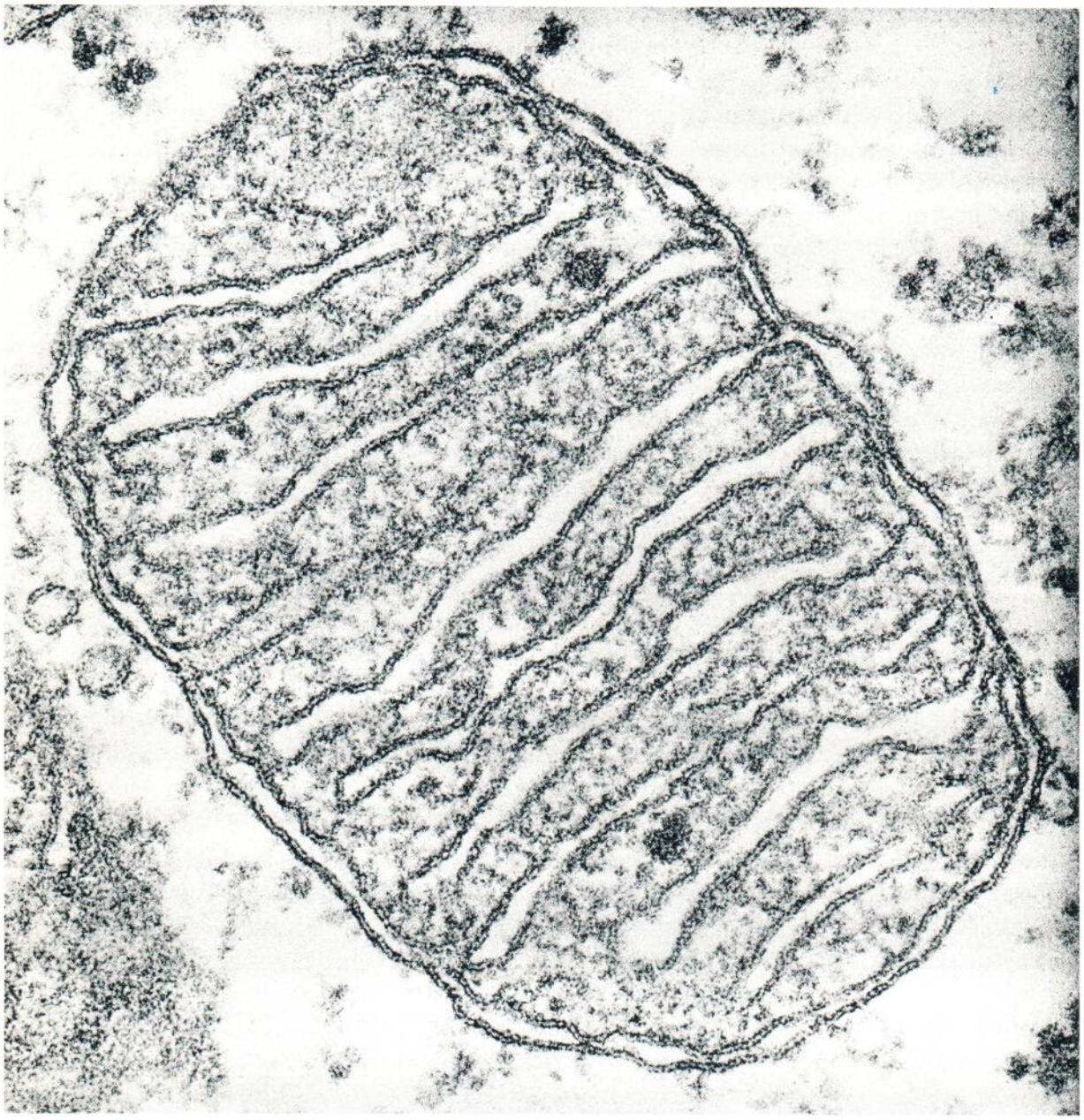
Boston Biomedical Research Institute

1993 Annual Report



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

Cover.
Electron micrograph of a mitochondrion in cross section, magnified 155,000-fold. Mitochondria have two sets of membranes, an outer membrane, which surrounds the entire organelle, and an inner membrane, which contains ATP synthase (see pages 12-13 of this report). Note that the inner membrane is extensively invaginated so as to increase the surface area available for converting the energy generated by burning foodstuffs into ATP. (The electron micrograph was taken by Nobel laureate George Palade at Rockefeller University.)

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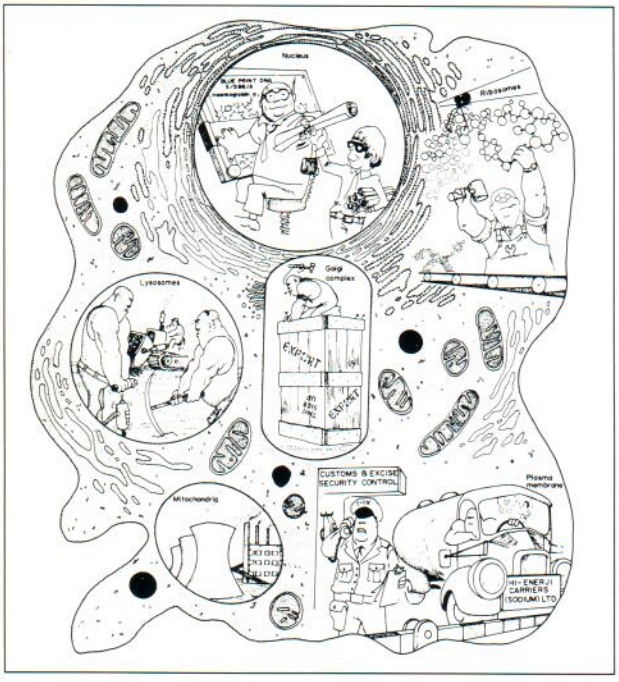


The cell viewed as a factory, with its various membrane-bounded compartments carrying out many different functions.

There have been a number of common themes running through my letters in the annual reports over the past years - issues of governance, the work of the Institute's scientists, and the ever present matter of finances, both government funding and private fund raising. These are obviously necessary and important matters, and I could expound on these again this year, as they remain both timely and ongoing. However, as this will be my last President's letter, I thought I would use it to express a more general thought.

It seems to me important that the Institute continually remind itself that it must be open minded and receptive to consider and deal with change in all aspects of its operation. We are not a static but a dynamic organization, and the environment for basic biomedical research is likewise not static but dynamic and constantly evolving. This is nothing new, change being probably the one constant in all the many things we have dealt with in the past and will face in the future - whether it be in the way research is funded, the way the Institute is governed, both internally through the scientific staff and externally through the Board of Trustees, and, of course, the way research is being conducted.

Certainly, over the years we have seen significant changes in the area of NIH funding, which has long been the backbone of our operations. While BBRI has been fortunate in surviving in the tougher funding environment, we must be alert and even aggressive in seeking out other sources for funding our research. There are or may be possibilities of various collaborative arrangements, with industry, with other research organizations, with hospitals, and these need to be looked at and explored, even if it changes to some degree the way in which some of our research is done. Likewise, our governance has evolved and will continue to evolve - as evidenced by the replacement of departments in the last year with more flexible research groupings which can shift as different research projects or lines of research are embarked upon. A new director when he or she is in place will undoubtedly have ideas and recommendations for other structural and scientific changes - and this is as it should be. Both staff and Trustees must be prepared for and willing to accept them. We also need to change the way we view our obligations as Trustees and Corporation members in the area of fund raising. There always seems a valid reason to postpone this, but the need for increased endowment funds cannot be ignored much longer, and there is no better way - no other way in fact - than for the Institute "family" to take on this task itself.

To suggest a few examples of changing circumstances is not to limit our thinking to these matters, nor is it to suggest that the scientists or the Trustees have heretofore resisted adapting to changes which affect the Institute. However, it bears repeating that we must continually be alert to consider in a positive and constructive manner suggestions for different or additional approaches to our endeavors, and not look only at the problems or drawbacks it might generate. Further, none of this is to suggest that we should accept uncritically all proposals or go along with all circumstances affecting our collective operation, without subjecting them to thorough analysis and scrutiny, which will assuredly lead to improved proposals. Knowing the caliber of the scientific staff and the Trustees, I am confident about the future of the Institute. I expect what I have said above is both obvious and not necessary, but if so, no harm is done other than it has taken your time to read it, but if not, I hope it may do some good as the Institute moves forward under new leadership.

I cannot close without noting with regret, but also with a collective expression of gratitude on behalf of the entire family of BBRI, that after twenty-two years as a senior officer of the Institute, Bill Tyler is relinquishing the post of Chairman which he has held since 1980. Bill was "present at the creation", being an original incorporator of BBRI in 1968, and he played a key role in the separation of the Institute from the Retina Foundation. He served as President from 1971 to 1979, and throughout all these years has been our chief spokesman to the outside world and has had a significant impact on the development activities of the Institute. He has been the guiding force behind many of the major foundation gifts which the Institute has received, and his tireless efforts on our behalf are both unequaled and, to a large degree, unrecognized. While this brief paragraph cannot adequately reflect all that Bill has done and meant to BBRI for the past 25 years, it can at least give public recognition of his many contributions. I am happy to report that Bill will remain a Trustee, and I am sure we will find many occasions to impose upon him further.

John B. French

Report of the Executive Director

This has been a year of beginnings at BBRI. It was the first year in which we have operated without formal departments and with the Director and Deputy Director nominated by the Faculty for appointment by the Board of Trustees last November. Scientific interactions among those who share common interests are maintained through informal discussions and group seminars. We anticipate that in the future interactions will continue to grow among investigators who used to belong to different departments.

A very important event was the launch of the Search Committee under the chairmanship of Ed Davis and with Albert Wang, Senior Scientist, as vice-chairman. This step was taken after the helpful report of an external review committee, consisting of distinguished scientists, was carefully studied by a Faculty/Trustee committee appointed by the President and after review by the full Board. Almost two hundred people have been contacted to recommend potential candidates, and advertisements in the premier international broad-range scientific journals - the U.S. *Science* and the British *Nature* - have appeared. More than two dozen candidates have been named and indeed, as of the writing of this report, a goodly number of applications have already been received. There is a consensus within the Faculty, the Board, and the Search Committee that the director will be a scientist of international reputation who will bring a vigorous research program to BBRI and who will possess over-all leadership skills and the ability to represent the Institute to the outside world.

We were extremely pleased that Nilima Sarkar, Senior Scientist, on applying for a competitive renewal of an NIH grant, received a score in the top six percent - an outstanding research proposal on an exciting problem dealing with the mechanism of decoding the genetic message. Two new investigators - Brenda Williams, who joined us last April from The National Institute for Medical Research in London to work on neural development; and Peter Prevelige, who came to BBRI a year ago from MIT to start work on the mechanism of virus assembly - are at present supported in part by Institute funds given for the very purpose of launching new investigators. Peter Prevelige is also aided by a Shannon Award from NIH to cover the period before a grant providing full support can be obtained. These two able investigators exemplify how difficult it is to obtain one's first grant, a difficulty, as we know from colleagues at various universities, experienced by many new appointees with the highest qualifications. This serves to emphasize the importance of maximizing the total return on funds already available to the Institute and of searching for new sources of philanthropic gifts specifically earmarked for start-up projects by new investigators at BBRI.

At the risk of sounding like a broken phonograph record, I can't refrain from again pointing to the need for bridging funds; that is, interim funds to support the ongoing work of valuable investigators on the staff while they are waiting for NIH funding. The average waiting period for NIH funds is progressively increasing as the funding situation for basic biomedical research continues to tighten. The appointment of Harold Varmus as Director of NIH has been greeted by scientists throughout the nation with great joy and expectation for improvement in the level of funding.

New opportunities for BBRI may arise from the appointment of a new research director at our sister institution, Schepens Eye Research Institute, in the person of Wayne Streilein, at present a professor of immunology at the University of Miami School of Medicine. The two institutes are eager to develop closer scientific interactions and to increase wherever possible the joint use of facilities including the use of specialized equipment and the improved utilization of animal facilities.

In closing I wish to thank, both personally and on behalf of the Faculty, Bill Tyler for his wise guidance and his successful efforts to obtain funds from foundations and other philanthropic sources. Bill was instrumental in launching BBRI when it separated from the Retina Foundation and has served the Institute as President and currently as Chairman. We are sorry to see him relinquish his chairmanship but expect to be able to benefit from his continued presence on the Board of Trustees.

We are also grateful to Jack French for his leadership as President for many years and who - together with Bill Tyler and Elkan Blout - was a tremendous help in bringing our previous search for a director to a realistic conclusion, after having expended an enormous amount of energy in the process. Jack has indicated his desire not to continue as President of the Board of BBRI, but we are pleased that he has agreed to accept the nomination for the position of Chairman. We are all elated by Ed Davis' willingness to accept the nomination for President and are looking forward to continuing the kind of relations we have had with both Jack and Bill when they were in the President's office. Again, thanks are due to all the members of the Board of Trustees and Corporation who have helped BBRI in various ways and without whom institutions like ours could not exist.

John Gergely

MEMBRANES AND MEMBRANE CHANNELS

INTRODUCTION

Membranes as barriers

All cells, be they those of animals, plants or bacteria, are covered with a thin envelope, the so-called *plasma membrane*. The major components of cell membranes are fat-like substances or lipids, which are arranged in a two-layered structure, the *lipid bilayer*, only two one-hundred thousandths of an inch thick but clearly visible in the electron microscope. Since lipids are water repellent (*hydrophobic*) molecules, the plasma membrane serves cells as a barrier which prevents a mixing of the aqueous interior of the cell (the *cytosol*) with the solution on the outside of the cell. Thus, the cells can maintain their own composition, the "milieu interne" in the words of the famous 19th century French physiologist Claude Bernard, protected from the external medium.

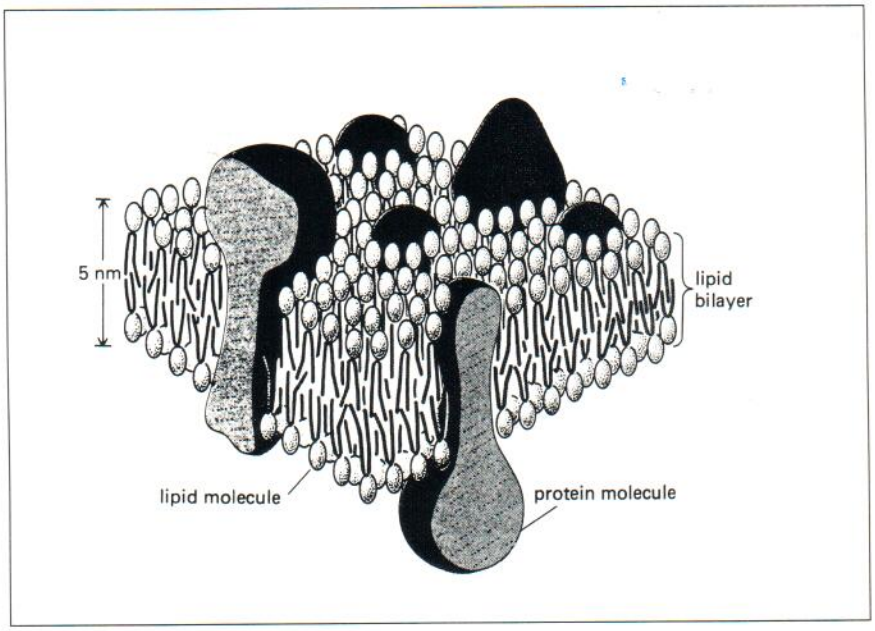


Diagram of the structure of a membrane, showing the lipid bilayer composed of the tadpole-like lipid molecules in which protein molecules are embedded. Note that the lipid molecules in the two layers are oriented in opposite directions, so that their tails, the most hydrophobic portion of the lipid, form the interior of the membrane bilayer.

Most cells also contain internal membranes that divide their interiors into various compartments, which have highly specific functions in the life of the cell. For example, the nucleus contains the genetic material and is concerned primarily with its duplication, care and maintenance; the *mitochondria* are the powerhouse of the cell where food fuels are burned and converted into useful energy; and the *sarcoplasmic reticulum*, found only in muscle cells, serves to control the cytosolic concentration of calcium and thereby regulates muscle contraction. The subdivision of the cell interior into various membrane vesicles or "organelles" allows chemical reactions which would ordinarily interfere with each other to go on at the same time and provides special environments where reactions that otherwise would not be possible can occur.

Walls must have doors

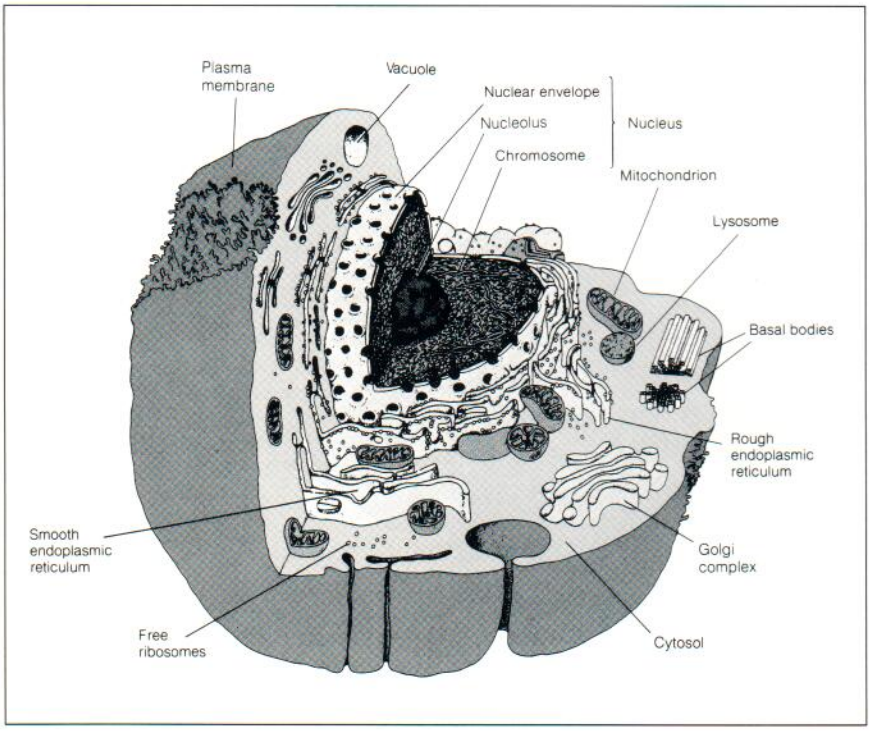
A house would be useless if it didn't have doors through which we could enter from the street and pass from room to room. In the same way, a cell would not be viable if there were no way of conveying substances from one side of its membranes to the other. Indeed, transport of many different types of molecules through the membranes that surround and subdivide cells is essential to the healthy function of the body. In order to sustain the living state, cell membranes therefore have two important attributes: they serve as protective barriers against the environment and yet they allow passage of specific ions and molecules for food and for communication between cells. Given that membranes are composed of hydrophobic lipids, which serve as barriers to water soluble (*hydrophilic*) molecules, whereas the molecules that the cell needs to bring through the membranes are hydrophilic, how can this be accomplished? The answer lies in specialized proteins that are embedded in the lipid bilayers of membranes and have been designed to provide highly specific pathways (or *channels*) for the transport of certain molecules through the membrane. A very important feature of all membrane channels is their extremely high

specificity. Each channel allows only one kind of molecule or ion to pass through, in the same way as the front door of a house only admits the person who has the correct key. A second critical feature of many transport pathways is that they can be modulated by other molecules and thereby be turned on or off.

The study of membrane transport is currently a "hot" field, as shown by the fact that last September the Federation of European Biochemical Societies (FEBS) organized a special course on this topic at Lake Balaton in Hungary, at which Dr. Hartmut Wohlrab, Senior Scientist at BBRI, was one of the instructors. As stated by Ernesto Carafoli of the Swiss Federal Institute of Technology in Zürich, another instructor in the FEBS course: "Nobody really knows how the various molecules are transported through these highly molecule-specific transport proteins." In this report, we describe some of the research at the Boston Biomedical Research Institute that addresses this important problem.

There are many different kinds of membrane channels

Although it would seem that all membrane channels should be basically the same, essentially submicroscopic pores in the double-layered membrane through which specific molecules can pass back and forth, nothing could be further from the truth. In fact, there are many kinds of membrane channels whose function differs fundamentally. For example, the research at BBRI which is described in this report deals with three quite different types of channel. The first, discussed on pages 6 - 9, is essentially a floodgate which can be opened or locked shut and through which a specific ion flows from an intracellular membrane compartment into the cytosol so as to control muscle contraction. The second type of channel to be discussed (pages 10 - 11) differs from the first in that it involves an active transport process rather than the passive flow of molecules. The molecule of interest is carried from one side of the membrane to the other by the flow of another substance in the same direction. Instead of a floodgate, this type of membrane channel resembles the lock in a canal through which a ship is conveyed by the flow of water in the appropriate direction. Finally, we will describe (pages 12-13) an even more complex kind of channel, in which the passage of hydrogen ions through the membrane actually drives the synthesis of an essential substance. Perhaps the best analogy for this third type of channel is a water wheel or steam turbine driving an electric generator.



Cut-open view of a typical animal cell. The gray outer coating is the plasma membrane. The cytosol is studded with many types of organelles - like a pudding with raisins - most of which are also surrounded by membranes. The sarcoplasmic reticulum, unique to muscle cells, is not present in the cell shown.

Perhaps the most immediate message that the reader will glean from reading these accounts is how surprisingly little we know about fundamental life processes, how many basic questions remain to be answered before we can fully understand the functions of even a single cell. As scientists our life is dominated by this search for knowledge, a search which has special urgency because it is clear that this knowledge will ultimately provide the power to understand, prevent, and cure disease. Many diseases are caused by defects in membrane channels, and this report describes some areas where research at BBRI may have a significant impact on future therapies.

HOW CALCIUM CONTROLS MUSCLE CONTRACTION

Muscle contraction is controlled by the movement of calcium in and out of the sarcoplasmic reticulum of muscle cells

It has been known for more than 30 years that calcium ions (i.e. calcium atoms carrying a positive electrical charge) control the contraction and relaxation of the specialized muscle cells known as muscle fibers. In relaxed muscle cells, the concentration of calcium ion in the fluid interior of the muscle fiber, the so-called cytosol, is only one ten-thousandth as much as in the fluid outside the cells and in the blood. The reason for the very low calcium levels in relaxed muscle fibers is that calcium ion is constantly being pumped from the cytosol into the interior of the sarcoplasmic reticulum. This active transport of calcium is mediated by a specific molecular pump, which is driven by the breakdown of ATP, an important biological molecule whose function and synthesis is discussed in more detail on page 12. For each molecule of ATP broken down, two calcium ions are moved from the cytosol into the sarcoplasmic reticulum. On the other hand, when muscle fibers are stimulated to contract, the calcium stored in the sarcoplasmic reticulum is suddenly released, and the cytosolic calcium ion concentration increases about ten-fold. The process of calcium release is extremely rapid, because calcium moves through an opened gate from an internal pool of high concentration to an extremely dilute calcium solution surrounding the fibrils in the muscle fiber, like water rushing through an opened floodgate.

Noriaki Ikemoto

A huge protein complex, the so-called *junctional foot protein*, has been identified as the floodgate for calcium release or - in technical language - a *gated calcium channel*. A useful tool for elucidating the role of the foot protein complex in excitation-contraction coupling is ryanodine, a potent insecticide isolated from the stems of a South American shrub. Ryanodine kills insects by paralyzing their muscles because it binds strongly to the calcium channel proteins.

Dr. Noriaki Ikemoto, the Amelia Peabody Senior Scientist at BBRI, and his colleagues have investigated the functions of sarcoplasmic reticulum, utilizing vesicles formed from isolated membranes as a model. One of the most important messages derived from their work is that these tiny vesicles of sarcoplasmic reticulum are capable of performing all of the functions essential for the regulation of the cytosolic calcium and for the regulation of contraction and relaxation of muscle cells. This makes it possible to study these processes in the test tube under highly controlled conditions.

Calcium release from the sarcoplasmic reticulum is activated *via* the plasma membrane

The calcium release channel of the sarcoplasmic reticulum is opened by a "remote control" mechanism *via* the plasma membrane. To understand this remote control mechanism, we must understand some electrical features of the muscle cell. In a resting muscle cell, there is a voltage difference across the plasma membrane, which makes the interior of the cell more negative than the outside, as if the inside and the outside of the muscle cell were connected to the - and + poles of a battery. This state, referred to as *"membrane polarization"*, is produced by an uneven distribution of several physiologically important ions, such as sodium and potassium, on the two sides the plasma membrane. As a matter of fact, the concentration of sodium is almost 100 times higher outside of the cell than inside, whereas the concentration of potassium outside is only one hundreth as high as that inside.

When a muscle is stimulated by its nerve, a local area of the plasma membrane around the nerve-muscle junction becomes highly permeable, particularly to sodium. This leads to a rapid and transient loss of the electrical potential across the membrane, as if the two poles of a battery were temporarily short-circuited, a process called *"membrane depolarization"*. By repeating this process successively in adjacent areas, a depolarization wave is propagated along the whole surface of the plasma membrane. But because the plasma membrane of skeletal muscle cells has special tubular extensions, called *transverse tubular membranes* or *T-tubules*, the

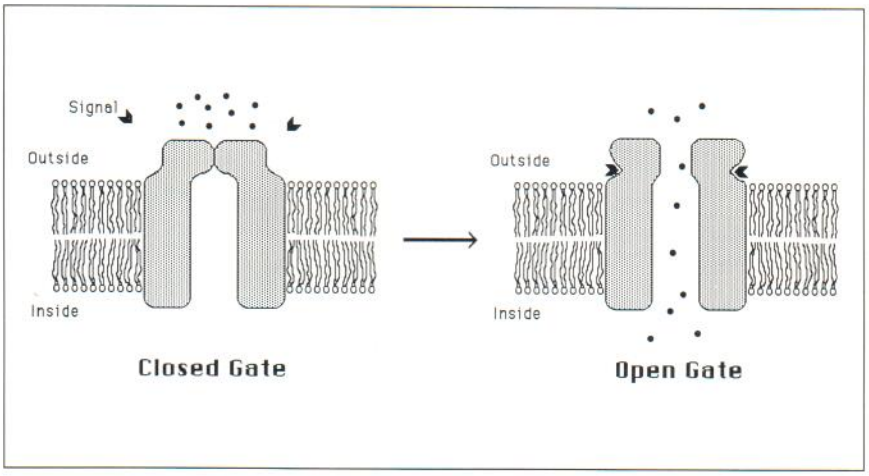


Diagram of a gated channel such as the calcium channel of the sarcoplasmic reticulum. The calcium floodgate is set in either an open or a closed position by a signal transmitted from the T-tubules to the junctional foot protein, shown in gray.

depolarization wave is also propagated into the interior of muscle cells. In the cell interior, the T-tubules make contact with the sarcoplasmic reticulum at junctions known as *triads*. When membrane depolarization progresses to the triads, the change in voltage causes the gated calcium channels, described in the preceding section, to open so as to flood the cytosol with calcium and initiate muscle fiber contraction. The above processes, initiated by the excitation of the plasma membrane leading to muscle contraction, are collectively called *excitation-contraction coupling* or *e-c coupling*, the terminology commonly used in today's muscle physiology laboratories.

Dr. Ikemoto has successfully isolated a membrane fraction consisting of closed vesicles of the sarcoplasmic reticulum in close apposition to vesicles of the T-tubule. A rapid change of the potential of the membrane of the T-tubule portion of the triad leads to the release of calcium from the sarcoplasmic reticulum at the same rate as that seen in the intact tissue, indicating that the excitation signal generated in the T-tubule membrane is in fact transmitted to the sarcoplasmic reticulum and opens the calcium channel's floodgate. Dr. Ikemoto believes that the isolated triad system, which is easier to study than the whole muscle cell yet retains the essential aspects of physiological e-c coupling, will resolve many of the unsolved questions relating not only to a fundamental mechanism but also to the causes of some muscle diseases.

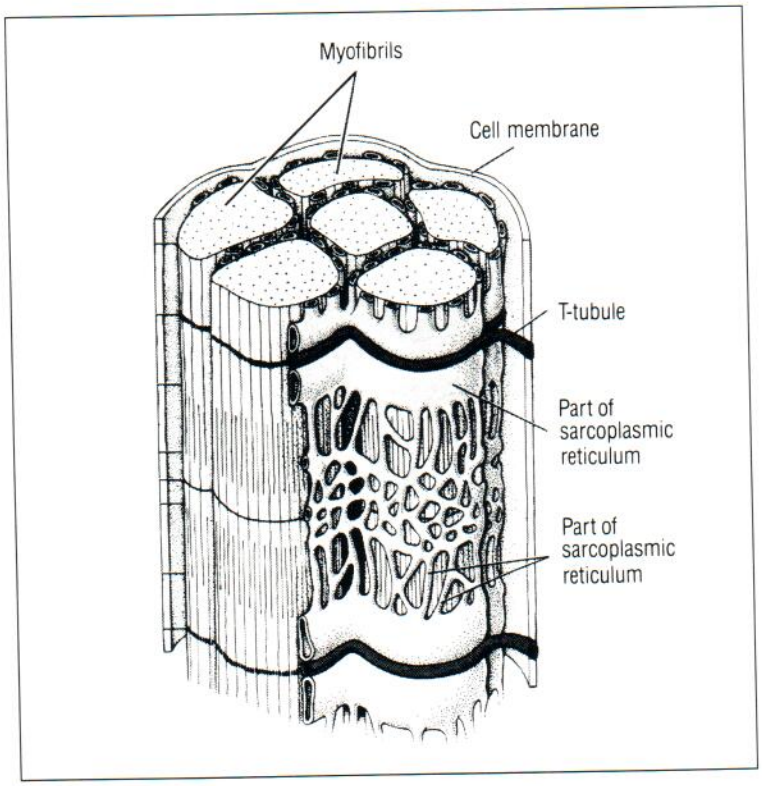


Diagram of muscle fibers with sarcoplasmic reticulum and T-tubules.

Answers to fundamental questions in biology

One of the most important unsolved basic questions in muscle physiology is how the excitation signal elicited in the T-tubule membrane is transmitted to the sarcoplasmic reticulum. Recent work by a number of investigators has identified a protein complex in the T-tubule membrane that acts as a voltage sensor and induces the opening of the gate in the foot protein. As a first step in elucidating the mechanism of this process, which at this time remains essentially a black box, Dr. Ikemoto and his colleagues have incorporated an optical probe into the junctional foot protein to monitor rapid changes occurring in the conformation of this protein during e-c coupling. Such studies will bring about a clearer picture of the actual mechanism by which calcium is released and muscle activation ensues. The solution to the complex biochemical problem of how a nerve impulse sent by our brain can bring about the movement of our arm or leg will then finally be within our grasp.

Understanding the cause of a potentially deadly muscle defect

Malignant hyperthermia is an insidious disease. Too frequently, otherwise normal carriers of this genetic disorder have been identified only upon their sudden "unexpected" death during surgery under anesthesia. In these patients certain commonly used anesthetics induce skeletal muscle rigidity and high fever (i.e. hyperthermia), which, if not immediately reversed by appropriate treatment, lead to severe tissue damage and death.

Fortunately, the discovery that certain strains of swine carry a corresponding genetic defect has allowed laboratory study of the underlying biochemical mechanism. Thus, it has been found that muscle fibers from patients or affected animals develop higher tension during contraction than the fibers of normal individuals and have a higher sensitivity to some contraction-inducing drugs such as caffeine and halothane. Earlier studies by Dr. Ikemoto's group with several outside collaborators showed that caffeine or halothane induced a much higher level of calcium release from vesicles of sarcoplasmic reticulum isolated from disease-positive swine muscle than those from normal muscle. Subsequent extensive studies of swine sarcoplasmic reticulum and isolated junctional foot protein by Charles Louis and his group at the University of Minnesota have shown that these abnormalities can usually be ascribed to altered properties of the foot protein. Indeed, according to recent studies by David MacLennan and his associates in Toronto, all cases of malignant hyperthermia in swine and many cases in humans are linked to mutations in the gene encoding the junctional foot protein. Not only will the identification of the biochemical defect in malignant hyperthermia allow physicians to screen individuals to determine whether they are afflicted with this potentially fatal genetic disorder, but the study of the malfunction of mutant foot proteins will also provide important insights into the mechanism underlying normal e-c coupling as well as the pathological processes in other muscle diseases.

Masafumi Yano, Roque El Hayek, Noriaki Ikemoto, Bozena Antoniu

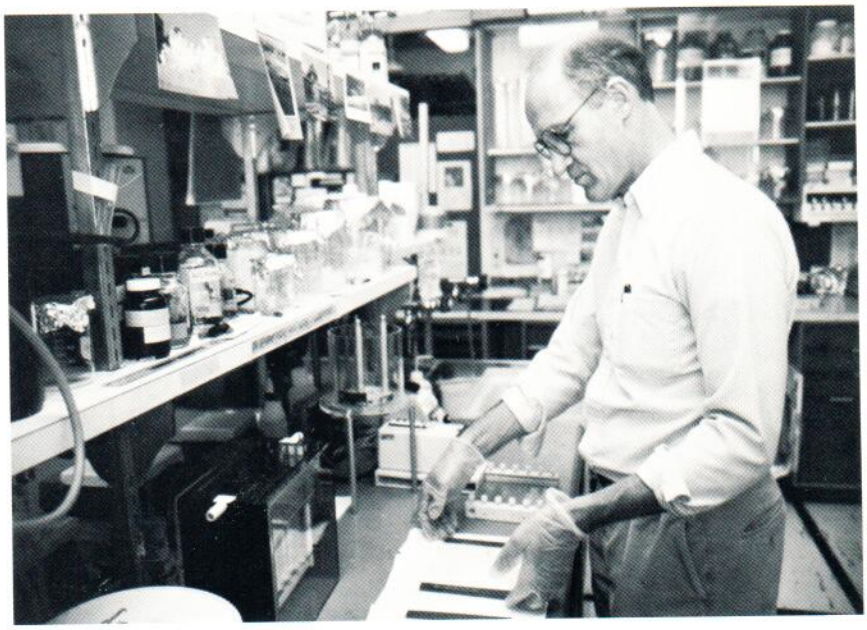
HOW SUBSTANCES ARE TRANSPORTED ACROSS MEMBRANES

The phosphate transport protein

The laboratory of Dr. Hartmut Wohlrab, Senior Scientist at BBRI, has concentrated for the last fifteen years on a protein that is responsible for the transport of inorganic phosphate. His laboratory was the first to identify this protein, to separate it from many other cellular proteins, and to incorporate it into membrane vesicles prepared from highly purified lipids. Studies of the phosphate transport protein were initiated for several reasons. The most important ones are that inorganic phosphate participates in many essential reactions in the human body and that it is a rather well-defined small molecule, making it easier to identify the chemical groups of the transport protein that interact with it. Another interesting feature of phosphate transport is that the phosphate molecule can be transported only if a proton is transported simultaneously through the protein. In other words, the transport of the phosphate is coupled to the transport of a proton (hydrogen ion). This type of coupled transport reaction occurs in many membranes of the body and also with other molecules, e.g., with various sugars, and its improper functioning is responsible for several human diseases.

Coupled transport processes

Very little is known about the molecular basis of such coupled transport mechanisms. But two recent advances provide a context in which Dr. Wohlrab's research can provide important insights into how the transport of protons and phosphate may be coupled in a single protein molecule. The first is the detailed studies in Gobind Khorana's laboratory at MIT on the light-driven transport of protons by a transport protein from purple bacteria. The second is the work by Florante Quiocho at Houston's Baylor College of Medicine on the three-dimensional structure of a bacterial protein that binds phosphate in a highly specific manner. Dr. Wohlrab hopes that the structural information provided by these studies will serve as guide for the genetic manipulation of the phosphate transport protein so as to determine which of the protein's components are essential for phosphate and which for proton transport and to determine how these separate functions are coordinated or "coupled".



Hartmut Wohlrab

Use of genetic engineering in the study of transport

With the aid of genetic engineering techniques, Dr. Wohlrab's laboratory has recently been able to identify the gene for the phosphate transport protein in the baker's yeast *Saccharomyces cerevisiae*, one of the organisms most highly developed for the manipulation of genetic material and thus also of proteins. These genetic and bioengineering studies have already led to basic new insights into pathways that protons can take in passing though a protein. Initial results suggest a pathway also for the phosphate, making speculations possible on how these two pathways are coupled. Dr. Wohlrab's findings have attracted considerable attention, and in recent months he was invited to give research seminars at the University of Pennsylvania, UCLA, Hong Kong Polytechnic, University of Bern, and the Biozentrum in Basel.

Most recently, Dr. Wohlrab's laboratory has been able to genetically modify bacteria so that they produce the phosphate transport protein normally found in yeast. Engineered bacteria can now produce sufficiently large amounts of this protein for the preparation of crystals. An analysis of X-rays diffracted by these protein crystals, carried out in collaboration with laboratories in Switzerland, Germany and Texas, will permit the determination of the three-dimensional structure of the phosphate transport protein.

Relevance of membrane transport for diseases

It is expected that the basic information gained from these studies will help identify molecular defects in bigger and more complex membrane proteins. Defects in such proteins are responsible for diseases such as cystic fibrosis, where a regulator of chloride ion transport seems to malfunction. Moreover, a large membrane protein encoded by the so-called multidrug-resistance genes is responsible for the development of drug resistance in many human tumors. This protein interferes with cancer chemotherapy by transporting the drugs out of the cancer cells before the cells can be killed. As we learn about the mechanisms by which proteins can carry specific molecules across membranes, we will also learn how to manipulate transport proteins so as to correct defects that cause disease or to block transport processes that interfere with cures.

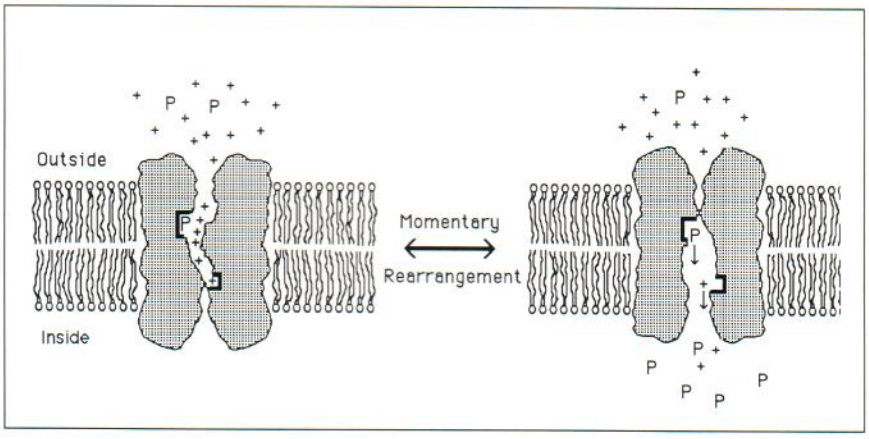
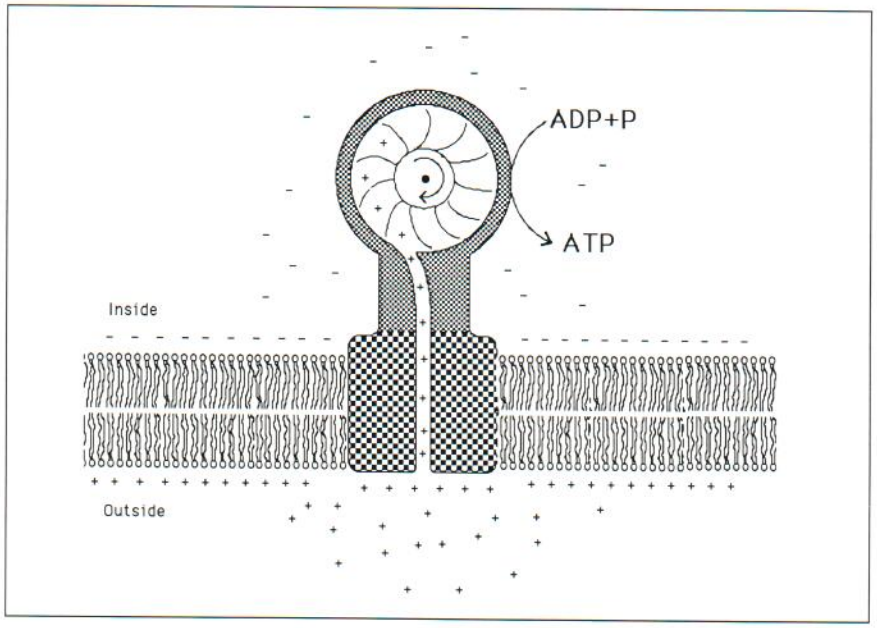


Diagram of a coupled transport system such as the phosphate transport protein. The protein channel (shown in gray) has separate sites (outlined in black) for binding phosphate (P) and protons (+). When both sites are occupied, the channel protein is thought to undergo a momentary rearrangement so as to allow phosphate and the proton to pass to the other side of the membrane bilayer.

HOW METABOLIC ENERGY IS HARNESSED BY MEMBRANE PROTEINS

Mitochondria are the power plants of the cell

There are two sides to metabolism. One is the chemical breakdown or "burning" of foodstuffs to generate energy; the other is the utilization of energy to synthesize essential body constituents and to drive many cellular processes such as muscle contraction, nerve conduction, and membrane transport. Within most animal cells, there are many small bodies called mitochondria, which are surrounded by a double membrane. An electron micrograph of a mitochondrion is shown on the front cover. It is in the mitochondria where food is ultimately "burned" to carbon dioxide and water with the release of chemical energy. But just as the burning coal in a power plant doesn't directly run the appliances in our homes, the energy generated by burning foodstuffs in the mitochondria cannot be used directly to drive cellular processes. Rather, the power plant burns coal so as to generate electric current which can be transmitted to our homes to run appliances; likewise, mitochondria convert the energy released during the metabolism of foodstuffs into the energy currency called ATP (adenosine triphosphate), which can be used elsewhere in the cell to do useful work. However, whereas the power plant uses a steam turbine for driving its electric generator, mitochondria use proteins embedded in their membranes for the synthesis of ATP.



Schematic representation of how ATP synthase associated with the mitochondrial membrane uses the flow of protons (+) to drive the synthesis of ATP.

How do mitochondria generate ATP?

In the last stages of the breakdown of foodstuffs in the mitochondria, positively-charged protons are driven out, leaving the interior of the mitochondria with a negative charge. The mitochondrial membranes are good insulators and hold the charge like a microbattery until ATP is needed for the cell's activities. Then a channel for protons opens in the membrane bilayer and as the protons reenter the mitochondrion, they somehow promote the synthesis of ATP from ADP (adenosine diphosphate) and inorganic phosphate. Essentially, the electrical energy of the "microbattery" is thereby converted to the chemical energy stored in ATP, which can be utilized by many cellular processes. This conversion occurs in a highly organized assembly of enzymes called ATP synthase, which is anchored in the mitochondrial membrane.

ATP synthase is an extremely complex membrane protein

ATP synthase is a very complex enzyme, which consists of at least eight protein subunits. Five of these subunits, collectively called F_1, contain the sites for the synthesis of ATP from ADP and phosphate. The remaining three or more subunits, the so-called F_o segment, form a channel through which protons can pass. The F_o segment is embedded in the membrane, with the proton

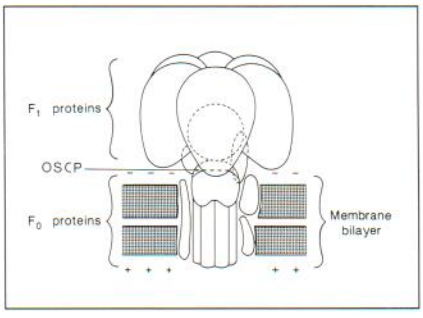
channel completely traversing the membrane, whereas the F_1 complex is attached to F_o and extends beyond the membrane into the interior of the mitochondrion, as shown in the accompanying diagram.

How does the passage of protons across the membrane through the F_o proton channel drive the synthesis of ATP by the F_1 complex? The answer to this central question must obviously lie in the communication between the F_o and the F_1 complexes. Dr. Saroj Joshi, a Senior Scientist at BBRI, has been studying this difficult and challenging problem by focussing on a protein with the seemingly arcane name "oligomycin-sensitivity conferring protein" or — more simply — OSCP. This protein lies at the F_o-F_1 interface, and, even though nominally part of the F_o segment, influences the properties of F_1, for example by conferring on F_1 sensitivity to inhibition by the antibiotic oligomycin, hence its name. OSCP is thus a potential candidate for the communication link between the F_o proton channel and the F_1 ATP synthase. Dr. Joshi was able to provide strong support for this notion by demonstrating that OSCP is not necessary for conduction of protons through F_o but is absolutely required for coupling the energy of the proton gradient to the synthesis of ATP by F_1. In order to study further the role of OSCP in the communication between the F_o and F_1 segments, Dr. Joshi has cloned the gene for OSCP in bacteria and found ways of producing large amounts of both the normal protein and mutant variants. It will thus be possible to study the effect of mutations on the ability of OSCP to couple the flow of protons to ATP synthesis and thereby subject this mysterious process to molecular analysis.

Saroj Joshi

Many degenerative diseases are caused by genetic defects in mitochondrial ATP synthesis

In view of the complexity of the process of mitochondrial ATP synthesis, which involves the concerted action of the products of many different genes, it is not surprising that genetic defects arise in which mitochondria can make ATP only inefficiently if at all. Since ATP is the organism's major energy currency, individuals with such genetic defects, known as *mitochondrial myopathies*, suffer from muscle weakness, tiring after even mild exercise, and often also from heart problems and central nervous system disorders. In many cases of inherited mitochondrial myopathies, the defective gene has been identified, and at least one is known to be caused by a mutation in one of the subunits of the F_o proton channel of the ATP synthase. In the light of this finding, Dr. Joshi's search for the biochemical link between the proton channel and the F_1 ATP synthase assumes special importance.



The arrangement of the protein subunits of ATP synthase. The F_o segment is embedded in the inner mitochondrial membrane, whereas the F_1 segment extends into the interior of the mitochondrion.

PUBLICATIONS

September 1992 - August 1993

An important part of research is the communication of scientific discovery so that the knowledge gained can help new research as well as benefit clinical studies directed towards curing or preventing disease. The dissemination of new research findings is achieved primarily by publication in scientific journals. Over the past year, BBRI investigators have published the following papers:

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Thank you!

In the year just past, BBRI's friends - individuals, foundations, and businesses - contributed well over $300,000 to help the Institute maintain momentum in its programs to attract and support outstanding investigators. In fiscal 1992 the costs of these commitments totalled just over $500,000 - less than 10% of our annual budget.

BBRI is fortunate to merit the trust and support of the many fine friends listed below. Each contributor shares our vision of a future that is better because of the new knowledge in basic medicine which they have helped us discover and disseminate.

This year I want especially to mention the exemplary support provided by Ernest and Mary Louise Henderson. Ernie is BBRI's Treasurer, and both Ernie and Mary Louise are members of our Corporation. Their creative support has made a wonderful difference, and we are most grateful!

We're proud to say that 100% of our Trustees and 88% of our Corporators have contributed this year. We hope that all of our Corporators and other friends will be on the list next year.

To each of you listed below, our heartfelt thanks.

Bill Tyler

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Boston Biomedical Research Institute, Inc.

BALANCE SHEETS

AUGUST 31, 1993 AND 1992

	1993	1992
ASSETS		
CURRENT ASSETS		
Cash	$ 209,681	$ 415,661
Grants receivable	5,553,563	3,409,939
Prepayments, deposits and other receivables	111,113	168,404
Investments, at market value (cost 1993 - $5,452,155 1992 - $4,927,072)	6,301,542	5,771,907
Total current assets	12,175,899	9,765,911
FIXED ASSETS		
Leasehold improvements	1,935,632	1,935,632
Research equipment	5,095,645	4,893,498
Total	7,031,277	6,829,130
Less accumulated depreciation	5,718,387	5,514,645
Net fixed assets	1,312,890	1,314,485
	13,488,789	$11,080,396
LIABILITIES AND FUND BALANCES		
CURRENT LIABILITIES		
Accounts payable and accrued expenses	$ 25,230	$ 31,032
Deferred grant income	5,900,306	3,643,024
Deferred fund (building)	115,702	115,702
Total current liabilities	6,041,238	3,789,758
FUND BALANCES		
Unrestricted	5,365,124	5,305,708
Restricted	769,537	670,445
Fixed assets	1,312,890	1,314,485
Total fund balances	7,447,551	7,290,638
	13,488,789	$11,080,396

Boston Biomedical Research Institute, Inc.

Statements of Revenues, Expenses and Changes in Fund Balances

For the Years Ended August 31, 1993 and 1992

	1993	1992
REVENUES		
Grants and contracts	$5,871,529	$4,256,384
Unrestricted contributions	126,293	137,432
Restricted contributions availed of in current period	121,402	203,829
Property and equipment purchased	202,147	111,014
Investment income		
Interest and dividends	175,731	211,138
Realized gains on securities	152,022	272,686
Unrealized gains on securities	4,252	95,173
Total	6,653,376	5,287,656
EXPENSES		
Salaries and benefits	4,025,907	3,433,771
Occupancy costs	760,000	600,000
General support and services	1,296,963	874,560
Fixed assets purchased	202,147	111,015
Development	69,280	74,931
Depreciation	203,742	235,855
Total	6,558,039	5,330,132
EXCESS OF REVENUES OVER EXPENSES (EXPENSES OVER REVENUES)	95,337	(42,476)
Restricted contributions	182,978	375,000
Restricted contributions availed of in current period	(121,402)	(203,829)
FUND BALANCES, BEGINNING OF YEAR	7,290,638	7,161,943
FUND BALANCES, END OF YEAR	$7,447,551	$7,290,638

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

Boston Biomedical Research Institute, Inc.

GRANTS, CONTRACTS AND FELLOWSHIPS

Principal Investigator	Title	Duration of Grant	Total Award
NATIONAL INSTITUTES OF HEALTH			
Program Project Grant			
Dr. Wang	Molecular mechanism of smooth muscle regulation	9/92 - 8/97	$ 6,000,000
Research Grants			
Dr. Badwey	Synergistic stimulation and priming of neutrophils	7/90 - 6/95	960,000
Dr. Badwey	Enzymes modulating second messengers in neutrophils	4/93 - 3/97	645,000*
Dr. Coleman	ATP binding site photoaffinity probes for F_1-ATPase	6/92 - 5/96	748,000
Dr. Gergely (MERIT)	Biochemistry of muscle contraction	7/89 - 6/94	2,844,000
Dr. Grabarek	Calcium binding protein/target interactions	6/92 - 5/95	600,000
Dr. Graceffa	Smooth muscle and non-muscle caldesmon	5/93 - 4/97	728,000*
Dr. Ikemoto	Structure and function of sarcoplasmic reticulum	7/92 - 6/96	1,674,000
Dr. Joshi	Molecular mechanisms of mitochondrial ATP synthesis	9/92 - 8/95	802,000
Dr. Lehrer	Tropomyosin and myosin interaction in muscle	12/90 - 11/95	1,546,000
Dr. Lu	Structure-function relation in myosin	9/91 - 8/95	819,000
Dr. Pande	Protein glycation: structure and stability of products	7/91 - 6/94	548,000
Dr. Paulus	Control of diaminopimelate and lysine biosynthesis	4/93 - 3/97	1,160,000*
Dr. Prevelige (Shannon)	Subunit interaction during icosahedral capsid assembly	9/92 - 8/94	100,000
Dr. Raso	Targeting toxins with acid-triggered hybrid antibodies	12/89 - 11/94	1,234,000
Dr. Raso	Model to test the therapeutic value of toxin conjugates	9/92 - 8/95	769,000
Dr. Stafford	Engineered anti-breast cancer single-chain Fv immunotoxin	6/90 - 5/95	646,000
Dr. Tao (MERIT)	Proximity relationship among muscle proteins	4/91 - 3/96	1,359,000
Dr. Volloch	Antisense intron as modulator of gene expression	12/88 - 11/93	1,289,000
Dr. Wang	Comparative study of troponin C and calmodulin	7/88 - 6/94	631,000
Dr. Wohlrab	Proton-coupled inorganic phosphate transport	4/92 - 3/96	1,181,000
Fellowships			
Dr. Kalapos	Identification of replication origin in the dystrophin gene	8/92 - 7/94	71,000
Dr. Roten	Characterization of Bacillus subtilis aspartokinase I	9/92 - 8/94	53,000
NATIONAL SCIENCE FOUNDATION			
Research Grant			
Dr. Paulus	Regulation of amino acid biosynthesis	3/92 - 8/94	160,000
AMERICAN HEART ASSOCIATION			
Research Grants			
Dr. Joshi	Role of OSCP in mitochondrial energy coupling	7/91 - 6/94	132,000
Dr. Tao	Structure and function of genetically engineered calponin	7/92 - 6/95	132,000
Dr. Wang	Caldesmon-myosin interaction in smooth muscle regulation	7/90 - 6/94	114,000
MUSCULAR DYSTROPHY ASSOCIATION			
Research Grant			
Dr. Ikemoto	Excitation-contraction coupling in malignant hyperthermia	7/91 - 6/94	126,000
OTHER			
Research Contract		3/93 - 2/94	424,000*
Dr. Codington	Carcinoma assay research project		
Fellowship		9/92 - 8/94	40,000*
Dr. Paulus	Support for predoctoral fellowship		

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