β-arrestin-1 as an important player."

The long-term effects of these stress hormones on DNA damage identified in the study represent a conceptual as well as a tangible advance, according to Robert J. Lefkowitz, a Duke University professor of medicine who led the study.

Since stress is not time-limited and can be sustained over months or even years, it is well appreciated that persistent stress may have adverse effects for the individual. These new findings not only uncover a novel pathway, but also have important practical implications.

"Our results provide a possible mechanistic basis for several recent reports suggesting that significant risk reductions for diseases such as prostate cancer, lung adenocarcinoma, and Alzheimer's disease may be associated with blockade of this particular stress-response pathway by beta blockers," Leftkowitz said. "Although there are most likely numerous pathways involved in the onset of stress-related diseases, our results raise the possibility that such therapies might reduce some of the deleterious DNA-damaging consequences of long-term stress in humans."

A Newly Discovered Mechanism

The newly uncovered mechanism involves β-arrestin-1proteins, β2-adrenoreceptors (β2ARs), and the catecholamines, the classic fight-or-flight hormones released during times of stress—adrenaline, noradrenaline, and dopamine. Arrestin proteins are involved in modifying the cell's response to neurotransmitters, hormones, and sensory signals; adrenoceptors respond to the catecholamines noradrenaline and adrenaline.

Under stress, the hormone adrenaline stimulates β2ARs expressed throughout the body, including sex cells and embryos. Through a series of complex chemical reactions, the activated receptors recruit β-arrestin-1, creating a signaling pathway that leads to catecholamine-induced degradation of the tumor suppressor protein p53, sometimes described as "the guardian of the genome."

The new findings also suggest that this degradation of p53 leads to chromosome rearrangement and a build-up of DNA damage both in normal and sex cells. These types of abnormalities are the primary cause of miscarriages, congenital defects, and mental retardation, the study noted.

The first author of the study, "Stress Response Pathway Regulates DNA Damage through β2-Adrenoreceptors and β-Arrestin-1," is Makoto R. Hara of Duke University. In addition to Duckett and Hara, other authors include Jeffrey J. Kovacs, Erin J. Whalen, Sudarshan Rajagopal, Ryan T. Strachan, Aaron J. Towers, Barbara Williams, Christopher M. Lam, Kunhong Xiao, Sudha K. Shenoy, Simon G. Gregory, Seungkirl Ahn, and Robert J. Lefkowitz of Duke University; and Wayne Grant of Scripps Research.

The study was supported by the National Institutes of Health.

Scripps Research Scientists Define Cellular Pathway Essential to Removing Damaged Mitochondria

In a joint research effort with researchers at St. Jude Children's Research Hospital, and with help from scientists at The University of Pennsylvania, The University of Minnesota, and the National Institutes of Health, investigators from the Florida campus of The Scripps Research Institute have defined a specific protein complex that allows cells to rid themselves of damaged mitochondria, which are the energy producing machines of the cell.

"This protein complex is already being targeted in cancer therapeutics," said John Cleveland, chair of the Department of Cancer Biology at Scripps Florida, "but now we understand why some of the therapies that target this complex work and this new knowledge will have tremendous impact on both current and potential cancer therapies."

In particular, the study, which appears in the current issue of the journal Molecular Cell, focuses on how the cell uses a process known as autophagy—the major recycling center of the cell—to

remove damaged mitochondria. The autophagy pathway is exploited by many tumors to survive stressful conditions and to remove damaged components.

The Cell under Stress

On a molecular level, the new study focuses on the role of the molecular complex known as "Hsp90-Cdc37 chaperone complex," which orchestrates various aspects of the cellular stress response. Although scientists had known that both the Hsp90-Cdc37 complex and autophagy help maintain the integrity of mitochondria, the exact relationship between Hsp90-Cdc37 and autophagy has not been well understood until the new study.

Hsp90, is a heat-shock protein, one of the cell's most abundant proteins, and assists in everything from protein folding and tumor repression to cell signaling. Cdc37, also a protein, is a co-chaperone to Hsp90 and is involved in cell signal transduction and connecting Hsp90 to the right kinases (kinases add a phosphate group to various molecules and can modify protein activity).

The study highlights the interaction between Hsp90-Cdc37 and Ulk1, a kinase that the authors show is required for the degradation and elimination of damaged mitochondria. Hsp90-Cdc37 stabilizes and activates Ulk1, which in turn phosphorylates its substrate Atg13, which is then released from the complex. Atg13 then eliminates damaged mitochondria via the autophagy pathway. Thus, the study links Hsp90-Cdc37-Ulk1-Atg13 in a direct pathway that is essential for efficient mitochondrial clearance.

"The new study shows that the key regulatory mechanism of this process is the Hsp90-Cdc37 chaperone, which functions as an on-off switch that is critical for the correct functioning of the Ulk1 kinase," Cleveland said. "Thus, if we can control this switch, we can significantly improve the therapeutic window."

The first authors of the study, "Hsp90-Cdc37 Chaperone Complex RegulatesUlk1- and Atg13-mediated Mitophagy," are Frank C. Dorsey of Scripps Research and Joung Hyuck Joo, Aashish Joshi, and Kristin M. Hennessy-Walters of St. Jude Children's Research Hospital. Other authors include Kristie L. Rose, Stephanie M. Prater, Meredith A. Steeves, and John L. Cleveland of Scripps Research; Chang-Hwa Jung, and Do-Hyung Kim of the University of Minnesota; Der-Fen Suen, Chia-Ying Yang, Craig B. Thompson of the University of Pennsylvania School of Medicine; and Richard Youle of the National Institutes of Health; and Kelly McCastlain, Rekha Iyengar, Paul A. Ney and Mondira Kundu of St. Jude Children's Research Hospital. For more information, see http://www.cell.com/molecular-cell/abstract/S1097-2765%2811%2900464-3 .

The study was supported by the National Institutes of Health, the Burroughs Welcome Fund, the American Society of Hematology, the Scripps Florida Funding Corporation, and the American Lebanese Syrian Associated Charities.

Scripps Research Scientists Establish New Class of Anti-Diabetic Compound

In a joint study, scientists from The Scripps Research Institute and Harvard University's Dana-Farber Cancer Institute have established a new class of anti-diabetic compound that targets a unique molecular switch.

The finding paves the way for the development of anti-diabetic therapeutics with minimal adverse side effects plaguing currently available drugs such as Avandia (rosiglitazone), scheduled to be removed from pharmacy shelves this fall due to concerns about increased risk of heart attack.

The new study, led by Patrick R. Griffin, professor and chair of the Department of Molecular Therapeutics at Scripps Florida, Bruce Spiegelman, professor of cell biology at the Dana-Farber Cancer Institute, and Theodore Kamenecka, associate scientific director of medicinal chemistry at Scripps Florida, was published September 4, 2011, in the journal Nature. The study describes a new compound known as SR1664.

"In this study, we demonstrate that we have discovered novel compounds that work effectively through a unique mechanism of action on a well-validated clinical target for diabetes," said Griffin. "This unique mechanism of action appears to significantly limit side effects associated with marketed drugs. This study is a great example of interdisciplinary, inter-institutional collaboration with chemistry, biochemistry, structural biology, and pharmacology."

"It appears that we may have an opportunity to develop entire new classes of drugs for diabetes and perhaps other metabolic disorders," said Spiegelman.

Diabetes affects nearly 24 million children and adults in the United States, according to the America Diabetes Association.

A Viable Therapeutic Target

The study follows previous research by the authors published last year in Nature (Volume 466, Issue 7305, 451-456) that suggested an obesity-linked mechanism that may be involved in the development of insulin-resistance. In that research, the team found disruptions in various genes when a protein known as PPARγ undergoes phosphorylation (when a phosphate group is added to a protein) by the kinase Cdk5, an enzyme involved in a number of important sensory pathways.

The new study confirms that blockage of Cdk5's action on PPARG is a viable therapeutic approach for development of anti-diabetic agents. The new SR1664 compound is a potent binder to the nuclear receptor PPARG, but does not activate gene transcription via the receptor's normal mechanism.

While Griffin stressed the difficulty of fully assessing side effects of new compounds such as SR1664, the new research is extremely positive in that it clearly demonstrated fewer of the major

well-documented side effects, such as weight gain or increased plasma volume, from SR1664 as compared to Avandia in diabetic mice.

While both the mice treated with Avandia and those treated with SR1664 demonstrated improved blood sugar levels, those treated with Avandia showed weight gain and increased fluid retention within a few days of beginning treatment; those being treated with SR1664 showed none of these side effects. In cell culture studies, SR1664 also appeared to have little effect on bone formation, nor did it increase fat generation in bone cells, another side effect of current therapies such as Avandia.

While S1664 likely will not be developed as a drug, it now serves as a molecular scaffolding for the creation of similar compounds with potential to treat diabetes. "With data in hand showing that our compounds are as efficacious as the currently marketed PPARG modulators, while demonstrating a significant improvement of side effects in limited studies, we are now advancing newer compounds with improved pharmaceutical properties into additional studies," Griffin said.

The first authors, denoted as equal contributors to this study, "Anti-Diabetic Actions of a Non-Agonist PPARG Ligand Blocking Cdk5-Mediated Phosphorylation," are Jang Hyun Choi and Alexander S. Banks of Dana-Farber Cancer Institute and Theodore M. Kamenecka and Scott A. Busby of The Scripps Research Institute. Other authors include Michael J. Chalmers, Naresh Kumar, Dana S. Kuruvilla, Youseung Shin, Yuanjun He, David Marciano, and Michael D. Cameron of Scripps Research; Dina Laznik of the Dana-Farber Cancer Institute; Michael J. Jurczak and Gerald I. Shulman of the Howard Hughes Medical Institute; Stephan C. Schürer and Dušica Vidović of the University of Miami; and John B. Bruning of Texas A&M University.

The study was supported by The National Institutes of Health.

Scripps Research Scientists Pinpoint Shape-Shifting Mechanism Critical to Protein Signaling

In a joint study, scientists from the California and Florida campuses of The Scripps Research Institute have shown that changes in a protein's structure can change its signaling function and they have pinpointed the precise regions where those changes take place.

The new findings could help provide a much clearer picture of potential drugs that would be both effective and highly specific in their biological actions.

The study, led by Patrick Griffin of Scripps Florida and Raymond Stevens of Scripps California, was published in a recent edition of the journal *Structure*.

The new study focuses on the β2-adrenergic receptor, a member of the G protein-coupled receptor family. G protein-coupled receptors convert extracellular stimuli into intracellular

signals through various pathways. Approximately one third of currently marketed drugs (including for diabetes and heart disease) target these receptors.

Scientists have known that when specific regions of the receptor are activated by neurotransmitters or hormones, the structural arrangement (conformation) of the receptor is changed along with its function.

“While it’s accepted that these receptors adopt multiple conformations and that each conformation triggers a specific type of signaling, the molecular mechanism behind that flexibility has been something of a black box,” said Griffin, who is chair of the Scripps Research Department of Molecular Therapeutics and director of the Scripps Florida Translational Research Institute. “Our findings shed significant light to it.”

The study describes in structural detail the various regions of the receptor that are involved in the changes brought about by selective ligands (ligands are molecules that bind to proteins to form an active complex), which, like a rheostat, run the gamut among activating the receptor, shutting it down, and reversing its function, as well as producing various states in between.

To achieve the results described in the study, the team used hydrogen-deuterium (HDX) mass spectrometry to measure the impact of interaction of various functionally selective ligands with the β2-adrenergic receptor. A mass spectrometer determines the mass of fragments from the receptor by measuring the mass-to-charge ratio of their ions. HDX has been used to examine changes in the shape of proteins and how these shape changes relate to protein function. The approach is often used to characterize protein-protein interactions that are critical for signal transduction in cells and to study protein-folding pathways that are critical to cell survival.

“At this early stage in understanding GPCR structure and function, it is important to view the entire receptor in combination with probing very specific regions," said Stevens, who is a professor in the Scripps Research Department of Molecular Biology. "Hydrogen-deuterium exchange mass spectrometry has the right timescale and resolution to asked important questions about complete receptor conformations in regards to different pharmacological ligand binding. The HDX data combined with the structural data emerging will really help everyone more fully understand how these receptors work."

“Using the HDX technology we can study the intact receptor upon interaction with ligands and pinpoint regions of the receptor that have undergone change in position or flexibility,” Griffin said. “By studying a set of ligands one can start to develop patterns that are tied to activation of the receptor or shutting it down. Once we get a picture of what a functional ligand looks like, it might be possible to develop a drug to produce a highly selective therapeutic effect.”

The lead author of the study, "Ligand-Dependent Perturbation of the Conformational Ensemble for the GPCR b2 Adrenergic Receptor Revealed by HDX," is Graham M. West of Scripps Research. Other authors include Ellen Y.T. Chien, Jovylyn Gatchalian, and Michael J. Chalmers of Scripps Research, and Vsevolod Katritch of the University of California, San Diego.

The study was supported by the National Institutes of Health.