

Scripps Florida Scientific Report 2009

• Scripps Florida Researchers Detail Kinetic Mechanism of Kinase Linked to Diabetes

Scripps Florida scientists scored something of a scientific hat trick last July in terms of determining exactly how a trio of potent, and potentially useful, kinases work.

The new study, which details the kinetic mechanism of the c-jun NH₂-terminal kinase 1 α (JNK1), follows on the heels of a study published last March. In that earlier study, the Scripps Florida scientists described the same type of kinetic or binding mechanism for the closely related JNK3

"A detailed picture of the kinetic mechanism involved in binding and activation is an absolute necessity for the design of specific and potent inhibitors," said Phil LoGrasso, senior director of drug discovery and Associate Professor of Molecular Therapeutics at Scripps Florida, and author of the new study with Research Associate Brian Ember. "Understanding the kinetic mechanism of protein kinases is important because it affects how we look for inhibitors—you develop your drug screening process differently depending on whether the binding is ordered or random."

The study found that like JNK2 and JNK3, JNK1 kinase binds randomly to two key substrates—ATF2, a transcription factor that regulates gene expression and ATP (adenosine triphosphate), often referred to as the energy currency of the cell; ATP could bind first, then ATF2 binds, or vice versa. Once the substrates are in place, a product is released from the active site. These products activate other processes, such as turning on other genes, for example.

The study also described the inhibition pattern of a known JNK binding protein, which is an endogenous inhibitor of JNK.

That inhibitor is a JIP-1 peptide, a naturally occurring protein that regulates the signaling activity of JNK1; JIP was found to compete for binding with ATF2, but was noncompetitive with ATP.

Diabetes Link

LoGrasso pointed out that some recent studies have directly implicated JNK1 in increased blood glucose levels, insulin resistance, and diabetes, which make further study of this enzyme highly relevant in terms of potential treatment development.

"That's the essential component of this particular kinase," LoGrasso said. "It has a link to type 2 diabetes, so inhibition of it could be a treatment. Most of this comes from knock out data in mice, where the gene is knocked out and expression of the protein is knocked out—the same effect you might get with a small molecule inhibitor. The result is that, in

animals, at least, the insulin resistance goes away, blood glucose levels go down, and insulin signaling returns to normal."

LoGrasso stressed that JNK 2 and JNK 3 inhibitors also have a future as potential therapeutics.

Recent studies have shown that mouse models became highly resistant to neuron damage when the neural-specific JNK3 gene was blocked, which opens the door to a lot of potentially good things, especially in neurodegenerative diseases like Parkinson's disease, a primary target of LoGrasso's drug discovery research program.

Relative Kinases

Like its relatives, JNK1 is a mitogen-activated protein (MAP) kinase that phosphorylates (adds a phosphate group to) protein transcription factors such as ATF2 and ATF1. JNKs are stress-activated by a number of things including cytokines, UV radiation, and hypoxia.

The JNK kinases are involved in a range of cellular signaling pathways. As a part of the mitogen-activated protein (MAP) kinase family, kinases like JNK1 have been implicated in a range of important processes, including metabolic reactions, gene regulation, and cell proliferation, all areas that when something does go wrong, things like cancer, diabetes, and inflammatory disease begin to happen.

As a result, small molecule kinase inhibitors are among the most sought after therapeutic proteins these days. At the moment, all kinase inhibitors under development target what is known as the ATP-binding pocket. This turns out to be somewhat problematic because the ATP pocket is common to all protein kinases. As a result, selectivity becomes difficult—it's hard to find specific inhibitors that can actually tell the difference among the 500 plus members of the protein kinase family.

"This new study is basic mechanism biochemistry," LoGrasso said. "What could have a great impact on drug discovery is that if you can understand the molecular features that give rise to substrate competitive inhibitors, you have a potential for a much more selective and much less toxic drug. Understanding these basic binding principles means you can potentially create substrate selective inhibitors that are more effective."

- **Scripps Florida Scientists Find Blocking a Receptor Significantly Decreases Nicotine Addiction**

Scientists at Scripps Florida, in a paper published in November, reported that they have found that blocking the receptor for a specific neuropeptide--short chains of amino acids found in nerve tissue--significantly decreases the desire for nicotine in animal models. In addition, these data may explain intriguing findings from human smokers who spontaneously quit smoking when they suffer brain damage restricted to a small portion of their frontal cortex.

The neuropeptide, hypocretin-1 (Orexin A), may initiate a key signaling cascade, a series of closely linked biochemical reactions, which maintains tobacco addiction in human smokers and could be a potential target for developing new smoking cessation treatments.

"Blocking hypocretin-1 receptors not only decreased the motivation to continue nicotine use in rats, it also abolished the stimulatory effects of nicotine on their brain reward circuitries," said Paul Kenny, the Scripps Research scientist at Scripps Florida who led the study. "This suggests that hypocretin-1 may play a major role in driving tobacco use in smokers to want more nicotine. If we can find a way to effectively block this receptor, it could mean a novel way to help break people's addiction to tobacco."

Cigarette smoking is one of the largest preventable causes of death and disease in developed countries, and accounts for approximately 440,000 deaths and \$160 billion in health-related costs annually in the United States alone. Despite years of health warnings concerning the well-known adverse consequences of tobacco smoking, only about ten percent of smokers who attempt to quit annually manage to remain smoke free after one year, highlighting the difficulty in quitting the smoking habit.

In the study, Kenny and a postdoctoral fellow in his laboratory, Jonathan Hollander, blocked the hypocretin-1 receptor using low doses of the selective antagonist SB-334867, a commercially available compound often used in research.

"While hypocretin 2 systems, otherwise known as orexin B, have been mainly implicated in regulating sleep," Kenny said, "hypocretin 1, also known as orexin A, appears to be more involved in regulating motivated behavior. Our previous studies in close collaboration with other Scripps Research scientists have shown that hypocretin-1 receptors play a central role in regulating relapse to cocaine seeking. With that in mind, it seemed reasonable to test whether it was involved in nicotine reward as well."

The new study indeed showed that blocking the receptor in rats significantly decreased nicotine self-administration and also the motivation to seek and obtain the drug. These findings suggest that hypocretin-1 receptors play a critical role maintaining nicotine-taking behavior in rats, and perhaps also in sustaining the tobacco habit in human smokers.

In addition, the study highlighted the importance of hypocretin-1 receptors in a brain region called the insula, a walnut size part of the frontal lobe of the brain. A highly conserved brain region, all mammals have insula regions that sense the body's internal physiological state and direct responses to maintain homeostasis. The insula has also been implicated in regulating feelings of craving. In a recent groundbreaking study, it was reported that smokers who sustained damage to the insula lost the desire to smoke, an insight that revealed the insula as a key brain region that sustains the tobacco habit in smokers. Until the new study, however, the neurobiological mechanisms through which the insula regulated the persistence of tobacco addiction remained unclear.

The new study sheds light on this question, showing that hypocretin-containing fibers project significantly to the insula, that hypocretin-1 receptors are expressed on the surface of neurons in the insula, and that blockade of hypocretin-1 receptors in the insula, but not in the adjacent somatosensory cortex region (which also records and relays sensory information), decreases nicotine self-administration. The effects of blocking hypocretin-1 receptors only in the insula, however, were less than blocking these receptors in the brain as a whole, suggesting that hypocretin transmission in other brain regions may also be playing a role in nicotine reward.

Working with scientists from Scripps Florida's Translational Research Institute, Kenny and his colleagues are now searching for new antagonists at hypocretin-1 receptors that are less toxic than the compound used in the published experiments in the hopes of furthering the development of a human therapy

- **Phil LoGrasso's Quartet of Important Papers**

Phil LoGrasso, Scripps Florida's senior director of drug discovery and an associate professor in molecular therapeutics, recently published four papers focused on the discovery of several potent and highly selective Rho Kinase (ROCK-II) inhibitors, not to mention the development of a cell-based assay that allows for the rapid functional assessment of these same inhibitors.

"In these papers, we're reporting on hundreds of inhibitors but we've actually produced thousands," LoGrasso said. "Which ones might be worth pursuing out of the hundreds identified in these papers? We hope two to three compounds per class will have the properties we want in an effective inhibitor."

Protein kinases like ROCK, a member of the serine/threonine kinase family, are enzymes that add phosphate groups to other proteins and modify their behavior, a process commonly known as phosphorylation. Kinases may modify as many as one-third of all proteins and, since kinases regulate most cellular pathways within the cell, especially signal transduction, this makes them serious contenders for the most favored drug targets of all time.

According to one 2006 report, described in Drugresearcher.com, the kinase-targeted drug market is set to grow from \$12.7 billion in 2005 to \$58.6 billion in 2010; a 2008 Reuters story pointed out that kinase inhibitors now account for 20-30 percent of all drug discovery programs.

In animal studies, Rho-kinase has been shown to be substantially involved in cerebral vasospasm, arteriosclerosis, ischemia/reperfusion injury, hypertension, pulmonary hypertension, stroke and heart failure, central sympathetic nerve activity, and, last but not least, glaucoma.

None of this surprises LoGrasso, since it's the very same area he's studying himself.

"We're really focusing on glaucoma with these new inhibitors," he said. "I think we might have a compound that we could put into a safety assessment, which is pretty deep in preclinical development, in the next six to eighteen months. From there, you could have an IND [Independent New Drug application] filed within a year."

A New Class of Kinases for Glaucoma

Of the four papers, the one published in the Journal of Medicinal Chemistry stands out. This study was a collaborative effort of Scripps Florida and the Duke University School of Medicine.

LoGrasso and his colleagues successfully identified an entirely new class of potent and highly selective ROCK-II inhibitors, one of which, labeled compound 5 (originally SR3677), turned out to be very potent indeed.

"Compound 5 is the most selective ROCK inhibitor ever published," LoGrasso said. "There might be another one in a laboratory somewhere, but this is most selective we've ever seen. Selectivity is critical because there are 518 other kinases that are ATP competitive inhibitors—ATP is the energy transport vehicle within the cell—and you want to make certain that you don't inhibit any of these. If you do, you may develop significant safety issues."

In terms of glaucoma, the study showed that compound 5 was efficacious both in increasing aqueous humor outflow and in modulating or reducing phosphorylated-myosin light chain, making it a good candidate for treating glaucoma. Myosin light chain phosphorylation, an event important for cellular contraction, is involved in the development of diseases like glaucoma, as well as hypertension and cancer.

"Generally speaking, the potency of a compound depends on how tight it binds to the target protein," said Yangbo Feng, the first author of the study. "We're delighted that the Rho kinase protein obviously likes the molecules we designed as its inhibitors. This is mainly due to several special structural elements we designed and built into our novel scaffolds, which differentiate our ROCK inhibitors from others."

Further optimization of the novel compound, plus additional pharmacology studies, are already under way.

More to Come

Another study, published in Biochemical and Biophysical Research Communications, describes a cell-based myosin light chain phosphorylation assay for the rapid functional assessment of novel ROCK inhibitors. The results showed that the measurement of changes in myosin light chain phosphorylation can be successfully used to screen a larger set of small molecule ROCK inhibitors.

"The throughput and ease of this functional assay enables it to be used for medicinal chemistry efforts, potentially not just ROCK but for other proteins and pathways regulating myosin light chain phosphorylation," LoGrasso said.

Finally, in a pair of studies, both published last October in the journal *Bioorganic & Medicinal Chemistry Letters*, LoGrasso and his colleagues identified a slew of new ROCK inhibitors, in two distinct classes.

In the first study, the scientists synthesized a series of chroman-3-amides with remarkable inhibition of ROCK-II, all of which favor further study. Assessment of the *in vivo* efficacy of these inhibitors, specific absorption rate studies and a detailed analysis of factors affecting affinity for both ROCK-I and ROCK-II are all under way.

In the second study, LoGrasso and his colleagues discovered an early lead compound, which they gave the name "amide 1," with a high affinity for ROCK-II, good selectivity over a related kinase, plus high potency. Of course, amide 1 is just the beginning; further work added a potent series of benzimidazole- and benzoxazole-based ROCK inhibitors from that initial amide lead.

"Ultimately all these inhibitors will have the same properties for drug development," LoGrasso said, "but since they're from different chemical series, they could later be differentiated by toxicities that we aren't aware of now but that may pop up during further testing."

As with the other discoveries, the group has already embarked on a effort to optimize the compounds with an eye on developing selective ROCK (I and II) inhibitors and the development of pharmacokinetic properties tailored to specific therapeutic applications.

"The compounds in the last two studies are companions in the sense that the same biological tests were conducted for both," he said, "but they're different structurally. I wouldn't even call them cousins, really; they're just friends. The chrom-3-amides hold more promise for glaucoma, while the benzimidazole and benzoxazole based inhibitors are probably better for peripheral applications."

• **Scripps Florida Scientists Find Novel Use for Old Compound in Cancer Treatment**

In January of this year, scientists from the Scripps Florida reported that they had found a potentially beneficial use for a once-abandoned compound in the prevention and treatment of neuroblastoma, one of the most devastating cancers among young children.

The compound, α -difluoromethylornithine or DFMO, targets the activity of a specific enzyme and, even in very limited doses, is effective in protecting against the malignancy in animal models.

"The drug, which was developed as a cancer therapy and later shelved because of toxicity concerns, has been around since the 1970s," said John Cleveland, Ph.D., chair of the Scripps Florida Department of Cancer Biology whose laboratory conducted the study. "But over the past five years, it has undergone a rebirth as a chemoprevention agent, first showing efficacy in animal models of human cancer and more recently in human prostate and colon cancer. Our study shows that it likely works in a large cast of tumors, even those having poor prognosis, like high-risk neuroblastoma."

Neuroblastoma is a childhood malignancy of the sympathetic nervous system (part of the nervous system that serves to accelerate the heart rate, constrict blood vessels, and raise blood pressure) that accounts for nearly eight percent of all childhood cancers and 15 percent of pediatric cancer-related deaths. Its solid tumors arise from developing nerve cells, most commonly in the adrenal gland, but also in the abdomen, neck, and chest. Neuroblastoma usually occurs in infants and young children, appearing twice as frequently during the first year of life than in the second.

Tragically, children with stage IV, high-risk neuroblastoma have a less than a 40 percent chance of long-term survival.

The best-known genetic alteration involved in neuroblastoma is the amplification of the proto-oncogene—a molecule that when overexpressed can cause cancer—called MYCN. Amplification of MYCN occurs in about 20 percent of all neuroblastoma and is associated with the high-risk form of the disease. Targeting this and related genes directly might be therapeutically tempting, the study noted, but highly problematic because the oncoproteins they produce are also required for the growth of most normal cell types.

As a result, Cleveland and colleagues focused on inhibiting ornithine decarboxylase (Odc), a protein that contributes to cancer cell growth and that is a target of the proto-oncogene MYCN. Increased levels of Odc are common in cancer, and forced Odc expression in animal models has been shown to lead to increased tumor incidence. Recent findings have shown that Odc overexpression is also an indication of poor prognosis in neuroblastoma. DFMO, the drug used by the Cleveland team, inhibits the activity of Odc.

To test the effect of DFMO on preventing neuroblastoma, the study used a transgenic mouse that faithfully models many of the hallmarks of MYCN-amplified neuroblastoma in humans.

"We were able to prevent neuroblastoma caused by MYCN, delaying the onset and incidence of this tumor type" said Cleveland. "What's even more compelling, we used low doses of the drug, and DFMO only had to be given for a moderate amount of time to prevent cancer."

While DFMO selectively impaired the proliferation of MYCN-amplified neuroblastoma, it had no appreciable effect on non-MYCN-amplified neuroblastoma cell lines, indicating that the growth of the former is "addicted" to Odc.

"Our study offers a strong suggestion to the clinical cancer community that they should keep an open mind about the Odc-polyamine pathway, and that this particular pathway might represent a novel therapeutic angle to tackle this malignancy." Cleveland said. "While there are valid safety concerns about giving DFMO to pediatric patients suffering from advanced stage MYCN-amplified neuroblastoma, it may be time to revisit the issue as our study showed that transient treatment with DFMO is sufficient to provide chemoprevention and may show benefit for this otherwise lethal malignancy."

• **Scripps Florida Scientists Uncover Potential New Target for Schizophrenia Treatment**

Also in January, scientists from Scripps Florida and colleagues for the first time linked a specific microRNA to behavioral problems frequently associated with psychiatric disorders such as schizophrenia. The finding presents new opportunities in the development of potential treatments.

Scientists had previously known that a number of brain disorders—including schizophrenia, autism, attention deficit hyperactivity disorder (ADHD), mood disorders, and other psychiatric illnesses can involve a disruption in a signaling process in the brain involving N-methyl-D-aspartate (NMDA) glutamate receptors, which are regulators of rapid neurotransmission and synaptic plasticity—the ability of neuronal connections to change strength. Yet the specific molecular components of this disruption have remained a mystery.

In the new study, however, the research team, led by Scripps Florida Professor Claes Wahlestedt, shed some light on the molecular mechanisms associated with NMDA-related behavior problems in mice. Specifically, the team discovered that disruption of NMDA signaling is associated with a reduction of a non-coding microRNA known as miR-219. Non-coding RNAs are small molecules that do not produce proteins, yet often play a vital role in gene expression.

"In the study we asked the question, 'Which noncoding RNA players might have something to do with NMDA signaling in the prefrontal cortex part of the brain?'" said Wahlestedt. "As we discovered, miRNA-219, which is a brain-specific microRNA, plays an integral part in the NMDA signaling process. Our findings strongly support the idea that this previously uncharacterized microRNA significantly modulates NMDA signaling and associated behavioral problems."

To learn more about the molecular components of these behavior problems, the scientists disrupted NDMA signaling using both pharmacological and genetic methods. For the pharmacological portion of the study, the scientists treated normal mice with dizocilpine, a known NMDA antagonist reported to cause changes in human patients that resemble acute psychosis. For the genetic portion of the study, the scientists studied mice that had a mutation in the gene *Grin1*, which results in a dramatic decrease in NMDA expression levels. The scientists found that, in both cases of NMDA disruption, levels of miR-219

were reduced in the prefrontal cortex, the area of the brain associated with memory, personality, and decision-making.

Interestingly, pretreatment of the mice with the antipsychotic drugs haloperidol and clozapine prevented the behavioral abnormalities and reduction of miR-219 in both groups.

The scientists went on to shed light on the actions of miR-219 in the NMDA signaling cascade (a chain of reactions where one reaction is consumed by the next). The team found that miR-219 targets a key component of the signaling cascade, specifically, CaMKII γ , a calcium/calmodulin-dependent protein kinase (kinases are enzymes that add a phosphate group to molecules and are important to signaling). The study showed that miR-219 repressed the levels of the kinase in vitro and that inhibition of miR-219 in vivo altered the expression of CaMKII γ . Calcium is important in signal transduction pathways, where it helps release neurotransmitters from neurons; calcium flow through NMDA is believed to play a vital role in synaptic plasticity.

"We have shown the involvement of miR-219 in the signaling cascade, and with this important calcium kinase, and that makes miR-219 even more of a target of interest for potential drug development," Wahlestedt said. "These two facts alone mean that we should look at this microRNA far more than we have in the past."

- **A Major Advance in Understanding Cell Differentiation**

Not a lot is known about the moment when cell division—proliferation—stops and when cell differentiation—when the cell decides what it wants to be when it grows up—begins. That lack of knowledge has been a problem for cancer research. If the cell goes into proliferative overdrive and skips exiting the cell cycle (the sequence of growth and division of a cell), you get a rapidly dividing cell or cancer.

"There must be a reason why cells exit the cell cycle at a certain point, but only when we understand it thoroughly will we be able to tweak the machine to get the cells to stop dividing—and that would have tremendous potential," said Nagi Ayad, assistant professor in the Scripps Florida Department of Cancer Biology. "It has been done with transcription factors, but from a pure cell cycle perspective it's still unclear."

Scientists do know that the transition from division to differentiation takes place after the anaphase stage of mitosis—the division of chromosomes in the cell nucleus into identical chromosomal twins. The anaphase promoting complex or APC is a large multi-protein assembly activated during the anaphase stage. The APC targets a number of proteins for destruction, triggering a process that leads to the destruction of cohesion, a protein complex that holds together the chromatids—those identical DNA twins—ushering in the cell's exit from mitosis and onto the next stage of cell division, when the twins separate completely and two new cells are formed.

Ayad, who is one of only a handful of researchers looking at APC outside the cell cycle, has added an important new twist to what little is known about this essential complex in cell cycle exit.

"APC is such a critical complex that if you remove it from the cell cycle in organisms from yeast to man, the cell will die," he said. "It is absolutely essential. Our new study (published in February) shows that this protein has a role in cell cycle exit, and that it responds to nerve growth factor in different cell types."

In the study, Ayad and his colleagues show that APC is required to coordinate differentiation programs with cell cycle exit, and that Skp2, a key APC substrate—the molecule acted upon—is critical to this response.

The study further suggests that the APC mediated degradation of Skp2 may be regulated by cues from nerve growth factor signaling. Nerve growth factor is a small secreted protein which induces the differentiation and survival of target neurons. (Newlyweds take note: In an interesting side study done in 2005, Italian scientists at University of Pavia found that when people first fall in love they exhibit high levels of nerve growth factor, but these levels return to normal after one year.)

"We found that APC is downstream from the nerve growth factor pathway, which is well known to induce differentiation into neuron-like cells," Ayad said. "If you inhibit APC activity you inhibit cell cycle exit and differentiation of the neuron. Our model is that the nerve growth factor activates APC, which then tags the APC substrate SKP2 for degradation. That action leads to an increase in the level of p27, a cell cycle inhibitor protein known to set the threshold for cell cycle exit and differentiation, particularly neuronal differentiation. The next big question is how does nerve growth factor actually talk to APC – we're working on that."

It has been a little over ten years since APC was first characterized as a highly regulated ubiquitin ligase—a protein that marks other proteins for destruction by the proteasome, a large protein complex in the cell that basically rip proteins into pieces, once described as garbage cans with teeth. APC appears to have multiple functions at different cellular locations, modulating diverse processes in addition to cell cycle regulation.

"What I hope our lab will be able to show over the next few years is that the decision to leave the cell cycle is made early on," said Ayad. "The decision point, prompted by nerve growth factor or some other kind of differentiation factor, can't be at the last mitotic division and it's out."

Ayad and his colleagues are also taking the next step in the process, looking for small molecule probes to inhibit degradation of certain proteins that work in the cell cycle and that might be useful in cancer therapy.

- **Scripps Research Team Identifies Key Molecules that Inhibit Viral Production**

A team from Scripps Florida has found a way to inhibit viral production of the Hepatitis C virus (HCV). The advance has the potential to accelerate future research on the virus life cycle and to aid in the development of novel HVC drugs.

The research, led by Professor Donny Strosberg of Scripps Florida, was published in March of this year.

In the new study, Strosberg and his colleagues describe peptides (molecules of two or more amino acids) derived from the core protein of hepatitis C. The team found that these peptides inhibit not only dimerization of the core protein (the joining of two identical subunits), but also production of the actual virus itself.

"We went for the simplest solution, taking a peptide from core to see if we could block the interaction," Strosberg said, "and it did."

The Problem with Hepatitis C

With over 170 million people infected worldwide by HCV, new therapeutic strategies for HVC—a blood-borne disease that affects the liver—are urgently needed.

But one of the critical problems in developing drugs for HCV is that it mutates at such prodigious rates. An RNA virus like hepatitis C can mutate at a rate estimated as high as one million times that of DNA viruses; in contrast, DNA viruses contain an enzyme (polymerase) that acts as something of a proof reader to ensure that newly transcribed DNA strands are the same as the original, helping to reduce mutations.

"In one sense, the ongoing issue with hepatitis C is that there are still so very few drugs to treat the virus and very few tools to study it," Strosberg said. "We set out to develop new tools and to identify a new target – core, the capsid protein. By targeting the interactions of core with itself or other proteins, we could reduce the problem of rapid mutation not only because the core protein mutates significantly less, but also because mutations that would affect the interface between core and itself or other proteins would often be more likely to deactivate the virus, in contrast to mutations in viral enzymes which often lead to increased resistance to drugs."

Recent efforts to develop therapeutic strategies against HCV have concentrated on designing inhibitors that target several of the 10 HCV proteins that comprise the virus, including mostly the non-structural proteins. However, as the study points out, the one HCV structural protein that has not been targeted yet is the core protein, the one responsible for assembly and packaging of the HCV RNA genome.

The Core of the Matter

Core, the most conserved protein among all HCV genotypes, plays several essential roles in the viral cycle in the host cell; studies have suggested that these core-core or core-other protein interactions can modulate various functions including signaling, apoptosis or programmed cell death, lipid metabolism, and gene transcription.

The core protein is particularly important in the assembly of the hepatitis C nucleocapsid, an essential step in the formation of infectious viral particles; the nucleocapsid is the viral genome protected by a protein coat—the capsid. This core protein plays an essential role in the HCV cycle during assembly and release of the infectious virus, as well as disassembly of viral particles upon entering host cells.

Looking closely at the core interaction with itself, Strosberg developed several novel quantitative assays or tests for monitoring these protein-protein interactions with the specific goal of identifying inhibitors of the core dimerization, which would block virus production.

"People have been dreaming about inhibiting protein-protein interactions, as a new El Dorado for finding novel drug targets," says Strosberg, "but few conclusive studies have emerged, except in the virus-host area."

Inhibition of HCV Production

The new research, however, led to the discovery of two peptides that inhibited HCV production by 68 percent and 63 percent, respectively; a third related peptide showed 50 percent inhibition. When added to HCV-infected cells, each of the three peptides blocked release but not replication of infectious virus; viral RNA levels were reduced by seven fold. Strosberg notes that the efficacy of small molecules like these can often be improved over initial levels.

"After we'd finished our work, we learned that Frank Chisari—one of the leading experts on HCV who works at Scripps Research in La Jolla—had been looking at similar peptides using a very different approach," said Strosberg. "One of his peptides was the same as ours—it also inhibited virus production. It's an incredible coincidence and a confirmation of our study."

• Scripps Florida Scientists Devise Accelerated Method to Determine Infectious Prion Strains

Current tests to identify specific strains of infectious prions, which cause a range of transmissible diseases (such as mad cow) in animals and humans, can take anywhere from six months to a year to yield results—a time-lag that may put human populations at risk.

In May, a group of scientists from Scripps Florida reported the development of a new method that cuts this critical time lag by several months.

"Because some prion strains are pathogenic for humans and some are not, it's vital that we know the difference when we find them in the field and when we study them in the laboratory," said Corinne Lasmézas, a professor in the Department of Infectology at Scripps Florida who led the study. "Currently, the identification process for mouse-adapted strains takes between six and eight months and can take as long as a year, depending on the strain. Our accelerated method reduces that time to around four months."

The new method for distinguishing among various strains combines a transgenic mouse model with a rapid and sensitive cell-based procedure, the Cell Panel Assay developed by Scripps Florida's Charles Weissmann (chair of the Department of Infectology) and Sukhvir Mahal (senior staff scientist), also investigators on the new study.

"There are about 20 prion strains known in mouse models," Lasmézas said. "We still don't understand what determines the difference among strains even though it's very important, especially for any potential therapeutic development. Our new method should help quicken the pace of research."

The Mysteries of Prions

Prion diseases, also called spongiform encephalopathies, are a group of closely related, fatal neurodegenerative disorders that affect mammals, including cows, sheep, and deer, as well as humans. Different strains of the infectious agent, called a prion, cause mad cow disease, chronic wasting disease, and different forms of scrapie and human Creutzfeldt-Jacob disease.

Mad cow disease has had devastating consequences for bovine livestock populations, particularly in Europe, and for humans who have consumed contaminated beef products.

To date, there have been more than 200 recorded human fatalities worldwide due to mad cow disease. Creutzfeldt-Jacob disease, a low-incidence but always fatal disease, affects humans in all countries.

Prions consist mainly or entirely of an abnormal form of a normal cellular protein. They multiply by converting their normal counterparts into a likeness of themselves, which may aggregate to form deposits called amyloid. Accumulation of different kinds of amyloid plays a role in a wide range of neurodegenerative diseases, including Alzheimer's and Parkinson's diseases.

Currently, different varieties of prion strains are identified in mouse models according to incubation time, clinical symptoms, and localization of brain lesions.

Accelerated Incubation

In the new test developed by Lasmézas and Weissmann, a transgenic mouse line called Tga20 plays an important role, because it succumbs rapidly to prion disease.

"The prions primarily target the brainstem and the thalamus of this transgenic mouse, explaining why these animals have a shorter incubation time than their normal counterparts," Lasmézas said. "The prion aggregates also don't spread evenly to other brain regions, and their distribution is characteristic for different strains."

Importantly, the brain tissue can be subjected to the Cell Panel Assay, which unlike the current histological method, doesn't require time intensive examination of brain lesions and can be completed within two weeks, Lasmézas added. The test has been partially automated.

Development of the method provided the scientists with the opportunity to make the observation that there was, in some brain regions, little relationship between the amount of abnormal prion protein deposition and the amount of normal prion protein. This was something of a surprise, Lasmézas said, but not totally unexpected.

"We and others believe that the prion protein may not be the sole player in these diseases," she said. "Perhaps there is a co-factor, or perhaps the protein structure differs somewhat from one brain region to the next. We don't know. Just like we don't really know the reason for the different behavior of the various prion strains—is this cause or effect? Our new method should help accelerate the process of discovery."