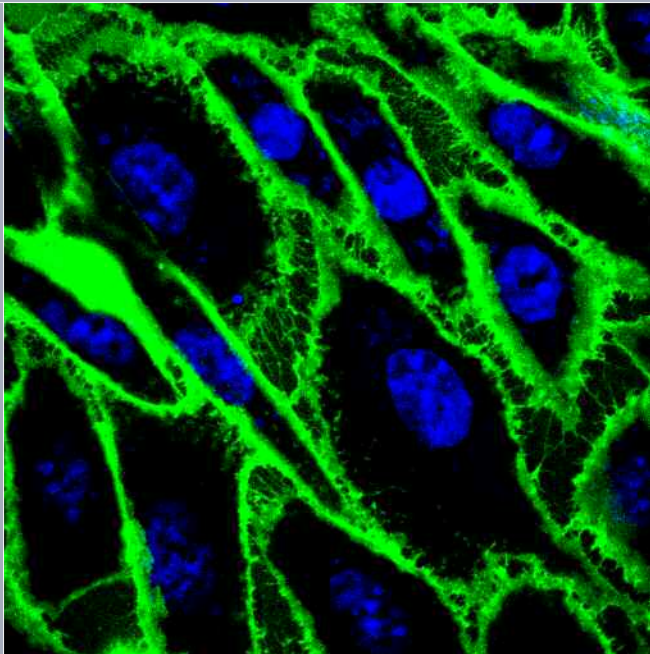


Chemical Physiology

Glycoproteins on Chinese hamster ovary cells were chemically tagged with biotin and illuminated with fluorescently labeled streptavidin. Biotin was introduced by using a novel and efficient labeling procedure developed by researchers in the laboratories of J. Paulson and P. Dawson. Selective periodate oxidation of cell-surface sialic acids was followed by an aniline-catalyzed oxime ligation. Nuclei were stained with TOTO-3 (blue). Photograph by Ramya Chakravarthy, Ph.D., research associate, in the laboratory of James Paulson, Ph.D., professor.





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** Joint appointments in the
Department of Chemistry, The
Skaggs Institute for Chemical
Biology, and the Helen L. Dorris
Child and Adolescent
Neurological and Psychiatric-
Disorder Institute*

*** Appointment completed, new
location shown*

**** Joint appointment in The Skaggs
Institute for Chemical Biology*

***** Joint appointment in the
Department of Molecular and
Experimental Medicine*

****** Appointment completed*

Chairman's Overview

The Department of Chemical Physiology aims to bring together researchers dedicated to developing and using cutting-edge chemical technologies to address biological problems of exceptional complexity and medical relevance. The success of genome-sequencing projects has propelled 21st century biologists and chemists into an era in which the focus has shifted from the discovery of new genes to the functional characterization of gene products. Indeed, of the more than 20,000 genes in the human genome, at least half lack functional annotation. This finding underscores how little we still understand about the molecular basis of life and its disorders, and at the same time highlights the tremendous opportunity that awaits postgenomic researchers interested in advancing new methods to characterize gene and protein function on a global scale.

The goal of the Department of Chemical Physiology is to discover, characterize, and eventually control the biochemical pathways that regulate higher order physiologic and pathologic processes. We are committed to creating innovative analytical and pharmacologic tools to address biological problems at multiple levels of inquiry, to move seamlessly from molecules to cellular pathways to living systems. Emergent from these studies will be a detailed understanding of the chemistry of life, along with the requisite tools to probe pathways and restore dysregulated states in human disease. Recent discoveries made by researchers in the department are highlighted in the following paragraphs.

Marty Fedor is studying the mechanisms of RNA catalysis, which controls many important cellular processes, including gene expression and mRNA stability. She and her group have used innovative biophysical methods to overturn generally accepted views of how RNA molecules cleave phosphodiester bonds. Natasha Kralli has discovered a network of protein regulators of nuclear receptors and is using a combination of chemical and genetic technologies to elucidate their roles in controlling mitochondrial biogenesis. Jeanne Loring has applied large-scale profiling technologies to map molec-



Benjamin Cravatt, Ph.D.

ular signatures that define the pluripotency of human embryonic stem cells. The signatures point to key genes and proteins that can be targeted to control the differentiation potential of these cells.

In his own group and as director of the Consortium for Functional Glycomics, Jim Paulson has developed innovative technologies for characterizing and controlling carbohydrate-protein interactions on a global scale, in particular, interactions that involve the siglec family of sialic acid-binding proteins. These proteins play critical roles in the immune system, and new methods to control their signaling could lead to new strategies to treat immune system disorders.

Kerri Mowen has discovered that arginine methylation, an unusual posttranslational modification, plays an important role in regulating the expression of cytokines in T cells. These studies may lead to the identification of new therapeutic targets to treat diseases associated with excessive cytokine signaling, such as arthritis.

In his own group and as director of the Scripps Research Molecular Screening Center, Hugh Rosen has created powerful new chemical probes to selectively perturb receptors in the sphingosine 1-phosphate signaling network. These probes have revealed important functions for the receptors in immunosuppression, inflammation, and maintenance of vascular integrity. Enrique Saez has determined that the liver X receptor, which is involved in maintaining cholesterol and triglyceride homeostasis, also is activated by the sugar glucose. The receptor thus serves as a key node for cross talk between lipid and sugar metabolic pathways and could play a role in both diabetic and atherosclerotic syndromes.

John Yates has continued to be a pioneer in the development and application of advanced mass spectrometry-based proteomics methods for large-scale analysis of proteins in biological systems. In particular, he and his group have used proteomics to identify proteins controlled by insulin, greatly expanding our knowledge of the pathways regulated by this key hormonal signal.

In my group, we have used active site-directed chemical probes to broadly profile enzyme classes in complex biological systems. Our results have led to the discovery of novel enzymes that regulate key lipid signaling molecules in the brain and enzymatic pathways that are dysregulated in human cancer cells.

Investigators' Reports

Chemical Physiology

B.F. Cravatt, D. Bachovchin, K.T. Barglow, J.L. Blankman, M.H. Bracey, E.E. Carlson, M. Dix, H. Hoover, W.W. Li, J.Z. Long, B.R. Martin, K. Masuda, M.K. McKinney, S. Niessen, G.M. Simon, J. Thomas, S.E. Tully, E. Weerapana, A.T. Wright

We are interested in understanding complex physiology and behavior at the level of chemistry and molecules. At the center of cross talk between different physiologic processes are endogenous compounds that provide a molecular mode for intersystem communication. However, many of these molecular messages remain unknown, and even in the instances in which the participating molecules have been defined, the mechanisms by which these compounds function are for the most part still a mystery.

We are investigating a family of chemical messengers termed the fatty acid amides, which affect many physiologic functions, including sleep and pain. Members of this family activate a range of signaling pathways, including the endocannabinoid system.

The *in vivo* levels of chemical messengers such as the fatty acid amides must be tightly regulated to maintain proper control over the influence of the messengers on brain and body physiology. We are characterizing a mechanism by which the level of fatty acid amides can be regulated *in vivo*. Fatty acid amide hydrolase (FAAH) degrades the fatty acid amides to inactive metabolites. Thus, the hydrolase effectively terminates the signaling messages conveyed by fatty acid amides, possibly ensuring that these molecules do not generate physiologic responses in excess of their intended purpose.

We are using transgenic and synthetic chemistry techniques to study the role of FAAH in regulating fatty acid amide levels *in vivo*. We found that transgenic mice that lack FAAH have highly elevated brain levels of fatty acid amide that correlate with reduced pain behavior, suggesting that FAAH may be a new therapeutic target for the treatment of pain and related neural disorders. In collaboration with R.C. Stevens, Department of Molecular Biology, we solved the first 3-dimensional structure of FAAH. We are using this structure as a template for the design of potent and selective inhibitors of the enzyme. In collaboration with D.L. Boger, Department of Chemistry, we have identified potent FAAH inhibitors, and

using a functional proteomic screen developed by our group, we showed that these inhibitors are highly selective for this enzyme. We are also interested in enzymes responsible for the biosynthesis of fatty acid amides and in enzymes that regulate additional classes of lipid signaling molecules in the nervous system and cancer.

Another area of interest is the design and use of large-scale technologies for the global analysis of enzyme function. The evolving field of proteomics, defined as the simultaneous analysis of the complete protein content of a given cell or tissue, encompasses considerable conceptual and technical challenges. We hope to enhance the quality of information obtained from proteomics experiments by using chemical probes that indicate the collective catalytic activities of entire classes of enzymes. Using activity-based probes that target the serine and metallo hydrolases, we have identified several enzymes with altered activities in human cancer. Using a combination of pharmacologic and molecular biology approaches, we are now testing the role that these enzymes play in cancer pathogenesis. Additionally, we are developing chemical probes that target many other enzyme families. Finally, we are developing advanced metabolomic and proteomic platforms to map the endogenous substrates and products for uncharacterized enzymes directly in living systems.

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Mechanisms of RNA Assembly and Catalysis

M.J. Fedor, J.W. Cottrell, L. Liu, L. Li, P. Watson, S. Zimmerman

Our goal is to understand how RNA enzymes catalyze biological transformations so we can gain basic insights into fundamental aspects of gene expression and build a framework for technical and therapeutic applications in which RNAs are used as targets and reagents. The hairpin ribozyme catalyzes a reversible phosphodiester cleavage reaction that involves in-line attack of the 2' oxygen nucleophile on the adjacent phosphorus to create a trigonal bipyramidal transition state and generates products with 5'-hydroxyl and 2',3'-cyclic phosphate termini. Structural studies have revealed a network of stacking and hydrogen-bonding interactions that align the reactive phosphate in the appropriate orientation for an S_N2 -type nucleophilic attack and orient nucleotide base functional groups near the reactive phosphate to facilitate catalytic chemistry (Fig. 1).

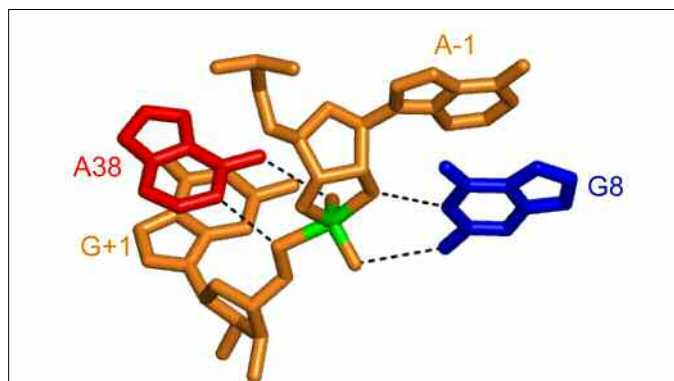


Fig. 1. Structure of the hairpin ribozyme active site in a ribozyme complex with a vanadate (green) mimic of the transition state that shows interactions with the catalytically essential G8 and A38 nucleotide bases.

Ribonuclease A is a protein enzyme that catalyzes the same chemical reaction as the hairpin ribozyme but has an active site composed of amino acids rather than nucleotides. Ribonuclease A provides a textbook example of concerted general acid-base catalysis in which a histidine acts as a general base to activate nucleophilic attack by removing a proton from the 2' hydroxyl while a second histidine protonates the 5' oxygen-leaving group to facilitate breaking the 5' oxygen-phosphorus bond. The positions of G8 and A38 nucleobases in the active site of the hairpin ribozyme resemble the orientation of the 2 histidine side chains in the active site of ribonuclease A, leading to the suggestion that G8 and A38 might also mediate general acid-base catalysis. With an acid ionization constant near 6.5, significant fractions of histidine residues are in both protonated and deprotonated states, so histidines are adept at accepting and donating protons at neutral pH. However, adenosine and guanosine undergo ionization only at pH extremes, at least as free nucleosides in solution, a characteristic that seems to make them poorly suited for mediating proton-transfer reactions at neutral pH.

We are using fluorescent nucleotide analogs to learn whether some feature of the ribozyme active site alters the ionization equilibria of adenosine or guanosine relative to the ionization behavior of the nucleotide bases in solution and enhances their ability to serve as general acid-base catalysts. An 8-azapurine analog of guanine has a high fluorescent quantum yield when the N1 position is deprotonated and a low fluorescent quantum yield when N1 is protonated (Fig. 2). A hairpin ribozyme in

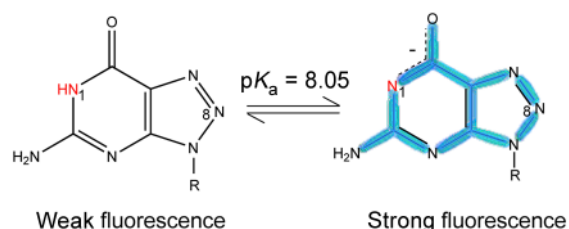


Fig. 2. 8-Azaguanine, an analog of guanine, has a high fluorescent quantum yield when the N1 position is deprotonated at high pH and low fluorescence intensity when N1 is protonated at neutral pH. Ribozymes with 8-azaguanine substituted for G8 enable us to measure any change in ionization state that might occur within the context of a functional active site.

which G8 has been replaced by 8-azaguanine has full catalytic activity, evidence that an 8-azapurine analog can indicate the ionization equilibria of G8 without perturbing the ribozyme active site. The protonation-depen-

dence of the intensity of fluorescence emission enabled us to calculate ionization equilibria from changes in emission intensity with pH.

We found that fluorescence is quenched 10- to 100-fold when 8-azaguanine is incorporated into base-paired RNA, as reported previously for other fluorescent nucleobase analogs. The apparent ionization equilibrium for 8-azaguanine that we determined from the pH dependence of fluorescence intensity in the context of a perfectly paired duplex RNA is shifted in the basic direction, consistent with the idea that removing a proton and introducing a negative charge is more difficult in a stacked RNA helix than in a free nucleotide in solution. Strikingly, the ionization equilibria of 8-azaguanine in the context of the hairpin ribozyme active site also is shifted in the basic direction relative to the ionization equilibria of 8-azaguanosine in solution. Thus, our results provide no support for the idea that G8 is more adept at general acid-base catalysis in a ribozyme active site than it would be as a free nucleoside in solution and suggest alternative roles for G8 in positioning and electrostatic stabilization.

Function of Nuclear Receptors in Stress and Mitochondrial Homeostasis

A Kralli, M.L. Gantner, B. Hazen, M.B. Hock, K. Machini, C. Tiraby-Nguyen

We are interested in the molecular mechanisms that relay metabolic and stress signals to a network of transcriptional regulators and the ensuing transcriptional outputs that mediate adaptive metabolic responses to these signals. In particular, we focus on peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α) and PGC-1 β and the orphan nuclear receptors of the estrogen-related receptor (ERR) subfamily, which control mitochondrial biogenesis and energy homeostasis. Our goals are to elucidate the biology of the PGC-1-ERR transcriptional network in skeletal muscle and the CNS, understand how deregulation of the network leads to disease, and ultimately identify the components of the network that are most suitable for drug intervention to counteract metabolic disease.

REGULATION OF THE PGC-1-ERR NETWORK

Levels of expression and activity of PGC-1 α and PGC-1 β are regulated by signals that relay changes in

metabolic needs. The coactivators then transmit such signals, via interactions with ERRs and other transcription factors, to regulate expression of target genes that mediate adaptation to the new energetic needs. We are interested in the mechanisms that regulate PGC-1s at the posttranslational level via covalent modifications or interaction with other proteins and thereby control the properties of the PGC-1-ERR network. In collaboration with M. Stallcup, University of Southern California, Los Angeles, we showed that PGC-1 α is regulated by arginine methylation via the action of the protein arginine methyltransferase 1. In collaboration with S.I. Reed, Department of Molecular Biology, we have shown regulation of PGC-1 α via phosphorylation-dependent ubiquitination by the ubiquitin ligase SCF^{Cdc4}. Currently, we are interested in understanding the interdependence of the different modifications (i.e., phosphorylation, acetylation, and ubiquitination) and how these modifications define PGC-1 activity.

THE PGC-1-ERR NETWORK IN MITOCHONDRIAL FUNCTION AND MUSCLE PHYSIOLOGY

We have shown that the orphan nuclear receptor ERR α is the major DNA-binding protein that enables PGC-1 α and PGC-1 β to regulate mitochondrial biogenesis and function. Accordingly, mice that lack ERR α have defects in mitochondrial function and cannot maintain body temperature when exposed to cold or sustain long periods of physical activity. Mice that lack ERR α have some but not all of the energetic deficiencies associated with loss of PGC-1 α or PGC-1 β , suggesting the existence of other pathways that compensate for the lack of ERR α in vivo. To elucidate these compensatory pathways, we are investigating 2 ERR α -like proteins, the orphan nuclear receptors ERR β and ERR γ . Using chemical tools and molecular genetic approaches, we are elucidating the specific roles and relative contributions of the 3 ERRs on energy homeostasis, skeletal muscle physiology, and adaptive metabolic responses. Mitochondrial dysfunction and deregulation of energy homeostasis in skeletal muscle have been implicated as underlying causes of insulin resistance and type 2 diabetes and as contributing factors in muscle degenerative diseases. Understanding the biology of ERRs can lead to new approaches for intervention in metabolic and degenerative diseases.

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Human Genome Project Meets Human Embryonic Stem Cell

J. Loring, R. Wesselschmidt, L. Laurent, F.J. Mueller, I.F. Ben-Nun, C. Lu, K. Andrews, L. Young, C. Lynch, S. Peterson

We arrived at Scripps Research in October 2007 to initiate a focus on human embryonic stem cells and other human pluripotent cells at the new Scripps Center for Regenerative Medicine. Human embryonic stem cells, first isolated about 10 years ago, are remarkably flexible cells derived from embryos donated by patients of in vitro fertilization who wish to support medical research. These stem cells have 3 qualities unique among cultured cells.

First, all primary somatic cells that come from adult human tissues eventually undergo senescence; after a limited number of cell divisions, the cells are no longer able to renew themselves. In contrast, embryonic stem cells are capable of unlimited self-renewal and are effectively immortal cell lines. Second, unlike other cells, embryonic stem cells are pluripotent, capable of forming every one of the distinctive cell types in adult tissues. Third, whereas most cells become chromosomally abnormal after a long period in culture, embryonic stem cells maintain a remarkably stable karyotype.

These qualities make human embryonic stem cells ideal for many basic and applied research applications. For drug development, they offer the opportunity to perform toxicology and screening assays on real human cell types. For example, embryonic stem cells can be differentiated into human neurons of multiple types, in quantities that make high-throughput screens feasible. Human embryonic stem cells are already in development as cell replacement therapies for a wide range of human diseases, including diabetes, heart disease, Parkinson's disease, and spinal cord injury. The major goal of the Center for Regenerative Medicine is to combine the superb expertise of scientists at Scripps Research with the potential usefulness of human embryonic stem cells.

We are studying the molecular biology of human embryonic stem cells and of a new type of genetically

engineered pluripotent cells called induced pluripotent cells. The new type of cells come from somatic adult tissues such as skin and are generated by overexpressing genes associated with human embryonic stem cells. In its earliest manifestation, the induction is triggered by using viral vectors to introduce transcription factors associated with human embryonic stem cells, but new methods that we and others are developing should simplify the process considerably within the next year. Our main objective is to understand the molecular basis of pluripotency, so that we can intelligently maneuver cells to become any cell type we require.

In the past few years, we have systematically created a large collection of highly quality-controlled human embryonic stem cells, induced pluripotent cells, somatic (adult) stem cells, and differentiated cells. We use precommercial technology in development by collaborative biotechnology groups for high-information content, high-throughput analyses of important molecules. We have completed the first global surveys of DNA methylation, protein-coding whole genome expression, and microRNA expression. In each instance, our large number of samples and powerful technology have given us an outline of what constitutes pluripotency. In each instance, we discovered a unique signature that characterizes pluripotent cells and clearly distinguishes them from all other cell types. Most recently, we discovered a unique protein-protein interaction network (the PluriNet) that is prominent in all pluripotent cells, including pluripotent cells from other species and early human and mouse embryos.

We think we have most of the pieces of the pluripotency puzzle, and we are beginning to test the key hypotheses that have come out of our large-scale analysis. We are also expanding our search for additional pieces of the puzzle, analyzing the chromatin structure of pluripotent cells, their copy number variation (with single nucleotide polymorphism genotype), and their phosphoproteome. If we can control pluripotency, then we can control differentiation, and if we can control differentiation of cells, we will have a whole new world to explore for the benefit of human health.

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Control of Cytokine Expression by Arginine Methylation

K.A. Mowen, S. Hemmers, S. Arandjelovic, A. Hallum, K. Bonham, Y. Kawakami, C. Thom

T helper cells can be divided into 2 distinct populations on the basis of their immune specificity and cytokine profiles. Type 1 helper T cells produce IFN- γ and are responsible for cell-mediated immunity; type 2 helper T cells secrete IL-4 and are associated with the humoral immune response. These 2 types of cells have been associated with susceptibility to malignant, infectious, allergic, and autoimmune diseases. The improper development of type 2 helper T cells can lead to allergy and asthma, and an overactive response by type 1 helper T cells can lead to autoimmune diseases such as type 1 diabetes.

Because of the opposing roles of the 2 types in immune function, the development and migration of helper T cells must be tightly regulated. Indeed, the discrete subsets, type 1 and type 2, reciprocally antagonize the maturation and behavior of each other in the immune response, resulting in a population of helper T cells that is primarily type 1 or type 2. Thus, manipulation of the ratio of type 1 to type 2 helper T cells provides an intriguing avenue of therapy, and understanding the molecular events that control lineage-specific cytokine expression may provide useful tools to modulate the helper T cell response.

Although several lineage-specific and nonspecific transcription factors are required for the development and function of type 1 and type 2 helper T cells, less is known about the events that occur after the reactivation of type 1 and type 2 effector populations and result in the disparate cytokine profiles of the 2 types of helper T cells. Signal transduction pathways use posttranslational modifications to translate changes in the extracellular milieu into environment-sensitive gene expression in a timely and efficient fashion.

Phosphorylation of serine, threonine, and tyrosine residues and protein ubiquitination have been widely studied. Although methylation of arginine residues was discovered more than 30 years ago, it has only recently aroused renewed interest. Arginine methylation of proteins by members of the protein arginine methyltransferase (PRMT) family regulates subcellular localization of the methylated proteins and modulates protein-protein interactions.

We discovered a unique contribution of arginine methylation to cytokine gene expression downstream of T-cell receptor signaling. Our goal is to investigate more broadly the role for arginine methylation in immune function, including further study of helper T cells and other immune cell types. We are determining upstream regulation of PRMT expression and activity and are characterizing the effects of ablation or suppression of PRMT expression. Understanding the role of posttranslational modifications, such as arginine methylation, of proteins that are key in regulating cytokine production will give us novel targets in diseases induced or exacerbated by the cytokine environment, such as inflammatory arthritis.

Chemical Glycobiology

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We investigate the roles of glycan-binding proteins that mediate cellular processes central to immunoregulation and human disease. We work at the interface of biology and chemistry to understand how the interaction of glycan-binding proteins with their ligands mediates cell-cell interactions, endocytosis, and cell signaling. Our multidisciplinary approach

is complemented by a diverse group of chemists, biochemists, cell biologists, and molecular biologists.

BIOLOGICAL ROLES OF SIGLECS

The siglecs are a family of 13 sialic acid-binding proteins that function as cell-signaling coreceptors. They are primarily expressed on various leukocytes that mediate acquired and innate immune functions, including B cells, eosinophils, macrophages, dendritic cells, and natural killer cells. Siglecs are a subfamily of the immunoglobulin superfamily that have a unique N-terminal Ig domain that binds sialic acid-containing carbohydrate groups (sialosides) of glycoproteins and glycolipids as ligands. The cytoplasmic domains of most siglecs contain tyrosine-based inhibitory motifs characteristic of accessory proteins that regulate transmembrane signaling and endocytosis of cell-surface receptor proteins. The diverse specificity for their sialoside ligands and their variable cytoplasmic regulatory elements provide siglecs with attributes for unique roles in the cell-surface biology of each cell that expresses them.

The best understood siglec is CD22 (siglec-2), an accessory molecule of the B-cell receptor (BCR) complex that has both positive and negative effects on receptor signaling. The carbohydrate ligand recognized by CD22 is the sequence Sia α 2-6Gal β 1-4GlcNAc found on both neighboring glycoproteins of the same B cell (*cis* ligands) and on cells that interact with B cells (e.g., T cells, *trans* ligands). Interactions of CD22 with *cis* or *trans* ligands regulate aspects of B-cell activation, proliferation, and development.

We found that CD22 is predominately associated with clathrin-coated pits in resting B cells, whereas BCRs are minimally associated with clathrin domains. Mice deficient in the ligand for CD22 have greater colocalization of CD22 and the BCR in fused raft-clathrin domains than do mice that have the ligand, accounting for the immunosuppression in the deficient mice. In wild-type mice, after antigen activation, the BCR is endocytosed via raft-clathrin domains, a logical site for the dampening of B-cell signaling by CD22. In resting cells, CD22 undergoes constitutive endocytosis, which can result in internalization of high-affinity ligands of CD22 (Fig. 1).

A major barrier to studying the ligand-binding properties of siglecs and their role in siglec biology is the difficulty in creating synthetic probes that compete with endogenous (*cis*) ligands. Only highly multivalent polymers containing high-affinity ligands will compete with the abundant natural *cis* ligands. Recently, we found

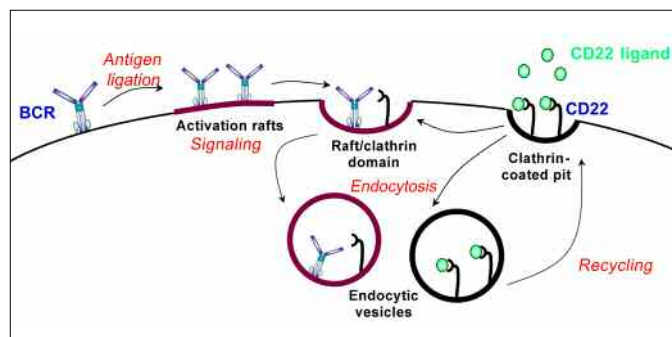


Fig. 1. Relationship between microdomain localization of the BCR and CD22 (siglec-2), a regulator of BCR signaling that binds glycan ligands.

that bifunctional molecules containing a high-affinity ligand of CD22 coupled to the antigen NP will dock an anti-NP antibody (IgM, IgA, or IgG) to CD22 on the surface of B cells. In effect, the antibody acts as a multivalent protein scaffold that promotes spontaneous assembly of an immune complex on the surface of B cells driven by the bifunctional ligand of CD22 (Fig. 2). We

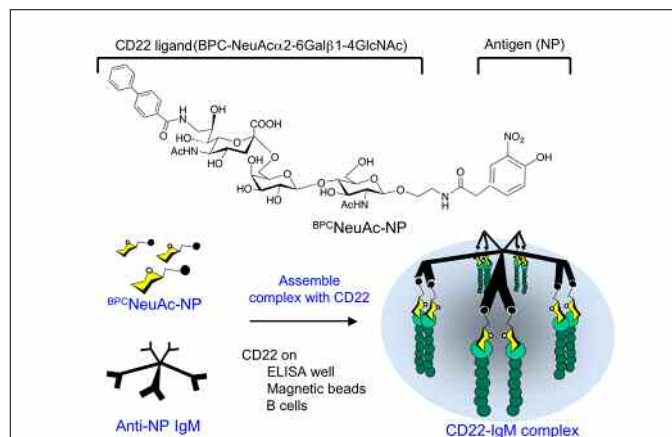


Fig. 2. Bifunctional ligands of CD22 mediate binding of IgM to CD22 on the surface of B cells.

are also collaborating with M.G. Finn, Department of Chemistry, on the use of viral capsids that can be functionalized to carry variable numbers of synthetic ligands. We are pursuing the use of such multivalent ligands for active targeting of B cells for therapy of B-cell leukemia and B-cell depletion therapy.

SIALOSIDE ANALOG GLYCAN ARRAYS

With the understanding gained from development of ligand-based probes of CD22, we are identifying high-affinity ligand analogs of each siglec to produce ligand-based tools to investigate the biological roles of the siglecs in innate and adaptive immunity. We have developed a robotically printed glycan array that displays sialoside analogs to assess the affinity of siglecs for

unnatural substituents at the C-9 and C-5 positions of sialic acids. Even in the initial experiments with 65 acyl substituents at the C-9 position of sialic acid, the method was a powerful one for identifying substituents that increase the affinity of the natural ligand for siglecs by 100-fold or more (Fig. 3). Results from the array

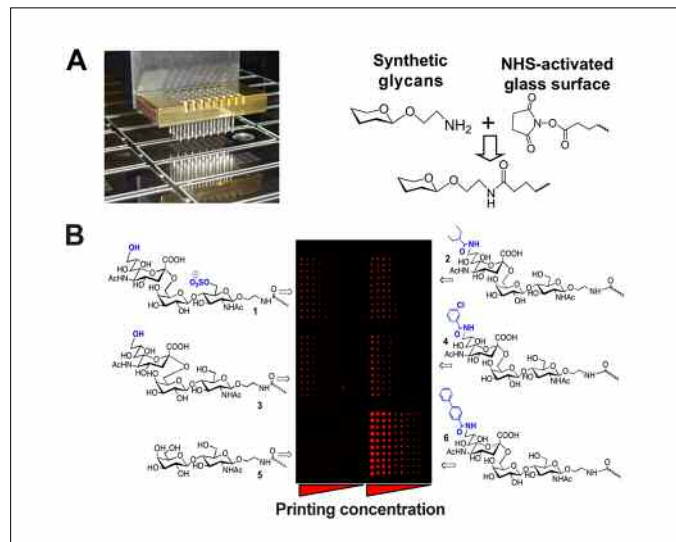


Fig. 3. Sialoside analog glycan microarray reveals high-affinity ligands for CD22. A, Sialoside ligands of CD22 with amino-terminated linkers are printed on *N*-hydroxyl succinimide (NHS)-activated glass slides, resulting in a covalent amide bond. B, The natural ligand (3) with various substituents (1, 2, 4, 6) and a nonligand control (5) are printed in 10 replicates at 10 twofold-diluted printing concentrations. Overlay with a fluorescence-labeled CD22-ig chimera reveals the increased binding to various substituents compared with the natural ligand.

can be rapidly assimilated into the synthesis of high-affinity ligands and ligand-based probes of the corresponding siglec by using flexible chemoenzymatic synthesis strategies.

CONSORTIUM FOR FUNCTIONAL GLYCOMICS

Members of our laboratory also staff 2 scientific cores for the Consortium for Functional Glycomics, organized to elucidate the mechanisms by which glycan-binding proteins mediate cell communication (<http://www.functionalglycomics.org/>). Scientists in the Mouse Transgenics Core, led by B. Ma, have created 8 novel mouse strains from C57Bl/6 embryonic stem cells that are deficient in key glycan-binding proteins that affect immune function. Scientists in the Glycan Array Synthesis Core, led by N. Razi, have produced a library of synthetic glycans by chemoenzymatic synthesis for use in numerous applications. In addition, scientists in the Scripps DNA Microarray Core, led by S. Head, have designed and conducted investigator-

initiated analysis with a custom-based microarray with genes of relevance for the consortium.

A major achievement by the Glycan Array Synthesis Core is the development of the world's largest glycan microarray, which currently has more than 400 unique structures, mostly synthetic glycans produced by chemoenzymatic synthesis. Now produced in collaboration with the DNA Microarray Core, the microarray is widely used by investigators around the world to assess the specificity of glycan-binding proteins that mediate a broad scope of biological interactions. In an exemplary collaboration with I.A. Wilson, Department of Molecular Biology, this array was used to investigate the specificity of the 1918 pandemic influenza and the more recent avian influenza (H5N1) viruses to identify mutations required to switch specificity from avian receptors to human-type receptors.

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The Scripps Research Institute Molecular Screening Center

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The Scripps Research Institute Molecular Screening Center is a national center for small-molecule screening and is part of the National Institutes of Health (NIH) Molecular Libraries Probe Production Centers Network of the NIH Roadmap. The Scripps center is distributed between the California and the Florida campuses; its component parts are assay implementation, chemistry, ultra-high-throughput screening, and pharmacokinetics. These 4 cores are unified by the informatics core in Florida, which provides a single data environment.

The mission of the Scripps center is to use the NIH library of more than 200,000 individual compounds to screen molecular and cell-based targets, which are accepted through an NIH-wide peer-reviewed application process, for proof-of-concept small-molecule probes. The Scripps center is a leading, full-production NIH center, and researchers at the center have successfully identified and published proof-of-concept molecules. Last year, the center completed 26 full-deck screens for 11 different types of molecular targets. Compounds discovered by this process are public information that can be accessed by all scientists through the PubChem database of the National Center for Biotechnology Information.

The Scripps center now integrates expertise in small-molecule discovery and optimization with state-of-the-art robotics and informatics. The center is well poised to provide new insights into the basic science of small-molecule probes of physiologic and pathologic function that can move scientific fields forward, and, we hope, over time provide new, significant insights into therapies for human diseases. Publications from the center have documented novel proof-of-concept chemical probes and provide a deeper understanding of which biological targets can be meaningfully modulated by chemical approaches in therapeutics.

Proof-of-Concept Chemical and Genetic Approaches to Signaling Lipids in Health and Disease

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IMMUNOSUPPRESSION AND VASCULAR INTEGRITY

Lymphocytes develop in the thymus (T cells) and bone marrow (B cells) and upon maturation egress from their sites of development to enter the bloodstream. Because the numbers of lymphocytes with specific receptors for antigen are limited, the probability of random productive collision of specific lymphocyte, antigen, and antigen-presenting cell in a permissive environment for an efficient immune response is low. In the immune system, this probability is enhanced by rapid recirculation of lymphocytes through secondary lymphoid organs, so that each lymphocyte has many opportunities to respond to its specific antigen. A sufficient number of blood lymphocytes are therefore essential for the development of efficient immune responses and are maintained by the recirculation of lymphocytes through the secondary lymphoid organs.

Using small synthetic druglike organic molecules, we elucidated specific molecular gatekeepers that control the number of recirculating lymphocytes. These compounds alter lymphocyte trafficking and induce clinically useful immunosuppression by activating a single sphingosine 1-phosphate (S1P) receptor subtype, S1P₁.

MOLECULAR CONTROL OF LEUKOCYTE MIGRATION AND ACTIVATION

Molecular control of migration of subsets of lymphocytes within the recirculation pathway is a fundamental issue of therapeutic importance. Although transplantation involves the sensitization of an immunologically naive host, treatment of most autoimmune diseases requires intervention in a sensitized host that already has autoreactive effector T cells in the periphery. We approached this problem by examining the role of the S1P system in the control of innate and adaptive responses and in linking coagulation and sepsis.

ROLE OF SIGNALING LIPIDS IN CONTROL OF IMMUNE AND INFLAMMATORY RESPONSES

Modulating immune and inflammatory responses is of high therapeutic interest. Enhanced integrity of

the capillary barrier protects against important inflammatory diseases of the lung, such as acute respiratory distress syndrome. We have used agonists, antagonists, genetic deletion of receptors, and biosynthetic enzymes to study the role of the signaling lipid S1P system. In collaboration with W. Ruf, Department of Immunology and Microbial Science, we defined a new critical contribution of the S1P₃ receptor on dendritic cells as a key step in the induction of disseminated intravascular coagulopathy in septic shock. In collaboration with M.B.A. Oldstone, Department of Immunology and Microbial Science, we defined a key dendritic-cell step in the immunopathology of influenza viruses that can be modulated chemically to define new therapeutic approaches that may be relevant to pandemic influenza infections.

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Transcriptional Regulation of Metabolic Pathways

E. Saez, N. Mitro, J. Pawlak, C. Godio, T. Jain, E. Dominguez

We are interested in how nutrients are sensed and in diet-modulated signaling pathways that alter gene expression to control energy balance in mammals. Proper regulation of nutrient-sensitive metabolic pathways is essential to ensure the health of an organism. Defective control of these pathways is associated with multiple serious disorders, including atherosclerosis, obesity, and diabetes. By understanding how diet influences gene expression, we aim to uncover novel therapeutic targets for treatment of metabolic diseases.

Nuclear receptors are ligand-activated transcription factors that modulate gene expression in response to endocrine and environmental signals. Several members of the family work as sensors of various dietary components, including lipids, fatty acids, retinoids, vitamins, cholesterol, bile acids, and xenobiotics. The liver X receptor (LXR) is a nuclear receptor that is activated by oxidized forms of cholesterol (oxysterols). It acts as a sensor of excessive intracellular accumulation of cholesterol and activates a program of gene expression to promote removal of harmful cholesterol. In the liver, LXRs also control triglyceride production by regulating expression of the enzymes responsible for fatty acid synthesis. In addition, LXRs modulate expression of key genes in glucose metabolism.

We recently discovered that glucose can bind LXRs and activate LXR target genes *in vivo*. This novel carbohydrate signaling pathway may be involved in determining the fate of glucose in the liver; excess glucose is sensed by the same transcription factor responsible for control of fatty acid synthesis. LXRs appear to be the molecular switch responsible for transforming surplus energy into triglycerides to be stored in fat tissue for times of deprivation.

Spread of the Western diet and a sedentary lifestyle have led to a spectacular increase in the incidence of obesity, type 2 diabetes, and cardiovascular disease. The risk for atherosclerosis is much greater in people with diabetes than in healthy individuals; more than 70% of patients with diabetes die of cardiovascular disease. The molecular details of how hyperglycemia facilitates atherogenesis remain poorly understood. Because

the LXRs can respond to both oxysterols and glucose, LXRs may be a molecular connection between diabetes and atherosclerosis. We are exploring this hypothesis.

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Advancing Applications in Mass Spectrometry–Based Proteomics

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Mass spectrometry has emerged as a powerful technique for cellular proteomics, complementing traditional gene-by-gene approaches with a comprehensive description of the molecular factors that contribute to a biologically relevant system. We remain at the forefront of this field, developing new strategies to address more sophisticated scientific questions through proteomics, such as how to measure global changes in protein abundance and how to characterize complex posttranslational modifications.

QUANTITATIVE PROTEOMICS BY MASS SPECTROMETRY

We used quantitative mass spectrometry to identify insulin-signaling targets in the worm *Caenorhabditis elegans*. DAF-2, an insulin receptor–like protein, regulates metabolism, development, and aging in *C. elegans*. In a quantitative proteomic study, we identified 86 proteins that were more or less abundant in long-lived *daf-2* mutant worms than in wild-type worms. Genetic studies on a subset of these proteins indicated that the proteins act in one or more processes regulated by DAF-2. In particular, we discovered a compensatory mechanism activated in response to reduced DAF-2 signaling that involves the protein phosphatase calcineurin.

We quantified the synaptosomal proteome of the rat cerebellum during postnatal development. Strategies

to efficiently quantify protein expression levels in the brain in a large-scale fashion are lacking. We used a novel quantification strategy for brain proteomics called stable isotope labeling of mammals. We used an ^{15}N metabolically labeled rat brain as an internal standard for quantitative multidimensional protein identification technology (MudPIT) analysis of the synaptosomal fraction of the cerebellum during postnatal development. We measured the protein expression level of 1138 proteins at 4 developmental time points; 196 protein alterations were statistically significant. This research was the first large-scale proteomic analysis of synaptic development in the cerebellum, and the method we used is a useful quantitative strategy for studying animal models of neurologic disease.

MASS SPECTROMETRY IN STUDIES OF PROTEIN PHOSPHORYLATION

We are developing new techniques for the analysis of protein phosphorylation. Protein phosphorylation is involved in many important cellular events such as cell signal transduction. We combined protein-based immobilized metal affinity chromatography (IMAC), peptide-based IMAC, and MudPIT for efficient phosphoproteomic analysis. IMAC is a common strategy used for the enrichment of phosphopeptides from digested protein mixtures. However, this strategy by itself is inefficient for analysis of complex protein mixtures. We assessed the effectiveness of using protein-based IMAC as a preenrichment step before peptide-based IMAC. Ultimately, we coupled the 2 IMAC enrichments and MudPIT in a quantitative phosphoproteomic analysis of the epidermal growth factor pathway in mammalian cells. We identified 4470 unique phosphopeptides containing 4729 phosphorylation sites.

We also developed a method for motif-specific sampling of phosphoproteomes. Phosphoproteomics, the targeted study of a subfraction of the proteome that is modified by phosphorylation, has become an indispensable tool for studies of cell signaling dynamics. We developed a method that links phosphoproteome and proteome analysis on the basis of barium-binding properties of amino acids. With this technology, motif-specific phosphopeptides can be selected independent of the system under analysis. Using MudPIT, we identified 1037 precipitated phosphopeptides from as little as 250 μg of proteins. Upon isoproterenol stimulation of HEK cells, we identified an increasing number of phosphoproteins from MAP kinase cascades and A-kinase anchor protein signaling hubs. We quantified changes in both protein

and phosphorylation levels of 197 phosphoproteins, including a critical kinase, MAP kinase 1. Integration of differential phosphorylation patterns for MAP kinase 1 with information from knowledge bases resulted in modules that correlate well with the role of MAP kinase as a node for cross talk between pathways.

BIOINFORMATICS IN MASS SPECTROMETRY PROTEOMICS

We developed Census, a quantitative software tool compatible with many labeling strategies and with label-free analyses, single-stage mass spectrometry and tandem mass spectrometry, and high- and low-resolution mass spectrometry data. Census uses powerful algorithms to address poor-quality measurements and improve quantitative efficiency, and it can support several input file formats. We tested Census with stable isotope labeling analyses and with label-free analyses.

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