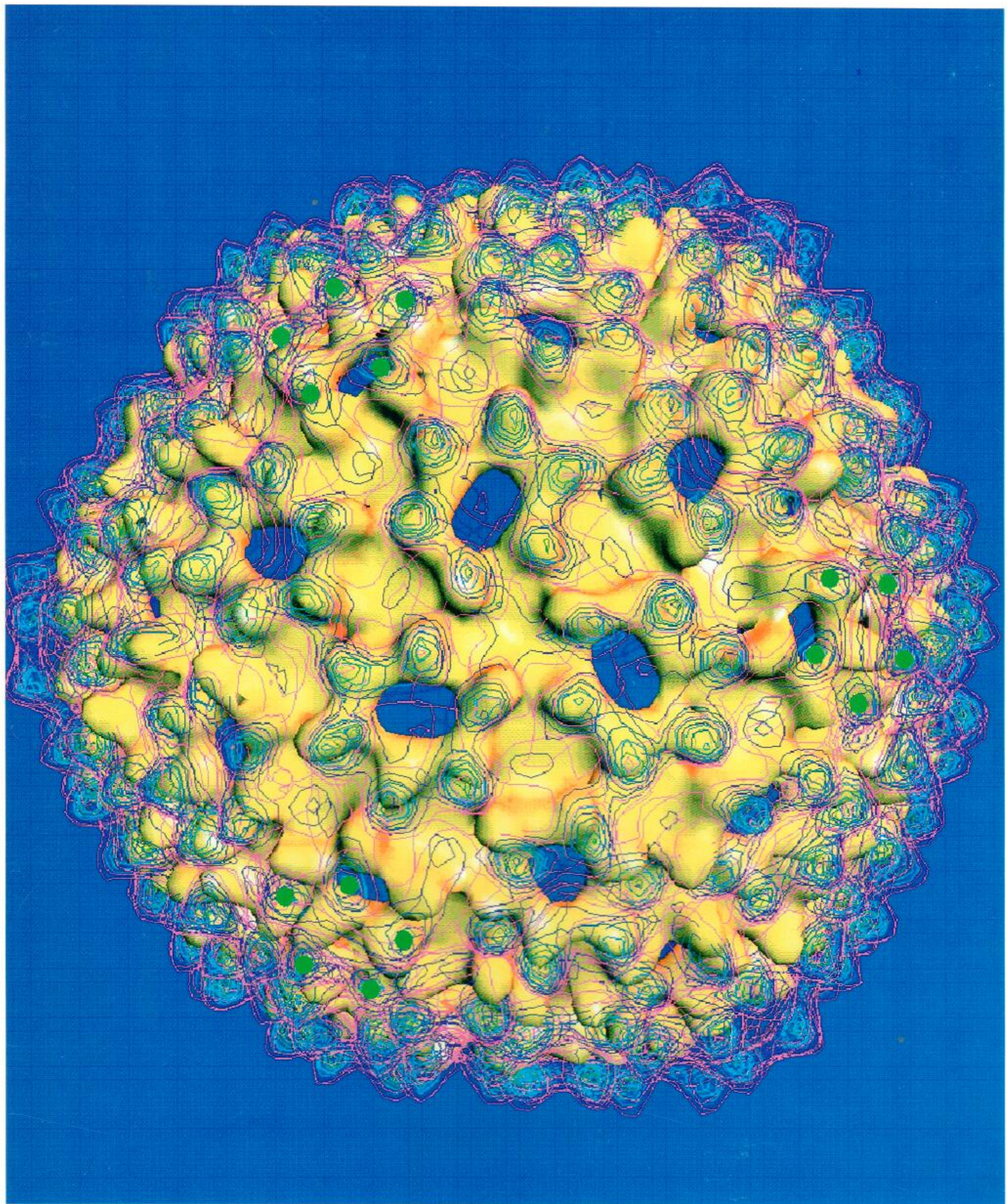
Boston Biomedical Research Institute

1994 Annual Report



Boston Biomedical Research Institute is dedicated to basic biomedical research, which promotes the understanding, treatment and prevention of specific human diseases. One major focus is muscle cell biology and its implications for neuromuscular and other muscle-related diseases such as asthma, hypertension, malignant hyperthermia and gastrointestinal disorders. Results of research are published in leading scientific journals. When appropriate, the Institute collaborates in clinical studies of patients to apply the results of basic research to problems of human health, the cure of disease and the development of new medicines. Boston Biomedical Research Institute is an independent, not-for-profit institution.

Cover:
Three-dimensional Representation of the Bacteriophage P22 Viral Capsid. The three-dimensional structure of the bacteriophage P22 Procapsid and Capsid was obtained by computer-aided reconstruction of electron micrographs of frozen hydrated samples. The viral DNA is contained within the capsid. The contour lines overlying the capsid represent the structural transformation which occurs on DNA packaging. Courtesy of Drs. B.V.V. Prasad and Peter E. Prevelige, Jr.

Table of Contents

BBRI Corporators and Trustees	2
Report of the President	3
Report of the Director	4
Research Activities of BBRI Faculty	5
Publications	18
BBRI's Staff	19
Contributors to the Annual Research Fund	20
Financial Data	22
Grants, Contracts, and Fellowships	24

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We hope you will find this Annual Report to be both an interesting and an inspiring review of the explorations and discoveries of BBRI scientists during the past year. Behind the crisp language of science lies the excitement of the quest to learn the unknown; to seek answers which enable one to ask more questions and to gain new insights. Such is the substance of life at BBRI. Again in 1994, our faculty has distinguished itself with numerous publications and achievements which reinforce their position of eminence in basic scientific research.

As we approach the year 2000 and a new century in the search for discovery, our challenge is to spread the word about this great Institute with vigor and enthusiasm to the widest possible circle of individual and institutional benefactors. By enlisting a growing base of support for our programs, we will enable our faculty to sustain and enhance its contributions to science and to society.

This challenge is intensified by the contradictory forces of promising new scientific frontiers on the one hand and constrained economic resources in both the public and private sectors on the other. To advance the conquest of cancer, heart disease, Alzheimer's disease, AIDS, and other tragic illnesses, the basic scientific world needs to accelerate the utilization of new technologies so as to enable the earliest possible application of new basic knowledge to treatment-specific drugs or devices. Thus, the successful research institution in the 21st century will be one which has combined a track record of prior excellence with a clear vision and a practical plan to acquire the financial and human resources necessary to achieve its goals of service and distinction in the future.

At BBRI we are gearing up to compete for the scarce funds of the future by pursuing a variety of organizational and programmatic initiatives. We do so from a basis of solid operating and financial practices developed over the past years by our Director, Dr. John Gergely, our distinguished faculty and a strongly committed Board of Trustees. The first step forward was taken in the past year when the Board of Trustees, at the recommendation of the faculty, appointed Dr. Kathleen Morgan to succeed Dr. John Gergely as Director of BBRI effective January 1, 1995. The Institute is fortunate that Dr. Gergely will carry forward his important scientific research as a Senior Scientist and member of the faculty. His experience and counsel will be valuable resources to Dr. Morgan as she builds upon the foundation which exists now at BBRI. We hope you share with us our enthusiastic faith in the future as a result of Dr. Morgan's leadership.

Other actions we are pursuing include the development of a strategic plan, specific in scientific focus and financial implications, with which to assure continued support of existing programs, new faculty recruitment and development, essential space and equipment requirements and continued efficient administrative support.

These efforts are already in good order because of an uncommonly strong bond of trust and collaboration amongst Board, Faculty, and Staff of the Institute. This sense of working together as stewards to the Institute is, I believe, our "secret weapon". For as we present our proposals for financial support in the future, I believe that BBRI will become even better known as a first rate basic scientific research center recognized and supported because of its ability to design imaginative scientific projects and to deliver significant findings which will advance scientific and medical knowledge throughout the world.

This is an exciting time in the history of BBRI. We are grateful for the support which so many have given to enable us to achieve our accomplishments to date. We are committed to retain the endorsement of our current community and to seek significant new numbers to comprise the BBRI Family of the Future. We hope to earn even greater support in 1995 and beyond.

Edgar G. Davis

Report of the Director

This is my last report as Director of BBRI, and the first thing I want to say is that I do this with a strong feeling of confidence in the Institute's future in light of the results of the search that came to a conclusion with Kathleen Morgan's appointment as Director effective January 1st, 1995.

She is an Associate Professor of Physiology in the Department of Medicine at Harvard Medical School and Director of the Program in Smooth Muscle Research, Department of Medicine, Beth Israel Hospital. After a Ph.D. in pharmacology at the University of Cincinnati College of Medicine in 1976 Kathleen spent seven years at the Mayo Medical School in Rochester, Minnesota, first as a Research Fellow and then as a faculty member rising to Associate Consultant in the Department of Pharmacology. Since 1983 she has been in her present location and during this period she became a highly respected member of the smooth muscle research community. She is the author of numerous papers in leading journals and has served on the Editorial Boards of premier professional journals including Circulation Research and the American Journal of Physiology. At present Kathleen is a member of the Experimental Cardiovascular Study Section of the National Institutes of Health. She will continue her affiliation both with Harvard Medical School and Beth Israel Hospital where she will maintain a small laboratory. Kathleen's research will add a new dimension to BBRI's program. Her vision of the importance of cell growth and cell signalling both as fundamental research areas and fertile grounds for clinically relevant investigations will represent an integrating force for much ongoing more molecularly-oriented research at BBRI. Her many years at one of the leading teaching hospitals in Boston will undoubtedly open up new opportunities for closer interaction between basic research and investigations more oriented towards clinical problems.

I am happy to report that Peter Prevelige, who came to BBRI from MIT two years ago, and who had received a Shannon Award while he was competing for a full NIH grant, has now received an award which will fund his research on viral structure for the next three years.

In the past year BBRI staff members have continued to take their share not only of being productive researchers as the publication list which is part of our annual report will show, but also have been good citizens of the scientific community in that they continued serving on NIH and NSF review boards, did their share of editorial work for various scientific journals and participated in a number of national and international conferences and symposia presenting the results of their research.

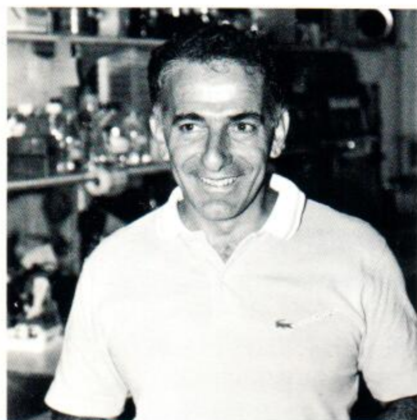
Throughout the year at many committee and board meetings there was considerable discussion concerning the relation of basic and applied research, both with respect to biotechnology and to clinical medicine. I cannot miss the opportunity of reiterating my strong conviction that whatever BBRI has achieved so far is due to the basic research that stems from the vision and enthusiasm of the investigators who choose fields of investigation that they find stimulating and in which there are fine puzzles that they consider worth solving. Needless to say, there is a healthy feedback control mechanism built into the system. Those research projects that appear worthy to the investigator of their spending many hours of the day and night also have to appeal to the peer groups that eventually decide whether they are worthy of funding. I say this to dispel the notion, which I hope is not held by too many people, that basic research is something that is done in an ivory tower detached from the hard realities of everyday life. Perhaps it is appropriate to point out that this year's Nobel Prize in Medicine was given to two investigators whose prize-winning research on the so-called G proteins - proteins that bind a cousin of the by now familiar ATP, the immediate fuel of most of our biological processes, including muscle contraction and nerve conduction - started out as an investigation of what fifteen or twenty years ago may have looked a rather obscure project on an, at that time, hardly known substance. I would also point out that this particular compound, GTP, is also at the center of a number of signal transduction processes which, as many readers of this report will recognize, is one of the central interests of Kathleen Morgan and a field that we hope will grow and develop at BBRI.

A word about this year's Annual Report. In the last few years we tried to encapsulate certain research areas in mainly lay terms, having selected one aspect of BBRI's work and describing that particular area in more detail. This year the individual scientists have described in a brief fashion their own interests, attempting at least in a few sentences to indicate in a language understandable by lay people what they are after, but leaving enough technical detail so that fellow scientists would get insight into some of the nuts and bolts of the work.

In closing, I first of all would like to express our pleasure at having been able to work with Ed Davis as President. He has brought an enormous amount of energy and imagination to his office and, although he is dividing his time between Boston, Oxford, and Indianapolis, in this age of easy communication we have no problem in keeping in touch with each other. We are also grateful to all other members of the Board of Trustees and Corporation for their support - both moral and financial - and and for their willingness to act as ambassadors for BBRI and its work. Particular thanks are due to members and chairs of various committees, who contribute so much to the ongoing work of BBRI.

John Gergely

BBRI's research is carried out by a faculty of 22 scientists with the support of government grants, which are awarded to individual investigators in nationwide competition with other scientists. The Institute might thus be viewed as a "scientists' cooperative" in which the faculty members are responsible for their own research projects. However, BBRI is more than that, for it provides an environment that fosters a spirit of informality, cooperation and close interaction among scientists, and much of the research carried out by its faculty has a common focus. The principal areas of investigation at BBRI concern the structure and function of muscle proteins, membrane and transport processes, and the control of cell growth and gene function. As you peruse the research summaries that follow, keep in mind that BBRI is more than the sum of its parts: even though the research projects described here reflect the individuality of its faculty members, each project contributes to a theme that connects it with one of the broader research goals of the Institute.



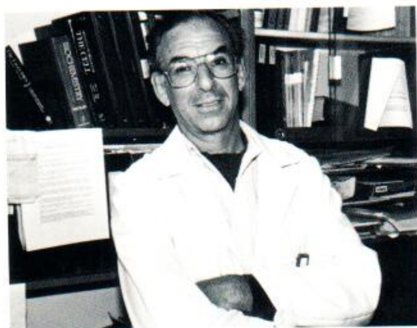
John Badwey

Our interests concern the molecular mechanisms by which certain types of white blood cells (leukocytes, e.g. neutrophils, monocytes) produce large quantities of superoxide during phagocytosis - the defensive process by which foreign material, including bacteria, is engulfed and destroyed. Superoxide is a major component of the oxygen-dependent antimicrobial arsenal of these cells. We are investigating both the nature of the oxidase enzyme complex that catalyzes the production of superoxide and the signal transduction pathways that lead to the activation of this enzyme system. The enzyme complex is dissociated in unstimulated cells into its cytoplasmic (p47-phox, Rac 2), cytoskeleton-associated (p67), and membrane-bound components. During cell stimulation there is a translocation of the cytoplasmic and cytoskeletal components to the cell membrane where the functional oxidase is assembled. This assembly generally requires multisite and hierarchical phosphorylation of p47-phox by protein kinase C and some novel protein kinases. A number of potent tumor-promoters (e.g. phorbol esters, calyculin A) can interact with either the protein kinases or phosphatases that control the phosphorylation of the protein. Particular attention is now being directed at characterizing the novel protein kinases and phosphatases that are acting on p47-phox and elucidating their regulatory mechanisms. These studies may lead to important practical applications with regard to defenses against infection and possibly cancer. ■

John Codington

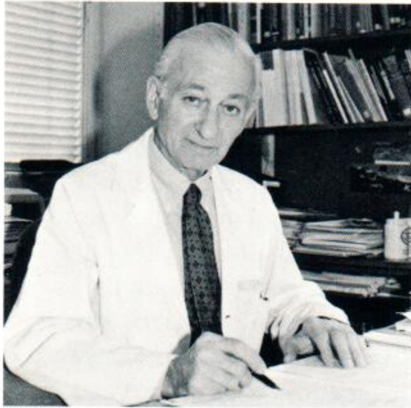
In the course of our investigation of the immunological properties of proteins with attached carbohydrate moieties (glycoproteins) at the surfaces of cancer cells, an interesting observation was made. Antibodies to epiglycanin, a large glycoprotein on the surface of a mouse breast cancer cell (TA3-Ha), strongly bind not only to the mouse cell but also to the human carcinoma antigen (HCA) shed

from the cells and found in the blood of patients. This finding that an antibody to a mouse glycoprotein can bind and identify the HCA in human cancer patients has led to a practical application, the development of a test (the Cod Test) to monitor the course of the disease in cancer patients, which is now in clinical trials. Monoclonal antibodies to epiglycanin are also being applied toward the achievement of two additional goals: the development of a therapeutic agent for carcinomas which represent over 80% of all human cancers, and the development of a radioactive monoclonal antibody that may serve as an imaging agent for locating a tumor mass in the body. The latter is a collaborative effort with Dr. Rashid Fawwaz of Columbia-Presbyterian Medical Center. Work on the characterization of the HCA from human carcinomas is in collaboration with Dr. Svein Haavik at the University of Oslo. ■



Peter Coleman

Current studies by my lab focus on one portion (the F_1 segment) of one of the most structurally complicated enzymes called ATPase or ATP-synthase This many-subunit protein complex is found in bacteria, plants and animals and is responsible for manufacturing most of the ATP (the universal energy donor molecule) needed to support biochemical life processes by "capturing" energy released upon the metabolic combustion of nutrients in a process called oxidative phosphorylation. Many details about how this enzyme operates remain obscure despite 35 years of concentrated investigation by some of the world's most noted biophysical chemists, even in the light of the recently obtained detailed structure of the enzyme by British scientists via X-ray diffraction analysis. F_1-ATPase is a very complicated structure constituting nine subunits of five different kinds: three α, three ß and one each of γ, δ and ϵ, adding up to a molecular weight of 371,000. There are six adenine nucleotide (ATP or ADP) binding sites, but only three of these participate in the catalytic process. The functional role of the other three nucleotide sites has so far eluded clarification. However, the most striking feature about F_1 catalysis is the fact that the binding of the nucleotides at its three catalytic sites is a highly cooperative and asymmetric affair. Binding a single nucleotide occurs with phenomenal tightness, but catalysis does not readily take place. Binding a second nucleotide occurs with much less tightness, but at the same time this event enormously stimulates catalysis (almost a million-fold). Binding the third nucleotide manifests even less tightness, with catalysis additionally enhanced to its maximum. Concomitant with the sequential, cooperative binding of these three nucleotides, the F_1-ATPase undergoes significant conformational changes in the folding of the many polypeptide subunits and alters the manner of association between them. To help clarify what happens at the three "catalytic" sites, and to determine, by peptide microsequencing, where these sites could be located within specific peptide domains on the F_1 complex, we employ low energy ultraviolet light to induce covalent binding to the enzyme of nucleotide analog compounds (photoaffinity probes). We synthesize these analogs, which can occupy various nucleotide sites on the F_1-ATPase. Our experiments use techniques for restricting the photoaffinity labeling of F_1 to each of the three catalytic sites on the enzyme in sequence. By means of such restricted selectivity of photoaffinity probes, we are currently attempting to identify what we classify the catalytically "priming" and "promoting" loci within the primary structure (the amino acid sequence) of the three b subunits of F_1-ATPase. Because F_1-ATPase is central to the cell's energy conversion processes, detailed knowledge about the structure and function of this crucial enzyme may help us better understand the irreversible loss in the capacity to generate energy that characterizes aging and neurodegenerative disease. ■



John Gergely

My long-standing interest has been the unraveling of the molecular mechanisms that underlie muscle contraction and its regulation. Although the question of how muscles work has been asked by scientists for centuries, and important work was done in the first half of this century on the mechanical properties of muscle, only the last fifty years or so brought insights into the molecular mechanisms. It is clear now that the production of force or movement by contracting muscle requires a great number of intricate interactions between a number of proteins each having a precise atomic structure and a clearly defined function. In addition to the ATP-powered moving machinery consisting of myosin and actin there is tropomyosin and the three-component-complex troponin. The latter two proteins, both located on the actin polymer, are responsible for the fast switching on and off of the interaction between myosin and actin initiated by calcium ions, in response to signals transmitted by the nerve. It is the complexity of the whole system that represents the major intellectual and technical challenge. In order to understand the function of the whole assembly one needs to know the detailed physicochemical characteristics of each of the components, map their interactions and, most importantly, to uncover how do they alter each other's function. A great deal of information has been obtained here and in other laboratories by studying optical and magnetic properties of proteins under various conditions, utilizing either their intrinsic properties or by tagging them with extrinsic marker molecules - so called probes. In recent years the use of molecular genetic engineering has been particularly useful in our work. These techniques make it possible to introduce changes in the chemical structure of a protein by substitution of one amino acid for another. In this way specific hypotheses about structural and functional features of a protein can be tested. Also the modification may make it possible to introduce specific probes at sites inaccessible in the native protein.

Future work will greatly benefit from the knowledge of the atomic structure of some muscle proteins including troponin C, actin, and a fragment of myosin obtained by X-ray diffraction techniques in several laboratories. This work has opened up new vistas for the future. A much more precise interpretation of the physicochemical observations becomes possible. While X-ray diffraction gives a precise "still" picture of a protein molecule, only chemical and physicochemical studies based on the information from X-ray work may give further insight into the details of the dynamics of the proteins as they function in the living cell. Knowledge of these functions in normal tissue may eventually lead to the discovery of the underlying molecular pathology of various diseases. ■

Zenon Grabarek

Calcium ions regulate intracellular processes and consequently the normal function of a living organism including the contraction of all types of muscles and the electrical activity of nerves. Calcium is also critical in many more fundamental biological processes such as egg fertilization, gene expression and cell division. The effect of calcium is always mediated by specific proteins that upon binding calcium undergo structural transitions enabling them to interact with and modulate the function of other proteins, including many enzymes. Calmodulin is an ubiquitous calcium binding protein regulating the activity of many enzymes, regarded as its targets, in all eukaryotic cells. It is one of a few well characterized members of a large family containing some 300 calcium binding proteins. Our goal is to understand the molecu-

lar mechanisms by which calmodulin and other related calcium binding proteins respond to calcium and subsequently interact with and modulate their targets. The primary tools used in my group are site-directed mutagenesis, supported by computer assisted molecular modeling and a broad array of biochemical and physico-chemical techniques. Most recently we have also started collaborative efforts to characterize some of our protein mutants with the use of high resolution techniques - nuclear magnetic resonance and crystallography. Currently our efforts are focused on characterizing the global conformation of calmodulin in complexes with various targets including intact enzymes and model synthetic peptides. Our studies clearly show that calmodulin is very flexible in the sense that it can utilize at least three different mechanisms to activate its various targets. This appears to be at odds with significant specificity of target recognition by calmodulin, which intuitively would require a well defined "rigid" structure. We need to know more about calcium binding proteins and protein structure in general to explain this phenomenon. Another exciting problem we address in our work is to understand the structural changes in calmodulin resulting from binding calcium. We expect that once we know how and why calcium changes calmodulin structure we will also know what may happen in other homologous calcium binding proteins and possibly predict their physiological role. ■

Philip Graceffa

Muscle converts chemical energy into mechanical energy in order to produce force and movement and it comes in a variety of types in order to fulfill specific functional requirements. For example, mammalian skeletal muscle can exert tremendous force or propel an animal at high speeds whereas insect flight muscle oscillates at very high frequencies. Mammalian smooth muscles are those which are part of the wall of hollow or tubular organs and play an important role in maintaining or changing their shape. For example, blood vessel smooth muscle regulates blood flow by either contracting to constrict vessel diameter or relaxing to increase vessel diameter. Once the vessel reaches the desired opening it must maintain this shape, sometimes for long periods, against the blood pressure impressed upon it. Smooth muscle has been uniquely designed to carry out this function very economically - that is, at the expense of very little energy. The mechanism of contraction of muscle involves the sliding of two sets of parallel filaments, thick and thin, past each other, thereby causing the muscle to shorten or produce force. Smooth muscle contains a protein called caldesmon which is bound to the thin filament and is thought to be involved in the regulation of contraction and the economic maintenance of force. Caldesmon is a long, thin molecule whose two ends have different properties. We have been studying the ends of caldesmon by placing a variety of crosslinking, fluorescent and magnetic probes at each end of the molecule. With these probes we are able to get information on the shape and arrangement of caldesmon on the thin filament and on its proximity to other thin and thick filament proteins. This structural information will be helpful in gaining an understanding of the molecular basis of caldesmon's function and may help to explain the unique properties of smooth muscle. ■

Jen-Shiang Hong

Our research deals with the regulation of the expression of the gene pgtP that encodes the phosphoglycerate transporter protein of the bacterium *Salmonella typhimurium*. This membrane-bound transporter protein mediates the transport into the cell of energy-rich phosphoglycerates, which are important metabolites

in glycolysis and include phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate. The pgtP gene is turned on only when phosphoglycerates are present in the medium. This sensing-triggered gene expression exemplifies a widely used cellular process termed signal transduction. The sensing mechanism involves three regulatory proteins serving as sensor-receptor and response regulator, each of which is encoded by a distinct gene. There are four regulatory sites, also termed operators, located right in front of the DNA strand that contains the transporter gene. When the response regulator is activated by phosphorylation as a result of the presence of phosphoglycerates in the external medium, it binds one of the four operators. This binding facilitates the binding of two other regulatory proteins to their respective operators, thereby activating the expression of the pgtP gene. A typical regulatory system requires only one or two operators for gene activation. The fact that four operators are necessary in the pgt system indicates unusual features in the arrangement and the structures of the pgt operators themselves. We are studying the structures of the operators, the proteins that bind these operators and the mechanisms by which these proteins interact and bend DNA to effect gene activation. DNA looping is a most plausible mechanism to bring together the proteins bound to the well-separated operators along a linear DNA molecule. ■

Noriaki Ikemoto

One of the most important unsolved questions in muscle physiology is how the excitation elicited in the surface membrane of the muscle cell induces Ca^{2+} release from an intracellular Ca^{2+} store which in turn leads to muscle contraction. This is the so-called excitation-contraction (e-c) coupling, and we are trying to elucidate its detailed molecular mechanism in normal and diseased muscle. According to our current views the muscle cell contains at least two membrane systems. One is the transverse tubular system (T-tubule), and the excitation signal elicited in it produces conformational changes in a large protein called the foot protein (FP). This activates the Ca^{2+} channel located in the FP, leading to Ca^{2+} release from a space enclosed by the second membrane, the sarcoplasmic reticulum (SR). To unravel the intimate details of this mechanism, we have established a simplified membrane system (triad) that is suitable for the studies of e-c coupling at the molecular level. Using this model, we are investigating several key events in e-c coupling, such as (a) rapid changes in the T-tubule membrane electric potential, (b) conformational changes in the FP induced by the T-tubule depolarization, and (c) Ca^{2+} release from the SR. The effects of site-specific pharmacological reagents, antibodies, and peptides on each of these sequential events are investigated to characterize the role of individual molecular components in e-c coupling. Animal models carrying diseases similar to those in the human will yield further insights of clinical importance.. For example, there is a strain of pig carrying a mutation of FP resulting in abnormal e-c coupling, mimicking a potentially lethal complication arising from the use of certain anesthetics in people genetically disposed to malignant hyperthermia. We are particularly interested in the mechanism by which the fundamental molecular event for activation of Ca^{2+} release, viz. the conformational change of the FP, is altered by the mutation in this protein. ■

Saroj Joshi

The synthesis of ATP, the immediate source of energy for most cellular functions, takes place in the mitochondria, particles found in cells of higher organisms. It results from the combustion of nutrients and occurs in two steps. The first step in the formation of

ATP is a movement of protons, positively charged H-atoms, driven out by the energy of oxidation from inside the mitochondrion. The second step, driven by the reversed flow of protons, is the actual synthesis of ATP. This process is catalyzed by an enzyme complex named ATP synthase, consisting of a catalytic segment F_1, a proton-translocating segment F_o, and an intervening segment, stalk, that brings about the coupling of F_1 and F_o segments. In order to have a better understanding of the communication between F_o and F_1 we have to know more about a protein - the oligomycin sensitivity conferring protein (OSCP) - located at their interface, which plays a key role in the transfer of energy between the two segments. As its name implies, OSCP renders the F_1 enzyme sensitive to the antibiotic oligomycin, which results in the inhibition of the activity of F_1. We have cloned the OSCP gene in bacteria and are using genetic engineering approaches in which various regions suspected of interacting with F_1 are modified to obtain mutant forms. We shall study the effect of these mutations on the ability to couple the flow of protons to ATP synthesis. Recent X-ray diffraction studies in the U.K. have established the structure of the F_1 component of the ATPase on the atomic level. This work will help to design genetically engineered mutants that pinpoint key sites in the interaction between the F_1 and F_o components of this important enzyme. It is hoped that this kind of analysis will lead to a better understanding of the mechanism of ATP synthesis in normal and diseased tissue, which may eventually help in diagnosing and possibly curing neurodegenerative diseases that involve defects in mitochondria. ■

Sherwin Lehrer

In skeletal, cardiac and smooth muscle, tropomyosin (Tm) - in conjunction with other protein components present in the thin filament of the contractile apparatus and with myosin - confers the cooperativity important for the regulation of contraction. It is an essential protein for normal muscle function and mutations in the α-Tm gene in human cardiac muscle have been implicated in the disease familial hypertrophic cardiomyopathy. Tm is a two-chain rod-like molecule that interacts with actin and with the troponin complex in striated muscle and in complexes with caldesmon and calponin in smooth muscle. Tropomyosin chains isolated from different kinds of muscles have different amino acid sequences which influence the different regulatory properties in the different systems. My long-range goal is to understand the molecular and submolecular mechanism of tropomyosin action. This involves studying the molecular and regulatory properties of different tropomyosins isolated from different muscles and of mutant Tms produced by recombinant DNA technology. Techniques used include circular dichroism to monitor Tm stability via studies of unfolding and folding produced by temperature changes and denaturants; and fluorescence, to monitor changes in the signal of a specific probe attached to Tm associated with the way it regulates actomyosin ATPase activity. We also use fluorescence microscopy to obtain information about changes in localization of fluorescent proteins incorporated into muscle fibrils associated with contractile activity and to measure the effects of regulatory proteins on the movement of actin filaments with an *in vitro* motility assay. Circular dichroism and fluorescence techniques are also used to study the rod portion of the myosin molecule, whose structure is responsible for assembly into thick filaments in smooth and striated muscle. In smooth muscle in particular, the configurational changes in the rod portion, which affect filament assembly as well as the contractile activity of the myosin-actin system, are also under study. ■

Renne Chen Lu

Myosin in smooth muscle is not only the motor but also plays a role in the regulation of contraction. Phosphorylation of the regulatory light chain of myosin is required to initiate force generation in smooth muscle contraction. It is intriguing that the function of a large molecule such as myosin, consisting of a pair of heavy and a pair of light chains, is controlled by the 5000 times smaller phosphate group. The major focus of my research group is to correlate the structural features of smooth muscle myosin from chicken gizzard with its functions. In particular, our goal is to resolve the unsolved question - how the effects of phosphorylation of the light chains are communicated to the regions in the heavy chains that are responsible for muscle contraction.

Our strategy is to map out the communication pathway in a systematic fashion by determining the effects of phosphorylation in turn on: (1) the structure of light chains; (2) light chain-heavy chain interactions; and (3) actin-myosin interactions. A number of chemical methods including cross-linking and assays of.proteolytic susceptibilities as well as optical methods such as fluorescence, resonance energy transfer, and circular dichroism measurements will be used to monitor the structural changes due to phosphorylation. Light chain mutants and chemically synthesized peptides corresponding to segments of the heavy chain, with cysteine residues at strategically important positions, are crucial tools that tell us about the structural changes taking place in various parts of the myosin molecule. ■

Katsuhide Mabuchi

How does a bicycle move? It's easy to see how a bicyclist's legs generate force that is transferred successively to the pedals, chains, gears and finally, the wheels. How do the legs move? Of course we know, through the action of muscles. The obvious next question then is, how do muscles produce movement? The answer, unfortunately, isn't as obvious. While we can see the parts of a bicycle and understand how they interact, we are not afforded this luxury with muscle.

Nevertheless, the bicycle analogy is useful as different muscle proteins are coordinated towards an end much as bicycle parts are. However, our knowledge of muscles is sketchy for two reasons. Firstly, proteins are fragile structures, precluding many reliable, yet harsh, methods of sample treatment that would expedite their study. Secondly, current technology sets limits to our ability to see a muscle's inner workings and consequently our detailed understanding of it. Nevertheless, it is crucial to study the shapes and locations of proteins, as we may be able to ascertain how proteins make the muscle work - much as one could deduce the workings of a bicycle by seeing how its parts fit together.

Our approach has been to study the shapes and location of protein molecules through electron microscopy, which provides details at a resolution 100 times higher than that obtainable with the light microscope. In the past decades a great deal of knowledge has been generated by the electron microscope, even though its resolution is still a far cry from that at the atomic level ultimately necessary to understand the details of the operation of the molecular machinery of muscle. Our recent technical improvements, including the use of readily visualizable specific antibodies as markers, should help achieve a better understanding of muscle movement and aid in the development of cures for muscle diseases and developmental defects. ■

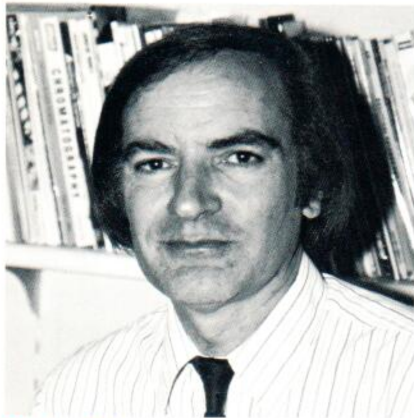
Henry Paulus

My research focuses on the regulation of a complex biosynthetic pathway with the aim of understanding the control processes that underly normal growth and development and the aberrations that lead to metabolic disorders. The pathway under study is the so-called aspartate pathway, one of the central metabolic pathways in bacteria, whose products include four of the eight amino acids that are essential for the nutrition of the adult human. The great complexity of the aspartate pathway, together with the fact that its products are of vital importance to human nutrition, give special significance to the study of its regulation.

Our studies of the regulation of the aspartate pathway in the gram-positive bacterium *Bacillus subtilis* have indicated that it involves rather unusual control mechanisms. These studies have focused primarily on the branch of the aspartate pathway that leads to the biosynthesis of the essential amino acid lysine. Lysine biosynthesis is unique among biosynthetic pathways in that one of its intermediates, diaminopimelate, is also an important constituent of the bacterial cell wall and of the spore cortex. In *Bacillus subtilis*, lysine biosynthesis is thus controlled not only by the end product lysine but also by the intermediate diamiopimelate. To understand how this dual control is mediated, the genes that encode the enzymes for the early steps in lysine biosynthesis were cloned and sequenced. A cluster of genes encoding the first three enzymes of the pathway was identified and found to be under rather unusual control. These genes are expressed not only during growth of *Bacillus subtilis* but also during the developmental process that leads to the production of bacterial spores. The regulatory mechanisms involved are unusual and complex and may involve a novel type of translational control element. Their detailed elucidation will be of considerable interest because it will not only throw light on the biosynthesis of an important class of substances essential to human nutrition but will also provide insights into developmental processes in prokaryotes. ■

Peter Prevelige

Viral infections, a major cause of disease worldwide, are difficult to treat clinically. The difficulty in treatment arises from the fact that the invading virus reprograms the machinery normally present in the cell so that it makes viral instead of cellular proteins. These viral proteins subsequently assemble within the cell into new virus particles, which are liberated in order to infect more cells, thereby perpetuating the cycle. In order to minimize side-effects, antiviral chemotherapeutics are necessarily targeted at biochemical steps unique to the viral life cycle. The currently available antiviral agents are targeted at steps involved in the synthesis of a viral protein. An alternative target is the series of steps involved in the repeated interaction between the viral coat protein subunits leading to an assembled capsid. These interactions are necessarily highly specific, making them an attractive target for antivirals. Research in my laboratory is focused on defining both the chemical nature and the sequence of interactions required to assemble a virus from its constituent protein subunits. The research takes advantage of the well developed bacteriophage P22 system, a virus whose host is the *Salmonella* bacterium. We have developed a system in which the viral proteins can be purified, and, when mixed together under appropriate conditions, are capable of forming viral particles in the test tube. Using this system we have been able to uncover the first steps in "initiating" assembly, to localize the regions of the viral proteins involved in the intersubunit interaction, and to demonstrate "proof of principle" for small molecule inhibitors of viral assembly. We expect these studies will be instrumental in the development of a new class of antiviral agents. ■



Victor Raso

Cancer is the second leading cause of death in the United States largely because the common solid malignancies remain resistant to conventional chemotherapeutic drugs. Recently, attempts have been made to treat cancer with large proteins such as monoclonal antibodies directed against malignant cells and immunotoxins that deliver poisons to selectively kill them. Although some success has been achieved in leukemias, solid tumors have proved refractory to these agents. This lack of success is commonly attributed to the limited localization and poor penetration of these macromolecules into solid tumors.

Our work aimed at developing an attack on solid tumors examines an "experiment of nature" to better understand this endeavor. Diphtheria toxin, a medium size protein, in small amounts produces cures of large, advanced stage, human tumors growing in mice that have genetically lost the ability to reject foreign tissue. This is possible because the toxin specifically targets a molecule that is present on the surface of the human cells, while the mice are naturally resistant to its poisonous action since their cells lack this target site. We have used this model of a specifically targeted toxin with tumor-curing ability to identify the conditions required to achieve such effective solid tumor dissolution. The inherent biological activity of diphtheria toxin allowed us to follow its rapid and uniform penetration into large, solid tumors. This toxin's capacity to rapidly dissolve these tumors was shown to involve both its abilities to selectively shut down protein synthesis, as revealed by autoradiography, and to induce apoptotic mode of cell death in the human target tissue, evident from its DNA fragmentation pattern. Our results dispel the established notion that targeted toxins must show pronounced accumulation at the target site to be effective antitumor agents. These findings should both encourage and guide the future development of therapeutically effective, targeted macromolecules. ■

Nilima Sarkar

One of the research projects in my laboratory concerns the mechanism of chromosome replication in mammalian cells. A key process in cellular growth is the orderly replication of all chromosomes. Perturbation of this process may be the cause of chromosomal abnormalities leading to abnormal growth as in cancer or to genetic disorders such as muscular dystrophy. My research aims to provide information on the molecular mechanisms that control the initiation of chromosome replication. We are investigating the temporal order of replication of specific mammalian genes and chromosomes using mercurated deoxy- ribonucleotide triphosphates as a tool for labeling newly synthesized DNA and its subsequent isolation. Specifically, we are mapping the replication start sites in the X-linked dystrophin gene, the largest human gene known, in order to correlate the replication pattern with the sites of the deletions that give rise to Duchenne muscular dystrophy. Other questions that we are addressing concern the factors that control the temporal order of gene regulation and their role in cellular differentiation and tumorigenesis.

Another research project in my laboratory concerns the polyadenylation of messenger RNAs (mRNA) in prokaryotes. We have found that polyadenylation of mRNA, which had long been thought to be a eukaryotic prerogative, also occurs in bacteria. Our discovery that polyadenylate sequences are also present at the termini of bacterial mRNAs provides the opportunity to subject the polyadenylation process to genetic analysis and to define its function and dissect its components by

genetic as well as biochemical means. We are elucidating the mechanism of mRNA polyadenylation by identifyng the enzymes and nucleic acid sequences that play an essential role in this process and by reconstituting theses components to yield an *in vitro* system capable of polyadenylating specific mRNAs. One major focus is the characterization of the poly(A) polymeras of *Escherichia coli*, of which there are two major isozymes, and the study of their genes, one of which has already been cloned. A second focus is the analysis of the essential sequence elements at the polyadenylation sites of specific *Escherichia coli* mRNAs by cloning and sequencing complementary DNAs and by examining the effects of site-directed mutagenesis. It is hoped that the proposed application of combined biochemical and molecular genetic approaches to the study of mRNA polyadenylation in *E. coli* will provide insight into the biological function of this process, which has thus far resisted elucidation in more complex organisms. ■

Walter Stafford

All living processes are mediated by interactions between different proteins or between proteins and other molecules or ions. For example, the expression of genes is controlled by binding of regulatory proteins called transcription factors to DNA. The immune response is mediated by the interaction of the immunoglobin (antibody) protein with an antigen molecule. Many metabolic processes are regulated by the binding of small molecules to enzymes. Muscle contraction is mediated by interaction of calcium ions with regulatory proteins that in turn interact with the contractile proteins to produce contractile work. Understanding the nature of these interactions is central to understanding the chemical and physical processes that comprise life. My main interests are in the application of physical methods to study protein-protein and protein-nucleic acid interactions that are relevant to the physiological function of normal and diseased states. In the last few years I have been involved in a project called The National Cooperative Drug Discovery Group funded by the National Cancer Institute, in which we are engineering single chain antibodies to breast cancer cells. These antibody molecules are being used as vehicles for the targeted delivery of therapeutic agents to kill breast cancer cells. The engineered antibody proteins are produced by using techniques of molecular biology to produce antibody molecules with the desired reactivity and properties. A very important step in designing protein molecules is the assessment of their properties in solution and their strength of binding to unique molecules found on the surface of breast cancer cells. The physical methods of analytical ultracentrifugation are being applied to the analysis of the strength of binding of the antibody molecules after changes are engineered into them. New highly sensitive analytical techniques for the analytical ultracentrifuge have been developed by me at BBRI and are being applied to these problems. ■

Terence Tao

The long term goal of my research is to understand how the contraction of mammalian muscles is regulated. There are three major types of muscles in the human body: cardiac, skeletal and

smooth. The contraction of all three muscle types is regulated by calcium ions. In cardiac and skeletal muscles, the calcium ions bind to a complex called troponin, which then transmits the regulatory signal throughout the entire muscle fiber. My research utilizes recombinant DNA techniques to produce mutants of the three troponin subunits, troponin C, I and T. These mutants contain unique sites onto which photocrosslinking or spectroscopic probes can be attached. After reconstitution of the functionally intact troponin complex, I can then study how the interactions of one subunit with the other troponin subunits or with other muscle proteins depend on calcium. In this manner I hope to reconstruct the events that occur subsequent to calcium binding and prior to initiation of muscle contraction.

Recently a protein with some similarity to troponin has been found in smooth muscles. Called calponin, this protein has been hypothesized to have a regulatory function in smooth muscle similar to that of troponin in cardiac and skeletal muscle. My laboratory has been intensely involved in examining the validity of this and other hypotheses. Although no firm conclusion can be drawn yet, early findings suggest that calponin may play a role in regulation of proliferation as well as contraction of smooth muscle cells. ■

Vladimir Volloch

The research in our laboratory is focused on the newly discovered process of RNA-dependent RNA synthesis in animal cells. Elucidation of this process, which involves antisense RNA, will contribute to the fundamental understanding of RNA processing in animal cells. It might also lead to the understanding of the molecular basis of certain diseases, since abnormalities of RNA-dependent RNA synthesis may result in cellular defects such as overproduction of a protein in its C-terminal fragment. Initially, the occurrence of RNA-dependent RNA-synthesis was suggested by the kinetics of incorporation of a radioactive label into globin mRNA in erythroid cells and by the detection of antisense globin RNA, a putative intermediate in RNA-dependent RNA synthesis. Subsequently we demonstrated that globin RNA is actually synthesized in enucleated erythroid cells. Recently we concentrated our efforts on defining the termini of the antisense globin RNA. To this end we developed the technique of ligation-mediated RNA amplification. In this approach the ligation of a defined ribooligonucleotide with cellular RNA unambiguously preserves the identity of RNA of interest as a sense or an antisense molecule and defines its termini. The development of this procedure made possible the detailed structural analysis of the antisense globin RNA in normal erythroid cells. The results of this study suggested a mechanism in which antisense RNA acts as an intermediate in the RNA-dependent RNA synthesis of new mRNA molecules. This process may be of great importance in terminally differentiated cells which lack a functional nucleus and hence a source of DNA-dependent RNA synthesis. Currently we are working on the purification of cellular RNA-dependent RNA polymerase and on the cloning of the gene encoding this enzyme. We are also evaluating the possible involvement of RNA-dependent RNA synthesis in the accumulation of ß-amyloid protein in Alzheimer's disease. ■

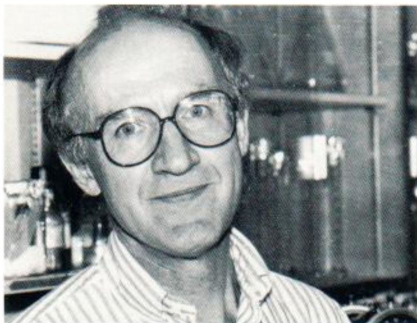
Albert Wang

The generally accepted mechanism of smooth muscle regulation involves phosphorylation at one of the myosin light chains by calmodulin-activated smooth muscle myosin light chain kinase. It has become increasingly evident, however, that the action of

crossbridges in smooth muscle is under the influence of additional regulatory mechanisms which might be associated with thin filament proteins. Caldesmon, a major actin-binding protein found in smooth muscle cells, is a good candidate for this suggested regulation, because when binding to actin it inhibits the acto-myosin ATPase activity, such inhibition being reversible by Ca^{2+}/ calmodulin. We are interested in the structure-functional relationships of caldesmon. We have set out to characterize the interactions between caldesmon and other smooth muscle protein components, as well as the mechanistic details of the inhibition of acto-myosin interactions by caldesmon and the reversal of such an inhibition by calmodulin. Our overall goal is to elucidate the functional role of caldesmon in the thin filament-based regulation of the smooth muscle system. The functional properties of various regions of caldesmon are studied by using genetically engineered fragments and synthetic peptides. Techniques involved include fluorescence spectroscopy, photo-crosslinking, and resonance energy transfer, in conjunction with electron microscopy, analytical ultracentrifugation and circular dichroism measurements. Similar biophysical approaches are used to characterize the intact caldesmon by specific modifications with various probes. In order to visualize the physical localization of caldesmon in the cell with respect to other smooth muscle proteins, molecular imaging is undertaken by immuno-fluorescence and immuno-electron microscopy with the aid of specific antibodies. Finally, the *in vivo* function of caldesmon in smooth muscle cells is probed by using fragments or peptides that prevent caldesmon from binding to the thin filaments. Monoclonal antibodies raised against key epitopes are also added into the cell to test the postulated functions of the molecule. These studies will enable us to assess the physiological role of caldesmon, and to better understand the regulatory process of smooth muscle contraction. Such an understanding may eventually lead to the design of new therapeutic drugs for smooth muscle-related diseases. ■

Brenda Williams

The focus of my research is to identify the signals that control the development of cortical precursor cells. I have used a special type of virus, called a retroviral vector, to mark individual precursor cells and follow their development in tissue culture, where it is possible to manipulate the environment to look for signals that influence their fate. Using this technique, I have identified three different precursor cell types in the developing rat cerebral cortex. (l) Neuroepithelial (NE) cells: these cells normally divide and generate more NE cells but can spontaneously generate small numbers of oligodendrocytes, astrocytes and neurons. (2) Precursor cells that generate two different cell types, either both neurons and oligodendrocytes or both neurons and astrocytes. (3) Precursor cells that generate only a single cell type, either neurons, astrocytes or oligodendrocytes. I have observed that development of these different precursor cell types depends on the type of cell-cell interactions that occur in the culture dish. I am now using this tissue culture system as an assay system to identify the signals involved in these interactions. At present my research focuses on two types of cell-cell interactions that influence precursor cell development: the interactions among NE cells (attained by growing these cells at high density) that promotes their own survival, and the interaction between astrocytes and neuronal precursor cells (neuroblasts) that inhibits the division of neuroblasts. My aim is to identify the survival factor(s) for NE cells and the astrocyte produced factor that inhibits neuroblast division. My tissue culture studies have also shown that basic fibroblast growth factor (bFGF) can promote the differentiation of NE cells into oligodendrocytes. Research is underway to investigate whether other known peptide growth factors can promote the differentiation of NE cells. ■



Hartmut Wohlrab

Inorganic phosphate is one of those anions required for the proper functioning of all cells in the body. More specificaly the cell uses it (l) to label metabolic intermediates that are required to extract energy from food (e.g. glucose) in a well controlled stepwise process; (2) as part of the cellular energy currency (adenosine triphosphate or ATP); (3) as an essential element in the intracellular signaling pathways of hormones, cellular growth factors, factors that command cells to die (apoptosis), and in cellular pathways that lead to cancer and other human diseases. Phosphate is also present in the extracellular fluids, e.g. blood. The concentrations of phosphate in the extracellular fluids and in the various compartments within a cell are exquisitely regulated. We have identified a protein that functions as a phosphate transport protein (PTP). It catalyzes the transmembrane transport of inorganic phosphate across an intracellular membrane (mitochondria). This transport is regulated by protons. Amino acid side chains within the PTP specify a gate which is highly specific for inorganic phosphate and does not allow passage of sulfate or tungstate, two anions very much like inorganic phosphate. The presence of a proton must move one or more of these amino acid side chains by less than one angstrom (about 10^{-10} yards) to permit a phosphate ion to pass through the membrane. We expect that with the use of bioengineering techniques as well as various instruments and methodologies required for protein structure and function studies, it will be posssible to identify the amino acid side chains that are primarily responsible for transmitting this signal and are located at the phosphate specific gate. Two other intrinsic membrane proteins that utilize the sodium ion as a signal for phosphate to pass through the cell membrane have more recently been identified by investigators at Eli Lilly (Indianapolis) in brain cells and at the ETH (Zurich) in kidney cells. Much exciting research thus lies ahead in elucidating this intramembrane signal pathway. ■

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Many thanks for your encouragement and support!

We are immensely grateful to all of you - individuals, foundations, and businesses - who in the year that ended on August 31, 1994, provided support totalling over $242,000 to help BBRI meet the costs of seed money for newly-recruited investigators who have yet to win major competitive grants, bridge support for any established investigators finding themselves between grants, and equipment to be shared by scientists working under different grants.

Ernie Henderson, BBRI's Treasurer, has again led the way with his extraordinary generosity. Businesses, too, have provided invaluable help.

State-of-the-art equipment for shared use was generously donated by The Millipore Foundation, and Rainin Instrument Company made an important contribution to our equipment needs. New England Biolabs provided fellowship support for a Ph.D. student. And more than a hundred other friends whose names appear below participated in our research programs through donations of cash or shares or stock.

Thank you for being on our team!

Edgar G. Davis
President

Foundations and Businesses which generously gave us their support and encouragement in fiscal 1994:

Analytical Biotechnology Services
The Boston Foundation/Leith Family Fund
The Roberta M. Childs Charitable Foundation
Combined Jewish Philanthropies/ Sumner and Carol Kaufman Fund
Connor Foundation
The Fuller Foundation
Hancock Venture Partners Inc.
Henderson Foundation
Hurdle Hill Foundation
Kenwood Foundation
June Rockwell Levy Foundation
The Evander Lewis Family Foundation
The Millipore Foundation
New England Biolabs
The Palmer Organization
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The Harold Whitworth Pierce Charitable Trust
Rainin Instrument Company
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Contributions to BBRI were given in memory of

Jack Seidel
by Mrs. Julius C. Ritter

Elizabeth Maclean Slayter
by Hugh Maclean

Matching gifts were provided by
Hancock Venture Partners, Inc.
The Millipore Foundation

Anne B. Stone, Corporator, and James R. Nichols, guest at BBRI's Evening of Discovery in May.

Charleen L. Johnson (center), Executive Director of The Millipore Foundation, with BBRI Director-designate Kathleen Morgan and Albert Wang. Earlier this year The Millipore Foundation donated the Millipore ConSep instrumentation seen in part on the bench between Ms. Johnson and Dr. Wang.

"It's very satisfying to see our equipment being used for research to improve the quality of life of our people. The employees at BBRI are totally committed to humankind. It's a pleasure to know and to work with them."

BOSTON BIOMEDICAL RESEARCH INSTITUTE, INC.

BALANCE SHEETS

AUGUST 31, 1994 AND 1993

	1994	1993
ASSETS		
CURRENT ASSETS		
Cash	$702,641	$209,681
Grants receivable	5,376,653	5,553,563
Pledges receivable	7,075	
Prepayments, deposits and other receivables	76,287	111,113
Investments, at market value (cost 1994 - $6,433,292 1993 - $5,452,155)	6,411,968	6,301,542
Total current assets	12,574,624	12,175,899
FIXED ASSETS		
Leasehold improvements	1,757,902	1,935,632
Research equipment	1,951,844	5,095,645
Total	3,709,746	7,031,277
Less accumulated depreciation	2,477,081	5,718,387
Net fixed assets	1,232,665	1,312,890
	$13,807,289	$13,488,789
LIABILITIES AND FUND BALANCES		
CURRENT LIABILITIES		
Accounts payable and accrued expenses	114,414	25,230
Deferred grant income	5,663,698	5,900,306
Deferred fund (building)		115,702
Total current liabilities	5,778,112	6,041,238
FUND BALANCES		
Unrestricted	6,101,236	5,365,124
Restricted	695,276	769,537
Fixed assets	1,232,665	1,312,890
Total fund balances	8,029,177	7,447,551
	$13,807,289	$13,488,789

BOSTON BIOMEDICAL RESEARCH INSTITUTE, INC.
STATEMENTS OF REVENUES, EXPENSES AND CHANGES IN FUND BALANCES
FOR THE YEARS ENDED AUGUST 31, 1994 AND 1993

	1994	1993
REVENUE		
Grants and contracts	$6,956,725	$5,871,529
Unrestricted contributions	160,674	126,293
Restricted contributions availed of in current period	130,499	121,402
Property and equipment purchased	108,108	202,147
Investment income		
Interest and dividends	123,900	175,731
Realized gains (losses) on securities	(147,626)	152,022
Unrealized gains (losses) on securities	(20,524)	4,252
Total	7,311,756	6,653,376
EXPENSES		
Salaries and benefits	4,607,890	4,025,907
Occupancy costs	600,000	760,000
General support and services	1,196,220	1,296,963
Fixed assets purchased	108,108	202,147
Development	75,532	69,280
Depreciation	188,333	203,742
Total	6,776,083	6,558,039
EXCESS OF REVENUES OVER EXPENSES	535,673	95,337
Restricted contributions	60,750	182,978
Restricted contributions availed of in current period	(130,499)	(121,402)
Transfer from deferred fund (building)	115,702	
FUND BALANCES, BEGINNING OF YEAR	7,447,551	7,290,638
FUND BALANCES, END OF YEAR	$8,029,177	$7,447,551

Copies of our complete, audited financial statements, certified by the independent accounting firm of John Vecchi, CPA, are available upon request from the Controller, Boston Biomedical Research Institute.

GRANTS, CONTRACTS AND FELLOWSHIPS

RESEARCH GRANTS

NATIONAL INSTITUTES OF HEALTH

Dr. Badwey	Synergistic stimulation and priming of neutrophils	7/90 - 6/95	$ 960,000	
Dr. Badwey	Enzymes modulating second messengers in neutrophils	4/93 - 3/97	645,000	
Dr. Coleman	ATP binding site photoaffinity probes for F_1-ATPase	6/92 - 5/96	748,000	
Dr. Gergely (MERIT)	Biochemistry of muscle contraction	7/89 - 6/95	2,844,000	
Dr. Grabarek	Calcium binding protein/target interactions	6/92 - 5/95	600,000	
Dr. Graceffa	Smooth muscle and non-muscle caldesmon	5/93 - 4/97	728,000	
Dr. Ikemoto	Structure and function of sarcoplasmic reticulum	7/92 - 6/96	1,674,000	
Dr. Joshi	Molecular mechanisms of mitochondrial ATP synthesis	9/92 - 8/95	802,000	
Dr. Lehrer	Tropomyosin and myosin interaction in muscle	12/90 - 11/95	1,546,000	
Dr. Lu	Structure-function relation in myosin	9/91 - 8/95	819,000	
Dr. Pande	Protein glycation: structure and stability of products	7/91 - 6/95	548,000	
Dr. Paulus	Control of diaminopimelate and lysine biosynthesis	4/93 - 3/97	1,160,000	
Dr. Prevelige	Subunit interactions during icosahedral capsid assembly	8/94 - 7/98	744,000	*
Dr. Raso	Targeting toxins with acid-triggered hybrid antibodies	12/89 - 11/94	1,234,000	
Dr. Raso	Model to test the therapeutic value of toxin conjugates	9/92 - 8/95	769,000	
Dr. Sarkar	Function of polyadenylate sequences in bacterial RNA	12/93 - 11/97	1,155,000	*
Dr. Stafford	Engineered anti-breast cancer single-chain Fv immunotoxin	6/90 - 5/95	646,000	
Dr. Tao (MERIT)	Proximity relationship among muscle proteins	4/91 - 3/96	1,359,000	
Dr. Volloch	Antisense intron as modulator of gene expression	12/88 - 11/94	1,289,000	
Dr. Wang (Pro. Proj.)	Molecular mechanism of smooth muscle regulation	9/92 - 8/97	6,000,000	
Dr. Wang	Comparative study of troponin C and calmodulin	7/88 - 2/95	631,000	
Dr. Wang	Caldesmon and transmembrane signaling	9/93 - 9/96	60,000	*
Dr. Wohlrab	Proton-coupled inorganic phosphate transport	4/92 - 3/96	1,181,000	

AMERICAN HEART ASSOCIATION

Dr. Joshi	Role of OSCP in mitochondrial energy coupling	7/91 - 6/95	132,000	
Dr. Szymanski	Interaction between calponin and smooth muscle myosin	7/94 - 6/96	59,000	*
Dr. Tao	Structure and function of genetically engineered calponin	7/92 - 6/95	132,000	

MUSCULAR DYSTROPHY ASSOCIATION

Dr. Ikemoto	Excitation-contraction coupling in malignant hyperthermia	7/94 - 6/97	130,000	*

NATIONAL MULTIPLE SCLEROSIS SOCIETY

Dr. Williams	Survival and differentiation of embryonic oligodendrocyte precursor cells	11/93 - 10/94	20,000	*

CONTRACT

Dr. Codington	Carcinoma assay research project (Epigen Inc.)	3/93 - 2/94	424,000

FELLOWSHIPS

Dr. Ao	American Heart Association, Mass. Affiliate	7/94 - 6/96	52,000
Dr. Banfalvi	Fulbright International Exchange for Scholars	6/94 - 10/94	16,000
Dr. Kalapos	Fogarty International Exchange for Scholars	8/92 - 7/94	71,000
Dr. Roten	Fogarty International Exchange for Scholars	9/92 - 8/94	53,000
Mr. Shao	New England Biolabs	9/92 - 8/95	60,000

* New grants and contracts awarded in Fiscal 1994

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