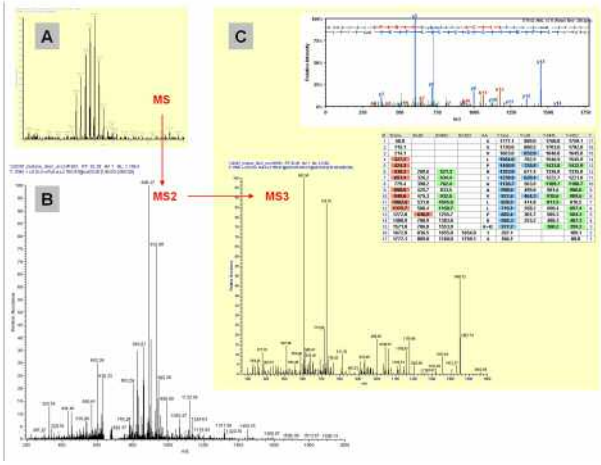
Molecular Therapeutics



Identification of posttranslational modifications on peptides by using high-resolution mass spectrometry and MS3 scanning for absolute assignment of site of modification. Work done in the laboratory of Jennifer Caldwell Busby, Ph.D., assistant professor.

Jennifer Caldwell Busby, Ph.D., Assistant Professor, and

Kristie Rose, Ph.D., Staff Scientist

DEPARTMENT OF MOLECULAR THERAPEUTICS

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Chairman's Overview

The Department of Molecular Therapeutics was established on the Florida campus of Scripps Research 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 on the Florida campus. These investigators have created strong research programs that take advantage of the unique high-throughput core facilities at the Florida campus, including genomics, cell-based screening, and proteomics.

Patrick R. Griffin, Ph.D.

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, including insulin resistance, obesity, and type 2 diabetes.

Paul Kenny and his group focus on the neuropharmacology of addiction and on establishing the role of several G protein–coupled receptors in addictive behavior. Phil LoGrasso and members of his laboratory are involved in the discovery of small-molecule therapeutic agents to be used as neuroprotective agents in diseases such as Parkinson's and are determining the role of rho kinase in vascular bed modulation and glaucoma. Thomas Burris and his group are studying the role of orphan nuclear receptors in circadian rhythms and metabolic disorders such as obesity. Scientists in Jennifer Caldwel Busby's laboratory use state-of-the-art mass spectrometry to identify, quantify, and characterize proteins and protein modifications to map the signaling pathways related to diabetes and cancer. Peter Hodder and coworkers focus on technology and assay development and novel chemical approaches to expand compound libraries. Michael Cameron and his group are involved in mechanistic studies of P450s and drug biotransformation mechanisms. Researchers in my group are dissecting the mechanism of ligand-dependent activation of orphan nuclear receptors implicated in cancer and metabolic disorders.

Investigators' Reports

Probing Protein Dynamics With Hydrogen-Deuterium Exchange Mass Spectrometry

P.R. Griffin, S.A. Busby, M.J. Chalmers, S.Y. Dai, J. Zhang, M. Istrate, R. Garcia-Ordonez, S. Novick, B. Pascal, J. Conkright, G. Zastrow-Hayes, K. Hayes, T. Schröter, F. Madoux, D. Minond, P.S. Hodder

We use a wide range of technologies to study ligand activation of nuclear receptors. During the past few years, we focused on the ligand-binding domains of the well-characterized nuclear receptors peroxisome proliferator–activated receptor γ (PPARγ) and the α and β estrogen receptors. Recently, we have focused on developing hydrogen-deuterium exchange (HDX) technology for probing the mechanism of activation of several orphan nuclear receptors. In addition, in collaboration with scientists at Xencor, Monrovia, California, we are studying the dynamics of TNF-α.

LIGAND ACTIVATION OF PPARγ

PPARγ is a multidomain ligand-dependent transcription factor. Ligands regulate PPARγ activation by binding to the receptor's ligand-binding domain, inducing a change in the conformational dynamics of the domain that leads to dissociation of corepressor molecules and formation of suitable neoepitopes for the binding of coactivator molecules. We used structural, biochemical, and cell-based techniques to examine the mechanism of ligand regulation of PPARγ transcriptional activity. We found that the magnitude of PPARγ agonism is regulated by coactivator recruitment selectivity of p160 coactivators. In mutagenesis studies, we determined the key residues on the receptor that facilitate these selective coactivator interactions.

In other studies, we are using coactivators as chemical tools to generate desired functional responses and to differentiate pharmacologically beneficial function from adverse function, a novel unexploited therapeutic avenue for treating insulin resistance. Our goals are to determine the structure-activity relationships between PPARγ ligands and their coactivator recruitment selectivity and to obtain PPARγ ligands with specific coactivator preferences by screening for agonists that favor specifically the association of a given cofactor. For a large-scale high-throughput screening to identify coactivator-selective agonists of the receptor, we have developed a validated time-resolved fluorescence resonance energy transfer assay for ligand-dependent recruitment of the coactivator to PPARγ. Scientists at the Scripps Research Institute Molecular Library Screening Center used these assays to examine the National Institute of Health small-molecule library. The results obtained from this research are providing molecular insight into coactivator recruitment and receptor activation and will result in chemical tools to dissect the biological role of specific coactivators in modulating PPARγ.

LIGAND ACTIVATION OF THE VITAMIN D RECEPTOR

In collaboration with scientists at Eli Lilly and Company, Indianapolis, Indiana, we are using HDX to characterize activation of the full-length heterodimer complex composed of the vitamin D receptor and its coreceptor retinoid X receptor α. This project is promoting further development of our HDX platform to facilitate the analysis of large transcriptional complexes. Although this research is in an early stage, we have data that suggest HDX is useful for probing dynamics of large transcriptional complexes.

PROBING G PROTEIN–COUPLED RECEPTORS

G protein–coupled receptors are an important family of transmembrane signaling proteins. Characterization of the structure and dynamics of these proteins is an analytical challenge because their transmembrane domains are hydrophobic. We have begun to expand the application of HDX to probe the dynamics of these receptors. This work is being done in collaboration with H. Rosen, Department of Chemical Physiology, and R.C. Stevens, Department of Molecular Biology.

PUBLICATIONS

Bruning, J., Chalmers, M.J., Prasad, S., Busby, S.A., Kamenecka, T., He, Y., Nettles, K.W., Griffin, P.R. Partial agonists activate PPARγ using a helix 12 independent mechanism. Structure 15:1258, 2007.

Chalmers, M.J., Busby, S.A., Pascal, B.D., Southern, M.R., Griffin, P.R. A two-stage differential hydrogen deuterium exchange method for the rapid characterization of protein/ligand interactions. J. Biomol. Tech. 18:194, 2007.

Dai, S.Y., Chalmers, M.J., Bruning, J., Bramlett, K.S., Osborne, H.E., Montrose-Rafizadeh, C., Barr, R.J., Wang, M., Burris, T.P., Dodge, J.A., Griffin, P.R. Prediction of the tissue-specificity of selective estrogen receptor modulators using a single biochemical method. Proc. Natl. Acad. Sci. U. S. A. 105:7171, 2008.

Madoux, F., Li, X., Chase, P., Zastrow, G., Cameron, M.D., Conkright, J.J., Griffin, P.R., Thacher, S., Hodder, P.S. Potent, selective and cell penetrant inhibitors of SF-1 by functional ultra-high-throughput screening. Mol. Pharmacol. 73:1776, 2008.

Mass Spectrometry for Identification of Proteins

J.A. Caldwell Busby, V. Cavett

Our general focus is the use of cutting-edge separation and mass spectrometry techniques to identify proteins involved in biological events. The biological applications are determined by the research needs of a large group of collaborators in various disciplines, with a wide variety of questions to be answered. We provide these collaborators access to powerful and novel approaches to examine posttranslational modifications and measure protein levels in multiple samples.

In addition to these collaborative efforts, we are developing a method to identify and temporally map chromatin proteins involved in transcriptional regulation. Gene regulation is a fundamental biological process that is studied from a variety of perspectives with a variety of methods; however, research to date has been highly gene centric, and only a few reports have been published on the proteomics of gene regulation. We target these missing proteomics components, particularly the components of the supermolecular complex of chromatin, including nucleosome substructure and regulatory and transcription complexes.

Methods for whole-system approaches are difficult to implement because traditional technologies tend to focus on the isolation and analysis of individual parts of the whole—DNA, RNA, or protein. Our techniques combine advances in molecular biology with the power of mass spectrometry to identify novel biomolecules involved in multibiopolymer complexes. In particular, we are modifying and combining techniques such as protein-protein and protein-DNA cross-linking, immunoprecipitation methods, chromatin immunoprecipitation, mass spectrometry, and liquid chromatography to determine the larger regulatory mechanisms involved in the fate of cells. The keystone of this method is a modified chromatin immunoprecipitation protocol that maintains the integrity of the DNA while allowing for the isolation, recovery, and analysis of the protein components of the nucleosome complex. This advanced method targets proteins that regulate chromatin function and correlates those proteins with histone modification states and gene occupancy. Incorporating proteomics into a traditionally DNA-based experimental protocol provides a new perspective and a novel approach to genetic regulation. Newly identified proteins and novel protein modifications associated with sites of transcription can be used as the basis for further experiments to determine the biological roles of the proteins in gene regulation and activation.

PUBLICATIONS

Amelio, A.L., Miraglia, L.J., Conkright, J.J., Mercer, B.A., Batalov, S., Cavett, V., Orth, A.P., Busby, J., Hogenesch, J.B., Conkright, M.D. A coactivator trap identifies NONO (p54nrb) as a component of the cAMP signaling pathway. Proc. Natl. Acad. Sci. U. S. A. 104:20314, 2007.

Neurobiology of Addiction

P.J. Kenny, P. Bali, C.D. Fowler, J.A. Hollander, H.-I. Im, P.M. Johnson,* Q. Lu, B.H. Miller

* Kellogg School of Science and Technology, Scripps Research

We focus on understanding the neurobiological mechanisms of addiction. This knowledge will be used to develop novel therapeutic agents for treatment of substance abuse. We use a multidisciplinary approach that includes mouse behavioral genetics, virus-mediated gene expression, RNA and protein analyses, and in vivo behavioral testing.

NICOTINE ADDICTION

We seek to identify the subtypes of nicotinic acetylcholine receptors and downstream signaling cascades through which nicotine promotes tobacco addiction. Currently, we are assessing the reinforcing effects of nicotine in mice with null mutations in various subunits of the receptors. In addition, we are testing the effects on nicotine reinforcement of using lentivirus-based short hairpin RNAs to silence the genes of targeted nicotinic acetylcholine receptor subunits in brain reward circuitries. Finally, we are using a proteomics approach to identify the intracellular proteins coupled to nicotinic acetylcholine receptors in the brains of mice. Our goal is to identify novel scaffold and signaling proteins involved in transducing the addictive actions of nicotine. These studies promise to yield significant new insights into the neurobiological mechanisms of nicotine addiction, with direct relevance for the treatment of the tobacco habit in humans.

BRAIN SYSTEMS INVOLVED IN ADDICTION

In collaboration with other scientists at Scripps Research, we found that the neuropeptide orexin (hypocretin) plays a critical role in drug reward. In ongoing studies, we are identifying the mechanisms through which orexin-mediated transmission regulates drug reward. We are also investigating the roles of

novel constitutive mechanisms of gene regulation in the neuroplasticity induced by drugs of abuse that may promote addiction. Further, we are testing the hypothesis that drug addiction and obesity share common reward and motivational mechanisms. These studies may identify novel targets for the development of therapeutics against addiction and obesity.

DEVELOPMENT OF NOVEL ANTIADDICTION MEDICATIONS

In collaborations with scientists in the Translational Research Institute, Scripps Florida, we are 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.

PUBLICATIONS

Faghihi, M.A., Modarresi, F., Khalil, A.M., Wood, D.E., Sahagan, B.G., Morgan, T.E., Finch, C.E., St-Laurent, G. III, Kenny, P.J., Wahlestedt, C. Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of β-secretase. Nat. Med. 14:723, 2008.

Johnson, P.M., Hollander, J.A., Kenny, P.J. Decreased brain reward function during nicotine withdrawal in C57BL6 mice: evidence from intracranial self-stimulation (ICSS) studies. Pharmacol. Biochem. Behav. 90:409, 2008.

Kenny, P.J., Chartoff, E., Roberto, M., Carlezon, W.A., Jr., Markou, A. NMDA receptors regulate nicotine-enhanced brain reward function and intravenous nicotine self-administration: role of the ventral tegmental area and central nucleus of the amygdala. Neuropsychopharmacology 14:723, 2008.

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

P. LoGrasso, M. Cameron, W. Chen, S. Clapp, D. Duckett, B. Ember, J. Habel, R. Jiang, T. Kamenecka, S. Khan, L. Ling, Y.-Y. Ling, M. Lopez, A. Pachori, C. Ruiz, Y. Shin, X. Song, T. Vojkovsky, 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. 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 inhibiting 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-1,2,3,5-tetrahydropyridine (MPTP), a compound used to induce parkinsonian signs in animal models of Parkinson's disease, whereas both wild-type mice 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 we will test for efficacy 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 1000 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 com-

pounds 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 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.

We have also begun investigating the role of JNK in myocardial infarction. We have set up animal models to test for the ability of JNK inhibitors to decrease infarct size and preserve cell function in these models.

Finally, we have determined the kinetic mechanism for JNK3 and have shown that it is a random sequential mechanism. We are investigating the kinetic mechanism of JNK1 and are examining differences substrate specificity that may exist between the isoforms. We plan to investigate the role played by different JNK isoforms and, more specifically, different splice variants in various apoptosis scenarios in different cell types. The purposes of these basic mechanistic studies is to understand structure-function relationships at the molecular level and to design specific inhibitors that may be selective for one isoform or splice variant.

PUBLICATIONS

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Jiang, R., Duckett, D., Chen, W., Habel, J., Ling, Y.-Y., LoGrasso, P., Kamenecka, T.M. 3,5-Disubstituted quinolines as novel c-Jun N-terminal kinase inhibitors. Bioorg. Med. Chem. Lett. 17:6378, 2007.

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