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NIGMS Protein Structure Initiative

STRUCTURAL GENOMICS: INSIDE THE CUTTING EDGE

by Alisa Zapp Machalek, NIGMS

Beyond the unfolding of complete genetic sequences lies the challenge of identifying and deciphering all the proteins that make up living organisms. Structural genomics—a new field catapulted into feasibility by the success of gene-sequencing projects and advances in the tools of structural biology—approaches that task through the large-scale determination of three-dimensional protein structures.

A protein's genetic sequence can provide clues about its function, but a protein's structure can better illuminate its biological action and its

See page 4 for explanations.

role in health and disease. A solved, high-resolution structure maps all the protein's atoms, exposes surface topology and inner architecture, reveals electrochemical properties, and presents a testing ground for possible molecular partners. It paves the way for advances in structure-based drug design and the development of new medical devices and materials.

Determining high-resolution protein structures is often difficult and time-consuming, however. The essential tools of structural biology—X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy—each have their drawbacks. The former requires crystallization of the proteins, a laborious task, and the latter, though it uses proteins in solution, is usually slower and is lim-

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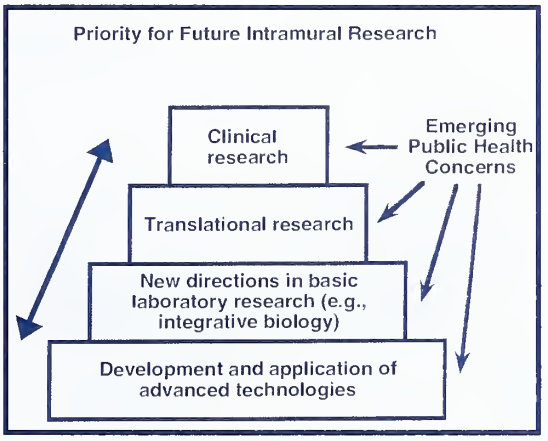
REFLECTIONS AND PROJECTIONS: TAKING STOCK OF THE INTRAMURAL PROGRAM

by Michael Gottesman
Deputy Director for Intramural Research

The intramural programs at NIH are in a period of transition. We are leaving a decade of restructuring and revitalization and moving forward to a period of evolution as the nature of how we conduct science changes.

To frame a context for exploring the directions of the next decade of intramural research, Ruth Kirschstein, NIH acting director, asked me to review for the institute and center directors the progress of the last decade and the challenges for intramural research that lie ahead. I presented my thoughts to the directors in two lively three-hour sessions April 12 and April 19.



First, let me summarize some of the statistics regarding personnel, budget, and space that informed the discussion.

■ There were 6,095 scientific personnel in 1990 and 7,728 in 2000, a 26 percent increase. That figure reflects a 53 percent increase in postdoctoral fellows—and a 25 percent decrease in PIs, allowing the recruitment of almost 200 PIs from outside NIH in the past five years.

■ There was a 78 percent increase in the intramural budget over the decade; adjusted for biomedical research inflation during the same period, however, it was really a 25 percent increase.

■ There was a 27 percent increase in usable space on and around the Bethesda campus, including the construction of Building 49 and the Vaccine Research Center and the acquisition of off-campus space.

With these figures as backdrop, I presented the following conclusions to the directors:

■ Despite only a modest growth rate in the past 10 years (about 2.2 percent a year on average), the intramural program has revitalized its scientific infrastructure, responded to public health and scientific needs, and made impressive scientific achievements (some examples follow, beginning next page).

■ Contributors to this success include rigorous scientific review to redirect resources for new or expanded programs, better-delineated career pathways, more emphasis on technology development and clinical research, and many shared resources to maximize available intramural funds, space, and personnel. (An overview of the shared resources and training programs of the IRP will appear in the next

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Taking Stock of the Intramural Program
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Michael Gottesman

issue of *The NIH Catalyst*, July-August 2001.)

- Space, budget, and personnel in the intramural program are closely linked—they have grown at the same rate over the past decade—suggesting that if one of these is no longer limiting, the others will quickly become so.
- Despite our scientific successes, there are continuing problems that demand our attention: increasing the diversity of scientific staff, making space more flexible and providing each person with more of it, and developing career pathways that reflect multidisciplinary needs of future scientific teams.

We would like to improve the infrastructure that responds to emerging scientific and public health needs. A schematic representation of the emerging paradigm for conducting research in the intramural program in the next decade is shown on page 1.

A strong base in technology development and utilization will continue to be needed to support both translational and basic biological research. Basic research here and elsewhere will build on current understanding at a molecular level to create an integrative biology that addresses how molecules interact to form cells, how cells interact to form tissues and organs, how organs form organisms, and how these organisms behave in the environment.

Translation of these basic concepts into research relevant to human health and disease, including animal models of human disease and clinical research, will remain the major goal of the intramural program.

We need the infrastructure to support this research paradigm: new, flexible spaces for research that allow interactions among different ICs and research disciplines, support for expensive instrumentation, new career tracks, and ways to recognize contributions of individuals in a multidisciplinary team. The physical infrastructure for future intramural research should focus on creation of multidisciplinary centers on and near the Bethesda campus. We already have a Vaccine Research Center, and a Neuroscience Research Center and a Musculoskeletal Center are being planned.

To optimize translational and clinical research, revitalization of the Clinical Center Complex (the existing Building 10 and the new Clinical Research Center) will be needed. The NIH Master Plan also accommodates the potential creation of a center that combines advanced technologies, integrative biology, and animal models of disease. The pace of development of this physical infrastructure and its ultimate extent depend heavily on the budget situation for NIH and maintenance of an appropriate balance between extramural and intramural funding. ■

Selected NIH Intramural Research Accomplishments 1993–2001

Organized According to Government Performance and Results Act Goals

Goal A: Add to the body of knowledge about normal and abnormal biological functions and behavior

Identification of disease genes

- AP-3, a major component of the protein trafficking system, and HPS-1: defective in two forms of Hermansky-Pudlak syndrome (NICHD)
- p16 and CDK4 mutations: responsible for familial melanoma (NCI, NHGRI)
- Transcription factor POU4F3, cadherin 23 (Usher syndrome 1D), and claudin 14: involved in familial deafness syndromes, including Pendred syndrome (NIDCD, NHGRI)
- Defect in the regulatory subunit of cAMP-dependent protein kinase: involved in Carney complex inherited cancer syndrome (NICHD)
- Defect in cholesterol synthesis pathway gene: involved in Smith-Lemli-Opitz syndrome (NICHD)
- Mutations in multifunctional ATM gene: responsible for ataxia telangiectasia (NHGRI)
- Mutations in Fas receptor and other steps in JAK-STAT pathway: responsible for autoimmune lymphoproliferative syndrome and other forms of immunodeficiency (NIAID, NHGRI, NIAMS, NCI)
- von Hippel–Lindau gene: responsible for a familial cancer syndrome (NCI, NICHD)
- MET: involved in papillary renal carcinoma (NCI)
- BRCA1 and BRCA2 mutations: major inherited causes of breast cancer (NIEHS); estimation of their contribution to risk of breast, ovarian, and prostate cancer (NCI)
- IL-1β and IL-RN polymorphisms: linked to gastric carcinomas (NCI)
- Mutations in the α-synuclein gene: associated with Parkinson's disease (NHGRI, NIMH)
- identification of transporter responsible for Niemann-Pick disease (NHGRI, NINDS, NIDDK)
- Identification of genes involved in familial Mediterranean fever and familial hybernium fever (NIAMS, NHGRI)
- Gene for multiple endocrine neoplasia (MEN) isolated and initially characterized (NIDDK, NHGRI, NINDS)
- Mutations in the *patched* gene: hereditary cause of nevoid basal cell carcinoma syndrome (NCI)
- Myosin 15 gene: responsible for nonsyndromic deafness (NIDCD)
- serotonin 1B receptor: candidate gene predisposing to alcoholism (NIAAA)
- ABC A1 cholesterol transporter: responsible for Tangier disease (NHLBI)
- FOXL2 transcription factor: defective in premature ovarian failure (NIA)
- Sarcomeric mutations: cause of familial hypertrophic cardiomyopathy (NHLBI)

Important new animal models

- Estrogen receptor α and β knockout mice (NIEHS)
- Cyclooxygenase 1 and 2 knockout mice (NIEHS)
- Transgenic mice developed and validated for use in identifying environmental carcinogens (NIEHS),
- Knockout mice for metabolic disorders including Gaucher (NIMH), Tay Sachs (NIDDK), and Fabry diseases (NINDS, NIDCR)
- Mice that develop breast cancer (NIDDK)
- Mice lacking fat (NIDDK, NCI)
- Mice with chronic granulomatous disease (NIAID)
- Mouse knockout of MEN1 gene (NIDDK, NHGRI, NCI)
- Mice with cleft palate (NICHD)
- Mice with glucose-6-phosphatase deficiency and classic glycogen storage disease type I (NICHD)
- Mouse models for Beckwith-Wiedemann syndrome and Wilm's tumor (NICHD)
- ABC A1 transporter knockout for studying cholesterol transport (NHLBI)
- Myosin light-chain knockout and transgenic mice for evaluating the role of this protein in tissue structure and development (NHLBI)
- p27, p21, and P27/p21 double knockout mice for evaluating vascular proliferation diseases and stem cell engraftment (NHLBI)
- Cyclin-dependent kinase inhibitor and apoE knockouts for analysis of atherosclerosis formation and progression (NHLBI)

Basic discoveries in cell, molecular, and structural biology with implications for the treatment of human disease

- Identification of taste and pheromone receptors (NIDCD)

- Improved understanding of signal transduction, including structure of adenylate cyclase (NIDDK) and signaling via G-α-2 through Jun kinase and Rho (NIDCR)
- Isolation and characterization of neural stem cells (NINDS)
- Role of co-receptors in HIV entry into cells (first description of fusin and demonstration that deletions in CCR5 lead to resistance to HIV infection) (NIAID, NCI)
- Mechanism of action of anthrax lethal factor through MAPKK signaling (NCI)
- Structure of HIV integrase (NCI)
- Structure and function of enzymes critical for HIV replication (NCI)
- Discovery of prions in yeast (NIDDK)
- Discovery that metalloproteinases increase after subclinical infection of the amniotic cavity, thereby weakening fetal membranes and causing 25 percent of premature births (NICHD)
- Demonstration that adult hematopoietic stem cells can give rise to cardiac muscle and blood vessels in damaged mouse myocardium (NHGRI, NINDS)
- Evidence that the dopamine transporter is the major molecular target for cocaine and that the serotonin transporter may also be involved (NIDA)
- Demonstration that endogenous marijuana-like substances (endocannabinoids) in the brain contribute to regulation of appetite (NIAAA)
- Purification and analysis of multiprotein complexes involved in faulty DNA repair in premature aging syndromes (NIA)
- Identification of nitroxides as a new class of antioxidants and protectors against radiation (NCI)
- Role of reactive oxygen species in cellular signal transduction in aging and hormone action (NHLBI)
- Demonstration that drug-associated cues that produce craving activate brain "memory" pathways and structures (NIDA)
- Ultrarapid visual reflexes that help people see clearly as they move are disrupted in patients with strabismus (NEI)
- Signal transduction pathways involved in the normal proteolytic processing of α-crystallins are altered in cataracts (NEI)

Basic discoveries in biology

- Clarification of iron metabolism regulation at the translational level (NICHD)
- Role of small RNAs in regulation of genes responsive to oxidative stress (NICHD, NCI)
- mechanisms of DNA recombination (NIDDK)
- Transcription factors can have acetylating and de-acetylating activity (NICHD)
- Discovery of natural killer cell receptor that interacts with HLA class I molecules (NIAID)
- Identification and characterization of novel human mitochondrial DNA polymerases (NIEHS)

Goal B: Develop new or improved instruments and technologies for use in research and medicine

Advances in imaging

- Improved methods to use NMR for structural determinations of proteins (NIDDK)
- New approaches for functional MRI applications to cardiac and brain imaging (NHLBI, NINDS, NICHD)
- New imaging contrast mechanism in MRI based on the exchange of magnetization of water with macromolecules, termed magnetization transfer contrast (now used in most commercial MRI scanners) (NHLBI)
- Improved 3-D tomographic reconstruction techniques for virology, small animal, and clinical imaging (CIT, NIAMS, CC, NCI)
- New optical reflectance spectroscope for clinical optimal imaging (NICHD)
- Hall effect imaging, a new modality based on the interaction of ultrasound with biological tissues in high magnetic fields (NHLBI)

Advances in bioinformatics

- Database development, including dbEST (NLM)
- Software development, including universal medical language (NLM)
- Web sites, including PubMed (NLM), visible human project (NLM), cGAP (NCI, CIT), human genome site (NHGRI, NLM), and the clinical trials database (NLM)

Advances in biotechnology

- Development of spectral karyotyping for all human chromosomes (NHGRI)
- Development of laser capture microdissection technology (NCI, BEPS, NICHD, CIT)

Goal C: Develop new or improved approaches for preventing or delaying the onset or progression of disease and disability

Vaccine development

- Clinical testing and FDA approval of vaccines against *Haemophilus influenza* (NICHD), hepatitis A (NIAID), and rotavirus (NIAID) (rotavirus vaccine use currently being evaluated)
- Polysaccharide conjugate vaccines against Salmonella (typhoid) and Shigella in successful clinical trials (NICHD)
- Successful use of acellular pertussis vaccine in Sweden (NICHD)
- New vaccine against *Escherichia coli* 0157, now being tested (NICHD)
- Preclinical work underway for a vaccine against papillomavirus (NCI) and B19 parvovirus (NHBLI)

Improved chemoprevention of disease

- Caloric restriction delays aging in nonhuman primates (NIA)
- Monoclonal antibody against respiratory syncytial virus (NIAID)
- Vitamin E supplementation reduces risk of prostate cancer (NCI)
- Antioxidant combination of β-carotene, vitamin E, and selenium reduces risk of stomach cancer (NCI)
- Diet high in carotenoids or other dietary antioxidants is associated with a decreased risk of neovascular age-related macular degeneration (NEI)

Goal D: Develop new or improved methods for diagnosing disease and disability

Gene Expression Patterns

- For diagnosis (and treatment) of breast and prostate cancer, among others (NHGRI, NCI, CIT)
- For diagnosis (and treatment) of anemias, hyperlipidemias, and vascular diseases (NHLBI)
- For evaluation of mechanisms of environmental toxicants (NIEHS)
- To analyze the aging process (NIA)

(Note: See "Goal A" for new diagnostic tests based on isolation of the disease gene)

Goal E: Develop new or improved approaches for treating disease and disability

Improved disease treatment

- Taxol to reduce smooth muscle hyperplasia after angioplasty (NIA)
- High-dose immunosuppression to treat autoimmune aplastic anemia (NHLBI)
- α-Glucosidase replacement therapy for Fabry disease (NCI)
- Immunotoxins to treat cancer (NCI)
- IL-2 with HAART to improve HIV and AIDS treatment (NIAID)
- Cysteamine to treat cystinosis (NICHD)
- Growth hormone to treat osteogenesis imperfecta (NICHD)
- Uteroglobin to treat IgA nephropathy (NICHD)
- Successful gene therapy for chronic granulomatous disease (NIAID)
- Plasmapheresis to treat pediatric autoimmune neuropsychiatric disorders associated with streptococcus (NIMH)
- Synthesis of novel cocaine analogs to treat cocaine dependence (NIDA)
- Stem cell allotransplantation to treat metastatic renal cell cancer and metastatic melanoma (NHLBI)
- Paclitaxel to treat Kaposi's sarcoma (NCI)
- Hydroxyurea to treat sickle cell anemia and inhaled nitric oxide to treat pulmonary hypertension in sickle cell anemia (NIDDK, NHLBI, CC)
- Treatment guidelines for diabetic retinopathy (NEI)
- New therapies for the ocular complications of AIDS (NEI)

Structural Genomics
continued from page 1

ited to solving the structures of small and medium-sized molecules.

Structural genomics focuses on cranking out, at industrial speed, thousands of carefully selected structures from which most others can be predicted computationally with a reasonable degree of accuracy.

This approach relies on a belief in nature's economy—that the countless different proteins in nature fold into a limited number of shapes and that all natural protein structures are a subset or combination of these shapes.

The key to structural genomics is to group proteins into families of similar structures based on their sequences. Then, based on the known structure of at least one protein in a family and using a computational technique called homology modeling, a good guess can be made about the shapes of other proteins in the family. Estimates of the number of protein structure families range from 30,000 to 50,000—orders of magnitude smaller than the total number of proteins in nature.

Andrzej Joachimiak, Argonne National Laboratory

Originally thought to look like a DNA-binding protein, this protein structure solved by Andrzej Joachimiak of Argonne (Ill.) National Laboratory, who leads the Midwest Center for Structural Genomics, turned out to be an enzyme with cyanase activity. The work illustrates how structural genomics can shed light on the evolution of protein function. This cyanase converts toxic isocyanide to ammonia and carbon dioxide, making it potentially useful as a detoxifying agent. It is a decamer composed of five dimers. The enzyme's amino acid sequence is not similar to any other known protein, and apparently neither is its structure. "The subunits of cyanase are arranged in a novel manner both at the dimer and decamer level," according to Joachimiak.

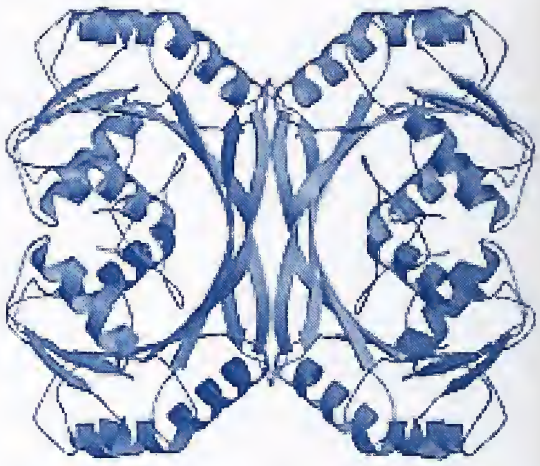
Thinking Globally, Acting Locally

Currently, there is funding for structural genomics projects in the U.S., the European Union, Japan, China, Canada, and Israel. (In early April, representatives from four continents gathered in Virginia to discuss goals, progress, and policy issues; see "International Airing," page 5.) Pharmaceutical companies and biotech start-ups are also committing to structural genomics, primarily to aid drug discovery.

The publicly funded U.S. effort is spearheaded by NIGMS, which last September launched the Protein Structure Initiative and will spend $150 million over the next five years on seven structural genomics pilot research centers, including one co-funded by NIAID (see "The First Seven," page 5). NIGMS expects to fund a few additional centers this September.

These pilot centers will develop new techniques to streamline and accelerate every step in structural genomics, from choosing which protein structures to solve to cloning and purifying the proteins, determining the structures, and depositing the data into the Protein Data Bank (PDB), an online database of macromolecular structures, maintained by the Research Collaboratory for Structural Bioinformatics.*

In five years, each of the centers will ramp up to a production level of 100 to 200 structures annually at a significantly reduced cost per structure. Using traditional techniques, it takes weeks to months—and an average of more than $100,000—to solve the structure of a single globular, soluble protein. More recalcitrant proteins, such as membrane proteins, are even more challenging.



Jacob Keller, Columbia University

A protein of Methanobacterium thermoautotrophicum, *MTO146 is one of the first structures determined by the Northeast Structural Genomics Consortium and reveals information that could alter the protein's original functional assignment. It is also known as precorrin-8W decarboxylase, a label that Columbia University (New York) crystallographer John Hunt, in whose lab the structure was solved, expects will change. "Enzymologists categorized the protein as a decarboxylase indirectly based on its sequence. We're not sure if it's a decarboxylase," he says, "but it's unambiguously a methyltransferase" based on the group's solved structure of the protein bound to S-adenosylmethionine. Recounts Jacob Keller, a research assistant in Hunt's lab, "I was simply playing around with the structure . . . superimposing structural homologs, when one of the homologs brought its AdoMet [S-adenosylmethionine] along, fitting it right into the homologous pocket in MTO146. Since all of the key contacts were in the right places, I co-crystallized the enzyme with AdoMet, and the new structure showed AdoMet density half an angstrom from where we expected it. It was very satisfying."*

Although they still don't know all the details of the enzyme's action, the group's structural data have convinced them that the protein adds methyl groups to a vitamin B_{12} precursor. Previous work from other groups shows that the enzyme is present in all organisms that make vitamin B_{12}. As an extra bonus, it contains a structural motif never seen before: a β-barrel tetramerization domain.

One long-term goal of the NIGMS project is to develop a public library of nature's protein shapes that integrates sequence, structural, and functional information. This library should enable researchers to use genetic sequences to predict the approximate structures—and possibly the function—of any protein.

To build this public resource, NIGMS is enlisting its pilot centers to determine the structures of one or two representative proteins from each of thousands of

* The Research Collaboratory for Structural Bioinformatics is a joint project of Rutgers University, Piscataway, N.J., the San Diego Supercomputer Center at the University of California at San Diego, and the National Institute of Standards and Technology, Gaithersburg, Md. It is supported by funds from the National Science Foundation, the Department of Energy, NIGMS, and NLM.

different structural families. Ten thousand unique protein structures should be solved over 10 years, which includes the current five-year scale-up phase, then five more years at full speed.

Currently, of the 15,000 structures that have been deposited in the PDB, less than 4,000 are of unique proteins, defined as those whose sequences are less than 90 percent identical. And the solved PDB structures represent only about 1,500 families. By determining 10,000 protein structures from almost as many families, the Protein Structure Initiative would more than triple the number of unique structures available and would provide more thorough coverage of structural families.

One catch at this early stage is that there are many different ways to group proteins into families. The five-year pilot period should provide time to determine whether any particular method is better than the others.

The project also seeks to identify new folds. Proteins with the same fold have similar overall shapes but no detectable sequence similarity. Such proteins have the same types of structural components connected in the same order. Studying folds could reveal the physical and chemical principles that determine how proteins form their three-dimensional structures.

Scientists estimate there are only a few thousand folds—considerably fewer than the number of structure families—and only 700 of these are represented in the PDB.

Just Data Gathering?

In its early days, structural genomics was criticized by those who believed it was a rote exercise devoid of the creativity and intellectual challenge that characterize high-quality scientific research. Although such concerns are less common now, says John Norvell, who directs the NIGMS initiative, "it's certainly true that structural genomics isn't hypothesis-driven. It's discovery-driven"—much like Darwin's detailed observations and descriptions of finches, barnacles, and other creatures, which led to his theory of evolution, Norvell observes.

Although it is clearly too early to predict the eventual impact of the Protein Structure Initiative, like its predecessor, the Human Genome Project, it promises to open a whole new chapter in biomedical research. ■

International Airing

For three days in early April, Airlie Conference Center, a restored estate in the Warrenton,Va., countryside, sounded like a miniature United Nations—but with a scientific twist. The voices speaking to each other in French, German, English, Italian, Chinese, and Japanese were those of participants in the Second International Structural Genomics meeting. They discussed policy issues, bottlenecks, and the status of their structural genomics projects. The Airlie Agreement, which is available online (**<http://www.nigms.nih.gov/news/meetings/airlie.html#agree>**), presents the consensus of the group on various policy issues.

Many of the discussions focused on balancing two different goals—timely release of all structural genomics data to the public and respect for intellectual property laws that vary significantly in different countries. The group was particularly concerned about the possibility that patents could be based solely on the submission of three-dimensional structural coordinates, without any identified nontrivial utility.

The participants agreed that for projects with public funding, researchers must deposit atomic coordinates and associated experimental data into the Protein Data Bank immediately after their determination and release most of these to the public soon thereafter. In some cases, the researchers may delay data release for up to six months to facilitate patent filing.

They also agreed that, although the goal of the field is to maximize efficiency, obtaining high-quality structures is of primary importance. Projects must not compromise quality for speed. Nor, however, should data release be "unduly delayed" while researchers endlessly refine their structures. They declined, however, to specify numerical criteria for when a structure is considered complete and ready to deposit.

—Alisa Zapp Machalek

The First Seven

The NIGMS Protein Structure Initiative is currently supporting projects at seven research centers to determine thousands of protein structures; study the relationship between genes, protein structure, and protein function; and develop new techniques. NIGMS will spend more than $150 million on these projects over five years, making it the world's single largest supporter of structural genomics. The centers, each a collaboration among multiple institutions, are:

- **Berkeley Structural Genomics Center** (**<http://www.strgen.org/>**). Will focus on two closely related bacteria with extremely small genomes—*Mycoplasma genitalium* and *Mycoplasma pneumoniae*—to study proteins essential for independent life. Aims to accelerate structure determination by X-ray crystallography.
- **The Joint Center for Structural Genomics** (**<http://www.jcsg.org/>**). Will initially focus on novel structures from the roundworm *Caenorhabditis elegans* and on human proteins thought to be involved in cell signaling and will determine the structures of similar proteins from other organisms to ensure the inclusion of the greatest number of different protein folds. Aims to develop high-throughput methods for protein production, crystallization, and structure determination.
- **The Midwest Center for Structural Genomics** (**<http://www.mcsg.anl.gov/>**). Will select protein targets from the domains Eukarya, Archaea, and Bacteria, with an emphasis on previously unknown folds and on proteins from disease-causing organisms. Aims to reduce the average cost of a protein structure from $100,000 to $20,000.
- **New York Structural Genomics Research Consortium** (**<http://www.nysgrc.org/>**). Aims to develop techniques to streamline every step of structural genomics and to solve several hundred protein structures from humans and model organisms.
- **Northeast Structural Genomics Consortium** (**<http://www.nesg.org/>**). Using both X-ray crystallography and NMR spectroscopy, will target proteins from various model organisms—including the fruit fly, yeast, and the roundworm—and related human proteins.
- **The Southeast Collaboratory for Structural Genomics** (**<http://www.secsg.org/>**). Emphasizes technology development, especially for automated crystallography and NMR techniques. Will analyze part of the human genome and the entire genomes of two model organisms genetically and biochemically similar to humans—the roundworm *Caenorhabditis elegans* and the high-temperature microbe *Pyrococcus furiosus*.
- **TB Structural Genomics Consortium** (**<http://www.doe-mbi.ucla.edu/TB/>**). A collaboration of scientists in six countries formed to determine and analyze the structures of about 400 proteins from *Mycobacterium tuberculosis*. Will optimize the technical and managerial underpinnings of high-throughput structure determination and will develop a database of structures and functions. NIAID, which is cofunding this project, anticipates this information will lead to new and improved drugs and vaccines for tuberculosis.

More information about the NIGMS Protein Structure Initiative is available at **<http://www.nih.gov/nigms/funding/psi.html>**.

'How the Social World Gets Under Our Skin': The Science of Mind-Body Interactions

by Esther Sternberg, Director
Integrative Neural Immune Program, NIMH

*The Science of Mind-Body Interactions conference, held at Masur Auditorium March 26–28, 2001, was hosted by the NIH Intramural Integrative Neural Immune Program (see box) and cosponsored by the John D. and Catherine T. MacArthur Foundation, NIMH, NINDS, and 13 other NIH institutes, centers, and offices: NCI, NHLBI, NIA, NIAID, NIAMS, NIDA, NIDCR, NLM, NCCAM, ORWH, OBSSR, OIR, and the National Center on Sleep Disorders Research, NHLBI. Hailed as a "demonstration of a paradigm shift in medicine," * the conference was attended by 500—and viewed simultaneously by 1,200 people in the United States and Canada, thanks to the web group at CIT.*

Concepts embedded in the popular culture for thousands of years were recast in the bright light of science earlier this year at a three-day conference here—the first of its kind at NIH to address the effects of such variables as individual social interactions and psychological responses on the molecular and cellular mechanisms of disease processes.

Emphasizing intervening brain pathways, molecules, and hormones, the conference on the "Science of Mind-Body Interactions" provided scientific mechanisms from the fields of neurobiology, immunology, and endocrinology to explain how the "social world gets under our skin"—a theme of the conference coined by Harvard science historian and MacArthur Foundation Mind-Body Network member, Anne Harrington.

Research directly connecting health effects observed at an epidemiological level to individual psychological and physiological health variables is not abundant. But conference speakers systematically highlighted cutting-edge research on the neurobiology of emotions; neural and neuroendocrine factors affecting autoimmune, inflammatory, allergic, and infectious diseases; and the very long arm of sleep or the lack of it.

Julian Thayer

Nancy Adler (University of California at San Francisco), director of the MacArthur Foundation Research Network on Socioeconomic Status (SES) and Health, presented evidence that lower SES is associated in a dose-related manner with adverse health outcomes. Among those SES factors that compromise health are poor living conditions, exposures to toxins and environmental irritants, stress, depression, and lack of medical care and follow-up. Most importantly, Adler said, perceived inequality leading to alienation and isolation could be factors in initiating a chain of negative health effects.

***Collegial Interactions** (left to right): Robert Rose, conference co-chair and director of the MacArthur Foundation Mind-Body Network; Esther Sternberg, conference co-chair and director of the NIMH Intramural Integrative Neural Immune Program (and author of this article), and acting NIH director Ruth Kirschstein.*

In his introductory remarks, Rose emphasized the importance of skeptical enthusiasm and solid scientific research to further the field of mind-body science. Much of the data presented during the meeting grew out of interdisciplinary projects jointly funded by NIH and the MacArthur Foundation Mind-Body Network.

In her conference-opening remarks, Kirschstein emphasized the increasing importance of interdisciplinary research, pointing to several NIH interdisciplinary initiatives in mind-body science research, including the university-based Mind-Body Centers funded through OBSSR and the newly established intramural Integrative Neural Immune Program at NIH.

Loneliness

The notion that social isolation predicts higher morbidity and mortality, especially in elderly, poor, and minority populations, was dramatically supported in three separate studies of the cardiovascular status of lonely individuals.

Reported by John Cacioppo (University of Chicago) and Julian Thayer (NIA, Baltimore), all three studies linked loneliness to greater sympathetic nervous system reactivity, higher blood pressure, or greater perceived stress. Components of this "threat" pattern (high impedance, low cardiac output), as opposed to "challenge" pattern (high cardiac output, low peripheral resistance) of cardiac reactivity, were seen in populations as varied as 2,600 undergraduate students, elderly individuals in Chicago, and elderly, isolated African Americans in inner-city Baltimore.

Preliminary findings from another study by Cacioppo and David Spiegel (Stanford [Calif.] University) provided additional support that feeling lonely can change psychological response patterns: When subjects who were not lonely were hypnotized into a lonely state, they experienced a shift in psychological variables from a challenge pattern to the threat pattern observed in truly lonely people.

Martha McClintock (University of Chicago) presented animal data showing that group-housed rats lived 40 percent longer than isolated animals. The cause of death in the isolated animals was related to opportunistic infections and tumors, suggesting that state of the organism rather than type of pathogen was a key factor.

Electricity

That emotional responses can be viewed as a transduction process by which social variables might affect health was further explored in a session on the neurobiology of emotions, chaired by Richard Davidson (University of Wisconsin at Madison). Davidson presented data combining the tools of PET and fMRI neuroimaging and EEG brain electrical activity mapping to show that differences in emotional circuitry are linked to differences in brain approach-and-withdrawal systems and differences in emotional style.

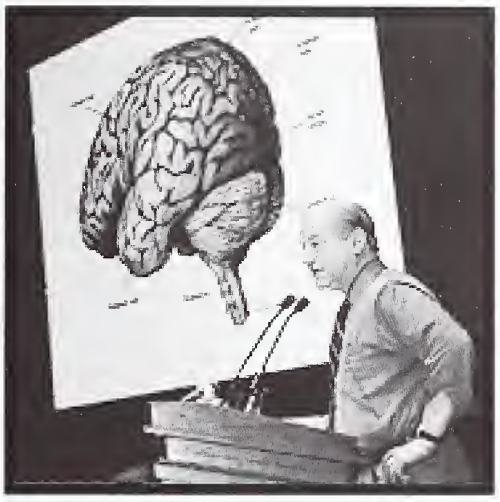
There is no single emotional center in the brain. Rather, many centers work together to detect conflict and recruit response centers that then generate be-

** Uttered by Emeran Mayer, director of the UCLA Mind Body CRC and CURE Neuroenteric Disease Program, who seemed to speak for many.*

haviors leading to mood alteration and ultimately to goal achievement.

Large-scale longitudinal studies carried out over several decades in Wisconsin show that individual differences in patterns of brain electrical activity, blood flow, and metabolism are associated with different emotional response patterns and differences in host resiliency.

Michael Meaney (McGill University, Montreal, Quebec) presented evidence that factors in early development, including maternal-offspring interactions, can influence hormonal and neuronal pathways at a molecular and cellular level and result in permanent alterations of the set-point of the hormonal stress response.



Gerald Fischbach, former NINDS director and currently vice president for health and medical sciences and dean of medicine, Columbia University, New York, returned to NIH to present his views on integrative research. During his tenure here, Fischbach was instrumental in supporting both the MacArthur/NIH Science of Mind Body Interactions conference and the Integrative Neural Immune Program.

John Sheridan (Ohio State University in Columbus) reported that mice subjected to the stress of social reorganization experienced higher mortality from viral infection and reactivation of herpesvirus. These outcomes were related to impaired immune responses and cell trafficking and changes in immune molecules that orchestrate these responses.

Perchance To Dream

The final session focused on an often- ignored fact of life that may account for many of the deleterious effects of emotions and stress on health: lack of sleep. This session, chaired by Eve Van Cauter (University of Chicago), surveyed the diverse consequences of sleep deprivation—from increased risk of motor vehicle accidents as a result of impaired cognition and motor skills and increased irritability to an array of hormonal changes—including increased blood cortisol, decreased growth hormone, and induced insulin resistance—that can impair immune function, retard growth, accelerate aging, and compromise sugar metabolism to the level of diabetes.

Interactive panel discussions throughout the conference, which engaged the audience in lively debate, also served as forums for related issues, including findings with implications for new therapeutic approaches. Among these were the use of an antidepressant phosphodiesterase inhibitor for immune suppression in the autoimmune disease multiple sclerosis and the therapeutic potential for pain management that can be deduced from the discovery of chemokine-opiate receptor interactions. ■

The archived videocast of the conference can be accessed through
<www.videocast.nih.gov>.

Integrative Neural Immune Program

The NIH Integrative Neural Immune Program is designed to foster intramural interdisciplinary research in the field of neural-immune interactions, the biological basis of the so-called "mind-body" interaction.

The program's structure also accommodates interagency, university, and private sector partnerships.

The program encompasses the study of molecular, cellular, and neuroanatomical mechanisms of neural-immune interactions, as well as systems-level analysis of communications between the central nervous, endocrine, and immune systems.

This research has relevance to the role of the immune system in neuronal cell death and repair, neuronal development and plasticity, and the role of the nervous and neuroendocrine systems in susceptibility and resistance to autoimmune, inflammatory, allergic, and infectious diseases.

Basic research in this area has clinical implications for understanding the pathogenesis of and developing treatments for diseases such as multiple sclerosis, Alzheimer's, AIDS, stroke, nerve trauma, and brain tumors.

It will inform our understanding of the effects of depression, stress, and beliefs on immune-mediated conditions, such as arthritis and allergic and infectious diseases.

This intramural research program will bridge neurobiology and immunology laboratories and related clinical branches through a series of cores—Administrative Core, Virtual Core, Scientific Communications Core, Laboratory Core, and Training Core.

A series of on-campus neural-immune lectures and intramural-extramural workshops and conferences is taking shape to foster interactions among participating labs, define the current state of the field, and create new research agendas. (The first major gathering was the "Science of Mind-Body Interactions" conference; see main story, opposite page.)

Resources to facilitate interdisciplinary collaborative research, as well as a neural-immune training program, should well serve the program's ultimate goal: to enable researchers to rapidly address cutting-edge multidisciplinary research questions and to translate basic research findings into tangible clinical health outcomes. More than 85 scientists currently participate in the program.

Directed by Esther Sternberg, chief of the NIMH Neuroendocrine Immunology and Behavior Section, the program is based at NIMH and is co-sponsored by NINDS, NCI, NIA, NIAID, and NIAMS and the OIR. ■

Hot Methods

Beyond Genomics to Clinical Proteomics: Part II: Protein Microarrays

In part one of this article we discussed the application of surface-enhanced laser desorption mass spectrometry to uncover disease-associated protein fingerprint patterns (The NIH Catalyst *March–April 2001). In part two, we describe the development of protein microarrays and discuss their potential for answering basic and applied questions.*

Protein Array Challenges . . .

Following the success of cDNA microarrays, protein arrays may seem straightforward. Unfortunately, the two types of molecules are vastly different, and the dynamic range required for proteomics may be a thousandfold more than for RNA transcripts.

cDNA interactions are governed by Watson–Crick base pairing, whereas protein-antibody interactions are determined by complex associations between epitopes on the target protein and the antigen-binding site on the antibody. These interactions are highly dependent on external environmental influences and hence may hinder the design and utilization of a generic protein array format. Moreover, sensitivities of individual antibody-antigen interactions depend on the relative abundance of the antigen-antibody species and the binding affinities.

This dependence is especially problematic if one considers the extremely broad range of protein concentrations that are present in living cells. For example, protein concentration differences between activated and nonactivated proteins in tissue may range from <2 logs to <6 logs in relative protein concentrations. Changes in a low-abundant protein, therefore, may be overshadowed by high-abundant proteins if these are analyzed on the same array.

Lastly, there is no PCR for proteins—so they cannot be amplified directly.

. . . And Solutions

About a year ago, we set out to develop a highly quantitative and precise protein array that would enable us to study defined signaling circuits within individual cell populations of clinically relevant material. We termed the robust technology that emerged "reverse-phase protein microarray" (RPPA). RPPA combines laser-capture microdissection (LCM) and cDNA microarray technologies.

First, histopathologically relevant cell populations are microdissected and lysed in a suitable lysing buffer; then, nanoliters of that lysate are arrayed with a pin-based microarrayer onto glass-backed nitrocellulose slides at defined positions.

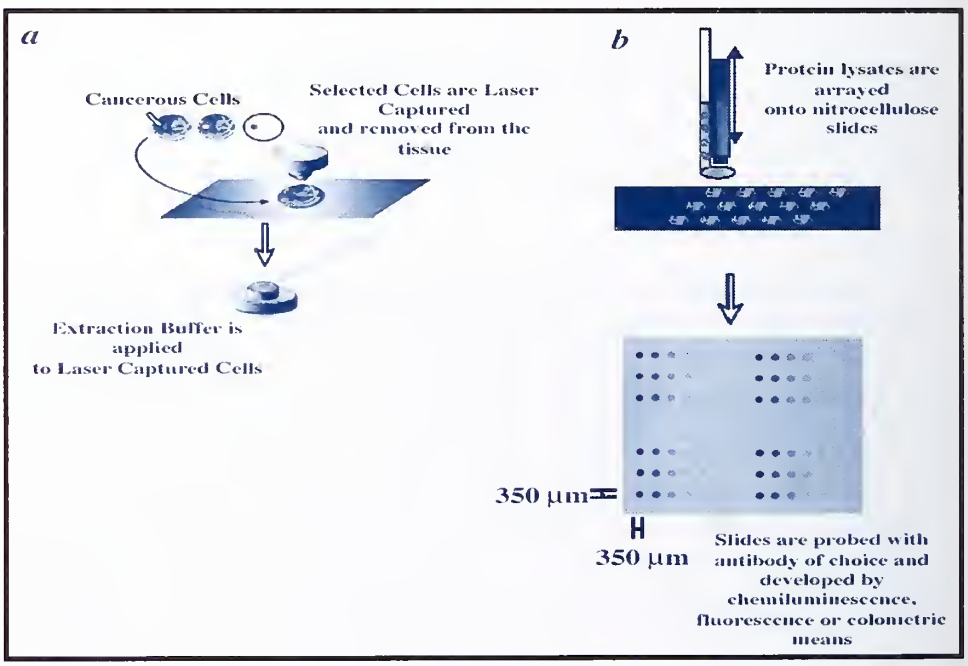
These applications result in spots that are 250–350 µm wide, each containing the *whole* cellular repertoire corresponding to a given pathologic state that has been captured. Subsequently, each slide can be probed with an antibody that can be detected by fluorescent, colorimetric, or chemiluminescent assays (see figure below).

The signal intensity of each feature is proportional to the concentration of analyte detected and therefore limited to the dynamic range of the detection method used. Thus, unlike constraints with immunohistochemical staining, it is now possible to quantify microscopic protein concentrations from one histopathologically relevant cell population to another by RPPA. To optimize the detection of each individual antibody-antigen binding pair, one arrays each cellular lysate sample in a miniature dilution curve and thus determines an optimized dynamic range for that particular antibody-antigen interaction.

RPPA differs from a complementary type of protein array, the antibody arrays—also developed by our laboratory for microdissected samples. Antibody arrays immobilize hundreds of antibodies on a solid matrix. The current limitation of antibody arrays is that the input protein sample must be labeled in a manner that does not interfere with the antibody-binding site.

In contrast, RPPA does not require labeling of the sample protein, yet has high sensitivity and precision. RPPA is best used to answer questions about specific candidate molecules and the state of critical, previously identified, nodes in the cellular circuitry. To wit:

- Is the candidate molecule actually present or altered in the human disease lesion?
- Is the candidate target within a pathway that can be verified to be aberrant in the diseased tissue chosen for treatment?



Cloud Paweletz

Schematic overview of the investigation of longitudinal cancer progression

Validation

As part of our format validation, we used this novel type of protein microarray to analyze the state of growth and apoptosis pathways at the invasion front of prostatic cancer in human tissue. We conducted extensive studies that demonstrated the linearity and reproducibility of RPPA.

Within and between slides, there was an excellent correlation between protein concentration and signal output (r^2

by Cloud P. Paweletz, NCI, FDA
Emanuel Petricion PhD FDA
Lance A. Liotta, MD, PhD, NCI

= 0.973 and r^2 = 0.952).

We analyzed phospho ERK and phospho Akt protein concentrations in patient-matched normal, PIN (premalignant), invasive carcinoma, and stromal cell populations and found that in every one of our 10 longitudinal cases, phospho ERK protein values were suppressed during the evolution of progression ($P < 0.02$), whereas phospho Akt protein values concomitantly increased ($P < 0.048$).

We validated the specificity of these results by western analyses of microdissected cells procured from the same cases.

Activation of Akt, a substrate of PI3K, may promote cell motility and survival as the invading cancer cells leave the gland and invade the stroma. Concomitantly, the suppression of ERK will prevent cell-cycle blockade and may thus deregulate cell proliferation.

Conclusion

The activated (phosphorylated) state of signal pathway checkpoints in vivo cannot be ascertained from gene expression alone. Nevertheless, the state of such pathways is a key determinant of the diseased cellular physiology and in evaluating therapeutic efficacy. To realize this goal, we are using RPPA to study changes in phosphorylated proteins and entire pathways in patient biopsy samples before, during, and after treatment. These clinical trials are being conducted within NCI. The clinical trial investigators are Elise Kohn and Susan Bates.

Application of proteomics to clinical trial monitoring may lead to patient-tailored therapy in which combinations of drugs are designed to match the proteomic profile of that patient's disease—the target will be the entire pathway itself. ■

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Materials and Methods

Microdissection and Cellular Lysate Arraying. Microdissection is carried out under careful direct pathological examination (as previously described; see *The NIH Catalyst*, November–December 1997, "Hot Methods") using a Pixcell 200 Laser Capture Microdissection system (Arcturus Engineering, Mountain View, Calif).

Before microdissection, paraffin-embedded tissue sections are deparaffinized by completely submersing the slide in Xylene three times for six minutes each.

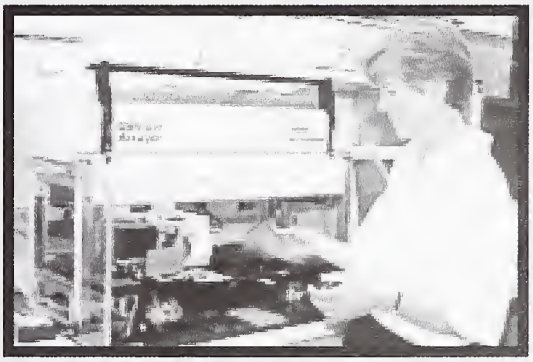
The sections are then stained according to a modified hematoxylin and eosin staining protocol that calls for treatment of tissue sections sequentially in 100%, 95%, and 70% ethanol, HPLC-grade water, hematoxylin (Sigma, St. Louis, Mo.), HPLC-grade water, blueing solution (Sigma, St. Louis), 70%, 95%, and 100% ethanol for 20 seconds each, and final dehydration in SubX. All staining baths contain 10 mmol Complete™ (Boehringer Mannheim, Germany) protease inhibitors. Between 500 and 3,000 LCM shots (approximately 2,500 and 15,000 cells, respectively) are acquired per investigated foci.

Microdissected cells are lysed in 30 µL of lysing buffer containing 1:1 mixture of 2×SDS electrophoresis buffer (125 mM Tris pH 6.8, 4% SDS, 10% glycerol, 2% β-mercaptoethanol) and Tissue Protein Extraction Reagent (Pierce, Rockford, Ill.) for 2 hours at 70 °C.

After cell lysis, samples are boiled between 3 and 5 minutes each, and 3 nL of the lysate are arrayed with a pin and ring GMSE 470 microarrayer (Affymetrix, Santa Clara, Calif.) using a 500-µm pin onto nitrocellulose slides with a glass backing (Schleicher and Schuell, Keene, N.H.). Spatial densities of 980 spots/slide and greater can easily be accommodated on a 20-nm X 30-mm slide.

Image and Statistics Analyses. Stained slides are scanned on a UMAX scanner with Adobe PhotoShop 5.5 at a resolution of 600 dpi for analyses. Scanned images (saved as "tif" files) are analyzed with ImageQuant (Molecular Dynamics, Sunnyvale, Calif.), using the "histogram" option as background correction of choice. The Wilcoxon rank-sum test is used to test group differences in the adjusted mean protein expression between histologically normal epithelium, PIN, stroma, and invasive lesions.

Two-sided statistical tests are used throughout; P-values < 0.05 are considered to be statistically significant. All analyses are performed using the statistical software package STATA (STATA Corporation, College Station, Texas). Linear regression analysis and graphing are carried out using Origin 4.1. ■



Lu Charboneau is manager of the Laser Capture Microdissection Core Facility supported under the NCI-FDA clinical proteomics initiative. The core lab conducts laser capture microdissection of tissue specimens and analyzes the microdissected cells with a variety of genomic and proteomic methods. Charboneau trains hundreds of scientists who travel to NIH to learn LCM and downstream molecular analysis. The protein microarrays described in the article are manufactured and constructed in this facility under her supervision. For more info or to set up an appointment, she can be reached at: **<lcharbon@mail.nih.gov>**.

Harbinger of the NIH Spring: Postbac Poster Day

text and photos by Valerie Judkins, OE

With posters, preceptors, and passersby crowded into the Building 10 Visitor Center reception area, 75 postbaccalaureate students presented their wares April 4 at the second annual postbaccalaurate poster session—officially signaling that this, indeed, will be an annual event.

Sponsored by the Office of Education, it was also the first display of the research done by postbacs in the inaugural class of the NIH Academy (see "The NIH Academy: Up and Running . . . , page 8, *The NIH Catalyst*, January–February 2001).

Recent college graduates who come to NIH to eat and breathe biomedical research for a year or two, many postbacs have deferred graduate or medical school to take advantage of what NIH has to offer; others—whose research interests are confirmed as they labor in an NIH lab—begin the postgraduate application process while in the throes of the program here.

In interviews with *The Catalyst* during poster day, some postbacs expressed astonishment at how much they had accomplished in a year's worth of full-time research. "It's really adding up," said Carlos Gonzalez, whose research, along with that of just a few of the other 74 postbacs who presented posters, is highlighted below.

Jessica Diggs
Perifosine, a Novel Alkylphospholipid, Blocks Cell Cycle Progression by Transcriptional Activation of $p21^{waf1/cip1}$

Jessica Diggs says her interest in health disparities led her to work in the lab of Adrian Senderowicz in the Oral and Pharyngeal Cancer Branch of NIDCR.

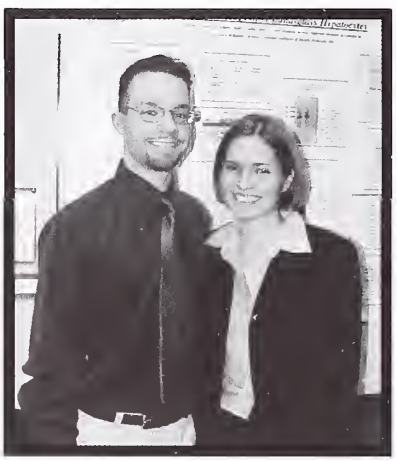
Jessica Diggs

Her research there focuses on perifosine, an alkylphospholipid that may be an important factor in controlling cell-cycle progression in head and neck cancers by activating $p21^{waf1/cip1}$, a protein that inhibits cyclin-dependent kinases.

"These cancers are often fatal and can be disfiguring for those who survive," says Diggs.

According to NIDCR data, there are about 40,000 new cases and 12,000 deaths from head and neck cancers each year. These cancers also disproportionately affect ethnic minorities and those of lower socioeconomic status.

"My research and NIH Academy activities I've participated in have greatly increased my understanding of health disparities," said Diggs, adding that African-American males have the highest incidence and mortality of head and neck cancers.



Carlos Gonzalez and Vanessa Muniz-Medina are married to one another but work in different NIH labs.

Diggs first came to NIH as a participant in the NIAID Introduction to Biomedical Research Program while she was an undergraduate at Carlow College in Pittsburgh. After graduation, she chose to return to NIH as a member of the first class of the NIH Academy, a postbac program specifically dedicated to the elimination of health disparities.

This year Diggs will attend Case Western Reserve University School of Medicine, Cleveland, where she will pursue an M.D./Ph.D. in health services research.

Carlos Gonzalez
Pathophysiology of Ground-glass Hepatocytes

Carlos Gonzalez's research is focused on ground-glass hepatocytes (GGH), a unique histological feature of chronic hepatitis B (HBV) infection.

His findings suggest that retention of a mutant form of the HBV PreS1 protein within the ER-Golgi intermediate space—either through abnormal folding or a lack of interaction with ER chaperone proteins—underlies GGH

Gonzalez graduated from the University of Puerto Rico, Rio Piedras. He came to NIH, with his wife, Vanessa Muniz-Medina (also a postbac—see below). Gonzalez says he transported his family to Bethesda because, "I honestly could not imagine a better place to get experience and insight into the world of biomedical research."

Although he did research in several labs as an undergraduate, he says nothing can compare to his experience at NIH.

"Working in the lab is *very* intense. There's always an experiment to do, results to review. . . . it's very fast paced."

He described his lab, headed by Jake Liang, in the Digestive Diseases Branch of NIDDK, as "a close-knit effort to understand the pathology of hepatitis B and C viruses."

It pleases him to think that the research he's done in a year here may contribute to the development of effective treatments of HBV infection—all the while he is gaining invaluable experience that he believes is preparing him for graduate studies.

Vanessa Muniz-Medina
Vascular Endothelial Growth Factor

Vanessa Muniz-Medina's research is concentrated on the study of vascular endothelial growth factor (VEGF), which she described as a small, soluble protein that transfers information regulating cellular growth.

Controlled growth factor release occurs when tissues are deprived of adequate oxygen. Cells in such a tissue will respond by releasing VEGF, which stimulates other nearby cells that specialize in constructing blood vessels.

Muniz-Medina's lab, headed by Carl Baker, chief of the Cellular regulation and Transformation Section in the NCI Basic Research Laboratory, is also interested in the study of the human papillomavirus (HPV). The goal in this study is to correlate the expression of the different VEGF isoforms with cancer tumors and with lesions caused by HPV.

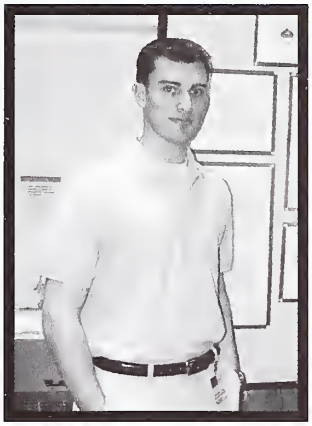
With this correlation, the investigators hope to identify which isoform(s) are present in the most aggressive cancers. Muniz-Medina believes this information could play a major role in treatment

monitoring and prognosis.

While she was an undergraduate at the University of Puerto Rico, Muniz-Medina had four years of lab experience in four different labs. But her experience here, she said, has also been more intense.

"In addition to my research, Dr. Baker is already preparing me for graduate school. He assigns readings and interviews me every day about what I'm learning."

She mentioned that she also "learned a lot about the human body and diseases" through attending six surgeries performed by Erik Kass, chief of otolaryngology head and neck surgery, NIDCD, and an autopsy performed by Baker.



Matthew Steinway

She expects to complete an MD/PhD program and have a career in teaching and research. "With the graduate school preparation I am receiving at NIH, I am well on my way," she said.

Matthew Steinway

The Determination of Variances in Exhaled Nitric Oxide Output in Normal Healthy Male Volunteers Consuming High and Low Nitrate/Nitrite Diets

Matthew Steinway has spent the year involved in clinical research in the lab of Joel Moss in the NHLBI Pulmonary-Critical Care Medicine Branch.

His research is a pilot study designed to determine whether dietary intake influences exhaled nitric oxide (NO) levels.

NO has been recognized for its multifaceted roles in such physiological processes as vasodilatation, host defense, neurotransmission, bronchodilation, and inflammation. Study results should allow researchers to determine the most effective method of monitoring nitric oxide synthase activity and could also aid in identifying activities responsible for maintaining nitric oxide levels.

Steinway recently graduated from the University of Michigan, Ann Arbor, and plans to attend medical school after he completes his research at NIH.

He was attracted to NIH, he said, because he had been involved in a "good amount of basic science research" during his undergraduate years and wanted to experience a "different view of biomedical research" that would be just as challenging—namely, clinical research. NIH, he said, "offers some of the best opportunities in clinical research." ■

Catalytic Reactions

On Recruiting Fellows

Here are few comments on Michael Gottesman's article on recruiting fellows to NIH (*The NIH Catalyst*, March–April 2001) on the difficulty of evaluating potential visiting fellows before they come here.

I am sure everybody agrees that this is very difficult, because letters of recommendation that future sponsors receive often largely exaggerate candidates' competence and obfuscate their true motivation.

It happens only rarely that a candidate makes it as easy for a future sponsor to decide to hire or not as a recent applicant did for me. He sent me his CV as an e-mail attachment, and in the short cover letter [included] a sentence to the effect that the most important thing for him was that he be admitted to the U.S. based on the H1b visa.

In my response, I let him know that I am looking for associates whose most important reason to come to the NIH is to learn a lot and do some good work. I also let him know that I did not bother to open the attachment.

Neither can one disagree with [Gottesman's] recommendation "that potential fellows be interviewed whenever possible, preferably in person in their home country or by bringing them to NIH for a visit, before committing a postdoc position at NIH." This, of course, would always be possible were the moneys to fund such trips available. Are they?

—Paul Kovac, NIDDK

—With respect to funding the interviewing of potential candidates, this is a decision that needs to be made as part of the recruitment process in each lab and in each intramural program.

Frequently, visiting fellows can be interviewed during scientific meetings or other scientific trips abroad, or fellows may be travelling to the United States and can be interviewed during their visits here.

—Michael Gottesman, DDIR

On Priorities of a New NIH Director

To improve the selection of lab directors so that they are not only very good scientists but properly trained at managing and leading people (with respect to their employees).

—Anonymous

How the genetic code was discovered

Cartoonist Brian Bradow, NICHD, can be reached at
<brianbradow@hotmail.com>

Problem-Solving Portal For NCI Postdocs

There's a new doorway in Building 31 through which NCI's hundreds of postdocs are invited to pass—either on the ground or in cyberspace—to find answers to the large and small dilemmas that may arise in the normal course of life here.

Donna Vogel, the first director of NCI's newly established Fellowship Office, is on the other side of that door—ready to help postdocs remove obstructions from their path to an exciting and useful postdoctoral experience at NIH.

Those obstructions can be personal, such as not having adequate childcare, or professional, such as feeling tethered to one's PI. The NCI Fellowship Office is viewed as a central portal to solutions to the diverse problems that can beset an NCI fellow and also, perhaps, as a prototype office for any NIH institute exploring ways to improve the lot of its research trainees.

Fran Pollner

Donna Vogel

"What was missing," Vogel said, recounting the evolution of the idea for the office—which originated in the deliberations in 1997 of the NCI Intramural Advisory Board—"was an office that exists solely to serve the needs of the fellows. A place that, first of all, could diagnose what those needs were and also diagnose the needs of the PIs vis-à-vis working with their post-docs. It was not just about getting postdocs here, but keeping them once they got here."

That a scientist was courted to head the office reflects the emphasis placed on enhancing the research environment for NCI fellows, observed Vogel, who came to NIH 20 years ago as an interinstitute endocrinology fellow and has been based at NICHD as a researcher and officer ever since her arrival. She officially assumed the position of director of the NCI Fellowship Office in January 2001, but she also continues as an adjunct scientist in the NICHD Developmental Endocrinology Branch.

Vogel's initial plan of action in a "place as huge as NCI" is to "get into the trenches [and] meet with small lab groups" to help the people there find like-minded individuals in other NCI regions with whom to collaborate or simply communicate. Almost instantly, she started taking field trips—visiting labs in Frederick, going on a research retreat with the NCI Laboratory of Pathology.

"I'm very interested in connecting the campuses (Bethesda, Frederick, Gaithersburg)—in building bridges" among people nominally based in different fields within the NCI community but sharing an interest in such pursuits as immunology, developmental biology, or basic biology, for instance. Postdocs, she said, ought not exist "in isolation, chained to a bench and a computer, with a taskmaster above telling them to 'produce results!' [They] need to be seen not as an isolated point in space and time but as part of a career trajectory that is building our next generation of scientists."

Indeed, she said, part of the "results" of any given lab is producing scientists, and scientists not only do research in the lab, they present research at meetings, they teach younger students, they develop business and writing skills—all legitimate activities away from the bench.

Vogel intends to develop IRP workshops tailored to the NCI population that will complement Office of Education survival skills and career seminars and "smooth the transition" out of NIH with training in how to find and secure a compatible job.

She hopes to conduct exit interviews with each outgoing postdoc to measure the success of the Fellowship Office in identifying and solving problems. "What were your problems, how were they resolved, were we helpful, did the fellowship meet your expectations, what did you like, where are you going now—these are the kinds of questions we want to ask each person before they leave." And the message she wants to convey to each postdoc right now is "do not hesitate to get in touch with us." The Fellowship Office web site has links to all its NIH partners—personal service organizations, research resources, and professional training opportunities.

The Fellowship Office is in Building 31, Room 3A44. Vogel's e-mail address is <**dv1h@nih.gov**>; the e-mail address of special assistant Viola Black is <**vb55k@nih.gov**>; the phone number is 496-4796; fax 496-0826; and web site: <**www.nci.nih.gov/fellowships**>.

—Fran Pollner

Microbial Milestone

Fran Pollner

Well-Read: *(standing, left to right): Celia Hooper (OD), Jerry Liddel (RFB&D), Andrea True (NHLBI), Nancy Sullivan (NIAID/VRC), Henry Metzger (NIAMS), Chris Smith (RFB&D); (kneeling, l. to r.): Ethel Schiff (RFB&D) and Bettie Graham (NHGRI); (not shown): Peggy Weston (NIAID) and Wanda Williams (NIDDK).*

They might not look like human microscopes, but this group of NIHers and friends spent more than 126 hours over the course of 14 months making microbes visible.

The group is made up of volunteers for the Washington-area chapter of Recording for the Blind and Dyslexic (RFB&D). On March 22, the group celebrated the completion of the taping of its first entire book, now known to visually impaired readers as Shelf #GC180, *Brock Biology of Microorganisms.*

Volunteers read at a Building 31 recording booth loaned by Calvin Jackson of the NIH Office of Communications and Public Liaison. Even as the group was recording the Brock text, it was being borrowed by six readers across the country.

Additional students had signed up for copies of the recordings as soon as the set of 32 tapes—covering more than 900 pages of text, figures, and appendices—was complete.

Because there is a large backlog of biomedical texts sought by visually impaired students, RFB&D is hoping to establish its own recording studio at NIH with extended hours, more volunteers, and digital recording equipment. RFB&D is currently recruiting volunteers to read computer manuals, statistics texts, and other technical materials at its main recording studio in Washington, D.C. (5225 Wisconsin Avenue, N.W., across the street from the Friendship Heights Metro stop on the red line).

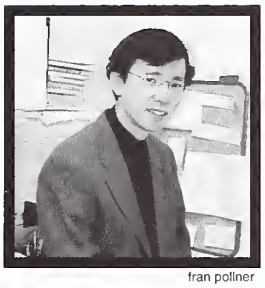
When expanded hours are available on the NIH campus, the group will recruit more biomedical readers to record here. For information, call Chris Smith at RFB&D at 202-244-8990 ■

—Celia Hooper

People

Recently Tenured

Jay Chung *received his M.D. and Ph.D. from Harvard Medical School in Boston in 1988. After his residency in internal medicine at Brigham and Women's Hospital, Boston, he joined NIDDK in 1990 as a clinical endocrinology fellow and in 1991 began doing research as a postdoctoral fellow in the Laboratory of Molecular Biology. In 1994, he moved to the Laboratory of Biochemical Genetics in NHLBI, where he is now a senior investigator.*



fran pollner

Jay Chung

My group is interested in understanding how the behavior of transcription factors is regulated in vivo by other transcription factors and signaling pathways. Although a great deal is known about transcription factor–DNA interactions in vitro, very little is known about how transcriptional factors are recruited in living cells.

To visualize transcription factor-DNA interaction in living cells, we developed the PIN*POINT (protein position identification with nuclease tail) method. This method is based on the idea of fusing the nuclease domain from the restriction endonuclease Fok1 to the transcription factor of interest and expressing the fusion protein in the cell.

Because the nuclease domain does not have sequence specificity of its own, the nuclease domain fused to the transcription factor cleaves DNA near where the transcription factor portion of the fusion protein binds. Detection of these cleavage sites with molecular biology techniques permits the visualization of the recruitment of the transcription factor to a region of DNA in vivo.

By applying PIN*POINT to understand how β-globin gene expression is regulated in vivo, we addressed several fundamental questions regarding transcription factor recruitment. We have discovered that the proximal promoter can also influence the recruitment of transcription factor EKLF to the distant regulator of β-globin expression (LCR, the locus control region). EKLF binds to a specific DNA sequence, the CACCC box, in vitro, and it has therefore been assumed that the EKLF-CACCC box interaction controls EKLF recruitment in vivo. Although both β-globin and γ-globin promoters contain the CACCC box, EKLF activates the β-globin promoter but not the γ-globin promoter. Our findings demonstrate that a novel suppressor that binds to the γ-globin promoter but not to the β-globin promoter suppresses EKLF recruitment to the γ-globin promoter. This finding provides evidence that proteins bound to the promoter have strong influence on recruitment of other proteins to the promoter.

In erythroid cells, the chromatin over the β-globin gene is in an "open" conformation but is in a "closed" conformation in other tissues. How the chromatin is remodeled in such a tissue-specific way is completely unknown. One possibility is that chromatin-remodeling complexes such as the BRG1 complex open the chromatin over the β-globin promoter in erythroid cells. However, the chromatin-remodeling complexes generally do not have DNA sequence specificity, and it is therefore not known whether these complexes are targeted to specific sites such as the promoter region.

Our findings indicate that EKLF and the general transcriptional machinery help recruit the BRG1 complex to the β-globin promoter, confirming that chromatin-remodeling complexes can be targeted to a specific site in vivo. This work will be important not only for understanding transcriptional mechanisms but may have implications in drug development for diseases such as sickle cell anemia.

Thyroid hormone receptor activates the transcription of genes with a TRE (thyroid hormone responsive element) in the presence of thyroid hormone. Using PIN*POINT, we have demonstrated that thyroid hormone triggers physical interaction between TRE and promoter.

More recently, our group has begun a new line of investigation in the area of DNA damage signaling. We cloned a kinase called hCds1, which plays an important role in DNA damage response. We discovered that one of the substrates of hCds1 is the BRCA1 tumor suppressor. We have discovered that hCds1 colocalizes with BRCA1 in nuclear foci and phosphorylates serine 988 of BRCA1 in vivo. Phosphorylation of serine 988 is increased after DNA damage and causes dispersion of BRCA1 to sites of its action. Mutation of serine 988 to alanine, which is not phosphorylated, destroys BRCA1's ability to confer DNA damage resistance to BRCA1-mutated cells, suggesting that serine 988 phosphorylation is important for BRCA1 function in DNA damage response. We have identified the consensus sequence for hCds1 substrates, which we are using to identify in vivo substrates of hCds1. We are now developing transgenic mouse models to extend these studies.

We are currently integrating our interests in transcription regulation and signal pathways by focusing on the regulation of transcription factor activity by various signal pathways. In the near future, we will use high-throughput functional genomics approaches in genetically tractable model organisms to help us discover disease-related transcription factors and signal pathway components in humans.

We are halfway through the analysis of a library of yeast strains in which each strain contains a single mu-tation in one of 6,000 different genes and will begin high-throughput gene knock-down analyses using RNAi in Drosophila cells and *Coenorhabditis elegans*. Our hope is to take full advantage of the genomics and proteomics approaches to answer mechanistic questions regarding transcription and signal transduction that have implications for human diseases and their treatments.

Victor Pike *received his Ph.D. from the University of Birmingham (U.K.) in 1976 and did postdoctoral work there before joining the Medical Research Council Cyclotron Unit in London in 1978 and becoming the head of its Chemistry and Engineering Group in 1996, with concurrent honorary appointments at Imperial College of Science, Technology and Medicine in London and the University of Surrey. In February 2001, he became chief of the PET Radiopharmaceutical Sciences Section in the Molecular Imaging Branch, NIMH.*

fran pollner

Victor Pike

My principal interests are in all chemical aspects of the design, synthesis, and biological evaluation of novel radioactive probes for use in pharmacological and clinical research with positron-emission tomography (PET). PET is now recognized as a powerful molecular imaging technique for unraveling the bio-

chemistry underlying various clinical disorders and for evaluating the efficacy of existing or proposed treatments. In particular, my work has focused on the challenge of radiochemistry with short-lived cyclotron-produced carbon-11 ($t_{1/2}$ = 20 min) and fluorine-18 ($t_{1/2}$ =110 min). This interest began at the MRC Cyclotron Unit, with the establishment of one of the world's first PET centers.

My first success was the development of a rapid automated procedure for the preparation of carbon-11–labeled acetate for the study of myocardial energy metabolism. This tracer can be used for the positive identification of transient myocardial ischemia in human subjects. After more than two decades, this tracer remains in widespread clinical use at several PET centers. At this time, I also labeled the antibiotic erythromycin A with carbon-11 in order to measure directly the antibiotic concentration at its site of action in human pneumonic lung, a pioneering example of the use of a PET tracer in drug research and development.

My group also developed a new class of labeling agents, [^{11}C]acid chlorides, which opened up the potential to prepare a wider range of labeled compounds; an early example is [^{11}C]diprenorphine, one of the first radioligands for imaging brain opiate receptors and for investigating the involvement of these receptors in neuropsychiatric conditions. This radioligand continues to be used for clinical research. My group also successfully developed radioligands for heart and lung adrenoceptors.

The pharmaceutical industry has appreciated the capability of PET to assist in drug discovery and development, and I helped develop several tracers for this purpose. An interesting example was ^{18}F-labeled HFA 134a, which was used to show that HFA 134a is safe for inhalation by human subjects. HFA 134a is now produced in enormous quantities to replace ozone-depleting chlorofluorocarbons in their large-scale applications and also as a drug aerosol propellant. Drug particles (for example, fluticasone propionate) administered by inhalation have also been labeled with positron-emitters to measure their regional lung deposition with PET. These measurements are crucial to a better understanding and optimization of drug-inhalation therapies.

The ability of PET to give precise data—the occupancy of a specific population of receptors by a given dose of drug—can be of immense benefit in drug discovery and development. However, such measurements depend on the availability of a selective radioligand for the receptor in question. My group has been instrumental in developing the first effective radioligand for brain serotonin type 1A (5-HT_{1A}) receptors, [^{11}C]WAY-100635. This radioligand has widened possibilities to examine the role of the serotonergic system in various neuropsychiatric disorders, including anxiety, depression, and schizophrenia. This radioligand is now used in clinical research worldwide and also has been widely used for receptor occupancy studies with established or candidate drugs. My collaborators and I received the Marie Curie Award and Springer Prize for our contributions to radioligand development.

Rashmi Sinha

Extensive medicinal chemistry and advances in radiochemistry have been at the core of my past success. I aim to continue work in these areas in support of radiotracer and radioligand development in the new Molecular Imaging Branch by developing probes for brain biochemistry of animal and human subjects to better understand neuropsychiatric disorders.

Rashmi Sinha *received her Ph.D. in nutritional sciences from the University of Maryland, College Park, in 1987. She joined NCI's Laboratory of Cellular Carcinogenesis and Tumor Promotion that year, moved to the Epidemiology and Biostatistics Program in 1992, and is now a senior investigator in the Nutritional Epidemiology Branch of the Division of Cancer Epidemiology and Genetics.*

My research focuses on the complex role of diet in cancer etiology, especially regarding carcinogens such as heterocyclic amines (HCAs) and aromatic hydrocarbons (PAHs) that are produced in meat cooked at high temperatures. Early epidemiologic studies found a suggestive association between meat-cooking techniques and cancer risk, but did not differentiate factors that influenced the production of HCAs and PAHs. For example, roast beef and steak were included within the same meat category, despite widely dissimilar cooking methods and very different levels of HCA formation. Following up on these initial leads, I designed an interdisciplinary research program to investigate the roles in human cancer etiology of mutagens and animal carcinogens formed during meat cooking.

First, I needed tools to estimate HCA and PAH intake in an accurate and reliable manner; I took two approaches to secure them—improving questionnaire-based measures of intake and evaluating biological markers of HCAs and PAHs. To improve intake estimates, I developed databases similar to those used for other dietary components (such as fats, proteins, and vitamins) that could be with a food frequency questionnaire (FFQ). In collaboration with several research laboratories, I measured mutagenic activity, HCAs (such as MeIQx, PhIP, and DiMeIQx), and PAHs (such as BaP) in meat samples cooked by different methods to varying levels of doneness.

Results from this study underscored the need to include questions on type or cut of meat, cooking technique, and doneness levels for individual meat items in order to capture the variability in HCA and PAH content. Using this information, I designed and validated a FFQ that takes account of these details. I also carried out a large metabolic study to evaluate HCA biological markers of internal exposure that could be used in etiologic studies and to assess the influence of polymorphic metabolic enzymes on these biomarkers.

I have used both questionnaire information and biomarkers in case-control studies of colorectal adenomas and lung, breast, and stomach cancers. I found an increased risk of colorectal adenoma associated with high intake of red meat. Most of this risk was attributable to high levels of doneness (well or very well done) and/or to high-temperature cooking techniques such as grilling. Linking the FFQ information to the mutagen-carcinogen database, I evaluated the effect on risk of mutagenic activity and exposure to several HCAs and to BaP. Mutagenic activity is an integrative measure of all mutagens contained in meat. I found an increased risk associated with higher levels of meat-derived mutagenic

activity; intake of MeIQx, a highly mutagenic HCA; and possibly PhIP. I am now investigating several polymorphic enzymes involved in metabolizing these compounds. I am also evaluating the relationship of BaP to colorectal adenoma risk using questionnaire data on diet and cooking and biologic measures.

Other studies in which I have collaborated also lend support to a causative role for HCAs in human cancer. For example, red meat, especially fried and/or well-done red meat, was associated with an increased risk of lung cancer in a population-based case-control study of Missouri women, and MeIQx was associated with elevated risk in both nonsmokers and moderate smokers. In a case-control study in Nebraska, intake of well-done red meat, a surrogate for HCA, and grilled red meat, a proxy for both HCAs and PAHs, were related to stomach cancer. And in a collaborative case-control study of breast cancer nested in the Iowa Women's Health Study, where well-done red meat was associated with increased risk of breast cancer, I found that high intake of PhIP, especially, was related to elevated risk.

Furthermore, the association of specific HCAs with different tumor sites—such as MeIQx and PhIP with colorectal adenomas, MeIQx with lung cancer, and PhIP with breast cancer—parallels to a certain extent the organ specificity of animal carcinogenicity studies. In rodent models, several HCAs produce colon tumors, MeIQx causes lung tumors, and PhIP leads to mammary tumors.

To extend my initial observations, I have established collaborations with multiple intramural and extramural investigators. I have contributed key components to dietary questionnaires on meat cooking in numerous case-control studies and in several cohort studies as well, for example, the Nurses' Health Study; the Health Professionals Follow-up Study; the NCI Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; and the Agricultural Health Study. These studies should help clarify the role that HCAs play in the etiology of several types of cancer in the United States. Should a consistent association between cancer and meat cooking mutagens be established, we can then make public health recommendations on the safest way to prepare meat to eliminate bacterial contamination and to minimize possible carcinogens.

Peter Sun *received his Ph.D. from the Institute of Molecular Biology, University of Oregon, Eugene, in 1990 and did postdoctoral work at NIDDK. He joined NIAID in 1994 and is now a senior investigator in the Laboratory of Immunogenetics.*

Peter Sun

My research focus has been on the structure and function of immunoreceptors. In particular, we study how the receptors recognize their ligands and initiate activation or inhibitory signals. We primarily choose to use the X-ray crystallography technique to define three-dimensional molecular structures of receptors and their ligand complexes.

By doing so, we can visualize the receptor-ligand molecular interface and thus map out the hot spots on the receptor—the regions critical for ligand recognition. In practice, knowing the shape of a receptor-ligand interface and the residues involved will help us not only in the diagnosis of diseases caused by the impairment of receptor function, but also in the design of potential new drugs that are capable of inhibiting the function of these receptors.

Recent advances in the molecular and cellular biology of innate immune functions have opened a new, exciting frontier for structural biology—to reveal the receptor activation mechanism involved in innate immunity.

Following the cloning of several natural killer (NK) cell surface receptors by others, we began to study their ligand recognition by X-ray crystallography and solution-binding methods. At the center of our interest is the mechanism by which NK cells distinguish between self and nonself and thereby direct their cytolytic killing against virally infected rather than healthy host cells.

Two families of inhibitory receptors, the killer immunoglobulin receptors (KIR) and the C-type lectin-like receptors, have been identified on human NK cells to interact with class I MHC antigens and thus may be critical in mediating this self vs. nonself recognition.

Members of C-type lectin-like CD94/NKG2 receptors recognize a nonclassical class I molecule—HLA-E—bound with peptides derived from the signal peptide of other classical class I MHC molecules; in contrast, KIR receptors recognize classical class I HLA molecules.

In an effort to elucidate the molecular interface between KIR receptors and their HLA ligands, we have determined the crystal structures of an HLA-Cw3–recognizing KIR and its complex with the HLA molecule. This is the first structure of a KIR-HLA complex, and it illustrates the resemblance between KIR and T-cell receptor recognition of HLA molecules. It also reveals the contribution of the class I peptide to KIR recognition and the nature of allotypic specificity of the receptor.

Through mutations and binding studies, we also demonstrated the importance of interface residues in KIR-HLA recognition.

Another receptor involved in NK cell–mediated innate immunity is an Fc receptor, FcgIII. Fcg receptors are expressed on the surface of macrophages, mast cells, and NK cells to mediate opsonization of antibody-coated pathogens and antibody-directed cellular cytotoxicity.

They are also implicated in certain autoantibody-mediated autoimmune diseases. In an effort to define how immunoglobulins bind and activate humoral immune systems through their Fc receptors, we crystallized and solved the X-ray structure of FcgIII in complex with an Fc fragment of IgG1.

The co-crystal structure revealed an unexpected binding of one receptor to the dimeric Fc ligand. This mode of receptor binding, although very different from other known ligands of Fc, explains the role of the antigen—specifically the need for immune complex formation—in receptor activation. Based on our crystal structure, we explored the possibility of using small molecular ligands that are capable of inhibiting the receptor function as potential useful therapeutic reagents.

We have designed four peptides and investigated their ability to bind to receptor FcgRIII. Using solution-binding experiments, we demonstrated that these peptides bind specifically to FcgRIII with $^1/_{20}$ to $^1/_{10}$ the affinity of IgG1 and are able to inhibit Fc binding to the receptor. The results of these peptide studies have opened new ways of designing therapeutic compounds. ■

CALL FOR CATALYTIC REACTIONS

In this issue, we are asking for your reactions in four areas: the past-10-year record of the intramural research program, IRP future directions, the pursuit of structural biology initiatives, and the Interest Group Directory.

Send your responses on these topics or your comments on other intramural research concerns to us via e-mail: <catalyst@nih.gov>; fax:402-4303; or mail: Building 2, Room 2W23.

In Future Issues...

- IRP Resources Inventory
- Gene Microarrays
- Eugenics

1) What is your evaluation of the success of the intramural research program over the past 10 years?

2) What directions should be taken by intramural research in the next decade?

3) How should NIH pursue and support structural biology initiatives in the intramural program?

4) This is not so much a question as **a request to Interest Group contacts.** Each July, the *Catalyst* runs an updated Interest Group Directory. Everyone who was listed as a first contact for any of the 81 Interest Groups included in the July–August 2000 issue will soon receive a copy of last year's listing to verify or change, as needed. If your new group is *not* on the list send the *Catalyst* its name and web address; regular meeting time and place; and the name, phone number, and e-mail of the contact person. **Changes and new information must be received by June 25th to be included in the July-August 2001 issue.**

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