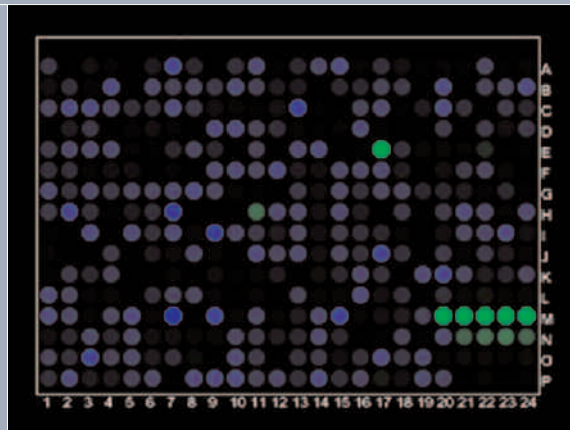


Biochemistry

High-throughput cell-based screening in the laboratory of M. D. Conkright, Ph.D., is used to uncover novel components of the cyclic AMP signaling pathway. Over 15,000 proteins were assayed for the ability to activate cAMP-responsive genes. Three out of 25 putative activators of the pathway are shown in green along with positive and negative controls in the bottom right 4 X 4 quadrant.





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Chairman's Overview

The Department of Biochemistry at Scripps Research was recently created to recruit faculty members to both the California and 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 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.

We have broad interests and 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 affect 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, N. Chen, J. Chu, A. deSchöpke, E. Farré, E. Hamilton, S.P. Hazen, A. Helfer, T. Imaizumi, B. Jenkins, W. Lewis, C.Y. Liu, A. Para, J. Pruneda, A.L. Quiroz, T.F. Schultz, H.G. Tran,* D.K. Welsh,** E. Zhang

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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 defect in circadian rhythms, 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 the 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 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 orphan 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

Farré, E.M., Harmer, S.L., Harmon, F.G., Yanovsky, M.J., Kay, S.A. Overlapping and distinct roles of *PPR7* and *PPR9* in the *Arabidopsis* circadian clock. *Curr. Biol.* 15:47, 2005.

Harmer, S.L., Kay, S.A. Positive and negative factors confer phase-specific circadian regulation of transcription in *Arabidopsis*. *Plant Cell* 17:1926, 2005.

Hazen, S.P., Borevitz, J.O., Harmon, F.G., Pruneda-Paz, J.L., Schultz, T.F., Yanovsky, M.J., Liljegren, S.J., Ecker, J.R., Kay, S.A. Rapid array mapping of circadian clock and developmental mutations in *Arabidopsis*. *Plant Physiol.* 138:990, 2005.

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, 2005.

Imaizumi, T., Schultz, T.F., Harmon, F.G., Ho, L.A., Kay, S.A. FKF1 F-box protein mediates cyclic degradation of a repressor of *CONSTANS* in *Arabidopsis*. *Science* 309:293, 2005.

Welsh, D.K., Imaizumi, T., Kay, S.A. Real-time reporting of circadian-regulated gene expression by luciferase imaging in plants and mammalian cells. *Methods Enzymol.* 393:269, 2005.

Welsh, D.K., Kay, S.A. Bioluminescence imaging in living organisms. *Curr. Opin. Biotechnol.* 16:73, 2005.

Mechanism of Activation of Nuclear Receptors

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

Peroxisome proliferator-activated receptor γ (PPAR γ), a ligand-dependent transcription factor and member of the nuclear receptor superfamily, is the molecular target of the drug class known as the glitazones. These compounds are used to improve muscle insulin resistance, a sign or cause of type 2 diabetes, and are referred to as insulin sensitizers. The glitazones are widely prescribed for treatment of type 2 diabetes.

However, they have limited usefulness in patients with mild insulin resistance or a history of cardiovascular disease because the drugs are associated with specific receptor-mediated side effects, such as weight gain, fluid retention, and plasma volume expansion.

Unfortunately, the incidence of cardiovascular disease is high in patients with type 2 diabetes. In addition, a clear link exists between body mass index and the incidence of insulin resistance and type 2 diabetes. Thus, the search continues for a PPAR γ modulator that increases muscle insulin sensitivity without causing weight gain and plasma volume expansion.

In addition, recent data indicate that thiazolidinedione-based PPAR γ agonists may be carcinogenic in rodents. Because of this finding, the Food and Drug Administration requires the completion of 2-year carcinogenicity studies in rodents before any new PPAR γ modulator can be tested in phase 2 studies in humans. Studies with animal models of insulin resistance have shown that weight gain and plasma volume expansion can be minimized without loss of insulin sensitization by using partial agonists of PPAR γ , although the mechanism of this dissociation of efficacy from unwanted events is unclear. Studies with preadipocytes revealed that these partial agonists do not induce adipogenesis as the full agonists do, and expression profiling has shown that the gene expression patterns differ for the different functional classes of activators.

Currently, we are studying the mechanism of activation of PPAR γ by partial agonists and the role of conformation-mediated recruitment of coactivators in adipogenesis. 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 coupled with mass spectrometry was used to characterize ligand-dependent structure and dynamic changes in PPAR γ . Ligand-induced protection of amide exchange in the transcriptional complex composed of PPAR γ , retinoic X receptor α , and steroid receptor coactivator-1 was probed by using automated solution-phase hydrogen-deuterium

exchange. We used fluorescence 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 genetic screen was used to probe the molecular determinants of ligand-dependent coactivator selectivity. The functional relevance of the screen was further confirmed in a 3T3-L1 preadipocyte differentiation assay as an *in vitro* model of adipogenesis.

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 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. The results obtained from this research will provide molecular insight into how recruitment of coactivators modulates activation of PPAR γ and shed light on the role of specific coactivators in the pharmacologic behavior of PPAR γ modulators.

Research is also under way to improve the technical aspects of the amide hydrogen-deuterium exchange method. We have developed a fully automated system for hydrogen-deuterium exchange experiments. The automation of the method created an absolute requirement for an integrated data management and analysis system. In collaboration with B. Pascal, Scripps Florida, we are developing software to control workflow, storage, and analysis of data obtained in hydrogen-deuterium exchange experiments. When the software solution, named HD Desktop, is completed, it will be offered to academic laboratories as an open source program.

PUBLICATIONS

Chalmers, M.J., Busby, S.A., Pascal, B.D., He Y., Hendrickson, C.L., Marshall A.G., Griffin, P.R. Probing protein ligand interactions by automated hydrogen/deuterium exchange mass spectrometry. *Anal. Chem.* 78:1005, 2006.

Hamuro, Y., Coales, S.J., Morrow, J.A., Molnar, K.S., Tuske, S.J., Southern, M.R., Griffin, P.R. Hydrogen/deuterium-exchange (H/D-Ex) of PPAR γ LBD in the presence of various modulators. *Protein Sci.* 15:1883, 2006.

Holt, T.G., Flick, R.B., Rohde, E., Griffin, P., Rohrer, S.P. Biomarker discovery in rat plasma for estrogen receptor- α action. *Electrophoresis* 26:4486, 2005.

Quint, P., Ayala, I., Busby, S.A., Chalmers, M.J., Griffin, P.R., Rocca, J., Nick, H.S., Silverman, D.N. Structural mobility in human manganese superoxide dismutase. *Biochemistry*, *in press*.

Insulin-Degrading Enzyme

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

AGING AND NEURODEGENERATION

We 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 β -protein, peptide substrates central to the pathogenesis of diabetes and Alzheimer's disease, respectively. We have shown that enhancing the proteolysis of amyloid β -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 for manipulating the activity of this protease in in vivo models. To meet this important need, we deduced the cleavage-site specificity of IDE by analyzing combinatorial mixtures of peptides. On the basis of this knowledge, we designed and synthesized peptide hydroxamates, which 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. The simplicity and versatility of this approach have enabled us to generate fluorescently labeled and affinity-tagged probes and cyclic peptide hydroxamates with exquisite selectivity for IDE.

The best compounds inhibit in the low nanomolar to high picomolar range, making them greater than 10,000 times more potent than any previously described small-molecule inhibitors for IDE. These new reagents promise

to yield new insights into the function of IDE and its homologs in vivo and could pave the way to a novel class of therapeutic agents based on slowing insulin clearance. Characterization of these compounds in cells and animal models is under way.

PUBLICATIONS

Leissring, M.A. Proteolytic degradation of the amyloid β -protein: the forgotten side of Alzheimer's disease. *Curr. Alzheimer Res.*, *in press*.

Leissring, M.A., Saido, T.C. $A\beta$ degradation. *In: Alzheimer's Disease: Advances in Genetics, Molecular, and Cellular Biology*. Sisodia, S.S., Tanzi, R.E. (Eds.). Springer, New York, 2006, p. 139.

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

P. LoGrasso, M. Cameron, D. Duckett, R. Jiang, T. Kamenecka, L. Lin

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 of 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.

This strategy 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 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 data, we are synthesizing potent, selective JNK 2/3 inhibitors that may be efficacious in MPTP animal models of Parkinson's disease. We have established homogeneous time-resolved fluorescence biochemical assays for JNK3 and counterscreens for JNK1 and p38. We have generated nanomolar inhibitors of JNK3 from 2 structural classes of compounds. 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. Moreover, we have screened 400,000 compounds in an effort to discover new structural classes that may be optimized as described. Current efforts are centered on (1) development of cell-based assays to monitor inhibition of c-Jun phosphorylation and survival of dopaminergic neurons and (2) 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.

PUBLICATIONS

Fricke, M., LoGrasso, P., Ellis, S., Wilkie, N., Hunt, P., Pollack, S.T. Substituting c-Jun N-terminal kinase-3 (JNK3) ATP-binding site amino acid residues with their p38 counterparts affects binding of JNK- and p38-selective inhibitors. *Arch. Biochem. Biophys.* 438:195, 2005.

Zhang, W.X., Wang, R., Wisniewski, D., Marcy, A.I., LoGrasso, P., Lisnock, J.M., Cummings, R.T., Thompson, J.E. Time-resolved Forster resonance energy transfer assays for the binding of nucleotide and protein substrates to p38 α protein kinase. *Anal. Biochem.* 343:76, 2005.

Genetic Determinants of the Efficacy of Selective Serotonin Reuptake Inhibitors

M.T. Pletcher, A. Gulati, B.H. Miller, B.M. Young,
G.M. Zastrow

Clinical 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 they 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 tests, 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 for these measurements 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 inhibi-

tors. Using this assay, we can screen the 18,000 full-length clone cDNA library of Scripps Florida for genes that modify the function of the transporter. Additionally, introducing a selective serotonin reuptake inhibitor into 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 improved diagnosis and therapies.

PUBLICATIONS

Fries, S., Grosser, T., Price, T.S., Lawson, J.A., Kapoor, S., DeMarco, S., Pletcher, M.T., Wiltshire, T., FitzGerald, G.A. Marked interindividual variability in the response to selective inhibitors of cyclooxygenase-2. *Gastroenterology* 130:55, 2006.

Ishimori, N., Li, R., Walsh, K.A., Korstanje, R., Rollins, J.A., Petkov, P., Pletcher, M.T., Wiltshire, T., Donahue, L.R., Rosen, C.J., Beamer, W.G., Churchill, G.A., Paigen, B. Quantitative trait loci that determine BMD in C57BL/6J and 129S1/SvImJ inbred mice. *J. Bone Miner. Res.* 21:105, 2006.

Reijmers, L.G., Coats, J.K., Pletcher, M.T., Wiltshire, T., Tarantino, L.M., Mayford, M. A mutant mouse with a highly specific contextual fear-conditioning deficit found in an *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis screen. *Lern. Mem.* 13:143, 2006.

Synthesis of Natural Products, Development of Synthetic Methods, and Medicinal Chemistry

W.R. Roush, R. Bates, Y.-T. Chen, E. Darout, A. DeBaillie, J. Dunetz, G. Halvorsen, M. Handa, J. Hicks, T. Hopkins, C.-W. Huh, W. Lambert,* R. Lira, C. Nguyen, M. Ober, R. Owen,* P. Orahovats,* R. Pragani, J. Qi,* T. Ryba, A. Sorg, K. Takao,** R. Thalji,* M. Tortosa, P. Va, A. Williams, S. Winbush

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Our research has 2 major themes. One is the synthesis of structurally complex, biologically active natural products (Fig. 1). These efforts in total synthesis are pursued in parallel with stereochemical studies and the development of new synthetic methods. We have been particularly interested in stereochemical aspects of intramolecular and transannular Diels-Alder reactions, in the development of methods for the diastereoselective and enantioselective reactions of allylmetal compounds with carbonyl compounds, and in the development of highly stereoselective methods for synthesis of 2-deoxyglycosides.

Recent studies to develop new synthetic methods included studies on the Diels-Alder reactions of

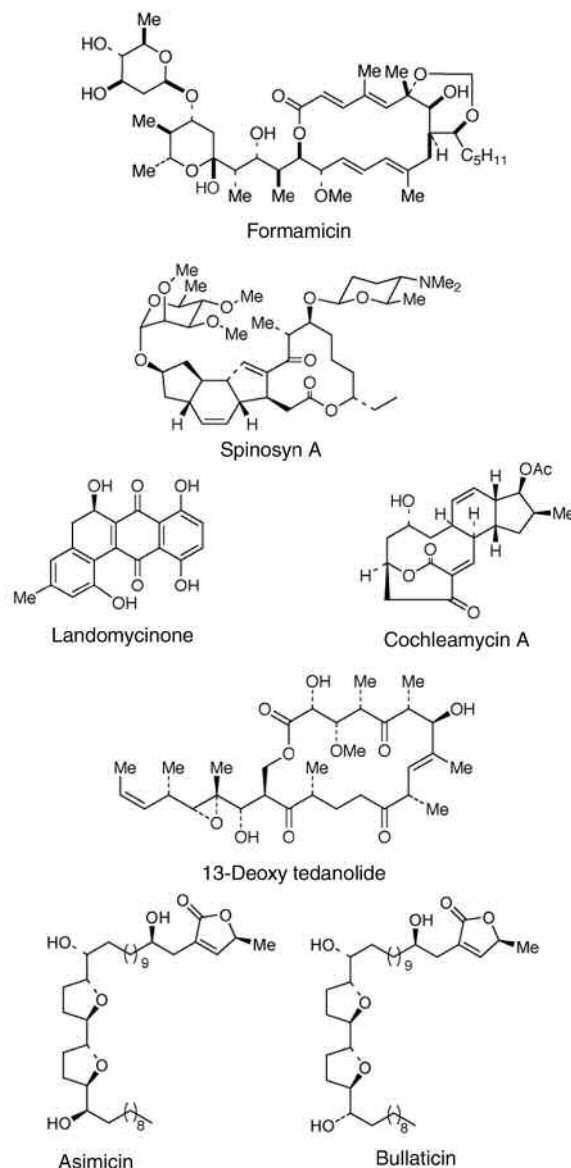


Fig. 1. Structures of recently synthesized natural products.

(*Z*)-dienes, double allylboration reactions of aldehydes with γ -boryl-substituted allylboranes for stereocontrolled synthesis of 1,5-endiols, synthesis of highly substituted tetrahydrofurans via [3 + 2]-annulation reactions of highly functionalized allylsilanes, and use of 2-deoxy-2-iodoglycosyl imidates and 2-deoxy-2-iodoglycosyl fluorides for the highly stereocontrolled synthesis of 2-deoxy- β -glycosides.

Natural products of current interest to us include amphidinolides C and F, amphidinol 3, angelmicin B, annonaceous acetogenin analogs, durhamycin A and analogs, integramicin, lomaiviticin A, peloruside A, psymbirin, quartromicin D1, reidispongolide A, spinosyn A biosynthetic intermediates, scytophycin C, superstolide

A, tedanolide, and tetrafibricin. We selected these molecules as targets because of their biological properties and their interesting and complex structures. We place a significant emphasis on the discovery, development, and/or illustration of new reactions and synthetic methods for achieving high levels of stereochemical control in each of these synthesis efforts.

Our second major area of interest focuses on problems in bioorganic chemistry and medicinal chemistry. One long-term project involves the design and synthesis of inhibitors of cysteine proteases isolated from tropical parasites, such as *Trypanosoma cruzi*, the causative agent of Chagas disease, and *Plasmodium falciparum*, the most virulent of the malaria parasites. This research is performed in collaboration with colleagues at the University of California, San Francisco. We have also participated in the development of proapoptotic benzodiazepines, in collaboration with G.D. Glick at the University of Michigan, and the design of novel heterocycles targeting HIV. New projects at Scripps Florida involve discovery of small molecules that affect cancer and other validated targets, studies of the structure-activity relationship of certain natural products, and optimization of the pharmacologic profiles of natural products.

PUBLICATIONS

Dias, L.C., Aguilar, A.M., Salles, A.G., Jr., Steil, W.J., Roush, W.R. Concerning the application of the ^1H NMR ABX analysis for assignment of stereochemistry to aldols deriving from aldehydes lacking β -branches. *J. Org. Chem.* 70:10461, 2005.

Hicks, J.D., Flamme, E.M., Roush, W.R. Synthesis of the C(43)-C(67) fragment of amphidinol 3. *Org. Lett.* 7:5509, 2005.

Lambert, W.T., Roush, W.R. Synthesis of the A-B subunit of angelmicin B. *Org. Lett.* 7:5501, 2005.

Thalji, R.K., Roush, W.R. Remarkable phosphine-effect on the intramolecular aldol reactions of unsaturated 1,5-diketones: highly regioselective synthesis of cross-conjugated dienones. *J. Am. Chem. Soc.* 127:16778, 2005.

Neuroscience Discovery and Pharmacogenomics

C. Wahlestedt, P. Kenny, P. McDonald, M.A. Faghihi, J. Kocerha, M. Przydzial, H. Thonberg,* H.-Y. Zhang,* T. Andersson,* O. Larsson,* L. Huminiecki,* C. Scheele,* P. Engstrom,* B. Lenhard,* C. Dahlgren,* K. Wennmalm,* Z. Liang, J.A. Timmons*

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Our research involves aspects of Alzheimer's disease, Parkinson's disease, depression, addiction, fragile X syndrome, autism, and aging. In

addition to drug discovery efforts, currently we are focusing on basic aspects of mammalian genomics and transcriptomics.

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF NONCODING AND ANTISENSE TRANSCRIPTS

Conventional protein-coding genes account for 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. Many of the noncoding sequences constitute natural antisense RNA transcripts. We have shown that the majority of noncoding RNAs identified to date have substantial conservation across species. We have also shown that many noncoding RNA and antisense transcripts have differential expression under various conditions and can affect conventional gene expression.

Antisense transcription, yielding both coding and noncoding RNA, is a widespread phenomenon in mammals. The mechanisms by which natural antisense transcripts may regulate gene expression are largely unknown. We recently provided direct evidence against the formation of sense-antisense RNA duplexes in the cytoplasm of human cells and subsequent activation of RNA interference associated with the nuclease Dicer.

Interestingly, however, a number of antisense transcripts regulate the expression of their sense partners, either discordantly (silencing of antisense transcripts results in increases in sense transcripts) or concordantly (silencing of antisense transcripts results in concomitant decreases in sense transcripts). We have proposed 2 new pharmacologic strategies based on the suppression of antisense RNA transcripts by small interfering RNA (siRNA). First, in discordant regulation, silencing of antisense transcripts increases the expression of the conventional (sense) gene, thereby conceivably mimicking agonist/activator action. Second, in concordant regulation, silencing of antisense transcripts alone or a synergistic concomitant silencing of antisense as well as sense transcripts results in reduction of the conventional (sense) gene expression.

RNA INTERFERENCE AND DEVELOPMENT OF HIGH-THROUGHPUT GENOMICS TECHNOLOGY

RNA interference has become one of the most important gene manipulation technologies. 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 portfolio of siRNA technology. In this package we have a powerful siRNA vector system, a validation system, and a design system, all of which are unique. Combining these technologies with the high-throughput chemistry for on-chip DNA synthesis, we have set up a system for constructing siRNAs. Finally, we have introduced the use of locked nucleic acids in siRNAs and have shown beneficial properties of these agents.

G PROTEIN-COUPLED RECEPTORS AS DRUG TARGETS

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 forming part of our drug discovery program.

HUMAN GENETICS AND PHARMACOGENOMICS

We are also pursuing drug discovery related to several human disorders that affect the brain. Our goal is to identify markers in the human genome that are associated with such common disorders. We wish to understand what makes certain individuals susceptible and how their responses to drug treatment may differ (pharmacogenomics).

PUBLICATIONS

Carninci, P., Sandelin, A., Lenhard, B., et al. Genome-wide analysis of mammalian promoter architecture and evolution. *Nat. Genet.* 38:626, 2006.

Dahlgren, C., Wahlestedt, C., Thonberg, H. No induction of anti-viral responses in human cell lines HeLa and MCF-7 when transfecting with siRNA or siLNA. *Biochem. Biophys. Res. Commun.* 341:1211, 2006.

Engstrom, P.G., Suzuki, H., Ninomiya, N., Akalin, A., Sessa, L., Lavorgna, G., Brozzi, A., Luzzi, L., Tan, S.L., Yang, L., Kunarso, G., Ng, E.L., Batalov, S., Wahlestedt, C., Kai, C., Kawai, J., Carninci, P., Hayashizaki, Y., Wells, C., Bajic, V.B., Orlando, V., Reid, J.F., Lenhard, B., Lipovich, L. Complex loci in human and mouse genomes. *PLoS Genet.* 2:e47, 2006.

Faghihi, M.A., Wahlestedt, C. RNA interference is not involved in natural antisense mediated regulation of gene expression in mammals. *Genome Biol.* 7:R38, 2006.

Fredman, D., Sawyer, S.L., Stromqvist, L., Mottagui-Tabar, S., Kidd, K.K., Wahlestedt, C., Chanock, S.J., Brookes, A.J. Nonsynonymous SNPs: validation characteristics, derived allele frequency patterns, and suggestive evidence for natural selection. *Hum. Mutat.* 27:173, 2006.

Frith, M.C., Wilming, L.G., Forrest, A., Kawaji, H., Tan, S.L., Wahlestedt, C., Bajic, V.B., Kai, C., Kawai, J., Carninci, P., Hayashizaki, Y., Bailey, T.L., Huminiecki, L. Pseudo-messenger RNA: phantoms of the transcriptome. *PLoS Genet.* 2:e23, 2006.

Timmons, J.A., Norrbom, J., Scheele, C., Thonberg, H., Wahlestedt, C., Tesch, P. Expression profiling following local muscle inactivity in humans provides new perspective on diabetes-related genes. *Genomics* 87:165, 2006.

Wahlestedt, C. Natural antisense and noncoding RNA transcripts as potential drug targets. *Drug Discov. Today* 11:503, 2006.

Wennmalm, K., Wahlestedt, C., Larsson, O. The expression signature of in vitro senescence resembles mouse but not human aging. *Genome Biol.* 6:R109, 2005.