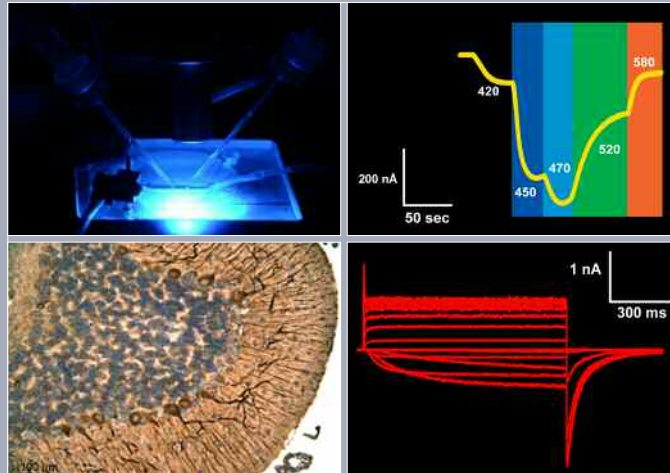


## Biochemistry



Upper left panel, Electrical signals are recorded from a *Xenopus* oocyte expressing the circadian photoreceptor melanopsin in response to illumination with blue light. Upper right panel, Photocurrent recorded from an oocyte expressing melanopsin and the TRPC3 ion channel in response to illumination at various wavelengths. Peak activation of melanopsin signaling occurs at about 470 nm, which corresponds to the peak light sensitivity of the circadian system. Lower left panel, Expression of the subthreshold potassium channel Elk2 in the soma and dendrites of cerebellar Purkinje neurons. Elk potassium channels are active at neuronal resting potentials and thus may have a strong influence on neuronal excitation. Lower right panel, Elk1 currents recorded from an inside-out patch in symmetrical potassium. Work done in the laboratory of Timothy Jegla, Ph.D., assistant professor, California campus.



*Timothy J. Jegla, Ph.D.,  
Assistant Professor,  
Department of Biochemistry,  
California Campus*

**DEPARTMENT OF  
BIOCHEMISTRY**
**STAFF**
**Steve A. Kay, Ph.D.\***

 Professor and Chairman  
California Campus

**Nicholas Gekakis, Ph.D.**

 Assistant Professor  
California campus

**Takato Imaizumi, Ph.D.**

 Assistant Professor of  
Biochemistry  
California Campus

**Timothy J. Jegla, Ph.D.**

 Assistant Professor  
California Campus

**Claudio A.P. Joazeiro, Ph.D.**

 Assistant Professor  
California Campus

**Malcolm A. Leissring, Ph.D.\*\***

 Assistant Professor  
Florida Campus

**Mathew T. Pletcher, Ph.D.\*\*\***

 Assistant Professor  
Florida Campus

**Thomas Schultz, Ph.D.**

 Assistant Professor of  
Biochemistry  
California Campus

**James Tam, Ph.D.**

 Professor  
Florida Campus

**Claes Wahlestedt, M.D.,  
Ph.D.**

 Professor  
Florida Campus

**RESEARCH ASSOCIATES**
**CALIFORNIA CAMPUS**
**Hedieh Badie, Ph.D.**
**Antoine Baudry, Ph.D.**
**Mario Bengston, Ph.D.**
**Ghislain Breton, Ph.D.**
**Brenda Chow, Ph.D.**
**Jessie Chu, Ph.D.**
**Sinead Clancy, Ph.D.**
**Eva Farré, Ph.D.**
**Elizabeth Hamilton, Ph.D.**
**Samuel Hazen, Ph.D.**
**Anne Helfer, Ph.D.**
**Warren Lewis, Ph.D.**
**Andrew C.-Y. Liu, Ph.D.**
**Julien Mamet, Ph.D.**
**Brooke Miller, Ph.D.**
**Alessia Para, Ph.D.**
**Jeffery Pitman, Ph.D.**
**Jose Pruneda-Paz, Ph.D.**
**Mariko Sawa, Ph.D.**
**Hein Tran, Ph.D.**
**Axel Ulbrich, Ph.D.**
**David Welsh, M.D., Ph.D.**
**Matthew Wheeler, Ph.D.**
**Jason Wilkes, Ph.D.**
**Eric Zhang, Ph.D.**
**FLORIDA CAMPUS**
**Shaun Brothers, Ph.D.**
**Jia Huang, Ph.D.**
**Ahmad Khalil, Ph.D.**
**Rebecca Kocerha, Ph.D.**
**Kristen Clarke Ware, Ph.D.**

*\* Joint appointments in the  
Department of Cell Biology and  
the Institute for Childhood and  
Neglected Diseases*

*\*\* Joint appointment in the  
Department of Molecular  
Therapeutics*

*\*\*\* Joint appointments in the  
Department of Molecular  
Therapeutics and the  
Translational Research Institute*



Steve A. Kay, Ph.D.

## Chairman's Overview

The Department of Biochemistry at Scripps Research was recently created to recruit faculty to both the California and the Florida campuses. The overall theme of the department centers on the need to understand physiologic processes from the molecular level to the whole organism. Our faculty members are generally multidisciplinary biologists and chemists who wield cutting-edge tools of structural biology, protein dynamics, biological chemistry, genetics and genomics, pathway analysis, and computational approaches to understand how organisms maintain homeostasis and respond to stress and disease.

We have broad interests, and we seek to answer contemporary questions in neurobiology, metabolic control, immunology, and cancer biology. By taking integrative approaches to substantial problems in modern biology, we will contribute to the understanding of a wide variety of diseases such as diabetes, cancer, and CNS disorders.

## INVESTIGATORS' REPORTS

### Genetics and Genomics of Circadian Clocks

S.A. Kay, A. Baudry, G. Breton, B. Chow, A. deSchöpke, E. Farré, E. Hamilton, S.P. Hazen, A. Helfer, T. Hirota, T. Imaizumi, S. Landais, W. Lewis,\* C.Y. Liu,\* D. Nusinow, A. Para, A. Priest, J. Pruneda, A.L. Quiroz, T.F. Schultz, D.K. Welsh,\*\* E. Zhang\*

\* Genomics Institute of the Novartis Research Foundation, La Jolla, California

\*\* University of California, San Diego, California

A vast array of cellular processes fluctuate with a 24-hour periodicity, and an endogenous circadian clock is responsible for generating these biological rhythms. Circadian rhythms are found in all kingdoms of life and control diverse events ranging from sleep-wake cycles in mammals to the overall rate of photosynthesis in plants. Many pathologic changes in humans, such as sleep disorders, most likely are due to a circadian defect, so understanding how the circadian clock operates within the cell will have significance for human health. To study how circadian clocks are built inside of cells, we use molecular, genetic, and genomic approaches in 2 model systems: mouse and *Arabidopsis*.

In mammals, the circadian clock plays an integral role in timing many physiologic rhythms, such as blood pressure, body temperature, and liver metabolism, in anticipation of dusk and dawn. The master circadian clock resides within a region of the brain that receives light information from the eyes. However, this brain region can still keep time even in the absence of light, as occurs in some who are visually blind. Mutations in the genes that encode components of the circadian clock are manifested as abnormal activity rhythms in rodents and as sleeping disorders in humans, although which photoreceptors set the clock is unclear. Thus, although marked advances have been made in understanding how the mammalian clock itself runs, little is known about how light transduces synchronizing signals to the clock.

To address this major question, we are using genetic and genomic approaches to identify new gene functions in circadian biology. We are producing a number of mouse strains with mutations in known and potential photoreceptors and are testing the mice for defects in circadian rhythm. Thus far, we have determined that one photoreceptor, melanopsin, is an important contributor in

maintaining synchrony between the clock and environmental light conditions. With the recently completed sequencing of the human and mouse genomes, we now know the sequences of more than 30,000 genes that can be investigated for potential roles in circadian function. We developed large-scale, in vitro, cell-based assays that can be used to rapidly determine if genes control clock activity. Combining this approach with genetic analysis will enable us to further dissect the connection between environmental stimuli, in the form of light, and the behavioral and physiologic events regulated by the circadian clock.

In recent years, researchers have found that intrinsic circadian clocks exist in various peripheral tissues and cell types, directly controlling local physiology and behavior. We are studying the circadian oscillators in the liver and in the vasculature. As the first step, we are investigating the heterogeneity and distinct functions of the central and peripheral oscillators. In particular, we are examining the distinct roles of the retinoid-related orphan nuclear receptors in the clock mechanism. These nuclear receptors were recently identified in our functional genomics studies.

Second, we are asking how environmental cues, mainly light-dark cycles and feeding time, entrain or synchronize peripheral oscillators. Peripheral oscillators most likely are synchronized by hormonal outputs of the suprachiasmatic nucleus or by physiologic inputs such as feeding-mediated metabolic changes. We are using transgenic mice, mice lacking certain genes, and immortalized hepatocytes and vascular smooth muscle cells in these studies along with real-time bioluminescence imaging and biochemical and genetic approaches. Furthermore, we are using high-resolution bioluminescence imaging to examine whether single neurons in the suprachiasmatic nucleus and peripheral cells are autonomous circadian clocks and to characterize the precise nature of synchronization of the molecular clockwork of individual cells.

Flowering is a major event in the life cycle of higher plants. Many plants use seasonal changes in the length of days as a signal to flower, and higher plants use their circadian clocks to perceive these changes. Recently, we defined a molecular link between the circadian clock and day length-dependent regulation of flowering. A flowering time gene known as *CONSTANS* was identified a number of years ago and is regulated by the circadian clock. We showed that clock regulation of *CONSTANS* expression is the key to seasonal control of flowering

in *Arabidopsis*. We are extending these studies by comparing gene expression profiles under conditions of long days and short days to identify other components involved in perception of day length.

By combining molecular, genetic, and genomic approaches, we are beginning to define a number of molecular links between the circadian clock and rhythmic regulation of behavior and development. Analysis of circadian rhythms in multiple organisms provides a unique opportunity to define molecular controls for the behavior of whole organisms. These results will provide targets for clinical and agricultural applications to improve the quality of life.

#### PUBLICATIONS

Allen, T., Koustenis, A., Theodorou, G., Somers, D.E., Kay, S.A., Whitelam, G.C., Devlin, P.F. *Arabidopsis* FHY2 specifically gates phytochrome signaling to the circadian clock. *Plant Cell* 18:2506, 2006.

Breton, G., Kay, S.A. Circadian rhythms lit up in *Chlamydomonas*. *Genome Biol.* 7:215, 2006.

Faigón-Soverna, A., Harmon, F.G., Storani, L., Karayekov, E., Staneloni, R.J., Gassmann, W., Más, P., Casal, J.J., Kay, S.A., Yanovsky, M.J. A constitutive shade-avoidance mutant implicates TIR-NBS-LRR proteins in *Arabidopsis* photomorphogenic development. *Plant Cell* 18:2919, 2006.

Hazen, S.P., Schultz, T.F., Pruneda-Paz, J.L., Borevitz, J.O., Ecker, J.R., Kay, S.A. *LUX ARRHYTHMO* encodes a Myb domain protein essential for circadian rhythms. *Proc. Natl. Acad. Sci. U. S. A.* 102:10387, 2006.

Imaizumi, T., Kay, S.A. Photoperiodic control of flowering: not only by coincidence [published correction appears in *Trends Plant Sci.* 11:567, 2006]. *Trends Plant Sci.* 11:550, 2006.

Sato, T.K., Yamada, R.G., Ukai, H., Baggs, J.E., Miragila, L.J., Kobayashi, T.J., Welsh, D.K., Kay, S.A., Ueda, H.R., Hogenesch, J.B. Feedback repression is required for mammalian circadian clock function. *Nat. Genet.* 38:312, 2006.

Weber, F., Hung, H.-C., Maurer, C., Kay, S.A. Second messenger and Ras/MAPK signalling pathways regulate CLOCK/CYCLE-dependent transcription. *J. Neurochem.* 98:248, 2006.

---

## Insulin Secretion and Action

---

N. Gekakis, J.J. Wilkes, J.L. Pitman, M.C. Wheeler

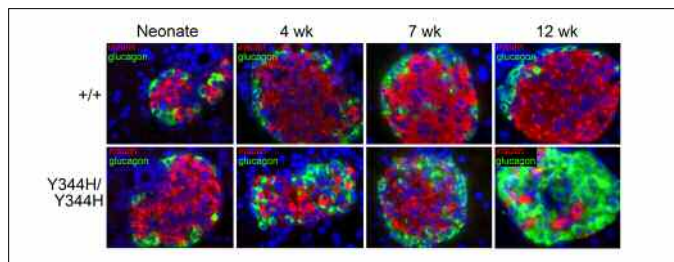
---

**T**ype 2 diabetes is a large and growing problem across the United States and around the world. Its incidence and prevalence have increased dramatically during the past several years. In addition to being the sixth leading cause of death in the United States, type 2 diabetes is also a major contributor to heart disease, blindness, limb amputation, and kidney failure. The development of this type of diabetes is a 2-hit process, involving both resistance of peripheral tissue (liver, muscle, fat) to the action of insulin and a failure of the insulin-secreting beta cells to adequately compensate for this insulin resistance. We are using

both forward and reverse genetic approaches to study both aspects of type 2 diabetes.

A major focus of current research in diabetes is the role of inflammation in insulin resistance that results from obesity and/or high-fat feeding. We have identified a G protein-coupled receptor, Gpr43, that plays a role in inflammation-induced insulin resistance. Gpr43 is activated by short-chain fatty acids and is expressed in adipocytes and macrophages. We have shown that silencing of *Gpr43*, the gene for this receptor, improves insulin sensitivity in whole animals and that this improvement is correlated with reduced production of cytokines in macrophages and other tissue. Further, this improvement in insulin sensitivity is mediated, at least in part, by *Gpr43* expression in bone marrow-derived cells, which include macrophages. Another reverse genetic approach we are using to understand insulin resistance is screening of cDNA overexpression libraries in a functional assay of insulin action in vitro to identify those cDNAs that can inhibit insulin action.

We have also taken an innovative forward genetic approach to understanding insulin secretion and action. In this approach, mutations are induced in the mouse genome, and mutant mice are screened for diabetes or insulin resistance. Once such a family of mice is found, the gene causing the defect is mapped and cloned. In



**Fig. 1.** Immunofluorescence detection of loss of beta cells in *Sec61a1* mutant mice. Top row, Normal distribution of insulin-secreting beta cells (red) and glucagon-secreting alpha cells (green) in wild-type (+/+) mice from neonate to 12 weeks of age. Bottom row, Mutant mice (Y344H/Y344H) begin with a normal distribution of alpha and beta cells but begin to lose beta cells between 4 and 7 weeks of age and have lost 80%–90% of their beta cells by 12 weeks of age, a time that correlates with the onset of diabetes.

this way, we have discovered a new gene, *Sec61a1*, required for beta-cell function (Fig. 1) that highlights the susceptibility of beta cells to “ER stress.” The endoplasmic reticulum is the site of synthesis of secreted proteins and as such plays an important role in quality control of those proteins, detecting and eliminating those proteins that are abnormal. ER stress is the accumulation of abnormal proteins in the endoplasmic reticulum

and can lead to cellular dysfunction or cellular death. *Sec61a1* is clearly important in this quality control function, and beta cells seem especially vulnerable to ER stress-induced cell death. Because beta cells are so central to the development of diabetes and are prone to ER stress, such stress may be a fundamental contributor to type 2 diabetes in humans.

#### PUBLICATIONS

Lloyd, D.J., Bohan, S., Gekakis, N. Obesity, hyperphagia, and increased metabolic efficiency in *Pc1* mutant mice. *Hum. Mol. Genet.* 15:1884, 2006.

## Regulation of Neuronal Signaling by Potassium Channels

T. Jegla, H. Badie, S. Clancy, B. Chen

A major goal of our research is to understand the fundamental mechanisms through which potassium channels regulate neuronal signaling. Potassium channels have long been recognized as key determinants of neuronal excitability. The channels have diverse roles in neurons, including setting of resting potentials, regulating subthreshold excitability, repolarizing action potentials, and setting firing patterns. Recent genetic advances have provided a strong link between abnormal potassium channel activity and diseases of the human nervous system, including epilepsy, ataxia, and retinopathies. A multitude of potassium channels with distinctly different properties has been identified via molecular cloning, but large gaps remain in our understanding of how this molecular diversity corresponds to the rich physiologic diversity of potassium channels in neurons.

We are using a combination of chemical genomics, mouse genetics, biochemistry, and electrophysiology to understand how orphan classes of channels function in neurons. We are focusing on 2 major families of genes that encode potassium channels: the Elk or Kv12 channels and the so-called silent Kv channels. Elk channels contribute to subthreshold conductance and are highly expressed within many key systems within the CNS. Silent Kv channels contribute to the functional diversity of the classic delayed rectifiers that repolarize action potentials in most neurons. Key outstanding questions about these 2 types of channels include subcellular localization, modulation during neuronal signaling, and contributions to circuit activity.

We have localized the most prominently expressed Elk channel, Kv12.2, to the soma and dendrites of exci-



tatory neurons in forebrain regions such as the hippocampus, amygdala, and cortex. The activity of Kv12.2 is highly regulated by multiple factors in heterologous systems, but its function in native neurons has been difficult to define because of a lack of genetic models or defining pharmacologic characteristics. We have identified specific inhibitors of Kv12.2 and are using them to characterize Kv12.2 function in the nervous system. Genetic approaches to Kv12.2 function are also in progress.

Silent Kv subunits are so named because they do not form functional channels when expressed as homotetramers in heterologous systems; instead they modify the functional properties of classic delayed rectifiers through the formation of heteromeric channels with Kv2 family subunits. Despite the importance of the delayed rectifier to neuronal excitation and substantial evidence for delayed rectifier diversity in vivo, the native biology of these heteromers has essentially remained unexplored. We are examining the function of silent Kv channels in photoreceptors, the circadian system, and motor neurons.

## Cellular Regulation by the Ubiquitin System

C. Joazeiro, M. Bengtson, W. Li, A. Ulbrich

The levels and duration of expression of intracellular proteins are determined by the proteins' rates of synthesis vs degradation. Degradation of most cellular proteins is mediated by posttranslational modification with the 8-kD protein ubiquitin, which signals recognition by the proteasome. In addition, ubiquitin can play nonproteolytic roles in signaling. Not surprisingly then, ubiquitylation is essential for most cellular processes, such as cell division, signaling, and DNA repair. In addition, deregulated ubiquitylation can cause a number of diseases, including neurodegeneration and cancer. We are interested in dissecting pathways (Fig. 1) and mechanisms that involve the ubiquitin system and in converting this information into tools for interfering with biology and disease.

### PROTEIN UBIQUITYLATION IN NORMAL CELLS

E3 ubiquitin ligases mediate substrate specificity in ubiquitylation. We and others previously discovered the largest family of E3s, which is characterized by a RING finger "catalytic" domain. Our goal is to assign function to E3s and to determine their role in disease. In one approach, we annotated the hundreds of genes that

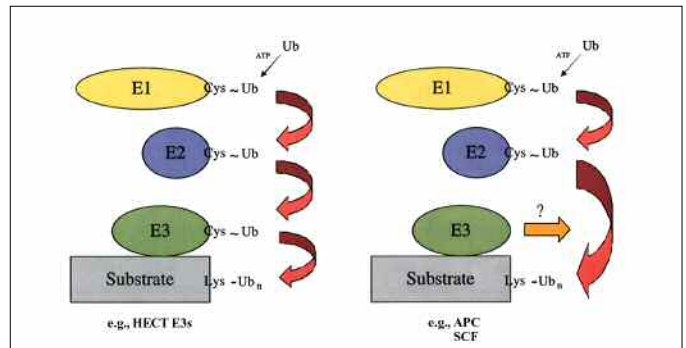


Fig. 1. Protein ubiquitination pathways.

encode E3s and E3 subunits in mammalian genomes and then built collections of short interfering RNAs and full-length cDNAs encoding most human and mouse E3s for use in functional genomic screens. For example, using an imaging-based phenotypic screen, we found a novel E3, MULAN, that localizes to mitochondria and regulates the organelle's trafficking, morphology, and signaling (Fig. 2). Currently, we are identifying the critical substrates and biological relevance of this ligase.

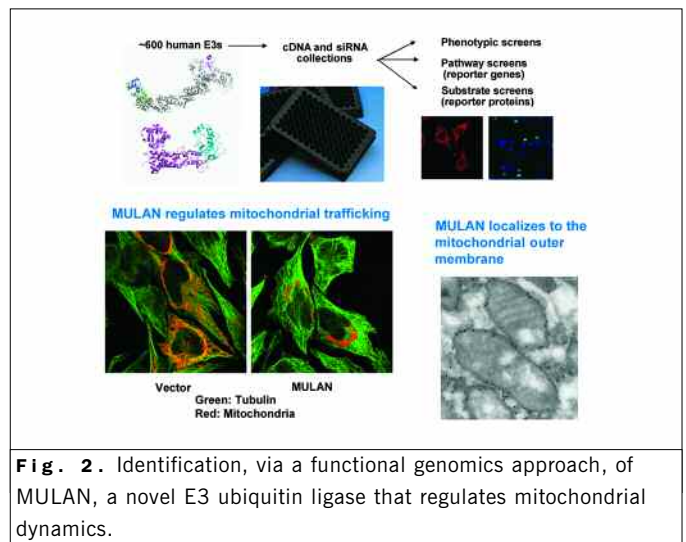


Fig. 2. Identification, via a functional genomics approach, of MULAN, a novel E3 ubiquitin ligase that regulates mitochondrial dynamics.

### PROTEIN UBIQUITYLATION IN DISEASE

Although several lines of evidence implicate the ubiquitin system in neurodegeneration, the pathogenic mechanisms involved remain unclear. To shed light on this, in collaboration with S.A. Kay, Department of Biochemistry, we have been studying a new mouse model of neurodegeneration caused by mutation of a novel RING finger E3. These mice, identified by using a high-throughput phenotypic screen for recessive mutations induced by *N*-ethyl-*N*-nitrosourea, have motor neuron loss in the spinal cord and progressive paralysis. We are using biochemistry and molecular genetics to determine the process or pathway that is defective in these mutant mice.

Inspired by the success of a proteasome inhibitor in the treatment of multiple myeloma, several researchers are searching for E3 inhibitors that might have similar druglike properties. We performed a high-throughput screen for compounds that inhibit the activity of the E3 ubiquitin ligase Mdm2 and that might be useful either in the clinic by leading to stabilization of the tumor suppressor protein p53 or for manipulating p53 levels and ubiquitylation in experimental models.

#### PUBLICATIONS

Joazeiro, C.A.P., Anderson, K.C., Hunter, T. Proteasome inhibitor drugs on the rise. *Cancer Res.* 66:7840, 2006.

Sierra, J., Yoshida, T., Joazeiro, C.A.P., Jones, K.A. The APC tumor suppressor counteracts  $\beta$ -catenin activation and H3K4 methylation at Wnt target genes. *Genes Dev.* 20:586, 2006.

Zhang, Q., Liu, Y., Gao, F., Ding, Q., Cho, C., Hur, W.-Y., Jin, Y., Uno, T., Joazeiro, C.A.P., Gray, N. Discovery of EGFR-selective 4,6-disubstituted pyrimidines from a combinatorial kinase-directed heterocycle library. *J. Am. Chem. Soc.* 128:2182, 2006.

## New Therapeutic Strategies in Alzheimer's Disease and Diabetes Mellitus

M.A. Leissring, J. Zhao, L. Li, Q. Lu

#### AGING AND NEURODEGENERATION

**W**e study diseases of the nervous system that occur during aging and the fundamental mechanisms that regulate aging. Currently, we are examining a fascinating but poorly understood zinc metalloprotease known as insulin-degrading enzyme (IDE). IDE is responsible for degradation of insulin and amyloid  $\beta$ -protein, peptide substrates central to the pathogenesis of diabetes and Alzheimer's disease, respectively. We showed that enhancing the proteolysis of amyloid  $\beta$ -protein by IDE or other proteases can completely prevent Alzheimer-type pathologic changes in a mouse model of Alzheimer's disease.

We are using techniques ranging from in vitro enzymatic assays to transgenic and gene-targeted rodent models to explore the normal biology and therapeutic potential of IDE and other proteases. In parallel, we are using high-throughput screening of compounds, solid-phase peptide synthesis, and medicinal chemistry to discover and rationally design pharmacologic modulators of IDE. These pharmacologic tools promise to deepen our understanding of IDE biology and could lead to novel therapeutic interventions for Alzheimer's disease or diabetes.

#### RATIONAL DESIGN OF POTENT AND SELECTIVE INHIBITORS OF IDE

Despite the potentially great importance of IDE in health and disease, no useful pharmacologic tools have been available to manipulate the activity of this protease in in vivo models. On the basis of proteomic analysis of the cleavage-site specificity of IDE, we designed and synthesized peptide hydroxamates that are potent IDE inhibitors. We next used solid-phase peptide synthesis to generate a series of *retro-inverso* peptide hydroxamates, which led to the identification of unnatural amino acid substitutions that improved potency by several 100-fold. Currently, our best compounds are inhibitory in low nanomolar to high picomolar concentrations, making them almost a million times more potent than any previously described small-molecule inhibitors for IDE. These inhibitors enhance the action of insulin at multiple levels in cultured cells, and we are working to develop variants suitable for use in vivo. These new reagents promise to yield new insights into the function of IDE and could pave the way to a novel class of therapeutic agents based on slowing insulin clearance.

#### ANIMAL MODELING

We have made significant strides in evaluating the importance of different enzymes that degrade amyloid  $\beta$ -protein in vivo. Most recently, we found that deletion of cathepsin D leads to a selective increase in amyloid  $\beta$ -protein 42, the species that accumulates in the amyloid plaques characteristic of Alzheimer's disease.

#### PUBLICATIONS

Farris, W., Schütz, S.G., Cirrito, J.R., Shankar, G.M., Sun, X., George, A., Leissring, M.A., Walsh, D.M., Qiu, W.Q., Holtzman, D.M., Selkoe, D.J. Loss of neprilysin function promotes amyloid plaque formation and causes cerebral amyloid angiopathy. *Am. J. Pathol.* 171:241, 2007.

Kim, M., Hersh, L.B., Leissring, M.A., Ingelsson, M., Matsui, T., Farris, W., Lu, A., Hyman, B.T., Selkoe, D.J., Bertram, L., Tanzi, R.E. Decreased catalytic activity of the insulin-degrading enzyme in chromosome 10-linked Alzheimer disease families. *J. Biol. Chem.* 282:7825, 2007.

Leissring, M.A. Proteolytic degradation of the amyloid  $\beta$ -protein: the forgotten side of Alzheimer's disease. *Curr. Alzheimer Res.* 3:431, 2006.

Leissring, M.A., Selkoe, D.J. Structural biology: enzyme target to latch onto. *Nature* 443:761, 2006.



## Identification of Genetic Determinants of Depression

M.T. Pletcher, K.J. Clarke, B.C. Long, B.H. Miller, L.E. Schultz, B.M. Young

**C**linical depression is a mood disorder with high morbidity and mortality that is estimated to occur in more than 15% of the adult population in the United States. Depression has a wide range of symptoms, including loss of energy, changes in weight, diminished interest or pleasure in daily activities, insomnia or excessive sleep, anxiety, slowness of movement, feelings of worthlessness, difficulty concentrating, and thoughts of death.

Depression can be a one-time occurrence but is often an ongoing problem throughout a person's lifetime. A total of 15% of patients with major depressive disorders die of suicide. The onset of depression is often linked to environmental factors such as life events that greatly increase stress. Despite this association, depression has a strong genetic component. Genetics accounts for 40%–50% of the risk for depression in a person's lifetime, and the risk for members of a family does not change if the members are raised separately.

We are using cell-based screening technology and a mouse model to identify the genes and pathways that contribute to depressive behavior. Currently, we are conducting a survey in 30 strains of mice for differences in molecular, biochemical, and behavioral traits that represent endophenotypes (i.e., single well-defined symptoms or traits of a much more complex disorder) associated with depression. In cataloging the variation in quantities of neurotransmitters and stress hormones in the blood, gene expression in regions of the brain such as the hippocampus and hypothalamus, and performance in behavioral models such as the tail suspension test and open field test, we are producing the data necessary to identify the genetic controls for each of these traits. Using the *in silico* genetic mapping method, we can correlate the natural variation of these measurements present in the inbred lines of mice with the underlying haplotype structure of the mice, thereby pinpointing biologically important genes.

In parallel, we are developing a cell-based assay to monitor the function of the serotonin transporter, the gene target of the most common pharmaceutical treatment for depression: selective serotonin reuptake inhibitors. Using this assay, we can screen the 14,500

full-length clone cDNA library of Scripps Florida for genes that modify the function of the transporter. Additionally, introducing a selective serotonin reuptake inhibitor to the screen will enable us to identify genes that modulate the efficacy of this important class of drugs. Cumulatively, we hope to provide a better understanding of the molecular basis of depression and its treatment to allow for improved diagnosis and therapies.

### PUBLICATIONS

Miller, B.H., Schultz, L.E., Gulati, A., Cameron, M.D., Pletcher, M.T. Genetic regulation of behavioral and neuronal responses to fluoxetine. *Neuropsychopharmacology*, *in press*.

Pinto, L.H., Vitaterna, M.H., Shimomura, K., Siepka, S.M., Balannik, V., McDearmon, E.L., Omura, C., Lumayag, S., Invergo, B.M., Glawe, B., Cantrell, D.R., Inayat, S., Olvera, M.A., Vessey, K.A., McCall, M.A., Maddox, D., Morgans, C.W., Young, B., Pletcher, M.T., Mullins, R.F., Troy, J.B., Takahashi, J.S. Generation, identification and functional characterization of the nob4 mutation of *Grm6* in the mouse. *Vis. Neurosci.* 24:111, 2007.

## Neuroscience Discovery and Pharmacogenomics

C. Wahlestedt, M.A. Faghihi, J. Huang, J. Kocerha, M. Przydzial, A. Khalil, S. Brothers, F. Modarresi, H.-Y. Zhang,\* C. Scheele,\* C. Dahlgren,\* J.A. Timmons\*

\* Karolinska Institutet, Stockholm, Sweden

**O**ur research involves aspects of Alzheimer's disease, Parkinson's disease, depression, alcohol addiction, fragile X syndrome, autism, and aging. In addition to drug discovery efforts, currently we are focusing on basic aspects of mammalian genomics, genetics, and transcriptomics.

### IDENTIFICATION AND FUNCTIONAL ANALYSIS OF REGULATORY RNA TRANSCRIPTS

We are among the few neuroscientists who for a number of years have been, and continue to be, involved in high-throughput sequencing of transcriptomes (i.e., all the RNA transcripts in a cell) in humans and mice. Such efforts have provided strong evidence that in contrast to earlier understanding, the majority of the mammalian genome is transcribed *in vivo*. Analysis of such data sets has indicated that most mammalian RNA transcripts are noncoding.

Thus, conventional protein-coding genes appear to account for only a minority of human RNA transcripts. A substantial component of the full-length mouse and human cDNA sets that we and others have analyzed does not contain an annotated protein-coding sequence and most likely corresponds to noncoding RNA. In

addition to small RNAs, many of the noncoding RNAs constitute natural antisense RNA transcripts. We have shown that many noncoding RNAs identified to date have substantial conservation across species. We have also shown that many small noncoding RNAs and antisense transcripts have differential expression under various conditions and can affect conventional gene expression.

#### **RNA INTERFERENCE AND DEVELOPMENT OF HIGH-THROUGHPUT GENOMICS TECHNOLOGY**

RNA interference has become one of the most important gene manipulation technologies. Short interfering RNA (siRNA), the inducer of RNA interference in mammals, can be used to elucidate gene functions by rapidly silencing expression of a target gene. Today, siRNAs are widely used as research tools and have potential for becoming therapeutic agents. We have built up a powerful and versatile portfolio of siRNA technology. Finally, we have introduced the use of locked nucleic acids as components of siRNAs (and antisense oligonucleotides) and have shown a range of beneficial properties of these modified agents.

#### **G PROTEIN-COUPLED RECEPTORS AS DRUG TARGETS FOR CNS DISORDERS**

More than half of known drugs bind to G protein-coupled receptors. We have continued our long-standing work on these receptors, particularly certain neuropeptide receptors. These efforts are now part of the drug discovery program at Scripps Florida.

#### **HUMAN GENETICS AND PHARMACOGENOMICS**

We also are pursuing genotyping related to several human disorders. Our goal is to identify biomarkers associated with human disorders. We wish to understand what makes certain individuals susceptible and how their responses to drug treatment may differ (pharmacogenomics).

#### **PUBLICATIONS**

Larsson, O., Perlman, D.M., Fan, D., Reilly, C.S., Peterson, M., Dahlgren, C., Liang, Z., Li, S., Polunovsky, V.A., Wahlestedt, C., Bitterman, P.B. Apoptosis resistance downstream of eIF4E: posttranscriptional activation of an anti-apoptotic transcript carrying a consensus hairpin structure. *Nucleic Acids Res.* 34:4375, 2006.

Scheele, C., Nielsen, A.R., Walden, T.B., Sewell, D.A., Fischer, C.P., Brogan, R.J., Petrovic, N., Larsson, O., Tesch, P.A., Wennmalm, K., Hutchinson, D.S., Cannon, B., Wahlestedt, C., Pedersen, B.K., Timmons, J.A. Altered regulation of the *PINK1* locus: a link between type 2 diabetes and neurodegeneration? *FASEB J.*, *in press*.

Scheele, C., Petrovic, N., Faghihi, M.A., Lassmann, T., Fredriksson, K., Rooyackers, O., Wahlestedt, C., Good, L., Timmons, J.A. The human *PINK1* locus is regulated in vivo by a non-coding natural antisense RNA during modulation of mitochondrial function. *BMC Genomics* 8:74, 2007.

Timmons, J.A., Scheele, C., Wahlestedt, C. Do mitochondria provide a common link between diabetes and Parkinson's disease? *Pract. Diabetes Int.* 24:337, 2007.

Timmons, J.A., Wennmalm, K., Larsson, O., Walden, T.B., Lassmann, T., Petrovic, N., Hamilton, D.L., Gimeno, R.E., Wahlestedt, C., Baar, K., Nedergaard, J., Cannon, B. Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proc. Natl. Acad. Sci. U. S. A.* 104:4401, 2007.

Zhang, H.Y., Du, Q., Wahlestedt, C., Liang, Z. RNA interference with chemically modified siRNA. *Curr. Top. Med. Chem.* 6:893, 2006.