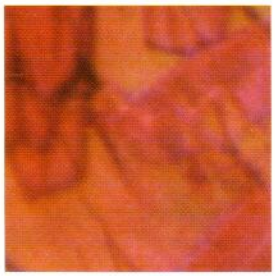
Boston Biomedical Research Institute

Annual Report 2001

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. One 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.



ON THE COVER Crystals of monomeric ADP-actin with a red fluorescent dye bound. The atomic structure of monomeric actin with bound ADP was solved by Dr. Roberto Dominguez's research group. The discovery is featured in this Annual Report along with recent research projects from labs of two other BBRI crystallographers, Dr. Celia Harrison and Dr. Andrew Bohm.

Message from the Director Henry Paulus, Ph.D.

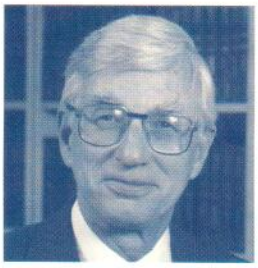
This has been our first full year in Watertown and before we begin to take our wonderful new surroundings for granted, we must not forget that just two years ago we were still working in the crowded and antiquated research facility at 20 Staniford Street. Before we forget even more, it behooves us to acknowledge our debt to David Gibbs and Kathleen Morgan, who not only helped us overcome our inertia by convincing us that moving to a new location was BBRI's only viable option, but who—through an incredible amount of work in which they were joined by many members of the Board—made our move possible.

In this great research environment, science cannot help but flourish. To give you a glimpse of the exciting progress that is being made, this report describes three recent breakthroughs achieved by our crystallography groups: Roberto Dominguez's structure of monomeric actin, which is being heralded in the scientific press as an important landmark because it provides critical insights into the mechanism of processes such as cell movement; Celia Harrison's solution of the structure of ephrin, a protein involved in cellular recognition, which may play an important role in blood vessel growth; and Andrew Bohm's structure of edema factor, one of the toxins produced by the anthrax bacillus to disrupt signaling pathways in the infected host. All of these research advances, besides being terribly exciting as pure science, are also highly relevant to the understanding and treatment of diseases ranging from cancer to bacterial infections.

BBRI's unique collaborative research environment was a very significant factor in these breakthroughs: the structure of edema factor is modulated by interaction with calmodulin, a protein whose structure is being investigated by Zenon Grabarek, and the form of monomeric actin used for crystallization was produced in a quite different context by Philip Graceffa. It is such collaborative interactions that make BBRI truly more than the sum of its parts.

Having moved into this larger research facility, we didn't believe in letting space lie idle. During the last year, a search committee—chaired by Michael Sherman and including Lynne Coluccio, Peter Erhardt, and myself—offered positions to four outstanding scientists among the more than 100 excellent applicants. Three scientists accepted our offers, Drs. Lucia Rameh, Jeffrey Miller, and Toshio Kitazawa. Their research programs will interface with and add new dimensions to BBRI's existing research programs. As you may know, BBRI is a "soft" research institution that expects its faculty to raise 100% of their salaries from research grants. Nonetheless, BBRI is so highly regarded in the scientific community that three of four candidates accepted the faculty positions that we offered them. I believe there must be something unique about BBRI that outstanding scientists turn down lucrative offers from universities in order to participate in our highly interactive and collegial research atmosphere. Yet, lest we become complacent, I must report that Michael Sherman has just accepted a full professorship at the Boston University School of Medicine. We have very much benefited from having Michael as a colleague for the past six years and wish him much success with his career at Boston University.

Of course, BBRI could not exist without the members of the Board of Trustees and Corporation, who generously donate their valuable time to provide advice and guidance, and who—together with other good friends and foundations—donate the funds that make our programs possible. May we continue this successful partnership into the future as we set and reach new goals!



Message from the President David A. Gibbs, Sc.D.

After almost seven years as Director of BBRI, Dr. Kathleen Morgan will return full-time to her scientific research. Since arriving at BBRI in 1995, Dr. Morgan has provided strong leadership that marked a period of significant advances at BBRI and led to the Institute's relocation to a new research facility in Watertown. She was an important player in the BBRI team of trustees, faculty and administrators who researched, planned and implemented this move.

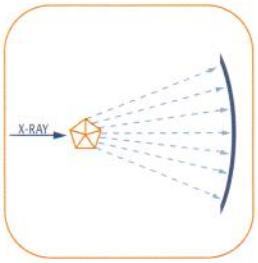
Dr. Morgan has helped to sharpen the Institute's scientific focus by encouraging the establishment of cell growth and cell communication programs to complement BBRI's traditional expertise in motility proteins. Under Dr. Morgan's leadership BBRI has recruited a group of very talented young scientists who have further strengthened our excellent faculty. In this Annual Report you will read in more detail about the flourishing crystallography research being conducted by three of these scientists — Drs. Roberto Dominguez, Celia Harrison, and Andrew Bohm.

Another of the many accomplishments during Dr. Morgan's Directorship was the strengthening of BBRI's Development effort. Indeed the bulk of the funding to start the crystallography research programs at BBRI came from individual gifts and foundation grants. The Annual Fund has continued to see significant growth each year, and we are also making good progress in our first major fundraising effort, *A Campaign for BBRI: Intellectual Partners for the Future of Science*. In the Development Report, you may read more about the Campaign and some exciting new Challenges in the year ahead — for which we will need help from each of you and many new friends!

Please join me and all members of the BBRI family in thanking Dr. Morgan and wishing her well as she devotes her full attention to her research as a Senior Scientist at BBRI. As we plan a comprehensive search for a new Director, we are fortunate indeed to have Dr. Henry Paulus to serve as Acting Director and Dr. Albert Wang as Deputy Director. Dr. Paulus has served as Deputy Director since 1990 and has been a member of the faculty since 1975 when he joined as a Department Director. Dr. Wang has been a faculty member at BBRI since 1979 and has served as the Principal Investigator on the Institute's Program Project Grant since its inception in 1992.

The past year has seen BBRI become more involved with the local community through several initiatives that seek to bring basic science to a wider public. Through our Education Committee, we have begun to plan science education programs with teachers and have supported the science education of local high school students. For the first time, BBRI has awarded scholarships to two very bright Watertown High School students who will be continuing their scientific studies at college — Claudia Steadman at MIT and Kristen Haskell at Providence College.

We also welcomed many new and old friends to the Institute. In February, we launched BBRI's Alumni Association with an evening of lab tours, speeches and much catching up! The gathering was part of the Biophysical Society Meeting for which thousands of scientists from around the world gathered in Boston. Special thanks to Dr. Albert Wang, faculty alumni representative, and Dr. Jim Potter, Professor & Chairman of the Department of Molecular & Cell Pharmacology at the University of Miami School of Medicine (BBRI 1970-74), who served as President in this inaugural year. We consider it very important that BBRI has trained more than 300 postdoctoral fellows as well as many other young scientists and look forward to staying in touch with as many as possible in the future!



Looking at Cells Through a Crystal

Scientists don't look at nature through a crystal ball but rather search for hard facts. Nevertheless, hard facts about nature can be discovered by looking through crystals using the powerful technique of X-ray crystallography. That is why the successful campaign in 1996 to meet the Peabody Challenge for the establishment of an X-ray Crystallography Facility has helped BBRI enter the era of Structural Biology with a flourish. By establishing a BBRI crystallography facility, we were able to recruit three outstanding crystallographers who have gone on to solve the structures of complex proteins that provide quite unexpected insights into the functioning of cells. Three of these structures and their biological implications are described in this report.

The structure of monomeric actin, solved by Roberto Dominguez's research team gives insights at the atomic level into the mechanism by which actin filaments assemble and disassemble. The addition of actin subunits to one end of an actin filament and their removal from the other is the basis of cell movement, which plays an important role in health and disease, be it the movement of a white blood cell towards a pathogenic bacterium which it is about to engulf or the movement of a cancer cell through the wall of a blood capillary in the initial phases of metastasis. The elucidation of the changes in actin structure that underlie these processes will help us understand how our cells control their movement by producing proteins that complex with actin and thereby modulate actin polymerization.

The movement of cells also plays an important role in the organization and shape of our bodies and the organs and tissues that compose it. This raises the question of how cells know when they have arrived at the right place so that they stop moving. One answer lies in the structure of the protein ephrin-B2, which has just been elucidated by Celia Harrison's research group. Ephrin-B2, which lies on the surface of certain cells, binds very specifically to certain Eph receptors, which are found on the surface of other cells. When a cell carrying ephrin-B2 encounters a cell with the right kind of Eph receptor, they establish a connection as the first step in the formation of a body structure, such as the vast network of cells that constitute our nervous system or the connection between arteries and veins that allow our blood to circulate. The atomic detail revealed by the ephrin-B2 structure opens the way to the discovery of small molecules that can modulate its interaction with the Eph receptors. Such drugs may give us the power to block this process when it becomes detrimental to our health, for example by preventing the formation of a circulatory system to feed a malignant tumor.

Parasites have evolved sophisticated mechanisms for invading our body and subverting its normal functions to their own advantage. They therefore know us better than we do ourselves and we can learn much about our cells by studying how parasites such as bacteria attack them. A case in point is the anthrax bacillus, which produces several different types of toxins. One of these, edema factor, has been crystallized and its structure has been solved by Andrew Bohm's group. Edema factor is actually an enzyme, which produces massive amounts of cAMP, an important cellular signaling molecule, and thereby throws cellular metabolism completely off kilter, ultimately leading to tissue destruction. A very interesting discovery is the mechanism by which this destructive enzyme is turned on when it enters a human cell. It involves a huge structural change brought about by the binding of calmodulin, a regulatory molecule that has been much studied by other scientists at BBRI in the context of the control of muscle contraction by calcium ions. This discovery provides interesting insights into cellular metabolism but also opens the door to finding drugs to treat anthrax and similar deadly bacterial infections.

How Actin Lets Our Cells Move

Roberto Dominguez
Scientist, Boston Biomedical Research Institute
M.S. Odessa University, ex-USSR, Theoretical Physics and Mathematics, 1987
Ph.D. Institute Pasteur and Paris-Sud University, Crystallography of Biological Macromolecules and Biochemistry, 1996

The ability to produce directed motion is a distinguishing attribute of all living organisms. Actin, the most abundant protein on earth, is central to most forms of movement. The contraction of our muscles involves the mutual sliding of two sets of filaments: thick filaments, formed by the molecular motor myosin, and thin filaments, consisting of long helical filaments of actin molecules. A more simple form of movement, which has existed for more than a million years, is brought about by the dynamic formation and reshaping of actin filaments alone. This form of movement allows unicellular organisms and human cells to migrate and divide. Both forms of movement depend on the continual input of chemical energy through the hydrolysis of ATP to produce ADP and P_i.

How the polymerization of actin produces directed movement has remained unclear. Part of the answer to this question resides with a phenomenon known as actin treadmilling (Figure 1). One of the ends of the asymmetric actin filament is enriched in actin with bound ATP; the other end consists mainly of actin with bound ADP, produced by the hydrolysis of ATP. While new actin molecules (with ATP bound) tend to associate to the ATP end of the filament, other actin molecules (with ADP bound) tend to disassociate from the ADP end of the filament, leading to simultaneous association and disassociation of actin molecules, hence the term treadmilling.

Treadmilling is controlled within the cell by various actin-binding proteins that "recognize" actin with bound ADP as different from ATP bound actin. Since only the atomic structure of actin with bound ATP, but not that of actin with bound ADP, was known thus far, it hasn't been clear how ATP hydrolysis is involved in treadmilling. A more complete understanding of the treadmilling process has now been achieved by the elucidation of the atomic structure of the actin monomer with bound ADP by X-ray crystallography in the laboratory of Roberto Dominguez with the help of a student, Ludovic Otterbein (Figure 2).

The new ADP-actin structure reveals crucial differences from previously determined actin structures with bound ATP, which may explain why these forms of actin dissociate (or associate) from the filament at different rates and are recognized differently by other proteins. The loss of P_i that results from the hydrolysis of ATP to

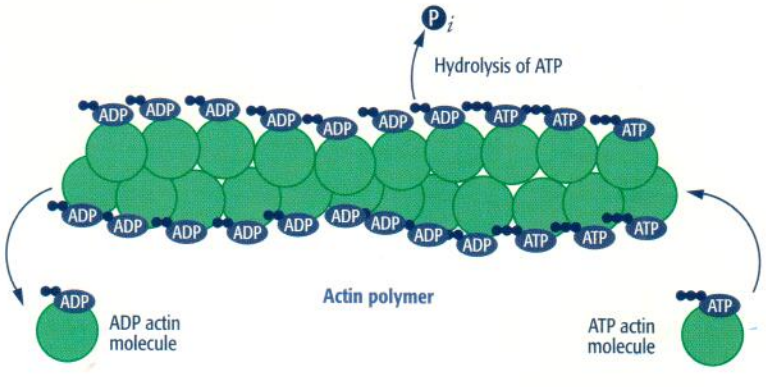


FIGURE 1 Actin treadmilling, i.e. simultaneous association and dissociation of actin molecules from an actin filament.

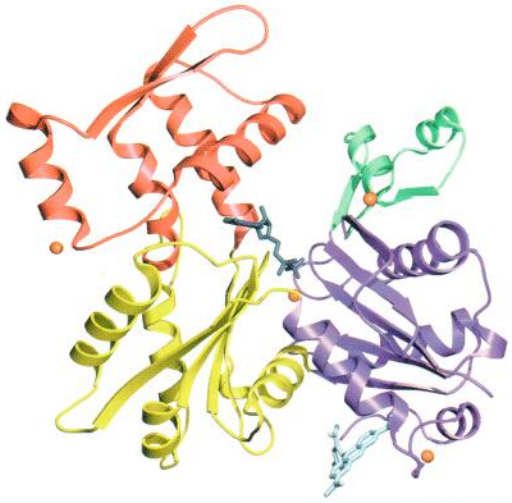


FIGURE 2 Atomic structure of monomeric actin with bound ADP.

ADP appears to set in motion a series of linked structural changes that are propagated from the nucleotide-binding site in the center of the molecule to its external surface. A particular region of actin, called subdomain 2, which is involved in important interactions within the actin filaments, has changed markedly by rotating about 10 degrees, and within it, a significant stretch known as the DNase I binding loop has undergone a rather unique β/α secondary structure transition.

In order to crystallize actin it is necessary to prevent its polymerization. To circumvent this problem in previous actin crystal structures with bound ATP, it was necessary to bind other protein that depolymerize actin. Unlike previous actin structures, in the new ADP-actin crystals (Figure 3) the actin is not associated with any other protein. The crystallization was achieved by the serendipitous discovery by Philip Graceffa that the attachment of a red fluorescent dye (TMR) prevented the polymerization of actin. The solution of the structure of monomeric actin also opens the way for co-crystallizing actin with any of the multitude of actin-binding proteins that exist in nature.

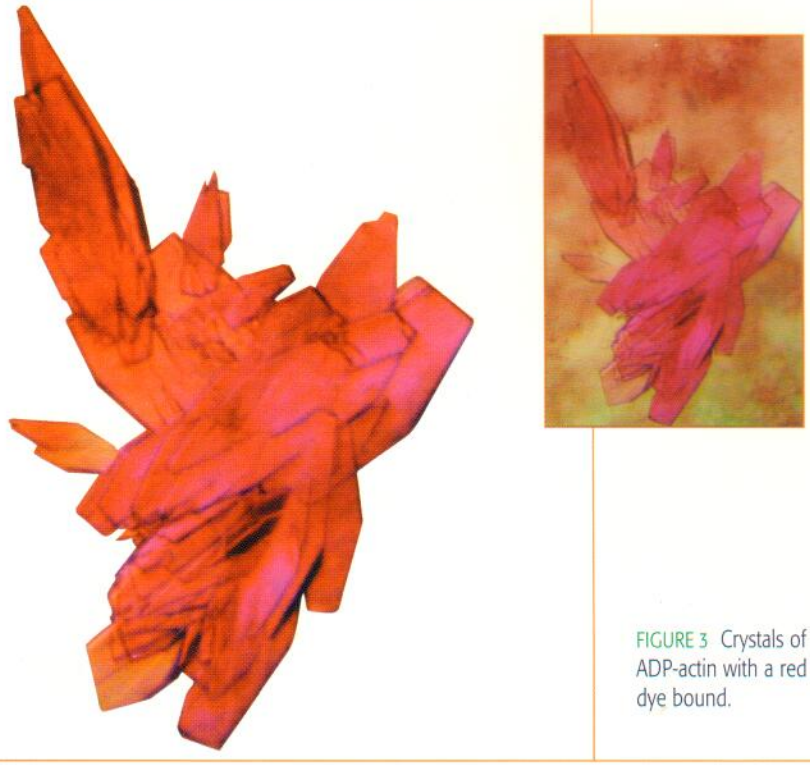


FIGURE 3 Crystals of monomeric ADP-actin with a red fluorescence dye bound.

How Ephrin Organizes Our Tissues

Celia Harrison
Principal Scientist, Boston Biomedical Research Institute
B.S. Pacific Lutheran University, Biology, 1988
Ph.D. University of California, Berkeley, Molecular and Cell Biology, 1994

We are only beginning to understand the process by which a fertilized egg develops into an adult organism consisting of a complex system of different tissues. Dividing cells need to organize into structures such as muscles, lungs and blood vessels in an accurate way; muscles would be useless if they were mixed together with skin cells. The organization of development is controlled by signals conveyed to and from cells by molecules on their surfaces, a process known as signal transduction.

The crystal structure of one of these signaling molecules, called ephrin-B2, has been determined by Joseph Toth, a postdoctoral fellow in Celia Harrison's group along with colleagues at Columbia University (Figure 1). Ephrin-B2 has been shown to help control angiogenesis, which is the formation of and organization of blood vessels. Angiogenesis is currently the focus of intense research by cancer biologists; malignant tumors must recruit blood vessels to deliver oxygen to the new and unwanted tissue. However, to provide the tumor with a functional circulatory system, the newly grown arterial and venous capillaries must be interconnected. This is where ephrin-B2 plays a critical role. Dimers of ephrin-B2 are displaced on the surface of arterial cells and Eph receptors on the surface of venous cells. As illustrated in Figure 2, the binding of ephrin-B2 to the Eph receptor makes contact between arteries and veins as the first step in providing a circulating blood supply to the tumor. Thus ephrin-B2 may serve as a target for

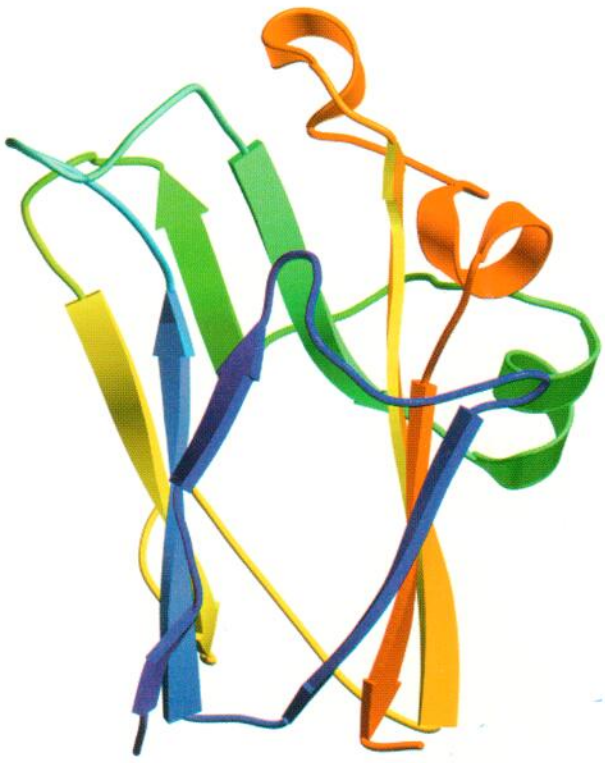


FIGURE 1 The solved crystal structure of the signaling molecule ephrin-B2 ectodomain.

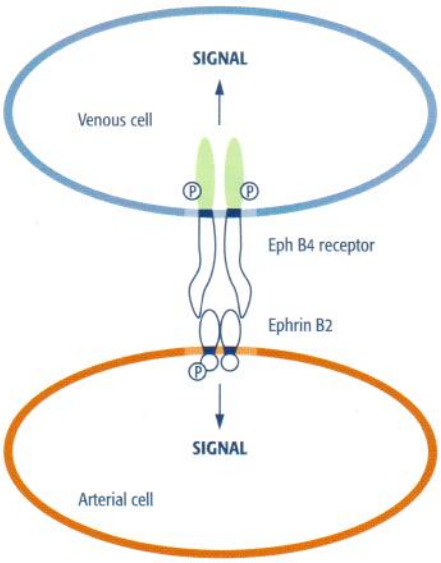


FIGURE 2 Ephrin-B2 plays a critical role in angiogenesis by helping to interconnect newly grown arterial cells and venous cells. The ephrin-B2 on the surface of the arterial cell binds to the eph B4 receptor on the surface of the venous cell, making contact between arteries and veins to build a capillary bed.

anti-angiogenesis therapeutics. Other ephrins, and their cognate molecules, the Eph receptors, are involved in axon guidance, which is the formation of correct connections between nerve cells, and segmentation, which is the separation of cells belonging to one tissue from another. Although the members of the ephrin family have been cloned in many species, there are still no atomic details of ephrin interactions with their receptors. This area of research is being actively pursued in the Harrison lab.

Several features of the ephrin-Eph receptor binding are just beginning to be understood. The ephrin ligands need to be multimerized in order to activate Eph receptors. The Harrison lab hypothesizes that the dimer of ephrin-B2 observed in the recently solved crystal structure is the same as the dimers that are known to form on the cell surface. Small variations in the sequences of the ephrin proteins have significant effects in binding to the Eph receptors; some Eph receptors bind many different ephrins while other Eph receptors are highly selective. The crystal structure of ephrin-B2 reveals a pocket that may contribute to receptor binding. The new structure is just a beginning for understanding the critical interactions between Eph receptors and ephrins.

How Anthrax Subverts Our Cells

Andrew Bohm
Scientist, Boston Biomedical Research Institute
Adjunct Assistant Professor of Biochemistry, Tufts University
Ph.D. University of California, Berkeley, CA, Biophysics, 1992
B.S. State University of New York at Binghamton, Biochemistry, 1987

Anthrax has long been a model for understanding the epidemiology and basic biology of infectious disease. In 1877, when Koch outlined the standard criteria with which to define an infectious agent, *Bacillus anthracis*, the bacterium which causes anthrax, was the first organism that met all of 'Koch's Postulates.' Subsequently, in 1881, Louis Pasteur formed the first recorded vaccine by heating anthrax spores (Figure 1) and inoculating livestock. 120 years later, anthrax is still teaching us about bacterial infection, now, by providing atomic resolution pictures of some of the molecules infectious organisms use to disrupt the cells of their victims.

BBRI scientist Andrew Bohm in collaboration with colleagues at the University of Chicago has determined the crystal structure of the cAMP-generating enzyme from anthrax. cAMP is a small molecule which regulates human metabolic rates, cell development, neuron activity and the maintenance of various ion levels. Many infectious organisms increase the internal cAMP levels of infected host cells. In doing so, these organisms profoundly disrupt these processes. The cAMP producing enzyme from anthrax is called "edema factor." It is roughly a thousand times more active than the human enzymes which perform the same reaction. An enzyme with such high catalytic activity would normally be harmful to the anthrax bacterium, but anthrax has evolved a mechanism to ensure that the enzyme is OFF when it is in the bacterium and turned ON only when it is within the host. Curiously, the key to this molecular switch is calmodulin, a molecule which has been studied at BBRI for years.

Calmodulin is a calcium-sensor molecule. When it binds calcium, it changes shape, and in its new conformation it binds to and regulates over 50 different enzymes. Though the three dimensional structure of calmodulin was known prior to the work in the Bohm lab, there was no clear explanation as to how calmodulin turns on and off its various enzyme targets. By comparing the atomic structure of edema factor's ON-state (with calmodulin) with structures of edema factor's OFF-state (without calmodulin), Dr. Bohm and his coworkers have shown how the switch works. The answer is surprisingly complex.

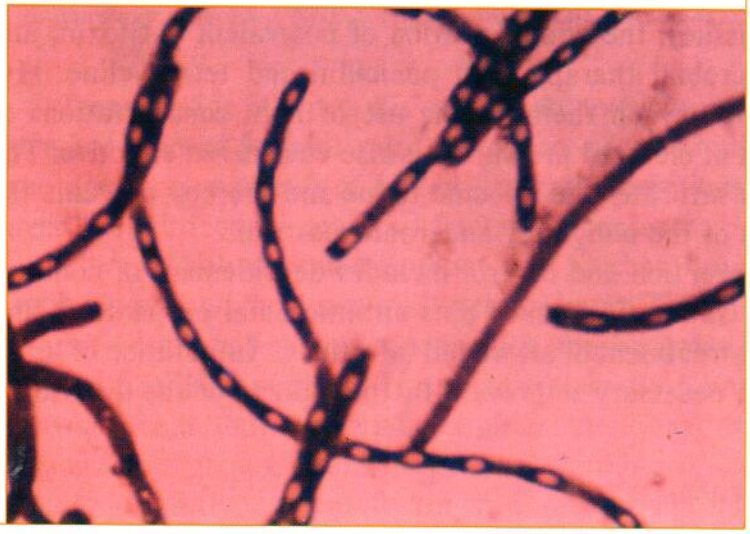


FIGURE 1 Microscope image of stained cells of anthrax bacillus. The egg-shaped objects are spores, which allow the bacillus to survive heat and dehydration for centuries.

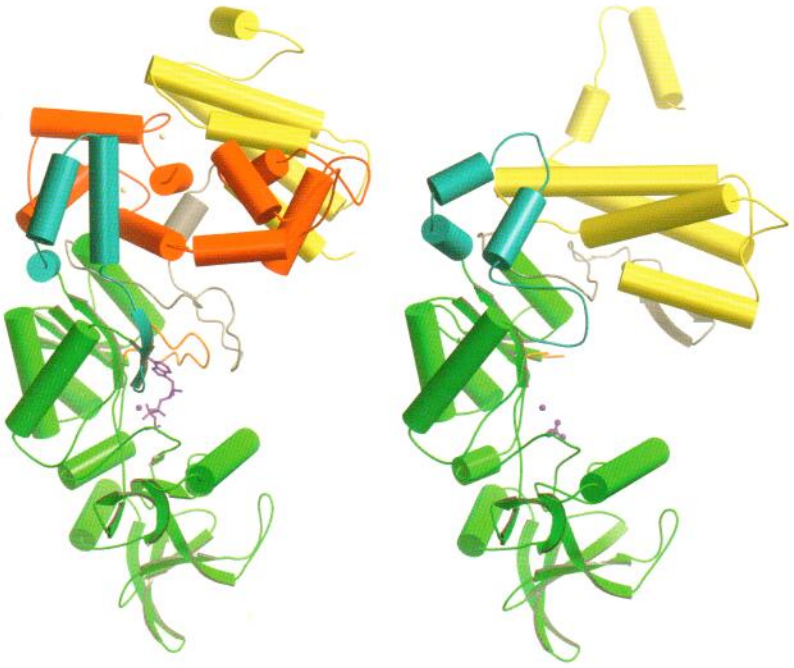


FIGURE 2 The solved atomic structure of edema factor in its ON state with calmodulin (left) and its OFF state without calmodulin (right) shows how calmodulin turns edema factor on and off.

Shown in Figure 2 is a "colored ribbons" representation of edema factor's OFF-state (right) and its ON-state (left). The amino acid chains have been drawn as a series of arrows, loops and cylinders (which is less complex than showing than showing the 9132 individual atomic coordinates determined by the experiments!). In the OFF state, the yellow segment of the edema factor enzyme is in contact with the turquoise segment. When edema factor binds calmodulin, the yellow segment opens up to receive the calcium-sensor molecule (red, with calcium ions in white). Though calmodulin itself is quite far from the site of cAMP formation, the linker region (grey), which connects the yellow segment of the protein to the rest of the molecules undergoes a huge structural change, moving from the back side of the enzyme in the OFF state to just on top of the catalytic center in the ON state. Once the linker moves, the amino acids beneath the linker (colored orange) fold to form the catalytic pocket, and the enzyme begins its work on the small molecule substrate (purple).

Now that we understand how edema factor makes cAMP, the molecular structure of this bacterial enzyme is being carefully analyzed so that drugs can be designed to prevent cAMP formation. Such drugs might be useful not only against anthrax, but also against other bacteria like *Bordetella pertussis* (which causes whooping cough) and *Pseudomonas aeruginosa* (the cause of various hospital-born infections), which harbor similar cAMP-producing enzymes. We now know that there are a variety of ways that these enzyme might be stopped. Drugs might block substrate binding, they might prevent the linker region from adopting the ON-state conformation, or they might block calmodulin binding. The challenge now is to determine which of these approaches might ultimately provide a useful therapeutic agent.

DEVELOPMENT REPORT JOHN R. LAYTON

In many ways, 2001 was quite a journey for BBRI. The move to Watertown has provided much improved space for existing scientists, and their research programs are flourishing. As a result of the enlargement of our lab facilities, BBRI was able to attract two highly accomplished cell biologists to the faculty. On the Development front, 2001 was quite a year as well.

In 2001, supporters of BBRI contributed a record amount of $466,000 to the Annual Fund, which helps to fund the postdoctoral fellowships, shared-use scientific equipment, seed money for new scientists, and bridge funding that together keep BBRI at the leading edge of biomedical research. Support for the Annual Fund has grown steadily over the past four years, as our donors have helped us surpass each year's target amount. BBRI would not be nearly as effective without the enormous generosity of our friends in helping us to provide these funds for the operation of the Institute. On behalf of the Board, I offer my sincere gratitude to each and every donor for their thoughtfulness. Many thanks, too, to the members of the Development Committee and those who were tenacious in helping us to move the Annual Fund to a new level. The Development staff also deserves great credit for keeping the Institute's volunteers focused on the task at hand. Let us all hope that BBRI's Annual Fund can continue to gain new ground as BBRI extends the boundaries of scientific discovery.

2001 was also a year in which we pushed the boundary for our comprehensive fundraising effort, *A Campaign for BBRI: Intellectual Partners for the Future of Science*. Thus far, BBRI has received $3.4 million in leadership gifts and pledges, and is well on its way toward success in moving the Institute to a new level in the biomedical research world. This effort seeks to enlarge the Institute's scientific staff, expand the postdoctoral fellowship program, initiate a Pilot Fund for truly innovative research projects, and provide resources for needed scientific equipment and educational outreach to local students.

Early response from individuals and foundations to our campaign goals has been enthusiastic. We are delighted to announce that two prominent national foundations have chosen to support our efforts. The Kresge Foundation and the Fidelity Non-Profit Management Foundation have each awarded BBRI significant challenge grants. We look forward to sharing the details of these challenges with our existing donors, as well as with new friends, in order to meet these challenges successfully. BBRI is already considered to have world-class research programs. To build on that excellent foundation, I urge you to join me in becoming a partner of the Institute in its ongoing efforts to improve human health through basic biomedical research.

BBRI's successes rely greatly on the generosity of the many people we are privileged to call friends, including those whose names appear in the following pages. Our sincere thanks and deepest gratitude to all who contributed to BBRI in the fiscal year just ended. We could not have come so great a distance without your extraordinary generosity. The Institute's journey is ongoing, and we hope to accomplish a great deal in the year to come. With your help, we will be certain to succeed.

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Our Heartfelt Thanks to the Individuals, Foundations and Businesses Who Generously Gave Us Their Support and Encouragement, Fiscal 2001

BBRI Founder Dr. Endre Balazs and Dr. Janet Denlinger, both members of the BBRI Corporation and Alumni, make remarks at the first annual Alumni Association meeting in February 2001.

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Vice President Allie Blodgett with newly elected Corporation member Donna Fisher at the 2000 Annual Meeting.

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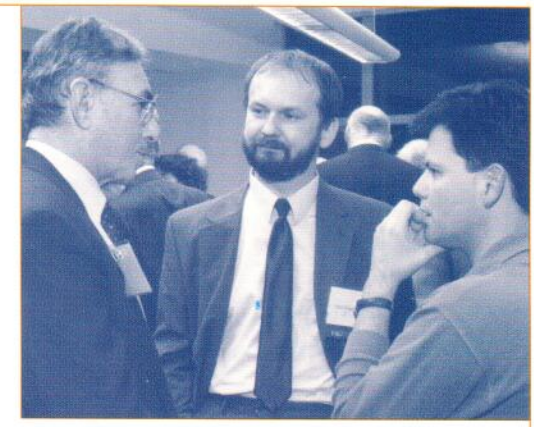

(L-R) Emeritus Scientist Dr. Peter Coleman shown with current Faculty members Dr. Peter Erhardt and Dr. Roberto Dominguez at the 2000 Annual Meeting.

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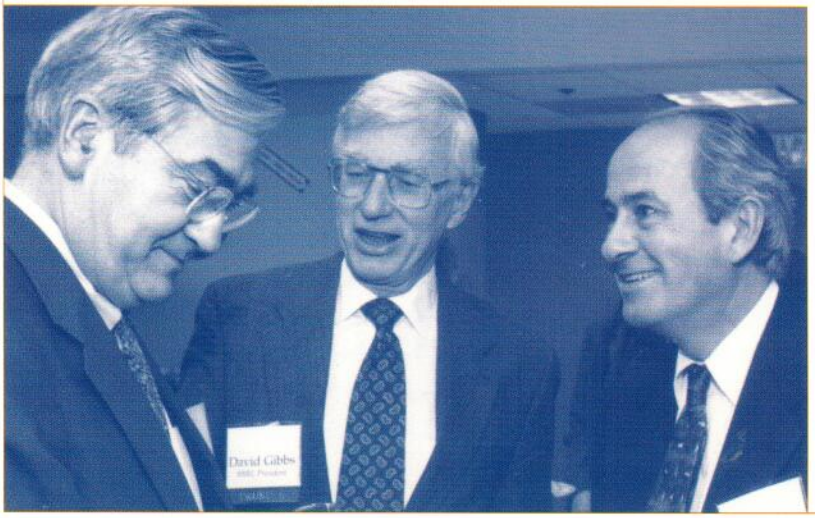

(L-R) Corporation member Peter Kliem with President Dr. David Gibbs and Watertown community leader John Airasian at the 2000 Annual Meeting.

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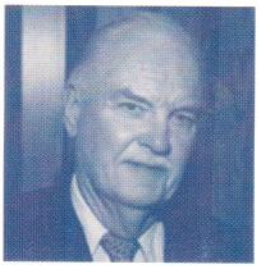
BBRI's Education Committee put its work into action in the community by awarding $2500 scholarships to two outstanding science students at Watertown High School. Pictured at the May 2001 awards ceremony are (L-R) Watertown High School Science Coordinator George Buckley, President Dr. David Gibbs, Claudia Steadman, Kristen Haskell, Director of Development and Public Affairs Simon Welsby.



Treasurer's Report

BBRI has enjoyed its first full year in its new state-of-the-art research facility in Watertown and has become an active member of the local community. During the past year, management and staff at BBRI have assessed and met the new challenges that are associated with owning and managing our own facility. A major challenge was a review of our existing relationships with vendors and the implementation of a significant cost savings program. While the administrative and support staff remained at stable levels, BBRI experienced a 16% growth rate in scientific personnel, including the addition of two new principal investigators and related staff. In fact, since the implementation of the strategic plan five years ago we have successfully recruited ten scientists.

A review of the Statements of Financial Position indicates that investments approximated $15,448,000 on June 30, 2001, a decrease of almost $2,591,000 over the prior year. As most investors are aware, this year was a particularly difficult period for the equity investment market. As a result, the portfolio suffered an annual total return of –11.3%.

A review of the Statements of Activities indicates that BBRI revenues from grants and contracts of approximately $7,942,000 experienced exceptional growth of 19% over the prior year. Revenue from federal agencies represents approximately 92% of revenue from grants and contracts. Unrestricted contributions of approximately $2,014,000 increased $1,329,000. Investment income primarily declined due to unrealized losses of approximately $3 million resulting from the downturn in the stock market. These unrealized losses caused BBRI's debt service coverage ratio (a target established in bond agreements) to be less than 110% for the fiscal year ended June 30, 2001. Trustees and management are formulating a plan to ensure the ratio stays well above 110%.

Total expenses of $10,101,000 increased approximately $1,188,000 over the prior year due primarily to research initiatives and a full year's depreciation costs associated with the research facility.

During the fiscal year the Institute was awarded four new grants from the National Institutes of Health. Additionally, BBRI received grants from the American Heart Association, CONRAD and the Hereditary Disease Foundation.

The Investment Committee reviews performance and asset allocation with our investment advisor, New England Pension Consultants, on a quarterly basis. Please allow me to extend my personal thanks to the members of the Investment Committee, who have been invaluable during the past year.

Philanthropic giving grew significantly as BBRI is in the leadership phase of *A Campaign for BBRI: Intellectual Partners for the Future of Science*. Fiscal year 2001 contributions of approximately $2,014,000 represent an increase of almost 157% over the prior year. As we progress along the path of the strategic plan, we hope you will continue your generous support of BBRI, assist us in expanding our growing circle of friends and supporters, and help us to benefit from exciting new opportunities!

Respectfully submitted,
Ernest Henderson III, Treasurer

Statements of Financial Position
June 30, 2001 and 2000

Assets	2001	2000
Cash	$520,284	$491,001
Grants receivable	4,981,270	5,648,808
Unconditional promises to give	417,140	355,802
Investments	15,447,786	18,039,094
Prepayments, deposits and other receivables	168,704	190,397
Trustee-held funds	1,281,381	1,211,332
Property and equipment	16,564,454	17,167,342
Deferred compensation investments	2,217,224	2,869,215
Total assets	**$41,598,243**	**$45,972,991**
Liabilities and net assets		
Accounts payable and accrued expenses	$240,524	$999,473
Accrued interest expense	391,140	393,628
Deferred income	4,798,037	5,526,557
Bonds payable	16,745,000	17,000,000
Deferred compensation payable	2,217,224	2,869,215
Total liabilities	**24,391,925**	**26,788,873**
Net assets		
Unrestricted	16,438,854	18,279,695
Temporarily restricted	187,904	259,522
Permanently restricted	579,560	644,901
Total net assets	**17,206,318**	**19,184,118**
Total liabilities and net assets	**$41,598,243**	**$45,972,991**

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, 2001 and 2000

Changes in unrestricted net assets	2001	2000
Revenues		
Grants and contracts	$7,942,481	$6,690,734
Contributions	2,014,487	684,879
Investment income	(1,743,133)	1,685,090
Other income including licensing fees	257	14,273
Total unrestricted revenues	**8,214,092**	**9,074,976**
Net assets released from restrictions	46,074	132,237
Total unrestricted support	**8,260,166**	**9,207,213**
Expenses		
Salaries and benefits	5,680,793	5,005,396
General support and services	1,677,110	1,794,753
Occupancy costs	812,632	1,216,540
Interest Expense	948,997	328,525
Depreciation	981,475	571,272
Total expenses	**10,101,007**	**8,916,486**
Increase (decrease) in unrestricted net assets	(1,840,841)	290,727
Changes in temporarily restricted net assets		
Investment income	(25,544)	39,345
Net assets released from restrictions	(46,074)	(132,237)
Decrease in temporarily restricted net assets	(71,618)	(92,892)
Changes in permanently restricted net assets		
Contributions	–	100,000
Investment income	(65,341)	46,935
Increase (decrease) in permanently restricted net assets	(65,341)	146,935
Increase (decrease) in net assets	(1,977,800)	344,770
Net assets at beginning of year	**19,184,118**	**18,839,348**
Net assets at end of year	**$17,206,318**	**$19,184,118**

GRANTS & FELLOWSHIP AWARDS

Research Grants

National Institutes of Health

Dr. Badwey	MAPK in the contractile phenotype of smooth muscle	3/96-2/02	$1,128,000
Dr. Bohm	Functional Studies of the Yeast Poly (A) Polymerase	9/99-2/02	165,000
Dr. Bohm	Catalytic Mechanism and Regulation of Mammalian Adenylyl Cyclase	9/99-8/03	198,000
Dr. Coluccio	Myosin-I mediated processes in liver cells	8/97-7/02	1,407,000
Dr. Dominguez	Atomic Structure of Smooth Muscle Caldesmon	3/00-2/05	1,620,000
Dr. Harrison	Structure / function analysis of molecular chaperones	7/98-6/03	1,206,000
Dr. Ikemoto	Structure and function of sarcoplasmic reticulum	9/96-8/02	2,416,000
Dr. Leavis	**Developmental proteins in the prevention and treatment of abnormal cell proliferation**	**12/00-5/01**	**154,000***
Dr. Lehrer	**Tropomyosin and myosin interaction in muscle**	**2/01-1/05**	**1,939,000***
Dr. Morgan	Regulation of contraction and growth of blood vessels	3/00-2/05	1,645,000
Dr. Morgan	**Contraction of vascular smooth muscle cells**	**4/01-3/05**	**940,000***
Dr. Morgan	Confocal Microscope Facility	4/00-3/01	293,000
Dr. Paulus	Mechanism of protein splicing in Mycobacterium	4/97-3/02	1,403,000
Dr. Raso	A binary system for cell-targeted delivery	3/99-2/02	246,000
Dr. Raso	Vaccine to elicit catalytic anti-cocaine antibodies	4/99-3/02	471,000
Dr. Raso	**Immunotherapeutic Agents to Treat Alzheimer's Disease**	**9/00-8/05**	**1,786,000***
Dr. Sarkar	Function of polyadenylate sequences in bacterial RNA	9/98-8/02	1,522,000
Dr. Sherman	Molecular chaperones and protein phosphorylation	5/96-4/01	1,081,000
Dr. Sherman	HSP72 and Regulation of Stress-Kinases in Tumor Cells	5/00-4/05	1,991,000
Dr. Tao (MERIT)	Proximity relationship among muscle proteins	5/96-3/02	2,201,000
Dr. Tao	Molecular Interactions of the Myosin Phosphatase Subunits	2/00-1/04	1,282,000
Dr. Wang (Pro. Proj.)	Molecular mechanism of smooth muscle regulation	12/97-11/02	7,868,000
Dr. Wohlrab	Phosphate path within homodimeric mitochondrial PTP	5/98-4/02	1,412,000

National Science Foundation

Dr. Erhardt	Mechanism of Cell Survival Mediated by the B-Raf Kinase	6/00-5/03	309,000

American Cancer Society

Dr. Bohm	Structure of G-Beta gamma / effector complex	1/99-12/01	342,000

American Heart Association

Dr. Dominguez	X-Ray Crystal Study of Recombinant Human Calcyclin and its Complex with a Target Smooth Muscle Caldesmon Fragment	7/99-6/01	77,000
Dr. Smith	**Cyclic GMP-Dependent Myosin Regulatory Light Chain Phosphorylation in Dictyostelium**	**7/00-6/02**	**99,000***

CONRAD

Dr. Leavis	**Contraceptive potential of novel embryo-derived peptides that modulate maternal immunity**	**12/00-11/01**	**126,000***

Hereditary Disease Foundation

Dr. Sherman	Role of stress kinase & HSPS in Huntington-induced apoptosis	2/99-1/01	111,000
Dr. Sherman	Screen for Compounds that prevent aggregation and toxicity of polyQ containing polypeptides using yeast	4/00-3/01	58,000
Dr. Sherman	**Mechanisms of Prevention of Cytoxicity in Huntington's Disease**	**2/01-1/02**	**121,000***

March of Dimes

Dr. Coluccio	Mechanochemical properties of mammalian myosin I's	6/98-5/03	310,000
Dr. Dominguez	Structural Biology of Caldesmon - based Thin Filament Regulation	2/00-1/02	100,000

The Medical Foundation-Charles A. King Trust

Dr. Yaglom (Dr. Sherman)	The role of Hsp 72 in regulation of stress - kinases	9/99-8/01	56,000

Sponsored Research

Dr. Paulus	Pharming Technologies BV	1/99-12/00	218,000

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