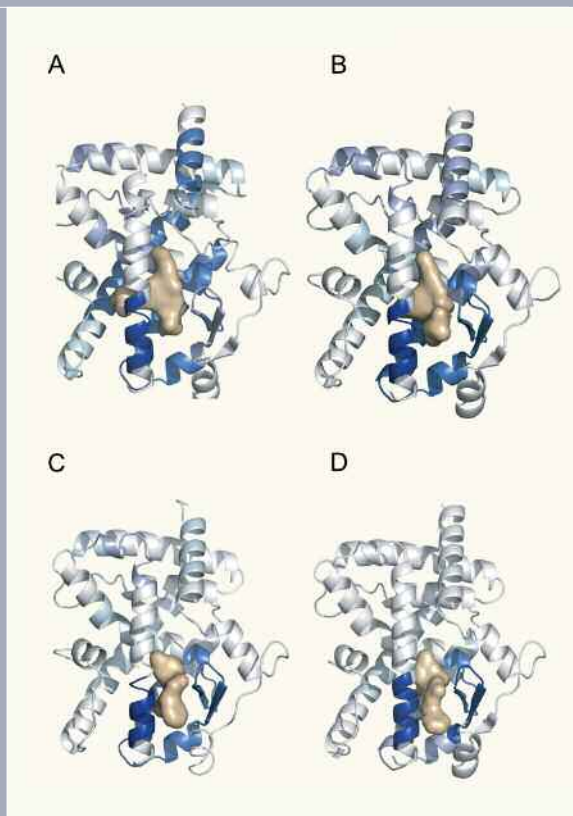
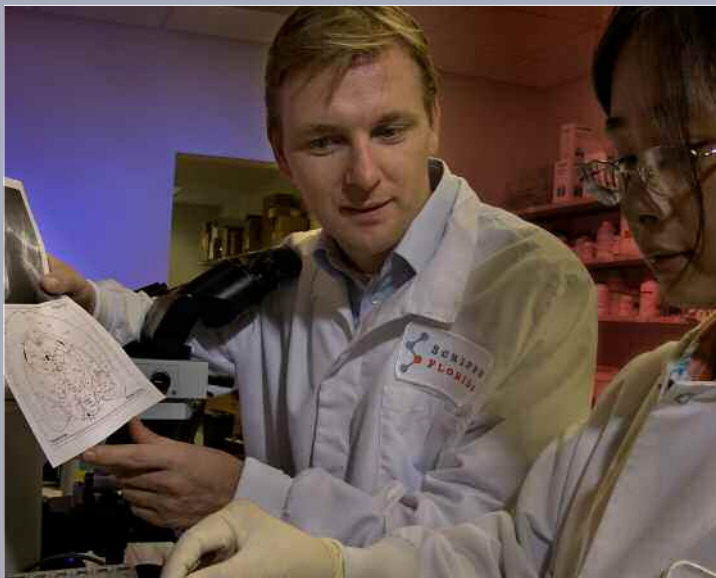


Molecular Therapeutics

Ribbon diagram of the ligand-binding domain of peroxisome proliferator-activated receptor γ (PPAR γ) overlaid with differential hydrogen-deuterium exchange data. Ligand density is displayed as a surface representation. The 4 ligand-receptor complexes are PPAR \pm MRL-20 (full agonist; A), MRL-24 (partial agonist; B), nTZDpa (partial agonist; C), and BVT.13 (intermediate agonist; D). The degree of stabilization of receptor dynamics as determined by hydrogen-deuterium exchange is shown in color; dark blue indicates the largest magnitude of stabilization. Cocystal structures were obtained by John Bruning, Ph.D. Hydrogen-deuterium exchange data was obtained by Michael Chalmers, Ph.D., who also generated the figure. These data refute the previous model of for ligand activation of PPAR, in which H12 alone acts as the molecular switch, and suggest a secondary coactivator epitope in the β -sheet region of the receptor. Work done in the laboratory of Pat Griffin, Ph.D., professor.





*Paul J. Kenny, Ph.D., Assistant Professor,
and Qun Lu, Research Assistant*

**DEPARTMENT OF
MOLECULAR
THERAPEUTICS**

STAFF

Patrick Griffin, Ph.D.
Professor and Chairman
Director, Translational
Research Institute

**Jennifer Caldwell-Busby,
Ph.D.***
Assistant Professor

Gregg Fields, Ph.D.
Adjunct Professor

Paul J. Kenny, Ph.D.
Assistant Professor

**Malcolm A. Leissring,
Ph.D.****
Assistant Professor

Philip LoGrasso, Ph.D.*
Associate Professor

**Matthew T. Pletcher,
Ph.D.*****
Assistant Professor

SENIOR SCIENTISTS

Scott Busby, Ph.D.

Michael Chalmers, Ph.D.

STAFF SCIENTIST

Lisa Cherry, Ph.D.

RESEARCH ASSOCIATES

Yuan Dai, Ph.D.

Brian Ember, Ph.D.

Kevin Hayes, Ph.D.

Jonathan Hollander, Ph.D.

** Joint appointment in the
Translational Research Institute*

*** Joint appointment in the
Department of Biochemistry*

**** Joint appointments in the Depart-
ment of Biochemistry and the
Translational Research Institute*



Patrick R. Griffin, Ph.D.

Chairman's Overview

The Department of Molecular Therapeutics was established at Scripps Florida in 2007. Faculty in the department use chemical biology approaches to dissect signaling pathways and transcriptional programs. We rely on state-of-the-art multidisciplinary

technology and methods and a variety of model systems for target identification, validation, and preclinical studies. Currently, the department has 5 tenure-track faculty members and several non-tenure track members who oversee key functional cores within Scripps Florida. These investigators have created strong research programs that take advantage of the unique high-throughput core facilities of Scripps Florida, including genomics, cell-based screening, proteomics, x-ray crystallography, informatics, and lead optimization.

Research activities include discovery and development of therapeutic agents for unmet medical needs in neurodegeneration, Parkinson's disease, acute respiratory distress syndrome, spinal cord injury, cardiovascular disease, cancer, addiction, and metabolic disorders such as insulin resistance and type 2 diabetes. Therapeutic areas and targets include G protein-coupled receptors, proteases, channels, and kinases.

Members of the department interact with researchers in the Translational Research Institute (TRI), which merges drug discovery expertise with advanced technology. The lead identification and high-throughput screening operation of TRI is headed by Peter Hodder of our department. Dr. Hodder and his group focus on technology and assay development and novel chemical approaches to expand compound libraries. The TRI discovery biology group is headed by Phil LoGrasso of our department. Dr. LoGrasso and members of his group focus on small-molecule therapeutic agents as neuroprotective agents in diseases such as Parkinson's. The TRI drug metabo-

lism and pharmacokinetics group is headed by Mike Cameron of our department. Dr. Cameron and his group are involved in mechanistic studies of P450s and drug biotransformation mechanisms.

Other research interests in the Department of Molecular Therapeutics include the neuropharmacology of addiction. Paul Kenny is establishing the role of several G protein-coupled receptors in addiction. Malcolm Leissring is investigating insulin-degrading enzyme as a drug target for treatment of diabetes and, perhaps, Alzheimer's disease.

Other faculty members in the department oversee technology cores. The genomics platform is headed by Mathew Pletcher. He and members of his laboratory use a systems biology approach to identify the determinants of antidepressant efficacy. Researchers in the genomics core oversee genotyping and gene expression profiling technology platforms. The services provided by this core allow Scripps Research investigators to query the genome at both the genetic and the transcriptional levels for the genes that underlie common diseases. In collaboration with Scripps Florida colleagues, members of the genomics core have been involved in projects to identify the genes responsible for pathologic states such as addiction and alcoholism, systemic lupus erythematosus, autism, obsessive-compulsive disorder, diabetes, obesity, and prion diseases.

The cell-based screening platform is headed by Julie Conkright. The faculty advisor to this core is Michael Conkright, Department of Cancer Biology. The cell-based screening platform leverages high-throughput technologies toward a systematic description of the function of genes encoded by the human genome and a more comprehensive understanding of the genetic basis for human disease. The cell-based screening group provides investigators with access to genome-wide collections of cDNAs and short interfering RNAs that can be used to examine cellular models of signal transduction pathways and phenotypes.

The proteomics platform is headed by Jennifer Caldwell-Busby. Liquid chromatography and state-of-the-art mass spectrometry are used to identify, quantify, and characterize proteins and protein modifications. Scientists in this core are involved in scientific collaborations in which novel technologies are used to identify biologically important proteins and protein modifications. Large-scale differential analysis is being used to map the pathways related to insulin sensitization and adipogenesis. In other projects, chromatographic enrichment techniques are used to identify sites of phosphorylation and other posttranslational

modifications. Researchers in the proteomics core collaborate with other scientists to create experiments that will provide meaningful mass spectrometric results.

INVESTIGATORS' REPORTS

Mechanism of Activation of Nuclear Receptors

P.R. Griffin, S.A. Busby, M.J. Chalmers, S. Prasad, S.Y. Dai

We use a wide range of technologies to study ligand activation of nuclear receptors. Previously, we focused heavily on peroxisome proliferator-activated receptor γ (PPAR γ ; see following), but we are beginning to study several orphan nuclear receptors that are implicated in a variety of disorders. Of particular interest are the orphan receptors liver receptor homolog-1 and steroidogenic factor-1. We are building the appropriate tools to begin comprehensive mechanistic studies of these 2 receptors.

MECHANISTIC STUDIES OF LIGAND ACTIVATION OF PPAR γ

PPAR γ is a multidomain ligand-dependent transcription factor. Activation of PPAR γ is regulated by binding of ligands to the receptor's ligand-binding domain, which induces a change in the conformational dynamics of the domain-mediated dissociation of corepressor molecules and forms suitable neopeptides for binding coactivator molecules. Ligands of PPAR γ are characterized as full or partial agonists on the basis of their ability to transactivate PPAR γ target genes. Full agonists have maximal transcriptional activity, whereas partial agonists have moderate activity. Although the structural and molecular determinants of full-agonist regulation of PPAR γ have been studied in detail, the determinants for partial agonists have not been completely characterized. We are using structural, biochemical, and cell-based techniques to examine the mechanism of regulation of PPAR γ transcriptional activity by partial agonists.

Hydrogen-deuterium exchange (HDX) coupled with mass spectrometry was used to characterize ligand-dependent structural and dynamic changes in PPAR γ . Ligand-induced protection of amide exchange in a transcriptional complex composed of PPAR γ , retinoic X receptor α , and either steroid receptor coactivator-1 or steroid receptor coactivator-3 were probed by using automated solution-phase HDX. We used fluorescence-based assays to determine recruitment of coactivators and to measure the affinity of the receptor heterodimer composed of PPAR γ and retinoic X receptor α .

A mammalian 2-hybrid genetic screen was used to probe the molecular determinants of ligand-dependent coactivator selectivity.

We found that the magnitude of PPAR γ agonism is regulated by coactivator recruitment selectivity of p160 coactivators. The functional relevance of the genetic screen was further confirmed in a 3T3-L1 preadipocyte differentiation assay as an *in vitro* model of adipogenesis. We found that conversion of the full-agonist phenotype to the partial-agonist phenotype and vice versa was a function of the availability of specific p160 coactivators.

Using combined proteomic and genomic differential analysis, we have extended these studies to examine the molecular components of PPAR γ -mediated insulin sensitization and adipogenesis after treatment of preadipocyte cells with full and partial agonists of PPAR γ . Our goal is to determine unique components of the insulin sensitivity pathway and dissociate them from components of the proadipogenic pathways that lead to the adverse side effects common after therapy with PPAR γ full agonists. To complete this study, we are developing new strategies to improve the detection of membrane- and lipid-associated proteins, and we are using new mass spectrometry methods to measure the samples.

In other studies, we are using coactivators as chemical tools to generate desired functional responses and distinguish beneficial functions from adverse functions, a novel therapeutic avenue for treating insulin resistance that has not yet been exploited. Our goals are to determine the structure-activity relationships between PPAR γ ligands and their coactivator recruitment selectivity and to obtain PPAR γ ligands with preferences for specific coactivators. To this end, we have developed a validated homogenous time-resolved fluorescence assay for ligand-dependent recruitment of the coactivator to PPAR γ for a large-scale high-throughput screen to identify coactivator-selective agonists of the receptor. We will do 3 different primary screenings of the National Institutes of Health small-molecule chemical library. In each screen, we will target PPAR γ association with a different coactivator to obtain coactivator-specific agonists. The results obtained from this research will provide molecular insight into how recruitment of coactivators modulates activation of PPAR γ and will shed light on the role of specific coactivators in the pharmacologic behavior of PPAR γ modulators.

MECHANISTIC STUDIES OF LIGAND ACTIVATION OF THE ESTROGEN RECEPTOR

In collaboration with scientists at Eli Lilly and Company, Indianapolis, Indiana, we used HDX to char-

acterize the estrogen receptor. As drug targets, estrogen receptors play important roles in the treatment of multiple diseases, including breast cancer and osteoporosis. Tamoxifen and raloxifene, modulators of estrogen receptors approved by the Food and Drug Administration, have intriguing mixed agonism and antagonism effects depending on the target tissue. Structural studies have revealed differences between complexes consisting of the estrogen receptor and an agonist and complexes consisting of the receptor and an antagonist and have provided insight into how these ligands interact with the receptor. We have used HDX to examine an array of chemical compounds that have different degrees of agonism/antagonism. We found an excellent correlation between HDX profiles and pharmacologic properties, and we were able to classify estrogen receptor ligands on the basis of HDX signatures. Discoveries derived from this study will help in understanding tissue-specific activities of drugs targeted to estrogen receptors and will facilitate future drug development programs.

PUBLICATIONS

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Mass Spectrometry for Analysis of Proteins

J.A. Caldwell Busby, V. Cavett

We work to develop methods compatible with mass spectrometry for the analysis of proteins. Our areas of interest are wide-ranging and include methods for subcellular compartmentalization for large-scale proteomics profiling and identification of proteins involved in gene regulation. Gene regulation, or epigenomics, is of particular interest. To date, the focus of epigenomics research has been patterns of DNA methylation and posttranslational modifications of the histone N termini. However, many other proteins are associated with chromatin, including transcription factors, enzymes, and scaffolding proteins. We are developing methods for isolating and analyzing the

supermolecular structure of chromatin, particularly the regulatory protein machineries, and we are using cutting-edge mass spectrometric techniques to characterize the proteins involved in gene regulation.

Study of the interactions of proteins and DNA biopolymers is not new; much work has been done on transcription and gene regulation. However, most of this research has relied on the analysis of DNA methylation patterns and chromatin immunoprecipitation methods. Although informative, these studies ignore the extensive protein components of the system, potentially a rich source of information about how epigenetic patterns are established, maintained, and modified in healthy tissue. Such studies also cannot be used to assess how changes in these protein components contribute to disease states, aging, or cell death.

Mass spectrometry is a widely used technique in proteomics. Powerful, high-resolution instruments, coupled with new fragmentation methods, including electron capture dissociation, are driving the identification of novel proteins and new sites of protein identification. This identification is something for which neither traditional molecular biology nor modern RNA/DNA analysis is well suited. The development of a mass spectrometry-based process to identify components of the chromatin regulatory network will yield a broadly applicable method that will contribute to a wide range of biological investigations, including chromatin regulation of gene expression.

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Neurobiology of Compulsive Behaviors

P.J. Kenny, J.A. Hollander, P. Johnson, Q. Lu, D. Pham*

* Florida Atlantic University, Jupiter, Florida

We focus on elucidating the neurobiological mechanisms of compulsive behaviors, in particular, the motivational processes that

drive compulsive drug consumption in addiction and overeating of palatable foods in obesity. Our findings will be used to facilitate the development of novel therapeutic agents for treatment of substance abuse disorders and obesity.

Recently, we investigated the role of *N*-methyl-D-aspartate (NMDA) receptors in the rewarding properties of nicotine. We found that pharmacologic blockade of NMDA receptors reversed the “hedonic valence” of nicotine in rats; animals were averse to previously rewarding doses of nicotine after treatment with an NMDA receptor antagonist. In addition, we found that nicotine increased NMDA receptor-mediated transmission throughout brain reward circuitries, and chronic intake of nicotine was associated with profound alterations in the expression of NMDA receptors throughout the brain. Taken together, these data support a critical role for NMDA receptors in nicotine reward.

In ongoing studies, we are investigating the subtypes of nicotinic acetylcholine receptors at which nicotine acts in the brain to elicit its rewarding properties. Specifically, we are assessing the rewarding effects of nicotine in mice with mutations in various subunits of the receptors. To compliment this genetic approach, we are also using a lentiviral-based short interfering RNA approach to silence the expression of the genes of targeted subunits of the nicotinic acetylcholine receptors in the brains of rats in a spatially and temporally controlled manner. Subsequently, we will assess the effects of the gene silencing on nicotine reward. These studies should provide important new insights into the neurobiological mechanisms of nicotine addiction, with direct relevance for the treatment of the tobacco habit in humans.

In collaborations with scientists in the Translational Research Institute, Scripps Florida, we are also developing small-molecule drugs that may be useful as novel therapeutic agents for treatment of substance abuse disorders. The targets for these drugs are G protein-coupled receptors that we previously showed play a role in drug dependence.

Finally, we have started a new program to investigate the possibility that neurobiological mechanisms similar to those that drive compulsive drug seeking in addicted rats may also contribute to overeating in obese rats.

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Inhibition of Jun N-Terminal Kinase 2/3 for the Treatment of Parkinson's Disease

P. LoGrasso, M. Cameron, W. Chen, D. Duckett, J. Habel, R. Jiang, T. Kamenecka, S. Khan, L. Lin, Y.-Y. Ling, M. Lopez, C. Ruiz, Y. Shin, L. Smith, X. Song, T. Vojtkovsky, D. Zadory

Apoptosis, or programmed cell death, plays a vital role in the normal development of the nervous system and is also thought to contribute to the aberrant neuronal cell death that characterizes many neurodegenerative diseases. Therefore, blocking neuronal apoptosis could be an approach for treating neurodegenerative diseases. A major pathway implicated in neuronal cell death and survival is the MAP kinase pathway, which controls cell proliferation and cell death in response to many extracellular stimuli. Recent studies have linked Jun N-terminal kinase (JNK) activity with the cell death associated with Parkinson's disease and Alzheimer's disease.

JNK is linked to many of the hallmark pathophysiologic components of Parkinson's disease, such as oxidative stress, programmed cell death, and microglial activation. Many pieces of evidence support JNK as a target for treatment of the pathologic changes that underlie Parkinson's disease. One attractive feature of JNK3 as a selective drug target is that this kinase is almost exclusively expressed in the brain; levels expressed in the kidney and testis are extremely low. In contrast, JNK1 and JNK2 are ubiquitously expressed. Despite the ubiquitous expression of JNK2, we are developing a therapy to prevent degeneration of dopaminergic neurons and halt the progression of Parkinson's disease by targeting JNK2/3.

Our strategy for inhibition JNK2/3 is based on the results of experiments with mice in which the gene for JNK3 or JNK2 was deleted and mice in which the genes for both JNK2 and JNK3 or both JNK1 and JNK2 were deleted. In contrast to mice lacking the gene for JNK1 alone, which had defective T-cell differentiation, mice lacking the gene for JNK2 alone had normal T- and B-cell development and normal T-cell proliferation. Moreover, mice lacking the gene for JNK2 alone and mice

lacking the gene for JNK3 alone were protected against the effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a compound used to induce parkinsonian signs in animal models of Parkinson's disease, whereas both wild-type and mice lacking the gene for JNK1 were not. In other research, compared with wild-type mice, mice lacking the genes for both JNK2 and JNK3 were dramatically protected against acute MPTP-induced injury of the nigrostriatal pathway. This protective effect resulted in a 3-fold increase in the number of neurons positive for tyrosine hydroxylase, an indication of the increase in survival of dopaminergic neurons.

On the basis of these in vitro and in vivo data, we are synthesizing potent, selective JNK 2/3 inhibitors that are efficacious in MPTP animal models of Parkinson's disease. We have established homogenous time-resolved fluorescence biochemical assays for JNK3 and counterscreens for JNK1 and p38. We have generated more than 500 compounds from 3 different structural classes; many of the compounds are inhibitory for JNK3 in nanomolar concentrations. Some of the compounds have a cellular potency of 40–60 nM and in vitro efficacy in promoting primary survival of dopaminergic neurons. We have tested compounds in vivo in rats and mice for drug metabolism and pharmacokinetic properties. Many of the JNK3 inhibitors have had good oral absorption, good brain penetration, and good pharmacokinetic properties that enable efficacy studies.

We have also developed an in vivo target modulation assay to monitor inhibition of c-Jun phosphorylation and an in vivo efficacy model with MPTP to create lesions in the substantia nigra. Moreover, we have solved the crystal structure of 10 complexes of JNK3 with inhibitor at approximately 2.2-Å resolution. This information is being used in structure-based drug design to help guide medicinal chemistry studies and optimize compounds for potency, selectivity, brain penetration, oral absorption, half-life, clearance, and efficacy.

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