SCRIPPS FLORIDA ANNUAL REPORT FOR THE YEAR ENDING JUNE 30, 2019

PART I: SCIENTIFIC ACHIEVEMENTS AND GRANT AWARDS

A: SCIENTIFIC ACHIEVEMENTS

Scripps Florida 2019 Scientific Report
October 1, 2018 – June 30, 2019

Part 1: New Faculty and Scientific Administration

Executive Appointments and Promotions

The Scripps Research Institute Announces New Board Member

Noted Mathematician Benedict Gross Joins Scripps Research Board of Directors

October 9, 2018: Benedict Gross, PhD, a professor emeritus of mathematics at Harvard University and former dean of Harvard College, has joined the Board of Directors at Scripps Research. Gross is widely recognized for his contributions to number theory and was named a MacArthur Fellow in 1986. Additionally, he is an elected member of the National Academy of Sciences.

"Dick brings extraordinary intellect and experience to the Board, both as a professor of mathematics and as dean of Harvard College," says Peter Schultz, president and CEO of Scripps Research. "We're very grateful that he's agreed to lend his time and energy to our institute."

Gross held faculty positions at Princeton University and Brown University before becoming a tenured professor at Harvard in 1985. He served as the dean of Harvard College from 2003 to 2007. Together with Don Zagier, he established the Gross-Zagier formula for the L-functions of elliptic curves, work that led to the Frank Nelson Cole Prize in Number Theory being awarded to both men in 1987. In addition to numerous academic publications, Gross has authored *Arithmetic on Elliptical Curves with Complex Multiplications* (2000) and co-authored *The Magic of Numbers* (2004), a book intended to introduce the "beauty of numbers" to non-math readers.

Gross earned his bachelor's and doctoral degrees from Harvard University, as well as a master of science degree from Oxford University. He is the George Vasmer Leverett Professor of Mathematics, Emeritus at Harvard, as well as a professor in the mathematics department at the University of California, San Diego.

Current TSRI Board Members

John D. Diekman, Ph.D., Chairman of the Board, The Scripps Research Institute; Founding Partner of 5AM Ventures
Peter G. Schultz, Ph.D., Vice Chair of the Board and President, The Scripps Research Institute; President, Calibr
Paul Schimmel, Ph.D., Hahn Professor, Department of Molecular Medicine and Chemistry, The Scripps Research Institute

Herb Boyer, Ph.D., Professor Emeritus of Biochemistry and Biophysics at the University of California, San Francisco
Gerald Chan, Ph.D., Co-founded Morningside, a private equity and venture capital firm
William R. Hearst III, Chairman of the Board of Hearst Corporation, President of the charitable William Randolph Hearst Foundation, Affiliated Partner of Kleiner Perkins Caufield & Byers (KPCB)
Mark Edwards, Founded Bioscience Advisors, Inc. (Biosci)
Isy Goldwasser, Co-Founder and Chief Executive Officer of Thync, Inc.
Ge Li, Ph.D., Founder, Chairman and Chief Executive Officer of WuXi AppTec
Christopher T. Walsh, Ph.D., Consulting Professor to the Stanford University Department of Chemistry
Peter C. Farrell, Ph.D., D.Sc., Founder and Chairman, ResMed
Claudia S. Luttrell, President, The Skaggs Institute for Chemical Biology
Mark Pearson, Co-Founder, Vice Chairman of the Board, Drawbridge Realty Trust
Bernard Saint-Donat, President, Saint-Donat & Co.
Ron Burkle, **Founder,** Yucaipa Companies and Philanthropist
Joel Marcus, Chairman, Chief Executive Officer and Founder of Alexandria Real Estate Equities, Inc. (NYSE: ARE)
John C. Martin, Chair, Gilead's Board of Directors
Sherry Lansing, Co-Founder, Stand Up To Cancer initiative; Founder, Sherry Lansing Foundation

The Scripps Research Institute Announces New Board of Overseers

October 10, 2018: Scripps Research has announced the establishment of a Board of Overseers, which significantly expands the institute's advisory network. Pete Schultz, president and CEO of Scripps Research, says the new Board will serve in an "advisory capacity to institute leadership and its Board of Directors regarding academic, scientific and business strategies, as well as provide support for the institute's philanthropic efforts." The 21 founding members of the Board, all of whom are listed on the institute's website, include influencers in biotechnology, pharmaceuticals, academia, law, science policy, and investment.

One of the new Overseers is Rajiv Kaul, a portfolio manager and research analyst for Fidelity Investments. Widely recognized for his leadership in the biotech industry, Kaul manages Fidelity's Select Biotechnology Portfolio and its Fidelity Advisor Biotechnology Fund. "We're in a golden age of innovation and medicine," he says, "with newly discovered strategies for addressing more and more of the diseases that affect the human population. Pete [Schultz] has both the scientific understanding and business expertise to accelerate delivery of these new medicines from Scripps Research and Calibr to the patient."

Another of the Overseers ready to lend expertise is Magda Marquet, PhD. She's the co-founder and co-CEO of ALMA Life Sciences LLC, an investment and consulting firm with a portfolio of 20 private investments. She says her three decades of experience in the biotechnology industry in both the United States and Europe will be readily shared with Scripps Research. "Let's not forget that if San Diego is a thriving cluster for life sciences," she says, "it all started with world-renowned academic institutions and innovative science breakthroughs. Scripps Research stands out since it combines very innovative science with the development tools and infrastructure to make it more accessible to patients faster, a very creative structure which is unique in the world."

Joe Panetta, president and CEO of Biocom, which advocates for the life sciences industry in California, agrees. “Scripps Research has been responsible, directly and indirectly, for the development of many cutting-edge therapies, as well as the training of top scientists. It has contributed significantly to the overall global reputation of San Diego as a leading life sciences cluster.” He, too, has joined the Board of Overseers at Scripps Research.

In recent years, Scripps Research has been broadening its scope from fundamental scientific research into drug discovery and development, as well as the utilization of genomics and digital tools to promote individualized medicine. Kaul, Marquet, Panetta and the rest of the Board of Overseers bring a great amount of expertise pertinent to these growth areas. The members were announced at the institute’s Board of Directors meeting on Sept. 28.

Scripps Research Receives Highest Possible Charity Rating

April 2, 2019: LA JOLLA, CA — Following a qualitative review of dozens of performance metrics valued by charitable givers, Scripps Research has been awarded an “exceptional” rating of four stars, indicating it exceeds industry standards and outperforms most charities in its cause. The four-star designation from Charity Navigator, an independent evaluator, is the highest rating possible.

“This recognition from Charity Navigator is so important to us because it acknowledges our dedication to financial stewardship,” says Jennifer Crosby, vice president of Philanthropy and Community Engagement for Scripps Research. “We’re grateful for all of the support we receive from our donors and our community, and they can expect the best from us in return.”

Financial gifts to Scripps Research enable scientific discovery that advances the field of medicine, ultimately to improve or save lives. Among the many FDA-approved drugs to result from ingenuity at Scripps Research are treatments for several cancers, leukemia, arthritis and respiratory distress syndrome. Dozens of additional drug candidates—targeting pain, multiple sclerosis, dementia and other disease areas—are currently undergoing analysis and refinement.

In addition to its reliance on philanthropic gifts and funding from the National Institutes of Health, Scripps Research has established a first-of-its-kind translational research model for funding nonprofit research institutes. Through industry partnerships and licensing agreements, the organization is advancing new drug candidates and bringing yet another layer of financial sustainability to fuel its mission.

“Being a four-star charity means that when we receive gifts, we put them to the best and most efficient use,” Crosby says. “We believe our new operating model will make our organization an even more appealing place for donors to invest in the future of health.”

Charity Navigator is the nation's largest and most-utilized evaluator of charities. Its professional analysts have examined tens of thousands of non-profit financial documents to develop an unbiased, objective, numbers-based rating system to assess over 9,000 of America's charities. The ratings help donors gauge how efficiently a charity will use their support, how well it has sustained its programs and services over time, and its level of commitment to accountability and transparency.

Scripps Research Ranked Second in the World Among Scientific Institutes for Biomedical Research

May 16, 2019: LA JOLLA, CA – *Nature Index* has ranked Scripps Research second in the world and first in the United States among stand-alone scientific institutes for biomedical research, based on discoveries by the institute's researchers published in leading scientific journals.

The ranking, released today, "highlights scientists and institutions prominent in the ongoing research effort that will further transform our ideals of a healthy human life in the coming decades," according to *Nature.*

"This new biomedical research ranking by *Nature Index*, on top of the other recent rankings, reflects the life-changing science that is taking place at Scripps Research, and the curiosity and tenacity of our scientists," says James Williamson, PhD, Scripps Research's executive vice president of Research and Academic Affairs. "One of our outstanding features is how we erase the barriers between disciplines such as chemistry, biology, drug discovery and genomics, and that is evident in the breadth and depth of the research taking place here and with our collaborators around the world."

In addition to ranking second globally and first in the United States among stand-alone institutes in the biomedical research rankings, Scripps Research ranked 29th globally among all scientific organizations, including universities.

The *Nature Index* is compiled by Nature Research, part of Springer Nature. The *Nature Index 2019 Biomedical Sciences* supplement released today is the latest in a series of focused rankings in areas such as collaboration, innovation and biomedical research. The new biomedical research rankings are based on papers in 27 fields of biomedical research published in 55 journals from 2012 to 2018. The tables rank institutes based on fractional count, which considers the share of authorship on each article.

Previously, Scripps Research was ranked the overall most influential research institution in the world by the *Nature Index 2017 Innovation* supplement, a separate focused ranking. That ranking was based on the Normalized Lens Influence Metric, which measures the influence an institution's research has on innovation by calculating the citations of its research articles in patents owned by third parties, rather than those owned by institutions themselves.

In addition to the special supplements, *Nature Index* publishes a more general set of rankings each year, called the *Annual Tables*, which show calendar year output in 68 high-quality journals for the last three years, and reveal the countries, institutions and companies that are leading the way in publishing high-quality global science.

Last year, the *Annual Tables* ranked Scripps Research the top stand-alone scientific institute in the United States. In addition to receiving the top overall U.S. ranking, the institute was ranked first in its class in the United States in both life sciences and chemistry research. The Annual Tables also ranked Scripps Research third globally in life sciences research and fourth in chemistry.

Scripps Research Selected High School Summer Interns for 15th Annual Kenan Fellows Program

Nine-week program to include laboratory skills "boot camp" and conclude with celebration reuniting former participants from as far back as 2005.

May 3, 2019*:* Eleven students from high schools across Palm Beach County will join biomedical research laboratories at Scripps Research in Jupiter, Florida this summer as part of the institute's 15th annual high school student internship program.

The Kenan Fellows, so named in acknowledgement of the longstanding support from the William R. Kenan, Jr. Charitable Trust, represent high schools throughout Palm Beach County, including Palm Beach Gardens, Riviera Beach, Wellington, Lake Worth, West Palm Beach and Boca Raton.

Working alongside Scripps Research scientists, Kenan Fellows make important contributions to the institute's research initiatives while learning critical scientific skills and gaining valuable laboratory experience. Interns assist with research on diverse topics ranging from neuroscience and cancer biology to medicinal chemistry and virology. Each Kenan Fellow is assigned a specific research project by their faculty mentor and presents the results of their studies at an all-day seminar on the final day.

Prior to their arrival on campus, all Kenan Fellows participate in a one-week laboratory skills boot camp, as part of a partnership with Palm Beach State College. Held at its Palm Beach Gardens campus, the boot camp is designed to better prepare students for the eight-week internship experience.

"The Biotechnology program at Palm Beach State College is pleased to partner with Scripps Research to provide laboratory skills training for the summer high school student interns," says Edison Mejia, Biotechnology Program Instructor at Palm Beach State. "This one-week, intensive training is the first step toward a productive internship. Interns become familiar with basic lab safety, scientific calculations, experimental design, along with data collection and analysis. This program helps make their time in the laboratory more efficient."

Since its inception, more than 180 high school students and science teachers have completed the prestigious summer internships. In addition to conducting research, interns attend special weekly seminars presented by Scripps Research postdoctoral scientists and graduate students, offering an in-depth look at the latest science taking place on the Florida campus.

"The summer internship program at Scripps Research provides high school students the opportunity to experience authentic, cutting-edge scientific research," says Jennifer Davis, Secondary Science Program Planner at the Palm Beach County School District. "The skills and relationships built through these experiences will serve these students in their future STEM careers. We are so lucky to be able to continue this partnership with Scripps Research."

In addition to student presentations, the final week of this summer's internship program will also include the first-ever Kenan Fellows reunion celebration, a special reception and dinner event open to all current and former participants of the program. Alumni are invited to return to the Scripps Research campus in Jupiter to reconnect with fellow alumni and faculty mentors, and to meet members of the Kenan Charitable Trust who have made this program possible since 2005.

Scripps Research Awards Doctorates to Largest Graduating Class in Institute's History

Biotechnology pioneer John C. Martin granted honorary degree

May 16, 2019: LA JOLLA, CA — Scripps Research's Skaggs Graduate School of Chemical and Biological Sciences will recognize the outstanding research accomplishments of more than 50 graduate students, comprising the largest graduating class in the school's history, on Friday, May 17, with the conferral of doctoral degrees at the 27th annual commencement ceremony.

"Each graduating student has demonstrated a deep understanding of their field, and has built upon this knowledge to advance biology and chemistry through their discoveries," says Philip Dawson, PhD, professor in the Department of Chemistry and dean of graduate and postdoctoral studies at Scripps Research. "They have all made a tremendous impact on our campus community. The conferral of doctoral degrees on such an accomplished group of young scientists illustrates how Scripps Research profoundly impacts the future of science. I am confident they will continue to learn, innovate and assume leadership roles in any career path they choose."

Joining the assembled students and faculty at this year's commencement will be Scripps Research Board of Directors member and former Gilead Chairman and CEO John C. Martin, PhD. Martin will deliver the keynote address and receive an honorary doctoral degree for his extensive contributions to the development of antiviral therapeutics and advancements in global health.

The record number of students earning doctoral degrees at this year's ceremony reflects a steady growth in the institute's keystone educational program, which was renamed the Skaggs Graduate School in 2018 in recognition of a significant gift by the Skaggs family toward the endowment of fellowships for all Scripps Research students. The program's growth can also be seen in a 30 percent enrollment increase on the institute's campus in Jupiter, Florida.

"In addition to our largest incoming class, we will also be graduating a record number of Florida-based students at this year's commencement," says Christoph Rader, PhD, associate dean of graduate studies for the Florida campus and associate professor of Immunology and Microbiology. "Whether in basic research or translational studies, research opportunities for graduate students on the Florida campus continue to expand, as do new courses taught by faculty here. It's an exciting time to be a part of this vibrant academic community."

Ranked among the top 10 doctoral programs of its kind in the nation by *U.S. News & World Report*, the Skaggs Graduate School at Scripps Research offers rigorous training in chemistry, chemical biology, neuroscience, immunology, cell biology and numerous other biomedical research areas. The program immerses students in intensive laboratory research while offering a customizable course curriculum that allows students to match individual research interests while exploring multidisciplinary topics at the interface of chemistry and biology.

Benefactor Mark Skaggs says the enduring values of hard work and education held by his late father, the food and drugstore pioneer L.S. "Sam" Skaggs, are exemplified by the graduate program that now bears his name. "The faculty and students here work at the pinnacle of their respective fields," he says.

Part 2: Grant Awards and Licensing Agreements

Potential Drugs for ALS, Alzheimer's and Parkinson's Garner $3 Million Grant

At least 14,000 Americans have ALS. Now a new grant from the National Institute of Neurological Disorders and Stroke could help advance a potential treatment for ALS and related neurological disorders.

September 18, 2018**:** A new four-year, $3 million grant will enable Scripps Research scientists to advance compounds that may protect neurons in diseases caused by toxic protein accumulation, including Parkinson's, ALS, Alzheimer's and Creutzfeldt-Jakob disease.

Those diseases appear to share a common mechanism, the clumping of improperly formed proteins, which leads to destruction of nerve cells' energy supply—and cell death. ALS, also known as Lou Gehrig's, is one such disease. The toxic protein accumulation in ALS leads to the death of the critical neurons that link the brain to muscles. In an animal model of ALS, the compounds developed at Scripps Research by professors Corinne Lasmézas, PhD, Thomas Bannister, PhD, and colleagues, improved the animals' strength and ability to move.

"In 2015, we discovered this new mechanism in these diseases, so we set up a drug discovery strategy to turn it into much-needed treatments," Lasmézas says. "We are now optimizing promising compounds."

Lasmézas, a specialist in neurodegenerative diseases, and Bannister, a medicinal chemist, will use the award from the National Institute of Neurological Disorders and Stroke to refine and optimize the compounds, which were identified with the help of the Scripps Research robotic high-throughput molecular screening center. Their quest now is to move those compounds toward the clinic.

To accomplish this, the scientists are studying nicotinamide adenine dinucleotide, or NAD, a metabolite necessary for energy production in cells, as well as for other important cellular processes. The compounds developed by Lasmézas, Bannister and colleagues protect neurons by restoring healthy NAD metabolism in the cells, Lasmézas says.

"We are going to optimize the compounds to make them more efficient and brain-penetrant," Lasmézas says. "Ultimately, they will become drugs able to treat these devastating neurodegenerative diseases."

The grant number is 1R01NS103195-01A1.

With New NIH Support, Florida Scientists to Develop Faster, Cheaper Way to Screen Potential Drugs

October 2, 2018: An innovative proposal to dramatically reduce the cost of drug discovery has won Scripps Research chemist Thomas Kodadek, PhD, one of ten Transformative Research awards from the Director of the National Institutes of Health, Francis Collins, MD, PhD.

Kodadek's award, worth more than $4 million over five years, is part of an initiative called the NIH Common Fund High-Risk, High-Reward program, which is intended to support unconventional approaches to major challenges in biomedical and behavioral research.

Kodadek's proposal harnesses the power of scale both large and small. It employs miniaturization technologies developed by Kodadek and others at Scripps Research, reducing the amount, and thus the expense, of compounds required to test drug candidates against cellular targets. It also envisions development of vast "libraries" of millions of biologically important compounds displayed on beads roughly the size of a human cell.

The system would allow millions of different chemical compounds to be tested for their ability to drive a desired change in cells. These might include tests for compounds that interfere with cancer cell proliferation, or compounds that can prevent toxic Alzheimer's plaques from accumulating in brain cells, Kodadek says.

"If the fundamental problems of high cost and target identification could be solved,

this new type of screening could provide a wealth of drug leads, even for diseases whose mechanisms are not sufficiently well understood to allow standard target-focused screens to be carried out," Kodadek says.

The platform has the potential to slash drug-discovery costs by 100-fold, he adds. The potential applications go beyond cancer and diseases like Alzheimer's, and include anti-bacterial agents, since "this system should be especially effective for finding novel compounds toxic to bacterial pathogens," Kodadek says.

The NIH's Transformative Research Award was established in 2009 to encourage interdisciplinary approaches to research problems. It funds scientific endeavors that, while inherently risky and untested, "could potentially create or challenge existing paradigms," the NIH says.

Combining technologies to speed discoveries

Kodadek's multi-year plan begins by creating the compound libraries, and encoding each chemical structure with a unique DNA segment—not the DNA that makes up human genes, but DNA used as information storage system.

A cell engineered to show the outcome of the test via a fluorescent tag would be pasted to a bead and then suspended in a gel, like stars in a galaxy. Releasing the chemicals from the beads will expose the affixed cells to the chemical on the particle. The cell will glow more or less brightly depending upon the test's setup. The DNA encoding the chemical "hits" can be sequenced, revealing the structure.

Kodadek says melanoma and pancreatic ductal cell carcinoma will be among the first cancer cells to be screened, with the intent of finding compounds that are selectively toxic to the cancer cells, but not to healthy cells.

Critical to the system, the compounds in the library will be equipped with "weakly reactive electrophiles," compounds whose charge will drive them to bind to target proteins.

"This tight connection between the active molecule and its target will greatly simplify finding this needle in a haystack," Kodadek says.

Once the basic platform is established, Kodadek says he plans to make the compound libraries he develops available to collaborators, in the hopes of advancing many areas of biological research.

"Application of this technology to a variety of different screens would hopefully lead to the discovery of dozens, if not hundreds, of new bioactive molecules," Kodadek says.

The NIH Director's High-Risk, High Reward grants, totaling $282 million over five years, will go to 89 investigators nationwide this year. They include 10 Pioneer, 58 New Innovator, 10 Transformative

Research, and 11 Early Independence awards. In addition to the 2018 Transformative Research award, Kodadek in 2006 received the NIH Director's Pioneer award.

The grant number is 1R01GM133041-01.

Scripps Research Chemist Matthew D. Disney Awarded the Sackler Prize

January 31, 2019: JUPITER, FL — Chemistry Professor Matthew D. Disney, PhD, of Scripps Research in Jupiter, Florida, has been awarded the Raymond and Beverly Sackler International Prize in Chemistry from Tel Aviv University. The prize recognizes outstanding scientists under age 45 and is intended to encourage dedication to science, originality and excellence.

Disney shares the 2019 prize with chemists Christopher J. Chang of the University of California, Berkeley, and Jason W. Chin, of Cambridge University in England.

Disney's laboratory has demonstrated that RNA can be a small-molecule drug target, countering prevailing thoughts among many scientists, who considered RNAs undruggable due to their small size, relative lack of stability and undetermined specificity.

Most drugs work by binding with proteins. While a small fraction of the human genome encodes proteins, much more of it, about 70 to 80 percent, is transcribed into RNA. Disney reasoned that finding ways to bind drug molecules to RNA could offer new ways of tackling incurable diseases, and many more potential targets.

Disney's group has proven that is the case. His group has developed broad approaches to the directed use of RNA genome sequence to inform the development of lead small-molecule medicines for multiple conditions with unprecedented potency and selectivity. Furthermore, his group recently developed methods to cleave RNAs in cells by locally awakening the immune system to seek out and destroy RNAs that are the root cause of disease.

These studies have already provided multiple lead medicines for incurable genetic diseases, including various forms of muscular dystrophy and ALS, and difficult-to-treat cancers. Because of this work, almost every drug company and many smaller biotechnology companies are pursuing RNA as a small-molecule drug target.

"I have been blessed with great teachers, especially my graduate advisor, Doug Turner, who taught me almost everything about science. I've also been blessed with an excellent environment at Scripps Research, where science is enabled by our staff," Disney says. "I am especially grateful for the wonderful students in the lab. I would never have imagined that we would have gotten as far as we have. None of this would have been possible without these people."

Chemistry Professor Ben Shen, PhD, co-chairman of Scripps Research's bicoastal Department of Chemistry, predicts Disney's work will benefit many.

"Matt's research has fundamentally changed how the scientific community approaches RNA as drug targets for diseases with no known cure or treatment options," Shen says. "He is richly deserving of the Sackler Prize."

Farzan Elected to the American Academy of Microbiology

January 31, 2019: The American Society for Microbiology has elected Scripps Research Professor Michael Farzan, PhD, to its American Academy of Microbiology. The academy's members are elected based on their record of scientific excellence and originality, scholarly achievement, leadership and high ethical standards. Fellows represent all subspecialties of the microbial sciences, including basic and applied research, teaching, public health, industry and government service.

Farzan, based at the Jupiter, Florida Scripps Research campus, co-chairs the bicoastal institute's Department of Immunology and Microbiology. His research focuses on immune responses to enveloped viruses including HIV-1. Farzan's lab conducts both basic and applied research, including investigating immune responses to viral entry, and developing gene therapy strategies for universal protection from HIV-1.

The American Academy of Microbiology is the honorific leadership group within the American Society for Microbiology, among the world's oldest and largest life science organizations. Composed of more than 30,000 scientists and health professionals, ASM promotes and advances the microbial sciences through conferences, publications, certifications and educational opportunities.

Janiszewska Named an AACR "NextGen Star"

January 31, 2019: The American Association for Cancer Research has named Michalina Janiszewska, PhD, a "NextGen Star" ahead of its 2019 Annual Meeting in Atlanta, Georgia March 29 - April 3. The honor provides early career scientists with career development support and increased visibility in the field.

Janiszewska, an assistant professor in the department of Molecular Medicine at Scripps Research in Jupiter, Florida, studies cancer heterogeneity, or the diversity of cancer mutations, gene expression patterns and cellular behaviors that may reside within a single patient's cancer. Her focus has been on resistance mechanisms of HER-2 positive breast cancers, and more recently on brain cancer, one of the most heterogeneous and aggressive of all cancer types.

The American Association for Cancer Research accelerates progress toward the prevention and cure of cancer by promoting research, education, communication, and collaboration. It publishes several peer-reviewed journals *including Cancer Discovery; Cancer Research; Clinical Cancer Research; Molecular Cancer Therapeutics; Molecular Cancer Research; Cancer Epidemiology, Biomarkers & Prevention; Cancer Prevention Research*, and *Cancer Immunology Research*.

Part 3: Scientific Accomplishments

Scientists Design New Metabolic Technology to Open Scientific Data for Everyone

September 13, 2018: Patients want to see their medical information. Researchers want to share their data.

Now, scientists at Scripps Research have released a new technology designed to make these measurements easier to perform and more accessible to practitioners, scientists and the general public.

"This is really about data sharing and accelerating the process of discovery," says Gary Siuzdak, PhD, professor at Scripps Research and co-corresponding author of the new XCMS/METLIN open data analysis platform, published recently in *Nature Methods*.

XCMS-MRM and METLIN-MRM represent a cloud-based analysis platform that allows scientists to quantify molecules from biological samples and make their results publicly available.

"When we say 'publicly available,' we mean it. Anyone with a computer would have access," says Siuzdak.

Directed by Siuzdak, the Center for Metabolomics and Mass Spectrometry at Scripps Research specializes in using a technique called mass spectrometry to identify and quantify small molecules, whether they are drugs or naturally occurring metabolites. Metabolites are critical as they interact with every level of a person's physiology: the genome, transcriptome and the proteome. One could say metabolites are master manipulators of physiology and reflect an individual's health signature.

Tens of thousands of labs around the world generate data using mass spectrometry, so the platform could be useful outside medicine as well, say study first authors Xavier Domingo-Almenara, PhD, and J. Rafael Montenegro-Burke, PhD, of Scripps Research. Any field using mass spectrometry could benefit from these new resources, such as environmental sciences, pharmaceuticals, forensics, food control and sports medicine, they add.

Siuzdak and his colleagues have long aimed to keep their research tools free and open to the public. Paul Benton, PhD, bioinformatics analyst at Scripps Research and co-corresponding author of the study, explains that hosting their platform on the cloud allows anyone, from collaborators to patients, to check the validity of the mass spectrometry results. "In an age where scientific results are being constantly questioned, open data has become an essential part the discovery process," Benton says.

The idea of open data sharing has caught on in recent years. Since its launch in 2004, the XCMS/METLIN platform has grown to over 25,000 users, and the molecules in its data repository have leapt from 14,000 to 150,000 in just the last year, partly thanks to a collaboration with Calibr, a division of Scripps Research.

The new resources take advantage of breakthroughs that let scientists speed up research—and increase precision—by identifying the key fragments of a molecule that set it apart.

"There's nothing else even close to this," Siuzdak says.

The study, "XCMS-MRM and METLIN-MRM: a cloud library and public resource for targeted analysis of small molecules," included additional authors from the University of Lausanne; Lausanne University Hospital, Geneva University Hospitals; Umeå University and Imperial College London.

The study was supported by the UK Medical Research Council (grant MC-PC-12025), the Department of Energy (contract number DE-AC02-05CH11231) and the National Institutes of Health (grants R01 GM114368-03, P30 MH062261-10 and P01 DA026146-02)

Opioid Users Could Benefit from Meth-Relapse Prevention Strategy, Study Finds

September 17, 2018: New research raises the possibility that a wider group of people in recovery from substance use disorders may benefit from a Scripps Research-developed relapse-prevention compound than previously thought.

The research, published recently in the journal *Learning and Memory*, shows that the compound appears to be effective even if multiple drugs of abuse are involved, such as methamphetamine in combination with either opioids or nicotine. Polysubstance use is common among people addicted to methamphetamine, in part because the rate of smoking is high among meth users. In addition, the meth available today is so potent that many users turn to opioids to dampen the high.

The potential medication, a modified form of the compound blebbistatin, works by breaking down methamphetamine-linked memories that can trigger craving and relapse. The opportunity to boost treatment success by modulating emotional memory is a novel concept, and a promising one, says Courtney Miller, PhD, associate professor on the Florida campus of Scripps Research and senior author of the study.

For people in recovery from substance use disorders, memories can drive uncontrollable urges to return to drug use. Handling money, tasting certain foods or returning to places linked to their drug use can all trigger those intense cravings.

"A lot of current users aren't even aware of what their triggers are until they encounter them. These triggers can maintain their ability to drive craving for a person's entire life, meaning a lifetime relapse risk. That's what makes this new finding exciting," Miller says. "This would be the first compound to directly target the motivational power of craving triggers."

Importantly, the compound doesn't appear to act on other forms of memory or motivations, and this selectivity is key to its powerful potential.

How the drug works to target cravings

The modified blebbistatin, tested in animal models for this study, works by interfering with storage of memories in the amygdala, the brain's emotional memory center, Miller says. The medication inhibits a protein called nonmuscle myosin II. That protein organizes another, called actin, which is involved in neural plasticity, the extension of brain cells' finger-like projections that form new connections.

Normally, actin and nonmuscle myosin II stabilize within minutes of learning, lending stability to long-term memory storage. However, the actin and myosin supporting meth memories behaves differently. They remain active and, therefore, uniquely vulnerable to a drug designed to inhibit nonmuscle myosin II. In animal models, treatment with the compound eliminated the animals' learned preference for a place where they had ingested addictive drugs.

"What they are losing is that drive to go and drug seek when they see the familiar place," Miller adds.

Miller hopes this sort of treatment could offer long-term relief from drug cravings. Miller explains that while many U.S. insurance plans now cover 30 days of inpatient drug treatment, spontaneous addiction cravings last significantly longer, and at present, no medications exist to reduce the pull of lifelong drug-linked memories.

"The height of drug cravings peaks around 30 days and goes out to about 180 days after cessation of use," Miller says. "That's when they are out of rehab and back in their environment, surrounded by the things that trigger their cravings, so it's a really problematic situation."

A closer look at the role of stress in relapse

Memories aren't the only problem. Studies show that encountering intense stress during that sensitive recovery period can boost relapse risk. For a related study, published recently in the journal *Addiction Biology*, Miller, research associate Ashley Blouin, PhD, and colleagues, developed a novel animal model of social stress-potentiated meth seeking that may provide a more accurate way to test the effectiveness of the modified blebbistatin compound and other therapies in development.

"There is data in humans that social stress—combined with using a small amount of meth—can drive a much stronger craving for the drug," Miller says. "We found we can recapitulate that in an animal model."

The new model measures relapse in animals that had been previously exposed to social defeat and meth. With the model, a rat is placed in the home cage of an aggressive resident rat. Some of these "intruders" handle the aggressive resident in an active way, defending themselves. Others take a more passive approach and rapidly acquiesce to the resident by laying down. The passive rats were consistently more likely to self-administer methamphetamine after the stressful encounter compared to rats that actively defended themselves, Miller says. Interestingly, passive coping strategies have been linked to a host of behavioral and mental disorders, including an increased likelihood of developing addiction, she adds.

"Having a reliable model of social stress in the context of drug use will be important to developing medication strategies to improve treatment response," Miller says.

Depending on many factors, relapse rates following treatment for methamphetamine and heroin addiction can hover between 40 percent and 60 percent, or as high as 90 percent. Studies suggest that people who abuse both methamphetamine and heroin are almost three times more likely to overdose, underscoring the need for innovative solutions. With these two new studies, Miller hopes to bring treatment options closer to patients as quickly as possible. With funding from the Blueprint Neurotherapeutics Network, a translational research program of the National Institutes of Health, the Miller laboratory is working with Scripps Research colleagues, Pat Griffin, PhD, and Ted Kamenecka, PhD, to move the modified blebbistatin to the clinic.

Additional authors of the study, "The role of nonmuscle myosin II in polydrug memories and memory reconsolidation" include Sherri B. Briggs, Madalyn Hafenbreidel, Erica J. Young and Gavin Rumbaugh. The study was supported by grants from the National Institutes of Health (R01DA034116, UH2/ 3NS096833, and R01DA034116-03S1 Diversity Supplement).

Additional authors of the study, "Social stress-potentiated methamphetamine seeking," include Swathi Pisupati, Colton G. Hoffer, Madalyn Hafenbreidel, Sarah E. Jamieson and Gavin Rumbaugh. The study was funded by grants from the National Institute on Drug Abuse (R03DA033499 and R01DA034116 and K01DA040737) and the Brain and Behavior Foundation (NARSAD Young Investigator Award to A.M.B.).

Newly Discovered Long Noncoding RNA Plays Critical Role in Brain Growth and Signaling

October 8, 2018: A new study from the Scripps Research laboratory of Sathyanarayanan Puthanveettil, PhD, peers deep within the nucleus of developing brain cells and finds that long noncoding RNAs play an important role in the healthy functioning and maintenance of synapses, the communication points between nerve cells in the brain.

"Long noncoding RNAs are often described as 'the dark matter of the genome.' So, systematic interrogation of their function will illuminate molecular mechanisms of brain development, storage of long-term memories and degradation of memory during aging and dementia," Puthanveettil says.

RNA are the master regulators of the cell, tiny chains of nucleotides that read, transcribe and regulate expression of DNA, and build proteins. While scientists have gained great insights recently into the genetics underpinning how brain cells reach out and communicate with each other, the role of noncoding RNA is poorly understood. Research suggests that the longest of these noncoding RNA, those over 200 nucleotides long, help determine which genes are activated and operating in brain cells at various times. But which ones?

Writing in the journal *Proceedings of the National Academy of Sciences*, Puthanveettil and his colleagues on Scripps Research's Florida campus report that a specific long non-coding RNA, GM12371, controls expression of multiple genes involved in nervous system development and functioning. Furthermore, it affects the developing neurons' shape and ability to signal.

In mouse hippocampal cells, learning-related signaling upregulates GM12371, while its reduction produces inactive neurons, ones with sparse branches.

Together, the results suggest that healthy growth and development of brain cells and brain circuits depends not just upon specific proteins but also upon specific long noncoding RNAs, which scientists are now beginning to explore.

What role GM12371 dysfunction may play in diseases of the brain and nervous system demands further study, Puthanveettil says.

"Both coding and noncoding RNAs are increasingly viewed as druggable targets. Identifying their specific roles in the fundamental biology of functioning of neural circuits might eventually open new ways of treating neuropsychiatric disorders, such as autism and Alzheimer's disease," Puthanveettil says.

Additional authors of the study, "Long noncoding RNA GM12371 acts as a transcriptional regulator of synapse function," include Bindu L. Raveendra, Supriya Swarnkar, Yosef Avchalumov, Xin-An Liu, Eddie Grinman, Kerriann Badal, Adrian Reich and Bruce D. Pascal of Scripps Research.

This research was supported by the National Institutes of Health (grants 1R21DA039417-01A1 and 5R21MH108929-02).

Team Gets a Closer Look at How Proteins Meet on the Cell Membrane

October 10, 2018: Scripps Research scientists have uncovered the workings of a critical process in cell survival. Their study, published recently in the *Proceedings of the National Academy of Sciences*,

is the first to show exactly how a protein called talin activates another critical protein, called integrin, to do its job on the cell membrane.

While the researchers focused on basic cell biology, the findings suggest targeting a protein like talin to interfere with this activation process, giving scientists a potential way to tackle cancer cells.

The research was led by the laboratory of Tina Izard, PhD, professor on Scripps Research's Florida campus. The laboratory focuses on understanding the structures and functions of proteins involved in a process called cell adhesion. Without these proteins, cells could not send signals or react to the surrounding environment—cells simply could not function effectively.

A key protein cell adhesion is protein integrin, which is involved in certain cancers and even bleeding disorders. "Integrin activation is a fundamental process in cell biology that also goes awry in important pathological states," says Izard. "Integrins play key roles in cancer progression and metastasis where certain tumor types exhibit higher levels of certain integrins."

One long-standing mystery in the field of cell adhesion was the question of how talin, a protein that interacts with integrin and makes cell adhesion possible, is activated. Researcher Rangarajan Erumbi, PhD, staff scientist at Scripps Research and co-author of the study, says past studies have suggested that talin interacts with the cell membrane to activate integrin, although the detailed molecular mechanisms were unknown.

Erumbi says it is especially important to understand talin's role because talin "glues" integrin to the cytoskeleton within the cells. "By gluing together multiple players, talin brings stability to the cell and helps with functions like cell migration and differentiation."

To answer the question of integrin activation, the researchers had to overcome technical hurdles that had previously blocked scientists from seeing how proteins bind the cell membrane. After many years of trial and error, the researchers crystallized the domain of talin that interacts with the cell membrane, letting them map out its structure in high resolution using x-ray crystallography.

Their structure reveals how talin binds to the cell membrane to activate integrin. Inside the cell, talin exists in a non-activated state defined by interaction between the two ends of talin, its "head domain" and its "tail domain." By combining several techniques, the scientists could show that the lipid activates talin by severing this head-tail interaction and exposing a region of talin that binds to integrin.

At last, the researchers have defined the molecular basis of the cell membrane in integrin activation.

Erumbi believes this closer look at talin could also open the door to future cancer therapies that could target the talin-membrane interaction to stop tumor growth. Although some experimental tumor therapies target integrin on the cell membrane, a potential issue with these therapies is that drugs tend to target the region external to the cell membrane. This means talin could be a better target in tumor cells to weaken adhesion and cell integrity at the same time.

"We now understand this aspect of lipid biology, which lays the foundation for the development of novel integrin therapeutic agents," Erumbi says.

Krishna Chinthalapudi is first author of the study, "The interaction of talin with the cell membrane is essential for integrin activation and focal adhesion formation."

The laboratory is supported by the National Institutes of Health (GM 094483), the Department of Defense (W81WH-18-100451), and the State of Florida on related membrane binding proteins.

Jupiter Chemist Matthew Disney Named BioFlorida's Entrepreneur of the Year

October 16, 2018: FORT LAUDERDALE, FL—Saying his discoveries had "launched a new front in the war on disease," Florida's statewide biotechnology industry organization, BioFlorida, today named Scripps Research Chemistry Professor Matthew Disney, PhD, the 2018 Weaver H. Gaines Entrepreneur of the Year.

"We are pleased to present Matthew D. Disney, Ph.D. with this award for his contributions to the Florida life sciences industry and his ongoing passion and commitment to find cures for those facing rare diseases, such as ALS," says Nancy K. Bryan, president and CEO of BioFlorida.

Disney is the scientific founder of Expansion Therapeutics, Inc., with business offices in San Diego, California and R&D offices in Jupiter, Florida. The company pursues oral medicines for diseases that involve specific types of damage to RNA, using technologies and methods Disney developed in his lab at the Florida campus of Scripps Research.

In January 2018, Expansion Therapeutics announced that it had raised $55.3 million in series A financing, and planned to pursue 30 RNA-based diseases including myotonic dystrophy type I, the most common form of muscular dystrophy in adults.

According to BioFlorida, "The Entrepreneur of the Year Award recognizes an entrepreneur who has made extraordinary contributions to the growth of life sciences in the leadership of a company or institution." The organization also cited Disney's work on "a mathematical model to predict which RNA could be bound with drug-like molecules, and now boldly intends to develop novel treatments for multiple diseases."

Disney's research has changed how scientists approach so-called "undruggable" diseases. Drugs currently on the market bind to proteins. However, of about 20,000 different protein types in the human body, many cannot be readily bound with drug molecules, leading some diseases to be described as undruggable.

By contrast, the human body contains around 200,000 types of RNAs, the molecules that that control genetic transcription, cellular regulation and protein construction itself. RNAs present vastly more potential targets for cures; however, conventional wisdom in the scientific community was that RNA presented a poor drug target, due to its tiny size, structural changeability and other challenges.

Disney, over a decade of research, proved it possible. He identified key RNA structures and found that their small size presented new possibilities to tackle difficult-to-treat, diseases including forms of muscular dystrophy.

"Our ultimate goal is to take compounds that can target RNA and engineer them to do things we want them to do, like cross the blood-brain barrier or more readily enter skeletal muscle," Disney says.

Disney and his team at Scripps Research are continuing to develop compounds intended to treat other rare "orphan" diseases with no known cure and more common disorders that show poor prognoses, such as drug-resistant cancers.

"Our central focus here is to get medicines to patients that have no treatments as effectively and quickly as possible. Our team is completely committed to this goal. We strive for this every day," Disney says.

Scripps Research Translational Institute and NVIDIA Partner to Advance Artificial Intelligence in Genomics and Digital Medicine

October 23, 2018: Scripps Research Translational Institute and NVIDIA today announced a collaboration to develop deep learning tools and methods to process and analyze genomic and digital medical sensor data.

The partnership seeks to accelerate the application of artificial intelligence (AI) for disease prevention, health promotion and the streamlining of biomedical research efforts. Scripps and NVIDIA will focus on advancing the use of deep learning, a subset of AI that is poised to play a key role in improving clinical outcomes and reducing healthcare costs.

Merging technology development with leading-edge scientific research on the use of data in medicine will be critical to this effort, according to Eric Topol, MD, founder and director of Scripps Research Translational Institute and professor at Scripps Research.

"AI has tremendous promise to transform the future of medicine," says Topol, who will lead the Scripps Research team on the project. "With NVIDIA, we aim to establish a center of excellence for artificial intelligence in genomics and digital sensors, with the ultimate goal of developing best practices, tools and AI infrastructure for broader adoption and application by the biomedical research community."

While the use of computer-aided diagnostics is not new in medicine, the use of AI systems is currently largely limited to diagnoses from medical imaging. Preliminary studies, however, suggest that deep learning techniques could also be applied to big data of whole genomic sequences and continuous physiologic sensors, with potential to prevent illness. In deep learning, machine learning happens in layers, forming neural networks with each layer adding to the knowledge of the previous layers.

NVIDIA has helped pioneer the spread of AI across a growing range of fields, including self-driving cars, robotics and healthcare. "AI is already transforming healthcare by using electronic health records and medical imaging to better diagnose and treat disease," says Kimberly Powell, vice president of healthcare at NVIDIA. "Our collaboration with Scripps expands these opportunities by tapping into the rapid accessibility of genomic and digital wearable data, and furthers the quest to better predict and prevent disease."

The team from NVIDIA and Scripps will initially focus on developing deep learning-based genetic and digital sensing prediction of atrial fibrillation, an irregular heartbeat which increases the risk of stroke, along with analytics of whole genome sequences, with later expansion to other diseases and datasets.

Synthetic Microorganisms Allow Scientists to Study Ancient Evolutionary Mysteries in the Laboratory

Scripps Research scientists use the tools of synthetic biology to engineer organisms similar to those thought to have lived billions of years ago

October 29, 2018: Scientists at Scripps Research and their collaborators have created microorganisms that may recapitulate key features of organisms thought to have lived billions of years ago, allowing them to explore questions about how life evolved from inanimate molecules to single-celled organisms to the complex, multicellular lifeforms we see today.

By studying one of these engineered organisms—a bacterium whose genome consists of both ribonucleic acid (RNA) and deoxyribonucleic acid (DNA)—the scientists hope to shed light on the early evolution of genetic material, including the theorized transition from a world where most life relied solely on the genetic molecule RNA to one where DNA serves as the primary storehouse of genetic information.

Using a second engineered organism, a genetically modified yeast containing an endosymbiotic bacterium, they hope to better understand the origins of cellular power plants called mitochondria. Mitochondria provide essential energy for the cells of eukaryotes, a broad group of organisms—including humans—that possesses complex, nucleus-containing cells.

The researchers report engineering the microbes in two papers, one published October 29, 2018 in the *Proceedings of the National Academy of Sciences (PNAS)* and another published August 30, 2018 in *Journal of the American Chemical Society (JACS).*

"These engineered organisms will allow us to probe two key theories about major milestones in the evolution of living organisms—the transition from the RNA world to the DNA world and the transition from prokaryotes to eukaryotes with mitochondria," says Peter Schultz, PhD, senior author on the papers and president of Scripps Research. "Access to readily manipulated laboratory models enables us to seek answers to questions about early evolution that were previously intractable."

The origins of life on Earth have been a human fascination for millennia. Scientists have traced the arc of life back several billion years and concluded that the simplest forms of life emerged from Earth's primordial chemical soup and subsequently evolved over the eons into organisms of greater and greater complexity. A monumental leap came with the emergence of DNA, a molecule that stores all of the information required to replicate life and directs cellular machinery to do its bidding primarily by generating RNA, which in turn directs the synthesis of proteins, the molecular workhorses in cells.

In the 1960s, Carl Woese and Leslie Orgel, along with DNA pioneer Francis Crick, proposed that before DNA, organisms relied on RNA to carry genetic information, a molecule similar to but far less stable than DNA, that can also catalyze chemical reactions like proteins. "In science class, students learn that DNA leads to RNA which in turn leads to proteins—that's a central dogma of biology—but the RNA world hypothesis turns that on its head," says Angad Mehta, PhD, first author of the new papers and a postdoctoral research associate at Scripps Research. "For the RNA world hypothesis to be true, you have to somehow get from RNA to a DNA genome, yet how that might have happened is still a very big question among scientists."

One possibility is that the transition proceeded through a kind of microbial missing link, a replicating organism that stored genetic information as RNA. For the *JACS* study, the Scripps Research-led team created Escherichia coli bacteria that partially build their DNA with ribonucleotides, the molecular building blocks typically used to build RNA. These engineered genomes contained up to 50 percent RNA, thus simultaneously representing a new type of synthetic organism and possibly a throwback to billions of years ago.

Mehta cautions that their work so far has focused on characterizing this chimeric RNA-DNA genome and its effect on bacterial growth and replication but hasn't explicitly explored questions about the transition from the RNA world to the DNA world. But, he says, the fact that *E. coli* with half its genome comprised of RNA can survive and replicate is remarkable and seems to support the possibility of the existence of evolutionarily transitional organisms possessing hybrid RNA-DNA genomes. The Scripps Research team is now studying how the mixed genomes of their engineered *E. coli* function and plans to use the bacteria to explore a number of evolutionary questions.

For instance, one question is whether the presence of RNA leads to rapid genetic drift—large changes in gene sequence in a population over time. Scientists theorize that massive genetic drift occurred quickly during early evolution, and the presence in the genome of RNA could help explain how genetic change occurred so quickly.

In the paper published in *PNAS*, the researchers report engineering another laboratory model for an evolutionary milestone thought to have occurred more than 1.5 billion years ago. They created a yeast dependent for energy on bacteria living inside it as a beneficial parasite or "endosymbiont." This composite organism will allow them to investigate the ancient origins of mitochondria—tiny, bacteria-like organelles that produce chemical energy within the cells of all higher organisms.

Mitochondria are widely thought to have evolved from ordinary bacteria that were captured by larger, single-celled organisms. They carry out several key functions in cells. Most importantly, they serve as oxygen reactors, using O_2 to make cells' basic unit of chemical energy, the molecule ATP. As crucial as mitochondria are to cells, their origins remain somewhat mysterious, although there are clear hints of descent from a more independent organism, widely assumed to have been a bacterium.

Mitochondria have a double-membrane structure like that of some bacteria, and—again, like bacteria—contain their own DNA. Analyses of the mitochondrial genome suggest that it shares an ancient ancestor with modern *Rickettsia* bacteria, which can live within the cells of their hosts and cause disease. Stronger support for the bacterial origin of mitochondria theory would come from experiments showing that independent bacteria could indeed be transformed, in an evolution-like progression, into mitochondria-like symbionts. To that end, the Scripps Research scientists engineered *E. coli* bacteria that could live in, depend upon, and provide key assistance to, cells of *Saccharomyces cerevisiae*, also known as baker's yeast.

The researchers started by modifying *E. coli* to lack the gene encoding thiamin, making the bacteria dependent on the yeast cells for this essential vitamin. At the same time, they added to the bacteria a gene for ADP/ATP translocase, a transporter protein, so that ATP produced within the bacterial cells would be supplied to their yeast-cell hosts—mimicking the central function of real mitochondria. The team also modified the yeast so that their own mitochondria were deficient at supplying ATP. Thus the yeast would be dependent on the bacteria for normal, mitochondria-based ATP production.

The team found that some of the engineered bacteria, after being modified with surface proteins to protect them from being destroyed in the yeast, lived and proliferated in harmony with their hosts for more than 40 generations and appeared to be viable indefinitely. "The modified bacteria seem to accumulate new mutations within the yeast to better adapt to their new surroundings," says Lubica Supekova, PhD, co-first author of the *PNAS* paper and a staff scientist at Scripps Research.

With this system established, the team will try to evolve the *E. coli* to become mitochondria-like organelles. For the new *E. coli* endosymbiont, adapting to life inside yeast could allow it an opportunity to radically slim its genome. A typical *E. coli* bacterium, for example, has several thousand genes, whereas mitochondria have evolved a stripped-down set of just 37.

The Scripps Research team rounded out the study with further gene-subtraction experiments, and the results were promising: they found they could eliminate not just the *E. coli* thiamin gene but also the genes underlying the production of the metabolic molecule NAD and the amino acid serine, and still get a viable symbiosis.

"We are now well on our way to showing that we can delete the genes for making all 20 amino acids, which comprise a significant part of the *E. coli* genome," says Schultz. "Once we've achieved that, we'll move on to deleting genes for the syntheses of cofactors and nucleotides, and within a few years we hope to be able to get a truly minimal endosymbiotic genome."

The researchers also hope to use similar endosymbiont-host systems to investigate other important episodes in evolution, such as the origin of chloroplasts, light-absorbing organelles that have a mitochondria-like role in supplying energy to plants.

Shape-shifting Ribosomes "Tune" the Cellular Response to Stress

November 6, 2018:

Ribosomes help your cells build proteins, based on the instructions provided in genes. So, when ribosomes malfunction, disease is not far behind.

To better understand how cells respond to stressors, scientists at Scripps Research are looking to a new yeast model that reveals how human ribosomes may function in both healthy and diseased states.

The scientists recently discovered that cells can manage stress through a process they dub "ribo-tuning." This means cells reprogram themselves by evolving their genes to bind to specialized ribosomes, which are produced under stress conditions. The study, which has implications for better understanding the role of ribosomes in cancer, was published recently in *Cell Chemical Biology*.

"We created the ribo-tuning model in the lab to study how ribosomes decipher information about the amount of protein produced from an mRNA. But it turns out there's evidence in naturally occurring yeast strains that ribo-tuning happens on its own in response to stress in the cell," says senior author Katrin Karbstein, PhD, an associate professor on Scripps Research's Florida campus. "This finding suggests to us that this adaptation may be a commonly used mechanism that's been previously underappreciated."

The protein behind 'ribo-tuning'

The scientists focused on a protein called Rps26, which the Karbstein lab had previously found helps ribosomes select individual genetic messenger molecules, called mRNAs, by recognizing the start of protein sequences and begin building proteins. People with Rps26 deficiencies can develop a bone marrow disorder called Diamond Blackfan anemia, which includes an increased risk of leukemia.

In the new study, the investigators wanted to systematically reprogram protein translation to alter the cellular response to deficiencies in the Rps26 protein. They discovered that with a single point mutation they were able to program yeast cells to change their cell wall composition, activate DNA repair, or differentiate in response to Rps26 deficiency, as well as biological stresses, which produce Rps26 deficiency. The simplicity of these adaptations via a single mutation, together with the finding that some of the changes they made were found in naturally occurring yeast strains, suggest that this "ribo-tuning" mechanism might be a widespread mechanism to adapt to cellular stress.

Although the research was done in yeast, it has important implications for understanding certain processes in cancer. Both cancer cells and yeast cells are known to change the composition of their ribosomes. "And because cancer cells grow and divide very rapidly, they are similar to the samples of yeast that we found that have these types of mutations," says Karbstein.

Karbstein's lab will continue to study other ribosomal proteins in yeast to further decode how proteins are manufactured—and mis-manufactured. In addition, they plan to look at the role of Rps26 and other ribosome-related proteins in mammalian cells in culture, with the goal of understanding more about how these proteins may contribute to cancer progression.

Students moved research forward

The new study was also a chance to train the next generation of biomedical researchers. The first author of the study was Max Ferretti, a graduate student in Karbstein's lab. The other author on the study was Jennifer Louise Barre, who at the time she conducted research was a high school student at the nearby Benjamin School.

"I knew that working at Scripps would be a once in a lifetime opportunity. I was fortunate enough to work with Dr. Karbstein and also Max who was my mentor," says Barre. "Going into this internship I had no idea that I would be fortunate enough to be published and I feel so lucky that I was. I was able to work with some incredible people and expand upon my knowledge of working in a lab environment, which is something I will forever be grateful for."

Karbstein's lab has hosted several high school students over the past few years, thanks to Benjamin School science teacher Renee Szeliga, and this is the second published paper with one of these students as a co-author. "This collaboration with Mrs. Szeliga and The Benjamin School has been extremely rewarding for us," says Karbstein. "For me personally, it is also important that we open doors for young women as they enter college, and hopefully the scientific workforce."

Florida Outstanding Mentor Blends Stoicism and Intensity to Advance his Field

One mentor taught him by example; one through encouragement and one through critical inquiry.

November 20, 2018:

The Society of Research Fellows at Scripps Research in Florida named chemist Hans Renata, PhD, the Outstanding Mentor for 2018 in recognition of his dedication to his students and postdoctoral fellows. Renata's lab members say he embodies both patience and high expectations.

“He is in the lab before most of us arrive and stays after most of us have left,” says graduate student Emma King-Smith. “Seeing your mentor so dedicated in his research inspires all of us to push ourselves.”

Renata recalls three important mentors during his studies and preparation to become a chemistry faculty member. His most recent mentor, Frances Arnold, PhD, of Cal-Tech, recently won the 2018 Nobel Prize in Chemistry. He worked as a post-doctoral fellow in her lab. It was a place of intense, tough questioning, he recalls.

“She always asked, ‘What’s interesting about the solution that you’ve worked out, and if it is successful, how will it change the field you are working in?’” Renata recalls. “I guess that caused me to be more critical in examining my own work.”

Renata’s lab develops tools to functionalize carbon-hydrogen bonds with enzymes. As he describes his work, an uncommon mixture of both stoicism and passion emerge – qualities that his lab members prize.

“If you can functionalize one of these carbon-hydrogen bonds in a selective manner you can begin to create something that will be more useful for people,” he explains.

Their work is producing interesting new techniques.

“I think we have developed a pretty nice set of tools to functionalize carbon-hydrogen bonds with enzymes, and we’re really excited about the applications of this process,” Renata says. “We have things in the pipeline that hopefully can become novel antibiotics and also, hopefully, anti-cancer agents.”

That cool intensity makes Renata both an exciting and supportive mentor, says Christian Zwick, III, a graduate student.

“I think his strongest quality is his patience. He’s stoic, meaning he’s never discouraged,” Zwick says. Yet, “Hans is very passionate about his work. We’ll often text late into the night discussing ideas or results.”

Good mentors frequently lead by example, Renata notes. During Renata’s undergraduate years at Columbia University, chemist and newly minted professor Tristan Lambert, PhD, gave Renata his first lab position. Amid all of those firsts, Renata absorbed many lessons about outfitting a lab and launching new research projects.

As a graduate student at Scripps Research in La Jolla, California, he worked alongside chemist Phil Baran. Renata calls Baran, “one of the leading figures in the world of organic synthesis.” It was an incredible place to learn, he says.

“Every time you’d talk to him about your ideas, as long as they seemed to be grounded in some sound fundamental concepts he would always encourage you to try them,” Renata says. “He would allow you to think through your idea and your process.”

As his own students go off into the world and take positions in industry and academia, he hopes they will take away that lesson of independent inquiry, as well as the drive to change their field.

"For the field to keep going, you want to make sure that the next generation of scientists will be better than you," he says.

Scripps Research Scientists Decode Mechanism of Remembering – and Forgetting

November 21, 2018: LA JOLLA, CA – It's a common expression to say that your brain is full. Although the brain doesn't literally fill up, in recent years researchers have discovered that the brain does sometimes push out old memories in order to take up new ones.

Now, a team at Scripps Research has shown for the first time the physiological mechanism by which a memory is formed and then subsequently forgotten. The research, which was done in fruit flies, looked at the synaptic changes that occur during learning and forgetting. The investigators found that a single dopamine neuron can drive both the learning and forgetting process. The study was published in *Cell Reports*.

"We believe this system is set up to remove memories that are unimportant and not necessarily supposed to last a long time," says first author Jacob Berry, PhD, a postdoctoral associate in the Department of Neuroscience on Scripps Research's Florida campus. "I find it elegant that all of this is done with the same neuron. Our paper highlights exactly how this is achieved."

To study memory in flies, the insects are conditioned to associate a particular odor with an electric shock. Once they've been trained, scientists observe that they subsequently avoid that odor, which confirms that the memory has been made. By monitoring the activity of neurons in the brain before and after the conditioning process, scientists can get an inside look at the physiological underpinnings of memory formation.

In earlier work, the Scripps Research team showed that there are specific dopaminergic circuits that are involved in both the formation of memory and the removal of memories. In the current study, the investigators used imaging techniques to look at the process in more detail. They discovered that when a behavioral memory is degraded, the cellular changes made during the learning process are reversed by the same dopamine neuron that helped form the changes in the first place.

The researchers also found that when this dopamine neuron is recruited to form a new memory, it also works to degrade older memories. "Whenever you learn something new, you're simultaneously forming a new memory while potentially interfering with or erasing old ones," Berry says. "It's a very important balancing act that prevents you from becoming overloaded."

"For decades now, neuroscientists studying learning and memory have focused on how the brain acquires information and how that information is made to be stable memory, a process called memory consolidation," says first author Ron Davis, PhD, a professor and chair of the Department of Neuroscience at Scripps Research. "Only recently have neuroscientists grasped the importance of active forgetting and begun to unravel the processes that causes the brain to forget."

Berry adds that this learning-and-forgetting process helps to explain retroactive interference, a common observation in psychology. Retroactive interference describes the situation when more recent information gets in the way of trying to recall older information—for example, calling your former boss by your current boss's name.

Although the research was done in fruit flies, the investigators expect that the findings will apply to higher organisms, including humans. “Evolution worked out a lot of important processes like this pretty early on,” Berry says, “so there’s a lot of relevance to studying these synaptic pathways in simpler organisms.”

“The study led by Berry not only provides new insights into the brain mechanisms for active forgetting but offers a wonderful example of how much we learn about brain function from laboratory animals like the fruit fly, Drosophila,” Davis adds.

Understanding the processes of both remembering and forgetting—and potentially how to manipulate them—has a number of implications for humans. For conditions like drug addiction or post-traumatic stress disorder, it may be beneficial to develop approaches that can boost active forgetting. Improving memory retention, on the other hand, could help to treat dementia and other forms of memory loss.

In addition to Davis and Berry, Scripps Research postdoctoral associate Anna Phan contributed to the paper, *Dopamine Neurons Mediate Learning and Forgetting Through Bidirectional Modulation of a Memory Trace*. This study was supported by the National Institutes of Health (grants 4R37NS019904-33, 4R01NS052351-10, and 5R35NS097224-02).

Families Aid Discovery of a Sensory-processing Disorder Gene in Children with Autism Traits

November 26, 2018: JUPITER, FL— A concerned Texas mom finally has answers about the cause of her son’s sensory-processing problems thanks to a special collaboration among her patient-advocacy group and the lab of Scripps Research Neuroscientist Gavin Rumbaugh, PhD.

Monica Weldon of Cypress, Texas, says her son Beckett, a 10-year-old fraternal twin, has never experienced pain the way his sister, or other children, do.

“His pain threshold was so high,” Weldon says. While he had signs of autism, he had other symptoms that were not typical. “He walked around with a broken finger for four days and we didn’t know it was broken.”

Beckett has a genetic mutation that results in deactivation of a gene called *SYNGAP1*, which is critical to healthy brain development. Rumbaugh’s research, published in the journal *Nature Neuroscience*, for the first time links disordered touch and pain processing to *SYNGAP1* mutations. Rumbaugh and his co-authors note that children born with only one working copy of the gene are known to exhibit a wide range of symptoms, including autistic traits, epilepsy and intellectual disability. Analysis of a patient registry that Weldon helped assemble, plus a series of experiments in an animal model of the condition, led to the discoveries.

“We knew *SYNGAP1* was critically important for synapse plasticity,” or experience-driven changes in neural circuitry, says Rumbaugh, the study’s senior and corresponding author. “What we found is in addition to that, it also seems to regulate how many connections are made in the brain’s primary somatosensory cortex, in how we process touch-related sensory information. That was a surprising finding.”

It was surprising, in part, because previous research showed that *SYNGAP1* mutations cause hyper-sensitivity in the other senses. The processing of hearing and sight may be on overdrive, and yet they found that touch awareness and pain sensation barely register in somatosensory circuits.

Weldon says that paradox describes her son's challenges exactly. While he ignored dog bites or skin burns, the sights and sounds of a simple trip to the grocery store overwhelmed him.

"Nothing could relieve him of the over-stimulation," she says. "He was constantly screaming in the car. You couldn't go anywhere because of the screaming. We had ear plugs in the house."

It also may explain why it's challenging for doctors to prescribe medications to regulate the children's symptoms, Rumbaugh says.

Many different mutations can lead to inactivation of one copy of the SYNGAP1 gene, Rumbaugh says. Two inactivated copies of the gene are lethal in the laboratory, he adds. The next step will be developing therapies to restore the missing SYNGAP1 gene product, he adds. That work is underway at Scripps Research now. He's hopeful that catching the disorder and treating it early may make it possible to prevent some of the developmental and intellectual disabilities, or to avoid the seizures.

"We think that it's likely that the sensory deficits that these children experience contribute to their altered learning and thinking," he says. "If the who, what, where, and when information is coming in incorrectly, you would expect it is going to affect behavioral responses to touch, as well as thought processes that are generated from touch experiences."

For Monica Weldon, learning the source of her son's developmental challenges has been life-changing. A former science teacher, she shares authorship of the *Nature Neuroscience* paper. The patient advocacy group she founded, Bridge the Gap – SYNGAP – Education and Research Foundation, has spent years working with Rumbaugh's lab to build a scientifically valid patient registry, and develop and distribute questionnaires.

Weldon recalls the difficulty of learning why Beckett was so different from his twin. The identification of the SYNGAP1 gene as disease-causing wasn't identified until Beckett was 1 year old. She visited more than a dozen doctors trying to find a cause. She finally had to take out a loan to pay the $13,000 whole genome exome test that her doctor ultimately suggested. On learning of Beckett's status, she created the first SYNGAP1 Facebook page in the hopes of finding others. That group eventually grew into the non-profit. Beckett and his sister are 10 years old now. Nearly 200 families have joined the patient registry used for the study, and 48 different families uploaded detailed medical history data, including answering detailed questions about sensory function in their children. Of those, 45 of 48 described obvious sensory processing impairments, Rumbaugh says.

SYNGAP1 mutations are rare, but recent studies have estimated that tens of thousands of individuals globally may live with it, Rumbaugh says. Families learn of their child's *SYNGAP1* status only after pediatricians notice missed milestones and then order genetic tests, he adds.

Thanks to the internet and Bridge the Gap, families are finding each other and sharing such vital information. The new findings show how powerful these connections can become when families partner with scientists like those at Scripps Research, Weldon adds.

"The families educate the scientists on the specifics of how their children are affected, and they educate us on how the gene mutations impair brain function at the cellular and molecular level—it's a win-win for everybody," Weldon says.

"This open communication is getting us closer to understanding what this gene really does and possibly developing precision medicine approaches for treating these kids."

In addition to Rumbaugh and Weldon, authors of the study, "SYNGAP1 heterozygosity disrupts sensory processing by reducing touch-related activity within somatosensory cortex circuits,"include first authors Sheldon D. Michaelson and Emin D. Ozkan of Scripps Research. They also include Massimiliano Aceti, Sabyasachi Maity, Nerea Llamosas, Elisa Mizrachi, homas Vaissiere and Courtney A. Miller, of Scripps Research; Michael A. Gaffield and Jason M. Christie of the Max Planck Florida Institute for Neuroscience, and J. Lloyd Holder Jr. of the Baylor College of Medicine.

This work was supported in part by NIH grants from the National Institute of Mental Health (MH096847 and MH108408 and MH105400), the National Institute for Neurological Disorders and Stroke (NS064079 and NS083894), and the National Institute for Drug Abuse (DA034116 and DA036376). J.L.H. is supported by a National Institute for Neurological Disorders and Stroke Mentored Clinical Scientist Research Career Development Award (NS091381). The SYNGAP1 Natural History Study and Patient Registry is supported by a grant from The National Organization of Rare Disorders.

Memory Strength Dependent on Abundance of 'Package' Deliveries, Study Shows

December 3, 2018: JUPITER, FL – Neurons communicate with each other by exchanging gifts — sending molecules back and forth across the synapse in tiny packages called synaptic vesicles. A team from Scripps Research has shown that the number of these vesicles, not only the number of synapses, plays an important role in how neurons share information. Moreover, increases in the number of vesicles at specific types of synapses lead to better memory formation.

The research, conducted using fruit flies as model organisms, focused on dopamine neurons. It was published online this week in the journal *Neuron*.

"The aim of this project was to figure out the mechanistic changes that can lead to constraints in learning and memory," says first author Anna Phan, PhD, a postdoctoral researcher in the lab of senior author Ron Davis, PhD, a professor in the Department of Neuroscience at Scripps Research in Jupiter, Florida.

Unexpectedly, the team also found that having a good or poor memory may be determined early in life.

"These changes occur at a very specific time during development, but not in adulthood," Phan explains.

The researchers focused on a protein called stromalin. Earlier research by the Davis lab identified stromalin as one of several proteins that act as a memory suppressor. That is, it seems to constrain memory formation when working normally. If the ability to make stromalin is knocked out in the fruit flies, memory is enhanced, Davis says. But until now, how this protein functions to regulate memory formation was not known.

In the current study, the investigators used a number of laboratory tools, including high-resolution imaging techniques, to determine the alterations that lead to the suppression—and enhancement—of memory. After ruling out changes in the form of the neurons or in their numbers, they eventually determined that knocking out the gene for stromalin had one very striking effect: It increased the number of the neurotransmitter vesicles, or "packages," that transfer information from dopamine neurons onto their downstream partners.

"This strengthens the communication at the synapses," Phan says. "Our studies indicate that this is what leads to the memory enhancement that we see."

Stromalin has many other functions. These include helping chromosomes find their way during cell division and regulating the expression of genes. The protein has been evolutionarily conserved from yeast all the way to humans, Phan says. Understanding stromalin's normal role in suppressing learning and memory is important, she says.

There are good reasons why memory may need to be constrained.

"Unconstrained memory is like a car without brakes," Phan says. "If it's the only car on the road, maybe you can get away with it. But in reality, there are multiple other cars trying to get to where they're going. You can think of the cars like different kinds of neural signals, controlling a variety of critical functions in the body. Without a way to regulate the system, you end up with a big mess and mass confusion."

In fact, mutations in the stromalin gene can lead to seizures, hyperactivity, and autistic behaviors, in addition to other, non-neurological problems, Phan says. The next step will be identifying drugs that act on this type of synaptic signaling, she says.

Ultimately, the discovery may offer a new path for developing treatments for Parkinson's disease, which also results from defects in the dopamine neurons, as well as therapies to improve learning and memory, Davis says.

"Our studies open an entrée into studying the cell biology of the neuron that was previously unrecognized," Davis says. "Stromalin seems to be the first identified protein that controls the number of synaptic vesicles in neurons without altering the number of synapses between neurons. It shows that the number of synaptic vesicles that form is controlled independently from synapse number."

Other contributors to this paper, Stromalin Constrains Memory Acquisition by Developmentally Limiting Synaptic Vesicle Pool Size, were Scripps Research investigators Molee Chakraborty and Jacob Berry. Connon Thomas and Naomi Kamasawa of the Max Planck Florida Institute were also contributors.

This study was supported by the National Institutes of Health (grants 5R37NS019904, 2R01NS052351, and 1R35NS097224) and a Canadian Institutes of Health Research postdoctoral fellowship.

Parkinson's-linked Gene Product may be Druggable, Structural Study Finds

December 13, 2018:

JUPITER, FL – Opposites often attract in the world of romance, but they also attract inside the human body when receptors located on or in cells bind to other molecules, including medications. In fact, some cellular receptors have special "pockets" that only bind molecules with a specific shape to fit them—kind of like finding the right hand to fit a glove.

A new Scripps Research study finds that receptors for a Parkinson's-associated gene product have a pocket needed to bind to other molecules, opening the ability to more accurately probe its role in disease, and possibly develop a new class of therapies, according to research published recently in *CellPress.* The gene, called Nurr1, may enable certain neurons to create the neurotransmitter dopamine, in addition to helping them survive various toxic stressors. Loss of dopamine is a cause of the tremors and rigidity associated with Parkinson's.

This research is the first to identify that Nurr1 receptors, which were previously thought to lack binding pockets for ligands, or binding molecules, actually do have pockets that make them potentially druggable, says Douglas Kojetin, PhD, an Associate Professor in the Department of Integrative and Structural Computational Biology and the Department of Molecular Medicine at Scripps Research.

"Our study showed that the pocket can actually open and close, and when an unsaturated fatty acid binds, such as DHA, it can actually induce a further opening of the pocket," Kojetin says. "We found that there are natural ligands that bind to the Nurr1 receptor, and that Nurr1 binds natural and synthetic drug-like ligands that way other receptors do."

To get a better idea of how the Nurr1 receptor responds to different binding molecules, Kojetin and his colleagues used several highly sophisticated technologies including nuclear magnetic resonance spectroscopy, which, similar to MRI instruments, uses high-field magnets to study the receptor on the atomic level, along with mass spectrometry and computer simulations to help them better observe the Nurr1 receptor's behavior when exposed to different solutions. They compared "before-and-after" images of the receptor and found the Nurr1 receptor changed shapes in altered environments.

"These results may surprise some people because many people in the pharmaceutical industry previously thought that Nurr1 could not bind a ligand in the same way other receptors do, but our study is proof-of-concept that the Nurr1 receptor binds ligands," Kojetin says. "The next step is to determine if these Nurr1-binding molecules can be used to target Parkinson's disease."

Additional authors of the study, "Defining a Canonical Ligand-Binding Pocket in the Orphan Nuclear Receptor Nurr1," were Ian Mitchelle S. De Vera, Paola Muñoz-Tello, Jinsai Shang, Travis Hughes, Pankaj Kumar Giri, Patrick Griffin, Edna Matta-Camacho,Jie Zheng, Venkatasubramanian Dharmarjan, and David Marciano of Scripps Research, along with Mark Rance from the Department of Molecular Genetics at the University of Cincinnati.

This research was made possible by the National Institutes of Health (NIH) grants R01GM114420 and K99DK103116; the National Science Foundation (NSF) funding to the Summer Undergraduate Research Fellows (SURF) program at Scripps Research in Florida (1659594); and the Academic Year Research Internship for Undergraduates (AYRIU) program at Scripps Research; A portion of this work was performed at the National High Magnetic Field Laboratory (NHMFL/MagLab), which is supported by the National Science Foundation (NSF; DMR-1157490).

Once-promising Diabetes Drugs' Side-effects can be Limited, Study Finds

December 13, 2018: JUPITER, FL – A group of diabetes drugs that have fallen out of favor due to side-effects may merit renewed attention after scientists at Scripps Research discovered a way to capitalize on the insulin-sensitizing talent of the drug receptor while limiting its ability to cause weight gain, brittle bones and other impacts.

The class of diabetes drugs called thiazolidinediones (TZDs) have proven highly effective in helping to manage blood sugar, but long-term use comes with the unacceptable consequences of increased risk for heart failure, brittle bones and weight gain, says structural biologist Douglas Kojetin, PhD, an associate professor at Scripps Research.

In a recent study published in *Nature Communications*, Kojetin and colleagues, including Scripps Research scientists Patrick Griffin, PhD, and Theodore Kamenecka, PhD, describe a method for keeping the drugs' therapeutic response and limiting the undesirable side effects by altering the activity of the receptor—essentially changing how the lock and key connect.

The drugs, known as TZDs, work by binding to a type of protein that can act as a switch for gene expression, called nuclear receptors. The specific receptor for TZDs, peroxisome proliferator-activated receptor gamma (PPARg), when activated by the drug, initiates a series of steps that fight high blood sugar. Unfortunately, if activated in bone, it can also stimulate the development of fat cells. That also causes bones to become brittle and boosts weight gain.

In their paper, Kojetin and colleagues lay out the steps by which PPARg receptors can be targeted to produce therapeutic benefits without degrading bone tissue. Specifically, PPARg activity can be modulated with specially designed molecules called "inverse agonists" that repress, or inactivate, the unwanted biological activities.

"There have been studies that show that repressive drug-like molecules allow bone formation to occur while maintaining diabetic efficacy," says Kojetin. "Our study is probably one of the first to give the molecular blueprint on how to design a new class of inactivating drug-like molecules that repress PPARg activity."

The researchers identified a compound that enabled the PPARg receptor to shift between two different shapes. One of these conformations enabled an opposite effect compared to anti-diabetic TZD drugs.

More studies are needed to investigate how these findings may influence the development of new anti-diabetic drugs with a bone-protective benefit, Kojetin says.

"Beyond diabetes, repressive molecules have been implicated as potential therapies in cancer and the differentiation of stem cells from cord blood," says Kojetin. "We simply don't know all the potential benefits of repressive PPARγ drug-like molecules because very few are available, and they have not been studied as extensively as the TZDs."

The authors of the study, "A structural mechanism for directing corepressor-selective inverse agonism of PPARg" were Richard Brust, Jinsai Shang, Jakob Fuhrmann, Sarah Mosure, Jared Bass, Andrew Cano, Anne-Laure Blayo, Patrick Griffin, and Theodore Kamenecka at Scripps Research in Jupiter, FL; Zahra Heidari, Ian M. Chrisman, Michelle Nemetchek, and Travis Hughes at the University of Montana in Missoula.

This research was made possible by the National Institutes of Health (NIH) grants R01DK101871, F32DK108442, R01DK105825, R00DK103116, and P20GM103546; and an American Heart Association (AHA) fellowship award (16POST27780018). A portion of this work was performed at the National High Magnetic Field Laboratory (NHMFL/MagLab), which is supported by the National Science Foundation (NSF; DMR-1157490).

Parkinson's-linked Gene Product May be Druggable, Structural Study Finds

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in *CellPress.* The gene, called Nurr1, may enable certain neurons to create the neurotransmitter dopamine, in addition to helping them survive various toxic stressors. Loss of dopamine is a cause of the tremors and rigidity associated with Parkinson's.

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To get a better idea of how the Nurr1 receptor responds to different binding molecules, Kojetin and his colleagues used several highly sophisticated technologies including nuclear magnetic resonance spectroscopy, which, similar to MRI instruments, uses high-field magnets to study the receptor on the atomic level, along with mass spectrometry and computer simulations to help them better observe the Nurr1 receptor's behavior when exposed to different solutions. They compared "before-and-after" images of the receptor and found the Nurr1 receptor changed shapes in altered environments.

"These results may surprise some people because many people in the pharmaceutical industry previously thought that Nurr1 could not bind a ligand in the same way other receptors do, but our study is proof-of-concept that the Nurr1 receptor binds ligands," Kojetin says. "The next step is to determine if these Nurr1-binding molecules can be used to target Parkinson's disease."

Additional authors of the study, "Defining a Canonical Ligand-Binding Pocket in the Orphan Nuclear Receptor Nurr1," were Ian Mitchelle S. De Vera, Paola Muñoz-Tello, Jinsai Shang, Travis Hughes, Pankaj Kumar Giri, Patrick Griffin, Edna Matta-Camacho,Jie Zheng, Venkatasubramanian Dharmarjan, and David Marciano of Scripps Research, along with Mark Rance from the Department of Molecular Genetics at the University of Cincinnati.

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Genetic Cause of ALS and Frontotemporal Dementia Blocked by RNA-binding Compound

December 17, 2018: JUPITER, FL – Since the ice bucket challenge went viral in 2014, raising awareness and funding for ALS research, scientists have learned much about a disease that disconnects muscles from nerves, leading to muscle atrophy and eventual death. Their ultimate goal is to create medications capable of stopping ALS in its tracks.

Writing in the journal *Cell Chemical Biology*, Scripps Research chemist Matthew Disney, PhD, and colleagues describe a new compound that blocks the most common genetic cause of both familial ALS

and frontotemporal dementia. Disney's group is assessing its potential to become a drug to treat both these diseases.

"Hopefully, this will be an accelerant not only for us but for all people in the field working toward a treatment for ALS," Disney says.

The compound works differently than most drugs on the market. Rather than binding with the toxic protein behind the disease, it binds with what's involved in making the protein, a specific form of RNA folded over like a hairpin. Since RNA molecules manage the expression of genes, intervening at the RNA level goes right to the apparent cause of that form of the disease, Disney says.

"There are zero therapies that address the root cause of this disease," Disney says. "Zero. Our goal is not to target the symptoms, it is to target the root cause, which is that RNA."

ALS, short for amyotrophic lateral sclerosis, is also known as Lou Gehrig's disease in honor of the late baseball legend. An estimated 20,000 people in the United States currently live with the neurodegenerative disorder, including former professional football player Tim Green, who recently announced his illness on *60 Minutes*.

There are likely multiple causes of ALS. Green's is a sporadic form. Meanwhile, for an estimated 1 in 10 people with ALS, it runs in the family. Around a third of them appear to have inherited the type of DNA damage that Disney's compound targets.

The damaged DNA lies in a non-coding section of the 9th chromosome. There, a stutter-like repeat of letters GGGGCC prompt the cell's protein building machinery to start production of a toxic substance, C9RAN. Disney credits his collaborator, Leonard Petrucelli of the Mayo Clinic in Jacksonville, FL, with co-discovery of the cause. That toxic substance appears to disrupt the nerve cell's normal metabolism, leading to its death, Disney says.

It is the death of the nerve connectors between muscles and the brain that leads to ALS symptoms of muscle atrophy, weakness, difficulty swallowing and breathing. In frontotemporal dementia, the toxic protein appears to be a cause of neuron death in parts of the brain that control behavior and personality, the frontal and temporal lobes. Disney's compound, a small molecule he designed, is simply referred to as "4," in the paper. It interferes with production of the toxic C9RAN protein, he says.

One important finding of the paper is that the form of the RNA being targeted by many research groups at present may not actually be the one driving neuron death, Disney says.

"There are two different shapes of the protein. The data supports that the shape people have not been looking at is actually the toxic one," he says.

Many questions must be answered before it's clear that the compound "4" could serve as a drug. The research is ongoing, Disney adds.

"We have a long and winding road to make this into a drug. You have to not only show that a molecule works, but that it is safe," he says. "Now that we have a target and we know how to bind it, this should accelerate making compounds that could become drugs in a much more streamlined way."

In addition to Disney and Petrucelli, the authors of the paper, "The Hairpin Form of r(G4C2)exp n c9ALS/FTD is Repeat-associated non-ATG Translated and a Target for Bioactive Small Molecules," are Zi-Fu Wang, Andrei Ursu, Jessica L. Childs-Disney, Rea Guertler, Wang-Yong Yang, Viachaslau Bernat, Suzanne G. Rzuczek, Rita Fuerst and Brendan G. Dwyer of Scripps Research; and Yong-Jie Zhang and Tania F. Gendron of the Mayo Clinic. Also, Ilyas Yildirim of Florida Atlantic University, and Joseph E. Rice of Rutgers University contributed.

This work was funded by the NIH (DP1 NS096898-02; P01 NS099114-01; R35NS097273, R21NS084528, P01NS084974, R01NS088689, and R01NS093865), Department of Defense (ALSRP AL130125; Mayo Clinic Foundation; Robert Packard Center for ALS Research at Johns Hopkins; Target ALS; Association for Frontotemporal Degeneration; Muscular Dystrophy Association [no. 416137]; Amyotrophic Lateral Sclerosis Association [ALSA; 15-TALS-297], Alzheimer's Drug Discovery Foundation [ID: 2014120] and Target ALS). The Deutsche Forschungsgemeinschaft Postdoctoral Fellowship also provided support, along with gifts from Marilyn and Jay Nelson.

Proofreading Mistakes Drive Autoimmune Disease Involving Key Protein, Scientists Find

December 18, 2018: JUPITER, FL – A team from Scripps Research has found a molecular cause of a group of rare autoimmune disorders in which the immune system attacks the body's own healthy cells.

The discovery, published Dec. 18 in *Nature Communications*, improves understanding of a protein's role in several autoimmune disorders, including Singleton–Merten syndrome (SMS), Aicardi-Goutières syndrome, familial chilblain lupus, proteasome associated autoinflammatory syndromes and many others which involve improper stimulation of interferon, says Patrick Griffin, PhD, professor and co-chair of the Department of Molecular Medicine at Scripps Research's Florida campus.

Interferon is a key component of our frontline defense against pathogens. Interferon earned its name because it literally interferes with virus' ability to make copies of themselves. The immune system relies on a gene called RIG-I, short for retinoic acid inducible gene-I, to signal for the release of interferon whenever certain viral markers are encountered. RIG-I has to determine if the markers are of foreign origin or are from its own body. The scientists demonstrated precisely how mistakes in a molecular proofreading system can lead to confusion and generate out-of-control interferon signaling, setting off development of autoimmune disease.

"This dysregulated molecular mechanism of RIG-I mediated RNA proofreading that we identified may help us understand and treat SMS and other autoimmune disorders," says Jie Zheng, PhD, a postdoctoral associate and the first author and co-corresponding author of the study.

The National Institutes of Health estimates more than 20 million Americans suffer from autoimmune disorders. They include rheumatoid arthritis, psoriasis, inflammatory bowel disease, multiple sclerosis, lupus, type 1 diabetes, and dozens of others. There are very few safe and effective treatments for such disorders, largely because so little is understood about how they arise and are sustained.

That is true for SMS, which is so rare that only a few cases have been described in the medical literature. Patients develop serious bone, heart, muscle and skin problems starting in early childhood, largely due to chronic inflammation from an overactive immune system. The scientists' aim was to understand how two RIG-I mutations linked to SMS end up triggering the autoimmunity.

Most viruses have genes made of ribonucleic acid, or RNA, a close chemical cousin of DNA. RIG-I works as an early-warning detector of viral RNA, capable of triggering a broad antiviral immune response, including interferon release. The scientists showed that mutations in RIG-I cause the sensor protein to activate even when it encounters non-viral, "self" RNA. The aim of the study was to discover the molecular details of how this happens.

RIG-I is a big protein with flexible elements, and thus is hard to study with standard techniques. But Griffin has helped pioneer the use of an advanced technology called hydrogen-deuterium exchange mass spectrometry (HDX-MS), which enables scientists to analyze the structures and dynamics of just such proteins. For the study, he and his team applied HDX-MS to normal and mutant RIG-I, and essentially solved the mystery of how these mutations cause a failure of discrimination between self and viral RNA.

Scientists have known that RIG-I has a particular segment that it keeps mostly covered and concealed. When RIG-I encounters and recognizes viral RNA, this segment is supposed to briefly swing open and thus become available for binding to another protein called MAVS, an event that triggers the immune response. Griffin and colleagues found that the two SMS-linked mutations, in subtly different ways, cause this key segment of RIG-I to become stuck open—making it much more likely to bind to MAVS and trigger an immune response.

The scientists now are using their data to try to find a way to target mutant RIG-I, to block its inappropriate signaling to MAVS and thus alleviate the autoimmunity it causes.

This new, detailed understanding of RIG-I's dysfunction may not only provide insights into the origins of more common autoimmune disorders, Griffin says, it clarifies how RIG-I works normally to detect viruses, a discovery that may enable development of new antiviral drugs.

The authors of the study, "HDX-MS reveals dysregulated checkpoints that compromise discrimination against self RNA during RIG-I mediated autoimmunity," were Jie Zheng, Mi Ra Chang, Gogce Crynen, Ruben Garcia-Ordonez, Bruce Pascal, Scott Novick, and Patrick Griffin at Scripps Research in Jupiter, Fla.; Chen Wang and Joseph Marcotrigiano at NIH; and Swapnil Devarkar, Brandon Schweibenz and Smita Patel at Rutgers Robert Wood Johnson Medical School at Rutgers University.

This research was made possible by NIH grants GM111959 and GM103368, and by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases.

Brain Cell Communications Fine-tuned through Multiple Couriers, Study Finds

December 19, 2018: JUPITER, FL – No brain cell is an island, you could say. Healthy neurons must communicate with their neighbors, and they rely on proteins called kinesins to do it.

In a new study published recently in *Scientific Reports,* scientists on the Florida campus of Scripps Research investigated the roles of different members of the kinesin protein family. Their research expands fundamental understanding of how neurons communicate. Among their findings is the discovery that individual neurons can have several kinesins in motion at the same time, which may give them a way to fine-tune communications.

"It is very, very important to study kinesins because of their role in early brain development and learning—and in neurodegenerative disorders such as ALS," says Sathyanarayanan Puthanveettil,

PhD, an associate professor at Scripps Research and senior author of the new study. “This study helps us understand the complex ways by which genes and proteins regulate communication between neurons.”

It's helpful to picture kinesins like trucks on a highway. The proteins move along cellular structures called microtubules as they transport proteins, RNAs and other gene products out of the center of a cell. Scientists knew this process was important for communication between neurons, called synaptic transmission, but the details were unclear.

Puthanveettil and his colleagues also wondered why around 40 different kinesins functioned in neurons. “No one had really studied their contributions in an elaborate way,” says Puthanveettil.

Using techniques in electrophysiology and fluorescence imaging, the scientists studied 18 representatives of the kinesin family in mice. They focused on how these proteins worked in neurons in a region of the brain called the hippocampus, which is critical in learning and memory. Right away, the scientists were surprised to find that seven of the kinesins had no critical role in neuronal communication. The other 11 kinesins did affect the amplitude or frequency of communications, called synaptic transmissions.

The scientists thought these kinesins likely helped with cellular communication, so they were surprised to find that three of the kinesins actually inhibited synaptic transmission.

Then came something likewise unexpected—the scientists found that at least three types of kinesins, including ones that inhibit communication, can be expressed in the same individual neurons. “This means neuronal communication is much more complex than we thought,” Puthanveettil says. “How do neurons incorporate these different kinesins? How are they controlled?”

One theory is that neurons may use the different kinesins to fine-tune their communications.

Next came a deep dive into the workings of a kinesin called Kif11. This was one of the odd kinesins that inhibited communications. The scientists found that reducing Kif11 levels in neurons allowed certain receptors to better help send signals. This was the first time scientists had shown part of the mechanism that kinesins use to inhibit communications.

“Because we do not know about mechanisms by which any Kif act as an inhibitory constraint of synaptic transmission, determining mechanisms driving Kif11 will be immensely helpful for understanding how neurons will be able to use Kif11 for calibrating synaptic function,” says Supriya Swankar, PhD, a postdoctoral associate at Scripps Research and study co-first author.

Puthanveettil says more work needs to be done to understand the mechanisms that drive kinesins. In the end, the goal is to understand the basic workings of the brain to shed light on diseases where communication goes wrong.

“The most important message to takeaway is that there are important molecules in the brain that regulate neuronal communication differently, and that their regulation is important for a healthy brain,” says Yosef Avchalumov, PhD, co-first author of the study and former research associate at Scripps Research. “The next crucial step in understanding the role of these molecules would be to study them in disease states, such as in Alzheimer's, Parkinson's and some other neurological disorders.”

Additional authors of the study, "Kinesin Family of Proteins Kif11 and Kif21B Act as Inhibitory Constraints of Excitatory Synaptic Transmission Through Distinct Mechanisms," were, Bindu L. Raveendra and Eddie Grinman of Scripps Research.

The research was supported by the National Institutes of Health (grants 5R01MH094607-05 and 5R21MH108929-02), the National Science Foundation (award number 1453799) and a training grant in Alzheimer's drug discovery from the Lottie French Lewis Fund of the Community Foundation for Palm Beach and Martin Counties, Florida.

Scientists Uncover How Protein Clumps Damage Cells in Parkinson's

December 20, 2018: JUPITER, FL – Biologists studying Parkinson's disease have long hoped to solve the mystery of the telltale "clumps." Scientists want to know how clumps of misfolded proteins damage brain cells and contribute to the disease.

Corinne Lasmézas, PhD, and her Scripps Research colleagues have now cracked the case of pα-syn*, a protein clump that is particularly toxic to the cells. Their recent study in the journal *Neurobiology of Disease* shows that pα-syn* causes damage by recruiting certain enzymes and an accomplice to damage cells. The accomplice is a protein called tau.

"We really felt like detectives in this study," says Lasmézas, a professor on the Florida campus of Scripps Research. "We hope that this research into the root cause of Parkinson's will bring us closer to finding a disease-modifying treatment."

Parkinson's disease is the most common neurodegenerative disease after Alzheimer's. The disease strikes when the brain starts losing the cells that produce dopamine, a critical neurotransmitter. Parkinson's can have many causes, from genetics to environmental factors, but a protein called α-synuclein (α-syn in short) is found to turn bad and form clumps in every case. The Lasmézas lab has discovered that a particular type of α-syn clumps, or "aggregates," that they called pα-syn*, starts to show up around cellular structures called mitochondria. This is a big problem for cells, which need mitochondria to produce their energy.

"We can see the mitochondria break into fragments in these cells," says Lasmézas. "We wanted to understand the mechanism behind this."

The investigation relied on a combination of cultured neurons and a mouse model of Parkinson's, as well as analysis of donated brain tissues from deceased Parkinson's disease patients.

The new research shows that pα-syn* hurts mitochondria by starting a cascade of events. First, pα-syn* activates a pathway in cells called the MAPK pathway. Enzymes of the MAPK pathway then modify the protein tau. This was a fascinating finding, since tau has long puzzled neuroscientists. Tau is known to form tangles inside neurons in the brains of Parkinson's disease patients. But scientists did not know how they got there or what they were doing.

Lasmézas and her team found that enzymes of the MAPK pathway modify tau through a process called phosphorylation. This version of tau then clumps together with pα-syn* on the mitochondrial membrane. The two protein aggregates grow bigger and bigger, destroying the mitochondria in the process.

At last, the researchers knew what pα-syn* was doing and how it hurt cells. “We’ve shown how pα-syn* works as the main trigger in mitotoxicity,” says Lasmézas.

Study first author Diego Grassi, PhD, a research associate at Scripps Research at the time of the study, stresses the importance of discovering tau’s role in destroying mitochondria. Scientists know tau is involved in Alzheimer’s disease, so this study suggests a mechanism behind how Alzheimer’s and Parkinson’s overlap at the molecular level. The presence of α-syn and tau aggregates is also a telltale sign of other forms of dementia, and now scientists know how this might occur.

“This is also important for its possible implications in other neurodegenerative disorders,” says Grassi.

Lasmézas and Grassi say the next step in this research is to study how to stop pα-syn*, with the ultimate goal of treating Parkinson’s disease.

“I know we are doing something that could make a meaningful difference in the quality of life of people affected by this condition,” says Grassi. “I can hardly imagine a place better than Scripps Research to perform this kind of translational activity."

Additional authors of the study, “Pα-syn mitotoxicity is linked to MAPK activation and involves tau phosphorylation and aggregation at the mitochondria,” were Natalia Diaz-Perez of Palm Beach Gardens and Laura A. Volpicelli-Daley of the University of Alabama at Birmingham.*

The research was supported by The Sandy Hill Foundation and by a generous gift from Dr. David P. Brown.

Drunken Flies Reveal Important Step to Intoxication

December 21, 2018: JUPITER, FL - As New Year’s Eve approaches, many people will experience the familiar buzz that comes from imbibing a favorite cocktail or glass of wine.

A new study from Scripps Research, published in the *Journal of Molecular Biology*, reveals a twist in how intoxication happens. When the alcohol from our cocktail reaches our nerve cells, the alcohol apparently employs intermediary molecules on the membrane surface of the neuron to produce the intoxicating effect, indirectly.

It turns out that both flies and mammals can get drunk on alcohol. So, for their study, Scott Hansen, PhD, associate professor in the Department of Molecular Medicine, and his team, enabled fruit flies to become inebriated to track ethanol’s path. The fly is a useful model to study gene activity because its genome is smaller than other animals and is easily manipulated, Hansen says.

“They act just like people,” Hansen says about the flies. “They start losing coordination. They literally get drunk.”

The alcohol in beverages acts much like an anesthetic. It creates a hyper “buzzed” feeling first, and then sedation, Hansen explains. But how? It turns out there is an important intermediate step that wasn’t previously known.

The scientists looked to a system they have seen at play in anesthesia to track alcohol’s effects, starting with an enzyme on nerve cell membranes called phospholipase D2, (PLD2). The enzyme links ethanol

molecules to lipid (fat) in the membrane of the nerve cell. They found the enzyme becomes a catalyst triggering multiple downstream activities within the cell. It creates a fatty alcohol metabolite called phosphatidylethanol (PEtOH). That metabolite builds up and causes nerves to fire more easily, resulting in more hyperactive flies.

“With hyperactivity you see the flies run around more, and this is what we equate to being buzzed,” Hansen says. When the scientists knocked out the gene for the enzyme that makes the PEtOH metabolite, thus eliminating the signal, the flies did not become more active.

This is the first time this pathway has been identified as a determinant of alcohol sensitivity, Hansen says. It remains to be seen whether the metabolite is involved in the full sedation experienced by the flies after the initial buzz and how this pathway may play a role in the hangover that many people experience later on. Hansen says that his current research is addressing these questions.

Knowing alcohol’s molecular targets could enable development of an antidote to intoxication, or even hangover, Hansen says.

“The fatty alcohol is known to linger in the brain for more than 16 hours making it a likely target,” Hansen says. “Also, understanding this pathway could give insight as to why people use alcohol for pain management.”

“It has definitely led to some different ways of thinking about alcohol intoxication at the molecular level,” Hansen says. “Most scientists thought alcohol had a direct effect. Blocking the enzyme in flies shows that’s not likely true.”

The lead authors on the study, “A Molecular Target for an Alcohol Chain Length Cutoff” were Hae-Won Chung and E. Nicholas Petersen with additional authors Cerrone Cabanos, William W. Ja, and Mahmud Arif Pavel at Scripps Research; Keith R. Murphy affiliated with Scripps Research and Florida Atlantic University; and Andrew S. Hansen with the HB Biotech, BioInnovations Gateway in Salt Lake City, Utah.

This work was supported by the National Institutes of Health’s Director's New Innovator Award 1DP2NS087943-01 as well as R01AG045036, and a graduate fellowship from the Joseph B. Scheller and Rita P. Scheller Charitable Foundation.

Scientists Discover Link in Brain Between Anxiety and Weight Loss

January 17, 2019: JUPITER, FL – Scripps Research scientists have published a study revealing a shared mechanism for both anxiety and weight loss. Their research, published in the journal *Cell Metabolism*, describes a key molecule that triggers anxiety in the brain, while also increasing metabolism and fat burning.

"We’ve found a relationship between anxiety and weight loss,” says Baoji Xu, PhD, professor on the Florida campus of Scripps Research and senior author of the study. “This research could guide new therapies for anxiety and help researchers design treatments for obesity.”

Anxiety disorders are the most common types of mental health disorders in the world. Along with the psychological effects, many people have noticed that weight changes accompany periods of anxiety and stress.

Xu, a long-time obesity researcher, noticed the same phenomenon in a group of mice engineered to lack a molecule called brain-derived neurotrophic factor (BDNF). These mice showed anxiety-like symptoms and stayed lean.

“Even on a high-fat diet, these mice were really lean,” says Xu. “Could the same thing be happening in humans?”

Answering that question required a study of how BDNF works. Normally scientists simply turn off a gene to find out what it does. There was a challenge with BDNF, though: Previous work had shown it is mandatory for brain development, learning and memory. They needed a mouse model that had normal BDNF in some areas of the brain, but not the areas they wanted to study.

When they deleted the BDNF gene only in the brain’s cortex, hippocampus and amygdala, their model worked as expected. The mice developed anxiety-like symptoms and that same tendency to stay lean.

With the new model, the researchers discovered that the lack of BDNF meant they could not dampen busy “excitatory” signaling in those brain circuits. They could not take advantage of an important neurotransmitter called GABA, a molecule that normally slows signaling in the brain and promotes relaxation.

Next, the researchers studied how a lack of BDNF kept the mice lean. They found that these anxious mice had an elevated basal metabolic rate, the rate of energy expended to keep the body functioning. In addition, these mice produced more brown fat—a kind of fat that releases more energy and leads to faster weight loss.

Interestingly, the researchers discovered that deleting BDNF just in the amygdala, a brain region that plays a primary role in anxiety, could increase energy expenditure in mice.

The researchers had found a molecule—and a brain region—that link anxiety and weight loss. Xu and his team are now considering how to apply this work to help patients. No one would ever want to trigger anxiety in humans, Xu says. But it may be possible to harness this knowledge to develop obesity therapies able to target just the parts of the pathway involved in energy expenditure, Xu says.

“In this way, we could help obese people lose weight,” says Xu.

He also hopes to further study the neurons that BDNF targets to relieve anxiety. This knowledge could be useful to design additional therapies for people with anxiety disorders.

Xu says the environment at Scripps Research is a great place to try to answer these basic questions about the brain. “At Scripps Research, we have the freedom to pursue any research direction we think is important,” says Xu.

Additional authors of the study, “Activation of Anxiogenic Circuits Instigates Resistance to Diet-Induced Obesity via Increased Energy Expenditure,” were Xiangyang Xie (first author), Juan Ji An, Jessica Houtz, Ji-Wei Tan, Haifei Xu, Guey-Ying Liao and Zhi-Xiang Xu of Scripps Research; and Haili Yang, previously at Scripps Research and now at Southwest University, Chong Qing.

The study was supported by the National Institutes of Health (grants R01 DK103335, R01 DK105954 and P40 RR018604) and a Training Grant in Alzheimer's Drug Discovery from the Lottie French Lewis Fund of the Community Foundation for Palm Beach and Martin Counties.

Sea Slug Study Illuminates How Mitochondria Move within Cells

January 16, 2019: JUPITER, FL – Your cells have an amazing ability—they can build their own energy factories, called mitochondria. Once built, mitochondria must move where needed in the cell. Defects in mitochondrial transport are a suspected cause of diseases including Alzheimer's, ALS, Huntington's and Parkinson's.

Scientists at Scripps Research have discovered how neurons manage this important process by studying cells from Aplysia californica, a type of sea slug used in neuroscience research. The paper, published this week as the cover story in the journal *Cell Reports*, may help open the door to new therapies to improve mitochondrial transport, says neuroscientist Sathyanarayanan Puthanveettil, PhD, an associate professor at Scripps Research and senior author of the new study.

"We are very interested in looking at this process in neurodegenerative diseases," Puthanveettil says. "If you can find potential drugs that can manipulate transport, that might be beneficial."

Puthanveettil's team set up the sea slug neurons to grow in dishes. Some neurons grew alone, and others grew alongside partners. In neuroscience lingo, the cell that sends a signal is called "pre-synaptic" and the cell receiving the signal is "post-synaptic." Because the slugs' brain circuitry is simple, and their cells are larger, they are a useful model organism to study, Puthanveettil explains.

Neurons have elongated projections called axons that allow messages from the pre-synaptic neurons to reach the post-synaptic neurons. At the end of each axon is a busy chamber called a synapse that transmits the message from one to the other. This system requires a great deal of energy to function, so cells transport mitochondria toward their synapses to provide that energy, Puthanveettil says. Older mitochondria move back to the cell body for recycling.

Puthanveettil, first author Kerriann Badal and their colleagues wanted to uncover the mechanism in cells that starts the transport process. To do this, the team monitored mitochondrial transport as they tried activating different signaling pathways. The experiments led the researchers to pinpoint a signaling pathway called cAMP as a major player. Once a neuron has grown a synapse, cAMP is activated and appears to step in to enhance mitochondrial transport.

Significantly, the team found that the pre-synaptic neuron alters expression of around 4,000 genes (possibly around 20 percent of the genes it has) as it makes new mitochondria.

"The pre-synaptic neuron's identity is almost completely changed," Puthanveettil says.

This identity shift appears persistent, too—the cell doesn't just make mitochondria in a quick burst. Instead, protein synthesis permanently changes to support the building and transport of new mitochondria. This supports the previous finding that a massive amount of energy is needed to maintain pre-synaptic function and keep the cell communicating with its neighbors.

"We've discovered a fundamental mechanism responsible for higher brain function," says Puthanveettil.

Puthanveettil says this discovery was surprising for two reasons: First, the realization that mitochondrial transport increased after the synapse was built, not before. Both processes require a lot of energy, so it was interesting to discover that maintaining the synapse seems to require more energy than the initial building process.

Also, the researchers did not expect to see the production of so many new mitochondria. Many people had assumed that enhancing transport would simply jump-start the movement of the many mitochondria that tend to stall along transport lines.

Puthanveettil says future studies could look at how to design a drug therapy to enhance transport in diseases where transport is defective. He is also studying how mitochondrial transport changes in response to learning and memory formation.

Additional authors of the study, " Synapse Formation Activates a Transcriptional Program for Persistent Enhancement in the Bi-directional Transport of Mitochondria," were Komol Akhmedov, Adrian Reich, Mohammad Fallahi-Sichani, Supriya Swarnkar of Scripps Research; Phillip Lamoureux and Kyle E. Miller of Michigan State University; and Xin-An Liu, previously at Scripps Research, now at Icahn School of Medicine at Mount Sinai.

This study was supported by the National Institutes of Health (grants 5R01MH094607-05 and 5R21MH108929-02) and the National Science Foundation (award no. 1453799).

Synapse Maintenance Depends on Network within Neurons, Study Finds

February 4, 2019: JUPITER, FL — Like our cars, our synapses require maintenance to run smoothly. While the scientific community has spent great time and effort learning how synapses drive linkages among our billions of nerve cells, comparatively little time has been spent discerning the process used to keep those connections humming once established.

New research from the lab of Brock Grill, PhD, at Scripps Research in Jupiter, Florida, reveals a role in synapse maintenance for a key protein. Called ATAT-2, short for alpha-tubulin N-acetyltransferase 2, the protein helps maintain the neuron's connections after they are assembled, explains Grill, associate professor in the Scripps Research Department of Neuroscience. Writing in the journal *eLife*, Grill and first author Melissa Borgen reveal ATAT-2's working relationship with another protein, called RPM-1, which serves as hub for a network that includes other molecules, such as PTRN-1 and DLK-1.

Understanding this genetic network may prove important to understanding multiple brain diseases, Grill says.

"We're putting together a missing piece of the puzzle here," he says. "Increasing evidence indicates synapse instability is a hallmark of many neurodegenerative diseases, including Alzheimer's disease."

So what does ATAT-2 do within nerve cells? ATAT-2 modifies molecular signatures on microtubules, which serve as the chassis of cells by providing structure. Microtubules also serve as a conduit for molecules and organelles that need to move to synapses, such as synaptic vesicles, Grill says. Without proper microtubule function it stands to reason that synapses could crash. In a series of studies involving the model organism *C. elegans*, Grill's team found that ATAT-2 functions with RPM-1 to modify and stabilize synapses made by mechanosensory neurons – to protect the cell's chassis from

rusting, so to speak. Without ATAT-2 and this genetic network, not only are synapses lost, but entire nerve cell extensions called axons degenerate as well, Grill notes.

Many questions remain to be studied, Grill notes. For example, the regulators of synapse maintenance in developing neurons as opposed to mature neurons appears to be somewhat different. Also, the order of protein activities needed to stabilize synapses needs further study. Learning these answers are important for adding to basic scientific understanding of nervous system biology, and could have important therapeutic implications, Grill explains.

In addition to Grill and Borgen, the authors of the study, "Synapse maintenance is impacted by ATAT-2 tubulin acetyltransferase activity and the RPM-1 signalling hub," include Andrew Giles and Dandan Wang, all of Scripps Research.

The research was made possible by a grant from the National Institute of Neurological Disorders and Stroke and support from the Esther B. O'Keeffe Charitable Foundation.

Breast Cancer Cells Shifted into HER2 Positive Status with Bold New Strategy

February 6, 2019: JUPITER, FL — When women or men receive the worrisome diagnosis of breast cancer, that news comes with an important piece of information, namely, whether their cancer is HER2-positive or HER2-negative. It can be especially difficult to hear that one's cancer is HER2-negative, because it means an effective group of targeted anti-cancer drugs isn't available.

A new study from the lab of Scripps Research chemist Matthew D. Disney, PhD, suggests that in the future, that might not be the final word on the matter. Writing in the *Journal of the American Chemical Society*, Disney's group describes shifting three different cancer cell lines from HER2-negative status to HER2-positive status with the addition of a selective micro-RNA binding molecule they referred to as TGP-515.

It's a revolutionary idea, that a cancer's genotype might not have to be the limiting factor in its range of targeted treatment options. However, this is just a first step in a long series of work ahead to enable the technology to benefit cancer patients, Disney notes.

The team designed their compound by using a mathematical system Disney developed called Inforna. The compound rendered the cancer cells vulnerable to both Herceptin (trastuzumab) and Kadcyla (ado-trastuzumab emtansine), both targeted therapeutics. Meanwhile, it left healthy breast cells unaffected.

"It's possible that precision medicines like Herceptin can be made available to a wider group of people by altering gene expression with therapeutics that bind not to the proteins but to noncoding RNA," Disney says.

About 20 percent of people diagnosed with breast cancer have the HER2-positive mutation, which means the surface of their cancer cells have more of the HER2 protein on them. While that mutation is associated with faster growing, more aggressive cancers, since 1998 it has come with good options for effective treatment. Since the monoclonal antibody drug Herceptin was introduced, 10-year disease-free survival rates for the HER2-positive breast cancer subtype surged to 84 percent.

For breast cancer patients with dwindling options, switching on HER2 sensitivity might be life-changing, Disney says.

"This study is proof of concept for the strategy of creating sensitivity to a drug where one otherwise wouldn't exist," Disney says. "It also validates the notion that transcription of genes can be modulated via small molecule compounds engineered to bind to relevant noncoding RNA. This means that supposedly untreatable diseases may, one day, be readily treatable. There is a long way to go for this to get to patients, however."

There were many challenges that had to be overcome to create the effective compound, says first author Matthew G. Costales, a Scripps Research graduate student. Unintended targets had to be identified and blocked, and selectivity engineered into the molecule. Disney's computational system, Inforna, guided the efforts.

The first compound the Inforna database highlighted bound to two different micro RNAs, both 885 and 515. That could have posed problems. Further refinement made it clear that a compound could be designed to be selective for one micro RNA but not the other. Eventually they created a molecule that successfully selected only for the desired microRNA, 515. The result was that the cells produced increased HER2 levels, making them sensitive to the drugs.

"The demanding synthetic, biochemical, and cellular experiments described in this paper were three years in the making," Costales says. "It required tremendous effort, but the work is by no means complete. I am looking forward to future work guided by the lessons we learned here."

Disney credits his colleague, Scripps Research chemist Alexander Adibekian, PhD, with helping understand the mechanisms of action of their compound, TGP-515, and demonstrating its selectivity.

It's important to note that this is a first step toward making drugs to boost HER2 sensitivity where there is none, Disney says. Significant additional research lies ahead, he says.

Successful tests in cultured cells must be followed with tests in mouse models of cancer, a process that will take several years. Financial support from the community, including Frenchman's Creek Women for Cancer Research, Alan and Susan Fuirst, and the R. J. Scheller Graduate Student Fellowship, has helped made the work possible.

"These studies would not have been realized without the local support graciously provided by the community," Disney says. "I am incredibly grateful for the community taking the time to invest in the future of biomedical research completed here at Scripps Research."

In addition to Costales and Disney, the authors of the study, "A Designed Small Molecule Inhibitor of a Non-Coding RNA Sensitizes HER2 Negative Cancers to Herceptin," were Dominic Hoch, Daniel Abegg, Jessica Childs-Disney, Sai Pradeep Velagapudi and Alexander Adibekian of Scripps Research in Jupiter, Florida.

The research was made possible by the R.J. Scheller Graduate Student Fellowship, the ACS Medicinal Chemistry Division Predoctoral Fellowship, the National Institutes of Health, Alan and Susan Fuirst, and Frenchman's Creek Women for Cancer Research.

Leading Cause of Muscular Dystrophy in Adults Responds to New Treatment

April 1, 2019: JUPITER, FL — People diagnosed with myotonic dystrophy type 1 have difficulty unclenching muscles due to a type of genetic defect that generates toxic material within their cells.

There is currently no treatment. In a new report published in the *Proceedings of the National Academy of Sciences*, a group at Scripps Research in Florida says they have made a potential drug that targets a disease-causing RNA at the root of the disease. In mouse and cellular models of myotonic dystrophy type 1, it improved the muscle defects with no apparent side-effects.

The study, "Precise small molecule cleavage of an r(CUG) repeat expansion in a myotonic dystrophy mouse model," appears in the March 29 issue of PNAS. More work lies ahead before testing in people will be possible, but "the results look better than we could have imagined," says lead author Matthew Disney, PhD, a Scripps Research chemistry professor.

"The results suggest that our technology can be used to treat myotonic dystrophy type 1 and similar categories of inherited diseases, and without unintended, off-target effects," Disney says, adding, "There is great potential for drugging RNA in all disease settings."

He notes that diseases with similar genetic causes include ALS and Huntington's disease.

The most common form of muscular dystrophy in adults, myotonic dystrophy type 1 has been estimated to affect around 1 in 8,000 people, although the Myotonic Dystrophy Foundation based in San Francisco reports that misdiagnosis is common, and the actual numbers appear to be significantly higher, according to recent research. Genetic studies suggest the actual numbers are about three times higher, around 1 in 2,500, says Molly White, CEO of the foundation.

The disease is inherited. Symptoms emerge in late teens or early adulthood as genetic changes accumulate. They include muscle clenching, lock-jaw, early-onset cataracts, brain fog, muscle wasting and weakness, digestive difficulties, and sudden cardiac death, White says. Severity and rate of progression depends on factors including the nature of the genetic defect.

Myotonic dystrophy type 1 occurs when a sequence of three nucleotides, CTG, is repeated too many times within a gene called DMPK. Toxic protein clumps generate further genetic defects, resulting in muscle weakness and other symptoms. A healthy person could carry between 5 and 35 repeats of CTG within that gene without experiencing obvious difficulty. But symptomatic people may have 50, 100 or even up to 4,000 repeats of the CTG sequence.

The drug Disney's group designed, called Cugamycin, works by recognizing the toxic RNA repeats and destroying the garbled gene transcript. Significantly, in treated animals, the drug left the healthy version of the gene transcript intact. The results were consistent in both the mouse model of myotonic dystrophy type 1 and in human patient-derived muscle fibers called myotubes.

Cugamycin was made by attaching an RNA-binding molecule to an existing drug called bleomycin, which cleaves nucleic acids.

"Analysis of the tissue from a pre-clinical disease model showed more than 98 percent of the disease defects are improved, with no detectable off-targets." Disney says.

So far, the results have been excellent, but these studies are still in their early stages, says Alicia Angelbello, the study's first author and a Scripps Research graduate student.

"A key next question will be to evaluate the effectiveness of our compound over a longer period of time," she adds.

In the current study, the treated mice experienced fewer instances of "myotonic discharges" in their muscles—occasions when it takes longer than usual for a contracted muscle to relax—compared to untreated mice, Angelbello says.

"Once given the Cugamycin at a dose of 10 milligrams per kilogram, the frequency of the myotonic discharges was reduced by 50 percent, which is a significant improvement," Angelbello says.

"The fact that we can improve the muscular and genetic defects in DM1 mice with the molecules we have made in the lab is a significant step in learning about how to treat this disease," she says.

The Myotonic Dystrophy Foundation has supported Disney's work for many years.

"Matt Disney is super-committed to making a treatment for this disease," says White, CEO of the Myotonic Dystrophy Foundation. "It's clear that many people think he's on the right track. We're thrilled at the progress he is making."

In addition to Disney and Angelbello, the authors of the study include Suzanne Rzuczek, Jonathan Chen and Michael Cameron of Scripps Research; Kendra McKee, Hailey Olafson and Eric Wang of the University of Florida; and Walter Moss of Iowa State University. Disney and Wang consult for Expansion Therapeutics. Suzanne Rzuczek is an employee of Expansion Therapeutics.

Disney's work was funded by the National Institutes of Health (grant DP1NS096898) and the Muscular Dystrophy Association (grant 380467). The Myotonic Dystrophy Foundation also provided support.

Gene Repair Improves Memory and Seizures in Adult Autism Model

April 29, 2019: JUPITER, FL – A new study challenges the presumption that people born with developmental brain disorders such as severe autism will benefit from medical interventions only if treated during a narrow window in infancy or early childhood.

Writing in the journal *eLife*, an open-access scientific journal, the Rumbaugh lab at Scripps Research in Florida reports improvement in measures of seizure and memory in adult mouse models of a genetic cause of autism, called *SYNGAP1* disorder.

Children born with only one working copy of the *SYNGAP1* gene don't make enough of the critical SynGAP protein. Two broken copies is lethal. Depending on the extent of their deficit, these children can develop a range of developmental challenges as they mature. This may include intellectual disability, autism-like behaviors, disordered sensory processing, and epileptic seizures that don't respond to medication. The disorder likely affects one to four individuals per 10,000, similar to the frequency of Fragile X syndrome, says Gavin Rumbaugh, PhD, an associate professor in the Department of Neuroscience at Scripps Research in Florida. However, patients can only be discovered through genetic tests. As a result, only a small fraction of patients with this disorder have been discovered.

To study whether treatment of *SYNGAP1* disorder in adulthood could be beneficial, Rumbaugh's team genetically restored levels of the mice's SynGAP protein to normal. The treated adult mice showed multiple improvements. It suggests that having one broken copy of the gene not only harms the brain

as it develops, but it also has effects in the adult brain, Rumbaugh says. There may be reason to treat at any stage of life once options become available, Rumbaugh adds.

"Our findings in mice suggest that neurodevelopmental disorders' disease course can be altered in adult patients," Rumbaugh says. "We can correct brain dysfunction related to seizure as well as memory impairments after restoring SynGAP protein levels in the adult animals."

Significantly, the paper offers a path to measure the effectiveness of potential medications or other therapies for neurodevelopmental disorders going forward. Electrographic spikes between seizures is an indicator of epilepsy. In their paper, the scientists looked at human EEG data collected from a *SYNGAP1* disorder patient registry and found that the appearance of these spikes were much more likely to occur during sleep. Similar findings were observed from mouse models of *SYNGAP1* disorder, offering a useful endpoint. Establishment of biomarkers that predict generalized improvements in brain function will be a critical step in advancing treatments for people with severe neurodevelopmental disorders, Rumbaugh says.

The need for a treatment option is clear, Rumbaugh says. Seizures typically become more frequent as children with *SYNGAP1* disorders mature, and for many patients, those seizures do not respond to anti-epilepsy drugs.

"Getting to know families affected by this severe disorder has been invaluable, and drives us to develop treatments that will improve the lives of both children and adults," Rumbaugh says. "It is encouraging that gene therapy techniques that increase pathologically low protein levels for other types of brain disorders are showing promise in the clinic now."

Additional authors of the study, "Re-expression of SynGAP Protein in Adulthood Improves Translatable Measures of Brain Function and Behavior," include first author Thomas K. Creson, Camilo Rojas, Thomas Vaissiere, Muratb Kilinc, and Courtney Miller of Scripps Research, Ernie Hwaun and Laura Lee Colgin of the Institute for Neuroscience at the University of Texas at Austin, and J. Lloyd Holder, Jr. and Jianrong Tang of the Baylor College of Medicine.

This work was supported in part by grants from the National Institute of Mental Health [MH096847, MH108408, MH105400, MH102450], the National Institute for Neurological Disorders and Stroke [NS064079, NS091381], and the National Institute for Drug Abuse [DA034116 and DA036376, T32DA01892].

Nanotubes Enable Travel of Huntington's Disease Protein

May 10, 2019: Jupiter, FL - A toxic protein linked to Huntington's disease can move from neuron to neuron through a nanotube tunnel whose construction is initiated by a protein called Rhes, say scientists at Scripps Research.

The finding, by Scripps Research neuroscientist Srinivasa Subramaniam, PhD, improves understanding of how and why this disease attacks and destroys certain brain cells. The research was published Friday, May 10 in the *Journal of Cell Biology*.

Scripps Research Neuroscience Associate Professor Srinivasa Subramaniam, PhD, and research associate Manish Sharma, PhD, review confocal microscope images of the Huntington's protein moving between neurons via nanotube.

"We are excited about this result because it may explain why the patient gets the disease in this area of the brain called the striatum," says Subramaniam, an associate professor in the Department of Neuroscience at Scripps Research-Florida.

People with Huntington's disease inherit a damaged protein that is somehow complicit in destroying brain cells. Scientists discovered this protein in 1993 but are still piecing together its role in this degenerative disease. Scans show Huntington's disease brains are shrunken and degraded. As the neurons deteriorate, people lose motor control, they can have emotional problems and their thinking and memory suffer. Symptoms usually begin around age 30 to 40 and last 15 to 20 years until death. A rarer and more aggressive form of the disease affects children, cutting their childhood and lives short.

About 3 to 7 people out of 100,000 have the disease and it has mostly affected those with European ancestry. However, Subramaniam believes the disease is underreported in other areas, such as India.

"There is a lot of stigma associated with the disease," says Subramaniam.

His laboratory investigates the molecular mechanics of Huntington's disease and other neurodegenerative illnesses, including Alzheimer's and Parkinson's disease, to find potential therapy targets.

"In the case of Huntington's, the question is can we block this transport, and does it have any benefit or effect?" says Subramaniam.

For this study, Subramaniam and colleague Manish Sharma, PhD, looked at mouse neurons under a confocal microscope and saw that the cells formed sticky, string-like protrusions around 150 microns long which floated above the cells, connecting them.

"When I saw Rhes making these tunnel-like tubes between the cells I was excited and at the same time perplexed," says Sharma, the first author of the study.

"They may have been missed before because they are on a different plane," says Subramaniam. "You have to be really looking for it. It's like a bridge over a lake. If you are on the lake, you may not see the bridge above, but if you are on shore, you can see the bridge."

Scientists first described another type of tunneling nanotube in rat neurons in 2004. Since then, a number of researchers have observed them in cancer and other types of cells. But how they form and what they do was less clear.

To find out, Subramaniam and Sharma tracked cell cargo moving through this tunnel bridge. They inserted the Huntington human disease protein into the mouse brain cells, tagged it with fluorescence and then watched as it crossed over and crawled up to enter the neighboring cell. Once the tunnel delivered its shipment it released and sprang back. Lysosomes and endosomes, cellular cargo bins that transport cell pieces or waste, also travel these intercellular highways, Subramaniam says.

The Rhes protein exists in both mouse and human brains sick with Huntington's disease. Knocking out the Rhes gene in diseased mice results in less brain damage. In 2009 study, Subramaniam found that Rhes also alters the Huntington disease protein's structure making it more toxic to brain cells.

"The Rhes protein makes its own road. That is what is surprising to us," says Subramaniam. "But it not only transports itself. Once the road is made, many things can be transported."

Subramaniam's group continues to investigate what other proteins may be helping with tunnel construction and if other disease proteins move along these membranous highways. His laboratory is also developing ways to identify how the Huntington's disease protein travels in the live brain.

The study, *"Rhes Travels from Cell to Cell and Transports Huntington Disease Protein Via TNT-like Cellular Protrusions," was supported in part by a training grant in Alzheimer's drug discovery from the Lottie French Lewis Fund of the Community Foundation for Palm Beach and Martin Counties, and from the National Institutes of Health NIH/NINDS R01-NS087019-01A1, NIH/NINDS R01-NS094577-01A1 and Cure for Huntington Disease Research (CHDI).*

Unexplored Neural Circuit Modulates Memory Strength

May 14, 2019: JUPITER, FL — Learning to avoid negative experiences requires an interplay of two distinct brain circuits, one to interpret "Yikes!" and drive learning, and the other, unexpectedly, to dial in the strength of that memory, a new fruit fly study shows.

"This kind of gain control is probably present in many levels of the nervous system, in many organisms," says Scripps Research neuroscientist Seth Tomchik, PhD, lead author of the study.

The human brain is comprised of a staggering number of neurons—about 86 billion, according to recent studies. Given that complexity, neuroscientists working to understand how learning and memory work begin with simpler model organisms. Humans and flies share a degree of fundamental biology, including reliance on the neurotransmitter dopamine in learning.

"We know with flies, just like in mammals, there are neurons involved in positive reinforcement, there are neurons involved in negative reinforcement—the valence neurons—and then there are this third set," Tomchik says. "Nobody really knew what they did."

The fruit fly brain contains eight groups of neurons that produce dopamine. Three of them can be found in what's known as the fly brain's "mushroom body." Humans don't have an exact analogous brain section, but other brain regions perform similar functions. In *Drosophila melanogaster*, aka the fruit fly, the mushroom body is an area highly responsive to odors.

Past fly brain studies have shown that one of the dopamine-producing groups projecting into the mushroom body handles desire-inducing memories connected to odors. ("Mmmm, rotten bananas!") while another guides avoidant behavior related to negative experiences. ("Yikes, dangerous banana smell!")

To find out the role of the third group, referred to as PPL2, research associate and first author Tamara Boto, PhD, trained the flies with an experiment that involved exposing them to fruit-like odors while simultaneously giving them a mild electric shock.

Their conditioned response could be visualized under a microscope by adding a green fluorescent protein that releases light upon reacting to calcium. Calcium ions are released when neurons communicate. Stimulating the PPL2 neurons during the odor experiments changed the brightness of the fluorescence when presented with the odor, an indication that the structures involved in learning and memory had altered the degree of response.

"When we activated this PPL2 set of neurons, it would actually modulate the strength of that memory," Tomchik says. "So we see there are dopaminergic neurons that encode the aversive stimulus itself, and then there is this additional set that can turn the volume up or down on that memory."

The ability of PPL2 to strengthen the response was a surprise, Boto adds.

"I think it is amazing that there is this physiological effect that translates to a behavioral effect," Boto says. "Dopamine is not likely to excite on its own, but the response is greater if it is paired with stimulation of this set of neurons."

The study, "Independent Contributions of Discrete Dopaminergic Circuits to Cellular Plasticity, Memory Strength and Valence in Drosophila," appears May 14 in the journal *Cell Reports*.

A next step will be exploring what stimulates PPL2 neurons and how their activity influences other neurons in the memory network, Tomchik says.

Studying the brain circuitry underlying experience, learning and memory in model organisms can offer insights into our own, more complex brains, insights that could help us understand issues like addiction, PTSD, depression and neurodevelopmental disorders, Tomchik says.

"We want to understand more about what their fundamental function is, what types of stimuli activate them under what conditions," he says. "Translating the learned information into behavioral execution, through the neurons in between, that's where I expect a lot of discoveries in the next few years are going to be."

In addition to Tomchik and Boto, the authors of the study include Aaron Stahl, Xiaofan Zhang, and Thierry Louis.

The work was supported in part by the National Institute of Mental Health, the National Institute of Neurological Disorders and Stroke (R00 MH092294), (R01 NS097237), and the Whitehall Foundation. Boto and Louis were Neuroscience Scholars of the Esther B. O'Keeffe Charitable Foundation.

Discovery of Bacteria's Protein Quality Control Agent Offers Insight into Origins of Life

May 30, 2019: JUPITER, FL — Our cells' process for transforming genes into useful proteins works much like an automobile factory's assembly line; there are schematics, parts, workers, motors, quality control systems and even recycling crews. If the cell's recycling process falters, abnormal protein fragments accumulate, potentially causing the cell's death. In nerve cells, the process is linked to a variety of neurodegenerative diseases, including ALS and dementia.

A new study from the lab of Claudio Joazeiro, PhD, published online in the journal *Cell* on May 30, uncovers how simpler organisms—bacteria and archaea—manage the recycling of incomplete

proteins. The discoveries not only offer new directions for fighting the virulence of some of humanity's most dangerous pathogens, including listeria, staph and streptococcus, they have implications for our understanding of how life itself evolved.

Scripps Research Molecular Medicine Professor Claudio Joazeiro, PhD.

Joazeiro's group found the mechanism isn't so different from one they previously uncovered in plant, animal and fungal cells.

"We know that as cells are making proteins, this process is occasionally halted due to errors," says Joazeiro, who has joint appointments in the Scripps Research Department of Molecular Medicine in Jupiter, Florida, and the Center for Molecular Biology of Heidelberg University, in Germany.

"One of the problems with this is that the accumulation of partially formed proteins may be toxic. So in our lab, we're asking how do cells sense this, and how do they disassemble these proteins and recycle the building blocks?"

Organelles called ribosomes serve as the protein-assembly motors within cells. If they stall during the process of piecing together the parts—amino acids—cells have a variety of systems for responding. In human and other eukaryotic cells, when a ribosome jams, rescue factors split it open. A protein called Rqc2, also known as NEMF, zooms in and recruits another protein—the ubiquitin ligase Ltn1, also called listerin. The Joazeiro lab previously discovered that Ltn1 marks the truncated protein fragment on ribosomes with a destruction tag called ubiquitin. Protease saws then handle the demolition.

Underscoring the importance of this recycling process, Joazeiro discovered in 2009 that mutations in Ltn1 can cause the death of nerve cells in mice, resulting in ALS-like symptoms.

Bacteria have related, but somewhat more direct systems for addressing halted ribosomes and their protein fragments, according to the *Cell* report. Studying the bacterium *B. subtilis*, the Joazeiro team found that Rqc2 itself marks the protein fragment with a flag—a polymer made of the amino acid alanine. Thus flagged, proteases come to cut up the bad fragment.

Previous studies had suggested that in some pathogenic bacteria, Rqc2 proteins had a different job, one that functioned outside the cell, helping attach the microbes to hosts.

"We have found this is not the complete story," Joazeiro says. "Rqc2 plays a more fundamental role inside of bacterial cells."

The next step will be to find out whether the defective virulence of strep varieties lacking Rqc2 is primarily a consequence of their failure to recycle protein fragments inside the cell. As increasing varieties of pathogens develop multi-drug resistance to antibiotics, understanding bacterial virulence may prove especially necessary.

Equally important to Joazeiro is the realization that Rqc2 serves as a "living" molecular fossil, illuminating new insights about the ancient ancestral organism that emerged some 4 billion years ago to form the very base of the tree of life that evolved into the planet's biodiversity today.

"Shortly after cells invented how to make proteins, they were also faced with determining how to deal with incompletely made proteins," Joazeiro says. "The analyses suggest that an Rqc2 homolog in the last universal common ancestor already carried out this task."

In addition to lead author Joazeiro, the authors of the study, "Alanine Tails Signal Proteolysis in Bacterial Ribosome-Associated Quality Control," were Tina Mueller, George Tsaprailis and Christina Chiang of Scripps Research in Jupiter, FL; Iryna Lytvyenko, Helge Paternoga, Anna Thrun and Simon Anders of Heidelberg University in Germany; Annika Balke and Christian Spahn of the Institute of Medical Physics and Biophysics in Berlin, Germany; Katja Nagler and Ilka Bischofs of Heidelberg University and the Max Planck Institute for Terrestrial Microbiology in Marburg, Germany, and Julie Maupin-Furlow of the University of Florida in Gainesville, FL.

The research was supported by the National Institutes of Health (NS075719, NS102414, GM57498), the Deutsche Forschungsgemeinschaft in Germany (SFB1036, SFB740) and the Max Planck Society in Germany.

B. GRANTS RECEIVED

Grant Number	Awarding Agency	New or Renewal Award		Amount Awarded
1R01 CA223823-01	National Cancer Institute	New	$	266,732
1R01 CA223823-01	National Cancer Institute	New	$	125,687
1R01 CA227073-01	National Cancer Institute	New	$	232,796
1R01 CA227073-01	National Cancer Institute	New	$	152,000
1R01 CA241816-01	National Cancer Institute	New	$	222,186
1R01 CA241816-01	National Cancer Institute	New	$	251,913
1R01 CA241816-01	National Cancer Institute	New	$	18,042
1R01 CA241816-01	National Cancer Institute	New	$	55,537
1R21 CA229961-01	National Cancer Institute	New	$	145,845
3R33 CA206949-03	National Cancer Institute	Renewal	$	138,631
4R00 CA201606-03	National Cancer Institute	Renewal	$	248,999
5R01 CA174844-05	National Cancer Institute	Renewal	$	377,590
5R01 CA174844-05	National Cancer Institute	Renewal	$	188,796
5R01 CA197944-04	National Cancer Institute	Renewal	$	416,138
5R01 CA197944-04	National Cancer Institute	Renewal	$	92,691
5R01 CA206493-03	National Cancer Institute	Renewal	$	109,800
5R01 CA206493-03	National Cancer Institute	Renewal	$	184,345
5R01 CA220284-02	National Cancer Institute	Renewal	$	152,285
5R01 CA220284-02	National Cancer Institute	Renewal	$	477,138
5R01 CA225890-02	National Cancer Institute	Renewal	$	355,789
5R01 CA225890-02	National Cancer Institute	Renewal	$	157,449
5R01 CA225890-02	National Cancer Institute	Renewal	$	27,469
5R01 CA225890-02	National Cancer Institute	Renewal	$	13,219
5R01 CA227849-02	National Cancer Institute	Renewal	$	430,851
5R33 CA206949-03	National Cancer Institute	Renewal	$	316,508
3R01 DA031927-08	National Institute on Drug Abuse	Renewal	$	7,448
3R01 DA033073-07	National Institute on Drug Abuse	Renewal	$	22,200
5DP1 DA043912-03	National Institute on Drug Abuse	Renewal	$	1,095,990

5K01 DA040737-03	National Institute on Drug Abuse	Renewal	$	201,631
5K02 DA026405-11	National Institute on Drug Abuse	Renewal	$	121,500
5R01 DA031927-09	National Institute on Drug Abuse	Renewal	$	688,041
5R01 DA031927-09	National Institute on Drug Abuse	Renewal	$	172,805
5R01 DA033073-07	National Institute on Drug Abuse	Renewal	$	423,613
5R01 DA033073-07	National Institute on Drug Abuse	Renewal	$	312,599
5R01 DA036596-05	National Institute on Drug Abuse	Renewal	$	519,222
5R01 DA038964-05	National Institute on Drug Abuse	Renewal	$	480,000
5R01 DA042746-03	National Institute on Drug Abuse	Renewal	$	132,776
5R01 DA042746-03	National Institute on Drug Abuse	Renewal	$	409,803
1R01 GM133041-01	National Institute of General Medical Sciences	New	$	832,213
1R21 GM131420-01	National Institute of General Medical Sciences	Renewal	$	285,000
3R01 GM094483-08	National Institute of General Medical Sciences	Renewal	$	95,291
5R01 GM086451-11	National Institute of General Medical Sciences	Renewal	$	1,064,172
5R01 GM087638-08	National Institute of General Medical Sciences	Renewal	$	836,000
5R01 GM094483-08	National Institute of General Medical Sciences	Renewal	$	374,552
5R01 GM097455-08	National Institute of General Medical Sciences	Renewal	$	658,456
5R01 GM097455-08	National Institute of General Medical Sciences	Renewal	$	52,001
5R01 GM102187-07	National Institute of General Medical Sciences	Renewal	$	951,236
5R01 GM120491-03	National Institute of General Medical Sciences	Renewal	$	375,697
5R35 GM128895-02	National Institute of General Medical Sciences	Renewal	$	950,000
1R01 NS103195-01	National Institute of Neurological Disorders and Stroke	New	$	508,830
1R01 NS103195-01	National Institute of Neurological Disorders and Stroke	New	$	247,000
5R21 NS105941-02	National Institute of Neurological Disorders and Stroke	New	$	191,670
3UH3 NS096833-03	National Institute of Neurological Disorders and Stroke	Renewal	$	180,291
5DP1 NS096898-04	National Institute of Neurological Disorders and Stroke	Renewal	$	1,348,066
5P01 NS099114-03	National Institute of Neurological Disorders and Stroke	Renewal	$	73,253
5P01 NS099114-03	National Institute of Neurological Disorders and Stroke	Renewal	$	193,369
5P01 NS099114-03	National Institute of Neurological Disorders and Stroke	Renewal	$	446,178
5R01 NS072129-09	National Institute of Neurological Disorders and Stroke	Renewal	$	1,062,010
5R01 NS087019-05	National Institute of Neurological Disorders and Stroke	Renewal	$	420,000
5R01 NS094577-04	National Institute of Neurological Disorders and Stroke	Renewal	$	420,000
5R01 NS097237-03	National Institute of Neurological Disorders and Stroke	Renewal	$	415,625
5R21 NS099417-02	National Institute of Neurological Disorders and Stroke	Renewal	$	340,016
5R21 NS105941-02	National Institute of Neurological Disorders and Stroke	Renewal	$	758,330
5UH3 NS096833-03	National Institute of Neurological Disorders and Stroke	Renewal	$	532,585
5UH3 NS096833-03	National Institute of Neurological Disorders and Stroke	Renewal	$	22,876
5UH3 NS096833-03	National Institute of Neurological Disorders and Stroke	Renewal	$	108,974
5UH3 NS096833-03	National Institute of Neurological Disorders and Stroke	Renewal	$	76,251
1R01 MH118444-01	National Institute of Mental Health	New	$	568,318
1R21 MH117485-01	National Institute of Mental Health	New	$	277,500
3R01 MH109957-03	National Institute of Mental Health	Renewal	$	459,586
5R01 MH096847-07	National Institute of Mental Health	Renewal	$	751,943
5R01 MH096847-07	National Institute of Mental Health	Renewal	$	51,215
5R01 MH105482-05	National Institute of Mental Health	Renewal	$	493,054
5R01 MH105610-05	National Institute of Mental Health	Renewal	$	480,000
5R01 MH108408-05	National Institute of Mental Health	Renewal	$	709,336
5R01 MH108519-04	National Institute of Mental Health	Renewal	$	570,557
5R01 MH109957-03	National Institute of Mental Health	Renewal	$	622,806
5R01 MH111116-03	National Institute of Mental Health	Renewal	$	83,326
5R01 MH111116-03	National Institute of Mental Health	Renewal	$	80,268

5R01 MH113648-03	National Institute of Mental Health	Renewal	$	667,513
5R01 MH117499-02	National Institute of Mental Health	Renewal	$	633,786
5R01 MH117499-02	National Institute of Mental Health	Renewal	$	211,174
5R21 MH113156-02	National Institute of Mental Health	Renewal	$	327,848
1R01 AI143821-01	National Institute of Allergy and Infectious Diseases	New	$	386,587
1R21 AI145575-01	National Institute of Allergy and Infectious Diseases	New	$	95,000
1R21 AI145575-01	National Institute of Allergy and Infectious Diseases	New	$	142,500
1R61 AI140439-01	National Institute of Allergy and Infectious Diseases	New	$	649,986
1R61 AI140439-01	National Institute of Allergy and Infectious Diseases	New	$	300,014
5R01 AI097012-07	National Institute of Allergy and Infectious Diseases	Renewal	$	1,523,353
5R01 AI110692-05	National Institute of Allergy and Infectious Diseases	Renewal	$	285,630
5R01 AI110692-05	National Institute of Allergy and Infectious Diseases	Renewal	$	21,874
5R01 AI118432-05	National Institute of Allergy and Infectious Diseases	Renewal	$	422,373
5R01 AI129868-02	National Institute of Allergy and Infectious Diseases	Renewal	$	1,413,893
5R01 AI130303-03	National Institute of Allergy and Infectious Diseases	Renewal	$	420,823
5R01 AI130303-03	National Institute of Allergy and Infectious Diseases	Renewal	$	46,356
5R01 AI130303-03	National Institute of Allergy and Infectious Diseases	Renewal	$	12,386
5R33 AI116226-05	National Institute of Allergy and Infectious Diseases	Renewal	$	562,273
3R01 EY028033-03	National Eye Institute	Renewal	$	128,305
5R01 EY028033-03	National Eye Institute	Renewal	$	359,447
5R01 EY028033-03	National Eye Institute	Renewal	$	142,942
1R01 HL144089-01	National Heart, Lung, Blood Institute	New	$	556,577
2R01 DK103335-05	National Institute of Diabetes and Digestive and Kidney Diseases	Renewal	$	593,066
3R01 DK105954-04	National Institute of Diabetes and Digestive and Kidney Diseases	Renewal	$	402,031
5F32 DK115099-02	National Institute of Diabetes and Digestive and Kidney Diseases	Renewal	$	60,132
5R01 DK105825-05	National Institute of Diabetes and Digestive and Kidney Diseases	Renewal	$	439,519
5R01 DK105825-05	National Institute of Diabetes and Digestive and Kidney Diseases	Renewal	$	176,140
5R01 DK105954-04	National Institute of Diabetes and Digestive and Kidney Diseases	Renewal	$	618,253
5R01 DK108801-02	National Institute of Diabetes and Digestive and Kidney Diseases	Renewal	$	463,125
5R01 DK113056-02	National Institute of Diabetes and Digestive and Kidney Diseases	Renewal	$	432,000
3R00 HD090292-02	National Institute of Chiuld Health and Human Development	Renewal	$	45,245
4R00 HD090292-02	National Institute of Chiuld Health and Human Development	Renewal	$	269,920
5R01 AG049037-04	National Institute on Aging	Renewal	$	255,138
5R21 AG050172-02	National Institute on Aging	Renewal	$	144,884
5R21 AG055049-02	National Institute on Aging	Renewal	$	442,241
5R21 EB024116-02	National Institute of Biomedical Imaging and Bioengineering	Renewal	$	139,775
1659594	National Science Foundation	Renewal	$	195,252
W81XWH-18-1-0451	Department of Defense	New	$	332,501
W81XWH-18-1-0782	Department of Defense	New	$	606,180
W81XWH-19-1-0063	Department of Defense	New	$	370,000
CCF 546172	Non-Federal, Other Awarding Parties	New	$	58,250
CHDI FDN/SFP2012	Non-Federal, Other Awarding Parties	Renewal	$	330,183
MDA/602541	Non-Federal, Other Awarding Parties	Renewal	$	100,000
BMS	Private and Pharmaceutical grantees	New	$	133,253
CASMATH/2018-077	Private and Pharmaceutical grantees	New	$	163,530

EXPAN TH/SFP2249	Private and Pharmaceutical grantees	New	$	1,368,125
EXPANSI/SFP-0504	Private and Pharmaceutical grantees	New	$	176,388
GSK/2018-0581	Private and Pharmaceutical grantees	Renewal	$	40,000
SHANG PHARMA SFP	Private and Pharmaceutical grantees	Renewal	$	208,698
SHANG PHARMA SFP	Private and Pharmaceutical grantees	Renewal	$	248,262
TSRI/SIMONS	Subcontracted Grants & Contracts	New	$	22,660
SBU/AI141592-01	Federal NIH Subcontracted Grants & Contracts	New	$	263,775
STONYBROOK/R01 C	Federal NIH Subcontracted Grants & Contracts	New	$	131,219
STONYBROOK/R01 C	Federal NIH Subcontracted Grants & Contracts	New	$	25,390
UCSD/DA047039-01	Federal NIH Subcontracted Grants & Contracts	New	$	174,926
VARI/GM129436-01	Federal NIH Subcontracted Grants & Contracts	New	$	29,775
BWH/DK107239-02	Federal NIH Subcontracted Grants & Contracts	Renewal	$	146,733
BWH/DK107239-02	Federal NIH Subcontracted Grants & Contracts	Renewal	$	180,945
DANA FR/CA154303	Federal NIH Subcontracted Grants & Contracts	Renewal	$	42,642
FHCRC/AI126623-3	Federal NIH Subcontracted Grants & Contracts	Renewal	$	287,525
JAX/5R01NS102414	Federal NIH Subcontracted Grants & Contracts	Renewal	$	300,491
JAX/5R01NS102414	Federal NIH Subcontracted Grants & Contracts	Renewal	$	263,042
LJAI/AI109976-05	Federal NIH Subcontracted Grants & Contracts	Renewal	$	301,569
LJAI/AI109976-05	Federal NIH Subcontracted Grants & Contracts	Renewal	$	85,180
MN/R01HL105550-8	Federal NIH Subcontracted Grants & Contracts	Renewal	$	210,358
MOFFITT/7RF1AG06	Federal NIH Subcontracted Grants & Contracts	Renewal	$	819,609
MSSM/UG3DA048385	Federal NIH Subcontracted Grants & Contracts	Renewal	$	475,000
NEU/DA045020-02	Federal NIH Subcontracted Grants & Contracts	Renewal	$	334,378
NSU/AR066676-04	Federal NIH Subcontracted Grants & Contracts	Renewal	$	52,500
NU/DA009158-19	Federal NIH Subcontracted Grants & Contracts	Renewal	$	194,219
RUTGERS/NS096032	Federal NIH Subcontracted Grants & Contracts	Renewal	$	326,774
STONY/R21AI13085	Federal NIH Subcontracted Grants & Contracts	Renewal	$	19,300
SUNY/DK112759-02	Federal NIH Subcontracted Grants & Contracts	Renewal	$	37,820
TSRI/AI119564-03	Federal NIH Subcontracted Grants & Contracts	Renewal	$	19,741
TSRI/DA046204-02	Federal NIH Subcontracted Grants & Contracts	Renewal	$	314,240
TSRI/GM088278-08	Federal NIH Subcontracted Grants & Contracts	Renewal	$	43,699
TSRI/GM103368-07	Federal NIH Subcontracted Grants & Contracts	Renewal	$	300,810
TSRI/TR002550	Federal NIH Subcontracted Grants & Contracts	Renewal	$	24,586
TSRI/TR002550	Federal NIH Subcontracted Grants & Contracts	Renewal	$	14,677
UF/AI119043-04	Federal NIH Subcontracted Grants & Contracts	Renewal	$	62,482
UF/AI119043-04	Federal NIH Subcontracted Grants & Contracts	Renewal	$	264,367
UMIAMI/MH110441	Federal NIH Subcontracted Grants & Contracts	Renewal	$	286,802
UMIAMI/MH110441	Federal NIH Subcontracted Grants & Contracts	Renewal	$	147,278
UPITT/AI127677-3	Federal NIH Subcontracted Grants & Contracts	Renewal	$	294,266
UWISC/AI121135-4	Federal NIH Subcontracted Grants & Contracts	Renewal	$	11,195
WSU/R01 DK076629	Federal NIH Subcontracted Grants & Contracts	Renewal	$	39,557
WSU/R01 DK105963	Federal NIH Subcontracted Grants & Contracts	Renewal	$	207,129
IAVI/HHSN2722017	Other Federal Subcontracted Grants & Contracts	Renewal	$	136,918
Total			**$**	**53,730,207**

(Note: In the above presentation, some awards are divided among more than one faculty member, and in those cases the individual award allocations are shown to provide a detailed accounting.)

PART II -MILESTONES AND UPDATES FROM AGREEMENT SECTIONS

Part II of the annual report addresses the milestones and provides updates to specific components of the formal agreement, listed by agreement section.

Section 9.3 **Annual Report. Scripps (The Scripps Research Institute) shall prepare the Annual Report for Scripps Florida each year and deliver such Annual Report to Funding by August 31st of each year. The Annual Report shall include, but not be limited to, the following information:**

Section 9.3(a) **An accounting of the expenditures of Grant Funds for the twelve months ended June 30th of each year (the "Report Year" [as amended]) and financial commitments made by Scripps during the Report Year.**

Report of SFFC Grant Fund Cash Disbursements from October 1, 2018 to June 30, 2019.

Salaries & Benefits	$3,369,159
Supplies	578,757
Scientific Equipment	1,413,476
External Affairs & Other Program Support	4,069,178
Project Commencement, Facilities, Administration & Other Capital Expenditures	6,390,808
Total	**$15,821,379**

The schedule above reflects cash expenditures charged to the grant from the State of Florida from October 1, 2018 to June 30, 2019. The expense categories set forth above reflect those used by Scripps to report grant activity to grantors. This schedule excludes unspent grant funds received of $3,852,072 restricted to support Scripps Florida. There were no new financial commitments made during the report year.

Section 9.3(b) **Data regarding the activities and performance of Scripps Florida during such Report Year and detailing the progress of Scripps in meeting its Business Plan, including but not limited to the information provided in responses to Sections 9.3(b) i-v below:**

Section 9.3(b)i **Information on the number and salary level of jobs created by Scripps within Scripps Florida, including the number and salary level of jobs created for residents of Florida;**

On June 30, 2019, Scripps Florida employed 549 people. Of those, 416 were full-time. The breakdown of those full-time employees is shown below.

Faculty	43
Staff Scientists	31
Research Associates	117
Scientific Support	122
Administrative Support	103
Total	416

In addition, on June 30, 2019 Scripps Florida employed an additional 133 employees who were part-time, research interns, or summer interns, for a total employee population of 549.

Of the 164 employees hired between July 1, 2018 and June 30, 2019, 71 were residents of Florida and 56 were residents of Palm Beach County.

The average salary/range for Scripps Florida employees as of June 30, 2019 is shown below.

Faculty	$47,486 - $340,018
Staff Scientists	$62,566 - $101,504
Research Associates	$7,071 - $100,099
Administrative Support	$68,367 Average

Section 9.3(b)ii A description of the status of the performance expectations set forth in Section 9.5 of this Agreement and the disbursement conditions set forth in Schedule 4.4(c) of this Agreement;

See responses to Sections 9.5 and 4.4(c), below.

Section 9.3(b)iii Information on the positions and funds required to be committed for equipment for such positions by means of the next annual disbursement of Grant Funds;

All grant funds have been disbursed. That said, the Scripps Research Institute will submit the budgets for Scripps Florida for the year ending September 30, 2020, to SFFC after approval by the Scripps Research Institute Board of Directors expected to occur on October 8, 2019. These budgets set forth all anticipated revenue and expenses for Scripps Florida for the next fiscal year. The equipment requirements for new positions will be incorporated into these budgets.

Fiscal year equipment reporting:

Approximately $1,988,819 of equipment - acquired with State grant funds was purchased from October 1, 2018 through June 30, 2019. In addition, $1,051,502 of equipment was purchased using non SFFC funds during the period from October 1, 2018 through June 30, 2019.

Section 9.3(b)iv Commencing with the Annual Report for 2006 Report Year and ending

with the Report Year after which Scripps has moved the Scripps Florida operations to its permanent facility and such facility is fully operational, a description of the status of Scripps' relocation to its second planned temporary facility and the progress of construction activities for its permanent facility, as described in the Business Plan, including a projected date for and status of Scripps' occupancy of its permanent facility.

Scripps Florida officially opened its Permanent Facilities in February of 2009.

Section 9.3(b)v **And commencing with the Annual Report for the Report Year during which Scripps commences activities at its permanent facility, a description of the status of Scripps' activities in its permanent facility, including its educational and outreach programs.**

TSRI's mission is to perform high impact basic biomedical research, improve the human condition by fostering translation of its discoveries into useful products and engaging in the highest quality graduate scientific education and post graduate scientific training.

Scripps Florida Scientific Reports for the years 2007-2018 can be found at https://www.scripps.edu/campuses/florida/reports/

Over the past 15 years (since 2004) Scripps Florida has placed considerable effort in community and education outreach programs. Palm Beach County K-12 students and teachers have participated in science education lessons and events designed and presented by Scripps Florida education outreach, graduate students, post-doctoral fellows, faculty and staff researchers. The programs described in Sections 4.4(c)6 and 4.4(c)7 define the goals of Scripps Florida's K-12 education programs: to work directly with students and teachers, to help develop instructional materials, and to contribute to science literacy in Palm Beach County and the State of Florida. Scripps Florida has taken a leadership role in science education since its inception; presenting at state and national meetings such as National Science Teachers Association, American Association for the Advancement of Science and the International Teacher/Scientist Conference. In Florida, education outreach has participated in education meetings with the Florida State Department of Education, Florida Council of 100, State University System of Florida Board of Governors, STEM Florida and the Sunshine State Scholars. More than twenty thousand students, teachers, and community members of Palm Beach County have participated in the Scripps Florida Education Outreach programs.

Scripps Florida community outreach has offered opportunities for the public to gain insight into cutting edge biomedical research while providing opportunities for Scripps Florida faculty and staff to respond to the social needs of Palm Beach County. Since 2004, Scripps Florida has hosted and/or participated in Community Outreach events that include symposia on Alzheimer's disease, breakthroughs in cancer research, and current discoveries in drug development, just to name a few.

Section 9.3(c) **A schedule of the shares of stock (or other securities) held by Scripps as payment of the royalty referred to in Section 10.2(a) and a report on**

any trades or activity concerning such stock (or other securities);

There were no new securities issued to Scripps Florida between the period October 1, 2018 and June 30, 2019. However, the schedule below shows such activity for prior periods.

Yr. Est.	Company	Business Description	
2018	**CALM Therapeutics**	CALM Therapeutics is developing novel therapeutics to treat Prader-Willi syndrome.	Ex. Lic 4/9/2018; 1,200,000 shares
2017	**Deluge Biotechnologies**	Deluge Biotechnology is focused on developing diagnostics by utilizing epitope surrogate technology with focus on tuberculosis, lung cancer and T1D.	Option Agreement 5/5/2017. Pre-negotiated license to execute Ex. Lic at 2nd anniversary with 20% equity to be maintained until $20M equity raise
2016	**Expansion Therapeutics**	Expansion Therapeutics is developing therapies targeting of RNA expanded sequences (e.g. Amyotrophic lateral sclerosis).	Ex. Lic 12/28/2016; 1,288,522 shares
2015	**Emmune**	Emmune is developing novel approaches for the prevention and treatment of HIV infection.	Option Agreement 8/26/2016. Negotiating Ex. Lic to be executed with 10% equity to be maintained at 5% after $10M equity raise
2014	**Hyconix**	Hyconix is developing and commercializing chemical processes and products useful in the petrochemical industry.	Ex. Lic. 10/21/14; 1,000,000 shares Preferred Series A
2014	**Padlock Therapeutics** (acquired by Bristol-Myers Squibb 3/16)	Padlock Therapeutics is focused on autoimmune diseases, specifically the role of modified auto-antigens in triggering and maintaining these pathologies.	Ex. Lic 12/5/14; $868,953.64 payout 3/02/17
2013	**Vova Ida Therapeutics**	Vova Ida Therapeutics is focused on developing therapeutics for neurodegenerative diseases with protein misfolding.	Option Agreement 9/30/2013; Ex. Lic 07/26/2018 with 13% equity to be maintained at 6.5% until $20M raise
2011	**Ember Therapeutics** (merged with Mariel Therapeutics 3/15)	Ember Therapeutics is continuing the development of a new class of PPARγ-binding compounds that have potent antidiabetic activity but lack the side effects associated with other PPARγ agonists.	Ex. Lic 12/12/11; License Terminated 2/27/14; 150,000 shares

2008	**Curna** (acquired by Opko Health 2/11)	Curna develops therapeutics based on non-coding RNA technology.	$314,029.20 payout 2/1/11
2007	**Xcovery**	Xcovery develops next generation kinase therapeutics for cancer and inflammatory diseases.	263 shares; Company is private; License terminated by failure to pay patent expenses 9/6/07

Section 9.4 **Annual Scientific Report. Scripps shall prepare the Annual Scientific Report that describes its scientific activities for Scripps Florida each year and deliver such annual report to Funding within one hundred twenty (120) days after the end of each fiscal year of Scripps. The form of the annual report will be substantially similar to the form Scripps uses at such time with respect to its California operations.**

The Annual Scientific Report is not due until January of 2020. However, annual scientific reports for Scripps Florida for years 2007-2018 can be found at https://www.scripps.edu/campuses/florida/reports/

Section 9.5 **Performance Expectations. Scripps, in cooperation with OTTED, shall report to Funding not less than annually on its progress in meeting certain performance expectations that reflect the aspirations of the Florida Governor and Legislature for the benefits accruing to Florida as a result of the Grant Funds. These reports shall include, but are not limited to, performance expectations addressing the following with respect to Scripps Florida;**

Section 9.5(a) **(Also see Section 9.5(h).) The number and dollar value of research grants obtained by Scripps with respect to Scripps Florida from the Federal Government or sources other than Florida;**

Scripps Florida scientists were awarded 126 research grants from non-Florida sources between July 1, 2018 and June 30, 2019. The total dollar amount of those grants for the first year of funding was $53,730,207.

Section 9.5(b) **The percentage of total research dollars received by Scripps from sources other than Florida, which is used to conduct research activities by Scripps in Florida;**

Between July 1, 2018 and June 30, 2019, scientists at Scripps Florida expended about $63,816,084 in research dollars to conduct research activities at Scripps Florida. During that same time, about $6,045,203 of SFFC funds were expended at Scripps Florida. Thus, about 91% of total research dollars came from sources other than Florida.

Section 9.5(c) **The number or value of patents obtained by Scripps with respect to Scripps Florida;**

Between July 1, 2018 and June 30, 2019, 20 foreign and domestic patent applications were filed and 9 U.S. Patents were issued. Since inception, 162 "families" of patent applications have been filed covering Scripps Florida technology, with each family containing 1-6 patent applications.

Section 9.5(d) **The number or value of licensing agreements executed by Scripps with respect to Scripps Florida;**

Two license agreements were executed between July 1, 2018 and June 30, 2019 with respect to Scripps Florida technologies.

Section 9.5(e) **The extent to which research conducted by Scripps Florida results in commercial applications;**

Because of the early stage of the technology being developed at Scripps Florida and the time delay attendant to further development, no commercial therapeutic applications have emerged to date. However, as noted in previous Annual Reports, several research reagents developed at Scripps Florida continue to be available commercially through various licensing arrangements.

Section 9.5(f) **The number of collaborative agreements reached and maintained with colleges and universities in Florida and with research institutions in Florida, including agreements that foster participation in research opportunities by public and private colleges and universities and research institutions in Florida with significant minority populations, including historically black colleges and universities;**

The Scripps Research Institute has developed a template entitled the Joint Cooperation Agreement (JCA) to encourage and support research collaborations with Florida institutions. Provisions are included to make it easier to collaborate on filing patents for jointly developed technologies and to share revenues from commercialized innovations. By executing these agreements in advance, we expect to streamline the scientific collaboration process between Florida organizations and Scripps Florida as they work together on biomedical research. Nine Florida institutions have currently executed this formal agreement with TSRI:

1. Florida International University;
2. University of Florida;
3. Florida Atlantic University;
4. University of Central Florida;
5. University of Miami;

6. Florida State University;
7. Nova Southeastern University;
8. University of South Florida;
9. Max Planck Florida Institute

Scripps scientists have also participated in formal scientific meetings with colleagues at Florida foundations, colleges and universities.

Section 9.5(g) **The number of collaborative partnerships established and maintained with businesses in Florida, including small businesses;**

Scripps Florida continues to maintain collaborative relationships with Florida based companies: CALM Therapeutics, Dyadic International, Deluge Biotechnologies, Emmune, Expansion Therapeutics, Florida Power and Light, Opko Health, Vova Ida Therapeutics and F1 Oncology.

CALM Therapeutics is developing novel therapeutics to treat Prader-Willi syndrome.

Deluge Biotechnologies, based in Jupiter, founded in 2017 based on technologies from Paegel and Kodadek labs at Scripps Florida employs DNA encoded epitope surrogate technology to develop disease diagnostics.

Dyadic International - A collaborative effort between scientists at Scripps Florida and Dyadic was established to provide a complete annotation of the genome of Dyadic's proprietary fungal organism, Chrysosporium lucknowense ("C1"). The knowledge gained from this effort is expected to facilitate further development of the C1 Host Technology as a robust platform for the discovery, development and production of various materials for medical and industrial applications. Furthermore, this collaboration promotes the development of a successful biotechnology cluster in South Florida.

Emmune, previously Immunathon, was founded in 2015 in Palm Beach County to commercialize technology from Mike Farzan's lab at Scripps Florida. The company is engaged in the development and commercialization of novel approaches for the treatment and prevention of HIV infection.

Expansion Therapeutics based on Disney lab technology at Scripps Florida with research facility in Jupiter is focused on development of therapeutics for RNA mediated diseases.

Florida Power and Light - Scripps is collaborating with Florida Power and Light, a Juno Beach, Florida-based power utility that is the principal subsidiary of NextEra Energy Inc., to develop novel and proprietary

technology which may yield cheaper and more effective ways at producing fuels and other commodities from natural gas.

Opko Health is a publicly traded healthcare company involved in the discovery, development, and commercialization of pharmaceutical products, vaccines and diagnostic products. Opko and Scripps are currently collaborating in three major areas: the development of novel diagnostic products to detect Alzheimer's and other diseases; the development of novel drug candidates to treat Parkinson's Disease; and the discovery of novel antibiotics.

Vova Ida Therapeutics is a Palm Beach County-based company founded in 2013 to commercialize research from the Corinne Lasmezas lab at Scripps Florida. The company is developing drugs to treat age-related neurodegenerative diseases, such as Alzheimer's disease.

F1 Oncology is a Palm Beach County-based company founded in 2015 focused on development of therapeutics for solid malignancies.

Others - During the past year Scripps Florida has established many partnerships with State of Florida businesses and small business entities. Below is a list of the current and past Scripps Florida business partners:

- 3-D MICROSCOPES INC
- ACE ENTERPRISES GROUP LLC
- ADVANCED CASE PARTS
- A-1 MOVING & STORAGE
- ACOUSTI ENGINEERING CO OF FLORIDA
- ADVANCE CASE PARTS INC
- ADVANCED PAINTING CONTRACTORS INC
- AERC RECYCLING SOLUTIONS
- AFFORDABLE DRY ICE
- AIR COMPRESSOR WORKS INC
- AIR ENERGY COMPRESSOR & VACUUM CO AIR EZE
- AIR EZE
- AIRCOMO
- AKRON BIOTECH
- ALKALI SCIENTIFIC LLC
- ALLSTATE RESOURCE MANAGEMENT INC
- ALPHAGRAPHICS OF THE PALM BEACHES
- ANDERSON MATERIAL HANDLING
- ANDREA CARTER
- ANGELAND CORP DBA VALBRIDGE PROPERTY ADVISORS
- ARCHIVES MANAGEMENT CENTERS INC
- BARNES INDUSTRIAL PLASTIC PIPING INC

- BEYEL BROTHERS INC
- BLINK BIO
- BOCA BUSINESS EQUIPMENT
- BOCA SCIENTIFIC
- BODY WELL INC
- BON TEMPS DINING LLC
- BRAZILIAN LIMO SERVICE
- BREAKERS PALM BEACH
- BRIERS BOBCAT INC
- BROADBERRY USA LLC
- CAFÉ CHARDONNAY
- CALGONATE CORP
- CANDACE WEST PHOTO
- CANVAS GFX INC
- CAPITAL CARPET AND TILE
- C'EST SI BON CATERING
- CHEMPEP INC
- CIH EQUIPMENT CO INC
- CITATION COMMUNICATIONS
- CKM CONSULTANTS LLC
- CLEAN EARTH SYSTEMS INC
- CLEAN FUELS OF FLORIDA
- CMH SOLUTIONS INC
- COMBINE SCIENTIFIC SERVICE LLC
- COMMERCIAL DOOR & ACCESS INC
- COMPONENT SUPPLY COMPANY
- CORS-AIR
- COURTYARD MARRIOTT JUPITER
- CRATERS & FREIGHTERS OF SE FLORIDA
- CRYO-TECH INC
- CUMMINS POWER SOUTH LLC
- CUSTOM SIGNS TODAY
- DASH DOOR AND CLOSER SERVICE INC
- DEBON AIR MECHANICAL INC
- DISKOVERY EDUCATIONAL SYSTEMS CORP
- DOOR SYSTEMS OF SOUTH FLORIDA INC
- EASY CAULKING & WATERPROOFING INC
- EMPIRE OFFICE INC
- ENCOR BIOTECHNOLOGY INC
- EXCELL CAULKING & WATERPROOFING
- FARMER & IRWIN CORP
- FERGUSON ENTERPRISES INC
- FIRE & SECURITY SOLUTIONS INC
- FLORIDA BEARINGS INC

- FLORIDA FLUID SYSTEM TECH INC
- FLORIDA LAMBDARAIL LLC
- FLORIDA PIPETTE CALIBRATIONS
- FLORIDA POWER & LIGHT
- FLORIDA SCIENTIFIC SERVICES INC
- FLOW CONTROL TECHNOLOGY
- FORTHRIGHT TECHNOLOGY PARTNERS
- FPL FIBERNET DIRECT
- GAS GENERATOR SOLUTIONS
- GOSSETT MARKETING COMMUNICATIONS
- GRAPHICS PLUS INC
- GREYSON COMMUNICATIONS INC
- GYMSOURCE
- HARDRIVES OF DELRAY INC
- HILL AUDIO VISUAL INC
- HOOVER PUMPING SYSTEMS CORPORATION
- HPE AUTOMATION
- HR DIRECT
- ID BADGE INC
- IMPERIAL FASTENER COMPANY INC
- INFINITY ROOFING & SHEET METAL
- J.P. GROWERS
- J.R. MANNO UNIFORM AND POLICE EQUIPMENT
- JACK WALSH CARPETS AND RUGS INC
- JACKSON LABORATORIES
- JC WHITE ARCHITECTURAL INTERIOR PRODUCTS
- JMB REPAIRS INC
- JUPITER AUTO BODY LLC
- JUPITER BEACH RESORT
- JUPITER ENVIRONMENTAL LABORATORIES INC
- JUPITER GOLF CART
- JUPITER MEDICAL CENTER INC
- JUPITER MEDICAL CENTER PHYSICIAN GROUP
- JUPITER PRINTING INC
- JUPITER STADIUM LTD
- K&M ELECTRIC SUPPLY INC
- KAUFMAN-DAENZER INSTRUMENTS
- KMARCUS RESOURCE GROUP LLC
- KMI INTERNATIONAL INC
- KO-MAR PRODUCTIONS INC
- LEO A. DALY CO
- LIFE SCIENCES ADVANCED TECH INC
- LILA PHOTO
- LJB EQUIPMENT SALES CO INC

- LOTSPEICH CO OF FLORIDA INC
- LUMIPROBE
- MARBLE KARE USA
- MAX PLANCK FLORIDA INSTITUTE
- MARK PLATING INC
- MC2 INC
- MEDREP TECHNOLOGIES INC
- MICHAEL B SCHORAH AND ASSOC
- MICRO OPTICS OF FLORIDA INC
- MOLECULAR DIMENSIONS
- MOLLIES TROLLIES
- MORROW ENTERPRISES
- MOSES & ASSOCIATES INC
- NEWTON SEATING CO
- NOLAN POWER GROUP LLC
- NOZZLE NOLEN INC
- ODUMS SOD INC
- OFFICE FURNITURE WAREHOUSE INC
- OJAY ENTERPRISES LLC
- OLD FLORIDA CATERING
- ONE BLOOD
- OVER THE TOP WINDOW CLEANING INC
- PACE MACHINE & TOOL INC
- P CHRISTAFARO'S INC
- PALM BEACH HOSE & FITTINGS
- PALM BEACH PARKING INC
- PALM BEACH POST, THE
- PARKLAND SCIENTIFIC INC
- PENTAIR AQUATIC ECO-SYSTEMS
- PGA NATIONAL RESORT AND SPA
- PRICEWATERHOUSECOOPERS LLP
- PRIORITY LIMOUSINE
- PREFERRED VALET PARKING LLC
- PROSHRED SECURITY
- RAPID ROOTER
- RCI CONTROLS LLC
- REGAL PAINTS
- RIGHT WAY PLUMBING INC
- ROOF B KLEEN
- SANFORD BURNHAM PREBYS MEDICAL DISCOVERY INST
- SENSATIONAL SIPS
- SHOES FOR CREWS LLC
- SHORELINE FLOORING SUPPLIES SIGN A RAMA
- SIMPLE BEHAVIORAL SYSTEMS SIR SPEEDY OF TEQUESTA

- SIR SPEEDY PRINTING CENTER
- SLATON & SONS ENTERPRISES INC
- SMELT FEED AND PET SUPPLY INC
- SMITHCO SERVICES
- SOLID WASTE AUTHORITY
- SOARING CHEFS INC
- SOUTH FLORIDA JANITORIAL AND POOL SUPPLY
- SOUTH POINTE SURGICAL
- SOUTHERN BIOMEDICAL
- SOUTHERN WATER SERVICES INC
- SPEEDY ROOTER INC
- STAR ELECTRIC
- STEINER – ATLANTIC CORP
- SULLIVAN ELECTRIC & PUMP INC
- SUNBELT RENTALS
- SUNCHASER SYSTEMS INC
- SYNQUEST LABORATORIES INC
- TECHNICO
- TIRE KINGDOM
- TM TOOLING INC
- TOO JAYS ORIGINAL GOURMET DELI
- TOP CUT LAWN SERVICE INC
- TROPIC ENERGY SERVICES
- TTNT LLC dba CORPORATE CATERERS
- TURBO VACUUM
- TURNKEY ROOF CONSULTING INC
- U & ME MOVING & STORAGE
- UNIVERSAL MEDICAL SYSTEMS INC
- UNIVERSITY OF FLORIDA
- UNIVERSITY OF MIAMI
- USA SCIENTIFIC INC
- VACTEK INC
- VACUUM SYSTEMS SPECIALISTS
- VISION DATABASE SYSTEMS
- WEST PALM BEACH PLASTICS
- WILDLIFE REMOVAL SERVICES
- WORKSCAPES SOUTH LLC
- WORLD ELECTRIC
- WORLD PRECISION INSTRUMENT INC
- XENOPUS EXPRESS INC

Section 9.5(h) **The total amount of funding received by Scripps with respect to Scripps Florida from sources other than Funding, including a breakdown of amounts received from Grants and other sources.**

Since inception through June 30, 2019, Scripps Florida has been awarded $619,427,858 in grants and sponsored research funding from state and federal agencies (including the NIH), foundations, pharmaceutical companies and other grantors. In addition, the County of Palm Beach provided $210 million to Scripps for construction of the permanent facility.

Funding received by Scripps Florida from sources other than Funding for the nine months ending June 30, 2019 are set forth below:

Other Revenue Sources	$	3,439,842	9 mos. Ending 6/30/19
Grant Awards	$	26,760,525	9 mos. Ending 6/30/19
Contributions*	$	4,051,928	9 mos. Ending 6/30/19

*The amount reported above was determined in accordance with generally accepted accounting principles. Therefore, certain non-cash items, such as promises to give, are reflected at their estimated net realizable value.

Section 9.5(i) **The number or value of spin off businesses created in Florida as a result of commercialization of the research of Scripps.**

Florida companies that were created to exploit licenses to technology developed primarily at Scripps Florida are described in Section 9.3(c) above. No attempt has been made by Scripps to assign a value to these spin offs, with the exception of a) Curna, which was purchased by Miami-based Opko Health for $10,000,000, b) Envoy Therapeutics, which was purchased by Japan-based Takeda for $140,000,000, and c) Padlock Therapeutics, which was purchased by New York-based Bristol-Meyers Squibb for up to $600,000,000.

Section 9.5(j) **The number or value of businesses that locate in Florida as a result of Scripps Florida.**

Scripps cannot determine the number or value of businesses located in Florida as a result of Scripps Florida.

Section 9.5(k) **The establishment and implementation of policies to promote supplier diversity using the guidelines developed by the Office of Supplier Diversity under Section 287 .09451, Florida Statutes, and to comply with the ordinances, including any small business ordinances, enacted by applicable local governments and which are applicable to Scripps Florida.**

The TSRI Procurement Department, led by Mr. Erik Duffey, Senior Sourcing Manager, continues to pursue opportunities to partner with the diverse business community. Scripps Florida continues to participate in

county, state and national diverse supplier shows. These shows help Scripps Florida to identify diverse businesses that can provide goods and services to the institute at a competitive price. Participation in these shows has resulted in partnerships with local companies that provide furniture, pipette calibrations, refrigeration services, relocation services, dry ice services, landscaping and irrigation services, building maintenance services, printing services, shredding services and more.

Section 9.5(l) **The designation by Scripps of a representative to coordinate with the Office of Supplier Diversity.**

Mr. Adrian Orozco serves in the position as the Scripps Supplier Diversity Coordinator. Mr. Orozco represents Scripps in working with small and minority business enterprises in the State of Florida and is actively involved in many state and local supplier diversity outreach programs.

Supplier Diversity Mission and Vision Statement:

Scripps Research is committed to maintaining an equitable and competitive business environment. As part of this commitment, we work to develop procedures and initiatives that will help ensure that all companies receive fair consideration. Scripps Research recognizes contractor and supplier diversity as an important component of its overall business effort and will continue to take all necessary affirmative steps to assure that small and minority businesses, women's business enterprises, and labor surplus area firms are used when possible for the procurement of goods and services.

Section 9.5(m) **The establishment and implementation of a program to conduct workforce recruitment activities at public and private colleges and universities and community colleges in Florida, regardless of their size, which request the participation of Scripps Florida.**

As Scripps Florida has reached a state of full-employment, other public and private institutions of higher education request our presence at their job fairs on an as needed basis to fill open positions.

Section 4.4(c)l **Scripps shall create new jobs at Scripps Florida, the number of which shall be measured at the end of each calendar year.**

On June 30, 2019, Scripps Florida employed 549 people (416 full-time and 133 part-time).

Section 4.4(c)2 **Beginning 18 months after Scripps' occupancy of its permanent facility, Scripps shall annually obtain $100,000 of non-state funding for each full-time equivalent tenured track faculty member employed at Scripps Florida.**

On June 30, 2019, Scripps Florida employed 43 tenure track Faculty. During the year beginning July 1, 2018 and ending June 30, 2019, Scripps Florida received 19 new grant awards and 94 renewal grant awards from the National Institutes of Health (NIH) and 7 new grant/contract awards and 6 renewal grant/contract awards from non-state entities other than NIH. The total initial (first year) award value for those awards was $53,730,207 or $1,249,540 per tenure track faculty member (43) at Scripps Florida.

Section 4.4(c)3 **No later than 3 years after occupancy of its permanent facility, Scripps shall apply to the relevant accrediting agency for accreditation of its Florida graduate program.**

The re-accreditation of the Scripps Research Graduate Program was successfully completed in early 2011. The Doctoral Program in Chemical and Biological Sciences is a bi-coastal Ph.D. program, reflecting the "two campuses, one institute" makeup of The Scripps Research Institute. Owing to the larger size and earlier date of establishment of the Ph.D. program on the La Jolla campus, the reaccreditation process was handled by WASC Senior College and University Commission (WSCUC). The re-accreditation process included a specific site visit and assessment of the Scripps Florida graduate program in October 2010, by Dr. Karen Holbrook, Senior Vice President for Research, Innovation & Global Affairs, University of South Florida, and President, University of South Florida Research Foundation. As a result of the overall review and re-accreditation process, Scripps' Graduate Program received re-accreditation for a nine-year period, effective March 7, 2011.
In February 2011, the WSCUC Commission considered the report of the evaluation team who conducted the reaccreditation visit to the Doctoral Program in Chemical and Biological Sciences of The Scripps Research Institute on October 25-27, 2010, including a report of a team member who visited the Jupiter, Florida campus. The Commission Action Letter dated March 7, 2011 stated that the evaluation team and Commission found many outstanding practices in place at TSRI. Among these was the development and use of learning rubrics designed to assess doctoral students in four outcome areas – scientific research, oral presentations, scientific writing, and critical thinking. The Commission also noted the continued growth of the Jupiter, Florida campus, and growing integration with the La Jolla campus. The Commission reaffirmed the accreditation of the Doctoral Program at Scripps Research and requested an Interim Report on the following four areas due in spring 2014: (1) strategic planning; (2) program review; (3) assessment of student learning; and (4) efforts to improve the diversity of faculty and students.

On July 10, 2014, a panel of the Interim Report Committee convened to consider the Interim Report submitted by The Scripps Research Institute (TSRI) on March 1, 2014. The panel reviewed the Interim Report and the

WSCUC action letter of March 7, 2011 asking for the report. The panel appreciated the opportunity to discuss the report with James Williamson, Dean of Graduate and Postdoctoral Studies; Dawn Eastmond, Director of Graduate Studies and Accreditation Liaison Officer; and Sharon Joyce, Evaluation and Accreditation Consultant. The conversation was informative and helped the panelists better understand Scripps Research's challenges and progress on addressing the concerns cited in the Commission's letter.

The panel commended Scripps Research for the thorough and comprehensive approach taken in the review. The report showed the strong commitment of the institution to being highly data driven in its program with a sophisticated level of attention to details and follow through based on what the data shows. The panel also commended Scripps Research for the sustained attention shown to student learning which may be a model of interest to the broader higher education community and especially graduate programs.

After discussion of the progress made by Scripps Research in addressing areas raised by WSCUC Commission, the following reaccreditation timeline was set for Scripps Research:

1. The Institutional Report and appendices are due on Nov. 27, 2018;
2. The Offsite Review of Scripps Research's materials by the evaluation team was conducted on February 5, 2019; and
3. The Accreditation Visits to Scripps Research by the evaluation team are scheduled for August 28-29, 2019 on the Florida campus and Sept 17-19, 2019 on the California campus.

Scripps Research began planning for the reaccreditation process in January 2017. A steering committee was appointed, a plan and timeline were adopted, and the first meeting was held in February 2017. The committee includes the Deans, Graduate Office staff and key faculty who serve as chairs on self-study committees from both campuses. On August 17, 2018, our WSCUC liaison, Richard (Dick) Osborn, visited the California campus and hosted a bicoastal workshop on preparing for the Institutional Report and the Accreditation Visit.

The Institutional Report addresses the WSCUC Standards and Criteria for Review through a narrative designed to convey Scripps Research's unique training environment and its commitment to graduate education. Scripps Research prepared by gathering materials and perspectives from ongoing self-study, qualitative feedback, formal surveys, and program review. Thus, the requirement of Section 4.4(c)3 has been satisfied, within the requirement of "no later 3 years after occupancy of its permanent facility".

Section 4.4(c)4 **Scripps shall purchase equipment for Scripps Florida [using State grant funds] according to an agreed upon schedule. Equipment purchases [acquired with State grant funds] are to be measured as of January 31st of each year.**

Equipment purchase expectations set forth in the agreed upon schedule were met several years ago. Fiscal year equipment purchases are reported in Section 9.3(b)iii.

Section 4.4(c)5 Doctoral Research. No later than 18 months after occupying its permanent facility, Scripps shall establish a program for qualified graduate students from Florida universities permitting them access to the facility for doctoral, thesis-related research.

Scripps Florida established a Ph.D. program in 2005 ahead of the September 2010 deadline, 18 months after the anticipated occupancy of the permanent facility.

In addition, Scripps Florida has entered into a Joint Education Agreement with Florida Atlantic University. In March of 2006, FAU and Scripps Florida signed a "joint education agreement" that provided a framework for planning and implementing a variety of programs to promote education and research in areas involving biomedical science and related fields. The programs envisioned include collaborations in the areas of graduate and professional education, including post-doctoral training; undergraduate education and training, including laboratory and administrative internships and, community outreach activities, including continuing education for credit and service activities. This agreement also provides a blueprint for partnerships with other educational institutions throughout the region and state to facilitate similar cooperative activities.

Section 4.4(c)6 Summer Internships. No later than 18 months after occupancy of the permanent facility, Scripps shall establish a summer internship for high school students.

The Education Outreach and Community Engagement (EOCE) Programs at the Florida Campus of Scripps Research were established in 2005, thanks to the great generosity of the William R. Kenan, Jr. Charitable Trust, to benefit the Palm Beach Community and the trainees. The High School Student Summer Internship Program (Kenan Fellows) is one of many initiatives implemented by Scripps Research EOCE Programs. These initiatives strongly support Palm Beach County's elementary, middle, and high school students, as well as teachers and other members of the community. Throughout the years, the program has evolved to also include outreach initiatives for undergraduate students, especially those attending Florida colleges and Floridians who move away for college. Lastly, Scripps Research graduate students and postdoctoral fellows have benefited from the EOCE Programs by obtaining science communication and teaching skills as well as mentoring experience.

The goals of Scripps Research EOCE Programs are to promote careers in biomedical research in younger generations, to create awareness in the community about the importance of supporting basic scientific research and drug discovery, and to train the next generation of scientists. This is the same foundation on which the program was originally built, and it is our promise that we will continue working diligently on delivering these goals.

2019 High School Interns

Name	School	Lab/Department
Diane Altidor	Palm Beach Central High School	Ja/Neuroscience
Lynn Deng	Suncoast Community High School	Scampavia/Molecular Medicine
Jacob Eisenberg	Palm Beach Central High School	Disney/Chemistry
Jonathan R. Hung	Suncoast Community High School	Huang/Molecular Medicine
Maxwell LeGates	Wellington High School	Renata/Chemistry
Elizabeth Lekah	Spanish River Community High School	Disney/Chemistry
Osinachi Nwosu	Lake Worth Community High School	Scampavia and Spicer / Molecular Medicine
Alexcia Plunket	Lake Worth Community High School	Bannister/Chemistry
Samay Saxena	William T. Dwyer High School	Tomchik/Neuroscience
Charlotte Silver	Saint Andrew's School	Valente/Neuroscience
Joel Yearick	Suncoast Community High School	Hansen/Molecular Medicine
Mulan Yin	Boca Raton Community High School	Karbstein/ISCB

Undergraduate Program – The ten-week undergraduate program continues to elevate the intensity and independence of the research experience. Working with faculty, graduate students, and postdoc mentors, interns are provided the research and laboratory experience needed to successfully compete in graduate school admissions and gain valuable experience outside the context of basic undergraduate laboratory instruction. The program culminates in a public research poster competition in which the top three are recognized with travel awards. As a result of the program, students return to their academic institutions able to participate in campus undergraduate poster sessions, act as ambassadors for the research and graduate programs offered at Scripps Research, and enjoy an enhanced knowledge base as they continue their classroom instruction. In addition, eight alumni of undergraduate internships are now pursuing doctorate degrees in the Graduate Program at The Scripps Research Institute.

2019 SURF/REU Interns

Name	School	Lab Host/Department
Hayden Anderson	Trinity University	Parker/Chemistry
Noah Bartfield	Florida State University	Huang/Molecular Medicine
Payton Demarzo	Eckerd College	Karbstein/ISCB
Marcel Elkouri	The College of Wooster	Miller/Molecular Medicine
William Gao	Vanderbilt University	Davis/Neuroscience
Karen Garcia	University of Puerto Rico, Cayey	Grill/Neuroscience
Aniyah Godwin	The Pennsylvania State University	Valente/Immunology and Microbiology
Alexandra Lish	Utah State University	Page/Neuroscience
Ashley Nichols	Chapman University	Solt/Immunology and Microbiology
Benjamin Oakes	University of Miami	Janiszewska/Molecular Medicine
Steffan Okorafor	University of Minnesota, Twin Cities	Puthe/Neuroscience
James Olsen	Grove City College	Disney/Chemistry
Alyssa Quintero	Syracuse University	Bohn/Molecular Medicine
Emily Shimizu	Harvey Mudd College	Renata/Chemistry
Adwoa Tweneboa	Wake Forest University	Paegel/Chemistry
Allison Weinstock	University of Florida	Kotejin/Molecular Medicine
Brittany Zengotita	University of Central Florida	Sundrud/Immunology and Microbiology

K-12 and Public Science Education Programs

In addition to the internship programs for high school and undergraduate students, Scripps

Research support the following K-12 and public education programs developed through the efforts of our Office of Education Outreach and Community Engagement, faculty, and research staff. Descriptions of all these programs as well as a list of the latest events are below:

School visits, partnerships, and community outreach – This section comprises many different types of educational outreach events and activities. Schools and non-profit organizations that run educational and career related programs for middle and high school students frequently reach out to us to lead a session or a hands-on activity. Significantly more students and members of the public are reached when we partner with these organizations and their initiatives. Many lessons have been created by the Education Outreach Office through the years and they are presented at these events. Examples of these lessons are: What Makes You 'You'- DNA Isolation, Drug Discovery, Model Organisms, Introduction to Science, The Basic You - An Introduction to Genetics, and Discovering the Brain and its Functions. Lessons are also offered in Spanish.

The Science Family Nights series, as well as programs created in partnership with Max Planck Florida Institute of Neuroscience (MPFI), Palm Beach State College, and the Women's Foundation of Palm Beach County are other outreach initiatives that we have led. We hope the William R. Kenan, Jr. Charitable Trust shares our vision of creating strong partnerships with other Palm Beach County organizations to raise awareness of the importance of science to our well-being as a society. Our plan is to continue to build upon these relationships in future years.

Diversity Visitation Event for Research and Graduate Education (DiVERGE) – This program that is geared toward undergraduate students, particularly from underrepresented and underserved backgrounds in the sciences, interested in biomedical research. It allows selected students from all over the nation to learn about ongoing research projects at TSRI, internship opportunities and the Graduate Program, crafting successful graduate school and internship applications, writing an effective personal and research statement, constructing and giving a compelling self-introduction, and science identity and the culture of science. In the future, we hope to continue to host this important program as we have found that underrepresented and underserved minorities are in need of additional training in order to become more competitive candidates for nationally ranked graduate programs.

Lending Library – This program offers teachers in the county and TSRI scientists the opportunity to borrow scientific equipment (e.g. microscopes, specimen slides, pipettes) that is safe to bring into the classrooms for science projects and hands-on activities. A few teachers have benefited from this new program thus far. In the summer of 2017, Suncoast High School teacher Brett Stubbs worked on creating curricula around the materials available and a website will be created this semester where teachers can see the inventory, request materials, and download lesson plans. In the future, we hope to create a blog where teachers can post questions to which our scientists can respond. The new website allows for blog posting. The education, training, and outreach programs will pilot this technology this summer.

Scripps Research Florida Education Outreach Events (September 2018-Present)

Date	Event
10/24/2018	Nova Southeastern College undergraduate students visit to Scripps Research Florida
11/13/2018	Classroom visit at Inlet Grove Community High School
1/03/19 - 1/05/19	Diversity Visitation Event for Research and Graduate Education (DiVERGE)
01/14/19	Atlantic Community High School chemistry class visit to Scripps Research – Florida campus
2/21/2019	Family Science Night at Inlet Grove High School
03/30/2019	Vero Beach STEAM Fest
4/13/2019	TLJMS Health and Science Career Symposium at Inlet Grove Community High School
4/25/2019	Barean High School visit to Scripps Research – Florida campus
5/14/2019	Biotech Broward County School science visit to Scripps Research – Florida campus
5/17/2019	Career Day at Poinciana Elementary, Boynton Beach

Section 4.4(c)7 Research Program. No later than three years after occupancy of the permanent facility, Scripps shall establish a research program for middle and high school teachers.

Scripps Florida Secondary Teacher Workshops

Scripps Florida is directing greater efforts to address the needs of classroom science teachers by establishing teacher workshops in basic science, math, and laboratory skills. The Instructional Support Program for Innovative Research Education (InSPIRE) programs offer direct interaction with the bioscience researchers at Scripps Research Florida and provide greater professional development opportunities for in-service middle and high school science teachers in a supportive and engaging environment. Recently, Dr. Brian Paegel, a faculty member in the Department of Chemistry, and his graduate students developed a new curriculum based on microscopy and image analysis applications. Portability of the lessons allows teachers to leverage the Institute's curriculum to their own classrooms during the course of the school year.

The program, which was supported by a grant from the National Science Foundation awarded to

Dr. Paegel, provides opportunities for teachers in Palm Beach County to attend the workshops at Scripps Research Florida. Through our partnership with the school district, we emphasize teacher recruitment from schools with limited resources in rural and urban Palm Beach County, particularly in areas with large underrepresented and disadvantaged student populations. The last workshop was held in July 2017, and ten middle and high school teachers attended it. Future workshops will be announced at our website.

Section 4.4(c)8 **Adjunct Professors. No later than 18 months after occupancy of the permanent facility, Scripps shall establish a program for adjunct professors.**

Many current Scripps Florida Faculty have received adjunct faculty appointments with the University of Florida, University of Miami and/or Florida Atlantic University. Such adjunct appointments are intended to provide a mechanism for graduate students enrolled in Florida research universities to collaborate with, to be co-mentored by, and to perform research in the laboratories of a Scripps Florida faculty member.

A mechanism has been established for faculty members at Florida institutions who have established collaborative research programs with Scripps Florida faculty to be appointed to an Adjunct Professor position. The process is initiated by a Scripps Florida faculty member who submits a nomination to his/her department chair. If the chair concurs, the chair submits the nomination to the Office of the President for review and approval.

Section 4.4(c)9 **Access for Science Projects. No later than 6 months after commissioning its high throughput technology, Scripps shall establish a program to allow open access for qualified science projects.**

Scripps Florida initiated the "Access to Technologies" program in January 2006 to invite scientists from Florida universities and other academic research institutions to use state-of-the-art screening technologies at Scripps Florida's facilities in Jupiter for qualifying projects. An additional "Core" platform is now available at the Scripps Florida facility that combines basic research with advanced technology.

Section 4.4(c)10 **Collaboration with Florida Colleges and Universities. Beginning June 2004, Scripps shall commence collaborative efforts with Florida public and private colleges and universities, and shall continue cooperative collaboration through the term of the Agreement.**

On-going and new scientific collaborations between Scripps Florida scientists and colleagues from Florida colleges, universities, and local companies were commenced in 2005.

Section 4.4(c)11 **Seminar Series. Beginning 18 months after Scripps occupies the permanent**

facility, Scripps shall establish an annual seminar series featuring a review of the science work done by Scripps and its collaborators.

Collaborative seminars feature prominent Florida-based speakers from the academic, biotechnology or pharmaceutical communities and focus on topics within the broad fields of biomedical science, advanced technologies applied to biomedical research, drug discovery, and energy. External seminars are part of the institute series, inviting prominent researchers from national and international institutions. Both serve as a major foundation for creating knowledge- and technology-sharing opportunities, team building, and collaborations among biomedical researchers between Scripps Florida, Florida, and other research and academic institutions and companies. The sessions are open to interested professionals within the Scripps Florida and Florida scientific communities.

In addition to summer intern day-to-day responsibilities, high school and college undergraduate interns attend specially-designed seminars throughout the course of the summer. The weekly summer intern series features faculty members and research associates from Scripps Florida. Each seminar highlights basic science principles and the research focus/application efforts of the Scripps Florida biology, chemistry, and translational research laboratories. Syllabus available upon request.

Section 4.4(c)12 **Collaboration with OTTED. Beginning June 2004, Scripps shall commence collaboration efforts with the Office of Tourism, Trade and Economic Development by complying with reasonable requests for cooperation in economic development efforts in the biomed/biotech industry, and no later than July 2004, Scripps shall designate a person who shall be charged with assisting in these collaborative efforts.**

Business outreach efforts include participation in meetings facilitated by local business and government agencies such as the Office of Tourism, Trade and Economic Development, Palm Beach County Business Development Board, and the technology Entrepreneurship & Capital Committee meeting. Similarly, community efforts involve presentations to local residential groups, various cultural organizations, and specialty groups. Numerous educational programs such as the Summer Research Internship, Science Saturday, and Introduction to Science series have been ongoing including presentation to elementary, secondary, and high schools, selecting high school students as interns, and hands-on workshops. Scientific outreach spans a variety of regional, state and international interactions from conferences, seminars and workshops facilitating peer-to-peer discussions.