Scripps Florida 2014 Scientific Report
July 1, 2013 – June 30, 2014

Part 1: New Faculty and Scientific Administration

TSRI Appoints Columbia University Scientist to Chemistry Department

The Scripps Research Institute (TSRI) has appointed Scott A. Snyder as an associate professor with tenure in the Department of Chemistry. Before coming to the Florida campus of TSRI, Snyder was a member of the Columbia University faculty.

A TSRI alumnus, Snyder, 36, joined the TSRI faculty this month.

"We want to welcome Scott back to TSRI," said Dale Boger, chair of the Department of Chemistry. "His career has been stellar, and his research into the synthesis of natural products has resulted in a number of important discoveries that will open the door to improvements in human health. Scott is a great addition to our department and to the institute."

"It's a great honor for me to return to TSRI—and to become part of the Scripps Florida faculty," said Snyder. "I'm looking forward to rejoining the collaborative science that is the heart of TSRI, something that will keep us on the cutting edge of science—and I'm excited to be part of the new campus and its pioneering spirit."

Snyder received his bachelor's degree in 1999 from Williams College. After finishing graduate studies at TSRI under the tutelage of Professor K. C. Nicolaou in 2004, he was a National Institutes of Health postdoctoral fellow in the laboratory of Professor E. J. Corey at Harvard University, the 1990 Nobel Laureate in Chemistry. In 2006, Snyder was appointed as an assistant professor at Columbia University, and in the ensuing seven years established a vigorous research program that was recognized by a number of honors. For instance, in 2010, he received an Alfred P. Sloan Foundation Research Fellowship and a Bristol-Myers Squibb Unrestricted Grant in synthetic organic chemistry; in 2011, a DuPont Young Professor Award; and, in 2012, the Arthur C. Cope Scholar Award from the American Chemical Society.

He was recently chosen to deliver *The Chemical Record Lecture* at the Japanese Chemical Society meeting in early 2014.

Snyder's research focus is on the total synthesis of natural products, materials widely used in the drug development process, either as medicines themselves or as progenitors to more highly bioactive and selective molecules.

"Total synthesis has produced a host of landmark achievements," Snyder said. "There are just as many equally important discoveries waiting to be made, and the aim of my research group is to unearth them."

For instance, at TSRI Snyder will continue his work on the chemistry and biology of resveratrol, an ingredient in red wine and grape skins thought to be behind the so-called "French paradox"– the notion that despite the consumption of significant amounts of fat and cholesterol within the typical French diet, citizens of that nation experience relatively few heart attacks because their diet also includes large amounts of red wine.

Resveratrol, however, is just the tip of the iceberg. “Resveratrol is Nature’s version of a Lego® building block that can be used to produce a large collection of complex and equally bioactive molecules,” he said. “In nature, the goal of that compound collection is preventing fungal infections. But if you give it to mice, they live longer, have better cardiovascular profiles and better cognitive abilities.” Snyder’s goal is to explore these more complex compounds and see what their effects might be in humans. His creative approach for the controlled synthesis of many members of the resveratrol family was recently published in the journal *Nature* (http://www.nature.com/nature/journal/v474/n7352/full/nature10197.html).

At TSRI, Snyder, who lives in Jupiter, FL, plans to further explore these molecules, as well as other compound collections, that are produced by the combination of a simple building block with itself again and again.

In addition to his research, Snyder is also a recognized leader in chemical education, having co-authored an advanced graduate text while a graduate student and, more recently, an undergraduate text used worldwide which is currently in its 11th edition. Snyder also plans to involve himself deeply in education and outreach at TSRI.

Part 2: Grant Awards and Licensing Agreements

Scripps Florida Team Awarded $10.6 Million to Decipher Root Causes of Human Aging

While aging may be one of the most familiar (and certainly one of the most discussed) aspects of human biology, it remains one of the least understood. We age but no one really knows precisely how we get there.

Thanks to a new $10.6 million National Institute on Aging grant to a team on the Florida campus of The Scripps Research Institute (TSRI), the puzzling questions of human aging may soon receive some answers.

TSRI Professor Paul Robbins will be principal investigator of the new five-year study, which will focus on identifying just how damage that accumulates over time drives the human aging process. Scientists from the University of Pittsburgh and the University of California, Riverside, will also participate in the study.

“Aging is thought to be caused by cellular damage triggered by things we are exposed to and produce as part of normal metabolism.” Robbins said. “But it is how the cells respond to that damage over time that we believe drives aging.”

Robbins compares that cellular response to calling 911 for help to put out a small fire. While the firefighting response could lead to a lot of collateral damage, if firefighters identify steps to minimize the damage—say, keeping water use to a limited area—the collateral damage can be minimized. Similarly, Robbins says, there will be a lot of new opportunities to minimize the widespread degenerative changes that occur with aging.

The scientists will focus their research on stress caused by DNA damage, specifically by looking at the effects of taking away a cell’s ability to repair this damage. TSRI Associate Professor Laura Niedernhofer, a co-investigator on the grant, showed several years ago that taking away a cell’s ability to repair DNA damage causes very rapid aging in humans and in animal models. The question to be addressed now is how? And how to stop it?

Although not the primary focus of the research, Robbins is also looking at compounds and even stem cells that could affect these stress response pathways in a therapeutic way. “It’s something that our research and the TSRI research environment lend themselves to—identifying pathways as potential therapeutic targets and screening potential drug candidates,” he said. “The ultimate goal isn’t to allow people to live longer, but to help them maintain good health as they age—what some are calling a person’s healthspan.” That in itself would be significant.

It is estimated that in the next 20 years, the number of individuals in the United States over the age of 65 will double, reaching over 70 million individuals, according to the U.S. Department of Health and Human Services. More than 90 percent of Americans over 65 years of age have at least one chronic disease, while more than 70 percent have at least two, according to the National Council on Aging. These chronic diseases account for three-quarters of our healthcare spending, amounting to approximately $3 trillion in costs last year alone.

The number of the National Institutes of Health grant is 1P01AG043376.

Team Awarded $2.3 Million to Unlock Mysteries of Long-Term Memory

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have been awarded approximately $2.3 million from the National Institute of Mental Health to study the processes involved in long-term memory and how deficits in those processes contribute to brain diseases.

Sathyanarayanan Puthanveettil, a TSRI assistant professor, will be principal investigator of the new five-year study (NIH grant number 1R01MH094607).

The study focuses on "axonal transport," the cellular process whereby gene products move to and from a nerve cell body along its axon, the narrow, cable-like structure critical for signaling other nerve cells, muscles and glands.

"This new grant will help us better understand the role of axonal transport in long-term memory storage and identify signaling pathways that regulate it," Puthanveettil said. "Once we identify the molecular regulators of axonal transport, we may be able to manipulate them to produce new and innovative approaches to the treatment of memory disorders."

In earlier studies, Puthanveettil and his colleagues have shown that kinesin, a molecular motor protein, plays a key role in learning and memory.

In the 10 years since Scripps Florida's founding in 2004, the campus has brought in more than $375 million in grants and support from the National Institutes of Health, foundations, individuals and sources outside of the State of Florida.

The campus has also produced:

- More than 900 publications in scientific journals
- More than 100 foreign and domestic patent applications
- 39 technology licenses
- 3 spin-off companies
- Collaborations with other local institutions, including Florida Atlantic University, Florida State University, Max Planck Florida and the University of Miami.

For more information, see www.scripps.edu/florida.

Team Awarded$2.3 Million to Study Dengue Fever and Related Viruses

The outbreak of dengue fever that infected some 20 people in Florida's Martin County late last year unnerved many who feared the tropical disease had once again established a foothold in Florida. The last outbreaks occurred in 2009 and 2010 in Key West—before that, the disease hadn't struck Florida in more than 70 years.

Now, scientists from the Florida campus of The Scripps Research Institute (TSRI) have been awarded $2.3 million from the National Institutes of Health to study a category of viruses that cause dengue fever, West Nile, yellow fever and other diseases spread by mosquitoes and ticks. These diseases can result in flulike symptoms, extreme pain (dengue has been called “bone-break fever”) and, in some cases, encephalitis.

This family of viruses, called “flavivirus,” affect some 2.5 billion people worldwide and cause hundreds of thousands of deaths each year. There are no antiviral treatments and a just handful of vaccines that provide protection against only a few of these diseases.

The principal investigator for the new five-year study is TSRI Associate Professor Hyeryun Choe, who will lead the effort to understand the virus’s mode of infection and how new therapies might interrupt it.

“Flavivirus uses a very clever method of infection,” Choe said. “It’s like using a side door to enter a house when the front door is locked.”

The viruses take advantage of the process that normally occurs during programmed cell death. During programmed cell death (“apoptosis”), a lipid usually found on the inner side of the cell membranes, specifically phosphatidylserine (PS), shifts to the surface, making itself readily available to any passing cellular stranger. This is where the trouble begins.

When cells are dying from a flavivirus infection, their freshly exposed PS is grabbed by the exiting virus, and phagocytes—cells that devour invading pathogens and dead and dying cells—engulf the virus as if it were a dying cell. Once engulfed by the phagocyte, the virus quickly turns the cell's own biology on its head, forcing it to produce copies of the virus.

While some viruses (influenza A for example) do not use PS in their life cycle, the flavivirus exploits this opportunity to the hilt. Infection of cells by dengue or West Nile viruses is markedly enhanced when phagocytes express receptors that recognize and bind PS.

It appears, however, that flaviviruses use only a subset of these receptors. The high selectivity, and the potency with which some of these receptors promote flavivirus infection, suggest only a small number of receptors might be effectively targeted to treat these diseases.

“We want to understand which PS receptors contribute the most to flavivirus infections and how we might block them,” Choe said. “Our studies are designed to offer insights useful in the development of new therapies.”

The number of the grant is 1R01AI110692.

Grant Funds Development of New Diagnostics for Cancer, Rheumatoid Arthritis, Colitis

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have been awarded a $2.3 million grant from the National Institute of General Medical Sciences of the National Institutes of Health to develop new technology to diagnose cancer, rheumatoid arthritis and colitis.

Paul Thompson, a TSRI associate professor, will be the primary principal investigator for the four-year study.

“We’ve already identified a number of biomarkers for rheumatoid arthritis that we’re in the process of validating,” Thompson said. “The platform we are developing is faster and more versatile than existing technologies.”

Such biomarkers could allow for better, more individually tailored treatments because they could be used to diagnose and monitor disease progression or remission as patients experience different treatment options.

At the heart of the new study is a unique group of enzymes known as protein arginine deiminases (PAD). An increase in PAD activity has been noted in a number of conditions, such as inflammatory diseases like rheumatoid arthritis as well as cancer and Alzheimer's disease.

PADs participate in reactions in the body that form the amino acid citrulline in proteins through a process known as citrullination, a modification that can have a significant impact on the structure and function of the modified proteins. While the exact role of citrullination remains unknown, inhibition of citrullination in animal models, using a compound developed by Thompson, has been shown to alleviate the severity of rheumatoid arthritis, ulcerative colitis, atherosclerosis, lupus, nerve damage and cancer.

In addition to developing biomarkers, the scientists will use the grant to better understand this modification and whether it provides a common link among these disparate diseases.

The number of the grant is 1R01GM110394-01.

New Grant Funds Study to Develop Better Diabetes Drugs

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have been awarded $2.1 million from the National Institutes of Health to study the therapeutic potential of safer and more effective alternatives to the current crop of anti-diabetic drugs, which have been limited in their use due to side effects including bone loss and congestive heart failure.

Douglas Kojetin, a TSRI associate professor, is the principal investigator for the new five-year study.

The study will take a cue from the two mainstays of type 2 diabetes treatment—pioglitazone (Actos) and rosiglitazone (Avandia). Both drugs raise the body's sensitivity to insulin, increasing the amount of glucose or sugar absorbed by the cells.

Studies have shown, however, that while these and other recently developed drugs are designed to bind to a specific site on the PPAR gamma (PPARG) nuclear receptor, they can also bind to an alternative site.

"This unexpected finding opens a lot of potential opportunities," Kojetin said. "We're looking to design a molecule that blocks both sites and can be used to probe what this alternative binding does on a molecular level—with the hope that this information will help us come up with a better drug model."

The number of the new National Institutes of Health grant is 1R01DK101871.

Scientists Wins $2 Million Grant to Study Impact of Early Nutrition on Lifespan

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have been awarded a $2 million grant from the National Institute on Aging of the National Institutes of Health to study the effects of early nutrition on lifespan and overall health.

William Ja, a TSRI assistant professor, is the principal investigator for the five-year study.

During critical periods of growth and development, particularly during early stages of life, animals are highly sensitive to nutrition—or lack of it—and modify their metabolism accordingly. This "nutritional-priming" phenomenon causes physiological changes that can persist throughout life and can have a marked impact on life-long health.

In humans, imbalanced nutrition during early childhood greatly influences future health and development, and poor diets during early growth periods can increase the likelihood of developing obesity, diabetes and heart disease in later life.

“This study will provide new strategies to develop drug candidates that could reverse the effects of a poor early diet—and that have the potential to improve human healthspan,” Ja said.

In addition, he noted, the cellular mechanisms underlying the effects of early diet on metabolism and aging have been largely unexplored. Unraveling those fundamental mechanisms are a primary aim of the new study.

Because multiyear, longitudinal studies—research done on a single group of individuals over an extended period of time—can be costly and difficult, Ja and his colleagues will first tackle the problem using the fruit fly,Drosophila melanogaster, a reliable and commonly used model, to investigate the role of early nutrition on lifespan and overall health.

“How poor nutrition affects aging is difficult to test in humans,” said Kimberley Bruce, a postdoctoral associate in the Ja laboratory who will spearhead the study. “But fruit flies live 80 days rather than 80 years, so we can rapidly see how different diets alter lifespan.”

For example, preliminary data generated by TSRI graduate student Sany Hoxha showed that even a brief exposure to a high-protein diet during early adulthood—a critical period of both fly and human development—reduces lifespan compared to animals fed a low protein diet during the same period.

“Protein stimulates the same aging pathways in flies as in humans,” Bruce added. “It has not been well studied, but we think excessive protein intake in early life may also have a negative effect on human aging.”

The number of the grant is 1R01AG045036-01A1.

Team Awarded $1.8 Million to Develop New Approaches to Lung Cancer Therapy

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have been awarded approximately $1.8 million from the National Cancer Institute of the National Institutes of Health to identify the signaling pathways that underlie lung cancer and to use this information to develop new therapeutic approaches.

Joseph Kissil, a TSRI associate professor, will be principal investigator of the new five-year grant, which extends a study that began in 2006.

Although death rates from lung cancer have fallen in the last 20 years, survival rates are still not good, largely because of a lack of effective treatments for advanced disease.

Kissil’s research into non-small cell lung cancer, the most common form of the disease, has shown that a well-known cancer-causing gene implicated in a number of malignancies plays a far more critical role than previously thought. Activating mutations of the K-ras gene are found in more than a third of lung cancers.

“There are clear links between K-ras-induced lung cancer and the receptor known as Notch 1,” Kissil said. “A loss of this receptor results in reduced tumor growth. The new grant will let us continue our research into Notch signaling pathways as potentially important therapeutic options for treatment of this disease.”

According to the U.S. Centers for Disease Control, lung cancer remains the leading cause of cancer deaths in the United States. In 2010, the most recent year these statistics are available, almost 300,000 American men and women were diagnosed with lung cancer.

The number of the new grant is 2R01CA124495.

Christoph Rader and William Roush Awarded $1.4 Million Leukemia Research Grant

Christoph Rader, an associate professor in the Department of Cancer Biology at The Scripps Research Institute (TSRI), and William Roush, TSRI professor, associate dean of graduate studies and executive director of medicinal chemistry, have been awarded more than $1.4 million from the National Cancer Institute of the National Institutes of Health (NIH) to create a potential new drug to attack the malignant cells that cause chronic lymphocytic leukemia (CLL), the most common leukemia in the Western world.

Rader will serve as principal investigator of the new three-year study, and Roush will be co-principal investigator.

The Scripps Florida scientists, who will be working with the NIH laboratories of Adrian Wiestner and Terrence Burke, plan to use the recently discovered cell surface receptor TOSO, which is overexpressed in leukemia cells, to create a rapid and effective entry point for delivering drugs to these malignant cells while bypassing normal cells as much as possible. In addition, the team plans to use an antibody fragment to add a second target to the treatment—the receptor tyrosine kinase ROR1, expressed exclusively in leukemia cells.

“This dual-targeting strategy will lay the foundation for further preclinical and clinical investigations in the treatment of this form of leukemia,” said Rader. “We also think that the novel biological and chemical components that come from this study can be easily exploited to develop combinations for diseases beyond CLL.”

Peter Hodder to Co-Lead Study to Find New Cancer Drugs

Peter Hodder, senior director of lead identification at TSRI’s Translational Research Institute in Florida, will act as a co-principal investigator for a new three-year study on potential new cancer therapies.

The project, funded by a $1.4 million grant (1RO1CA178143) from the National Cancer Institute of the National Institutes of Health and co-directed by Bruce Clurman of the Fred Hutchinson Cancer Research Center in Seattle, will focus on identifying small molecules that restore the function of ubiquitin ligases that are mutated in cancers.

The ubiquitin-proteasome system targets proteins for degradation and controls many biological processes. Disruption of this activity has profound consequences and contributes to many diseases, including cancer, making the system an important therapeutic target.

“We’re contributing the screening to the project to help find new probes for this target,” Hodder said. “We’re expecting to gather an interesting portfolio of candidates and, once that happens, we plan to do some of the drug optimization work at Scripps Florida as well.”

Grant Funds Development of New Methods to Identify Drug Candidates

A scientist from the Florida campus of The Scripps Research Institute (TSRI) has been awarded just over $1 million from the National Institute of General Medical Sciences of the National Institutes of Health to develop a series of tests (“assays”) that could point the way to potential new ways to find therapies for a host of debilitating diseases, including Alzheimer’s and Parkinson’s disease, heart disease, stroke and diabetes.

Philip LoGrasso, a professor in the Department of Molecular Therapeutics and senior scientific director in the Translational Research Institute at TSRI, is the principal investigator for the new three-year study.

The link between these seemingly disparate diseases is a protein known as jun-N-terminal kinase (JNK), an important contributor to stress-induced cell death in key cell types, including neurons, heart muscle cells and beta-islets (which store and release insulin).

LoGrasso's goal is to develop novel assays that will point to new drug candidates and a better understanding of how inhibiting JNK can prevent mitochondrial dysfunction (disrupting the energy source of the cell) and cell death.

"This grant will help us take kinase assay development and drug discovery in a bold new direction," LoGrasso said. The new tests, he added, should produce inhibitors that could be much more selective and have the potential for reduced toxicity.

One of LoGrasso's discoveries related to JNK is already in development with OPKO Health, Inc., a Miami-based biotechnology company, for the treatment of Parkinson's disease.

The number of the grant is 1R01GM103825-01A1.

Grant Funds Study of How Exposure to Pain Meds in the Womb Affects Developing Brain

Scientists from the Florida campus of The Scripps Research Institute have been awarded a $472,500 Cutting Edge Basic Research Award (CEBRA) by the National Institute on Drug Abuse (NIDA) of the National Institutes of Health to study models of the brain development of newborns who have been exposed—and become addicted—to prescription pain medication while still in the womb.

Courtney Miller, a TSRI associate professor, is the principal investigator for the new two-year study.

"We don't really know the long-term effects of exposure to pain pills—how well will these babies function in adolescence and adulthood," Miller said. "Given the fact that there is one baby born every hour to mothers that abused pain pills during pregnancy—NICUs [neonatal intensive care units] are filled with infants going through withdrawal—we really need to understand the effects of this epidemic on mental function and brain wiring."

While previous research has suggested that such addiction at birth may lead to impulse control disorders such as ADHD, schizophrenia and addiction, little is known about the underlying biology of that chain of events.

Thanks to funding through CEBRA—designed to foster highly innovative research related to drug abuse and addiction and how to prevent and treat them—Miller and her colleagues, including TSRI Associate Professor Gavin Rumbaugh, will use rodent models to understand which areas of the brain are affected by this condition and how subtle changes in brain wiring might lead to later changes in behavior.

This research has special significance for Florida. At its peak, doctors in Florida prescribed 10 times more oxycodone pills than every other state in the country combined, and people traveled from all over the Southeast to visit the state's pain clinics because of their reputation as "pill mills," according to a 2011 report on National Public Radio. The state has since cracked down on the clinics and abuse has dropped off somewhat, but prescription drug abuse remains a serious public health threat.

The number of the new National Institutes of Health grant is 1R21DA036376.

Part 3: Scientific Accomplishments

Study Shows a Solitary Mutation Can Destroy Critical 'Window' of Early Brain Development

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have shown in animal models that brain damage caused by the loss of a single copy of a gene during very early childhood development can cause a lifetime of behavioral and intellectual problems.

The study, published recently in the *Journal of Neuroscience*, sheds new light on the early development of neural circuits in the cortex, the part of the brain responsible for functions such as sensory perception, planning and decision-making.

The research also pinpoints the mechanism responsible for the disruption of what are known as "windows of plasticity" that contribute to the refinement of the neural connections that broadly shape brain development and the maturing of perception, language, and cognitive abilities.

The key to normal development of these abilities is that the neural connections in the brain cortex—the synapses—mature at the right time.

In an earlier study, the team, led by TSRI Associate Professor Gavin Rumbaugh, found that in mice missing a single copy of the vital gene, certain synapses develop prematurely within the first few weeks after birth. This accelerated maturation dramatically expands the process known as "excitability"—how often brain cells fire—in the hippocampus, a part of the brain critical for memory. The delicate balance between excitability and inhibition is especially critical during early developmental periods. However, it remained a mystery how early maturation of brain circuits could lead to lifelong cognitive and behavioral problems.

The current study shows in mice that the interruption of the synapse-regulating gene known as SYNGAP1—which can cause a devastating form of intellectual disability and increase the risk for developing autism in humans—induces early functional maturation of neural connections in two areas of the cortex. The influence of this disruption is widespread throughout the developing brain and appears to degrade the duration of these critical windows of plasticity.

"In this study, we were able to directly connect early maturation of synapses to the loss of an important plasticity window in the cortex," Rumbaugh said. "Early maturation of synapses appears to make the brain less plastic at critical times in development. Children with these mutations appear to have brains that were built incorrectly from the ground up."

The accelerated maturation also appeared to occur surprisingly early in the developing cortex. That, Rumbaugh added, would correspond to the first two years of a child's life, when the brain is expanding rapidly. "Our goal now is to figure out a way to prevent the damage caused by SYNGAP1 mutations. We would be more likely to help that child if we could intervene very early on—before the mutation has done its damage," he said.

The first author of the study, "SYNGAP1 Links the Maturation Rate of Excitatory Synapses to the Duration of Critical-Period Synaptic Plasticity," is James P. Clement of TSRI. Other authors include Emin D. Ozkan, Massimiliano Aceti and Courtney A. Miller, also of TSRI. For more information, see http://www.jneurosci.org/content/33/25/10447.full

This work was supported by the National Institute for Neurological Disorders and Stroke (grant R01NS064079), the National Institute for Mental Health (grant R01MH096847) and the National Institute for Drug Abuse (grants R01 DA034116 and R03 DA033499).

Scientists Turn Muscular Dystrophy Defect On and Off in Cells

For the first time, scientists from the Florida campus of The Scripps Research Institute (TSRI) have identified small molecules that allow for complete control over a genetic defect responsible for the most common adult onset form of muscular dystrophy. These small molecules will enable scientists to investigate potential new therapies and to study the long-term impact of the disease.

"This is the first example I know of at all where someone can literally turn on and off a disease," said TSRI Associate Professor Matthew Disney, whose new research was published recently in the journal *Nature Communications*." This easy approach is an entirely new way to turn a genetic defect off or on."

Myotonic dystrophy is an inherited disorder, the most common form of a group of conditions called muscular dystrophies that involve progressive muscle wasting and weakness. Myotonic dystrophy type 1 is caused a type of RNA defect known as a "triplet repeat," a series of three nucleotides repeated more times than normal in an individual's genetic code. In this case, a cytosine-uracil-guanine (CUG) triplet repeat binds to the protein MBNL1, rendering it inactive and resulting in RNA splicing abnormalities.

To find drug candidates that act against the defect, Disney and his colleagues analyzed the results of a National Institutes of Health (NIH)-sponsored screen of more than 300,000 small molecules that inhibit a critical RNA-protein complex in the disease.

The team divided the NIH hits into three "buckets"—the first group bound RNA, the second bound protein, and a third whose mechanism was unclear. The researchers then studied the compounds by looking at their effect on human muscle tissue both with and without the defect.

Startlingly, diseased muscle tissue treated with RNA-binding compounds caused signs of the disease to go away. In contrast, both healthy and diseased tissue treated with the protein-binding compounds showed the opposite effect—signs of the disease either appeared (in healthy tissue) or became worse.

The new compounds will serve as useful tools to study the disease on a molecular level. "In complex diseases, there are always unanticipated mechanisms," Disney noted. "Now that we can reverse the disease at will, we can study those aspects of it."

In addition, Disney said, with the new discovery, scientists will be able to develop a greater understanding of how to control RNA splicing with small molecules. RNA splicing can cause a host of diseases that range from sickle-cell disease to cancer, yet prior to this study, no tools were available to control specific RNA splicing.

The first authors of the study, "Induction and Reversal of Myotonic Dystrophy Type 1 Pre-mRNA Splicing Defects by Small Molecules," are Jessica L. Childs-Disney of TSRI, Ewa Stepniak-Konieczna of Adam Mickiewicz University (Poland) and Tuan Tran of TSRI. Other authors include Ilyas Yildirim and George C. Schatz of Northwestern University; HaJeung Park of TSRI; Catherine Z. Chen, Noel Southall, Juan J. Marugan, Samarjit Patnaik, Wei Zheng and Chris P. Austin of the NIH; Krzysztof Sobczak of Adam Mickiewicz University; and Charles A. Thornton and Jason Hoskins of the University of Rochester. For more information, see
http://www.nature.com/ncomms/2013/130628/ncomms3044/abs/ncomms3044.html

The study was funded by TSRI; the Muscular Dystrophy Association (158552); the National Institutes of Health (3R01GM079235 and 1R01GM079235; AR049077 and U54NS48843); the National Cancer Institute (1U54CA143869); the Molecular Libraries Initiative of the National Institutes of Health Roadmap for Medical Research; the Marigold Foundation and the Foundation for Polish Science-TEAM program co-financed by the European Union within the European Regional Development Fund.

Researchers Design a Potential Drug Compound that Attacks Parkinson's Disease on Two Fronts

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have found a compound that could counter Parkinson's disease in two ways at once.

In a new study published recently online ahead of print by the journal *ACS Chemical Biology*, the scientists describe a "dual inhibitor"—two compounds in a single molecule—that attacks a pair of proteins closely associated with development of Parkinson's disease.

"In general, these two enzymes amplify the effect of each other," said team leader Phil LoGrasso, a TSRI professor who has been a pioneer in the development of JNK inhibitors for the treatment of neurodegenerative diseases. "What we were looking for is a high-affinity, high-selectivity treatment that is additive or synergistic in its effect—a one-two punch."

That could be what they found.

This new dual inhibitor attacks two enzymes—the leucine-rich repeat kinase 2 (LRRK2) and the c-jun-N-terminal kinase (JNK)—pronounced "junk." Genetic testing of several thousand Parkinson's patients has shown that mutations in the LRRK2 gene increase the risk of Parkinson's disease, while JNK has been shown to play an important role in neuron (nerve cell) survival in a range of neurodegenerative diseases. As such, they have become highly viable targets for drugs to treat disorders such as Parkinson's disease.

A dual inhibitor ultimately would be preferred over separate individual JNK and LRRK2 inhibitors because a combination molecule would eliminate complications of drug-drug interactions and the need to optimize individual inhibitor doses for efficacy, the study noted.

Now the team's new dual inhibitor will need to be optimized for potency, high selectivity (which reduces off-target side effects) and bioavailability so it can be tested in animal models of Parkinson's disease.

The first author of the study, "A Small Molecule Bidentate-Binding Dual Inhibitor Probe of the LRRK2 and JNK Kinases," is Yangbo Feng of TSRI. Other authors include Jeremy W. Chambers, Sarah Iqbal, Marcel Koenig, HaJeung Park, Lisa Cherry, Pamela Hernandez and Mariana Figuera-Losada. For more information see http://pubs.acs.org/doi/abs/10.1021/cb3006165

The study was supported by the National Institutes of Health grant NS057153.

Drug Candidate Leads to Improved Endurance

An international group of scientists has shown that a drug candidate designed by scientists from the Florida campus of The Scripps Research Institute (TSRI) significantly increases exercise endurance in animal models.

These findings could lead to new approaches to helping people with conditions that acutely limit exercise tolerance, such as obesity, chronic obstructive pulmonary disease (COPD) and congestive heart failure, as well as the decline of muscle capacity associated with aging.

The study was published July 14, 2013, by the journal *Nature Medicine*.

The drug candidate, SR9009, is one of a pair of compounds developed in the laboratory of TSRI Professor Thomas Burris and described in a March 2012 issue of the journal Nature as reducing obesity in animal models. The compounds affect the core biological clock, which synchronizes the rhythm of the body's activity with the 24-hour cycle of day and night.

The compounds work by binding to one of the body's natural molecules called Rev-erbα, which influences lipid and glucose metabolism in the liver, the production of fat-storing cells and the response of macrophages (cells that remove dying or dead cells) during inflammation.

In the new study, a team led by scientists at the Institut Pasteur de Lille in France demonstrated that mice lacking Rev-erbα had decreased skeletal muscle metabolic activity and running capacity. Burris' group showed that activation of Rev-erbα with SR9009 led to increased metabolic activity in skeletal muscle in both culture and in mice. The treated mice had a 50 percent increase in running capacity, measured by both time and distance.

"The animals actually get muscles like an athlete who has been training," said Burris. "The pattern of gene expression after treatment with SR9009 is that of an oxidative-type muscle— again, just like an athlete."

The authors of the new study suggest that Rev-erbα affects muscle cells by promoting both the creation of new mitochondria (often referred to as the "power plants" of the cell) and the clearance of those mitochondria that are defective.

The study, "Rev-Erbα Modulates Skeletal Muscle Oxidative Capacity by Regulating Mitochondrial Biogenesis and Autophagy" was led by Estelle Woldt and Yasmine Sebti (first authors) and Bart Staels and Hélène Duez (senior authors) of Institut Pasteur de Lille, France. Other contributors include Christian Duhem, Jérôme Eeckhoute, Charlotte Paquet, Stéphane Delhaye and Philippe Lefebvre of Institut Pasteur de Lille, France; Laura Solt, Youseung Shin, Thomas Burris and Theodore M. Kamenecka of TSRI; Steve Lancel and Rémi Nevière of Université Lille Nord de France; and Matthijs K.C. Hesselink, Gert Schaart and Patrick Schrauwen of Maastricht University Medical Center, Maastricht, the Netherlands. For further information, seehttp://www.nature.com/nm/journal/vaop/ncurrent/full/nm.3213.html

The study was supported by a Marie Curie International Reintegration Grant (FP7), the European Commission (FP7) consortium Eurhythdia, Région Nord Pas-de-Calais/FEDER, a CPER "starting grant," the European Genomic Institute for Diabetes (ANR-10-LABX-46), an unrestricted ITMO/Astra Zeneca grant, a joint Société Francophone du Diabète MSD research fellowship, Research Grant from the European Foundation for the Study of Diabetes, National Institutes of Health grant (MH093429 and DK080201) and a VICI Research grant for innovative research from the Netherlands Organization for Scientific Research (918.96.618).

Scientists Devise New Way to Dramatically Raise RNA Treatment Potency

Scientists from the Jupiter campus of The Scripps Research Institute have shown a novel way to dramatically raise the potency of drug candidates targeting RNA, resulting in a 2,500-fold improvement in potency and significantly increasing their potential as therapeutic agents.

The new study, published recently online ahead of print by the journal *Angewandte Chemie*, confirms for the first time that a small molecule actually binds to a disease-causing RNA target—a breakthrough that should help scientists identify precise RNA targets within living cells, profile their interactions, and predict drug candidates' side effects.

"We're trying to make tools that can target any RNA motif," said Matthew Disney, a TSRI associate professor who authored the research with a research associate in his lab, Lirui Guan. "This study completely validates our design—it validates that our compound targets the desired RNA sequence in a complex cellular environment that contains many hundreds of thousands of RNAs."

While targeting DNA has been used as a therapeutic strategy against cancer, few similar approaches have been attempted for disease-associated RNAs.

In the new study, the scientists created a small molecule that binds to the genetic defect in RNA that causes myotonic dystrophy type 1 and improves associated defects in cell culture.

Myotonic dystrophy type 1 involves a type of RNA defect known as a "triplet repeat," a series of three nucleotides repeated more times than normal in an individual's genetic code. In this case, the repetition of the cytosine-uracil-guanine (CUG) in the RNA sequence leads to disease by binding to a particular protein, MBNL1, rendering it inactive and resulting in a number of protein-splicing abnormalities.

To achieve the increase in the drug candidate's potency, Disney and his colleagues attached a reactive molecule (a derivative of chlorambucil, a chemotherapy drug that has been used to treatment a form of leukemia) to the small molecule they had identified. As a result, the new compound not only binds to the target, it becomes a permanent part of the target—as if it were super glued to it, Disney said. Once attached, it switches off the CUG defect and prevents the cell from turning it back on.

Disney was surprised at the approximately 2,500-fold improvement in potency with the new approach.

"I was shocked by the increase," he said. "This takes the potency into the realm where one would like to see if the compound were to have real therapeutic potential."

As a result, the new compound, known as 2H-4-CA, is the most potent compound known to date that improves DM1-associated splicing defects. Importantly, 2H-4-CA does not affect the alternative splicing of a transcript not regulated by MBNL1, demonstrating selectivity for the CUG repeat and suggesting that it might have minimal side effects.

"We can now use this approach to attach reactive molecules to other RNA targeted small molecules," Disney said.

The reactive molecule model also provides a potentially general method to identify cellular targets of RNA-directed small molecules. Such probes could also identify unintended targets, information that could be used to design and identify compounds with improved selectivity in an approach similar to activity-based profiling, Disney said.

The study, "Covalent Small-Molecule–RNA Complex Formation Enables Cellular Profiling of Small-Molecule–RNA Interactions," (DOI: 10.1002/anie.201301639) was supported by the National Institutes of Health (grant RO1- GM079235) and TSRI. For more information on the paper, seehttp://onlinelibrary.wiley.com/doi/10.1002/anie.201301639/full

New Findings Could Help Improve Development of Drugs for Addiction

Scientists from the Florida campus of The Scripps Research Institute have described findings that could enable the development of more effective drugs for addiction with fewer side effects.

The study, published in the August 2, 2013 issue of the *Journal of Biological Chemistry*, showed in a combination of cell and animal studies that one active compound maintains a strong bias towards a single biological pathway, providing insight into what future drugs could look like.

The compound examined in the study, known as 6'- guanidinonaltrindole (6'-GNTI), targets the kappa opioid receptor (KOR). Located on nerve cells, KOR plays a role in 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.

"There are a number of drug discovery efforts ongoing for KOR," said Laura Bohn, a TSRI associate professor, who led the study. "The ultimate question is how this receptor should be acted upon to achieve the best therapeutic effects. Our study identifies a marker that shows how things normallyhappen in live neurons—a critically important secondary test to evaluate potential compounds."

While KOR has become the focus for drug discovery efforts aimed at treating addiction and mood disorders, KOR can react to signals that originate independently from multiple biological pathways, so current drug candidates targeting KOR often produce unwanted side effects. Compounds that activate KOR can decrease the rewarding effects of abused drugs, but also induce sedation and depression.

The new findings, from studies of nerve cells in the striatum (an area of the brain involved in motor activity and higher brain function), reveal a point on the KOR signaling pathway that may prove to be an important indicator of whether drug candidates can produce effects similar to the natural biological effects.

"Standard screening assays can catch differences but those differences may not play out in live tissue," Bohn noted. "Essentially, we have shown an important link between cell-based screening assays and what occurs naturally in animal models."

The first author of the study, 'Functional Selectivity of 6'-guanidinonaltrindole (6'-GNTI) at Kappa Opioid Receptors in Striatal Neurons," is Cullen L. Schmid of TSRI. Other authors include John M. Streicher, Chad E. Groer and Lei Zhou of TSRI; and Thomas A. Munro of the Mailman Research Center and Harvard Medical School. For more information about the study, seehttp://www.jbc.org/content/early/2013/06/17/jbc.M113.476234.full.pdf+html

The study was supported by the National Institutes of Health (grant R01 DA031927).

Researchers Detail Critical Role of Gene in Many Lung Cancer Cases

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have shown that a well-known cancer-causing gene implicated in a number of malignancies plays a far more critical role in non-small cell lung cancer, the most common form of the disease, than previously thought.

These findings establish the gene as a critical regulator of lung cancer tumor growth. This new information could turn out to be vital for the design of potentially new therapeutic strategies for a group of patients who represent almost half of non-small cell lung cancer cases.

In the study, published online ahead of print by the journal *Cancer Research*, the scientists found that presence of known oncogene Notch1 is required for survival of cancer cells. In both cell and animal model studies, disabling Notch1 leads to a rise in cancer cell death.

"While Notch signaling has emerged as an important target in many types of cancer, current methodologies that target that pathway affect all members of the Notch family, and this has been associated with toxicity," said Joseph Kissil, a TSRI associate professor who led the study. "We were able to identify *Notch1* as the critical oncogene to target, at least in a common form of lung cancer."

The new findings show that Notch1 is required for initial tumor growth, as it represses p53, a well-known tumor suppressor protein that has been called the genome's guardian because of its role in

preventing mutations. The p53 protein can repair damaged cells or force them to die through apoptosis—programmed cell death.

Using animal models, the study shows that inhibition of Notch1 signaling results in a dramatic decrease in initial tumor growth. Moreover, disruption of Notch1 induces apoptosis by increasing p53 stability—substantially increasing its biological half-life, for example.

These findings provide important clinical insights into the correlation between Notch1 activity and the poor prognosis of non-small cell lung cancer patients who carry the non-mutated form of the p53 gene. "If you look at lung cancer patient populations, Notch signaling alone isn't a prognostic indicator, but if you look at p53-positive patients it is," Kissil said.

The first author of the study, "Notch1 is Required for Kras-Induced Lung Adenocarcinoma and Controls Tumor Cell Survival via P53" (doi:10.1158/0008-5472.CAN-13-1384), is Silvia Licciulli of TSRI. Other authors include Jacqueline L. Avila, Linda Hanlon and Ellen Pure of The Wistar Institute; Scott Troutman and Smitha Kota of TSRI; Matteo Cesaroni of Temple University School of Medicine, Brian Keith and M. Celeste Simon of the University of Pennsylvania School of Medicine; Fred Radtke of the Ecole Polytechnique Fédérale de Lausanne, Switzerland; and Anthony J. Capobianco of the University of Miami Miller School of Medicine. For more information on the study, seehttp://cancerres.aacrjournals.org/content/early/2013/08/13/0008-5472.CAN-13-1384.abstract

This research was supported by the National Institutes of Health (grant number CA124495).

Findings Suggest Possibility of Selectively Erasing Unwanted Memories

The human brain is exquisitely adept at linking seemingly random details into a cohesive memory that can trigger myriad associations—some good, some not so good. For recovering addicts and individuals suffering from post-traumatic stress disorder (PTSD), unwanted memories can be devastating. Former meth addicts, for instance, report intense drug cravings triggered by associations with cigarettes, money, even gum (used to relieve dry mouth), pushing them back into the addiction they so desperately want to leave.

Now, for the first time, scientists from the Florida campus of The Scripps Research Institute (TSRI) have been able to erase dangerous drug-associated memories in mice and rats without affecting other more benign memories.

The surprising discovery, published by the journal *Biological Psychiatry*, points to a clear and workable method to disrupt unwanted memories while leaving the rest intact.

"Our memories make us who we are, but some of these memories can make life very difficult," said Courtney Miller, a TSRI assistant professor who led the research. "Not unlike in the movie Eternal Sunshine of the Spotless Mind, we're looking for strategies to selectively eliminate evidence of past experiences related to drug abuse or a traumatic event. Our study shows we can do just that in mice — wipe out deeply engrained drug-related memories without harming other memories."

Changing the Structure of Memory

To produce a memory, a lot has to happen, including the alteration of the structure of nerve cells via changes in the dendritic spines—small bulb-like structures that receive electrochemical signals from other neurons. Normally, these structural changes occur via actin, the protein that makes up the infrastructure of all cells.

In the new study, the scientists inhibited actin polymerization—the creation of large chainlike molecules—by blocking a molecular motor called myosin II in the brains of mice and rats during the maintenance phase of methamphetamine-related memory formation.

Behavioral tests showed the animals immediately and persistently lost memories associated with methamphetamine—with no other memories affected.

In the tests, animals were trained to associate the rewarding effects of methamphetamine with a rich context of visual, tactile and scent cues. When injected with the inhibitor many days later in their home environment, they later showed a complete lack of interest when they encountered drug-associated cues. At the same time, the response to other memories, such as food rewards, was unaffected.

While the scientists are not yet sure why powerful methamphetamine-related memories are also so fragile, they think the provocative findings could be related to the role of dopamine, a neurotransmitter involved in reward and pleasure centers in the brain and known to modify dendritic spines. Previous studies had shown dopamine is released during both learning and drug withdrawal. Miller adds, "We are focused on understanding what makes these memories different. The hope is that our strategies may be applicable to other harmful memories, such as those that perpetuate smoking or PTSD."

The first author of the study, "Selective, Retrieval-Independent Disruption of Methamphetamine-Associated Memory by Actin Depolymerization," is Erica J. Young; other authors include Massimiliano Aceti, Erica M. Griggs, Rita A. Fuchs, Zachary Zigmond and Gavin Rumbaugh of TSRI. For more information, see http://www.biologicalpsychiatryjournal.com/article/S0006-3223(13)00727-0/abstract

The research was supported by the National Institute on Drug Abuse (grant numbers R00 DA024761 and R01DA034116) and the National Institute for Neurological Disorders and Stroke (R01NS064079).

Scientists Pinpoint Proteins Vital to Long-Term Memory

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have found a group of proteins essential to the formation of long-term memories.

The study, published online ahead of print on September 12, 2013 by the journal *Cell Reports*, focuses on a family proteins called Wnts. These proteins send signals from the outside to the inside of a cell, inducing a cellular response crucial for many aspects of embryonic development, including stem cell differentiation, as well as for normal functioning of the adult brain.

"By removing the function of three proteins in the Wnt signaling pathway, we produced a deficit in long-term but not short-term memory," said Ron Davis, chair of the TSRI Department of Neuroscience. "The pathway is clearly part of the conversion of short-term memory to the long-term stable form, which occurs through changes in gene expression."

The findings stem from experiments probing the role of Wnt signaling components in olfactory memory formation in Drosophila, the common fruit fly—a widely used doppelgänger for human memory studies. In the new study, the scientists inactivated the expression of several Wnt signaling proteins in the mushroom bodies of adult flies—part of the fly brain that plays a role in learning and memory.

The resulting memory disruption, Davis said, suggests that Wnt signaling participates actively in the formation of long-term memory, rather than having some general, non-specific effect on behavior.

"What is interesting is that the molecular mechanisms of adult memory use the same processes that guide the early development of the organism, except that they are repurposed for memory formation,"

he said. “One difference, however, is that during early development the signals are intrinsic, while in adults they require an outside stimulus to create a memory.”

The first author of the study, “Wnt signaling is required for long-term memory formation,” is Ying Tan of the Baylor College of Medicine. Other authors include Germain U. Busto of TSRI and Curtis Wilson of Baylor College of Medicine. For more information, see http://www.cell.com/cell-reports/abstract/S2211-1247(13)00431-2

The study was supported by the National Institutes of Health (NS19904).

Scientists Develop New Process to Create Artificial Cell Membranes

The membranes surrounding and inside cells are involved in every aspect of biological function. They separate the cell’s various metabolic functions, compartmentalize the genetic material, and drive evolution by separating a cell’s biochemical activities. They are also the largest and most complex structures that cells synthesize.

Understanding the myriad biochemical roles of membranes requires the ability to prepare synthetic versions of these complex multi-layered structures, which has been a long-standing challenge.

In a study published by *Nature Chemistry*, scientists at The Scripps Research Institute (TSRI) report a highly programmable and controlled platform for preparing and experimentally probing synthetic cellular structures.

“Layer-by-layer membrane assembly allows us to create synthetic cells with membranes of arbitrary complexity at the molecular and supramolecular scale,” said TSRI Assistant Professor Brian Paegel, who authored the study with Research Associate Sandro Matosevic. “We can now control the molecular composition of the inner and outer layers of a bilayer membrane, and even assemble multi-layered membranes that resemble the envelope of the cell nucleus.”

Starting with a technique commonly used to deposit molecules on a solid surface, Langmuir-Blodgett deposition, the scientists repurposed the approach to work on liquid objects.

The scientists engineered a microfluidic device containing an array of microscopic cups, each trapping a single droplet of water bathed in oil and lipids, the molecules that make up cellular membranes. The trapped droplets are then ready to serve as a foundation for building up a series of lipid layers like coats of paint.

The lipid-coated water droplets are first bathed in water. As the water/oil interface encounters the trapped droplets, a second lipid layer coats the droplets and transforms them into what are known as unilamellar or single-layer vesicles. Bathing the vesicles in oil/lipid deposits a third lipid layer, and followed by a final layer of lipids that is deposited on the trapped drops to yield double-bilayer vesicles.

“The computer-controlled microfluidic circuits we have constructed will allow us to assemble synthetic cells not only from biologically derived lipids, but from any amphiphile and to measure important chemical and physical parameters, such as permeability and stability,” said Paegel.

The study, “Layer-by-layer Cell Membrane Assembly,” was supported by a National Institutes of Health Pathway to Independence Career Development Award (GM083155) and a National Science Foundation CAREER Award (1255250). For more information on the research, seehttp://www.nature.com/nchem/journal/vaop/ncurrent/abs/nchem.1765.html

Researchers Shed Light on Body's Master Energy Regulator

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have discovered some key features that explain just what turns on a protein that is considered to be a master regulator of how the human body uses and stores energy.

The new discoveries could help in the design and development of new therapeutics to treat metabolic disease such as diabetes and obesity—and perhaps some cancers as well.

The new study, led by Patrick R. Griffin, chair of the TSRI Department of Molecular Therapeutics, was published recently online ahead of print by the journal *Structure*.

All cells need energy to function, and a molecule known as ATP stores what the cells use for fuel. The balance between stored energy and energy consumption is in constant flux, so the ability to sense changes in those energy supplies is essential for survival. When energy reserves run low, a molecule known as AMP activated protein kinase (AMPK), which monitors cellular energy levels, stimulates the generation of ATP and increases those energy stores.

As a result, AMPK has become an attractive target for the treatment of metabolic diseases like diabetes and obesity. But the development of molecular activators of kinase has lagged behind inhibitors because while inhibition is straight forward, less is understood about the mechanism of hyper-activation of kinases.

"Other research has revealed compounds that activate AMPK," said Griffin. "However, until our study no one has been able to confirm exactly where those compounds bind, nor how binding of these molecules leads to an increase in the activity of the kinase."

In the new research, Griffin and his colleagues not only identified the subunit where binding took place, but were able to show that binding by a known AMPK activator fully activates the protein—specifically through biochemical communication with the other subunits, a process that allows AMPK to respond quickly to changes in the cellular energy levels.

"Confirming the activation site and how it communicates with the rest of the protein means that others can start to model AMPK activators to see how effective they might be as potential drugs," Griffin said.

The first authors of the study, "Activation of AMP-Activated Protein Kinase Revealed by Hydrogen/Deuterium Exchange Mass Spectrometry," are Rachelle R. Landgraf and Devrishi Goswami of TSRI. Other authors include Francis Rajamohan, Melissa S. Harris, Matthew Calabrese, Lise R. Hoth, Rachelle Magyar and Ravi Kurumbail of Pfizer Worldwide Research and Development; and Bruce D. Pascal, Michael J. Chalmers and Scott A. Busby of TSRI. For more information on the study, seehttp://www.cell.com/structure/abstract/S0969-2126(13)00344-4

The research was supported Pfizer, Inc.

Team Identifies Potential New Drug for Inherited Cancer

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have identified a new drug candidate for an inherited form of cancer with no known cure.

The new study showed the drug candidate—known as FRAX97—slowed the proliferation and progression of tumor cells in animal models of Neurofibromatosis type 2. This inherited type of cancer, caused by mutations in the anti-tumor gene NF2, leads to tumors of the auditory nerve that connects the inner ear to the brain.

The new compound, originally developed to treat neurodegenerative disease, targets a protein family known as p21-activated kinases or PAKs. These kinases (enzymes that add a phosphate group to other proteins and change their function) play a critical role in the development of Neurofibromatosis type 2. PAK1 has also been implicated in the growth of breast and lung cancers.

"Our study shows that if we inhibit these kinases we can counter the formation of tumors in this brain disease," said Joseph Kissil, a TSRI associate professor who led the study.

In the new study, published in the October 4, 2013 issue of *The Journal of Biological Chemistry*, Kissil and his colleagues showed that the inhibitor slows down progression of Neurofibromatosis type 2 in animal models and reduces more than 80 percent of PAK1 activity.

Kissil notes a key challenge in developing drug candidates is finding potential agents that are both potent and highly selective for their targets—limiting its action to the desired arena and reducing unwanted side effects.

"This inhibitor turned out to be both potent and highly selective," he said. "The real question is why. We were able to show that it works through a unique mechanism."

While the binding site on PAK1 is quite large, it also contains a smaller pocket, a kind of backroom that juts off the larger site. The inhibitor not only takes up space in the larger site, but enters the back pocket as well. That extra binding gives the inhibitor its strong selectivity.

The first authors of the study, "FRAX597, a Small Molecule Inhibitor of The P21-Activated Kinases, Inhibits Tumorigenesis of NF2-Associated Schwannomas," (The Journal of Biological Chemistry, Vol. 288, Issue 40, 29105-29114) are Silvia Licciuli and Scott Troutman of TSRI; and Jasna Maksimoska and Chu Zhou of The Wistar Institute (Philadelphia, PA). Other authors include Smitha Kota of TSRI; Qin Liu and Ronen Marmorstein of The Wistar Institute; Sergio Duron and David Campbell of Afraxis, Inc. (San Diego); Jonathan Chernoff of Fox Chase Cancer Center (Philadelphia, PA) and Jeffrey Field of University of Pennsylvania Perlman School of Medicine. For more information on the paper, see:http://www.jbc.org/content/early/2013/08/19/jbc.M113.510933.abstract

The study was supported by the National Institutes of Health (grant numbers CA124495, CA114046 and CA148805); the American Cancer Society (RSG-10-018-01-CDD) and Afraxis, Inc.

Team Links a Protein to Initial Tumor Growth in Several Cancers

A team led by scientists from The Scripps Research Institute (TSRI) have shown that a protein once thought to inhibit the growth of tumors is instead required for initial tumor growth. The findings could point to a new approach to cancer treatment.

The study was published recently as the cover article of the journal *Science Signaling*.

The focus of the study was angiomotin, a protein that coordinates cell migration, especially during the start of new blood vessel growth and proliferation of other cell types.

“We were the first to describe angiomotin’s involvement in cancer,” said Joseph Kissil, a TSRI associate professor who led the studies. “ And while some following studies found it to be inhibiting, we wanted to clarify its role by using both cell studies and animal models. As a result, we have now found that it is not an inhibitor at all, but instead is required for Yap to produce new tumor growth.”

Yap (Yes-associated-Protein) is a potent oncogene that is over-expressed in several types of tumors.

In addition to identifying angiomotin’s critical role in tumor formation, Kissil and his colleagues found the protein is active within the cell nucleus. Earlier cell studies focused on the function of the protein at the cell membrane.

“This pathway, which was discovered less than a decade ago, appears to regulate processes that are closely linked to cancer,” Kissil said. “The more we study it, the more we see its involvement.”

The first authors of the study, “The p130 Isoform of Angiomotin Is Required for Yap-Mediated Hepatic Epithelial Cell Proliferation and Tumorigenesis,” are Chunling Yi of Georgetown University Medical Center and Zhewei Shen of the University of Pennsylvania.

Other authors include Anat Stemmer-Rachamimov of Massachusetts General Hospital; Noor Dawany, Louise C. Showe and Qin Liu of The Wistar Institute; Scott Troutman of TSRI; Akihiko Shimono of TransGenic, Inc.; Marius Sudol of Geisinger Clinic; Lars Holmgren of Karolinska Institutet, Stockholm; and Ben Z. Stanger of the University of Pennsylvania. For more information, seehttp://stke.sciencemag.org/cgi/content/abstract/sigtrans;6/291/ra77

This study was supported by the National Institutes of Health (grant numbers DK083355 and DK083111; CA142295 and NS077952; and CA0180815 and CA132098), the Commonwealth of PA (66651-01), the PA Breast Cancer Coalition (60707 and 920093), the Abramson Family Cancer Research Institute, the Geisinger Clinic, the Pew Charitable Trusts, the Children’s Tumor Foundation, the Georgetown Lombardi Cancer Center, a Cell and Molecular Biology training.

Compound Dramatically Reduces Joint Inflammation

An experimental compound synthesized and developed by scientists from the Florida campus of The Scripps Research Institute (TSRI) has the capacity to significantly reduce joint inflammation in animal models of rheumatoid arthritis, an autoimmune disease that affects more than two million Americans.

The study was published recently online ahead of print by the journal *Arthritis & Rheumatism*.

The study showed the compound, known as SR2211, blocked development of virtually all symptoms of rheumatoid arthritis in mice within the first eight to ten days of treatment. The mice also showed significantly reduced bone and cartilage erosion compared to animals that did not receive treatment.

The experimental compound targets the nuclear receptor RORγ, a key regulator of TH17 cells, one of a family of white blood cells that play a role in the immune system. Identified only a decade ago, TH17 cells have been implicated in numerous autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease and lupus.

“This compound, and its precursors, showed the ability to block the release of specific inflammatory mediators from Th17 cells in culture, so we were confident that SR2211 would demonstrate good efficacy in rodent models of autoimmune disease,” said biochemist Patrick R. Griffin, chair of the TSRI Department of Molecular Therapeutics. “Our newest study strongly supports the idea that by targeting

the RORγ receptor, we can therapeutically repress inflammation and joint destruction associated with rheumatoid arthritis."

While several treatments are currently available for rheumatoid arthritis, Griffin noted their use is associated with the increased risk of infections and pneumonia. Since they have to be taken by injection, they are optimized for long, sustained immunosuppressive action, which is a disadvantage in managing opportunistic infections. An oral medication could be taken daily and stopped immediately to allow the drug to leave the body in the case of a potentially life-threatening infection.

"This study with SR2211 shows that repressing the activity of the RORγ receptor alone works to reduce joint erosion and inflammation," Griffin said. "It's an alternative mechanism of action that can provide doctors with additional treatment options for patients who do not respond well or cannot tolerate current therapies."

"We wanted to develop a compound with the potential to help treat patients suffering from a range of autoimmune diseases, including rheumatoid arthritis," said Staff Scientist Mi Ra Chang, the first author of the study and a member of the Griffin lab. "Compounds such as SR2211 work directly and specifically on at least two immune cell types directly involved in the pathogenesis of autoimmune disease."

In addition to Griffin and Chang, other authors of the study, "Pharmacological Repression of RORγ Is Therapeutic in the Collagen-induced Arthritis Experimental Model" (doi: 10.1002/art.38272), include Brent Lyda and Theodore M. Kamenecka of TSRI. The Griffin and the Kamenecka labs have a long-standing collaboration to develop novel therapeutics for autoimmune and metabolic disorders. Seehttp://onlinelibrary.wiley.com/doi/10.1002/art.38272/abstract

The work was supported by the National Institutes of Health (grant number MH084512).

Scientists Design Targeted New Drug Candidates Based on Detailed Picture of Muscular Dystrophy Defect

Scientists from the Florida campus of The Scripps Research Institute have revealed an atomic-level view of a genetic defect that causes a form of muscular dystrophy, myotonic dystrophy type 2, and have used this information to design drug candidates with.

"This the first time the structure of the RNA defect that causes this disease has been determined," said TSRI Associate Professor Matthew Disney, who led the study. "Based on these results, we designed compounds that, even in small amounts, significantly improve disease-associated defects in treated cells."

Myotonic dystrophy type 2 is a relatively rare form of muscular dystrophy that is somewhat milder than myotonic dystrophy type 1, the most common adult-onset form of the disease.

Both types of myotonic dystrophy are inherited disorders that involve progressive muscle wasting and weakness, and both are caused by a type of genetic defect known as a "RNA repeat expansion," a series of nucleotides repeated more times than normal in an individual's genetic code. The repeat binds to the protein MBNL1, rendering it inactive and resulting in RNA splicing abnormalities—which lead to the disease.

Many other researchers had tried to find the atomic-level structure of the myotonic dystrophy 2 repeat, but had run into technical difficulties. In a technique called X-ray crystallography, which is used to find detailed structural information, scientists manipulate a molecule so that a crystal forms. This crystal is

then placed in a beam of X-rays, which diffract when they strike the atoms in the crystal. Based on the pattern of diffraction, scientists can then reconstruct the shape of the original molecule.

Prior to the new research, which was published in an advance, online issue of the journal *ACS Chemical Biology*, scientists had not been able to crystallize the problematic RNA. The Scripps Florida team spent several years on the problem and succeeded in engineering the RNA to have crystal contacts in different positions. This allowed the RNA to be crystallized—and its structure to be revealed.

Using information about the RNA's structure and movement, the scientists were able to design molecules to improve RNA function.

The new findings were confirmed using sophisticated computational models that show precisely how the small molecules interact with and alter the RNA structure over time. Those predictive models matched what the scientists found in the study—that these new compounds bind to the repeat structure in a predictable and easily reproducible way, attacking the cause of the disease.

"We used a bottom-up approach, by first understanding how the small components of the RNA structure interact with small molecules," said Jessica Childs-Disney of TSRI, who was first author of the paper with Ilyas Yildirim of Northwestern University. "The fact that our compounds improve the defects shows that our unconventional approach works."

In addition to Disney, Childs-Disney and Yildirim authors of the study, "Myotonic Dystrophy Type 2 RNA: Structural Studies and Designed Small Molecules that Modulate RNA Function," include Jeremy Lohman, Lirui Guan, Tuan Tran, and HaJeung Park of TSRI; Partha Sarkar of the University of Texas Medical Branch and George C. Schatz of Northwestern University. For more information on the study, seehttp://pubs.acs.org/doi/abs/10.1021/cb4007387

The study was supported by the National Institutes of Health (grant R01 GM079235), the Muscular Dystrophy Association (grant 254929), TSRI and the PS-OC Center of the NIH (grant 1U54CA143869-01

Researchers Identify Possible Key to Drug Resistance in Crohn's Disease

Two-thirds to three-quarters of the estimated 700,000 Americans living with Crohn's disease, an autoimmune condition that can disrupt the entire gastrointestinal tract, will require surgery at some point during their life. Patients and physicians often turn to this surgical intervention after a patient develops resistance to current treatments, such as steroids.

Now scientists from the Florida campus of The Scripps Research Institute (TSRI) have identified a normally small subset of immune cells that may play a major role in the development of Crohn's disease generally and in disease-associated steroid resistance specifically.

The study, published recently in an advance, online edition of the *Journal of Experimental Medicine*, focused on Th17 cells, part of a family of white blood cells that have been implicated in numerous autoimmune diseases, including Crohn's disease.

In the new study, the researchers found that a subset of TH17 cells in humans expresses the multidrug transporter MDR1 and that these cells are linked to inflammation in Crohn's patients. MDR1—a protein famous for promoting drug-resistance in tumors—may also act as a survival and steroid resistance factor in T cells, particularly in harsh environments such as the inflamed gut mucosa of Crohn's disease patients.

“Our study is the first to identify and characterize this uniquely pro-inflammatory T-cell subset,” said biologist Mark Sundrud, a TSRI assistant professor who led the study. “We were able to sort these cells directly out of damaged tissue resected from Crohn’s patients and found that these pro-inflammatory cells are over-expressing genes that contribute to disease.”

Within healthy individuals, only approximately 5 to 10 percent of CD4+ T cells are MDR1-expressing TH17 cells. In contrast, the study found that of the CD4+ T cells found in actively inflamed tissue taken from Crohn’s patients, nearly 60 percent were MDR1+ TH17 cells.

The study also showed that these cells are resistant to both natural and synthetic steroids, a class of drugs considered a first-line defense against most autoimmune diseases.

“If a T cell expresses MDR1, it is likely to have an unfair growth advantage over surrounding T cells,” Sundrud said. “When exposed to steroids, it’s this subset of cells that will survive and thrive.”

Although it is unclear whether these pro-inflammatory cells become more prominent in patients over time, these findings suggest that steroid treatment itself may be directly responsible for the accumulation of these cells in Crohn’s patients. Sundrud and his colleagues continue to investigate.

The first author of the study, “Pro-Inflammatory Human Th17 Cells Selectively Express P-Glycoprotein and are Refractory to Glucocorticoids,” is Radha Ramesh of Tempero Pharmaceuticals. Other authors include Lina Kozhaya and Derya Unumaz of New York University; Kelly McKevitt of TSRI; Ivana M. Djuretic and Thaddeus J. Carlson of Tempero Pharmaceuticals; Maria A. Quintero, Jacob L. McCauley and Maria T. Abreu of the University of Miami.
Seehttp://jem.rupress.org/content/early/2013/12/30/jem.20130301.full

The study was supported by the National Institutes of Health (Grants R01AI065303, R21AI087973 and 1R01CA137869), Crohn’s and Colitis Foundation of America and The Micky & Madeleine Arison Family Foundation Crohn’s & Colitis Discovery Laboratory at the University of Miami.

Team Develops Promising Drug Candidates for Pain, Addiction

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have described a pair of drug candidates that advance the search for new treatments for pain, addiction and other disorders.

The two new drug scaffolds, described in a recent edition of *The Journal of Biological Chemistry*, offer researchers novel tools that act on a demonstrated therapeutic target, the kappa opioid receptor (KOR), which is located on nerve cells and plays a role in the release of the neurotransmitter dopamine. While compounds that activate KOR are associated with positive therapeutic effects, they often also recruit a molecule known as βarrestin2 (beta arrestin), which is associated with depressed mood and severely limits any therapeutic potential.

“Compounds that act at kappa receptors may provide a means for treating addiction and for treating pain; however, there is the potential for the development of depression or dysphoria associated with this receptor target,” said Laura Bohn, a TSRI associate professor who led the study. “There is evidence that the negative feelings caused by kappa receptor drugs may be, in part, due to receptor actions through proteins called beta arrestins. Developing compounds that activate the receptors without recruiting beta arrestin function may serve as a means to improve the therapeutic potential and limit side effects.”

The new compounds are called “biased agonists,” activating the receptor without engaging the beta arrestins.

Research Associate Lei Zhou, first author of the study with Research Associate Kimberly M. Lovell, added, “The importance of these biased agonists is that we can manipulate the activation of one particular signaling cascade that produces analgesia, but not the other one that could lead to dysphoria or depression.”

The researchers note that the avoidance of depression is particularly important in addiction treatment, where depressed mood can play a role in relapse.

The two drug candidates also have a high affinity and selectivity for KOR over other opioid receptors and are able to pass through the blood-brain barrier. Given these promising attributes, the scientists plan to continue developing the compounds.

In addition to Bohn, Lovell and Zhou, other authors of the study, “Development of Functionally Selective, Small Molecule Agonists at Kappa Opioid Receptors,” include Angela M. Phillips, John M. Streicher, Edward Stahl, Cullen L. Schmid, Michael D. Cameron, Peter Hodder and Franck Madoux of The Scripps Research Institute; the chemistry was led by Kevin J. Frankowski, Stephen R. Slauson, Thomas E. Prisinzano and Jeffrey Aubé of the University of Kansas. For more information on the study, seehttp://www.jbc.org/content/288/51/36703

This work was supported by the National Institutes of Health (grant R01 DA031927).

Researchers Find Regulator of Amyloid Plaque Buildup in Alzheimer’s Disease

Scientists from the Florida campus of The Scripps Research Institute have identified a critical regulator of a molecule deeply involved in the progression of Alzheimer’s disease.

The new study, published in an advance, online edition of the *Journal of Biological Chemistry*, shows for the first time that levels of this regulating protein are decreased in the brains of Alzheimer’s disease sufferers and that this decrease could be a significant factor in the advance of the disease.

The regulator is known as Rheb, a protein that many believe may be active in neural plasticity, the ability of the brain to change in response to learning.

In the new study, the scientists found that Rheb binds and regulates activity of a molecule known as BACE1, an important enzyme in Alzheimer’s disease pathology, establishing for the first time a new molecular link between Rheb and BACE1.

“We found that Rheb regulates BACE1, which is a major drug target in Alzheimer’s disease,” said Srini Subramaniam, a TSRI biologist who led the study. “Studies of the autopsied brains of Alzheimer’s patients have found a significant reduction in Rheb, so it is possible that an increase in Rheb could reverse the buildup of amyloid plaque.”

The study noted that in some genetically modified animal models, an increase of Rheb has already been shown to reduce BACE1 levels and the production of amyloid plaque.

“If we can uncover the mechanism by which Rheb alters BACE1 levels, that would be a very good drug target,” said Neelam Shahani, a first author of the study with William Pryor, both research associates in the Subramaniam lab.

The new study indicates that Rheb degrades BACE1 through a number of pathways, but more research needs to be done before drug candidates can be developed.

“We’re very interested in the disease process and plan to keep moving forward to understand precisely how Rheb regulates BACE1,” said Pryor.

In addition to Subramaniam, Shahani and Pryor, other authors of the study, “Rheb GTPase Regulates β-Secretase Levels and Amyloid β Generation,” include Supriya Swarnkar of TSRI; Nikolai Kholodilov and Robert E Burke of Columbia University; and Gopal Thinakaran of The University of Chicago. For more information, seehttp://www.jbc.org/content/early/2013/12/24/jbc.M113.532713

This work was supported by the O’Keeffe Neuroscience Scholar Award and by the State of Florida.

Scientists Offer New Insight into Neuron Changes Brought About by Aging

How aging affects communication between neurons is not well understood, a gap that makes it more difficult to treat a range of disorders, including Alzheimer’s and Parkinson’s disease.

A new study from the Florida campus of The Scripps Research Institute (TSRI) offers insights into how aging affects the brain’s neural circuitry, in some cases significantly altering gene expression in single neurons. These discoveries could point the way toward a better understanding of how aging affects our cognitive ability and new therapeutic targets for the treatment of neurodegenerative disease.

“Although we don’t know exactly why, we do know there is a signaling imbalance as we age, and we’ve captured these changes at the single neuron level,” said Sathyanarayanan V. Puthanveettil, a TSRI assistant professor who led the work. “If we could identify the underpinnings of this mechanism, we may be able to target the specific mechanism to affect or reverse the aging process in human neurons.”

To record the electrical and physiological properties of single neurons, the scientists created a new method and applied it to the marine snail *Aplysia californica*, a widely used animal model. Many *Aplysia* gene expression signatures have counterparts in the human genome.

Using this methodology, which was published in the *Journal of Visualized Experiments*, the scientists were then able to focus on neuron R15, a burst firing neuron that is implicated in the regulation of water content and reproduction, showing how its response to the neurotransmitter acetylcholine and gene expression changed with age.

In a study published in the journal *PLOS ONE*, the team described specific changes in burst firing and action potentials—which play a central role in cell-to-cell communication—during the aging of R15, suggesting that changes in the response to acetylcholine during aging has been conserved during evolution in organisms from snails to mammals.

In another study, published in published in *BMC Genomics*, the team revealed unexpected information about gene expression during R15 aging.

“Aging brings bidirectional changes in the gene expression,” said Puthanveettil. “Some gene expression goes up; some goes down. This was surprising, particularly that some gene expression went up—something you don’t necessarily associate with aging.”

The study also noted that more than 1,000 DNA sequences are regulated differently in mature versus old R15 neurons. Among the specific biological pathways that are altered are networks involved in: cell signaling and skeletal muscular system development; cell death and survival; cellular function

maintenance and embryonic development; and neurological diseases and developmental and hereditary disorders.

To confirm these findings, Puthanveettil and his colleagues also isolated and examined three other *Aplysia* neurons. Interestingly, while all the neurons showed changes in gene expression with age, these changes weren't necessarily similar among the neurons. Also the magnitude of change was specific to individual neurons.

The scientists are now investigating how and why aging affects neurons differently.

The first author of the *Journal of Visualized Experiments* study, "AplysiaGanglia Preparation for Electrophysiological and Molecular Analyses of Single Neurons," is Komol Akhmedov of TSRI. Other authors include Beena M. Kadakkuzha, also of TSRI. For more information, see http://www.jove.com/video/51075/aplysia-ganglia-preparation-for-electrophysiological-molecular

The first authors of the *PLOS ONE* study, "Decreased response to acetylcholine during aging of Aplysia neuron R15," are Komol Akhmedov and Valerio Rizzo of TSRI. Other authors include Beena M. Kadakkuzha of TSRI, Christopher J. Carter and Neil S. Magoski of Queen's University, Canada, and Tom R Capo of the University of Miami. For more information, see http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3874043/

The first author of the *BMC Genomics* study, "Age-Associated Bidirectional Modulation of Gene Expression in Single Identified R15 Neuron of Aplysia," is Beena M Kadakkuzha of TSRI. Other authors include Komolitdin Akhmedov, Mohammad Fallahi and Anthony C Carvalloza of TSRI and Tom R Capo of the University of Miami. For more information, seehttp://www.biomedcentral.com/1471-2164/14/880/abstract

The studies were supported by the National Institutes of Health (grant Number 1 R21 MH096258), the Whitehall Foundation and the State of Florida.

Breakthrough Approach Quickly Identifies New Drug Candidates from Genome Sequence

In research that could ultimately lead to many new medicines, scientists from the Florida campus of The Scripps Research Institute (TSRI) have developed a potentially general approach to design drugs from genome sequence. As a proof of principle, they identified a highly potent compound that causes cancer cells to attack themselves and die.

"This is the first time therapeutic small molecules have been rationally designed from only an RNA sequence—something many doubted could be done," said Matthew Disney, an associate professor at TSRI who led the study. "In this case, we have shown that that approach allows for specific and unprecedented targeting of an RNA that causes cancer."

The technique, described in the journal *Nature Chemical Biology* online ahead of print on February 9, 2014, was dubbed Inforna.

"With our program, we can identify compounds with high specificity," said Sai Pradeep Velagapudi, the first author of the study and a graduate student working in the Disney lab. "In the future, we hope we can design drug candidates for other cancers or for any pathological RNA."

In Search of New Approaches

In their research program, Disney and his team has been developing approaches to understand the binding of drugs to RNA folds. In particular, the lab is interested in manipulating microRNAs.

Discovered only in the 1990s, microRNAs are short molecules that work within virtually all animal and plant cells. Typically each one functions as a “dimmer switch” for one or more genes; it binds to the transcripts of those genes and effectively keeps them from being translated into proteins. In this way microRNAs can regulate a wide variety of cellular processes.

Some microRNAs have been associated with diseases. MiR-96 microRNA, for example, is thought to promote cancer by discouraging a process called apoptosis or programmed cell death that can rid the body of cells that begin to grow out of control.

As part of its long-term program, the Disney lab developed computational approaches that can mine information against such genome sequences and all cellular RNAs with the goal of identifying drugs that target such disease-associated RNAs while leaving others unaffected.

"In recent years we've seen an explosion of information about the many roles of RNA in biology and medicine," said Peter Preusch, PhD, of the National Institute of Health's National Institute of General Medical Sciences, which partially funded the research. “This new work is another example of how Dr. Disney is pioneering the use of small molecules to manipulate disease-causing RNAs, which have been underexplored as potential drug targets.”

‘Unprecedented’ Findings

In the new study, Disney and colleagues describe their computational technique, which identifies optimal drug targets by mining a database of drug-RNA sequence (“motif”) interactions against thousands of cellular RNA sequences.

Using Inforna, the team identified compounds that can target microRNA-96, as well as additional compounds that target nearly two dozen other disease-associated microRNAs.

The researchers showed that the drug candidate that inhibited microRNA-96 inhibited cancer cell growth. Importantly, they also showed that cells without functioning microRNA-96 were unaffected by the drug.

“This illustrates an unparalleled selectivity for the compound,” Disney noted. “In contrast, typical cancer therapeutics target cells indiscriminately, often leading to side effects that can make these drugs difficult for patients to tolerate.”

Disney added that the new drug candidate, which is easy to produce and cell permeable, targets microRNA-96 far more specifically than the state-of-the-art method to target RNA (using oligonucleotides) currently in use. “That’s unprecedented and provides great excitement for future developments.”

In addition to Disney and Velagapudi, Steven M. Gallo of the University of Buffalo was an author of the study, “Sequence-Based Design of Bioactive Small Molecules That Target Precursor MicroRNAs.” For more information,
seehttp://www.nature.com/nchembio/journal/vaop/ncurrent/full/nchembio.1452.html

The work was supported by the National Institutes of Health (grant R01GM097455) and the Camille and Henry Dreyfus Foundation.

Scientists Devise New, Lower Cost Method to Create More Usable Fuels

As the United States continues to lead the world in the production of natural gas, scientists from the Florida campus of The Scripps Research Institute (TSRI) have devised a new and more efficient method with the potential to convert the major components found in natural gas into useable fuels and chemicals—opening the door to cheaper, more abundant energy and materials with much lower emissions.

The research, which was led by TSRI Professor Roy Periana, uses clever chemistry and nontraditional materials to turn natural gas into liquid products at much lower temperatures than conventional methods.

“We uncovered a whole new class of inexpensive metals that allows us to process methane and the other alkanes contained in natural gas, ethane and propane, at about 180 degrees centigrade or lower, instead of the more than 500oC used in current processes,” said Periana. “This creates the potential to produce fuels and chemicals at an extraordinarily lower cost.”

The research was described in the March 14, 2014 edition of the journal *Science*.

The Challenge

Methane is the most abundant compound in natural gas. However, converting methane into a useable, versatile liquid product remains a costly and complicated process that has changed little from the original process developed in the 1940s. But with the boom in natural gas discovery growing every day, new processes are needed to convert methane to fuel and chemicals that can compete economically with production from petroleum.

Methane, ethane and propane, the major components in natural gas, belong to a class of molecules named alkanes that are the simplest hydrocarbons and one of the most abundant, cleanest sources of energy and materials. However, transportation can be expensive and converting these alkanes into other useful forms such as gasoline, alcohols or olefins is expensive and often inefficient.

At the core of technologies for converting the alkanes in natural gas is the chemistry of the carbon-hydrogen. Because of the high strength of these bonds, current processes for converting these alkanes employ high temperatures (more than 500oC) that lead to high costs, high emissions and lower efficiencies.

The development of lower temperature (less than 250oC), selective, alkane carbon-hydrogen bond conversion chemistry could lead to a major shift in energy and materials production technology.

An Elegant Solution

Periana has been thinking about this type of problem for decades in pursuit of lower-cost, environmentally friendly energy solutions and has designed some of the most efficient systems (Periana et. al., Science 1993, 1998 and 2003) for alkane conversion that operate at lower temperatures.

However, when Periana and his team examined these first-generation systems they realized that the precious metals they used, such as platinum, palladium, rhodium, gold, were both too expensive and rare for widespread use.

"What we wanted were elements that are more abundant and much less expensive that can carry out the same chemistry under more practical conditions,” said Brian G. Hashiguchi, the first author of the study and a member of Periana’s lab. “We also wanted to find materials that could convert methane as well as the other major components in natural gas, ethane and propane.”

Approaching the problem both theoretically and experimentally, the team hit on inexpensive metals known as main group elements, some of which are byproducts of refining certain ores. For example, one of the materials can be made from common lead dioxide, a synthetic compound used in the production of matches and fireworks.

“The reaction of alkanes with this class of materials we’ve identified is novel,” Periana said. “They can react with methane, ethane as well as propane at lower temperatures with extraordinarily selectivity—and produce the corresponding alcohols as the only the desired products. These products are all major commodity chemicals and are also ideal, inexpensive sources for fuels and plastics."

If successfully developed, new process using these metals could potentially allow the large reserves of natural gas in the United States to be used as alternative resources for fuels and chemicals.

In addition to Periana and Hashiguchi, authors of the study, “Main-Group Compounds Selectively Oxidize Mixtures of Methane, Ethane, and Propane to Alcohol Esters,” are Michael M. Konnick, Steven M. Bischof and Niles Gunsalus of The Scripps Research Institute; and Samantha J. Gustafson, Deepa Devarajan and Daniel H. Ess of Brigham Young University. For more information, seehttp://www.sciencemag.org/content/343/6176/1232.abstract?sid=09c04312-544e-469d-b01b-bca5ebd9daac

This study was supported by the U.S. Department of Energy (DE-SC0001298).

In Mapping Feat, Scientists Pinpoint Neurons Where Select Memories Grow

Memories are difficult to produce, often fragile, and dependent on any number of factors—including changes to various types of nerves. In the common fruit fly—a scientific doppelganger used to study human memory formation—these changes take place in multiple parts of the insect brain.

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have been able to pinpoint a handful of neurons where certain types of memory formation occur, a mapping feat that one day could help scientists predict disease-damaged neurons in humans with the same specificity.

“What we found is that while a lot of the neurons will respond to sensory stimuli, only a certain subclass of neurons actually encodes the memory,” said Seth Tomchik, a TSRI biologist who led the study, which was published March 27, 2014, online ahead of print by the journal *Current Biology*.

The researchers examined a type of neuron called dopaminergic neurons—which respond to dopamine, a well-known neurotransmitter—and are involved in shaping diverse behaviors, including learning, motivation, addiction and obesity.

In the study, the scientists followed the stimulation of a large number of these neurons when an odor was paired with an aversive event such as a mild electric shock. The scientists then used imaging technology to follow changes in the brains of live flies, mapping the activation patterns of signaling molecules within the neurons and observing learning-related plasticity—in which neurons change and develop memory traces.

The scientists found that the neurons that did encode memories responded to a cellular signaling messenger known as cAMP (cyclic adenosine monophosphate) that is vital for many biological processes. cAMP is involved in a number of psychological disorders such as bipolar disorder and schizophrenia, and its dysregulation may underlie some cognitive symptoms of Alzheimer's disease and Neurofibramatosis I.

In fact, the study pointed to a specific location in the brain—a particular lobe with a region known as the mushroom body—where the neurons appear to be particularly sensitive to elevated amounts of cAMP.

According to Tomchik, that's an important finding in terms of human memory because olfactory memory formation in the fruit fly is very similar to human memory formation.

"We have a good model in these two classes of neurons, one that encodes and one that doesn't," he said. "Now we know exactly where the memory formation should be and where to look to see how disease may disrupt it."

Tamara Boto, the first author of the study and a member of Tomchik's laboratory, added, "We know where, but we don't yet know the mechanism of why only these subsets are affected. That's our next job—to figure that out."

In addition to Tomchik and Boto, authors of the study, "Dopaminergic Modulation of Camp Drives Nonlinear Plasticity Across the DrosophilaMushroom Body Lobes," are Thierry Louis and Kantiya Jindachomthong of TSRI; and Kees Jalink of The Netherlands Cancer Institute, Amsterdam. For more information, see https://www.cell.com/current-biology/abstract/S0960-9822(14)00313-3

The study was supported by the National Institutes of Health (grant MH092294).

Team Identifies a Novel Biomarker for Head and Neck Cancer, Non-Small Cell Lung Cancer

A team led by a scientist from the Florida campus of The Scripps Research Institute (TSRI) has identified a new biomarker linked to better outcomes of patients with head and neck cancers and non-small cell lung cancer. The work could help scientists develop new diagnostics and therapies and help physicians determine the best long-term treatments for patients with these cancers.

The findings, which were published recently online ahead of print by the journal *Cancer*, focus on a protein called Choline phosphate cytidylyltransferase-α CCT-α or CCTα, an "antigen" that prompts the immune system to produce antibodies against it.

"Based on what we found, a high CCTα expression appears to be indicative of survival, making CCTα a promising biomarker," said Laura Niedernhofer, a TSRI associate professor who led the study with Gerold Bepler of the Karmanos Cancer Institute. "Our findings suggest that CCTα may, in fact, be more important in determining outcomes in patients with both types of cancer than the already established ERCC1."

The new study, in fact, turns previous studies on ERCC1 on their heads. Dozens of large clinical trials are being conducted using expression of the ERCC1 DNA-repair protein as a determinant of whether patients with lung, pancreatic, gastric, colorectal, esophageal or ovarian cancer should be treated with platinum-based therapy, a very potent but toxic DNA-damaging agent.

However, the new research suggests that these positive results were not actually due to ERCC1, but to CCTα—which also binds to the antibody most frequently used to measure ERCC1 expression. "Our results show CCTα may be a better predictor of patient outcomes than expression of ERCC1," said Niedernhofer.

While ERCC1 is associated with DNA repair, CCTα is involved in the synthesis of a major component of cell membranes, active in membrane-mediated signaling and embryo survival.

The new results were based on an examination of samples from 187 patients with non-small cell lung cancer and 60 patients with head and neck squamous cell carcinomas.

CCTα expression was associated with longer survival rates, including for patients with non-small cell lung cancer who were treated with surgery alone—without the use of platinum-based chemotherapy drugs and associated toxic side effects.

The first author of the study, "Choline Phosphate Cytidylyltransferase-α is a Novel Antigen Detected by the Anti-ERCC1 Antibody 8F1 with Biomarker Value in Patients with Lung and Head and Neck Squamous Cell Carcinomas," is Alec Vaezi of the University of Pittsburgh.

In addition to Niedernhofer, Bepler and Vaezi, other authors include Agnes Malysa and Wei Chen of the Karmanos Cancer Institute; and Nikhil Bhagwat, Jennifer Rubatt, Brian Hood, Thomas Conrads, Lin Wang and Carolyn Kemp of the University of Pittsburgh. For more information, seehttp://onlinelibrary.wiley.com/doi/10.1002/cncr.28643/abstract

The study was supported by National Institute of Environmental Health Sciences (grant R01-ES016114), the National Cancer Institute (grants R01-CA129343 and P50-CA097190) and the American Head and Neck Society.

Findings Show 'Best Practices' Nutrition Measurement for Researchers

At first glance, measuring what the common fruit fly eats might seem like a trivial matter, but it is absolutely critical when it comes to conducting studies of aging, health, metabolism and disease. How researchers measure consumption can make all the difference in the accuracy of a study's conclusions.

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have developed what amounts to a best practices guide to the most accurate way of measuring fruit fly food consumption that could lead to more informed research and better decisions about directions in further studies.

"While our study isn't the final technical reference on measuring fly food consumption, it will help guide researchers to think more carefully about nutrition and nutrient intake in their own studies," said TSRI Assistant Professor William Ja, who led the study, which was published online ahead of print on March 30, 2014 by the journal *Nature Methods*.

Researchers, Ja said, generally haven't given sufficient thought to feeding and nutrient intake when it comes to measuring fruit fly behavior, metabolism and health.

"If you're making a huge effort to change an animal's diet and trying to draw conclusions about what nutrition and nutrients do to animal health and lifespan," he said, "then one of the most fundamental parameters is accurately measuring food intake."

TSRI Research Associate Sonali Deshpande, a first author of the study with graduate student Ariadna Amador and former TSRI Research Associate Gil Carvalho, underlined the importance of using the best measurement methods. "Drug studies, in particular, where compounds are added to fly food, are difficult to interpret without proper measurement of food and drug intake," she said.

In the study, the team determined that radioisotope labeling food is the most sensitive and consistently accurate feeding method now available—levels of accumulated isotope are later measured in the animals. This method's main limitation appears to be underestimation of consumption due to excretion.

For the most accurate measurement, the study suggested pairing radioisotope labeling with a more low-tech approach, such as the capillary feeder (CAFE). The CAFE assay, introduced by Ja in 2007, is similar to a water dispenser used for pet hamsters, but on a smaller scale.

“In a significant number of studies, we found that researchers appeared indifferent to the impact feeding might have on the experiment,” Ja said. “This doesn’t seem like good science to me. Can you imagine doing a mouse experiment, saying that you watched mice for four hours and saw no difference in feeding, then make conclusions about total caloric intake over days or longer?”

Other authors of the study, “Quantifying Drosophila Food Intake: Comparative Analysis of Current Methodology,” include Angela M Phillips, Sany Hoxha and Keith J Lizotte of TSRI. For more information, seehttp://www.nature.com/nmeth/journal/vaop/ncurrent/abs/nmeth.2899.html

The study was supported by the National Institutes of Health (grants R00AG030493 and R21DK092735), the Ellison Medical Foundation and the Glenn Foundation for Medical Research.

Researchers Uncover Startling New Functional Details of Common Anti-Diabetic Drugs

Scientists thought they basically knew how the most common drugs used to treat type 2 diabetes worked, but a new study from the Florida campus of The Scripps Research Institute (TSRI) reveals unexpected new aspects of the process. These findings could eventually lead to more potent anti-diabetic drugs with fewer serious side effects.

The study was published in the April 7, 2014 issue of the journal *Nature Communications*.

The most common type 2 diabetes treatments are known as insulin-sensitizing drugs, which improve how the body responds to glucose or sugar. These drugs mimic naturally occurring compounds that bind to a specific intracellular receptor (peroxisome proliferator-activated receptor-g or PPARG), altering its activity.

While these drugs were widely thought to bind to a single site on the receptor, the new study shows they also bind to an alternative site, leading to unique changes in receptor shape, which affects interaction with co-regulating protein partners and gene expression.

Douglas Kojetin, an associate professor at TSRI who led the study, called the discovery serendipitous—and revealing.

“It turns out that binding to PPARG is far more complex than anyone previously understood,” he said. “You don’t have to displace the naturally occurring ligand [binding partner] with a synthetically designed drug to regulate the receptor because you have this alternative site.”

Kojetin and his colleagues made the alternative binding site discovery using a far simpler mapping technique than had previously been applied to determine the receptor’s structure.

“We used a technique that yields easy-to-interpret results, one that you wouldn’t normally use to look at how drugs bind a receptor,” said Research Associate Travis Hughes, the first author of the study and a member of Kojetin’s lab. “Instead of finding one site, we realized we had two and wanted to know what the second one was doing.”

The scientists note that while they don’t yet know the full effect of the alternate binding site’s function, it might provide a clue to insulin-sensitizing drugs’ adverse effects, which include risk of bone loss and congestive heart failure.

“The question going forward is ‘Does this alternative site contribute to side effects, beneficial effects or both?’” said Kojetin. “Knowledge of this alternate binding site may help produce a new generation of anti-diabetic drugs.”

In addition to Kojetin and Hughes, authors of the study, “An Alternate Binding Site for PPARg Ligands,” include Pankaj Kumar Giri, Ian Mitchelle S. de Vera, David P. Marciano, Dana S. Kuruvilla, Youseung Shin, Anne-Laure Blayo, Theodore M. Kamenecka and Patrick R. Griffin of TSRI; and Thomas P. Burris of St. Louis University. For further information about the study, seehttp://www.nature.com/ncomms/2014/140407/ncomms4571/full/ncomms4571.html

The study was supported by the state of Florida, the James and Esther King Biomedical Research Program, the Florida Department of Health (grant number 1KN-09) and the National Institutes of Health (grant numbers DK101871 and DK097890).

Researchers Find Connection Between Gene Mutation, Key Symptoms of Autism

Scientists have known that abnormal brain growth is associated with autism spectrum disorder. However, the relationship between the two has not been well understood.

Now, scientists from the Florida campus of The Scripps Research Institute (TSRI) have shown that mutations in a specific gene that is disrupted in some individuals with autism results in too much growth throughout the brain, and yet surprisingly specific problems in social interactions, at least in mouse models that mimic this risk factor in humans.

“What was striking is that these were basically normal animals in terms of behavior, but there were consistent deficits in tests of social interaction and recognition—which approximate a major symptom of autism,” said Damon Page, a TSRI biologist who led the study. “This suggests that when most parts of the brain are overgrown, the brain somehow adapts to it with minimal effects on behavior in general. However, brain circuits relevant to social behavior are more vulnerable or less able to tolerate this overgrowth.”

The study, which focuses on the gene phosphatase and tensin homolog(PTEN), was recently published online ahead of print by the journal *Human Molecular Genetics*.

Autism spectrum disorder is a neurodevelopmental disorder involving a range of symptoms and disabilities involving social deficits and communication difficulties, repetitive behaviors and interests, and sometimes cognitive delays. The disorder affects in approximately one percent of the population; some 80 percent of those diagnosed are male.

In a previous study, Page and colleagues found that mutations in Ptencauses increased brain size and social deficits, with both symptoms being exacerbated by a second “hit” to a gene that regulates levels of the neurotransmitter serotonin in the brain. In the new study, the TSRI team set out to explore whether mutations in Pten result in widespread or localized overgrowth within the brain, and whether changes in brain growth are associated with broad or selective deficits in tests of autism-relevant behaviors in genetically altered mice. The team tested mice for autism spectrum disorder-related behaviors including mood, anxiety, intellectual, and circadian rhythm and/or sleep abnormalities.

The researchers found that Pten mutant mice showed altered social behavior, but few other changes—a more subtle change than would have been predicted given broad expression and critical cellular function of the gene.

Intriguingly, some of the more subtle impairments were sex-specific. In addition to social impairments, males with the mutated gene showed abnormalities related to repetitive behavior and mood/anxiety, while females exhibited additional circadian activity and emotional learning problems.

The results raise the question of how mutations in PTEN, a general regulator of growth, can have relatively selective effects on behavior and cognitive development. One idea is that PTEN mutations may desynchronize the normal pattern of growth in key cell types—the study points to dopamine neurons—that are relevant for social behavior.

"Timing is everything," Page said. "Connections have to form in the right place at the right time for circuits to develop normally. Circuitry involved in social behavior may turn out to be particularly vulnerable to the effects of poorly coordinated growth."

The first author of the study, "Pten Haploinsufficient Mice Show Broad Brain Overgrowth but Selective Impairments in Autism-Relevant Behavioral Tests," is TSRI Research Associate Amy E. Clipperton-Allen.

For more information on the research, seehttp://hmg.oxfordjournals.org/content/early/2014/02/27/hmg.ddu057.abstract?sid=291702ba-6aac-4ade-aae8-504129fed814

Scientists Identify Critical Protein Complex Involved in Learning and Memory

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have identified a protein complex that plays a critical but previously unknown role in learning and memory formation.

The study, which showed a novel role for a protein known as RGS7, was published April 22, 2014 in the journal *eLife*, a publisher supported by the Howard Hughes Medical Institute, the Max Planck Society and the Wellcome Trust.

"This is a critical building block that regulates a fundamental process—memory," said Kirill Martemyanov, a TSRI associate professor who led the study. "Now that we know about this important new player, it offers a unique therapeutic window if we can find a way to enhance its function."

The team looked at RGS7 in the hippocampus, a small part of the brain that helps turn short-term memory in long-term memory.

The scientists found the RGS7 protein works in concert with another protein, R7BP, to regulate a key signaling cascade that is increasingly seen as a critical to cognitive development. The cascade involves the neurotransmitter GABA, which binds to the GABAb receptor and opens inhibitory channels known as GIRKs in the cell membrane. This process ultimately makes it more difficult for a nerve cell to fire.

This process turned out to be critical to normal functioning, as the research showed mice lacking RGS7 exhibited deficits in learning and memory.

Martemyanov believes the findings could ultimately have broad therapeutic application. "GIRK channels are implicated in a range of neuropsychiatric conditions, including drug addiction and Down's syndrome, that result from a disproportionate increase in neuronal inhibition as a result of greater mobilization of these channels," he said. "Now that we know the identity of the critical modulator of GIRK channels we can try to find a way to increase its power with the hopes of reducing the inhibitory overdrive, and that might potentially alleviate some of the disruptions seen in Down's syndrome. It is possible that similar strategies might apply for dealing with addiction, where adaptations in the GABAb-GIRK pathway play a significant role."

Targeting the RGS7 protein could allow for better therapeutic outcomes with fewer side effects because it allows for fine tuning of the signaling, according to Olga Ostrovskaya, the first author of the study and a member of Martemyanov's lab, who sees many ways to follow up on the findings.

"We're looking into how RGS7 is involved in neural circuitry and functions tied to the striatum, another part of the brain responsible for procedural memory, mood disorders, motivation and addiction," Ostrovskaya said. "We may uncover the RGS7 regulation of other signaling complexes that may be very different from those in hippocampus."

In addition to Ostrovskaya and Martemyanov, other authors of the study, "RGS7/Gβ5/R7BP Complex Regulates Synaptic Plasticity and Memory by Modulating Hippocampal Gababr-Girk Signaling," include Keqiang Xie and Ikuo Masuho of TSRI; Ana Fajardo-Serrano and Rafael Lujan of the Universidad de Castilla-La Mancha, Albacete, Spain; and Kevin Wickman of the University of Minnesota. To view the paper, visitelifesciences.org/lookup/doi/10.7554/elife.02053

The study was supported by the National Institutes of Health (Grants DA021743, DA026405, MH061933, DA034696, HL105550) and by grants from the Spanish Ministry of Education and Science (BFU-2012-38348) and CONSOLIDER (CSD2008-00005).

Scientists Reveal Molecular Secrets Behind Resveratrol's Health Benefits

Resveratrol has been much in the news as the component of grapes and red wine associated with reducing "bad cholesterol," heart disease and some types of cancer. Also found in blueberries, cranberries, mulberries, peanuts and pistachios, resveratrol is associated with beneficial health effects in aging, inflammation and metabolism.

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have now identified one of the molecular pathways that resveratrol uses to achieve its beneficial action. They found that resveratrol controls the body's inflammatory response as a binding partner with the estrogen receptor without stimulating estrogenic cell proliferation, which is good news for its possible use as a model for drug design.

The study was recently published as an accepted manuscript in the online journal *eLife,* a publication supported by the Howard Hughes Medical Institute, the Max Planck Society and the Wellcome Trust.

"Estrogen has beneficial effects on conditions like diabetes and obesity but may increase cancer risk," said Kendall Nettles, a TSRI associate professor who led the study. "What hasn't been well understood until now is that you can achieve those same beneficial effects with something like resveratrol."

The problem with resveratrol, Nettles said, is that it really doesn't work very efficiently in the body. "Now that we understand that we can do this through the estrogen receptor, there might compounds other than resveratrol out there that can do the same thing—only better," he said.

"Our findings should lead scientists to reconsider the estrogen receptor as a main target of resveratrol—and any analogues," said Jerome C. Nwachukwu, the first author of the study and a research associates in the Nettles laboratory. "It has gotten swept under the rug."

In the new study, Nettles, Nwachukwu and their colleagues found that resveratrol is an effective inhibitor of interleukin 6 (IL-6), a pro-inflammatory protein that is part of the immune system (although IL-6 can be anti-inflammatory during exercise). High levels of IL-6 are also associated with

poor breast cancer patient survival. According to the study, resveratrol regulates IL-6 without stimulating cell proliferation by altering a number of co-regulators of the estrogen receptor.

In addition to Nwachukwu and Nettles, other authors of the study, "Resveratrol Modulates the Inflammatory Response via An Estrogen Receptor-Signal Integration Network," include Sathish Srinivasan, Nelson E. Bruno , Travis S. Hughes, Julie A. Pollock, Olsi Gjyshi, Valerie Cavett, Jason Nowak, Ruben D. Garcia-Ordonez , Patrick R. Griffin, Douglas J. Kojetin and Michael D. Conkright of TSRI; Alex A. Parent and John A. Katzenellenbogen of the University of Illinois; and René Houtman of PamGene International, The Netherlands. For more information, seehttp://elifesciences.org/content/early/2014/04/24/eLife.02057

The study was supported by the National Institutes of Health (grants PHS 5R37DK015556; 5R33CA132022, 5R01DK077085, 1U01GM102148, R01DK101871 and F32DK097890), the Ballen Isles Men's Golf Association, the Frenchman's Creek Women for Cancer Research, the State of Florida and the James and Esther King Biomedical Research Program, Florida Department of Health (1KN-09).

Revealing the Molecular Secrets of Short, Intense Workouts

In the last few years, the benefits of short, intense workouts have been extolled by both researchers and exercise fans as something of a metabolic panacea capable of providing greater overall fitness, better blood sugar control and weight reduction—all of it in periods as short as seven minutes a few times a week.

Now, in a new study, scientists from the Florida campus of The Scripps Research Institute (TSRI) confirm that there is something molecularly unique about intense exercise: the activation of a single protein.

The study, published recently by *The EMBO Journal*, revealed the effects of a protein known as CRTC2.

The scientists were able to show that following high-intensity exercise, which enlists the sympathetic nervous system's "fight or flight" response, CRTC2 integrates signals from two different pathways—the adrenaline pathway and the calcium pathway, to direct muscle adaptation and growth only in the contracting muscle.

Using mice genetically modified to conditionally express CRTC2, the scientists showed that molecular changes occurred that emulated exercised muscles in the absence of exercise.

"The sympathetic nervous system gets turned on during intense exercise, but many had believed it wasn't specific enough to drive specific adaptations in exercised muscle," said Michael Conkright, a TSRI assistant professor who led the study. "Our findings show that not only does it target those specific muscles, but it improves them—the long-term benefits correlate with the intensity of the workout."

Mobilizing Resources

In the genetically altered animal models, this resulted in a muscle size increase of approximately 15 percent. Metabolic parameters, indicating the amount of fuel available to the muscles, also increased substantially—triglycerides went up 48 percent, while glycogen supplies rose by a startling 121 percent.

In an exercise stress test, the genetically altered animals improved 103 percent after the gene was activated, compared to an 8.5-percent improvement in normal animals.

“If you think of the adrenaline system as something that mobilizes resources when you encounter, say, a bear on your way to work, what we found is that the system also gets you ready for your next bear encounter,” Conkright said.

The new findings open the door to a range of potential exercise enhancements.

“Nothing can supplant exercise; however, just by activating one protein, we clearly improved performance in animal models,” said Staff Scientist Nelson E. Bruno, the first author of the study and a member of the Conkright laboratory. “We are now searching for molecular therapeutics that will activate the CRTC2 protein so that even an average exercise routine could potentially be enhanced and made more beneficial.”

In addition to Conkright and Bruno, other authors of the study, “Creb Co-Activators Direct Anabolic Responses and Enhance Performance of Skeletal Muscle,” include Kimberly A. Kelly, Richard Hawkins, Mariam Bramah-Lawani, Antonio L. Amelio, Jerome C. Nwachukwu and Kendall Nettles of TSRI. The study can be accessed athttp://emboj.embopress.org/content/33/9/1027

The research was supported by the National Institutes of Health (R01 DK081491) and by the State of Florida.

Scientists Find New Targets that Could Increase Effectiveness, Reduce Side Effects in Breast Cancer Treatments

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have found new targets for potential intervention in breast cancer. These new targets could eventually increase effectiveness and reduce the undesirable side effects associated with current treatments.

The study was published online ahead of print on June 5, 2014 by the journal *Structure*.

Approximately two out of three breast cancers are driven by receptors that bind the hormones estrogen and progesterone—when the hormones bind to these receptors in cancer cells, they signal the cancer cells to grow. What makes the progesterone receptor therapeutically interesting is that it has two activation domains—AF1 and AF2. Normally, both are needed for full activation of the receptor.

“Using hydrogen-deuterium exchange technology, our study pinpoints just how AF2 communicates with AF1—the first evidence of the long-range interaction between these two functional domains,” said Patrick R. Griffin, chair of TSRI’s Department of Molecular Therapeutics who led the study. “These findings support further research to look for promising small molecules that block that interaction.”

The findings are especially important because in some mutations AF2 is deleted, yet the receptor still drives the cancer using its AF1 domain. Current drugs used for treating these cancers only target the AF2 domain, so with nothing to bind to, they do not work at all. While several studies have shown the importance of AF1, its binding domain is remarkably dynamic, frequently shifting shape and making it difficult to target with drugs.

In the new study, the scientists used an advanced technology known as hydrogen-deuterium exchange mass spectrometry (HDX) to measure the intricate interactions between the AF1 and AF2 domains of the progesterone receptor.

HDX mass spectrometry is a high-precision, high-sensitivity mapping technique that enabled the scientists to determine the specific regions of the receptor that are altered upon interaction. This

information was used to infer structural changes that result from the interaction and to probe the conformational flexibility of intact multidomain proteins.

In addition to exploring potential new drugs for breast cancer, the researchers also hope to investigate the implications for prostate cancer, another hormone-driven disease.

“Many features of the androgen receptor are similar to progesterone receptor, as they belong to the same subfamily of steroid receptors,” said Devrishi Goswami, the first author of the study and a member of the Griffin laboratory. “It could work the very same way. So these new insights may also help in finding new approaches to treating hormone-therapy-resistant prostate cancer.”

In addition to Goswami and Griffin, other authors of the study, “Influence of Domain Interactions on Conformational Mobility of the Progesterone Receptor as Detected by Hydrogen/Deuterium Exchange Mass Spectrometry,” include Bruce Pascal of The Scripps Research Institute; Celetta Callaway and Dean P. Edwards of the Baylor College of Medicine; and Raj Kumar of The Commonwealth Medical College, Scranton, Pennsylvania. The study can be accessed athttp://www.cell.com/structure/abstract/S0969-2126(14)00137-3

The study was supported by The National Institutes of Health (grants CA046938 and GM84041) and the National Cancer Institute (grant P30CA123125).

Scientists Pinpoint How Genetic Mutation Causes Early Brain Damage

Scientists from the Florida campus of The Scripps Research Institute (TSRI) have shed light on how a specific kind of genetic mutation can cause damage during early brain development that results in lifelong learning and behavioral disabilities. The work suggests new possibilities for therapeutic intervention.

The study, which focuses on the role of a gene known as *Syngap1*, was published June 18, 2014, online ahead of print by the journal *Neuron*. In humans, mutations in *Syngap1* are known to cause devastating forms of intellectual disability and epilepsy.

“We found a sensitive cell type that is both necessary and sufficient to account for the bulk of the behavioral problems resulting from this mutation,” said TSRI Associate Professor Gavin Rumbaugh, who led the study. “Because we found the root biological cause of this genetic brain disorder, we can now shift our research toward developing tailor-made therapies for people affected by *Syngap1* mutations.”

In the study, Rumbaugh and his colleagues used a mouse model to show that mutations in *Syngap1* damage the development of a kind of neuron known as glutamatergic neurons in the young forebrain, leading to intellectual disability. Higher cognitive processes, such as language, reasoning and memory arise in children as the forebrain develops.

Repairing damaging *Syngap1* mutations in these specific neurons during development prevented cognitive abnormalities, while repairing the gene in other kinds of neurons and in other locations had no effect.

Rumbaugh noted prenatal diagnosis of some infant genetic disorders is on the horizon. Technological advances in genetic sequencing allow for individual genomes to be scanned for damaging mutations; it is possible to scan the entire genome of a child still in the womb. “Our research suggests that if *Syngap1* function can be fixed very early in development, this should protect the brain from damage and permanently improve cognitive function,” said TSRI Research Associate Emin Ozkan, a first author of the

study, along with TSRI Research Associate Thomas Creson. “In theory, patients then wouldn’t have to be subjected to a lifetime of therapies and worry that the drugs might stop working or have side effects from chronic use.”

Mutations to *Syngap1* are a leading cause of “sporadic intellectual disability,” resulting from new, random mutations arising spontaneously in genes, rather than faulty genes inherited from parents. Intellectual disability affects approximately one to three percent of the population worldwide.

Rumbaugh and his colleagues are continuing to investigate. “Our findings have also identified exciting potential biomarkers in the brain of cognitive failure, allowing us to test new therapeutic strategies in our *Syngap1* animal model,” said Creson.

In addition to Rumbaugh, Ozkan and Creson, other authors of the study “Reduced Cognition in *Syngap1* Mutants Is Caused by Isolated Damage within Developing Forebrain Excitatory Neurons” include Courtney Miller and Camilo Rojas of TSRI; Enikö A. Kramár, Ron R. Seese, Alex H. Babyan, Yulin Shi, Xiangmin Xu and Gary Lynch of the University of California, Irvine; and Rocco Lucero and Jeffrey L. Noebels of Baylor College of Medicine. For further information on the study, seehttp://www.cell.com/neuron/abstract/S0896-6273(14)00401-2

The study was supported by the National Institute for Neurological Disorders and Stroke (grants R01NS064079, NS29709 and NS045260), National Institute for Mental Health (grant R01MH096847), National Institute for Drug Abuse (grants R01 DA034116 and R03 DA033499), the University of California, Irvine, and the State of Florida.