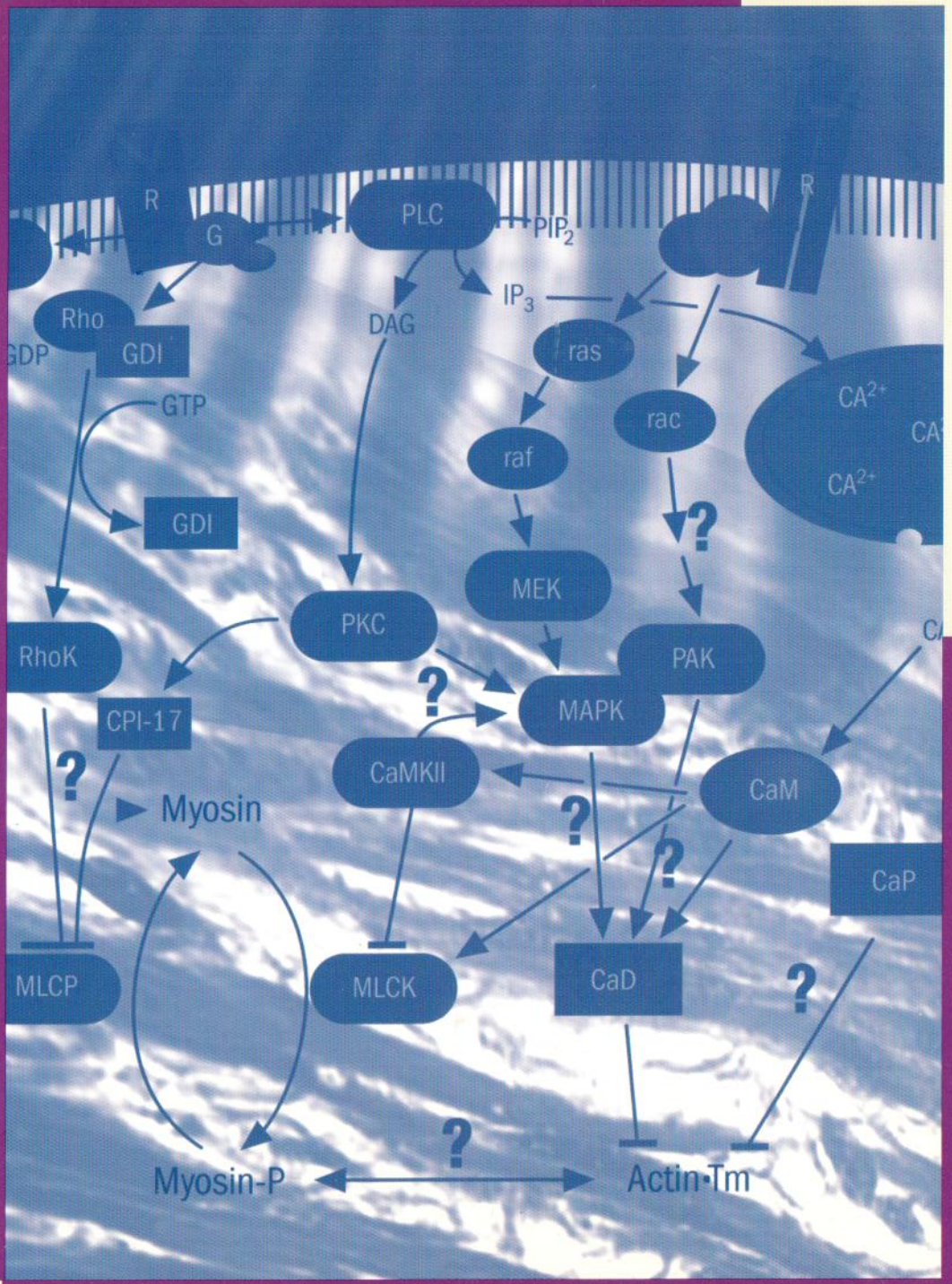
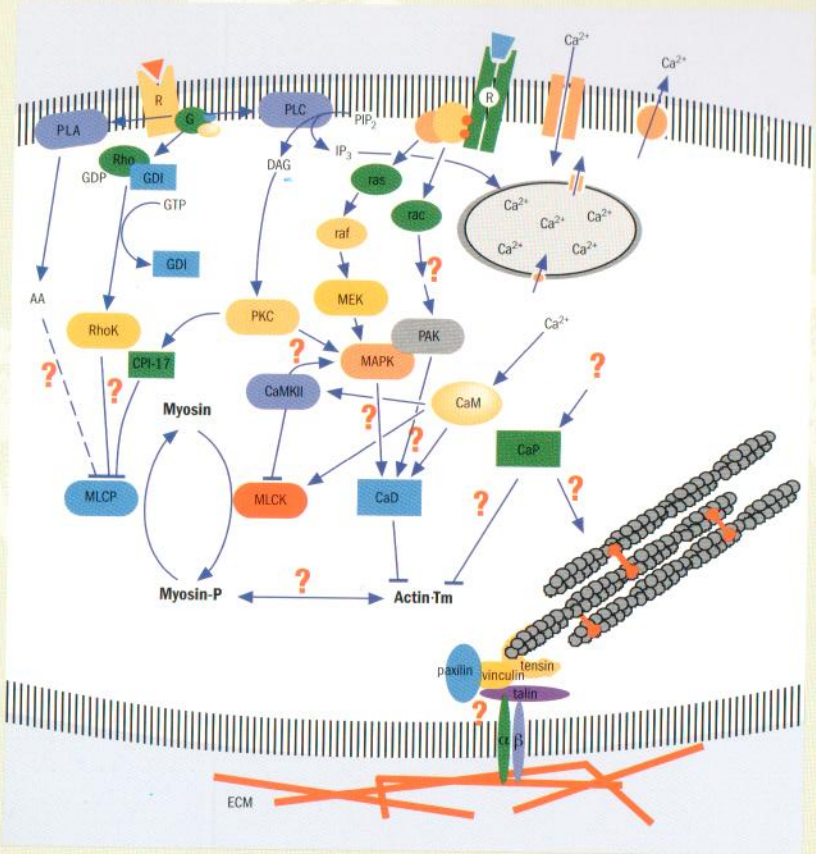


03 annual report

BOSTON BIOMEDICAL
RESEARCH INSTITUTE

on the cover



The regulation of smooth muscle contraction, as illustrated in the figure above, is a very complex process involving many interacting elements.

BBRI's effort to understand how this process works is the subject of the 2003 Annual Report. In these pages, you will learn about the Program Project Grant, a collaborative research effort being carried out by nine BBRI faculty members who are approaching this question from the angle of their respective research expertise. A better understanding of smooth muscle contraction has great relevance to numerous diseases including hypertension, stroke, asthma and premature labor.

our **mission**

The Boston Biomedical Research Institute (BBRI) is dedicated to basic biomedical research to promote the understanding, treatment and prevention of specific human diseases. The areas of investigation concern the structure and function of muscle proteins, mechanisms of cell communication, and the control of cell growth and gene function. A major focus is muscle cell biology, which has implications for muscle-related diseases such as asthma, stroke, and heart failure. When appropriate, the Institute collaborates in clinical studies of patients to apply the results of basic research to problems of human health and the cure of disease. BBRI is an independent, not-for-profit institution.

BOSTON BIOMEDICAL RESEARCH INSTITUTE

message from the president

John R. Layton

You helped to enable BBRI scientists to **gain new knowledge** and to make the **scientific advances** that will lead to a better understanding of how to prevent human disease.

BBRI had another year of outstanding accomplishments and success, due in large measure to the spirit of collaboration and partnership which is a hallmark of our Institute.

In June, 2003 we successfully completed the search for a new Director of BBRI. Prior to joining us in September, Dr. Charles P. Emerson, Jr. was the Professor and Chair of the Department of Cell & Developmental Biology at the University of Pennsylvania School of Medicine and Director of the Penn Center for Developmental Biology. One of the reasons that Charlie was eager to lead BBRI was that his area of scientific expertise meshes very nicely with the research that is currently ongoing at the Institute. Certainly, the quality of the faculty was also a key enticement for him. Another very important factor was the sense of community and the commitment that each of us—Faculty, Trustee, Corporator and Friend alike—has to our Institute. Having someone with Charlie's scientific achievements, broad scientific knowledge and administrative experience will be immensely important in ensuring the Institute's continued success going forward. We are confident that we are placing BBRI's scientific future in very capable hands.

We have been very fortunate to have Henry Paulus as the Interim Director of BBRI. We extend our deep gratitude to him for leading the Institute and working so effectively with all constituencies within BBRI. We wish him well with his ongoing research at BBRI.

This Annual Report focuses on a Program Project Grant (PPG) at BBRI, which is led by Albert Wang and is a wonderful example of collaborative science at its best. Conceptually, PPG's are designed to foster inter-disciplinary scientific research on a particular subject in order to gain efficiency and effectiveness. Under Dr. Wang's leadership, the PPG group at BBRI consists of nine Principal Investigators at BBRI who are supported by twelve assistants and associates. Additionally, the PPG has collaborations with two other Faculty members at BBRI and a research group in the UK. The result of this broadly inter-disciplinary effort is that important knowledge is being gained about the regulatory mechanisms in smooth muscle that control vital functions in our body such as blood pressure, breathing, digestion and child birth.

The year also saw the successful conclusion of *A Campaign for BBRI: Intellectual Partnerships for the Future of Science*. As you all know, there is only one way that an Institute like ours could raise $6.5 million in three years—through the collaborative efforts of every member of BBRI's community! You can read much more about the Campaign in the Development Report. On behalf of the Trustees, Faculty and Staff, I want to say a very deep and heartfelt thank you to each and every donor whose generosity made this Campaign such a success. We see the success of *A Campaign for BBRI: Intellectual Partners for the Future of Science* as the foundation on which to build an even stronger Institute going forward.

As part of our commitment to encourage young people to embark on careers in scientific research, BBRI has continued to build on its special partnership with the staff and pupils at Watertown High School. Dr. Jim Cavanaugh, the Principal of Watertown High School made the following remarks at Watertown High School's 2003 Awards Night: "Boston Biomedical Research Institute is a fine example of an organization that supports science education in Watertown through its partnership with the High School. They do this through a number of programs, including the two Scholarships they will be presenting this evening and through internships for students and teachers. We are very appreciative of this partnership and for the role that BBRI plays in the Watertown community." The recipients of this year's BBRI Scholarships were Rebecca Chase who is studying biology at the University of Southern California and Mark Steadman who is attending Cornell University to study biophysics.

In a very real sense, you helped to enable BBRI scientists to gain new knowledge and to make the scientific advances that will lead to a better understanding of how to prevent human disease. BBRI has an exceptionally bright future, especially with the arrival of Charles Emerson and given the spirit of collaboration and the sense of community that makes BBRI such a jewel. I encourage you to visit BBRI as often as possible, so you can share in the excitement of the scientific discovery that is happening on a daily basis.

message from

Henry Paulus, Interim Director 2001-2003

The past year has been a **very successful one** not only for our **new scientists** but also for our established faculty members and saw the **award of thirteen new grants** or competing grant renewals.

In the past year, BBRI continued to reap the benefits that accrued from our move to Watertown, which was the result of the long-term vision of Dr. Kathleen Morgan as BBRI's Director and the energetic support of our Board of Trustees. In the course of 2003, all five faculty members who were recruited after the move to our new facility succeeded in obtaining major NIH funding. This speaks not only to the quality of scientists that BBRI can attract, but also to the wisdom of investing in new scientists—the investment of $2 million in start-up funds for these scientists has yielded $15 million in NIH grants, a number which will certainly grow.

The past year has been a very successful one not only for our new scientists but also for our established faculty members and saw the award of thirteen new grants or competing grant renewals. Among the grant renewals, the second renewal of BBRI's Program Project Grant on smooth muscle, which extends this extremely productive research program to a 15-year period, deserves special mention, because it involves the close collaboration of nine BBRI faculty members. Under Albert Wang's leadership, the smooth muscle research program at BBRI has been exceptionally productive for more than a decade. Indeed, the peer review panels that evaluated the application for renewal gave it nearly the highest possible rating, not only with respect to the excellence of the science but in consideration of the high level of teamwork that drives this project forward. The theme of this Annual Report, "The whole is more than the sum of its parts," is taken from the grant reviewers' comments. In the pages that follow, Albert Wang and his colleagues describe the interdisciplinary team approach that has made our Institute a world leader in smooth muscle research.

BBRI has now nearly approached its capacity in terms of the number of faculty members, and the challenge will be to provide the means for each research group to grow by diversifying its research programs rather than relying on a single NIH grant. An important step in this direction was made in the past year through BBRI's successful completion of the challenge grant to endow a Pilot Fund for innovative research ideas. This fund generates an award which is given competitively every year to a BBRI scientist for pursuing a novel line of research to the point that it can qualify for NIH funding. The completion of the Pilot Fund challenge was the culmination of *A Campaign for BBRI: Intellectual Partners for the Future of Science*, which was launched in 2000 during Kathleen Morgan's tenure as Director and under the leadership of Allie Blodgett and Jake Layton as Co-Chairs of the Campaign Committee. The campaign, which was enthusiastically supported by members of the BBRI family and friends, raised $6.5 million for the expansion of the Institute's research and educational programs.

For me, one of the great pleasures of serving as Interim Director has been the privilege of working with an enthusiastic group of individuals—Trustees, Corporators, Friends, Faculty and Staff—who understand the importance of basic biomedical research, appreciate that BBRI offers a unique research environment, and are partners in making research at BBRI flourish. The only pleasure which can exceed this is to hand over the directorship to Dr. Charles Emerson with the complete confidence that he has the scientific vision and leadership to make BBRI an even greater institution than it is now. For this, I thank you all, both personally and on behalf of the Institute.

introductory remarks from BBRI's new director

Charles P. Emerson, Jr., Ph.D.

BBRI's research facility provides an **exceptional environment** for doing **first class collaborative** and **individual research.**

I am honored to be the new Director of the Boston Biomedical Research Institute and excited by the opportunities for the Institute to further excel in its research mission and for my research program to flourish. I am coming to BBRI with a diversity of research, educational and academic administrative experiences at research universities and research institutes. With this experience, I look forward to the stimulating scientific and administrative challenges that lie ahead of us at BBRI. I look forward to contributing to the success of BBRI as a leading research institute.

During my recruitment this spring, I came to the conclusion that BBRI is an excellent research and administrative fit for me. Research wise, my career has focused on investigations of skeletal muscle, specifically on muscle proteins and particularly on the discovery of regulatory genes and signals that control the formation of muscle stem cells and differentiated muscles in embryos. BBRI has a unique institutional research signature in areas of cardiovascular and skeletal muscle biology, which has been a great attraction for me to join BBRI. With its early research focus in connective tissue and muscle biochemistry, begun under the leadership of Endre Balazs and John Gergely, and the more recent focus in structural biology and cellular signaling, under the leadership of Kathleen Morgan and Henry Paulus research at BBRI clearly complements my own interests and expertise in developmental biology, gene discovery and muscle stem cell biology.

Another strength of BBRI is the excellent research Faculty, at all career levels, and their strong commitment to the pursuit of innovative research. Research institutes such as BBRI provide research Faculty unique opportunities to pursue individual and collaborative research without many of the academic constraints, institutional pressures and distractions common at large research universities. BBRI has a well established tradition of collaborative research, which I see as providing unique opportunities for research productivity and innovation. This research collaboration is well demonstrated by Albert Wang and the members of the Program Project Grant, which continues to be an amazingly successful model for the development of additional collaborative research activities at BBRI in the coming years. I look forward to working with my faculty colleagues to foster new collaborative research initiatives as well as to supporting individual research projects in their groundbreaking stages. BBRI Faculty are also strongly committed to participating in the governance and activities of the Institute, and this strong involvement reflects for me the commitment and support of the faculty to not only maintain BBRI's special qualities, but to work together to enhance these qualities in order to see BBRI flourish even more in the future.

It is terrific to be moving my research lab into our impressive modern research facility which ranks in quality with the best I have seen at academic research centers, including the research building where my lab previously was at Penn. BBRI's research facility provides an exceptional environment for doing first class collaborative and individual research. The building and the talented administrators who are stewarding the valuable resources of the Institute, provide another clear demonstration of the commitment of Faculty, Trustees, Corporators and Friends to the research future of BBRI. The location of the facility in Watertown also provides a unique opportunity for BBRI to have a visible and active role in the business and educational activities of this vital community.

The success of *A Campaign for BBRI: Intellectual Partners for the Future of Science* and the research resources that have been developed from these new funds have been an impressive signal to me that BBRI is proactive in defining its research future and also provides a clear demonstration of the amazing level of cooperation, good will and collegiality among the BBRI Trustees, Faculty and Friends. Your incredibly strong commitment to BBRI and your passion for its future as a leading research institute, as evidenced by the remarkable success of this campaign, was another major factor that attracted me to come to BBRI.

I am looking forward to working with each and every one of you to continue the job of building BBRI as a first rank research institute in this exciting era of biomedical science.

INTRODUCTION The Smooth Muscle Program Project Grant

Albert Wang, Ph.D., Senior Scientist and Program Director

"The whole is more than the sum of its parts." This is not only true for the PPG but it is also what BBRI is all about!

Smooth muscle is present in the wall of all hollow organs in our body, including blood vessels, the G-I tract, bladder, uterus and lungs. Through properly regulated contraction and relaxation of smooth muscle cells, the diameter of these tubular organs is controlled. Malfunctions of smooth muscle contraction lead to many common diseases such as hypertension, digestive disorders, miscarriage and asthma. It is therefore very important to learn how smooth muscle contraction is regulated. Compared to striated muscles (Figure 1), which are the muscles that move our limbs and pump our heart, smooth muscles (Figure 2) are relatively poorly understood, primarily because of their complexity and experimental difficulties. It takes more than the resources of a single laboratory to study such a complicated subject. That is why a group of investigators at BBRI teamed up to tackle the question of how smooth muscle contraction is regulated. This concerted effort formed the Program Project Grant (PPG), which is supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the NIH.

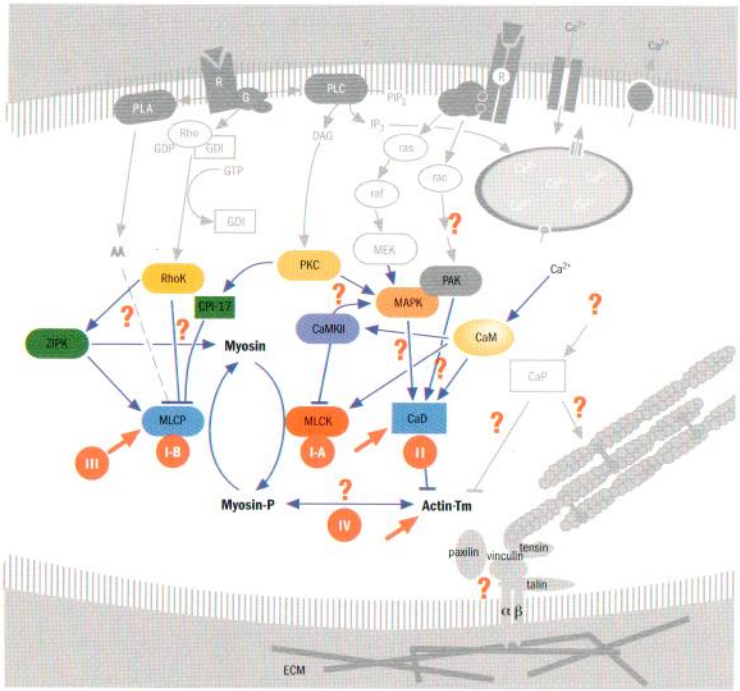


FIGURE 3. The Program Project Grant addresses some key questions in this very complicated regulatory scheme in smooth muscles.

The PPG at BBRI (entitled "Molecular Mechanism of Smooth Muscle Regulation") has been in operation since 1992, and will continue to at least 2007, with an annual budget of about $1 million. The current Program contains five projects, each focusing on one of the key aspects in the smooth muscle regulatory system (Figure 3). Dr. Zenon Grabarek is examining the process of myosin phosphorylation (Project I-A). Dr. Terence Tao is concerned with the reverse process, myosin dephosphorylation (Project I-B). Both I-A and I-B are looking at the so-called thick (or myosin) filament-based regulation. I myself have been interested in the thin (or actin) filament-based regulatory protein, caldesmon (Project II). Project III is a new project, in which Drs. Roberto Dominguez and Phil Graceffa set out to determine the atomic structures of several smooth muscle proteins. Finally, Project IV, led by Dr. Sam Lehrer, is devoted to the cooperative aspects of the integrated smooth muscle system. In addition, there are two cores, one Administrative Core, through which I deal with the day-to-day management of the PPG, and one Biophysical Facility Core (Drs. Renne Lu, Katsuhide Mabuchi and Walter Stafford), which provides technical support for all projects. Besides the nine Principal Investigators there are twelve associates and assistants working toward the PPG's aims. Dr. Mike Geeves of the University of Kent, UK, and BBRI scientists Drs. Kathleen Morgan and Toshio Kitazawa, also participate in this program as collaborators. Obviously, there are extensive interactions among all projects and cores. Not only are materials and methodologies shared and exchanged, but the cross-fertilization of ideas is in fact the greatest gain in bringing these investigators together under the program.

What follows are short summaries of all projects and the core of the PPG written by the Project Leaders. I hope you will find this information useful and interesting. As it has been said, "the whole is more than the sum of its parts." This is not only true for the PPG but it is also what BBRI is all about!

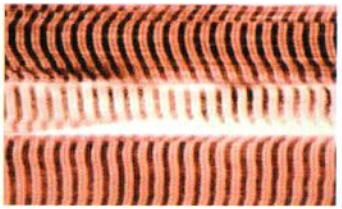
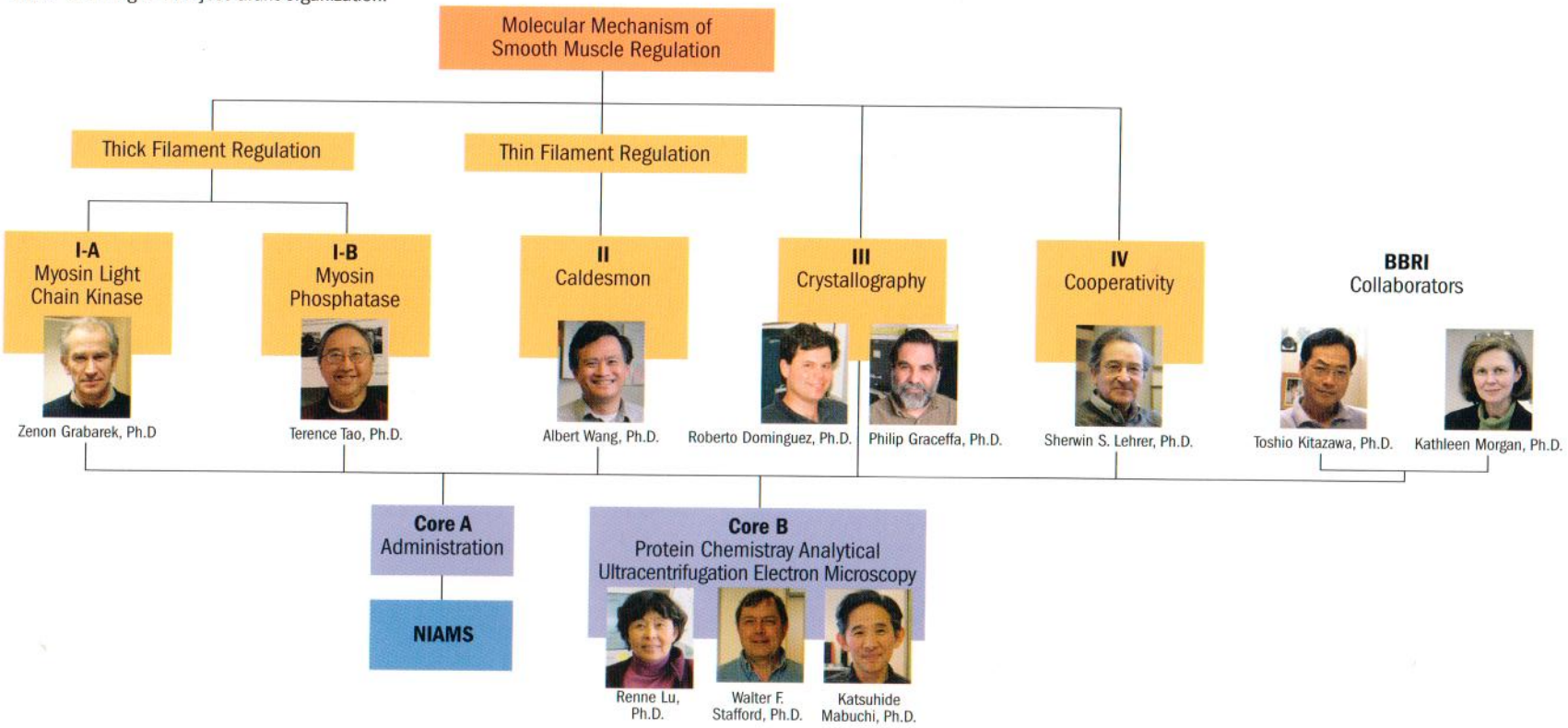


FIGURE 1. Both skeletal and cardiac muscles have striations.

FIGURE 2. Smooth muscles don't have striations.

CHART. The Program Project Grant organization.



Glossary of Terms

Actin The protein that, when *polymerized*, makes up the thin filament in muscle and non-muscle cells. Actin filaments often serve as the "track" of the motor protein *myosin*.

Angstrom A length unit for objects of atomic scale. One angstrom (Å) equals one hundred millionth of a centimeter.

Caldesmon A protein that is associated with the *actin* filament and interferes with the interaction between *actin* and *myosin*, and thereby acts as a "molecular brake."

Calmodulin The most important protein that binds calcium ions in biological systems and transfers the signal to other events.

Gene Knockout An experimental approach to study the function of a protein by disrupting its gene so that the protein is no longer made in the animal.

Kinase A protein (enzyme) that facilitates the process of *phosphorylation*.

MLCK A *kinase* that specifically targets the light chain of *myosin* and adds a phosphate group to it.

MLCP An enzyme that reverses the action of *MLCK* and removes the phosphate group from the light chain of *myosin*.

Myosin A protein that converts energy into movement (the "molecular motor.") The most abundant form of myosin *polymerizes* and forms the thick filament in muscle cells.

Phosphorylation The chemical reaction that adds a phosphate group to a protein. Since the phosphate group is highly negatively charged, this would alter the local electric property of the protein. Phosphorylation is a common mechanism used by nature to turn on or turn off certain events.

Polymerization A phenomenon of many small units being added to each other repetitively to make a larger or longer entity.

Smooth Muscle In contrast to *striated muscle*, this muscle doesn't exhibit striations under the microscope, and is therefore, smooth. Smooth muscle is present in the walls of all hollow organs in our bodies. Smooth muscle and cardiac muscle are both involuntary muscles, in that we cannot control their contraction and relaxation.

Striated Muscle Muscle that shows striations under the microscope; this includes the muscle attached to the bones (skeletal muscle) and that in the heart (cardiac muscle).

Tropomyosin A protein that is associated with the *actin* filament, and by braiding itself with it, makes the filament stronger and better connected.

PROJECT IA

Myosin Light Chain Kinase

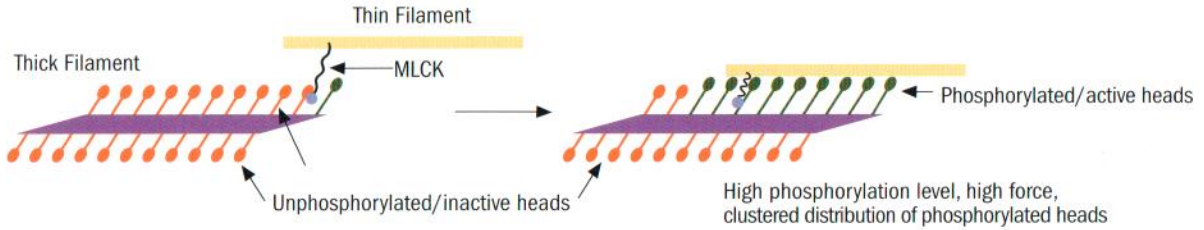
Zenon Grabarek, Ph.D., Principal Scientist and Project Leader

All **muscle machines** work by having two proteins interact with each other: myosin, which acts as a "**motor**," and actin, which acts as a "**track**." In smooth muscle, the trigger that activates the actin-myosin machinery consists of a kinase (MLCK).

When we try to describe how muscles work, we often use the word "machine." Indeed, the precise ways in which the protein components of the muscle fit together and move in harmony are reminiscent of a high precision mechanical device. The analogy works just as well when we think of switches that turn the muscle on and off. It would be counterproductive and very dangerous to turn on a machine, the muscle machine included, at a wrong time or in a wrong place. That is why organisms have developed highly sophisticated mechanisms that control muscle function.

All muscle machines work by having two proteins interact with each other: myosin, which acts as a "motor," and actin, which acts as a "track." In smooth muscle, the trigger that activates the actin-myosin machinery consists of a kinase (MLCK), which is an enzyme capable of putting a phosphate group on one of the myosin light chains. In ways that are not completely understood, the phosphate group unlocks the myosin molecule and enables its force-producing cyclic interactions with the actin thin filaments. The purpose of our studies is to understand how MLCK performs its triggering function.

MLCK is a complex molecule. The central segment of MLCK, spanning approximately 1/3 of the molecule, is sufficient to perform the phosphate transferring function. It is not clear what the functional role of the other 2/3 is, which harbors an actin-binding site at one end and a myosin-binding site at the other. We found that MLCK is a very long and flexible molecule. Compared to the myosin head, which is similar in mass, MLCK is more than 6 times longer. We believe that these unusual properties must serve some important physiological function. Our working model is that in smooth muscle MLCK molecules link the thin and thick filaments, and are thus well positioned to activate just those myosin molecules that need to work at any particular time. In Project I-A we have designed experiments to test this model.



A hypothetical mechanism that may explain how MLCK activates the myosin heads in smooth muscles.

Myosin Light Chain Phosphatase

Terence Tao, Ph.D., Senior Scientist and Project Leader

PROJECT 1B

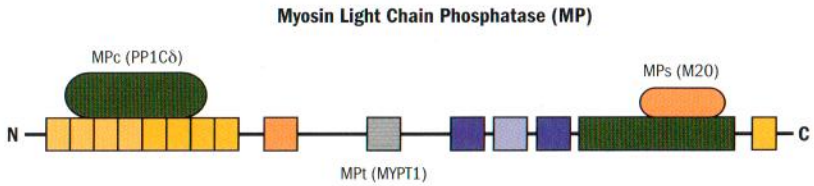
The protein myosin is often referred to as a "molecular motor" that powers the contraction of smooth muscle. In order to turn on the motor, myosin needs to be phosphorylated, a chemical process that attaches a phosphate group to the regulatory light chain component of smooth muscle myosin. To turn off the motor (thereby relaxing the muscle), the phosphate group needs be removed. My project in the PPG concentrates on the protein that mediates this latter process. This protein is called myosin light chain phosphatase, which I'll abbreviate as MLCP.

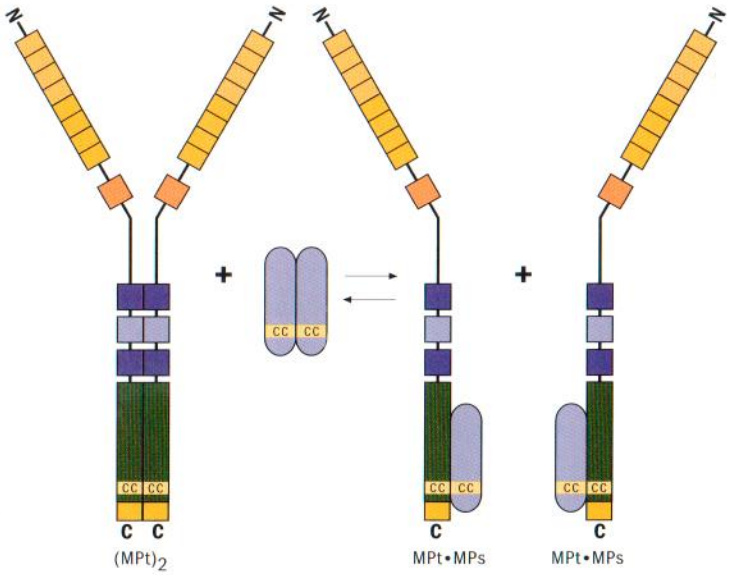
MLCP has three components (or subunits): the catalytic subunit which carries out the actual dephosphorylation, the targeting subunit which is thought to direct the catalytic subunit to myosin, and a small subunit whose function is as yet unknown. What's interesting is that MLCP is itself regulated by a variety of processes, including phosphorylation of the targeting subunit and interactions with proteins that inhibit its activity. This apparently is how certain hormones, such as adrenaline, effect changes in various bodily functions.

We began this project by looking at the shape of the targeting subunit using electron microscopy and found that it is a long flexible protein with three globular regions connected by flexible strands. We then found that the small subunit and the targeting subunit interact by coiling around each other in their terminal regions. We have determined that both the beginning and the terminal regions of the targeting subunit bind to myosin and that the catalytic subunit is required for the binding.

Still to be deciphered are how phosphorylation of the targeting subunit either diminishes or enhances the activity of MLCP, how the activity of the catalytic subunit is enhanced by the targeting subunit, how the various regulators of MLCP interact with the targeting subunit etc. In the meantime, we have become interested in some of these regulators. One of them is the protein called CPI-17, which is known to diminish the activity of MLCP. We have begun to characterize the physical properties of CPI-17 and its interactions with MLCP. We are also beginning to study the regulatory protein known as protein kinase G, which may bind to the targeting subunit at the same region as the small subunit.

To turn off the motor (**thereby relaxing the muscle**), the phosphate group needs be removed. The protein that mediates this process is called **myosin light chain phosphatase.**





Domain structure of the targeting subunit (MPt) of myosin phosphatase and its interactions with the catalytic (MPc) and small (MPs) subunits. Each colored box represents a structural motif of the linearized MPt polypeptide chain. Right: Interactions between MPt and MPs.

PROJECT II

Caldesmon: Its Role in the Regulation of Smooth Muscle Contraction

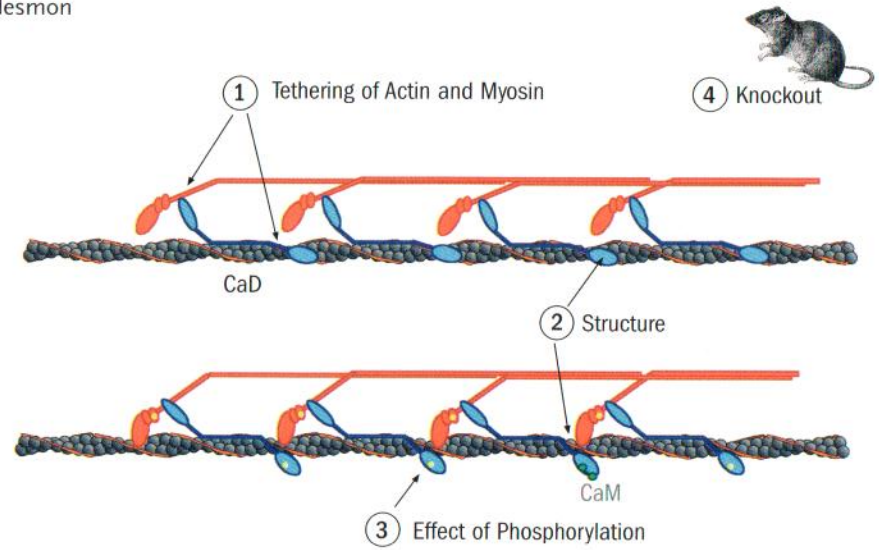
Albert Wang, Ph.D., Senior Scientist and Program Director

If myosin is a "**molecular motor**," caldesmon can be seen as a "**molecular brake**." This actin-based inhibition is distinctly different from the one based on myosin phosphorylation.

Caldesmon is a major actin-binding protein found in smooth muscle cells. It exerts an inhibitory effect on the actin-myosin interaction and, therefore, on smooth muscle contraction. If myosin is a "molecular motor," caldesmon can be seen as a "molecular brake." This actin-based inhibition is distinctly different from the one based on myosin phosphorylation. Recent studies indicate that, by binding to myosin and actin simultaneously through its two ends, caldesmon can also "tether" the thick filament to the thin filament and stabilize the network. It remains unclear how these actions are regulated. Our long-term goal is to understand the thin filament-based regulation of smooth muscle contraction. In this project, efforts are focused on defining the regulatory mechanism of smooth muscle caldesmon and to test the hypothesis that caldesmon is necessary for smooth muscle to function properly.

First, we study the binding of caldesmon to actin and myosin to test whether caldesmon is able to keep myosin filaments close to actin without preventing them from sliding against each other during contraction. Secondly, since structural information on caldesmon is lacking, we are mapping the topographical relationship between landmarks on this molecule. Thirdly, to test the hypothesis that caldesmon itself can be regulated by phosphorylation, we will determine both the level and the sites of modification. Finally, we will disrupt the production of caldesmon in the mouse by genetic means (gene knockout) to test whether removal of caldesmon will affect the animal's viability or its smooth muscle contractility.

These interdisciplinary studies use a wide range of biophysical and molecular biology techniques. Information so generated allows us to assess the functional role of caldesmon in smooth muscle contraction and will eventually afford proper diagnosis and future development of therapeutic measures for smooth muscle related diseases.



A model illustrating our plans to study the functions of caldesmon (the blue molecule). At resting state (upper) caldesmon acts as a brake and maintains the filamentous structure. When the muscle is stimulated (lower), the brake is released to allow filaments to slide.

X-ray Crystallographic Investigation of Smooth Muscle Regulation

PROJECT III

Roberto Dominguez, Ph.D., Principal Scientist and Project Leader

Two parallel mechanisms regulate the contraction of smooth muscle cells: the phosphorylation/dephosphorylation of myosin by myosin light chain kinase and myosin phosphatase, and a secondary mechanism involving caldesmon, a protein that tethers the thin actin filaments to the thick myosin filaments. Project III aims to investigate the three-dimensional high-resolution structures of some of the key proteins and complexes of proteins involved in smooth muscle regulation.

To understand the primary regulatory mechanism of smooth muscle contraction, we are investigating the structure of myosin phosphatase in collaboration with Dr. Terry Tao. Myosin phosphatase consists of three protein subunits: the catalytic, targeting, and small subunits. Binding of the targeting subunit to the catalytic subunit changes the properties of the phosphatase, conferring a higher affinity and an increased reactivity toward smooth muscle myosin. There is an enormous interest in knowing the structure of this complex, both because of its role in smooth muscle regulation and as a paradigm of the general concept of phosphatase targeting. We were able to crystallize and determine the structure of a complex between the catalytic and an N-terminal fragment of the targeting subunit to 4.0 Å resolution (Figure A). At this resolution, important details of the phosphatase function can not be fully understood. We are currently working on obtaining a higher resolution structure of this complex.

To understand the second path of smooth muscle regulation based on actin-caldesmon interactions, we are studying the structure of smooth muscle actin and its ATP-dependent conformational transitions in collaboration with Dr. Phil Graceffa. We also use crystals of a non-polymerizable form of actin, obtained after coupling actin to the fluorescence probe TMR, as a scaffold for the crystallization of actin-binding fragments of caldesmon (in collaboration with Dr. Albert Wang). We have made significant progress in getting the high-resolution crystal structures of TMR-modified actin in two different states (Figure B), which reveal a nucleotide-dependent conformational transition in actin. Currently, we are focusing our efforts on the study of smooth muscle actin and an actin-caldesmon complex.

Project III aims to investigate the **three-dimensional** high-resolution structures of some of the **key proteins** and complexes of proteins involved in **smooth muscle regulation.**

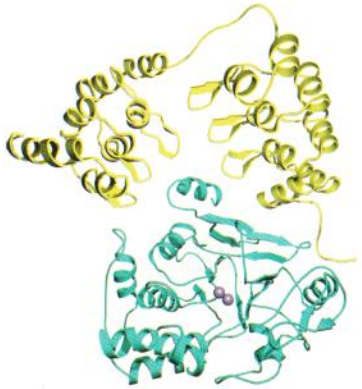


FIGURE A. Ribbon representation of the structures of a complex of the myosin phosphatase catalytic and targeting subunits.

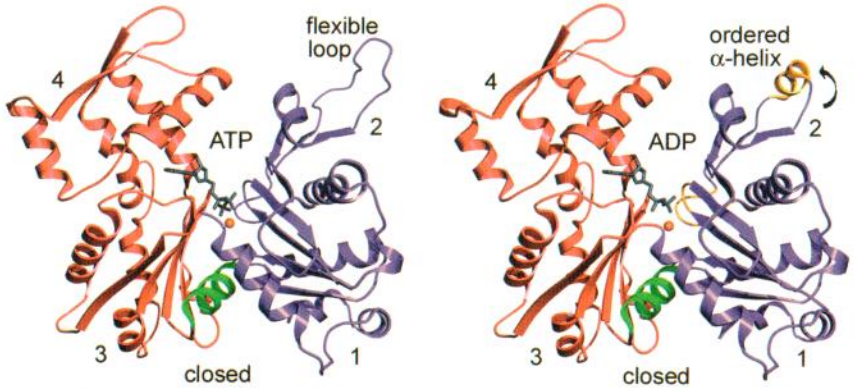


FIGURE B. Monomeric TMR-actin in the ATP and ADP states.

PROJECT IV

Cooperative Effects in Smooth Muscle Regulation

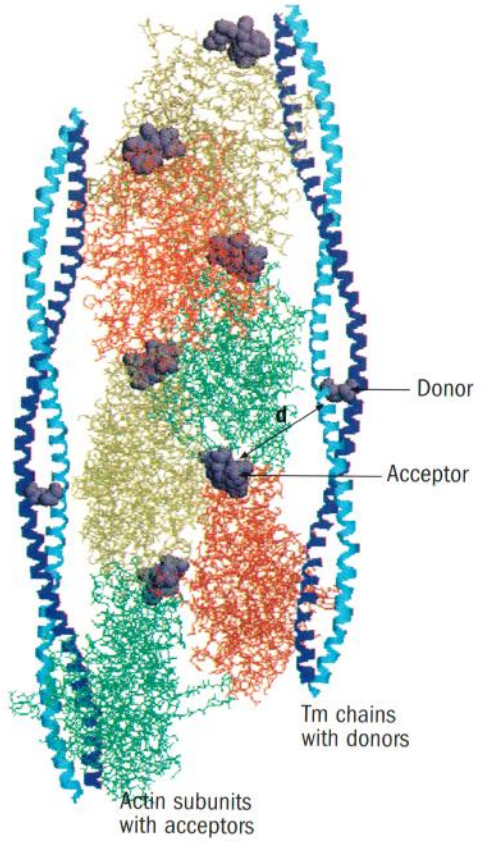
Sherwin S. Lehrer, Ph.D., Senior Scientist and Project Leader

Since each **tropomyosin** molecule interacts with seven actin subunits, the movement of tropomyosin activates **seven actin subunits** for interaction with myosin. This activation is therefore **highly cooperative.**

Tropomyosin is an essential protein component of both striated and smooth muscle because it is involved in the "turning-on" of muscle contraction. In smooth muscle, contraction is initiated by the phosphorylation of myosin. However, tropomyosin binds to a region on actin filaments from which it must move away before myosin can produce contraction. Caldesmon, another thin filament protein is also involved in the turning-on. Since each tropomyosin molecule interacts with seven actin subunits, the movement of tropomyosin activates seven actin subunits for interaction with myosin. This activation is therefore highly cooperative. We are investigating the role of caldesmon and the tropomyosin movement over actin with the use of fluorescence techniques.

One of the methods we use is Fluorescence Resonance Energy Transfer (FRET), a technique to measure distances between sites on proteins. This requires a fluorescent donor label to be attached to tropomyosin and an acceptor label to be attached to actin. The tropomyosin molecule is a dimer of two chains, α and β. Each chain conveniently contains a unique amino acid at a specific place in its entire sequence of 284 amino acid residues. We have been able to attach a single donor at this unique position on each chain. Acceptors are attached to actin using a toxin that binds at every subunit.

By measuring the lifetime decay of the donor fluorescence in the absence and presence of the acceptor label, we have been able to monitor distance changes associated with the binding of myosin. Analysis of the data showed that activation of contraction via myosin binding to actin results in a movement of Tm such that the β-chain moves about 8 Å closer to the actin and the α-chain moves about 1 Å further from actin. This movement is consistent with a uniform rolling of tropomyosin over the actin surface. The role of caldesmon in the tropomyosin movement and how myosin phosphorylation aids in this movement are also under investigation.



Model for part of an actin-tropomyosin muscle thin filament of tropomyosin labeled with fluorescent probes.

Biophysical Core

Renne C. Lu, Ph.D., Senior Scientist and Core Leader

While each of the PPG projects focuses on one aspect of the Molecular Mechanism of Smooth Muscle Regulation, a number of specialized techniques are shared by more than one project. The Biophysical Facility Core provides advice and support to all five projects with required expertise in the areas of analytical ultracentrifugation, electron microscopy and chemical approaches for protein characterization.

Dr. Walter Stafford assists the studies involving sedimentation velocity, sedimentation equilibrium, and other hydrodynamic analyses. BBRI is equipped with two analytical ultracentrifuges and each instrument has been optimized for a specific type of analysis. Data analyses are carried out using sophisticated computer programs written by Dr. Stafford and others. These measurements provide information on the size and shape of molecules as well as their dynamic behavior such as dimer- or complex-formation. (Figure 1)

Dr. Katsuhide Mabuchi offers his expertise in visualizing proteins by electron microscopy or immuno-cytochemical approaches. These techniques allow us to visualize the shape of protein molecules, to identify interacting regions of the molecules in a protein complex, and to determine the localization of various proteins in cells. (Figure 2)

I myself support the studies that require chemical characterization of proteins or peptides. BBRI is equipped with state-of-the-art equipment that allows us to synthesize peptides and to determine amino acid sequences and molecular weights. Frequently when the entire molecule is too large to be used for certain studies, synthetic peptides that represent functional regions are used to mimic the effect of the whole molecule in tissues or to identify binding partners. Synthetic peptides are also used in complexes in the crystal structures to reveal interacting regions. Partial sequence determination allows us to confirm the identity or the purity of a given protein or to identify its interacting partners.

Mass spectrometry is used to determine the masses of molecules precisely, to monitor their modification (such as phosphorylation), and to identify the components of complexes. The core thus represents a valuable collaboration that facilitates the advancement of the entire Program.

The core represents a valuable collaboration that facilitates **the advancement** of **the entire Program.**

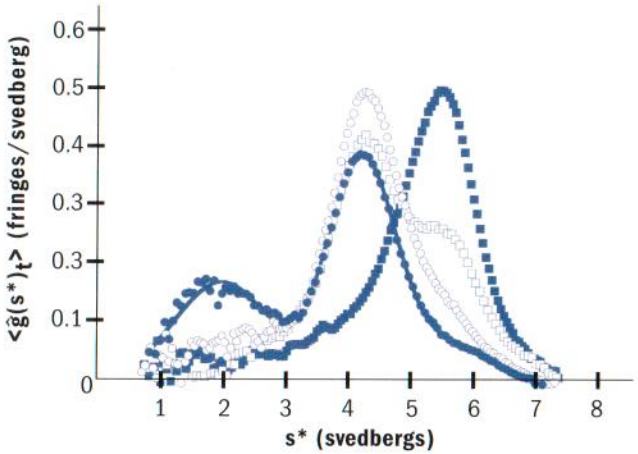


FIGURE 1. Demonstration of complex formation between myosin light chain phosphatase subunits by analytical ultracentrifugation. Peak on the left is the small subunit, the right-hand peak is the larger subunit and the middle peak is the complex.

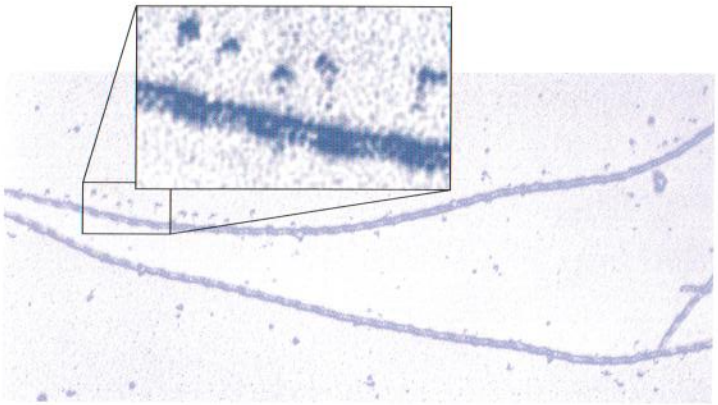


FIGURE 2. Molecules of myosin light chain kinase attached to long threads of actin filaments as observed in electron microscope.

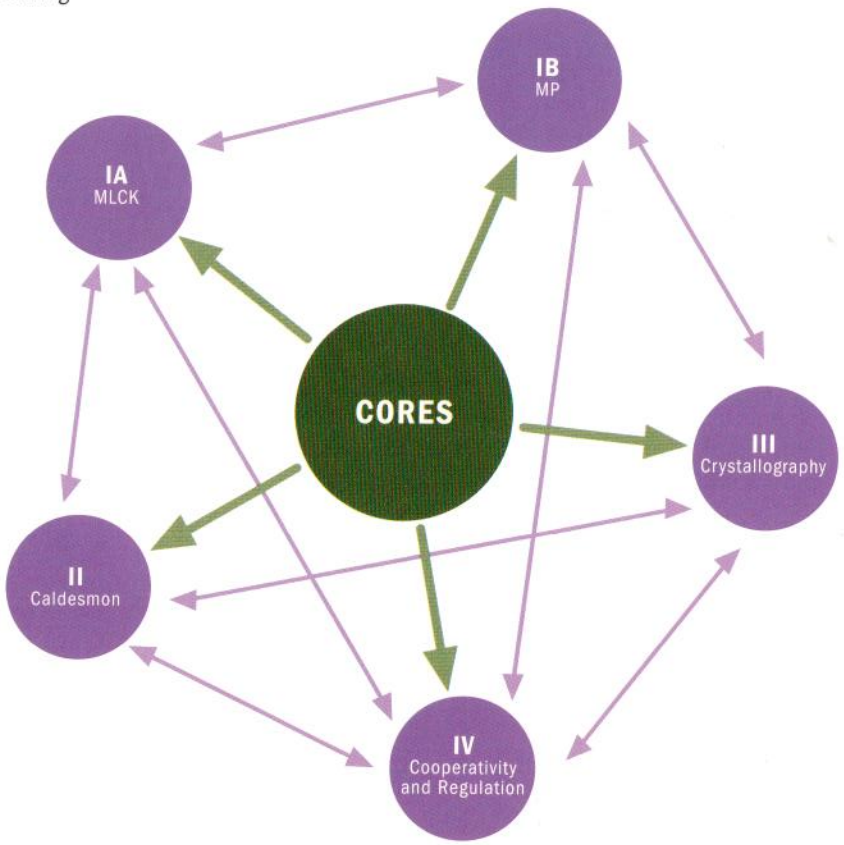
Epilogue

Albert Wang, Ph.D., Senior Scientist and Program Director

As the Program Project Grant enters its third five-year period, it has indeed evolved into a truly coherent, focused, and highly interactive enterprise. We would like to thank all of BBRI's donors for their role in supporting our work, and we are also grateful for the long and insightful support we have received from the NIAMS. With biophysics, biochemistry and molecular biology as our strength, we have further benefited from the expertise of smooth muscle physiology and protein crystallography. Such a highly multi-disciplinary program, representing both solid growth and bold exploration, is only possible under the funding scheme of a Program Project Grant. Along the way, our concerted efforts will undoubtedly lead to a better understanding of how smooth muscle contraction is regulated. Only from such an understanding can we then hope to develop new therapeutic drugs for smooth muscle-related diseases, such as hypertension, asthma, digestive and reproductive disorders.

"BBRI is an **outstanding group of internationally recognized smooth muscle scientists**. Under the direction of Dr. Wang, the members of the Program Project have provided some of the most important findings on the biochemical regulation of smooth muscle contraction. They have **shown great innovation** in their development of reagents and methodology for study of the component molecules of smooth muscle contraction."

FROM THE NATIONAL INSTITUTES OF HEALTH SUMMARY STATEMENT OF BBRI'S PROGRAM PROJECT GRANT RENEWAL



The PPG is a highly interactive enterprise.

development report 2003

John R. Layton, Chair

We are very appreciative that our **supporters believe strongly** enough in BBRI **to help provide the resources** for its groundbreaking research programs.

Through the tireless teamwork of the Board of Trustees, the Faculty and the Development staff, 2003 has been another very productive year in fundraising for BBRI. We are fortunate to have met some important goals in an economically very challenging environment. Two things have made our successes possible. As always, the excellence of the basic biomedical research programs at BBRI was the driving force behind our efforts. But we would not be where we are without the generosity of our dedicated family of contributors, those individuals, foundations and businesses recognized in the following pages. We are very appreciative that our supporters believe strongly enough in BBRI to help provide the resources for its groundbreaking research programs.

The end of Fiscal Year 2003 marked the successful completion of *A Campaign for BBRI: Intellectual Partners for the Future of Science*, a comprehensive fundraising effort that was launched in 2000. Thanks to the wonderful generosity of 232 donors and the hard work of our volunteer campaign committees, a record setting $6.5 million was raised for critical needs at BBRI. These funds already have had a significant impact on BBRI's programs: They enabled the recruitment of five new scientists, the establishment of a state-of-the-art cell biology core facility (thanks to a challenge grant from the Kresge Foundation), the expansion of the BBRI Scholar postdoctoral fellowship program, and the growth of our educational outreach program to the local high school. A very special thanks to my Campaign Co-Chair Allie Blodgett for her wonderful work, and to Challenge Campaign Co-Chairs Donna Fisher and Ty Howe for their outstanding leadership efforts that helped make this campaign such a success.

As part of this campaign, BBRI was awarded a challenge grant in support of the Pilot Fund Initiative from a prominent local foundation, which required BBRI to raise $750,000 by December 31, 2002 in order to receive a grant of $250,000. Thanks to the generosity of the thirty five donors listed in this report, BBRI established a $1 million Pilot Fund, which generates $50,000 annually for a competitive research award to the BBRI scientist proposing the most novel and promising scientific idea for further exploration. Supporters of the Pilot Fund Initiative have helped ensure that the innovative ideas of BBRI's scientists, even if outside mainstream scientific thinking, can be developed to their fullest potential. The 2003 Pilot Fund Award winner is Dr. Zenon Grabarek, who will pursue a very exciting project that involves the crystallization of the thin filament protein complex from cardiac muscle. If Dr. Grabarek's Pilot Fund project succeeds, the knowledge gained will have enormous impact on the muscle and motility field and help to further our understanding of the human heart.

In addition to the successes outlined above, BBRI raised $450,000 for the 2003 Annual Fund. Although we fell short of our $525,000 target, we are very pleased to have raised such significant funds which help to offset costs not covered by research grants. Our loyal and consistent Annual Fund donors are truly the lifeblood of BBRI, and we are grateful for their generosity. It is impressive to note that the amount contributed to BBRI in 2003, when the Annual Fund is combined with the campaign, totaled $1.2 million. To all who made this possible, thank you!

Finally, I would like to thank all of you for making my three years as Chair of the Development Committee a very fulfilling experience. I will be passing the torch to Jillian Hosford Darling, an insightful and dedicated supporter of BBRI, who brings an impressive array of skills with which to lead the Development effort at BBRI. I look forward to working with Jillian on the many new goals and challenges that lie ahead.

I thank every one of you for your generosity to BBRI!

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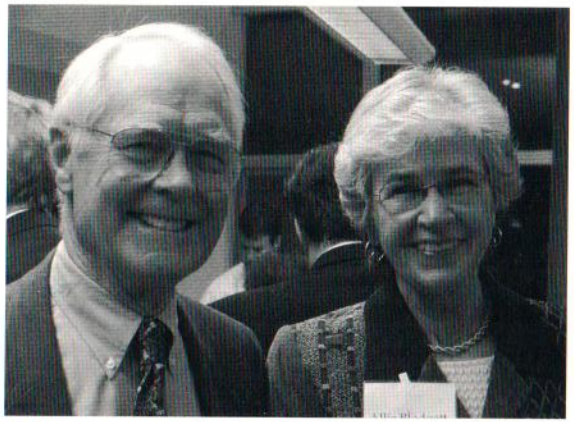

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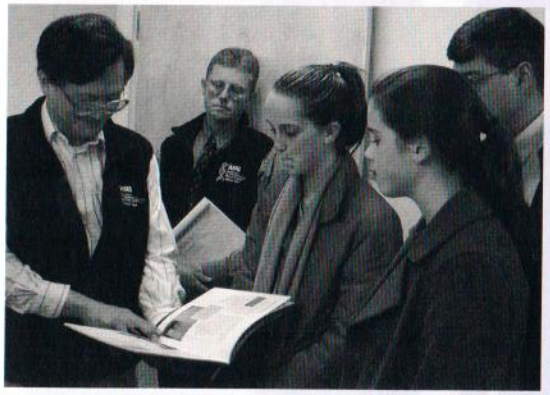
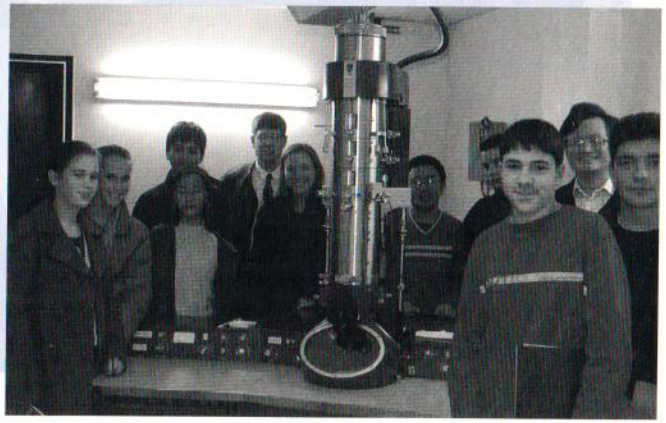
BBRI's Educational Outreach



BBRI's Annual Fund donors help to support BBRI's educational outreach efforts which include offering scholarships and internships to outstanding science students at Watertown High School, and hosting visits from local high school science classes. Pictured above and below are students from Westwood High School who toured BBRI's facilities with Dr. Albert Wang and Simon Welsby, and heard a presentation from Dr. Wang last fall.



Pilot Fund Initiative Challenge

Thanks to the generosity of the following very generous donors, BBRI met an important challenge grant from an anonymous Boston foundation in December, 2002 and raised a total of $1 million to establish a Pilot Fund for the development of innovative scientific ideas proposed by BBRI faculty members. These 'seed funds,' awarded on a competitive basis to the most promising research project each year, will aid the scientists tremendously by enabling them to develop the ideas to the point where they may apply for outside funding. Many thanks to all donors who made the Pilot Fund Initiative a success!

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BELOW AND RIGHT: Donors enjoy BBRI's celebration event for the successful conclusion of *A Campaign for BBRI: Intellectual Partners for the Future of Science.*

Campaign leadership gathers in front of the wall of donors: (L-R): Chairman Jack French, President and Campaign Co-Chair Jake Layton, Vice President and Campaign Co-Chair Allie Blodgett, Director Dr. Charles Emerson, and Trustee and Challenge Campaign Co-Chair Donna Fisher.

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treasurer's report

Geoffrey Nunes, Treasurer

We hope you will continue your **generous support** of BBRI and **help us to expand our friends, advocates** and **supporters** as we prepare to meet the strategic challenges formulated by our new Director.

BBRI's collaborative research continues to gain momentum in its state-of-the-art facility in Watertown MA. In fact, this new facility was a real advantage to BBRI in its recruitment of our new Director, Dr. Charles P. Emerson, Jr. The Board of Trustees is looking forward to working together with Dr. Emerson and the faculty to review their strategic initiatives that will enable BBRI to move ahead both scientifically and financially.

At June 30, 2003 total assets had increased to approximately $42,300,000 from $41,000,000 at June 30, 2002. Grants receivable increased to almost $7,800,000 as of June 30, 2003, an increase of $1,700,000 or 27% over the prior year. During the last fiscal year the Institute was awarded nine new grants from the National Institutes of Health, of which five awards became effective between March 1 and June 1, 2003 and account for the majority of the increase in the grants receivable. Noteworthy is the fact that three of these were first time awards for scientists recruited over the last three years. Investments approximated $14,181,000 on June 30, 2003, an increase of approximately $431,000 over the prior year. The stock market began to show signs of recovery during the fourth quarter of fiscal year 2003, resulting in an annual total return of 3.3%.

In the year ended June 30, 2003, BBRI revenues from grants and contracts of approximately $9,455,000 reflected growth of 8% over the prior year. Revenue from federal agencies represents approximately 95% of such revenue. Additionally, BBRI received nonfederal grants from Conrad, EMD Pharmaceuticals, the Muscular Dystrophy Association and the Wendy Will Case Cancer Fund.

Philanthropic giving once again was significant as BBRI successfully completed *A Campaign for BBRI: Intellectual Partners for the Future of Science*. Fiscal year 2003's total contributions approximated $1,187,000. We hope you will continue your generous support of BBRI and help us to expand our friends, advocates and supporters as we prepare to meet the strategic challenges formulated by our new Director. For the fiscal year unrestricted contributions approximated $407,000. As part of the campaign BBRI raised $731,000 as permanently restricted contributions thus meeting a challenge grant from a prominent local foundation. As a result, BBRI commenced its first pilot project which we anticipate will lead to future funding from both federal and private sources.

Total expenses of $11,240,000 increased approximately $298,000 over the prior year due to growth in research initiatives. The success of our cost containment program, which includes reviewing existing relationships with vendors, is evident in the Statements of Activities which indicates that general support and services (inclusive of administrative costs) and facilities costs were flat in fiscal year 2003 when compared to 2002 levels. We are pleased that BBRI experienced growth in scientific personnel while the administrative and support staff remained at constant levels.

BBRI improved its overall operating performance as total expenses increased by only 2.7%. As previously noted, the investment portfolio generated a 3.3% return, a great improvement over the previous two years, but still below BBRI's targeted return level. Despite these improving financial conditions BBRI was unable to meet its debt service coverage ratio (a target established in bond agreements at 110%) for the fiscal year ended June 30, 2003. Trustees and management, together with an independent consultant, continue to monitor a plan put in place to allow the Institute to achieve a ratio well above 110% in the current fiscal year.

The Investment Committee continues to review the performance and asset allocation of our portfolio with our investment advisor, New England Pension Consultants, on a quarterly basis. The Committee continued to reposition the portfolio during the year to help reduce risk and preserve principal given the bond covenants that focus on short-term results. I would like to thank Tom DiBenedetto for his leadership of the Investment Committee, as well as the other members for their valued expertise during the past year.

Respectfully submitted,
Geoffrey Nunes, Treasurer

statements of

Financial Position June 30, 2003 and 2002

	2003	2002
ASSETS		
Cash	$ 921,496	$ 809,876
Grants receivable	7,794,153	6,101,263
Unconditional promises to give	722,652	889,522
Investments	14,181,202	13,749,823
Prepayments, deposits and other receivables	150,861	125,895
Trustee-held funds	1,244,688	1,245,148
Property and equipment	15,517,841	16,269,056
Deferred compensation investments	1,813,629	1,798,647
TOTAL ASSETS	**$ 42,346,522**	**$ 40,989,230**
LIABILITIES AND NET ASSETS		
Accounts payable and accrued expenses	$811,577	$640,141
Accrued interest expense	385,202	391,140
Deferred income	7,608,262	5,988,342
Notes payable	10,056	—
Bonds payable	16,190,000	16,475,000
Deferred compensation payable	1,813,629	1,798,647
TOTAL LIABILITIES	**26,818,726**	**25,293,270**
NET ASSETS		
Unrestricted	13,183,469	14,054,961
Temporarily restricted	82,541	140,504
Permanently restricted	2,261,786	1,500,495
TOTAL NET ASSETS	**15,527,796**	**15,695,960**
TOTAL LIABILITIES AND NET ASSETS	**$42,346,522**	**$40,989,230**

Copies of our complete, audited financial statements are available upon request from the Chief Financial Officer, Boston Biomedical Research Institute.

statements of

Activities for the years ended: June 30, 2003 and 2002

CHANGES IN UNRESTRICTED NET ASSETS	2003	2002
REVENUES		
Grants and contracts	$9,454,776	$8,726,563
Contributions	406,723	578,473
Investment income	392,107	(1,127,514)
Other income including licensing fees (net)	5,921	147,245
TOTAL UNRESTRICTED REVENUES	**10,259,527**	**8,324,767**
Net assets released from restrictions	109,324	233,227
TOTAL UNRESTRICTED SUPPORT	**10,368,851**	**8,557,994**
EXPENSES		
Salaries and benefits	6,710,931	6,338,189
General support and services	1,661,649	1,655,951
Occupancy costs	947,199	969,985
Interest Expense	919,635	938,735
Depreciation	1,000,929	1,039,027
TOTAL EXPENSES	**11,240,343**	**10,941,887**
Decrease in unrestricted net assets	(871,492)	(2,383,893)
CHANGES IN TEMPORARILY RESTRICTED NET ASSET		
Contributions	50,000	200,000
Investment income	1,361	(14,173)
Net assets released from restrictions	(109,324)	(233,227)
Decrease in temporarily restricted net assets	(57,963)	(47,400)
CHANGES IN PERMANENTLY RESTRICTED NET ASSETS		
Contributions	730,651	956,755
Investment income	30,640	(35,820)
Increase in permanently restricted net assets	761,291	920,935
Decrease in net assets	(168,164)	(1,510,358)
NET ASSETS AT BEGINNING OF YEAR	**15,695,960**	**17,206,318**
NET ASSETS AT END OF YEAR	**$15,527,796**	**$15,695,960**

boston biomedical research institute

Grant and Fellowship Awards JUNE 30, 2003

RESEARCH GRANTS – NATIONAL INSTITUTES OF HEALTH

Dr. Bohm	Functional Studies of the Yeast Poly (A) Polymerase	9/99-8/03	198,000
Dr. Bohm	Catalytic Mechanism and Regulation of Mammalian Adenylyl Cyclase	9/99-2/05	510,000
Dr. Bohm	Mechanism of Poly (A) Polymerase Processivity	7/02-6/07	1,839,000*
Dr. Coluccio	Myosin-I Mediated Processes in Liver Cells	8/97-7/03	1,407,000
Dr. Dominguez	Atomic Structure of Smooth Muscle Caldesmon	3/00-2/05	1,761,000
Dr. Dominov	Enhancement of Myoblast Chemotactic Migration	9/02-6/04	210,000*
Dr. Erhardt	Prevention of Myocardial Ischemic Injury by RAF/ERK	4/03-3/07	1,471,000
Dr. Graceffa	Smooth Muscle Thin Filament	8/01-7/05	1,892,000
Dr. Hansen	Rnd Effector Molecules in Epithelial Cell Transformation	3/03-2/08	2,365,000*
Dr. Harrison	Structure/Function Analysis of Molecular Chaperones	7/98-6/03	1,237,000
Dr. Harrison	Structure-Function Study of Angiogenic Protein, Ephrin	7/01-6/05	1,261,000
Dr. Ikemoto	Structure and Function of Sarcoplasmic Reticulum	4/02-3/07	3,030,000
Dr. Ikemoto	Regulation of Normal & Diseased Cardiac Ca2+ Channels	4/03-3/07	1,682,000*
Dr. Kitazawa	G Protein Mediated Ca2+ Sensitization in Smooth Muscle	4/02-11/02	320,000
Dr. Kitazawa	Mechanism of Ca2+ Sensitization in Smooth Muscle	6/02-5/07	2,876,000
Dr. Lehrer	Tropomyosin and the Regulation of Muscle Contraction	2/01-1/05	2,106,000
Dr. Lehrer	Calorimetry Work Station	3/02-2/03	151,000
Dr. Lu	Procise Protein Sequencing System	5/02-4/03	147,000
Dr. Miller	Molecular Physiology of Respiratory Muscles	11/01-6/05	1,921,000
Dr. Miller	Neurotoxicogenomics and Child Health	11/01-8/06	1,506,000
Dr. Miller	Gene Arrays in Developmental Neurotoxicology	11/01-9/02	119,000
Dr. Miller	Pathogenesis of Laminin-Alpha2 Deficiency	9/02-8/06	1,291,000*
Dr. Morgan	Regulation of Contraction and Growth of Blood Vessels	3/00-2/05	1,762,000
Dr. Morgan	Contraction of Vascular Smooth Muscle Cells	4/01-3/05	1,023,000
Dr. Rameh Plant	The Role of PtdIns-5-P in Cell Function and Signaling	6/03-3/08	1,752,000*
Dr. Raso	Vaccine to Elicit Catalytic Anti-cocaine Antibodies	4/99-3/03	471,000
Dr. Raso	Immunotherapeutic Agents to Treat Alzheimer's Disease	9/00-8/05	1,958,000
Dr. Sarkar	Function of Polyadenylate Sequences in Bacterial RNA	9/98-8/03	1,567,000
Dr. Tao	Molecular Interactions of the Myosin Phosphatase Subunits	2/00-1/04	1,282,000
Dr. Tao	Mechanism of Calcium Regulation in Striated Muscle	6/03-5/08	3,109,000*
Dr. Wang (Pro. Proj.)	Molecular Mechanism of Smooth Muscle Regulation	12/02-11/07	9,132,587*
Dr. Wang	Regulation of MLCK by Phosphorylation	1/02-12/04	96,000
Dr. Wohlrab	Phosphate Path within Homodimeric Mitochondrial PTP	5/98-4/03	1,412,000

NATIONAL SCIENCE FOUNDATION

Dr. Erhardt	Mechanism of Cell Survival Mediated by the B-Raf Kinase	6/00-5/04	309,000
Dr. Smith	Role of Two Novel cGMP Binding Proteins in Dictyostelium	9/01-6/03	205,000

DEPARTMENT OF ARMY

Dr. Rameh Plant	The Role of Novel Phosphoinositide Pathways in Breast Cancer	10/01-7/04	473,000

US DEPARTMENT OF AGRICULTURE

Dr. Miller	Muscle Cell Growth and Development	11/01-4/04	236,000

AMERICAN CANCER SOCIETY

Dr. Bohm	Structure of G-Beta Gamma / Effector Complex	1/99-12/02	342,000

AMERICAN HEART ASSOCIATION

Dr. Dominguez	X-Ray Study of Smooth Muscle Actin and its Complex with a Caldesmon Fragment	1/02-12/05	300,000
Dr. Foster	Structural Investigation of Muscle Thin Filaments using 3D Helical Reconstruction of Electron Micrographs	7/01-6/03	57,000
Dr. Smith	Cyclic GMP Signaling by GBP-A and B in Dictyostelium	1/02-12/05	260,000

CONRAD

Dr. Gonzalez	Leptin Peptide Antagonists	12/02-11/03	120,000*

EMD PHARMACEUTICALS

Dr. Stafford	Characterization of EMD72000	10/02-10/03	60,000*

MARCH OF DIMES

Dr. Coluccio	Mechanochemical Properties of Mammalian Myosin I's	6/98-5/03	310,000

MERCK FOUNDATION

Dr. Ikemoto	Banyu Fellowship Award in Cardiovascular Medicine	4/02-3/04	80,000

MUSCULAR DYSTROPHY ASSOCIATION

Dr. Miller	Dysferlinopathy Model Studies	7/02-6/05	105,000*

WENDY WILL CASE CANCER FUND

Dr. Erhardt	Regulation of MDM2 Acivity by Phosphorylation	7/02-6/03	25,000*

*New grants in fiscal 2003

boston biomedical research institute

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