VII.
National Institute of Arthritis and Musculoskeletal and Skin Diseases

INTRODUCTION

The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) is a component of the National Institutes of Health (NIH), the principal biomedical research agency of the U.S. Government. NIAMS, which was established in 1986, conducts and supports basic, clinical, and epidemiologic research and research training and disseminates information on many of the most debilitating diseases affecting the Nation's health. These diseases include the many forms of arthritis and diseases of the musculoskeletal system and the skin.

The Institute also conducts and supports basic research on the normal structure and function of joints, muscles, bones, and skin. Basic research involves a wide variety of scientific disciplines, including immunology, genetics, molecular biology, structural biology, biochemistry, physiology, virology, and pharmacology. Clinical research includes rheumatology, orthopaedics, dermatology, metabolic bone diseases, heritable disorders of bone and cartilage, inherited and inflammatory muscle diseases, sports medicine, and rehabilitation medicine.

The Extramural Program of NIAMS supports research and research training at universities and medical centers through research grants and contracts. The Program consists of four scientific Branches: Rheumatic Diseases, Muscle Biology, Musculoskeletal Diseases, and Skin Diseases. The Intramural Research Program supports research and research training on the NIH campus, in Bethesda, Maryland. This Program has eight main components: the Arthritis and Rheumatism Branch, the Autoimmunity Branch, the Bone and Connective Tissue Biology Branch, the Laboratory of Physical Biology, the Protein Expression Laboratory, the Laboratory of Skin Biology, the Laboratory of Structural Biology Research, Research and the Office of the Clinical Director.

HIGHLIGHTS OF RECENT SCIENTIFIC ADVANCES RESULTING FROM INTERNATIONAL ACTIVITIES

In an ongoing study, the Skin Diseases Branch of NIAMS supports a grantee at the University of North Carolina, Chapel Hill, who is collaborating, under a subcontract, with investigators at the University of São Paulo, Brazil. This joint research team has focused on the blackfly species present in the areas of endemic pemphigus foliaceus in Brazil as the likely trigger of autoimmune events in susceptible populations. They have compared the subspecies present in villages where there is high prevalence of disease with genetically similar subspecies in nearby villages where there is little disease. The scientists discovered a wider variation in subspecies than had been suspected. They are now characterizing the antigens from these blackfly subspecies to identify the antigen(s) most likely to be the environmental trigger(s) for the disease.

SUMMARY OF INTERNATIONAL PROGRAMS AND ACTIVITIES

Extramural Programs

Muscle Biology Branch

The Muscle Biology Branch is supporting two collaborative international studies. In one study, the University of Ottawa, Ontario, and the University of Pennsylvania, Philadelphia, are examining genetic regulation of skeletal muscle repair. The other study, by Eastern Virginia Medical School, Norfolk, and the University of Leeds, West Yorkshire, England, involves cryoelectron microscopy of muscle contraction.

Skin Diseases Branch

The Skin Diseases Branch has several European joint studies:

1. University of Colorado Health Sciences Center, Denver—Scientists are exploring the genetic basis of vitiligo, by using cohorts in the United Kingdom and the United States to locate the gene(s) for this disease.

2. University of Michigan, Ann Arbor—Investigators are testing the genetic basis of psoriasis with European and U.S. cohorts, to locate the susceptibility gene(s).

3. University of Pennsylvania, Philadelphia—Researchers are conducting an epidemiologic study of risk factors for susceptibility to chronic lower-leg ulcers in cohorts in the United Kingdom and the United States.

International Meetings

The Director, NIAMS, was invited to address residents at the University of Milan, Italy, on February 18, 2000. His subject was Dermatology—Recent Advances. On February 19, 2000, he gave a lecture entitled The Skin Immune System—Clinical Relevance, at the Dermatological Day of Milan. The Director was invited to give the opening address at the Clinical Dermatology 2000 meeting, in Vienna, Austria, on May 17–19, 2000. He also participated in a seminar on recent advances in pathogenesis and diagnosis in dermatology, in Mexico City, Mexico, on June 29–July 1, 2000. While there, he gave three talks: (1) The Skin Immune System—Recent Advances; (2) Recent Advances in Connective Tissue Diseases; and (3) The Impact of Basic Science on Clinical Dermatology. In addition, the Director was invited to give three lectures at the XVth Argentine Congress of Dermatology, in Buenos Aires, on August 9–11, 2000. He spoke about The Skin Immune System—Why Do We Study It?; Erythem Elevatum Diutinum; and Advances in Our Understanding of Autoimmune Connective Tissue Diseases.

Extramural Programs

Rheumatic Diseases Branch

The Epidemiology Program Officer, Rheumatic Diseases Branch, participated in a World Health Organization Scientific Group Meeting, in Geneva, Switzerland, on

January 13–15, 2000. The meeting marked the launching of the Bone and Joint Decade. Scientific Group members are working to develop a World Health Organization Global Report on the Burden of Musculoskeletal Conditions.

Intramural Research Programs

The Scientific Director, NIAMS, was an invited lecturer at the 45th Anniversary Celebration at Instituto National de Cardiologia, in Mexico City, Mexico, on October 10–12, 1999, where he gave a talk on Modifying the Immune Response in Rheumatoid Arthritis. He participated in the International Advisory Committee for the Japan Rheumatism Foundation/Association, in Kobe, Japan, on March 16, 2000, and he gave an Overview of Clinical Immunology in Rheumatoid Arthritis, at the 9th Japan Rheumatism Association/Foundation Symposium, in Kobe, on March 17–18, 2000. The Scientific Director attended the 73rd Annual Meeting of the Japanese Orthopaedic Association, also in Kobe, on April 6–9, 2000, where he gave a plenary lecture on New Developments in Pathogenesis and Treatment of Rheumatoid Arthritis. At the Annual General Meeting of the British Society for Rheumatology, in London, England, on May 9–12, 2000, the Scientific Director made a presentation entitled Rheumatoid Arthritis: New Perceptions in the New Century. On May 18, 2000, he also spoke at the University of Alberta, Edmonton, on New Insights Into the Pathogenesis and Treatment of Rheumatoid Arthritis. At the APLAR (Asia-Pacific League of Associations for Rheumatology) Congress of Rheumatology, in Beijing, China, on May 21–26, 2000, the Scientific Director delivered a plenary lecture on the Pathogenesis of Rheumatoid Arthritis. Also, he gave a talk on Cell-to-Cell Interactions Regulating Lymphocyte Function in the Rheumatoid Synovium, at the meeting on Cell Contact Interactions in Rheumatology, in London, England, on June 1–2, 2000.

The Scientific Director spoke on A New Frontier in the Treatment of Rheumatoid Arthritis, at the 2000 EULAR (European League Against Rheumatism) Congress, and on the Patient Partners Program, in the EULAR Postgraduate Course in Rheumatology, in Nice, France, on June 21–27, 2000. At the VIIth World Conference on Clinical Pharmacology and Therapeutics, in Florence, Italy, on July 15–20, 2000, he gave an address on Unresolved Issues in the Role of Cyclooxygenase-2 in Normal Physiology and Disease. In Mexico City, Mexico, on July 25, 2000, he gave talks on (1) The Impact of TNF (tumor necrosis factor) Blockade in Rheumatoid Arthritis, (2) COX 2–Specific Inhibitors: Principles and Practice, and (3) B Cell Abnormalities in Autoimmune Disease, at the Annual Meeting of the Immunology and Rheumatology Society and the Mexican Society of Rheumatology. He talked about COX 2—What Are the Clinical Implications, at the 28th Annual Meeting of the Japanese Society of Clinical Immunology, in Tokyo, Japan, on September 28–29, 2000.

Arthritis and Rheumatism Branch

Chemical Immunology Section. The Chief of the Chemical Immunology Section, Arthritis and Rheumatism Branch, gave a lecture at the University of Birmingham, England, on November 29, 1999. He gave a plenary talk and participated in a workshop at the joint meeting of the British Societies of Biochemistry, Immunology, and Clinical Immunology, in Harrogate, England, on November 30–December 3, 1999. The Chief also gave a plenary address at the Congress of Latin American Associations of Immunology, Punta del Este, Uruguay, which he attended on December 11–14, 1999. On June 13–14, 2000, he participated in a meeting of the International Council for Science's Committee on Governance, in Paris, France.

Connective Tissue Diseases Section. The Chief, Connective Tissue Diseases Section, was an invited lecturer at the 2000 EULAR Congress, in Nice, France, on June 22–25, 2000. In Luxembourg, Belgium, he gave a lecture on Differential Diagnosis and Treatment of Myositis, at the 4th Belgian Congress for Rheumatology, on September 28–29, 2000.

Genetics Section. The Chief, Genetics Section, chaired a session and gave three invited lectures at the 2nd International Workshop on Hyper-IgD (immunoglobulin D) and Periodic Fever, in Nijmegen, the Netherlands, on December 10–11, 1999. He served on the advisory board, chaired a session, and gave four invited lectures at the 2nd International Conference on Familial Mediterranean Fever, in Antalya, Turkey, on May 3–7, 2000. Two Senior Staff Fellows from the Genetics Section also gave invited lectures at this conference. At the 8th TNF Congress, in Trondheim, Norway, on May 14–18, 2000, the Chief gave an invited lecture. He also made a presentation at Institut de la Genetique Humaine, in Montpellier, France, on May 22, 2000.

Inflammatory Joint Tissue Diseases Section. The Chief, Inflammatory Joint Tissue Diseases Section, presented a lecture on Genetic Dissection of Experimental Autoimmune Disease in Inbred Rats, at the XIIIth International Workshop on Genetic Systems in the Rat, in Göteborg, Sweden, in June 2000. At the Nobel Minisymposium on Complex Genetic Disorders, in Stockholm, Sweden, in September 2000, he gave the same lecture.

Lymphocyte Cell Biology Section. The Chief, Lymphocyte Cell Biology Section, attended the Cytokine Signaling Workshop, in Aachen, Germany, on March 24, 2000. On March 27, he delivered a lecture at Institut Pasteur, Paris, France.

Signal Transduction Group. The Head, Signal Transduction Group, was an invited lecturer at the GSF (Forschungszentrum für Unwelt und Gesundheit) Institute for Clinical Molecular Biology and Tumor Genetics, in Munich, Germany, on October 5, 1999. He was also invited to speak at the University of Udine, Italy, on October 12, 1999, and at the University of Puerto Rico Medical Sciences Center Campus, in San Juan, on February 3, 2000. At the 23rd Collegium Internationale Allergologicum Symposia, in Hakone, Japan, on May 17–23, 2000, the Chief was a plenary speaker.

Autoimmunity Branch

A Research Fellow from the Autoimmunity Branch was invited to give a lecture in the Immunology Seminar Series, at the University of Glasgow, Scotland, and to collaborate, in late September and early October 2000, on the study of activation of mitogen-activated protein kinase after CD40 binding on human B cells.

Bone and Connective Tissue Biology Branch

Craniofacial Development Section. The Chief of the Craniofacial Development Section, Bone and Connective Tissue Biology Branch, gave the keynote address at the International Dental Exhibition and Meeting, in Singapore, on April 14, 2000. The address was entitled Future Trends in Dentistry: Paradigm for the New Millennium. The Chief was also an invited speaker at the 1st Smile Train Cleft Lip and Palate Symposium, in Beijing, China, on March 2–4, 2000. He was also invited to speak at the 22nd Asia Pacific Dental Congress, in Tokyo, Japan, on May 28, 2000, where he conducted a seminar entitled Prospects for Dental Science, Education, and Practice.

Two Research Fellows in the Craniofacial Development Section presented their work at international meetings. One spoke on Functional Analysis of the Dach Gene During Tooth Morphogenesis, at the International Association for Dental Research, in Washington, D.C., in April 2000. The other made a presentation entitled Lovastatin Promotes Cartilage Growth but Inhibits Endochondral Ossification of the Cranial Base in Organ Culture, at the annual meeting of the American Society for Bone and Mineral Research, in Toronto, Ontario, in September 2000.

Laboratory of Physical Biology

The Chief, Laboratory of Physical Biology, was invited to speak at the Institute of Chemistry, Academia Sinica, Taipei, Taiwan, on August 14, 2000. He presented a lecture entitled Molecular Elasticity: Two Decades of Titin Research. The Chief also made a presentation entitled Proteomics: Profiling Molecular Interfaces by Chemical Cross-Linking, at the Institute of Biological Chemistry, Academia Sinica, on August 18, 2000.

Macromolecular Biophysics Section. The Chief of the Macromolecular Biophysics Section, Laboratory of Physical Biology, was an invited speaker at the University of London Institute for Neurology and at the Gordon Conference on Macromolecular Organization, at Oxford University, England, on August 6–11, 2000.

Laboratory of Skin Biology

Molecular Biology of Keratinization Section. The Chief of the Molecular Biology of Keratinization Section, Laboratory of Skin Biology, was an invited participant at the meeting of the Korean Society for Investigative Dermatology, in Seoul, on February 14–18, 2000. At the 6th International Conference on Transglutaminases, in Lyon, France, on September 16–18, 2000, he was the keynote speaker. The Chief also presented invited lectures at L'Oreál, Clichy, France; Chonnam University Hospital, Kwangju, Korea; Seoul National University, Korea; and the Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand.

A Visiting Scientist and a Senior Staff Fellow in the Molecular Biology of Keratinization Section made poster presentations at the 6th International Conference on Transglutaminases, in Lyon, France, on September 16–18, 2000.

Laboratory of Structural Biology Research

The Chief of the Laboratory of Structural Biology Research gave a seminar at the Institute of Molecular Virology, Chinese Academy of Preventive Medicine, Beijing, on April 13, 2000. He was an invited lecturer at the International Conference on Life Sciences and Clinical Medicine, in Beijing, on April 18–20, 2000, and at the European Congress on Electron Microscopy, in Brno, Czech Republic, on July 9–14, 2000. At the EMBO (European Molecular Biology Organization) Advanced Course on Cryo-Electron Microscopy, in Heidelberg, Germany, on September 10–20, 2000, the Laboratory Chief was an instructor, and a Visiting Fellow from Spain was a student. Another Visiting Fellow, from Japan, delivered the platform presentation at the annual meeting of the Japanese Biophysical Society, in Sendai, Japan, on September 10–13, 2000.

In FY 00, the Laboratory also hosted seminars at the NIH. The seminars were conducted by foreign scientists from Prague, Czech Republic; Grenoble, France; Martinsried, Germany; Jerusalem, Israel; Glasgow, Scotland; and Basel, Switzerland.

Office of the Clinical Director

A Visiting Associate in the Clinical Investigations Section made two poster presentations at the 20th European Rheumatology Research Workshop, at St. Catherine's College, in Oxford, England, on March 21–27, 2000. The posters were entitled (1) T_H1-Dependent Activation and Response of Monocytes From Patients With Lupus Nephritis and (2) Interleukin 10 Improves Skin Disease and Modulates Endothelial Activation and Leukocyte Effector Function in Patients With Psoriatic Arthritis.

Intramural Programs and Activities
Arthritis and Rheumatism Branch

Connective Tissue Diseases Section

The Connective Tissue Diseases Section, Arthritis and Rheumatism Branch, continues an exchange of ideas and reagents with the University of Rotterdam, the Netherlands, concerning the treatment of glycogenosis type II. The Section also provided serum from patients with autoimmune diseases to the University of Sheffield, South Yorkshire, England, for studies of autoimmunity.

A Visiting Fellow from China continues to study transcriptional control of the α-glucosidase gene, which is mutated in glycogenosis type II. A former Visiting Fellow from India continues active collaboration with the Section as a Special Volunteer doing studies on a transgenic model of myositis that he developed earlier in the laboratory.

Genetics Section

The Genetics Section continued collaborations to study the genetic basis of the dominantly inherited periodic fevers with groups at Women's and Children's Hospital, Adelaide, Australia; National University of Ireland, Cork; and St. Bartholomew's and the Royal London Hospital School of Medicine and Dentistry, England. This research was summarized in a review article by a Postdoctoral Fellow from France, entitled "*TNFRSF1A* mutations and autoinflammatory syndromes," in Current Opinion in Immunology (August 2000).

The Section also continues joint research efforts with groups in Israel to study the genetics of familial Mediterranean fever (FMF) at Sheba Medical Center, Tel Hashomer. A Visiting Fellow from Armenia and a Visiting Fellow from Israel also participated in these studies. Some of this work was reported at the 63rd Annual Meeting of the American College of Rheumatology, in Boston, Massachusetts, on November 13–17, 1999.

A Visiting Fellow from Korea conducted studies on the mouse and rat homologues of the FMF gene. He is now developing various lines of FMF knock-in mice. The investigator published an article entitled "Isolation, genomic organization, and expression analysis of the mouse and rat homologues of MEFV, the gene for familial Mediterranean fever," in *Mammalian Genome* (June 2000).

Inflammatory Joint Diseases Section

A Visiting Fellow from Bulgaria in the Inflammatory Joint Diseases Section coauthored an article entitled "Ligand activation of the adenosine A2a receptors inhibits IL-12 [interleukin 12] production by human monocytes," published in the *Journal of Immunology* (January 2000), and a book chapter, "The sympathetic nerve—an integrative interface between two supersystems: the brain and the immune system," published in *Pharmacological Reviews* (December 2000).

A Visiting Fellow from India presented two reports at the annual meeting of the American Society for Human Genetics, in San Francisco, California, in October 1999. The reports were on (1) development of a genetic-linkage map for the rat and (2) identification of quantitative trait loci on Rno 10, which regulate experimental arthritis. The investigator was the primary author of an article entitled "Genetic dissection of collagen-induced arthritis in chromosome 10 quantitative trait locus speed congenic rats: evidence for more than one regulatory locus and sex influences," which was published in *Immunogenetics* (September 2000). She was a coauthor of a report on "Identification of four new quantitative trait loci regulating arthritis severity and one new quantitative trait locus regulating autoantibody production in rats with collagen-induced arthritis," in *Arthritis and Rheumatism* (June 2000). Together with two Visiting Fellows from Japan, she coauthored another article, entitled "Genetic dissection of a rat model for rheumatoid arthritis: significant gender influences on autosomal modifier loci," in *Human Molecular Genetics* (September 2000).

A Visiting Fellow from Japan, who was coauthor of the same three reports, also made a presentation at the 63rd Annual Meeting of the American College of Rheumatology, in Boston, Massachusetts, in November 1999.

Lymphocyte Cell Biology Section

The Lymphocyte Cell Biology Section is collaborating with a research team at the University of Brescia, Italy, to define the genomic organization of the Jak-3 gene and to identify its mutations. This work resulted in two publications: "Complete genomic organization of the human Jak-3 gene and mutation analysis in severe combined immunodeficiency by single-strand conformation polymorphism," in *Human Genetics* (January 2000), and "Complex effects of naturally occurring mutations in the Jak-3 pseudokinase domain: evidence for interactions between the kinase and pseudokinase domains," in *Molecular Cellular Biology* (February 2000). Further collaboration with the Italian research team led to the publication of "Molecular modeling of the Jak-3 kinase domains and structural basis for severe combined immunodeficiency," in *Clinical Immunology* (August 2000). Collaborative work on a new molecule associated with Jak-3, this time with a Japanese group, was published in the article "STAM2, a new member of the STAM family, binding to the Janus kinases," in *FEBS Letters* (July 2000).

An Austrian Postdoctoral Fellow in the Lymphocyte Cell Biology Section wrote a review article on "Janus kinases and their role in growth and disease," which was published in *Life Sciences* (May 1999), and an article entitled "STAT4 [signal transducer and activator of transcription 4] is expressed in activated peripheral blood monocytes, dendritic cells, and macrophages at sites of TH1 [type 1 helper T cell]-mediated inflammation," which was published in the *Journal of Immunology* (May 2000). A Belgian Postdoctoral Fellow published an article on "Inhibition of T_H1 immune response by glucocorticoids: dexamethasone selectively inhibits IL-12–induced STAT4 phosphorylation in T-lymphocytes," which appeared in the *Journal of Immunology* (February 2000).

A Postdoctoral Fellow from Italy wrote a review article, "Janus kinases and signal transducers and activators of transcription: their roles in cytokine signaling, development, and immunoregulation," which was published in *Arthritis Research* (February 2000). She was also the first author on an article entitled "Importance of the MKK6/p38 pathway for interleukin-12–induced STAT4 serine phosphorylation and transcriptional activity," which appeared in *Blood* (September 2000).

Signal Transduction Group

A collaboration between the Signal Transduction Group and scientists at the Institute for Molecular Genetics, National Academy for the Sciences, Prague, Czech Republic, explored the conditions necessary for activation of Fc receptors. These studies revealed that activation of the receptors occurs independently of the presence of the activating kinase Lyn in lipid rafts. This finding indicates that protein–protein interactions between Lyn and Fc receptors are sufficient for the activation of the Fc receptors. Thus, interfering with this interaction may be a practical means of stopping the activation of Fc receptors, which participate in inflammation.

In joint research with the Signal Transduction Group, a scientist from a laboratory of the French National Institutes of Health and Research (INSERM), at Hôpital Cochin, Paris, France, examined the role of a hematopoietic, cell-specific protein in regulating gene expression in T cells and mast cells. These completed studies show that the movement of this protein to the nucleus is necessary for formation of a complex that drives transcription from specific cytokine genes. These studies served to define a hematopoietic cell-specific complex that may serve as a therapeutic target to modulate TH1-type responses.

A Postdoctoral Fellow from France participated in studies to localize components of the process that regulates exocytotic mechanisms in mast cells. These studies localized many of the proteins to both granule and plasma membrane, demonstrating that in degranulating cells, the mechanisms for degranulation reside at both of the sites that participate in fusion of membranes leading to degranulation.

A Visiting Scientist from Israel conducted studies on regulation of transcription factors that determine cell differentiation and growth. These studies have identified several novel steps in transcription factor activation that are essential for a mast cell's growth response to the mast cell growth factor interleukin 3.

A Visiting Fellow from Italy has been studying how Fc receptors initiate events inside the cell. These studies have demon-

strated the existence of a novel intracellular protein complex that contributes to the function of the cells on which these receptors reside.

A Visiting Fellow from Mexico has discovered that the profile of the cytokines produced in an activated mast cell is determined by the strength of the stimulus that activates the cell. These findings have important implications for improving understanding of the mechanisms that govern a cell's response to a stimulus.

Bone and Connective Tissue Biology Branch

Craniofacial Development Section

The Craniofacial Development Section, Bone and Connective Tissue Biology Branch, continues to collaborate with scientists at Tohoku University, Sendai, Japan, to investigate the effects of regulatory mechanisms of extracellular matrices on the phenotypic expression of chondroblasts and osteoblasts.

In FY 00, two Research Fellows started joint research with scientists at the Institute for Molecular Bioscience, the University of Queensland, Brisbane, Australia, to study the molecular regulation of the Sox9 gene, a cartilage-specific early transcription factor.

A Visiting Associate from Japan studied the molecular regulation of recovery of cranial neural crest cells from retinoic acid embryopathy when treated with sonic hedgehog, a signaling molecule. The study was presented at the Laser Capture Microdissection Symposium, on the NIH campus, in Bethesda, Maryland, in June 2000.

Another Visiting Associate from Japan is investigating the molecular regulation of cartilage formation by bone morphogenetic protein (BMP) and epidermal growth factor (EGF). His work, entitled "Convergence of the BMP and EGF signaling pathways on Smad1 in the regulation of chondrogenesis," was published in the *International Journal for Developmental Biology* (November 1999), and the article "Positionally dependent chondrogenesis induced by BMP4 is co-regulated by Sox9 and Msx2" was published in *Developmental Dynamics* (April 2000).

A Research Fellow received a short-term visiting research grant from the NIH through the John E. Fogarty International Center for Advanced Study in the Health Sciences and the Japanese Society for the Promotion of Sciences Joint Program. The research consisted of a 2-week study to determine the fate of mandibular mesenchymal cells exposed to BMP2 in culture. The study was conducted at Tohoku University, Sendai, on March 3–17, 2000. These fellowships are intended to enhance U.S.-Japanese collaboration in biomedical and behavioral research by providing flexible opportunities for capable U.S. scientists to work with colleagues in leading Japanese laboratories on substantive projects of mutual interest. During her visit to Japan, the Research Fellow presented the seminar Cartilage Morphogenesis: Genetic and Biomechanical Factors at three universities—Tohoku University, Sendai; Tsurumi University; and Kyushu University, Fukuoka.

A Research Fellow and an Intramural Research Training Award Fellow are working with scientists at the DNA/Cell Bank and Gene Research Laboratory, Hacettepe University, Ankara, Turkey, on the mapping of genetic mutations for the genetic disorder postaxial polydactyly type A2. Results from this study have been submitted for publication in a manuscript entitled "A postaxial polydactyly type A locus maps to chromosome 13q21–32."

Laboratory of Physical Biology

Macromolecular Biophysics Section

The Macromolecular Biophysics Section, Laboratory of Physical Biology, continues to cooperate in research projects with Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec; Boehringer-Ingelheim, Montreal; and McMaster University, Hamilton, Ontario.

Muscle Biophysics Section

The Muscle Biophysics Section is working with the Imperial College, London, England, to determine the three-dimensional structure of the sound-producing muscle in fish. The Section is also collaborating with Academia Sinica, Taipei, Taiwan, on structural studies of the conformational basis of titin elasticity.

A Visiting Scientist from China worked in the Muscle Biophysics Section on x-ray diffraction from living skeletal muscle and successfully identified the structure of myosin weakly bound to actin in the biochemical state of actin–myosin–ADP (adenosine diphosphate) in the process of converting chemical energy of ATP (adenosine triphosphate) hydrolysis to mechanical energy for muscle contraction.

A Visiting Fellow from China analyzed the x-ray diffraction patterns of skeletal muscle and pursued molecular modeling to establish the binding patterns of the myosin cross-bridges to the actin filaments when the affinity between the two molecules is low. The low-affinity states signify the initial stage of force generation by muscle.

A Research Fellow from China is conducting structural studies of titin elasticity, and a Visiting Fellow from Germany is profiling interfaces of muscle. An Intramural Research Training Award Fellow from Nepal is working on muscle regulation.

A Senior Scientist from the University of Bristol, England, was a Guest Researcher in the Muscle Biophysics Section. He was pursuing dynamic molecular modeling on the effects of binding various nucleotides in the active site of the myosin molecule. This research is expected to lead to a better understanding of why some nucleotides are hydrolyzed and some are not hydrolyzed. Such an understanding will give further insight into the energy transduction involved in muscle contraction.

Protein Expression Laboratory

A Visiting Fellow from Canada worked in the Protein Expression Laboratory to study Rev, an essential protein of the human immunodeficiency virus (HIV), the causative agent of the acquired immunodeficiency syndrome (AIDS). Viral replication involves Rev, which permits the export of unspliced and partially spliced messenger RNAs (mRNAs) from the nucleus and thereby facilitates the switch from the late phase of viral replication. Rev functions by binding to viral mRNAs at a high-affinity site and by directing them to a nonsplicing export pathway. Rev has both an export signal and a nuclear localization signal and consequently functions as a nucleocytoplasmic shuttle protein. The investigator demonstrated the capacity of Rev to interfere with microtubule polymerization in *Xenopus* egg extracts. A report of this work, "HIV-1 Rev depolymerizes microtubules to form stable bilayer rings," was published in the *Journal of Cell Biology* (June 2000). Preliminary results from immunofluorescence of induced Rev expression in HeLa cells have demonstrated

co-localization of Rev with microtubules in both mitotic and interphase cells. Hence, regulation of Rev expression may be important in modulating the cell cycle.

Laboratory of Skin Biology

Molecular Biology of Keratinization Section

The Molecular Biology of Keratinization Section, Laboratory of Skin Biology, continues cooperative efforts in the following international research efforts:

- studies of the function of transglutaminases on membrane surfaces, with the University of Debrecen, Hungary;
- investigation of the expression and function of transglutaminases in epidermal differentiation and apoptosis, with the University of Rome, Tor Vergata, and Istituto Dermopatico dell'Immacolata, Rome, Italy;
- studies of the biophysical and solution structures of transglutaminase substrates, as visualized by nuclear magnetic resonance imaging and x-ray crystallography, with the Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand;
- exploration of the structure of intermediate filaments, with the Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand; and
- studies of the biophysical and solution structures of intermediate filament coils, as visualized by nuclear magnetic resonance imaging and x-ray crystallography, with the Maurice Müller Institute, University of Basel, Switzerland.

A Staff Scientist from Bulgaria studied the postsynthetic modifications of several epidermal structural proteins. A Visiting Fellow from China investigated the expression of the gene for trichohyalin. A Visiting Scientist and five Visiting Fellows from Korea studied the expression and function of transglutaminase substrates in normal and degenerative tissues, the regulation of epidermal gene expression, and genes encoding enzymes involved in postsynthetic modifications of skin proteins. A Visiting Fellow from Russia and another from Ukraine examined the structures of protein involved in barrier function and their interactions with membranes.

Laboratory of Structural Biology Research

The Laboratory of Structural Biology Research continues to collaborate in several international research efforts, including studies with the following institutions:

- Commonwealth Scientific and Industrial Research Organization, Geelong, Australia, and Massey University, Palmerston North, New Zealand—investigations of the structure of trichocyte intermediate filaments;
- University of Graz, Austria—structural study of ATG2 ATPase (adenosine triphosphatase);
- Ebel Institute of Structural Biology, Grenoble, France—high-resolution structural analyses of viruses;
- Max Planck Institute for Biochemistry, Martinsried, Germany—implementation of electron tomography and preparation of a review of energy-dependent proteases;
- Medical Research Council, Institute of Virology, Glasgow, Scotland—investigations of the capsid structure of oyster herpesvirus, an evolutionarily remote herpesvirus; and
- Centro Nacional de Biotecnologia, Madrid, Spain—electron microscopy studies of human astrovirus.

In FY 00, a Visiting Associate from Canada studied the interaction between the Rev protein of HIV and tubulin and is also working on assembly of hepatitis B virus. A Visiting Fellow from Japan analyzed the three-dimensional structure of the energy-dependent ClpAP protease of *Escherichia coli.* Another Visiting Fellow from Japan completed a structural analysis of a virus and is now studying transport vesicles. A Visiting Fellow from Russia has been visualizing yeast prions inside cells, and another Visiting Fellow from Russia has been expressing fragments of the filamentous hemagglutinin of *Bordetella pertussis* and studying their properties. Also in FY 00, a Visiting Fellow from South Africa studied herpesvirus associated with Kaposi's sarcoma, and a Visiting Fellow from Spain studied interactions of the ClpXP protease with substrate proteins.