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Cell Biology:
Basic Research and Applications

Panel on Cell Biology

Board on Basic Biology

Commission on Life Sciences

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This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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Cover: An animal cell, as interpreted from electron micrographs. From *Biology* by Helena Curtis, Worth Publishers, Inc., New York, with permission. See Appendix A for a labeled diagram and a comprehensive caption.

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APR 6 1988

PANEL ON CELL BIOLOGY

GEORGE E. PALADE (*Chairman*), Yale University School of
Medicine, New Haven, Connecticut

GIUSEPPE M. ATTARDI, California Institute of Technology,
Pasadena

LAWRENCE BOGORAD, Harvard University, Cambridge,
Massachusetts

ROBERT T. FRALEY, Monsanto Company, St. Louis, Missouri

NORTON B. GILULA, Research Institute of Scripps Clinic, La Jolla,
California

THOMAS D. POLLARD, The Johns Hopkins University Medical
School, Baltimore, Maryland

FRANCIS H. RUDDLE, Yale University, New Haven, Connecticut

WALTER G. ROSEN, *Project Director*

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ARTHUR C. UPTON, New York University Medical Center, New
York, New York

JOSEPH E. VARNER, Washington University, St. Louis, Missouri

ALVIN G. LAZEN, *Executive Director*

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PREFACE

During the summer of 1985, a group of state-of-the-art reviews was initiated by the National Research Council (NRC) at the request of the National Science Foundation. The purpose of these reviews is to assess and monitor world trends, relative strengths, and competitiveness of the United States in rapidly evolving areas of science and technology. Particular emphasis was placed on developments that influence the rate at which these fields evolve. Three study areas—cell biology, pure and applied mathematics, and materials science—were chosen for review. The study on cell biology was conducted by the Panel on Cell Biology under the auspices of the Board on Basic Biology of the NRC's Commission on Life Sciences.

Cell biology had its beginning three centuries ago, but it developed little until the late 1940s. Since that time, advances in cell research have proceeded at an unusually rapid rate, on a broad front, and in many diverse directions, ranging from genes to extracellular matrices. As a result, we have at present a much deeper understanding of basic life processes than we ever had in the past.

From the beginning of its modern history, cell biology has stressed convergent structural, biochemical, and functional approaches in the study of cells and subcellular components. This type of integrated research was largely responsible for the merger of traditionally distinct biological disciplines and for the creation of a continuum of knowledge that stretches at present from biochemistry to organismic biology. In this continuum, cell biology has merged over the last decades with molecular biology and molecular genetics to create a common conceptual and methodological base for practically all other life sciences. Notwithstanding these mergers, cell biology has retained a central position in the realm of biological sciences: it has been the area in which many new research topics were identified, and it has provided the natural context for the validation of essentially all new findings.

The spectacular advances in cellular and molecular biology have already led to numerous practical applications in medicine and in animal

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and plant agriculture. Moreover, it is clear that we are now only at the beginning of the process by which knowledge gained from basic research is transferred to various applications.

The report of this panel comes at a time in the development of cell biology when opportunities for further advances and new discoveries are greater than they ever have been. But this is also a time when further growth of fundamental knowledge is not necessarily given a high priority in national policy. The crucial issue we are facing is how to maintain momentum in discovery and training and how to use to advantage the impressive body of knowledge and the powerful technologies we have already created. If we fail, there can be little doubt that our recent advances will be used to full advantage by our competitors.

The panel is grateful to the following people for their contributions of scientific expertise: J. Brackett, Robert Collier, Daniel Connolly, Gerald Galluppi, Alfred Gilman, Luis Glazer, Ira Herskowitz, Thomas Kasser, C. Kotts, Gwen Krivi, Paul Lazarow, Alan Levine, Pamela Manning, Robert Manning, Philippa Marrack, Arthur McGrath, Howard Rasmussen, Gottfried Schatz, and Joseph Varner.

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In particular, the panel wishes to acknowledge Walter Rosen, Frances Peter, and Kathy Marshall of the National Research Council staff for their able assistance in the preparation of this report. Thanks are also due to the science councillors at various U.S. and foreign embassies, who provided information on cell biology in their countries, and to William Walsh, who collected and analyzed data.

George E. Palade, *Chairman*
Panel on Cell Biology

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EXECUTIVE SUMMARY

A panel of seven experts examined developments, trends, and issues in cell biology. Faced with limited time and resources, the panel did not attempt to provide a comprehensive coverage of contemporary cell biology, but instead focused on selected areas in which research appears to be remarkably active, productive, and promising. The panel also examined applications in medicine and agriculture; facilities, support, and instrumentation needs; and the status of cell biology and its support in other countries.

BASIC RESEARCH

Cell biology is the study of the structure, chemistry, and function of cells and cell organelles (see cell diagram in Appendix A). It is a large field of research that has undergone dramatic growth in the last three decades and promises to continue to grow impressively for at least two or three more. It was only in the middle of the last century that cells, first seen much earlier, were recognized as the fundamental units of biological structure and function. Since then, studies have provided a description of the cell's major parts, defined their chemistry, and identified their functions. Current research is leading to an ever more complete understanding of the molecular basis for those functions and their regulation in terms of molecular interactions.

As our knowledge of cell biology has grown, the boundaries of the discipline have become less sharply defined. At the molecular level, where an ever-increasing amount of research activity is directed, cell biology interacts and often overlaps with chemistry, biochemistry, biophysics, and molecular biology, including molecular genetics. At a higher level of biological organization, cell biology functions as the starting base for neurobiology, immunology, and organ physiology, to name a few of its interfaces. Therefore this report covers issues relevant to many different disciplines.

The first section of this report describes the current status of our knowledge regarding the organization and the control of expression of the

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cell's genome as well as the structure and function of cell membranes and cell organelles (e.g., ribosomes, endoplasmic reticulum, mitochondria, and chloroplasts). Cell motility and cell-to-cell interactions are also discussed.

Knowledge of how the cell's genome is organized and how the expression of its genes is controlled during the cell's life cycle and during embryonic development (including cell differentiation) is advancing much more rapidly than anticipated 10 or 15 years ago. Our understanding of how cell membranes function to control the internal cell environment, to regulate interactions with the environment, and to control protein traffic (and hence, growth and differentiation) is growing rapidly. We are advancing steadily toward a full understanding of the production of adenosine triphosphate from sunlight in photosynthesis and from chemical sources in respiration.

We are also gaining an understanding of the ways in which cellular architecture is established and maintained and the way it relates to cell motility and to tissue and organ structure. The mediation and regulation of cellular interactions both within and between organisms are also being elucidated.

Following are examples of the kinds of questions currently being addressed at the molecular level by cell biologists:

- How do cells regulate the activity of their tens of thousands of genes?
- How do signal molecules coming to a cell from the other cells of an organism or from the environment exert their regulatory influence?
- How do such molecules affect the activity of specific genes in target cells?
- How is gene activity controlled during the process of differentiation of specialized cells (e.g., neuronal, muscular, epithelial, or glandular cells) in a multicellular organism?
- How do macromolecules synthesized in one part of a cell find their way to a site elsewhere in the cell, or in another cell, where they perform their biological function?
- What is the molecular basis and what are the molecular dynamics of cellular motility?
- What is the molecular basis for the capture, storage, transport, transformation, and release of the chemical energy required to power various life processes?

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- What processes regulate the cell-to-cell interactions responsible for the immune response, sexual reproduction, and development?
- What processes regulate cell division, and what processes lead to cell aging and death?

APPLICATIONS

The results of basic research in cell biology are already being applied on a large and expanding scale in medicine and in plant and animal agriculture. They have also been used extensively in the development of monoclonal antibodies, in cell culture for crop improvement, and in commercial biotechnology, e.g., in the production of human hormones and enzymes (in bacteria, yeast, or higher eucaryotic cells).

FINANCIAL AND LOGISTIC SUPPORT

The United States leads the world in research and training in cell biology. However, in a few areas of research (e.g., on mitochondria and on photosynthetic membranes), the United States is lagging behind other countries (e.g., Sweden, Switzerland, the Federal Republic of Germany, and Australia). Unfortunately, data on national and foreign support of cell biology could not be effectively analyzed because of the lack of uniformity in the use of the term by U.S. agencies and its absence from the lexicons of most other countries.

The panel identified the following factors as matters of particular concern with respect to the continued vigor of the U.S. research effort in molecular and cell biology:

- Support for training has declined in recent years, posing what is perhaps the most immediate threat to maintenance of research progress.
- Research on photosynthesis and other aspects of molecular and cell biology of plants is inadequately supported when one considers the basic role of plants in human welfare and the potential for progress based on the present state of knowledge.

The panel agreed that contemporary cell biology does not require and would not be well served by the development of new, large, national or regional centers supplied with extensive arrays of heavy instrumentation. Unlike fields such as radioastronomy, particle physics, and biological oceanography, basic research in cell biology benefits from the present decentralized system, whereby collaboration is initiated by individual

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investigators when they perceive the need. However, there is a need for state-of-the-art facilities providing advanced technologies, such as those required for protein and nucleic acid chemistry and monoclonal antibody production, for shared use by small groups of active investigators in cell biology. Such facilities will require not only up-to-date instrumentation but also competent personnel, for which there are currently no adequate sources of support.

Research in cell biology increasingly requires access to transgenic organisms, gene libraries, cDNA libraries, computerized data banks, high resolution microscopy, and high performance computers. Consideration should be given to providing access to such costly resources by organizing them as national or regional service facilities.

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INTRODUCTION

Cell biology is the study of the structure, chemistry, and function of cells, cell organelles, and other subcellular components (see cell diagram in Appendix A). It is a multifaceted, yet coherent discipline that originated at least 300 years ago when Robert Hooke first described cells. The boundaries of this discipline are not always clearly delineated, however. For example, the molecular events that occur within cells are clearly a part of the biology of cells, but they are often studied by investigators who may call themselves biochemists, molecular biologists, or molecular geneticists. The biology of nerve cells may be studied by neurobiologists, and cells of the immune system are investigated by immunologists. Moving from the cell to higher levels of biological organization, we find that there are also ill-defined boundaries between cell biology and such disciplines as physiology, immunology, and endocrinology. In this report, we have chosen to define cell biology broadly—that is, to include within its boundaries appropriate segments of the disciplines with which it interfaces. Thus, we have included molecular biology within the scope of this report, primarily because work at the molecular and cell levels has provided a continuous body of knowledge based on a common set of concepts and technologies.

Basic biological sciences, including cell biology, are in the midst of a true revolution that started about three decades ago and proceeded over the years with vigor. The revolution continues, fueled by the recruitment of bright young scientists and by the prospects of rapid application of some of the most fundamental discoveries.

The fundamental biological unit of all living organisms—plant and animal—is the cell. To a surprising degree, all cells are similar in design and function, whether in humans or oaks or in simple single-cell organisms such as bacteria. One major difference, however, is the presence or absence of a distinct compartment, the nucleus, for the genome. Cells with a nucleus, called eucaryotes, are found in advanced single-cell organisms and multicellular organisms. Those without nuclei, called procaryotes, include such simple single-cell organisms as bacteria.

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The importance of the cell as a biological unit is made clear when we consider the life cycle of advanced multicellular organisms such as human beings. The cycle begins with the fusion of egg and sperm, themselves single cells of specialized types, to form the one-cell embryo. At the earliest stage of our life cycle, therefore, we exist as a single cell. This cell divides into two cells, each of those into four, and so on to give rise to the adult organism, which may contain as many as 1 million billion cells. Every one of these cells is autonomous in some functions and is dependent or interdependent in others.

The entire developmental process is regulated by the myriad interactions within and among individual cells. These interactions regulate the capacity of cells to multiply, to differentiate into the hundreds of different cell types that make up our bodies, and to organize themselves into tissues, organs, and finally the human body itself, according to a specific, well-defined architectural plan. Even when adulthood is attained, cellular development continues. For example, blood cells are continually produced, and they differentiate, function, senesce, and die. The same process characterizes many other cells types that continually renew themselves. As aging continues, these renewal processes also continue, but at declining rates, eventually resulting in the overall aging of the organism and ultimately death. A generally similar developmental process is found in all multicellular organisms—both animals and plants.

This cycle of activity is characteristic of normal healthy individuals, but as we are aware, the process can go awry, leading to abnormal states characterized by sickness and disease in humans, animals, and plants. Since both normal and abnormal progression of the life cycle is governed in a fundamental way by cells, it is logical that to understand and to exert control over these phenomena and, in a real sense, over our own lives and biological environment, it is necessary to learn all we can about the cell and how it conducts its activities.

The early, largely descriptive phase of cell biology was later augmented by a reductionist strategy similar to that generally followed in biochemistry. The complex cell was fractionated into its subcellular components, and the chemistry and functions of the separate organelles and other fractions were studied. The analysis of cell organelles progressed in time to the level of the macromolecular assemblies, macromolecules, and molecules (e.g., nucleic acids, proteins, and lipids) that they contain. The approach has been remarkably productive, primarily because cell organelles are so well constructed that they emerge from the trauma

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of separation still able to perform complex, highly integrated functions such as contraction, transport of molecules across membranes, photosynthesis, the production of adenosine triphosphate, protein synthesis, and gene transcription, to cite only a few important examples. This strategy thus yielded a biochemical inventory and, in addition, a rather detailed functional characterization of each organelle showing that functional specialization and division of biochemical and biophysical labor prevail at the subcellular level.

At the same time, studies of reconstituted cell fractions demonstrated that most macromolecular assemblies are generated by the self-assembly of their component molecules. More recently, this approach has been used to demonstrate that cell organelles can interact *in vitro* to reconstitute a specific intracellular process. A striking feature of the current state of research in molecular and cell biology is the remarkable degree to which the effectiveness of the reductionist strategy has been confirmed by the reconstitution approach.

As in many other instances in the history of science, recent progress in cell biology has depended on newly developed instrumentation and technologies. The first of these to affect the field were electron microscopy and cell fractionation procedures. Both have subsequently been improved and diversified. They were followed by efficient procedures for the separation, purification, and partial characterization (amino acid composition and sequence) of proteins, and by novel procedures for the production of highly specific, monoclonal antibodies by hybridoma technology. Such antibodies (together with polyclonal antibodies produced in animals) are providing exquisite chemical tools for use in exploring a large variety of problems.

Further advances were made possible by the more recent development of procedures for isolating and sequencing DNA and RNA molecules, for copying messenger RNA (mRNA) into complementary DNA (cDNA), for cloning the latter in procaryotic cells (for mass production), and for producing (via appropriate nuclease treatments) cDNA and gene libraries. cDNAs can be used as probes to identify specific genes in gene libraries, and these genes can be modified *in vitro* by genetic engineering (i.e., through deletions, recombinations, or point-specific chemical mutations) to create probes that upon transfection in intact cells make possible the study of gene functions and gene products (proteins, RNAs) down to a resolution of one nucleotide or one amino acid substitution.

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At present, these and other associated procedures constitute a technological matrix that can be entered either from the gene or the protein end to provide specific, powerful tools for the analysis of a wide variety of fundamental problems in molecular and cell biology. Moreover, the amount of information obtained is already large enough to be incorporated into computerized data banks that are useful for further analysis and characterization of genes and their products.

Thus, the information revolution in molecular and cell biology has occurred in parallel with a supporting technological revolution. The power and breadth of the potential applications of these combined technologies guarantee high productivity in the field for at least two decades. But further technological advances in manipulating transfection and gene expression, among other techniques, can definitely be anticipated.

The following sections cover the current state of our knowledge of certain cell organelles and intracellular systems. The subjects discussed by the panel in this section are representative of the dynamic research being conducted in many areas of molecular and cell biology.

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STATUS OF BASIC RESEARCH

NUCLEAR GENES: REGULATION AND FUNCTION

Every cell carries within its DNA all the genetic information required to perform its normal functions. The DNA itself is composed of biochemical building blocks called nucleotides. In humans, there are approximately 3 billion nucleotides of DNA per cell. The nucleotides are strung end to end as long double helices of DNA, which are complexed with proteins. The resulting structures are called chromosomes. In all human body (somatic) cells, there are 46 chromosomes. The germ cells (sperm in the male; eggs in the female) that contribute to the embryos of the next generation contain only half the number of chromosomes—23.

The chromosomes of higher organisms are enclosed in a special cellular structure called the nucleus, which is surrounded by a double membrane (or nuclear envelope), the outer membrane of which is continuous with the endoplasmic reticulum of the cytoplasm (see section on Cytoplasm: Organelles and Functions). The inner and outer membranes of the nuclear envelope are continuous with one another at the periphery of numerous nuclear pores, which provide openings from the nuclear space to the cytoplasmic space. Many details of nuclear function are still obscure. In general, the nucleus appears to provide attachment sites for the chromosomes and influences the relationship among chromosomes, their movement, and possibly their function. The nuclear pores themselves appear to influence the traffic of molecules in and out of the nucleus, and as such may exert a profound influence on the regulation of cellular function. Currently, the composition of the nuclear envelope and the nuclear pores is under investigation, and important advances have been made concerning the identity of proteins associated with them.

The chromosomes are highly complex entities, each consisting of a long single DNA double helix complexed with a multitude of various proteins. If extended to its full length, the DNA strand of a single chromosome would be approximately 1 to 2 inches long. This is remarkable, since most nuclei are about 10,000 times smaller than this in diameter,

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meaning that chromosomes must be highly folded and condensed in their native state and in their location within nuclei. The ability of chromosomes to fold up in an orderly way is mediated primarily by the histones, a major class of nuclear proteins. A second major class of nuclear proteins is responsible for the precise replication of chromosomal DNA during cell division. These replication factors are still being identified and characterized, and represent an important area of current research. The third category of nuclear proteins encompasses those that govern gene expression. The nuclear gene regulatory proteins are being analyzed by many laboratories with great success, as described below in some detail.

The DNA in each cell encodes a complete set of genes, which individually specify the constitution and behavior of each individual cell. Every cell of the human body contains an identical set of an estimated 100,000 genes. In recent years it has become possible to assign specific genes to particular chromosome sites in both plants and animals. This activity, called gene mapping, is improving our understanding of the biological properties of cells.

Gene Mapping

Gene mapping in humans and selected mammalian species has advanced impressively during the past decade. In humans, approximately 1,000 genes of known function have been mapped to some degree of precision and another 1,000 DNA markers of unknown function have been localized (de la Chapelle, 1985). Both physical and Mendelian recombinant methods have been used.

Physical mapping makes use of somatic cell hybrids and *in situ* hybridization techniques. The method is particularly effective in the assignment of genes to chromosomes and to parts of chromosomes. Moreover, the ordering of genes can be achieved with a precision of approximately 1 million base pairs (of 3 billion postulated).

Research Opportunities. If the number of human genes is indeed approximately 100,000, only 1% of them have been mapped to date. Over the past decade, the rate of gene mappings has doubled every second year. Currently, new genes are being mapped at the rate of one every day. The acceleration in data acquisition will probably continue as many new investigators enter the field and new methods are introduced. Two important new mapping activities are just now beginning: the formulation of maps based on overlapping DNA segments (cosmids

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or plasmid inserts) and the DNA sequencing of the nucleotide bases contained in these segments. These appear to be formidable undertakings, considering the total size of the gene complement (or genome): 3×10^9 base pairs in humans. However, improved technologies, such as automated DNA sequencing, the growth of computer data bases for information analysis, and the subdivision of the gene mapping activities among many laboratories, should permit completion of the mapping of the human genome within two to three decades. A number of scientists have recently advocated the creation of a national effort to ensure the completion of this project within the next decade.

The development of an ultimate resolution map should benefit research in many ways. It would contribute to an understanding of chromosome behavior in the context of DNA and chromosome replication, somatic cell division (mitosis), and germ cell division (meiosis). It would facilitate the isolation and analysis of any gene or DNA segment. It would contribute to an understanding of gene expression control and hence of developmental mechanisms. It would also probably provide insight into our evolutionary origins and knowledge of the evolutionary process itself. It would enhance our ability to predict risk for genetic disease and to understand the molecular causation of these conditions. The argnments for preparing a complete map of the human genome are applicable to the nuclear genome of major crop plants and domesticated animals raised for food.

Gene Mapping and Genetic Diseases

The introduction of restriction fragment length polymorphism (RFLP) DNA markers has led to a resurgence of interest in Mendelian or family study techniques for mapping human genes (Ruddle, 1981). RFLPs are variants in the nucleotide sequence of DNA that can be used as genetic markers. They are transmitted from one generation to the next in the same way as the genes that govern, for example, eye color. RFLP DNA markers can be used with great facility and simplicity for genetic analysis, and therein lies their great advantage and power. The RFLP approach permits a somewhat higher precision of gene ordering than the current physical methods, but its unique advantage is that it permits the mapping of genetic traits, such as eye color, that can only be studied in the whole organism.

The value of a human gene map has become apparent in the last several years. The map serves as a body of data that can be used

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to formulate and answer a broad range of biomedical questions. Its usefulness in generating and resolving hypotheses becomes more powerful as the data base grows. Recently, the map has been useful in establishing the connection between chromosome rearrangement and protooncogene activation. Protooncogenes are normal genes generally involved in cellular growth control, but they have the potential, usually through mutation, to cause cells to become cancerous. The gene map has also enabled investigators to detect linkage relationships between RFLP markers and specific genes that carry a risk for genetic disease. This can now be accomplished for many genetic diseases, such as various anemias, phenylketonuria, hemophilia, Huntington's disease, and many others.

The identification of linkage relationships between genes causing genetic disease and DNA markers can also be used to physically isolate genes whose mutants cause genetic disease. This has been accomplished recently for Duchenne's muscular dystrophy (Monaco *et al.*, 1985), and substantial progress is being made using the same approach for the Huntington's disease and cystic fibrosis genes. However, the molecular basis of these diseases is as yet unknown. It is likely that this information will become available through the isolation of the respective genes through a so-called reverse genetic analysis (Ruddle, 1982). In this new procedure, one first locates a particular gene by mapping, and then on the basis of its map position, one isolates and clones the gene of interest. An examination of the gene enables one to deduce the protein structure of the gene product and then, armed with this information, to determine its function. It is clear that by the application of the gene mapping and reverse genetics approaches, the underlying molecular basis of more than 1,000 human genetic conditions can be ultimately understood. Moreover, since a substantial number of these loci can be expected to affect biochemical pathways that regulate growth, differentiation, and morphogenesis, it can be predicted that human linkage analysis will also provide crucial insights into the developmental processes of human beings and their vertebrate kin.

Gene Regulation

Gene expression must in some manner be properly controlled during the life cycle of any organism. For example, hemoglobin is characteristically produced in red blood cells but not in other cells. Yet it is well established that the hemoglobin gene is present and potentially active

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in all cells of the body. Proteins of the photosynthetic apparatus are characteristically expressed in specialized leaf cells but not in cells of the root, for example. By what mechanisms are the genes in individual cells differentially expressed? Much new information is now available to help answer this question. It appears that genes are activated in two steps. First, the chromosome region that contains the gene in question is unfolded. This allows the gene to interact with factors and molecules that affect its expression. Gene expression in this context is frequently called *transcription* for the following reason. When genes are expressed, their nucleotide sequence (DNA strand) is first copied, or transcribed, into a complementary stand of ribonucleotides called messenger RNA (mRNA). The resulting mRNA is called a transcript. The transcript will later be *translated* into a protein molecule. This translational activity is discussed in a later section of this report.

In recent years, much has been learned about the life of a transcript from its formation, its participation in translation, and finally, its degradation. Recent research findings have shown that transcripts are significantly modified during their lifetimes and that such modifications importantly influence gene expression. When a transcript is first formed, its free end is chemically modified, or capped. Such capping is believed to stabilize the transcript. Immediately following formation, many transcripts are modified by the addition of a string of adenylnucleotides that are believed to influence the life expectancy of transcripts. Most but not all mRNA transcripts are polyadenylated. Recently it has been shown that transcripts are extensively modified by the precise deletion of internal segments. This has been termed mRNA splicing or transcript splicing. The splicing mechanism, still poorly understood, must proceed at a high level of fidelity, since an error of even a single nucleotide could lead to the formation of a defective transcript. Again, the biological strategy of transcript splicing is not completely clear. In general, however, the process may lead to greater flexibility in gene design and formation and provide schemes whereby the expression of genes may be more flexibly regulated, both quantitatively and qualitatively.

Promoters and Enhancers. For a gene to be transcribed, it must possess certain nucleotide sequences arranged in serial order along the DNA chain. The minimal elements are—starting at one end and reading from left to right—a promoter sequence, which binds an enzyme (RNA polymerase) that facilitates transcription, a coding region that specifies

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the mRNA transcript, and terminal sequences that serve to terminate and stabilize the transcript. Experiments in bacteria showed that the promoter region also serves as a binding site for specific proteins. These transregulatory proteins are coded by genes at distant sites and serve to regulate the transcription activity of specific genes.

New techniques, especially gene cloning and DNA sequences, have enabled investigators to demonstrate that a similar system of gene regulation exists in our own body cells as well (Brown, 1984). The promoter regions of the genes of higher organisms, including humans, have recently been shown to possess sites for DNA-binding proteins. A subject of active research and rapid advance is the characterization of these proteins and an explanation of their functional roles.

One class of binding site usually in the vicinity of the promoter region of genes has been called the enhancer. It has been given this designation, since if removed, the activity of gene expression is lessened. An important property of the enhancers of higher organisms is their tissue-specific action. Sequences in the promoter region of the human insulin gene, for example, bind proteins found in insulin-producing cells but not in cells that do not produce insulin (Ohlsson and Edlund, 1986). Such results suggest that tissue-specific gene expression may at least in part be regulated by DNA-binding proteins.

It will be extremely important to follow up these early leads, since such a mechanism could be fundamental to the processes of normal cell differentiation and tissue morphogenesis. The inappropriate expression of transregulatory proteins could lead to abnormal responses associated with disease. The demonstration of enhancers goes a long way toward explaining how cells can coordinately express large sets of genes physically separated on different chromosomes. That is, specific DNA-binding proteins may recognize and bind with a similar nucleotide sequence present in the promoter region of a number of different genes. In this way, a particular set of genes typical for a certain cell type would be activated or inhibited in a coordinated, programmatic fashion.

Transposons. Not only is gene regulation important in the normal course of the life cycle, but the inactivation and reactivation of genes are integral processes in the evolutionary history of organisms. It has been long understood that genes may be inactivated or reactivated by mutation that is mediated by chemical or physical processes. More recently, we have come to realize that a similar event can be mediated

biologically. Some DNA elements that have been detected, e.g., in maize, can move from one site to another in the chromosomes. These have been termed transposons or transpositional elements. When such elements insert themselves into or near a gene, they may completely inactivate it or alter its expression. Frequently, the process is completely reversible, resulting in gene reactivation. Transpositional elements have obvious implications for the pace and character of evolutionary change. In recent years, studies on the structure and functions of transposons have revealed similarities between these elements and certain RNA viruses. These comparative studies may enhance our understanding of both kinds of elements with resulting benefits, especially in the control and prevention of human disease, since it is known that certain human cancers may be induced by certain strains of RNA tumor viruses.

Research Opportunities. Future exploration of the transcriptional control of gene expression is likely to enhance our basic understanding of growth and differentiation in plants and animals and of the abnormalities in these processes that are manifested as cancer, birth defects, immunological disease, and other conditions. Complex relationships in the coordinated expression of genes in nuclear, mitochondrial, and plastid genomes of single eucaryotic cells are likely to be revealed as well (see other sections of this report). Important functional interactions between microbial pathogens and symbionts with their plant hosts appear to be exercised through transcriptional controls induced by one organism in the other. This research will lead to an understanding of important plant diseases and their regulation.

Gene Transfer

Our ability to analyze the mechanisms that govern gene control has been dramatically advanced by the recent development of different types of gene transfer techniques that permit various kinds of analyses. It is now possible, for example, to transfer specific genes into specific body cells either in tissue culture or in the animal itself. The genes can be introduced into the nucleus either mechanically by microinjection or chemically through the outer membranes of the cells (Gordon and Ruddle, 1985). In both instances, the transferred genes then reside in a chromosome, where they function in a manner surprisingly similar to that of native genes.

The crown gall disease of plants has been the basis of an effective gene transfer system for a number of plants. The treatment of plant

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cells with electricity is also a promising method for transferring genes into plant cells. Plants of a few crop species can be regenerated from single cells; introducing a new gene into the cells of such plants easily leads to a plant with new characteristics.

The gene transfer system provides many opportunities to study in detail the manner by which genes function. For example, one can remove the control region (promoter) of a gene a bit at a time to identify segments that are critical for normal function (McKnight and Kingsbury, 1982). One can switch the control from gene to gene, or within a gene, to cause genes to be expressed under circumstances in which they would normally be extinguished. In addition to telling us how genes function, these experiments also provide us with a means of correcting certain genetic diseases. For example, certain life-threatening anemias result from the absence or underproduction of hemoglobin in the red blood cells, which in a number of instances is due in turn to genetic defects in the globin genes. By gene transfer analysis, it has been possible to gain a much better understanding of the molecular bases of these conditions.

Research Opportunities. This information sets the stage for the invention of new drugs that may either overcome the initial defect or facilitate the activation and expression of related genes that may substitute for the genetically damaged ones. Another possibility is the substitution of defective genes by gene transfer directly into the patient's cells. This new form of therapeutic substitution of genes by the transfer of functional genes into a patient's body cells appears to be imminent. Tests in experimental animals have already shown its feasibility, and preliminary trials in human subjects can be expected in the near future. In plants, gene transfer systems have begun to be used to study the control of gene expression as well as to develop crops with new features.

Transgenic Animals and Regulation of Development

A second kind of new gene transfer system is very different from the method described above and has thus far been limited to laboratory and agricultural animals. Instead of using body cells (i.e., somatic cells) as recipients of the transferred genes, this system makes use of one-cell embryos (Gordon et al., 1980). In this procedure, the one-cell embryos are removed from the fertilized female, and genes are then inserted into their nuclei with microinjection needles. Immediately following injection, the embryos are returned to foster mothers, where

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they develop to term. The resulting animals are in many instances surprisingly normal and are known as *transgenic* animals, since they carry a transplanted gene. Studies of transgenic animals have increased our understanding of how genes function and promise to shed light on one of our greatest scientific challenges—development itself.

Although every cell contains a complete gene set, only part of that set is expressed in any one cell. For example, the set of genes expressed by muscle cells is different from that of liver cells, the set in seeds is different from that in leaves, and so on. Studies of transgenic animals are now providing important information on the mechanisms that regulate differential gene expression. They have shown, for example, that the promoter regions of certain genes contain information required for the tissue-specific expression of genes. If the promoter for a pancreas-specific gene is coupled to a pituitary gland gene, then that gene is expressed inappropriately in the pancreas (Ornitz *et al.*, 1985). If a promoter for a seed-specific gene is used, the gene will be expressed in a transgenic plant. These kinds of gene rearrangement studies are also providing detailed information on how the promoter induces tissue-specific gene expression. This is being accomplished by deleting, adding, and rearranging subsegments within promoters and then testing the results of these rearrangements in transgenic animals.

Transgenic Systems as Models for Animal Diseases

Another important application of the transgenic experimental system is the creation of animal disease models. For example, certain human oncogenes, or cancer-causing genes, can be introduced into transgenic animals where they induce specific tumors (Hanahan, 1985). This provides a convenient animal system in which the genesis and progression of the tumor can be studied. The same model may also be used to develop new methods for diagnosing and treating the human version of the tumor. This same approach is now being considered for experimental studies on acquired immunodeficiency syndrome (AIDS). In this disease, the viral agents appear to regulate host cell genes through a transregulatory gene product encoded by the virus. If this is an important mechanism in the causation of the disease, it might be possible to simulate AIDS in animals by transferring the viral gene into animal embryos. The construction of animal models for AIDS would represent a significant step in furthering our understanding of the disease process and in formulating an effective therapy and prophylaxis.

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CYTOPLASM: ORGANELLES AND FUNCTIONS

In a eucaryotic cell, the nucleus is surrounded by the cytoplasm, which in animal cells usually accounts for nine-tenths of the cell's volume and which itself is surrounded in turn by a cell membrane or plasmalemma (see Appeudix A). The cytoplasm contains within a matrix or cytosol a large number of minute, functionally specialized cell organs, or organelles, most of them present in multiple copies. The current state of knowledge concerning most of these cytoplasmic components is reviewed later in this report.

Many of the metabolic reactions of the cell are carried out in the cytosol. Foremost among them are the synthesis and degradation of amino acids and sugars and especially the synthesis of proteins.

Protein Synthesis and Regulation of Protein Traffic

Protein Synthesis. For all cells, protein synthesis is a major, continuous activity needed for the production of intracellular enzymes, contractile and cytoskeletal assemblies, membranes, ribosomes, chromosomes, and many other types of functionally important macromolecular assemblies. In more complex multicellular organisms, it is also needed for the production of export proteins, which are either enzymes, hormones, growth factors, blood proteins, antibodies, or components of the extracellular matrix, i.e., collagens, proteoglycans, and adhesive molecules such as laminin and fibronectin.

In all cells, proteins are synthesized on ribosomes, which translate into amino acid sequences the instructions received from active genes in the form of mRNAs. The ribosomes themselves are macromolecular assemblies of ribosomal RNA (rRNA) and protein molecules. The two sets of components are produced separately in the cytoplasm (proteins) and in the nucleus (rRNAs) and are modified and assembled in a special intranuclear organelle, called the nucleolus, before being transferred to the cytoplasm. The ribosomes are outstanding examples of macromolecular complexes produced by self-assembly; all the information needed for their production is already present in their component molecules. Self-assembly is a recurrent theme in the production of many other subcellular components.

In procaryotic and eucaryotic cells, ribosomes are basically similar and protein synthesis proceeds along similar steps. In eucaryotic cells, however, the ribosomes are larger, have more protein and rRNA molecules, and require more factors for their activity. These differences

are probably related to the emergence of more versatile regulatory pro-cesses, which in eucaryotes control translation of mRNAs in addition to gene transcription (Ochoa and de Haro, 1979). In procaryotes, only gene transcription is regulated.

Protein Traffic Control. In eucaryotic cells, ribosomes and protein synthesis occur primarily in a single-cell compartment, the cytosol, to which mRNAs have direct access from the nucleus and in which the pool of amino acids and all ancillary factors required for protein synthesis are located. Only 2% of the total protein production is accounted for by small mitochondrial ribosomes whose products are used exclusively in mitochondria (see section on Mitochondria: Function and Biogenesis). A somewhat larger fraction of the cell's protein is produced by chloroplasts (or other differentiated forms of plastids) in plant cells.

From the cytosol, their quasiunique site of synthesis, proteins are accurately directed to more than 20 different sites of final functional residence. These sites are membranes or compartment contents, each endowed with chemical specificity. It is clear that eucaryotic cells have evolved a highly efficient protein traffic control system, but the molecular mechanisms involved in its operation are only partially elucidated at present. This topic is discussed in more detail in a subsequent section (see section on ER Targeting System).

Membranes and the Regulation of Cell Function

The cell membrane is an extremely thin, sheet-like assembly of lipid and protein molecules that provides a boundary to the cell's body. This exquisitely delicate skin, called plasmalemma, is a diffusion barrier for water-soluble substances. In the plasmalemma, the cell assembles all the molecular equipment needed for its exchanges and interactions with the environment.

Procaryotic cells, such as bacterial cells, have only one or two mem-branes at the cell surface. In contrast, the eucaryotic cells of animals and plants have, in addition to their plasmalemma, at least a dozen dif-ferent types of chemically specific membranes that separate intracellular compartments in which they create the different microenvironments re-quired by various processes, such as respiration, photosynthesis, protein synthesis, and intracellular digestion.

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Membrane Structure

The basic structure of all membranes is the same, irrespective of cell source or location. It relies on the use of a continuous bimolecular layer of lipids, the diffusion barrier, which is fluid at the temperature of the environment in which the cell lives (Singer and Nicolson, 1972). The barrier is traversed by transmembrane proteins that subserve a variety of functions and is reinforced by a fibrillar infrastructure made up of other different proteins. Depending on cell type, these infrastructures are built either for imparting tensile strength to a delicate membrane or for controlling the lateral mobility of transmembrane proteins, which if not restrained would move rapidly in the membrane because of the fluidity of the lipid bilayer. The first type of infrastructure has been studied extensively in the red blood cells of humans. Its molecular components and their mode of assembly are well known. Their function is to reinforce the membrane and to give the cell its characteristic shape. More recent work shows that the same or related proteins are used by many other cells to solve similar problems (Marchesi, 1985).

Most of the studies on the second type of infrastructure (the one controlling lateral protein mobility) have focused on the miniature geodetic cages formed by clathrins and other associated proteins. These clathrins are found on structures called coated pits and coated vesicles associated with the plasmalemma as well as with certain intracellular membrane systems. Coated pits function as trapping devices for functionally important molecules from the environment or from intracellular compartments (Pearse and Bretscher, 1981).

Permeability Modifiers

Transmembrane proteins facilitate the transport of water-soluble molecules across the lipid bilayer, which by itself has very low permeability. Many of these proteins transport nutrients such as glucose and amino acids. Others are channels for ions, and still others function as energy-driven pumps that move sodium, potassium, hydrogen, or calcium ions in or out of the cells against concentration gradients. These molecular pumps are called adenosine triphosphatases (ATPases), because they obtain the energy needed for their work by splitting adenosine triphosphate (ATP), a high energy, molecular fuel the cell uses to conduct practically all its activities. The main function of the pumps is to maintain stable intracellular ionic concentrations at levels that are optimal for the cell's activities.

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During the last few years, many transporters, channels, and pumps have been moved from their previous status as postulated physiological entities to that of well-defined protein molecules. Moreover, the amino acid sequence of many of them was deduced from the nucleotide sequence of the cognate complementary DNAs (cDNAs). Knowledge of the amino acid sequence of these proteins is needed as a first step toward understanding their function and the way in which they are fitted into membranes.

Channels and pumps generate differences in molecule and ion concentrations (chemical and electrochemical gradients) as well as electrical charge separations (membrane potentials) across the corresponding membranes. These gradients and potentials are used by cells to propagate signals along the cell surface, as in nerve and muscle cells, and to drive the transport of many molecules and ions, as in the cells of the intestine and the kidney.

Receptors and Signal Transducers

Another important class of transmembrane proteins, called receptors, enables the cell to respond to specific molecules (ligands) coming from the environment. To date, specific receptors have been recognized for peptide hormones, growth factors, neurotransmitters, and macromolecular nutrients. Some of them generate a signal upon interacting with their ligands and are able to transmit (or transduce) this signal across the cell membrane. Others depend on other membrane proteins that interact with the receptor-ligand complex to generate a second messenger in the cytosol. (The ligand, in this case, is the first messenger). Other receptors, such as the receptor for the neurotransmitter acetylcholine, are ion channels that open upon ligand binding (Stroud and Finer-Moore, 1985). Still others, such as the low density lipoprotein (LDL) receptor and the transferrin receptor, trigger endocytosis at coated pits and coated vesicles, apparently as a result of ligand binding. It is clear that cells have evolved an elaborate communication system with their environment, which enables them to respond and adapt to changing conditions while maintaining their own identity. In multicellular organisms like ourselves, and under normal conditions, this communication system is used to integrate the activities of each cell into the general functions of the entire cell community. Inherited or acquired defects of either receptors or ligands disrupt the integration and often lead to overt diseases.

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Many receptors have recently been isolated and characterized, as have transporters and channels. The amino acid sequences of these receptors have been established directly or deduced from the nucleotide sequence of their cognate cDNAs. Moreover, genetic manipulations, i.e., deletions or substitutions in the cognate genes or mRNAs, are used to learn in detail the effect of such man-made mutations on the physiological competence of the receptors.

Cell-cell interactions most probably involve mechanisms comparable to those mentioned above, except that receptors as well as ligands must be affixed to cell membranes. Such interactions are of crucial importance in fertilization, in developmental processes, in the construction of epithelial tissues, and in the regulation of immune responses. But this type of membrane protein is only now attracting the attention needed to encourage the undertaking of future studies comparable to those currently conducted on channels, pumps, and usual receptors. (For further detail, see section on Cell-Cell Interactions.)

Research Opportunities. Future research in this area is likely to add to our information concerning transporters, channels, pumps, and different types of receptors, many of which are still at present physiological postulates. Another challenging, unanswered question concerns the nature of signal transduction by a single receptor protein. Also still largely unknown is the intracellular chain of reactions and reactants that is triggered by the interaction of a ligand with its receptor at the cell surface. This chain has been elucidated in detail only for a few simple metabolic responses, such as mobilization of glucose or fatty acids from intracellular reserves. In such cases, it involves second messengers, such as cyclic adenosine monophosphate (cAMP), and a series of enzymes (protein kinases) that activate or deactivate other effector enzymes. For complex processes, such as the activation of a new gene-transcription program or the triggering of a new cycle of genome and cell replication, the reactions and the reactants remain unknown. One of the reasons it is important to pursue new knowledge about these processes is because cancer genes, or oncogenes, appear to be the mutated forms of normal genes (or protooncogenes) that regulate cell division. Some of them have been identified as defective genes for either a growth factor or a growth factor receptor. But the products of many other protooncogenes remain to be identified among the reactants of the complex chains that connect receptors to relevant intracellular targets.

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We have acquired an impressive body of knowledge and are now ready to tackle new and challenging problems connected with the proteins of the plasmalemma. Given the diversity and specificity of protein-membrane and membrane-membrane interactions within the cell body, it is likely that future research will lead to the identification of new pumps, transporters, and receptors in intracellular membrane systems.

Biosynthesis

In procaryotic cells, the plasmalemma is the site of a number of important biosynthetic activities, including the synthesis of membrane lipids, ATP, and cell wall components. The procaryotic plasmalemma is also the main site for protein traffic regulation, especially in cells with two membranes separated by an intervening periplasmic space. Bacterial cell membranes have their own transporters, receptors, and sensors for chemotactic movements.

In eucaryotic cells, comparable activities are differently distributed. Some of them (e.g., those of the transporters and receptors) remain in the plasmalemma, but others are relocated to different intracellular membrane systems. Lipid synthesis, for instance, occurs only in the endoplasmic reticulum (ER), a network of channels that pervades the cytoplasm in animal cells, and only in plastids in plant cells. From the ER or plastids, newly synthesized lipids are distributed to all other cellular membranes. The ER membrane also becomes the site of important steps in protein traffic regulation (discussed later in this section). Moreover, it acquires a complex set of enzymes that modify aromatic compounds imported by the cell from the environment. The modifications increase the water solubility of these compounds, thereby facilitating their elimination. Since sterols, drugs, herbicides, toxins, and chemical carcinogens are among these compounds, the relevant enzymes constitute an intracellular detoxifying system.

Research Opportunities. Issues awaiting resolution in this area include the means by which lipids are transported from a unique site of synthesis to a multiplicity of destinations and the way in which differences in lipid chemistry are established and maintained in different membranes. We must also advance our understanding of the ER-detoxifying system to shed light on problems related to chemically induced cancers and toxic effects of chemical pollutants in the environment.

In addition to the activities already mentioned, the ER functions prominently in the intracellular regulation of protein traffic. In fact, it

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sorts out and directs to their final destination the largest amount and the widest variety of proteins produced in the cytosol.

ER Targeting System. The traffic control of proteins directed to the ER membrane is based on mutual recognition between signals (called signal sequences) within the amino acid sequence of the protein to be transported and a signal recognition complex, which involves a ribonucleoprotein particle (called signal recognition particle, or SRP) and at least one transmembrane protein (called SRP receptor) in the target ER membrane.

The ER targeting system recognizes and processes secretory proteins, lysosomal proteins, as well as membrane proteins for many intracellular compartments. Secretory and lysosomal proteins are fully translocated across the ER membrane into the ER cisternal space. Membrane proteins are partially translocated and remain anchored in the membrane. Recent experiments indicate that a membrane protein can be converted into a secreted polypeptide, and conversely, a secretory protein can be converted into a membrane protein by modifying the genetic information encoded in their cognate mRNAs.

The mode of operation of the ER targeting system has been elucidated in reconstituted *in vitro* systems in which ribosomes were allowed to translate into proteins the genetic information encoded in specific mRNAs in the presence or absence of microsomes (i.e., ER-derived vesicles) *in vitro*. The results proved that the components of the systems are functionally equivalent in different species, phyla, and even kingdoms, which implies that this part of the traffic regulation system originated early in evolution and has been conserved all the way through mammals (Walter *et al.*, 1984).

Post ER Steps in Traffic Control. Once past the ER, proteins are moved within the cell through the Golgi complex (see Appendix A), where they are further modified by glycosylation, sulfation, and proteolysis and are sorted while in transit to lysosomes, secretion vacuoles, or different membranes (Farquhar, 1985). Transport from the ER to Golgi subcompartments, from one subcompartment to another in the Golgi complex, and finally, from the last Golgi elements to the plasmalemma requires energy and is effected by vesicular carriers, which implies that past the ER, protein traffic is regulated, at least in part, by controlling the traffic of vesicular carriers. These carriers apparently recycle continuously between the compartments with which they are

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connected (Palade, 1982). The best known among these vesicular carriers are the secretion granules or secretion vacuoles of various glandular cells. They transport products to the cell surface and discharge them in the extracellular medium by a process known as exocytosis. Sorting of the proteins to their ultimate destinations probably involves mutual recognition between a signal and a receptor, but so far only the signal for lysosomal proteins has been chemically defined, and its receptor has been isolated and partially characterized (Sly and Fischer, 1982). Reactants involved in the sorting of other proteins remain unknown, as are the signals and receptors that regulate the traffic of vesicular carriers. Studies to identify them are being actively pursued.

Other Traffic Control Systems. The ER targeting system is undoubtedly the most complex component of the overall protein traffic control system of the cell. The other components are simpler, and most of them probably transport the protein in a single step: from the cytosol directly to the final destination. The amino acid sequence of the signal that directs certain proteins to the nucleus is known in a few cases, but the corresponding receptor remains to be identified. There is already a substantial body of information about traffic regulation of proteins targeted to mitochondrial and chloroplast membranes (see sections on Mitochondria: Function and Biogenesis, and on Chloroplasts: Photosynthesis and Biogenesis). Among the protein products of plant nuclear genes are some that function in the mitochondria and some in chloroplasts; it is not known how the systems differ.

In a simpler form, protein traffic regulation occurs in procaryotic cells and has been studied extensively in gram-negative organisms, which are provided with two successive membranes at the cell surface. The number of final destinations is considerably less—only three or four (i.e., the two membranes and the intervening space). A signal, generally comparable to that found in eucaryotic proteins targeted to the ER membrane, has been identified and analyzed in detail by sequencing and by extensive genetic modifications (Emr and Silhavy, 1982). This line of work has led to the recognition of functionally critical residues in the signal sequence, but the other components of the system are still unknown.

Research Opportunities. We can anticipate considerable activity in this fertile and exciting area, especially in relation to the identification and characterization of signals and their receptor partners and to the intracellular location of receptors and sorters, i.e., molecules that

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control the selection and movement of proteins from one compartment to another. Although the picture already appears to be profusely rich in detail, many discontinuities and uncertainties remain to be resolved by further research. The reasons for removing the signal sequence are not clear nor are the reasons for the complexity of the enzyme that effects the removal. The enzyme may have additional functions, since it consists of six different proteins. The translocation process itself is still a mystery. Traffic-relevant information is most probably read by receptors and signals in three dimensions; at the moment, however, we can read it only in one dimension, and this may be the reason for many uncertainties.

Structural biology is likely to yield three-dimensional information on such interactions if large enough quantities of relevant proteins can be obtained. The main goal is to understand how cells succeed in effecting the parallel but distinct processing of their many proteins and how they achieve and maintain the chemical specificity of their membranes.

Another basic process that remains to be understood in molecular terms is membrane fusion. The process is critical for cell division, cell fusion in egg fertilization, and vesicle fusion along different pathways of intracellular transport. Membrane fluidity is a prerequisite for membrane fusion. It is also a prerequisite for membrane growth, which appears to proceed by expansion of preexisting membranes. The rationale behind these apparently disparate processes is the need for maintaining intact diffusion barriers in highly dynamic membrane systems.

Endocytosis

Eucaryotic cells in animals have evolved elaborate means for incorporating and digesting macromolecules and particulate matter from the environment and for using the digestion products for their own metabolic needs. Incorporation itself operates either nonspecifically, i.e., droplets of fluid and solutes are taken in bulk from the environment (a process called bulk or fluid phase endocytosis), or specifically, i.e., macromolecules are bound or adsorbed to the cell membrane, and thereby concentrated, before uptake (a process called adsorptive endocytosis). In many cases, the binding membrane molecules are receptors for metabolic macromolecules (e.g., LDL), metal-carrying proteins (e.g., transferrin), or hormones and growth factors. In this variant of the process, which is known as receptor-mediated endocytosis (Goldstein et al., 1985), the receptors either reside permanently in coated pits or

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become clustered in such pits upon ligand binding. The pits detach from the plasmalemma as coated vesicles that transport the ligand-receptor complex to a system of polymorphic acidic vacuoles called endosomes.

What happens at this level is an example of the versatility of the entire process: some ligands fall off their receptors at acid pH; the receptor is recycled by a vesicular carrier back to the cell surface for another binding event; and the ligand, such as LDL, is transported to and digested in lysosomes. Lysosomes are acid compartments that contain a complete set of degrading enzymes for proteins, nucleic acids, carbohydrates, and lipids. In other cases, e.g., transferrin, the ligand-receptor complex does not dissociate in endosomes; it loses only the iron carried by the ligand, and the complex returns promptly to the cell surface to pick up new iron. In still other cases, the receptor-ligand complex passes through endosomes without dissociation and is forwarded to lysosomes where both ligands and receptors are inactivated and digested. This appears to be the way in which cells regulate the plasmalemmal concentration of their receptors and the duration of their exposure to certain hormones and growth factors (Helenius et al., 1983).

In humans, many inherited diseases are caused by defective receptors. The best studied example is the LDL receptor in familial hypercholesterolemia (FH), the most severe form of arteriosclerosis known. In such cases, both receptor genes are defective, and the resulting cardiovascular disease is fatal before the age of 20. In milder forms, a single gene is defective, and heart attacks begin at age 40. The LDL receptor has been studied in considerable detail, and its role in regulating cholesterol production in cells and LDL concentration in the blood plasma are reasonably well understood (Brown and Goldstein, 1986). The knowledge derived from such studies is expected to help control excess blood LDL and arteriosclerotic lesions in forms of cardiovascular diseases other than FH. Many other diseases are caused by missing or defective lysosomal enzymes. Interest in this system is further enhanced by the fact that receptor-mediated endocytosis is used by some nonspecific ligands, primarily enveloped viruses, and by toxins in gaining access to cells. They escape from endosomes into the cytosol before the cell has a chance to degrade them in lysosomes (Helenius et al., 1983).

Research Opportunities. Further research in this area is needed to elucidate the molecular mechanisms responsible for sorting receptors and ligands in endosomes, to understand traffic regulation in the recycling

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of vesicular carriers between endosomes and the plasmalemma, and to understand viral membrane fusion to endosomal membranes as a model of the general process of membrane fusion-fission, which is crucial to so many cellular activities.

CELL-CELL INTERACTIONS

Unicellular eucaryotic as well as procaryotic cells have developed mechanisms for interacting with other cells for such important functions as sexual reproduction, colony formation, and attachment to various substrates. In multicellular organisms, cell-cell interactions have become greatly diversified. They ensure the integration of large cell populations into structurally coherent and functionally controlled tissues, organs, and organisms.

Many of these mechanisms function over short distances: from a cell to its immediate neighbors or from a cell to the adjacent extracellular matrix. The latter consists of fibrillar and laminar assemblies of glycoproteins (collagens, laminin, fibronectin) and proteoglycans secreted by the cells themselves but assembled into large structures only in the intercellular spaces.

Other cell-cell communication mechanisms function over long distances and involve the production of soluble primary messengers, i.e., hormones and growth factors, which are transported throughout the organisms by the circulating blood but affect only the activities of cells provided with cognate receptors (target cells) in their plasmalemma (see above section on Receptors and Signal Transducers). Soluble messengers acting over relatively short distances are found in the chemical synapses of the nervous system. Other messengers include steroid (hydrophobic) hormones. These hormones are also transported by the circulating blood, but since they readily permeate cell membranes, their target cells have receptors in the cytosol, rather than in their plasmalemma. These receptors bind the hormone and move it to the nucleus, where the hormone can affect the gene expression program of the cell.

The critical importance of cell-cell interactions is clearly illustrated by the developmental history of multicellular organisms, which, as mentioned in the introduction to this report, begin their life cycle when two haploid gametes (a sperm cell and an oocyte) recognize each other through species-specific receptors and fuse to generate a one-cell embryo in the process called fertilization. This type of cell-cell interaction maintains species specificity.

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As the one-cell embryo begins to divide and as cell differentiation starts, mechanisms of short-range cell-cell communications emerge. They consist of gap (or communicating) junctions that link a cell to its neighbors through common transmembrane channels. These junctions create common intracellular environments in cell subpopulations and ensure rapid cell-to-cell propagation of membrane permeability changes and intracellular messengers. Short-range interaction mechanisms also include junctional complexes between cells, which allow the developing organism to build walls of cells, called epithelia, that separate the different parts of its body. In addition, cells in general and epithelial cells in particular participate in short-range interactions with the newly formed extracellular matrix. These interactions are mediated by mutual recognition between cell membrane receptors and specific parts of matrix proteins. Cell membranes have multiple receptors for many matrix proteins, which are large, monomeric or polymeric protein molecules with specific sites for binding to the plasmalemma as well as to other matrix proteins. The result is the progressive construction of a mechanically coherent body in which the cells are kept in place by their attachment to one another as well as to structural differentiations (e.g., basement membranes and collagen fibers) of the extracellular matrix.

These attachments involve rigid plates on the plasmalemma to which are affixed bundles of fibrils from the extracellular matrix and from the cytosol (see discussion on intermediary filaments in the later section on Cell Motility and the Cytoskeleton). The rigid plates are maintained in relatively fixed positions by the fibrillar cables, which are under tension because they generally follow the lines of stress propagation within the entire system. This type of construction allows the cells to retain their shape, resist pressure, and recover from deformations.

During embryonic development, the production of matrix proteins follows a sequential program presumably matched by the production of plasmalemmal receptors for specific matrix proteins. Cell migrations, which are important processes in embryonic development, are controlled by cell-matrix interactions and can be experimentally blocked by antibodies to (or small peptides derived from) relevant matrix proteins. Cell migration is apparently controlled by a process that activates secretion of matrix proteins and concomitant production of cognate receptors. The cells move along tracks laid down by themselves or their immediate predecessors.

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As embryonic development progresses, mechanisms of short-range communication are extended and diversified, but long-range interactions through hormones and growth factors become progressively more important. In the adult organism, gap junctions control the propagation of contraction waves in the heart muscle and in the smooth muscles of the intestine and uterus (where large numbers are produced just before parturition). Modulations in the construction of junctional complexes control the permeability of epithelia in the intestine, lung, and kidney as well as the permeability of the vascular endothelium.

Long-range communication mechanisms are well understood for certain processes, such as target cell response to steroid hormones and to certain peptide hormones and growth factors. But many areas of great interest have been brought to light recently as we have learned more about the differentiation of red and white blood cells and especially about the network of interactions that control the differentiation and function of the cells of the immune system such as lymphocytes and macrophages. As in other cases already studied, these mechanisms involve specific factors (called interleukins) and cognate receptors now under active investigation.

Cell-cell communication based on peptide hormones serving as primary messengers involves the generation of intermediaries, known as second messengers, in the cytosol. These second messengers are cyclic nucleotides, e.g., cAMP or cyclic guanosine monophosphate (cGMP), or breakdown products of membrane phospholipids (phosphoinositides). They rely on enzymes that control the phosphorylation and dephosphorylation of proteins to propagate and amplify their effects. Many of them also alter the calcium concentration in the cytosol by opening calcium channels in the plasmalemma or by mobilizing calcium from intracellular components such as the endoplasmic reticulum and mitochondria. Calcium itself seems to function in such cases as a second messenger.

A multicellular organism, whose functions have been integrated by this network of cell-cell communications, reaches sexual maturity under the control of steroid hormones. At that time, the gametes it produces are ready to meet the gametes of the opposite sex, recognize them, and start the cycle of cell-cell interactions all over again.

Research Opportunities. The molecular mechanisms involved in cell-cell communications are at present under active investigation along

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lines already discussed in the preceding sections, i.e., isolation and characterization of factors and receptors, and cloning of their cDNAs and genes for detailed genetic analysis.

Areas of great future interest concern cell-cell interactions in the immune system and cell-matrix interactions in cell differentiation, embryonic development, and tumor metastasis. At present, work is concentrated primarily on the active elements (agonists) involved in these processes, but the system probably relies on balanced interactions between agonists and antagonists. The latter are of obvious importance in the control of cell differentiation, cell population size, neoplastic growth, and specific areas of cell differentiation, such as those involved in the immune system. Physiological antagonists remain a biological postulate. They will undoubtedly be the object of keen interest in the future.

Cell-Cell Interactions in Plants

Heuser and coworkers (Goodenough and Heuser, 1985) have used ultra-rapid freezing techniques followed by freeze fracturing and antibody labeling to visualize cell proteins in their native states in cell walls and surface membranes of the green alga *Chlamydomonas*. This procedure makes it possible to observe the actual structures, in their normal locations, of sexual agglutinins, clathrin, dynein, coated vesicles, and membrane surface proteins. The method will provide information on the molecular interactions responsible for mating behavior and other cell-cell interactions in plants.

CELL MOTILITY AND THE CYTOSKELETON

Cell motility is necessary for the survival of virtually all living species. For example, the egg would not be fertilized without a motile sperm, and every cell division that occurs thereafter requires a degree of motility in some cell parts. Thus, an understanding of the basis of cellular motility is central to our understanding of the functioning of all organisms—from the amoeba to both the whale and the sequoia. Without active changes in cell shape and cellular migrations, embryos would not form. Without cellular motility, white blood cells would neither accumulate at sites of inflammation nor ingest invading microorganisms. Without active and rapid movements of organelles in axons and large plant cells, the peripheral parts of these cells would not be nourished. In fact, cell motility is so vital that embryos would not progress past the single cell stage without it.

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Filaments and Contractile Proteins

Cells from both animals and plants contain three different types of fibers—actin filaments, intermediate filaments, and microtubules—each of which is formed by the polymerization of distinct protein molecules. Together these fibers provide mechanical support for the cell and thus are considered to be a cytoskeleton, but the actin filaments and microtubules also participate in cellular movements, including locomotion of whole cells, cell division, and movement of subcellular components. This combined ability to maintain form against mechanical forces and to produce and transmit force means that this cytoskeletal-motility system can determine cell shape and hence the structure of both tissues and whole organisms. A clear understanding of this system will be essential for unlocking the secrets of embryology.

This field is still in an explosive growth phase during which investigators have isolated and started to characterize the major molecular components of these systems. This inventory includes not only the protein subunits of the polymers themselves but also a surprising number of regulatory proteins. For example, in the actin system alone, one cell has already been shown to have almost 20 accessory proteins, which together with the actin constitute 25% of the total cell protein. In the developing brain, the microtubule system may include a similarly large proportion of the total cell protein. In skin, the keratin molecules that form the intermediate filaments constitute the major protein in the cells.

The Cytoskeleton

The polymeric nature and intracellular distributions of the filaments and microtubules suggest that they may mechanically stabilize the cytoplasmic matrix. Recent physical studies on purified cytoskeletal fibers and analysis of the mechanical properties of live cells support this conclusion. Other work has shown that some of the glycolytic enzymes bind to actin filaments and that polyribosomes are associated with isolated cytoskeletons. Thus, beyond imparting mechanical integrity, the cytoskeleton may provide scaffolding for enzymes that participate in cellular metabolism and protein biosynthesis. In this way, the cytoskeleton, like membranes, may be an essential integrator of cellular processes. In early embryos, the cytoskeleton seems to localize macromolecules, including regulatory proteins and selected mRNAs, in defined areas of the cytoplasm to provide signals for spatially determined developmental processes.

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Actin filaments and intermediate filaments are bound to the plasma membrane in most cells. It is now possible to trace the macromolecular connections from actin in the cortical cytoplasm through the proteins spectrin and ankyrin to protein molecules that span the lipid bilayer of the plasma membrane. Less complete information indicates there are also other mechanisms connecting actin filaments to membranes. Intermediate filaments in epithelial cells are connected to the plasma membrane on the cytoplasmic side of intercellular junctions called desmosomes, which fasten cells together or bind them to the extracellular matrix (see section on Cytoplasm: Organelles and Functions). The intermediate filaments serve as intracellular tendons to prevent excessive stretching of the cells, and when linked from cell to cell by desmosomes, they ensure the mechanical integrity of whole tissues like the skin. Similarly, the rigidity of microtubules appears to provide mechanical support for asymmetric cells and projections from the cell surface.

It is now possible to describe, in broad outline, how these protein polymers assemble and how some of the steps in the assembly process may be regulated, at least for actin. To a large extent, the construction of the system of cytoplasmic fibers can be explained by the process of self-assembly, where the protein subunits are driven by chemical attraction for each other to polymerize without external direction. This spontaneous process is controlled by a variety of regulatory proteins, some of which must react to signals from the external environment that direct the organization of the cytoskeleton. Cells also contain organelles, such as the centrosome, that help to organize the cytoskeleton. The centrosome is the site where the assembly of microtubules is initiated. Both biochemical and cellular experiments indicate that the mechanisms controlling the assembly and organization of these fibers in the cell are both complex and subtle, befitting a system that has such an important influence on cell architecture. To gain a better understanding of how form is determined in biology, considerable new work will be required at the molecular level to elucidate the molecular composition, regulation, and dynamics of the cytoskeleton and at the cellular level to determine how external stimuli affect the assembly of the cytoskeleton.

Mechanisms of Cell Movements

In parallel with studies on the structural elements of the cytoplasm, work on mechanisms of cell movements has pushed forward rapidly

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during the last 15 years on two main frontiers, and during the past year, a third and possibly a fourth frontier have begun to open up.

The first involves the microtubule-dynein system found in cilia and flagella—whip-like organelles (such as sperm tails) that beat rapidly. Cilia are found in groups on the surface of epithelial cells such as those lining the air passages in our lungs, where they are responsible for sweeping mucus and inhaled foreign material out of the lungs. If the cilia are not active, serious infection is inevitable. Flagella form the tails of sperm and propel them toward their meeting with the egg. In cilia and flagella, microtubules interact with a giant enzyme molecule called dynein to convert the chemical energy stored in ATP into a force that bends the cilia and flagella. Since the chemical steps in this process have now been identified, studies on the molecular mechanism that produces the motion can now be pursued vigorously.

The assembly of microtubules and dynein in the flagella is held together by no less than 140 different protein molecules. By skillfully combining genetic analysis and the characterization and ultrastructural localization of these proteins (as studied in *Chlamydomonas*), it will be possible to define the molecular architecture of the flagella. A key to understanding the assembly and function of cilia and flagella will be elucidation of the molecular architecture of basal bodies—specialized structures found at the base of these organelles. Basal bodies appear to provide the template for the assembly of the microtubules and other components in cilia and flagella, and they may help integrate the mechanical events during the beating of these organelles.

The second frontier is the characterization of myosin—the force-producing enzyme long known to cause contraction in highly specialized muscle cells and more recently recognized to exist along with actin in most other cells, even those not specialized for contraction. Superficially, most myosins are similar, and it seems likely that all myosins produce force by interacting with actin filaments and ATP in the same fundamental way. The steps in this process have been studied in detail by using muscle as an especially favorable test system, but investigations using spectroscopy, x-ray diffraction, electron microscopy, and biochemical methods are still under way to locate the site in the myosin molecule where motion is produced. Myosin and actin are widely believed to be responsible for many forms of cell movements in addition to muscle contraction. For cytokinesis (the final step in cell division), there is direct experimental evidence validating this assumption. Similar experiments

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on other types of movements are still required to explain their molecular basis.

In the past, most cell biologists suspected that either dynein or myosin was responsible for most cell movements, including the ubiquitous rapid movements of cellular organelles in the cytoplasm, but this now seems to be incorrect. During the past year, several investigators have demonstrated that movements of organelles in nerve cells are powered by another type of molecule that drags them along the surfaces of microtubules. The work is so new that the identity of the power source is still being debated, but a new frontier has been established. Even newer evidence suggests that an unusual form of myosin may pull some organelles along actin filaments. These new breakthroughs have raised the hope that we may soon understand how the mitotic apparatus works and how the traffic of organelles is directed to the proper destinations in the cell.

Each of these motile systems operates under exquisite controls that allow cells to respond to internal or external stimuli and to produce a coordinated motile response. In body muscles and heart, the contractile proteins are turned on by calcium, which activates regulatory proteins bound to the actin filaments. In the smooth muscle cells found in internal organs and in nonmuscle cells, the myosin is activated chemically by the attachment of phosphate to the protein. It is not yet understood how these chemical reactions are coordinated in the living cell to produce the complex patterns of movement that are essential for life. The purse string-like contraction that splits two daughter cells apart at cell division is an example of a movement in response to an internal stimulus, the mitotic spindle. The rapid locomotion of white blood cells to sites of infection and their ingestion of bacteria are examples of complex movements in response to external signals. In these examples, the stimuli and the responses are well documented, but little is known about the mechanisms that convert the stimulus into the response. Regulation of microtubule-based movements presents a similar challenge.

Research Opportunities

If work on cell motility continues with its current momentum, progress is likely in the following areas:

Molecular Inventory and Characterization. The complete catalog of the molecular components of the cytoskeletal-motility system must be finished for a few cell types that are particularly favorable

for use as model systems. These include, for example, the slime molds *Dictyostelium discoideum* and *Physarum*, the protozoan Acanthamoeba, sea urchin eggs, macrophages, platelets, and the intestinal epithelial cell. Completion of this molecular inventory may require new functional assays for proteins that have yet to be discovered. The primary structures of the major components need to be determined by sequencing cloned DNA, and the three-dimensional structures of the major components must be determined by x-ray crystallography. This has yet to be done for any of the components in this system, although good progress is being made on actin, myosin, and profilin—an actin-binding protein. To identify the steps in the reactions between the various components in these systems, it will be necessary to conduct studies based on transient state kinetics and other traditional biochemical methods.

Cellular Organization and Dynamics. Electron microscopy should lead to more precise localization of the components of these systems inside whole cells. Vastly improved probes consisting of antibodies coupled to colloidal gold and better methods to prepare cells should give us a clearer picture of macromolecular architecture. Furthermore, it should be possible to characterize the dynamics of the cytoskeleton in live cells by using new fluorescence techniques. Purified protein molecules can be tagged with fluorescent dyes and then injected into live cells. Remarkably, these fluorescent proteins can integrate into their normal cellular structures, and once inside the cell, their distribution and diffusion rates can be followed microscopically.

Functions and Regulation of the Cytoskeletal-Motility System. Perhaps the major challenges in the field will be to determine decisively the functions of the various components inside living cells and to learn how these functions are regulated by the cell. One approach is the use of *in vitro* assays with purified components. It is remarkable that functions as complex as the contraction of actin and myosin, the movement of an organelle on a microtubule, or the growth of microtubules from the centrosome to the kinetochore of a chromosome can all be reproduced today *in vitro*. These assays should become more sophisticated, enabling cell biologists to test for the ability of purified components to carry out complex functions outside living cells. Certainly the use of classical or modern methods to produce mutant cells with defects in single components will also be valuable in demonstrating functions and identifying regulatory mechanisms (see later section

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on Yeast as a Model for Cell Biology). This may be a laborious process, because there are multiple genes for many components, and even where there is a single gene, the protein itself may be part of a highly redundant system that will function normally without any given component. The microinjection of inhibitory antibodies to inactivate a single component inside a cell and the inactivation of relevant genes are also promising approaches. These and other creative new approaches will be necessary to test current ideas regarding the physiological functions of the cytoskeleton-motility system. A long-term challenge will be to characterize the mechanisms by which an external stimulus, such as a chemoattractant, causes a cell to move in a particular direction.

Mechanical Properties. Analysis of the mechanical properties of the cytoskeleton and its component molecules is essential but has only just begun, partly because few cell biologists are familiar with the engineering techniques required for the work. This is an area of potential collaboration between cell biologists and engineers.

For more information on the cytoskeleton and cell motility, see Borisy *et al.* (1984), Johnson (1985), Pollard and Cooper (1986), and Vale *et al.* (1985).

MITOCHONDRIA: FUNCTION AND BIOGENESIS

The mitochondrion is characterized in high school biology texts as the power plant of the cell. This refers to the fact that within this organelle, the cell performs the enzyme-catalyzed, stepwise oxidation of nutrients (such as sugars, fats, and amino acids) in a process called respiration (Tzagoloff, 1982). The products of this process include carbon dioxide and water along with ATP, the direct source of the energy required for all the chemical work the eucaryotic cell must perform to power its growth, movement, synthesis of new components, and other functions. A relatively small amount of ATP is produced in the cytosol during sugar catabolism (glycolysis), but by far the largest amount is generated in mitochondria. The mechanism of mitochondrial ATP synthesis has stubbornly resisted full elucidation, but progress continues to be made as a result of our insistent probing into this fundamental energy-transducing process. The synthesis of ATP in chloroplasts during photosynthesis—the energy transduction that sustains all but a tiny fraction of life—is discussed in the later section on Chloroplasts: Photosynthesis and Biogenesis.

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Mitochondria are also of interest because they contain their own complement of DNA, which cooperates with the DNA of the nucleus in the control of mitochondrial formation (Tzagoloff, 1982). The origin and evolution of the mitochondrion are linked to the origin and evolution of eucaryotic cells. Understanding of mitochondrial function in turn sheds light on a wide array of fundamental and practical issues, ranging from certain metabolic and genetic diseases to evolution itself.

Mitochondrial Structure and Function

Mitochondria have two membranes (inner and outer) that define two concentric separate spaces. The inner space houses hundreds of enzymes, including those involved in the oxidative reactions that supply the energy needed for cell function. The inner mitochondrial membrane contains the energy-conversion apparatus, i.e., the four enzyme complexes of the respiratory chain and the ATP-synthesizing enzyme as well as specific carriers that transport metabolites into and out of the organelle (Tzagoloff, 1982). The majority of the mitochondrial proteins are specified by nuclear genes; they are synthesized on ribosomes in the cytoplasm, and then imported into the organelle. A limited set of proteins of the inner mitochondrial membrane, namely, some protein subunits of the oxidative-phosphorylation apparatus, are encoded in DNA molecules located within the organelle itself and are synthesized by an organelle-specific translation system (Slonimski *et al.*, 1982). The distinctive structural RNA components of this system, i.e., RNAs and transfer RNAs (tRNAs), are also encoded in mitochondrial DNA. Mitochondria do not arise *de novo* in the cell by self-assembly of their constituent molecules, but are formed by growth and division of preexisting mitochondria.

After the first isolation of intact mitochondria in the late 1940s, mitochondrial research for about 15 years was almost entirely focused on function, especially the ATP-synthesizing role of these organelles. This research has resulted in a general understanding of the mechanism of energy conversion in mitochondria. According to the commonly accepted chemiosmotic model, the energy released by electron transport in the respiratory chain is utilized to create a pH gradient and a potential difference across the inner mitochondrial membrane, and the energy stored in these gradients is used for the synthesis of ATP and for driving the active transport of certain metabolites into and out of the organelle.

Following the discovery of mitochondrial DNA and of respiration-deficient mutants in yeast and *Neurospora*, the emphasis of mitochondrial research shifted, in the middle 1960s, to the genetic control and assembly of functional mitochondria (Quagliariello *et al.*, 1985). In the past 10 years, striking progress has been made in our understanding of the structure and function of mitochondrial DNA through the use of modern, powerful DNA, RNA, and protein analytical techniques and a refined genetic analysis in yeast (Quagliariello *et al.*, 1985; Slonimski *et al.*, 1982). The mitochondrial DNA from several organisms, including humans, has been completely sequenced, and much of its informational content has been elucidated. Furthermore, all mitochondrial gene products in humans have been functionally identified. A dramatic outcome of these studies has been the discovery that the mitochondrial genetic system in most organisms, except plants, uses a code that differs in several respects from the universal code and, in addition, utilizes for reading the code a novel mechanism, which requires only a restricted set of tRNAs. Present research is focused on several areas, which are discussed below.

Evolution of Mitochondrial DNA

The information content, gene organization, and mode of gene expression of mitochondrial DNA vary considerably from organism to organism (Quagliariello *et al.*, 1985). Therefore, studies of mitochondrial DNA are yielding not only data that are invaluable for understanding the origin and evolution of the mitochondrial DNA itself but also information of use in studying evolutionary relationships between organisms.

Research Opportunities. This line of research is likely to provide important information on the evolution of the genetic code and decoding mechanisms as well as on the evolution of basic processes, such as DNA replication and transcription, RNA processing, and protein synthesis.

Mechanisms of Expression and Replication of Mitochondrial DNA

Studies of the enzymes and ancillary proteins responsible for mitochondrial DNA replication, DNA transcription into RNA, and RNA processing to mature RNA species are making rapid progress, aided by the development of soluble *in vitro* preparations derived from broken mitochondria and by the use of recombinant DNA technologies

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(Quagliariello *et al.*, 1985). Specific proteins have already been identified and, in some cases, isolated in partially or completely pure form.

As in the case of many nuclear gene transcripts, the coding sequences of several mitochondrial gene transcripts in lower eucaryotes, especially yeast and filamentous fungi, are interrupted by "nonfunctional" segments or introns. These introns have to be removed by RNA splicing to produce the mature RNA. Recent work has revealed that the transcripts of some mitochondrial genes have the remarkable capacity to excise their own introns *in vitro* in the absence of protein, i.e., they function as enzymes acting on themselves. A unique property of some mitochondrial introns is that they encode proteins that facilitate their own excision from the RNA copies of the gene or the transposition of the introns from one gene to another (Quagliariello *et al.*, 1985).

Research Opportunities. It may soon be possible to reconstruct in the test tube the processes involved in the expression and replication of mitochondrial DNA. Furthermore, through the use of these *in vitro* systems and with the help of classical genetics and modern gene manipulation techniques, it may be possible to identify proteins or other factors that have a regulatory role in these processes in relation to the cell cycle, cell energy requirements, and possible hormonal influences. The discoveries of mitochondrial introns have opened up a very active field of research, which is expected to provide deep insights into the mechanisms of RNA splicing in general and into the origin and evolution of introns.

Nuclear Control and Nuclear-Mitochondrial Interactions

The dual (nuclear and mitochondrial) control of mitochondrial formation is most dramatically illustrated by the chimeric structure of nearly all the enzyme complexes of the oxidative-phosphorylation system: each such complex contains some subunits encoded in the nucleus and some encoded in mitochondrial DNA (Quagliariello *et al.*, 1985). Because of this dual control, the formation of functional mitochondria requires a quantitative and temporal coordination of expression of the relevant nuclear and mitochondrial genes.

Two main classes of nuclear genes are the object of intensive investigation based on recombinant DNA techniques and on structural and functional approaches: (1) genes coding for subunits of the enzyme complexes of the oxidative-phosphorylation system and for mitochondrial carriers used in metabolite transport and (2) genes coding for

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proteins involved in the expression and replication of the mitochondrial genome (Quagliariello *et al.*, 1985). Most of the latter genes are probably distinct from those that code for the homologous components of the nuclear-cytoplasmic apparatus. However, interesting exceptions have recently been reported. They concern the possible existence of common nuclear genes for cytoplasmic and mitochondrial components, which could account for at least some of the reported influences of the mitochondrial genome on the remainder of the cell.

Research Opportunities. Research now under way promises to elucidate the mechanisms and signals involved in the interactions between the nuclear and mitochondrial genomes in the formation of functional mitochondria. Furthermore, research in the area of nuclear-mitochondrial interactions should help us understand how the assembly and function of mitochondria are integrated with those of the rest of the cell, and how these processes can be modified in relation to cell respiratory demands, cell differentiation, and senescence.

Protein Import into Mitochondria

The hundreds of distinct polypeptides that make up a mitochondrion are distributed in a specific way in four compartments: the outer membrane, intermembrane space, the inner membrane, and inner mitochondrial space. After their synthesis on cytoplasmic ribosomes, the nuclear gene-coded polypeptides are imported to their correct location within the mitochondrion (Schatz and Butow, 1983) (see section on Cytoplasm: Organelles and Functions).

Biochemical studies and the application of recombinant DNA technology have shown that proteins destined for one of the three innermost compartments are usually made from precursors with extensions at the amino-terminal end, which can be as long as 100 amino acids and function as signals directing the proteins to the proper location. The amino-terminal extension penetrates across both mitochondrial membranes in a process requiring an electrical potential across the mitochondrial inner membrane. When the precursor is partially translocated, the extension is removed by a specific protease located inside the mitochondrion.

Research Opportunities. Following are some of the major unanswered questions: Which molecules are involved in the import of polypeptides into the mitochondria? What is the role of cytosolic factors in the import process? How do mitochondrial signal sequences

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perform their function? Why does translocation of proteins across the mitochondrial inner membrane require a gradient of electrical potential across that membrane? Do contact or fusion points between the two membranes function as ports of entry for protein import?

Structure and Function of the
Oxidative-Phosphorylation Apparatus

The subunit composition of the enzyme complexes of the oxidative-phosphorylation system and the location, nuclear or mitochondrial, of the genes specifying these subunits are largely known (Quagliariello *et al.*, 1985). From the nucleotide sequence of these genes, the amino acid sequence of the subunits is being inferred and their secondary and tertiary structure is being tentatively deduced. Definitive knowledge about their tertiary structure should eventually come from crystallographic studies.

Research Opportunities. This information, together with data derived from other approaches to studies of the spatial relationship of the subunits in each enzyme complex, is likely to provide useful models of the three-dimensional structure of the complex and of its topology in the inner mitochondrial membrane. These models will help in interpreting the results of ongoing functional studies on the individual complexes in intact mitochondria and in reconstructed systems (Quagliariello *et al.*, 1985). A powerful approach in these structural and functional studies is provided by gene manipulation techniques, especially by the ability to introduce suitably modified genes into the nucleus and, possibly in the near future, also into mitochondria.

The generation of ATP by photophosphorylation is discussed briefly in the section on Chloroplasts: Photosynthesis and Biogenesis.

GENERAL PLANT CELL BIOLOGY

Cells of plants and animals have many common features, e.g., nuclei, endoplasmic reticula, Golgi complexes, mitochondria, plasmalemmas, coated vesicles, microfilaments, and microtubules, but in some features and processes, cells of the two kingdoms are distinct from one another. For example, plant but not animal cells contain plastids (chloroplasts, amyloplasts, and chromoplasts), large vacuoles that contain mostly water, and relatively rigid cell walls composed of strands of cellulose embedded in amorphous carbohydrate polymers together with a small amount of protein.

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At higher levels of organization, plant cells are connected to one another by strands of cytoplasm (plasmodesmata) rather than by junctional elements of the type found in animal cells. The tissues and organs of plants and animals are not organized along similar functional lines. Leaves, stems, roots, and buds have no conspicuous counterparts among hearts, livers, skins, and lungs. With perhaps one exceptional class, plant and animal hormones are unrelated to one another chemically.

At many fundamental levels, biosynthetic pathways and structures of proteins and nucleic acids of plant and animal cells are often, but not always, the same. Therefore, each aspect of each organism must be studied separately. Differences, when they occur in very similar systems, often reveal a great deal about the basic nature of a process. It is often profitable to determine whether newly discovered features of plants occur in animals and *vice versa*.

The preceding paragraphs emphasize that some features of plant cells and tissues are distinctively different from those of animals, and each requires direct investigation. Examples include plant and animal mitochondria. In plants, mitochondria perform the same respiratory functions (and perhaps some other, as yet undiscovered functions) as in animals, but they generally contain a much greater amount of DNA. They also appear to use the standard genetic code, but the mitochondria of animals and yeast use a nonstandard one.

In addition to photosynthesis, there are a number of phenomena unique to plants, and some are targets of active research. For example, there are three rather than two gene-containing compartments in each plant cell (the nucleus, mitochondria, and plastids) thus greatly complicating protein targeting and the integration of genome expression. Other research is focused on the rigid plant cell walls, which are constituted in different ways at different stages of development and for various specific functions of different cell types. The composition of the cell walls is reasonably well understood, but many questions remain unanswered.

Self-incompatibility, the inability of some plants to self-pollinate, is an important mechanism for regulating fertilization and reproduction. To elucidate this phenomenon, genetic and biochemical studies are being conducted in the United States, Australia, Holland, and the United Kingdom. The expression of plant genes can be affected by symbiotic or pathogenic microorganisms through complex interactions. In this area, important advances have been made, especially in studies of

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symbiotic nitrogen fixation, but the total effort is still very small. Some transposable genetic elements have been characterized molecularly, but how they are integrated into and excised from the genome remains to be understood.

Research Opportunities

As in many other areas of cell biology, the newly available molecular tools are opening the way to getting answers, at a new level, to questions recognized long ago as well as to elucidating processes exposed by the use of these tools. Each research area given as an example of work leading to understanding of a specific area of plant cell biology is one in which rapid progress is now being made through molecular approaches. Because of the new ideas and new tools and the many new questions that have been brought to light through studies of plant physiology, agronomy, plant breeding, and plant pathology, an increasing number of people are being attracted to these areas. The possibilities for progress are great.

CHLOROPLASTS: PHOTOSYNTHESIS AND BIOGENESIS

Photosynthesis is the biological process that connects life on earth to the sun. Through this process, light energy is converted to chemical bond energy stored in sugar molecules. In higher plants, photosynthesis is carried out in chloroplasts—organelles containing vesicles bounded by energy-transducing membranes in which the chlorophyll is localized. In primitive, noncompartmentalized cells, i.e., procaryotes, these vesicles lie free in the cytoplasm.

The work of Joseph Priestly, in the last quarter of the 18th century, is generally regarded as the beginning of the serious scientific investigation of photosynthesis. Priestly discovered that all the free oxygen in the atmosphere is produced by photosynthesis in plants. Since that time, progress in understanding photosynthesis has been intertwined with advances in chemistry, photophysics, and biology. For example, photosynthesis was one of the first biological processes to be studied through the use of stable isotopes. In these experiments, heavy oxygen atoms were used to determine that the oxygen produced in photosynthesis comes from water. Tracing the path of carbon in photosynthesis was one of the earliest uses of radioisotopes to elucidate biochemical pathways. The analysis of the fine structure of the chloroplast and

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its photosynthetic membranes by electron microscopy was one of the first applications of this technique in biology. Investigations based on recently developed physical and chemical techniques have led to greater understanding of the component processes of photosynthesis. Modern tools such as recombinant DNA technology, x-ray diffraction for the study of molecular structure, immunochemistry, and protein separation techniques are being used to increase our knowledge of the photosynthetic apparatus and thus of the process of photosynthesis.

The path followed by carbon during photosynthesis—from carbon dioxide to sugar—is now known in detail, and most of the enzymes have been identified. The enzymes themselves are now being studied, and surprisingly, a number of them are found to be regulated, in ways yet to be understood, by light and by certain of the small molecules that are intermediates in the biosynthetic chains of photosynthetic carbohydrate production. How the effects of these segments of the carbon fixation and metabolism system are modulated and how each enzyme interacts with other proteins and with its substrates are questions being addressed now.

Ribulose Bisphosphate Carboxylase and Other Enzymes for Carbon Fixation

One of the best studied enzymes of carbon fixation is ribulose bisphosphate carboxylase (RuBPC). With a few exceptions, all photosynthetic carbon fixation on earth occurs through the intervention of this enzyme, which is judged to be the most abundant protein on earth. It can constitute as much as 50% of the total soluble protein of a leaf. RuBPC is made up of one large and one small protein. Genes for these proteins have been isolated and sequenced. As a result, the order of amino acids in each protein is also known. The enzyme is unable to discriminate between oxygen and carbon dioxide and is believed to be less efficient than it would be if it could discriminate. Genetic engineering techniques are being used by a number of research groups to understand how the parts of the enzyme work and to determine whether its efficiency can be improved through modifications.

Research Opportunities. Genes for other enzymes of carbon fixation are being isolated and sequenced, and we are gaining information about their three-dimensional structure. We should soon have some answers to the fascinating question of how the molecules are changed from inactive to active forms as part of the system for regulating the flow of

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carbon in photosynthesis. All this will give us a better understanding of how we are fed by the sun. It will also guide us to possible means of modifying the enzymes for the genetic engineering of crops.

Energy-Transducing Protein Complexes

A major focus of research on photosynthesis is the organization and operation of energy-transducing membranes. We know now that these chlorophyll-containing membranes are half protein and half lipids. Multiprotein complexes are floating in the lipid of the membrane.

Two of the multiprotein complexes contain reaction centers, which are the sites of the energy-conversion process that is at the heart of photosynthesis. Some protein molecules associated with these complexes carry large numbers of chlorophyll molecules that harvest light energy that is then funneled, as molecular excitation energy, to the charge-separation apparatus at the reaction center. Here it is converted to the potential energy of electrons.

There are two types of charge-separation complexes, designated PS I (photosystem I) and PS II (photosystem II). They are connected to one another by a series of electron-carrying molecules. Overall, not only is light energy converted into the potential energy of electrons, but a gradient of protons separated by the photosynthetic membrane is also built up. The energy in this gradient is converted into chemical bond energy in ATP (see discussion of oxidative phosphorylation in the section on Mitochondria: Function and Biogenesis). By the action of some other chemical intermediates and the intervention of enzymes of the carbon fixation pathway, light energy is ultimately converted to relatively stable carbon-bond energy in sugars.

What protein molecules constitute the chloroplast membranes? What fatty materials? What proteins make up each of the light-harvesting, reaction-center complexes? What are the connecting electron carriers? How do all the parts interact to convert light energy to chemical-bond energy? How are the parts of this complex apparatus produced in appropriate relative amounts on a timetable that works? Of course, these questions did not occur to Priestly nor, surprisingly, did the most profound of them cross the minds of students of photosynthesis even 20 or 25 years ago. It is only within the last 10 years or so that we have known enough to formulate such questions and to realize that some have become answerable.

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Complexes have been isolated from membranes and characterized with respect to partial reactions of photosynthesis. Furthermore, the size and some other properties of their protein components have been determined. Knowledge of the role of each protein component in the overall process ranges from thorough to nonexistent. Genes for some of these proteins have now been isolated and sequenced. Knowledge of the derived amino acid sequences has, in turn, been the basis of attempts to understand the three-dimensional structures of the individual protein components. How these proteins interact within a complex may be learned through the use of genetic engineering techniques to replace natural genes with retailored genes, as is being done in cyanobacteria, and to introduce modified genes into chloroplasts in higher plants.

All these lines of study—isolation of complexes, identification of their components, sequencing of the genes, alteration and reconstruction of the system—are converging in a very exciting way, enabling us to gain a better understanding of energy transfer and charge separation mechanisms in photosynthesis as well as of the structure and function of biological membranes of other kinds. The report by a German research group describing the three-dimensional structure of the reaction center of a photosynthetic bacterium obtained by x-ray diffraction analysis (Deisenhofer *et al.*, 1985) was a milestone, because it brought not only a full understanding of photosynthetic functional units but also of membrane protein complexes generally.

Research Opportunities. In the course of sequencing chloroplast DNA, some genes have been discovered for hitherto unrecognized components of the photosynthetic membranes. It seems likely that through the discovery of such additional proteins, DNA sequencing of plastid and nuclear genomes will change some aspects of our understanding of the photosynthetic apparatus not only by revealing amino acid sequences of its already known components but also by adding links that were not even known to be missing. Identification, isolation, and sequencing of genes for proteins of the photosynthetic machinery should lead to the use of the gene-modification tools of genetic engineering to understand the relationship of membrane components to one another, how they function, and how their production is regulated. The information will influence how we think about biological membranes in general in addition to revealing specific features of photosynthetic membranes.

An understanding of photosynthesis at the level of molecules and

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atoms will be gained through the genetic modification of experimental plants. Methods for introducing foreign genes into the nuclei of plants are well developed; those for introducing genes into organelles are just beginning to be developed. This approach will also open the way to gaining an understanding of the extent to which crops can be modified to increase productivity and the utility of agricultural products.

Chloroplast Gene Mapping

Approximately 20 to 30 genes for plastid proteins have been sequenced to date, and the number grows steadily. Restriction maps of the chloroplast chromosomes have been completed for at least 40 to 50 species of plants. In many cases, the first of the chloroplast genes—e.g., genes for ribosomal RNAs, the large subunit of RuBPC, and the 32,000 dalton quinone and triazine herbicide-binding proteins—have been located on the chromosome of each species. The mapping process continues mostly with genes that were located and initially mapped and sequenced in maize, spinach, tobacco, the green algae *Chlamydomonas* and *Euglena*, and a few other species (Steinback *et al.*, 1985).

Research Opportunities. The entire chloroplast chromosome of some species will probably be sequenced within a year or so. There will then most likely be an intense interest in making the effort required to identify the products of open reading frames for unrecognized proteins; their identification should move us toward a better understanding of photosynthesis and plastid metabolism.

This DNA sequencing will also reveal features of plastid genes. At least one type of promoter sequence—resembling the procaryotic type—has been recognized. The existence of other kinds of control sequences remains to be established. The identification of these and of trans-acting elements of plastid or nuclear-cytoplasmic origin that constitute the parts of the machinery for control of differential gene expression is, without question, the most interesting and outstanding problem in this research area. Its resolution is likely to illuminate the mechanisms underlying the transcriptional control of chloroplast gene expression and to reveal research approaches leading toward an understanding of intergenomic integration.

Gene Expression

How is the machinery of photosynthesis assembled? What are the sources of the parts? Studies of the development and assembly of

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chloroplasts led to the discovery that although plastids (the generic term that includes chloroplasts, amyloplasts that produce and store starch, and other DNA-containing organelles) contain genetic material, not all their components are products of these genes. Many proteins of the chloroplast are imported from the surrounding cytoplasm, and these are encoded in nuclear genes. For example, the larger of the two subunits of plant RuBPC is the product of a chloroplast gene, whereas the smaller protein is the product of a nuclear gene. Some ribosomal proteins are derived from nuclear genes and others from plastid genes. Some membrane components are products of nuclear genes and others of chloroplast genes.

Research Opportunities. How the photosynthetic apparatus of chloroplasts is produced, i.e., its biogenesis, is a question that is fundamental to understanding of the life of a plant cell. The answers will not be simple nor will they be easy to obtain, but it is remarkable that they can be sought seriously. A number of related questions are under active investigation: What are the special characteristics of chloroplast genes? What are the enzymes and mechanisms for their replication? What are the mechanisms for controlling the expression of sets of developmentally regulated chloroplast genes? How is the expression of these genes integrated with the expression of nuclear genes for chloroplast components? How are nuclear gene-coded, cytoplasmically synthesized proteins targeted for plastids, and what are the mechanisms for their uptake and integration into the life of the plastid? (See the discussion on protein targeting in the section on Cytoplasm: Organelles and Functions.) How does the chloroplast gene expression machinery interact with the machinery of the nuclear-cytoplasmic compartment?

The answers to these questions will reveal some principles of the mechanisms for regulating gene expression in all eucaryotic cells and in some procaryotes. The questions can be addressed now because of the new knowledge and technology that has come from molecular genetics in the last decade.

Among the most interesting aspects of the plant eucaryotic cell is the integration of the activities of its multiple compartmentalized genomes—of nuclei, plastids, and mitochondria. The nature of the integrating mechanisms can now be investigated.

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Origin of Plastids

One of the great puzzles of modern biology is how eucaryotic cells originated (Bogorad, 1982). Compared with the procaryote, a unique characteristic of eucaryotes is the presence of multiple compartmentalized genomes. In plants, these include the nucleus, mitochondria, and plastids. The question of how the expression of these genomes is integrated has already been raised. There is also an older unanswered question: How did the multiple genomes come into existence? There are two obvious possibilities. One is that membranous compartments formed in the structural equivalent of a modern procaryotic cell and some genes then became sequestered in each compartment. A second possibility is that two types of cells developed after the initial origin of life on earth. One type evolved to have all its genes in a membrane-limited compartment, the nucleus, and to have various other membrane structures, whereas the other cell type remained structurally simple and unnucleated. According to this endosymbiont model, eucaryotic cells arose by the fusion of these two cell types or perhaps by the nucleated cell taking up one of the nonnucleated types. To bring the latter hypothesis in line with fact, it has been necessary to postulate the movement of genes or of gene functions from the symbiont to the host genome. Detailed exploration of the molecular biology of plastids has emphasized that although their genetic system has many features of procaryotes, it is distinctive.

What evolutionary pressures led to the existence of the different information-processing systems now found in the nuclear-cytoplasmic and organelle compartments? Are the different systems relics of the independent evolution of two progenitor cell types that subsequently joined to be the ancestral form of the modern eucaryotic cell? Alternatively, did two or three information storage and processing systems evolve within a single cell? What we learn about the molecular biology of the organelle and the nuclear-cytoplasmic systems may lead to a better understanding of the origin and evolution of eucaryotic cells as well as of the forces and mechanisms underlying the shifts of genes among genomes.

Research Opportunities. Evolutionary relationships among genomes are understood very much better now than they were half a dozen years ago through the accumulation of nucleic acid sequence data. However, the forces that have led to the segregation of genes in nuclei,

mitochondria, and plastids are unknown. Gene transfer methods are beginning to be used to explore these questions from the nuclear genome focus. Efficient organelle gene transfer (transformation) methods that would greatly aid such investigations remain to be developed.

CONTROL OF THE CELL CYCLE

Although references to the cell cycle appear repeatedly in the previous sections, a short description of the current status of this research area and of its perspectives may be useful.

Research on the cell cycle is still fragmented; active interest is primarily focused on growth factors, growth factor receptors, and events (primarily protein phosphorylation) that follow growth factor binding to receptors. Some oncogenes appear to be mutations (in structure or position) of normal genes coding for growth factors, growth factor receptors, or proteins involved in initial phosphorylation. The mutations apparently remove them from the regular control mechanisms of the cell.

Research Opportunities. The chain of events that eventually lead from activated growth factor receptors to genome replication, mitosis (separation of the two daughter genomes), and cell division is expected to be extremely complex and remains largely unknown. But progress is being made in understanding chromosome condensation and nuclear envelope chemistry and behavior during mitosis. The spectacular events of mitosis and meiosis (the reduction of the two chromosomes sets in a usual cell to the single set in a gamete, i.e., oocyte or sperm) remain just as intriguing as they were a few decades ago and just as far from elucidation in terms of molecular interactions. Work on the cell cycle and its regulation deserves to be encouraged for its importance in general cell biology as well as for the insights it could provide in understanding neoplastic cell transformation. Two promising research areas can be discerned at present: work on oncogenes and work on yeast (in which a number of mutants affect the cell cycle). Important clues may be found when the cognate genes and gene products are identified.

YEAST AS A MODEL FOR CELL BIOLOGY

To a great extent, progress in molecular and cell biology depends on finding cell types that are suitable for experiments. Fortunately, because of the parisimony of nature, mechanisms for determining the most

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important cellular processes are shared by cells all along the phylogenetic tree. For example, myosin from muscle can bind functionally to actin filaments from all animals, plants, and protozoa. Similarly, protein synthesis can be reconstituted *in vitro* with different components from plant and animal cells.

In each field of cell biology, particularly favorable cells have been identified for experimental work. Fruit flies, sea urchins, and chickens have all been excellent sources of model systems for analyzing cell behavior during embryonic development. Frog eggs, because of their large size, are ideal for the production of foreign proteins by microinjection of cognate mRNAs. Highly motile protozoa that can be grown in large quantities have yielded much of the basic information about cytoplasmic contractile proteins. *Chlamydomonas*, an alga with two flagella and well-characterized genetics, has provided much of what we know about the molecular biology of flagella and cilia.

There are, of course, many other examples, but among all the organisms now under investigation, yeast has recently emerged as an excellent experimental system for many studies in cell biology. Yeasts are unicellular fungi that have attracted the attention of biologists because of their efficiency in fermentation and consequent applications in brewing and baking as well as for their role as disease agents. They are also of interest as food or food supplements and as industrial feedstock.

Advances in optics, along with progress in yeast genetics and molecular and cell biology, led to the recognition that yeasts share many features with other eucaryotic organisms. They have a nucleus, mitochondria, and cytoplasmic membrane systems akin to those found in other eucaryotes, albeit much less abundantly. Moreover, yeast cells have multiple chromosomes and undergo mitosis and meiosis. In other words, they exhibit the standard characteristics of eucaryotic organization and genetics.

The occurrence in yeast of three specific cell types, each of which plays a distinctive role in the cell's life cycle, makes the organism suitable for investigations in several important areas of cell biology, including genetic programming for cell differentiation, control of the cell cycle, exocytosis (secretion), endocytosis, and biogenesis of cell organelles, e.g., mitochondria.

Recognition of the commonality of subcellular entities and functions has been recently extended to macromolecules of defined function in other eucaryotic cells. This process has grown in parallel with the

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development of biochemical, cell biological, and genetic techniques and strategies for use in eucaryotic cell biology. All these can be applied to yeast, which in addition offers the advantages of a short cell cycle and, hence, prompt response and low cost.

The most important recent advance in yeast biology is the technique of gene replacement. With this technique, it is possible to produce mutations in cloned genes and to introduce these mutations into the yeast genome to study their *in vivo* expression.

Research Opportunities

These developments indicate that yeast has emerged as a valuable model for the study of many problems in eucaryotic cell biology. At the very least, they move yeast into the mainstream of cell biology, as evidenced by a recent symposium dedicated exclusively to yeast cell biology (Herskowitz, 1985).

Yeast has traditionally been a model for investigating regulation of the initiation, progression, and exit of cells from the reproductive cycle. The genes regulating the cell division cycle (CDC genes) of yeast may be functionally homologous to the cellular oncogenes of animal cells, illustrating yet another commonality that heightens interest in yeasts as models in cell biology.

CELLULAR AND MOLECULAR IMMUNOLOGY

Our understanding of the immune system has increased dramatically during the last few years, largely because of the application of several new technologies developed in molecular and cell biology. These include improved methods for growing cells in tissue culture, the development of methods for preparing monoclonal antibodies, and powerful recombinant DNA technologies. Although progress has occurred in almost all aspects of immunology, only a few highlights can be mentioned here.

It has been known for some years that humans and higher verte-brates use two different systems to recognize when foreign materials (antigens)—such as infectious bacteria or virus or even a host-derived cancer cell—are present in their bodies. Pioneering work in the 1960s and 1970s has led to a good understanding of one of these systems, that of antibody molecules produced by B lymphocytes in response to an antigen. It is clear, however, that the immune defenses of these organisms also depend on recognition of an antigen by another set of lymphocytes called T cells. Recently, after a long search, the T-cell

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receptors for antigens have been discovered and characterized. They resemble antibodies, and like them, they are made up of two polypeptide chains, which vary in amino acid sequence from one T cell line to another. This variability enables one T cell to respond to, for example, influenza virus, and another T cell to react with tuberculosis bacilli. Once the receptors on a particular T cell have bound to an antigen, they stimulate the cell to participate in a series of reactions that help the host deal with the infection. T cells, for instance, regulate the response of B cells by enhancing or moderating it.

The discovery of the genes and proteins of the T cell receptors is a major advance that with further research will lead to a much greater understanding of how the immune system accomplishes the difficult feat of attacking foreign invaders without destroying the tissues of the hosts themselves. (This does occur, however, in cases of so-called autoimmune diseases.) Practical applications are already in use or are certainly in sight. For example, identification of T cell receptors leads to a better diagnosis of and a more rational therapy for human leukemias. It is possible that T cells and their receptors may be manipulated so that they can play an increased role in cancer therapy. An understanding of the function of these receptors is likely to lead to improved diagnosis and therapy of various autoimmune diseases, such as juvenile diabetes or rheumatoid arthritis.

The immune system depends not only on the recognition of foreign material (molecules or cells) by the T and B cells of the invaded organism, but also on the ability of some T cells, called killer cells, to mount a destructive attack on such antigens. The rejection of grafted cells or transplanted organs is caused by such reactions. The attacks are driven in large part by hormone-like molecules, called lymphokines or interleukins, which are produced by the cells of the immune system in response to antigens. Other interleukins apparently coordinate the interactions of different types of lymphocytes. Much current research is focused on the isolation and characterization of interleukins because biologists are interested in their function and because clinical investigators are ready to use them in human and animal diseases. In the past 5 years, the genes and proteins for a number of lymphokines have been isolated. Moreover, it is now possible to produce some interleukins in large quantities for basic research and, in a few cases, for clinical trials. For example, some years ago investigators described a lymphokine, called interleukin 2, which is essential to T-cell proliferation and (in

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at least some cases) to B-cell production of antibodies. Current work is focusing on the signals generated by interleukin 2 binding to target cells and on the reactions that follow this event. Meanwhile, the large scale production of interleukin 2 has made it possible to conduct first stage clinical trials on the application of the hormone to various human diseases.

One of the greatest contributions of medicine to human health has been the development of vaccines. Although many diseases can now be prevented in humans and animals by immunization, other illnesses such as malaria and other diseases due to parasitic protozoa remain scourges. Progress in controlling such parasitic diseases by vaccines has been spotty. An encouraging recent development concerns malaria. Immunological research has shown that soon after invading an organism, the malarial parasite passes through a stage in which it is very susceptible to immune attack. The genes coding for the major protein antigen on the surface of the parasite at this stage have been isolated and sequenced. They indicate a very interesting structure for the proteins they encode, and also, of course, for the molecules to be used for the production of a successful vaccine. Vaccines based on these discoveries have been synthesized and will soon be tested for efficacy.

It would be difficult to discuss recent advances in immunology without bringing up the subject of AIDS. This disease is caused by viral infection and destruction of a particular kind of human T cell, the helper T cell. Much work has been done on the role of these cells, and it is now known that such cells are essential for antibody production by B cells and also that they participate in responses to infection by other T cells. These recent investigations have facilitated our very rapid understanding of the symptoms of AIDS patients.

APPLICATIONS OF RESEARCH RESULTS

Human health care has long been dependent on the broad base of knowledge resulting from our understanding of cell structure and function. In turn, studies of the molecular mechanisms in cancerous and diseased cells and the mode of action of drugs, hormones, and toxins has helped to illuminate fundamental cellular processes and to stimulate a steady progression of new discoveries in cell biology.

This interface between cell biology research and human health care has been clearly recognized in medicine and in the pharmaceutical industry. In many instances, there have been advances ranging from the definition and explanation of abnormal cellular dynamics to the rational design of new drugs, treatments, and medical practices. Now the rapidly expanding base of knowledge in cellular and molecular biology, together with newly developed biotechnology techniques, promises many new pharmaceutical products and innovative health care practices that could have a major impact on the prevention, detection, and treatment of human disease.

Knowledge of the biochemical and molecular features of cellular processes has not been used in the development of plant and animal agricultural products to the same extent that it has for human health care. Now, however, this knowledge is being exploited for the development of agricultural products. In the following pages, some of these applications are summarized along with uses in the commercial development of diagnostic and therapeutic products.

HUMAN HEALTH CARE

As a result of the wide and rewarding overlap between cell biology and other fields, such as immunology, endocrinology, neurobiology, and cardiovascular research, it is impossible to separate their relative contributions to the development of diagnostic and therapeutic pharmaceutical products. Instead, it is more useful to discuss key examples where fundamental knowledge of cellular functions and mechanisms has led or is likely to lead to commercial applications.

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Immunology

Undoubtedly, the cellular techniques and assays used in immunological research have made the most visible and productive contributions to the development of commercial applications. For example, cell fusion and selection techniques have provided the basis for monoclonal antibody (MAb) production by hybridomas, i.e., hybrid cells produced by the fusion of mouse myeloma cells with spleen cells obtained from immunized mice. Hybridomas produce large quantities of uniformly specific antibodies. MAbs are being used as reagents in more than 100 *in vitro* diagnostic products. The use of MAbs is faster and more reliable than traditional methods for identifying infectious diseases (e.g., chlamydiosis, gonorrhea, hepatitis B, acquired immunodeficiency syndrome [AIDS]), hormone levels, and the presence of cancer. In addition, MAbs may prove to be potentially useful as passive vaccines against viral diseases, as targeting agents for cytotoxic compounds in cancer therapy, and as cell-specific delivery agents.

Cellular and molecular dissection of the components of the major histocompatibility complex (MHC) over the last decade has elucidated the critical role that MHC genes and their protein products play in the rejection of organ transplants, the causation of autoimmune diseases, and the control of the immune response. Identification of the MHC gene-encoded antigens that trigger the immune response following organ or tissue transplants and the availability of specific molecular probes for their detection have been major contributors to the rapidly increasing success rate for organ transplants. Similarly, they have aided in the successful transplantation of pancreatic B cells as a treatment for diabetes in animals and are currently being evaluated for application to humans.

Finally, research leading to well-defined stem cell lines and cellular assays has been responsible for major advances in the identification, isolation, and characterization of various immune system regulators, such as interferons, interleukins, and other lymphokines. Cell biology techniques have enabled investigators to characterize viruses (e.g., HTLV III) that cause diseases of the immune system. The recent reports of successes in cancer treatment using lymphocytes activated *in vitro* with interleukin 2 demonstrate the major impact that cell biology has had in therapeutic immunology.

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Endocrinology

Study of the endocrine system is largely based on cellular models for studying hormone release, binding, and mechanism of action. The recent development of angiotensin-converting enzyme (ACE) inhibitors, and their success in the treatment of hypertension, is a notable example of how basic understanding of cellular function and biochemical action can lead to the logical design of new drugs. From the results of detailed studies of the ACE active site, it was possible to design nonpeptide inhibitors, such as Captopril, that have high specificity and can be orally administered. There is a growing list of similarly designed antihypertensives now in clinical trials.

Cellular research has also contributed enormously to the characterization of hormones such as human insulin and human growth hormone, which are the major first generation pharmaceutical products of the biotechnology industry. The discovery of second generation growth hormone derivatives as well as somatomedins and growth hormone releasing factors (GRF) has been dependent on the isolation of hormone-responsive cell lines and the development of sensitive cellular assays. These growth regulators appear promising for the treatment of hypopituitarism as well as for promoting the healing of burns and fractures. In turn, gene cloning and large-scale gene expression have made it possible to produce large amounts of the pure GRF form of the protein, which was previously unavailable for cell biology research.

Neurobiology

Cellular research in neurobiology has been heavily focused on the identification and characterization of neurotransmitter peptides and other molecules that carry signals between nerve cells. Among these are the endorphins and enkephalins, which are members of a family of biologically active neuropeptides termed the endogenous opioid system. The opiates themselves are small organic molecules (alkaloids) that are derived from the opium poppy and are most widely known for their analgesic or pain-alleviating effects. Evidence is accumulating that they as well as the endogenous opioid peptides may also influence the activity of the central nervous system, feeding behavior, the release of various endocrine hormones, learning and memory, and perhaps even social behaviors and interactions.

These peptides were first discovered in 1975, and since that time, more than 15 different species have been identified. Through recombinant

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DNA technology, we have learned that all these peptides are made from three different precursors. It has also been found that these precursors are distributed widely throughout the brain and various other parts of the body. By using cell culture techniques, tissue-specific peptide products of these common precursors have been identified and shown to be necessary for the functioning of, or for communication between, certain neuronal and other cell types. These peptides exert their effects on various cell types by interacting with specific receptors on the surface of target cells. Studies on the interaction of neuropeptides and neuroactive drugs with cellular receptors are expected to lead to the design of new tranquilizers.

The study of neurotrophic factors—factors that promote the survival, maintenance, growth, and regeneration of various types of neurons—and the study of the cell-cell interactions that occur either during regeneration in the peripheral nervous system or during the failure of regeneration in the central nervous system are perhaps two of the best examples of the importance of cell biology research in medical neurology. The nerve growth factor, for example, is a protein that functions in some as yet undefined way to keep the neuron alive and functioning normally. Although the precise mechanism governing this function is not known, much information on its physiological and pharmacological effects has been gained from studies of sympathetic and sensory neurons in culture as well as of neuronal tumor cell lines.

Since other neurotrophic agents required by other cell types are present only in minute amounts, their study has been difficult. Recombinant DNA technology combined with well-defined *in vitro* bioassay systems should enable investigators to purify and characterize these as yet undefined, but biologically important molecules.

Research on the regeneration of damaged nerves has advanced greatly because of results from cell biological experiments. Although peripheral neurons have the capacity to regenerate when damaged, central neurons do not. It has been determined in several systems that Schwann cells, which normally surround and insulate peripheral axons, appear to aid in regeneration. In fact, if Schwann cells are cultured *in vitro* and then placed in a damaged region of the central nervous system, the central neurons will regenerate through this Schwann cell graft. Further experimentation in this and related areas may provide the information required to promote functional recovery following spinal cord damage and other insults to the central nervous system.

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Cardiovascular Research

Studies on thrombolysis, angiogenesis, and cardiac hormones have recently become a focal point for the application of technologies associated with modern cell biology. Although coronary bypass surgery and angioplasty have emerged as effective treatments for occluded arteries, they lack the speed and ease of administration necessary when a person suffers a myocardial infarction. It now appears that irreparable damage to the ventricle of the heart occurs if clots are not cleared within 4 to 6 hours. This knowledge has driven the search for a treatment that is safe and can be rapidly administered, and thrombolysis, the enzymatic dissolution of a blood clot, has been studied as a possible solution.

Through the 1970s, two enzymes, streptokinase and urokinase, were advanced for use in thrombolytic therapy. Both catalyze the activation of human plasminogen to plasmin, which in turn catalyzes the digestion of the fibrin matrix associated with blood clots. Both have been tested clinically and both proved to have unwelcome side effects (e.g., bleeding).

Tissue plasminogen activator (tPA) is synthesized in and released from endothelial cells, but it has not been possible to culture these cells in quantities sufficient for studying the structural aspects of tPA. The screening of established mammalian cell lines has led to the identification of several sources of the molecule, notably Bowes melanoma cells and Hela cells. Work in the 1970s began to unravel the biochemistry of tPA and the peculiar features that made it especially suited to fibrinolysis *in vivo* and ultimately led to its purification in 1982.

Even in the highest-yielding cell lines, tPA production is not adequate if projected needs for its therapeutic use are correct. However, with a cell source and a supply of the pure enzyme, it became possible to isolate messenger RNA (mRNA), to complete the cloning of a double-stranded, complementary DNA (cDNA) encoding full length tPA, and to insert the gene into a suitable host-vector system to produce tPA in larger quantities. Although attempts have been made to produce tPA in bacterial and yeast cells, the research emphasis has been placed on mammalian cells as a system for large-scale production. Clinical testing of tPA is currently under way at centers throughout the United States.

Angiogenesis, the growth of new blood vessels, does not normally occur in adults, except during wound healing. From a scientific standpoint, angiogenesis provides an important model for studying the interactions between various cell types in the body, as well as the regulation of their

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various functions. Further study of angiogenesis may hold considerable medical and commercial potential. For instance, wound healing could be enhanced by substances that promote angiogenesis. Such treatments could provide obvious benefit to millions of surgical patients, accident victims, and burn victims. Millions of others who suffer from ischemic heart disease, in which coronary arteries are blocked by atherosclerosis, or from diabetes, often accompanied by retarded wound healing, could also possibly benefit from a method that promotes the growth of new blood vessels. Angiogenesis plays a significant role in a number of pathological conditions. Since solid tumors require angiogenesis for growth, the prevention of this process could increase the efficacy of certain cancer therapies. Angiogenesis also appears to be an unwanted and serious complication in certain other diseases, such as diabetic retinopathy.

Recent progress in the study of angiogenesis has been made in two areas. First, proteins that promote angiogenesis have been purified in a number of laboratories. One such family of proteins, the heparin-binding growth factors, promotes angiogenesis by stimulating the proliferation and movement of the endothelial cells that line the blood vessels. Another protein, angiogenin, promotes angiogenesis by an unknown mechanism. A second area of research that holds great potential stems from the discovery that combinations of certain steroids and heparin inhibit angiogenesis. This type of therapy has caused tumor regression in animals. Clinical studies will soon be undertaken.

A great deal remains to be learned about the mechanisms of angiogenesis. Although some of the chemicals that regulate this process have been identified, very little is known about their normal functions in the body. Furthermore, since angiogenesis is an extremely complex process involving the interaction of numerous cell types in different tissues and organs, other angiogenic substances undoubtedly remain to be discovered. It is therefore anticipated that future research on angiogenesis could be very rewarding scientifically, medically, and commercially.

Investigators have recently discovered a new class of cardiac hormones, comprising atriopeptins or atrial peptides, which possess potent natriuretic and diuretic activity and may be associated with fluid imbalance, altered vascular tone, and hypertension. Atriopeptides are synthesized and stored in cardiac atrial myocytes. Recent immunocytochemical studies have demonstrated that these peptides are localized specifically in atrial granules. High affinity atriopeptin binding sites have been found on cell membranes prepared from aorta as well as from

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adrenal zona glomerulosa. Specific receptors have also been identified in the central nervous system.

A variety of atriopeptides ranging in size from 21 to 126 amino acid residues in length have been isolated from rat and human tissues. The complete amino acid sequence of the precursor atriopeptigen (APG) has been deduced from cDNA clones of human and rat. Human APG is 151 amino acids long and contains a hydrophobic signal sequence like other secretory proteins. The active hormone is located at the extreme C-terminal end of APG. The expression of APG in the atrium and other tissues is being examined to determine its correlation with disease states, to identify processing and release mechanisms, and to learn more about its mode of action in cells.

Atriopeptides are potent and selective renal vasodilators. Since biological activity is retained in peptides containing as few as 21 amino acids, the synthesis and testing of more potent, longer lasting, and more selective derivatives appear to be quite possible. Clinical trials are under way to evaluate therapeutic benefits for hypertension and cardiovascular and renal diseases.

Mitochondrial Studies

In recent years, an increasing amount of evidence has implicated deficiencies of the oxidative-phosphorylation system as the primary cause of myopathies (muscular diseases), central nervous system abnormalities, and multisystem diseases in humans. Biochemically, the best characterized of these are the so-called mitochondrial myopathies, which exhibit deficiencies of one or more of the enzyme complexes of the oxidative-phosphorylation apparatus. In many cases, a hereditary genetic defect seems to be involved. The continuously increasing availability of molecular probes may eventually provide crucial evidence concerning the molecular basis and genetic origin of the primary defects responsible for these diseases.

PHARMACEUTICAL PRODUCTS

The use of cellular techniques and experimental approaches has had an obvious impact on human health care, as discussed above for immunology, endocrinology, neurobiology, and cardiovascular research. In addition, various cell biology techniques and discoveries have contributed to improvements in the pharmaceutical industry's approaches to the

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identification and development of new products. These include, for example:

- differentiated, responsive, or hybrid cell lines for screening and isolating active biological agents or derivatives;
- large-scale mammalian cell cultures, which have been developed to produce large quantities of MAbs and to produce proteins that are extensively modified posttranslationally and are not active when produced by bacterial or yeast fermentation;
- receptor modelling and active site elucidation in the design of rational drugs and derivatives; and
- delivery and targeting of drugs through increased understanding of receptor internalization, endocytosis, and intracellular trafficking (see discussion on protein synthesis in the section on Cytoplasm: Organelles and Functions).

ANIMAL AGRICULTURE

There is an enormous potential for applying basic knowledge of cellular processes in animal agriculture, such as for growth promotion, disease treatment, and genetic improvement. Recent advances in cell biology, including those made in human health care, provide new opportunities for improving the efficiency with which domestic livestock is produced.

Growth Promotion

Animal growth is regulated by interactions among polypeptides produced in the hypothalamus, pituitary gland, and liver. The growth hormone (GH), or somatotropin, is synthesized by the pituitary gland, and its release is regulated by two hypothalamic peptides—somatostatin and the growth hormone releasing factor (GRF), which inhibit or stimulate GH release, respectively. GH binds to receptors on the plasma membrane of liver cells as well as other tissues of the body. Using isolated cells and radiolabeled GH, investigators have observed specific binding to lymphocytes, adipocytes, chondrocytes, and hepatocytes. The growth stimulatory effects of GH are generally believed to be manifested indirectly by increases in circulating levels of insulin-like growth factors.

The enhancement of growth rate and milk production by GH has been studied for several decades, but commercial application has been

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impossible because of the limited supply of hormone that could be extracted from pituitary glands. Through advances in biotechnology, large quantities of GH can be produced for the first time. Recent studies have shown that treatment with exogenously administered recombinant bovine GH can increase milk production in dairy cows by as much as 23% to 41%. Injections of pigs with porcine growth hormone have been shown to stimulate growth rate by approximately 10% and to improve feed efficiency dramatically. Efforts to develop commercial formulations of GH are currently under way in several companies.

Other classes of hormones coming under intensive study include placental lactogens, which appear to play a role in the development of the mammary gland during gestation. These lactogens were originally identified by coculturing placental tissue with mammary tissue and evaluating lactogenic activity. Proteins of placental lactogen are approximately 35,000 daltons in mass and are immunologically related to prolactin. They are major products secreted by placental tissue during gestation. Through immunohistochemical techniques, these proteins have been localized to secretory granules in the fetal portion of the placenta.

The functions of placental lactogens require further definition. Indirect evidence in cows, sheep, and goats suggests that they may be involved in the development of mammary tissue during pregnancy, and the degree of this growth directly influences the milk output of a cow during lactation. Placental lactogen may be a potent growth hormone, since it can bind to the GH receptor. Genes coding for placental lactogen have recently been cloned from the rat, mouse, and cow. Studies of homogenous hormones should lead to a clear understanding of their function, mode of action, and commercial utility.

Disease Treatment and Prevention

The diagnosis of diseases in animals is likely to be substantially improved by the development of MAbs to infectious virus and bacteria. Such MAb-based diagnostic tests are already available for the detection of several human diseases, as discussed above. Because of the low value-added nature of farm animals, MAb-based tests may be initially limited to household pets and high profit animals (e.g., select breeding lines, race horses, and zoo animals). Currently, diagnostic tests are being developed for bovine bluetongue, equine infectious anemia, and bovine leukosis virus.

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The diagnosis of pregnancy in domestic animals is another potential market for MAb products. Several companies plan to market kits for conducting such tests on farms in the near future.

Attempts are being made to find rDNA (recombinant DNA) subunit vaccines—vaccines that contain only a portion of a pathogen—to prevent such viral diseases as foot-and-mouth disease, rabies, gastroenteritis, and Rift Valley fever. It is generally believed that subunit vaccines may be more stable, more easily stored, of greater purity, and safer than conventional vaccines. The genes encoding the major surface antigens of several important viruses, including rabies and foot-and-mouth disease, have been cloned, characterized, and genetically engineered for production by using microbial expression systems. Efforts continue to identify conserved, highly antigenic peptides that will promote consistent immune response to viral infections in animals.

MAbs may also provide passive immunity to viral and bacterial diseases. For example, MAbs directed against flagellar antigens of bacteria that cause colibacillosis (scours) protect young calves when administered orally within 36 hours of birth. This MAb-based product has already been approved for commercial use. Similarly, MAb products that prevent rabies are being developed and are likely to be available commercially in the near future.

In long-range programs to improve the treatment of diseases in animals, cellular and molecular biological techniques are being used to elucidate the molecular basis of genetic resistance to disease. Studies are currently under way to map the MHC genes in swine and poultry, which are resistant to specific diseases. These studies are likely to lead to information on animal immune system modulators and to the identification of genes that will be useful in transformation studies.

Genetic Improvement

Over the last 30 years, animal production has markedly increased because of the use of new techniques for manipulating and controlling reproductive processes. Methods developed for storing semen and culturing embryos have facilitated artificial insemination (AI) and embryo transfer—techniques now used routinely for genetic improvement in cattle, swine, and poultry. The power of these breeding tools is illustrated by the doubling of the average milk yield of dairy cows in the United States over the past 30 years. For turkeys, AI has been so successful that it is used for nearly 100% of the commercially produced turkeys.

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The feasibility of using molecular genetic techniques to modify animal growth has been established recently by the demonstration that foreign genes can be injected directly into fertilized mouse eggs and transplanted into surrogate females, which produce transgenic offspring. Transgenic mice containing either rat, human, or bovine GH genes display high levels of GH mRNA, especially in the liver and intestine. These mice have high concentrations of circulating GH and elevated levels of insulin-like growth factor 1. In addition, their growth rates are markedly enhanced, and their mature body size is almost twice as great as that of nontransgenic mice. The chimeric GH gene was shown to be inheritable. Thus all offspring also exhibited enhanced growth.

More recently, transgenic mice containing chimeric GRF genes have been produced. The increased body weight of these animals correlates with the presence of GRF mRNA, thereby unequivocally establishing the role of GRF in regulating growth. Interestingly, there is a dramatic increase in the size of the pituitary glands in transgenic mice containing MT(mouse metallothionein)-GRF genes. This resembles a condition in humans with pancreatic carcinomas that secrete GRF. Transgenic animals may therefore serve as useful models for growth disorders as well as providing a powerful experimental system for studying mechanisms that regulate growth.

Technical difficulties have been encountered in applying gene transfer techniques to livestock, but recently embryos have been successfully injected and implanted into sheep, pigs, and rabbits. The production of transgenic sheep and pigs is currently inefficient. Further experimentation will be required to optimize factors that influence DNA integration and embryo survival.

Animal Agricultural Products

Many of the promising new commercial developments in animal production are extensions of technical advances made in human health care, partly stemming from knowledge acquired during animal tests of pharmaceutical products intended for human use. In many cases, however, extrapolation to animals from research in humans is not possible. For example, early studies in humans and other primates have shown that the primate placental lactogens are structurally related to growth hormone. In contrast, bovine placental lactogen appears to be related to prolactin. The role of these relationships and their effect on the ability of various placental lactogens to interact with different receptors and

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elicit different functions are unknown. Other areas requiring more cell biology research include the following:

- differentiated, responsive animal cell lines for screening and isolating growth factors and other biological agents;
- efficient, low-cost, slow-release, long-term delivery systems for proteins and peptides; and
- receptor identification, modeling, and active site elucidation for animal hormones and other regulatory molecules.

PLANT AGRICULTURE

The discovery of agrochemicals, e.g., herbicides, pesticides, and plant growth regulators (PGRs), and genetic improvement of crops by plant breeding have historically been largely empirical processes. However, it is now considered very likely that the application of techniques such as cell culture, cell fusion, gene transfer, and MAb production will have a major impact on plant agriculture. Many have predicted that the long-range impact of biotechnology and cell biology on plant agriculture may be even more substantial than their effects on animal agriculture and human health. Potential crop improvements include increased yield, improved quality, and reduced labor and other production costs.

Increasing Yield

For most crop species, yield is determined by a complex interaction between genetic and environmental components. The remarkable increases in yield recorded for most U.S. crop plants over the last 50 years reflect the major impact of breeding programs on improving plant growth, reproduction, and harvest index (i.e., the percentage of plant biomass converted to harvestable grain or other materials). In addition, agronomic losses due to environmental conditions have been greatly reduced by the introduction of superior cultivation practices, agrochemicals, and cultivars with improved resistance to diseases and pests. Biotechnology is likely to increase crop yield even further by improving the efficiency of traditional breeding processes in much the same way that artificial insemination and embryo transfer have enhanced animal husbandry programs. Furthermore, the recently developed gene transfer systems for plants may provide a new approach to increasing yield by both accelerating the rate of genetic modification and expanding the diversity of plant traits that can be manipulated.

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The ability to culture plant cells and to induce regeneration of intact plants from a single cultured cell provides an elegant system both for studying cellular processes and for improving crops. Tissue culture has been used successfully for the selection of mutants displaying agronomically important traits as well as for the aseptic propagation of disease-free plants. Embryo culture and haploid cell culture techniques are being used with increasing frequency to accelerate breeding of selected cultivars. Protoplasts from related species are being fused to expedite the matching of different genomes of nuclei and organelles (e.g., chloroplasts and mitochondria). This technique may be particularly valuable for the rapid introduction of traits such as male sterility or herbicide resistance.

The efficiency of traditional breeding techniques is likely to be enhanced by the development of rapid methods for monitoring the transmission of agronomic traits. This has already occurred in some areas. For example, a nematode-resistant (Mi) gene from a wild tomato has been introduced into new commercial tomato cultivars. Genetic and biochemical studies have demonstrated that the nematode resistance trait is closely linked to an alkaline phosphatase gene. Thus, the progeny of crosses having the resistance gene could be easily identified by using phosphatase gel assays. Today the majority of the hybrid tomato commercial cultivars contain this gene. A major effort is under way to identify isoenzyme or DNA polymorphism markers linked to other important agronomic traits to facilitate their rapid transfer into new cultivars of major crop species.

The ability to manipulate chloroplasts through their own genes or nuclear genes is likely to be important in future genetic engineering processes. In photosynthesis, light energy is converted to chemical bond energy in the form of adenosine triphosphate. Accordingly, plants have evolved to use the chloroplast as the site not only of photosynthesis but also of many other processes essential for the life of the plant, such as the synthesis of amino acids required for protein synthesis and the synthesis of fatty acids required for membrane synthesis. Chloroplasts are thus attractive sites in which to introduce, by genetic engineering, energy-intensive, novel biosynthetic pathways.

Photosynthetic carbon dioxide fixation via the C4 pathway of maize and certain other plants that can take advantage of high light intensities is a reminder that it would be worthwhile either to improve

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ribulose bisphosphate carboxylase (RuBPC)—the key enzyme in photosynthetic carbon fixation—or to find some other mechanisms for altering photosynthetic carbon fixation to reduce energy losses through photorespiration—the light-stimulated respiration of chlorophyllous tissue. Information about plastid differentiation and the regulation of plastid gene expression is now sufficient for beginning an intensive investigation.

One of the most exciting prospects for increasing crop yields by genetic improvement is the recently developed gene transfer system in plants. The discovery that crown gall disease caused by the soil bacterium *Agrobacterium tumefaciens* results from the transfer and integration of a segment of bacterial plasmid DNA into plant cells has led to the design of vectors and markers for plant transformation. The growing list of transgenic plants now includes the tomato, potato, poplar, petunia, tobacco, carrot, alfalfa, oil seed rape, lettuce, flax, and asparagus. Gene transfer techniques have already been used to modify plants in ways that may eventually result in yield increases. For example, genes encoding the small protein subunit of RuBPC have been transferred into petunia and tobacco plants. More recently, genes for chlorophyll *a* and *b* binding proteins have also been introduced into transgenic plants. Expression of these important photosynthetic genes in transgenic plants is normal. These results establish the feasibility of long-range efforts to modify the photosynthetic process in plants to increase yield.

In research that parallels the interests pursued in studies on oncogenes and hormones in mammalian systems, genes encoding enzymes that synthesize phytohormones have been recently identified and characterized in plants. Phytohormones, including auxins, cytokinins, gibberellic acid, ethylene, and abscisic acid, are small molecules that regulate plant growth and development. They are often used commercially as plant growth regulators to improve seed set and yield, control ripening, and increase storage life of fruits and seeds. Expression of auxin and cytokinin biosynthesis genes in transgenic plants dramatically alters normal plant morphology and development. Petunia and potato plants containing cytokinin genes display increased branching due to the loss of apical dominance as well as other effects consistent with cytokinin overproduction. A major challenge now is to modify phytohormone levels and expression patterns to increase yield and to enhance other agronomically important traits.

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Gene transfer techniques may also increase agricultural productivity by improving disease control. It has been known for more than 50 years that infection of certain plants with a virus strain producing mild effects will often protect them from subsequent, more severe infection. This phenomenon, called cross protection, is used commercially to reduce losses from viral disease in certain vegetable and citrus crops. The mechanism responsible for cross protection is unknown. Recently it has been shown that both tobacco mosaic virus (TMV) coat protein mRNA and the coat protein itself are expressed by transgenic tobacco plants containing the coat protein gene from TMV as a result of genetic engineering accomplished under the control of a strong plant promoter. When these plants are challenged by inoculation with TMV, they exhibit delayed, reduced, or in some cases no viral symptoms. Whether the observed cross protection results directly from the expression of TMV coat protein mRNA, or the protein, or as a result of an induced cellular factor is not clear—but this exciting result demonstrates an approach for increasing crop yields by reducing losses due to viral diseases.

Improving Qualitative Traits

Qualitative traits of plants, such as oil and protein content, fruit color, fatty acid composition, and nutritional value, are important targets of most plant breeding programs. They are also traits that may be amenable to molecular manipulation. Inhibitors of amino acid biosynthesis have been used successfully in tissue culture to produce plants with altered protein or amino acid compositions. In tomatoes and carrots, induced mutagenesis followed by selection has been used to obtain cultivars with enhanced color or storage properties. With alfalfa, mutagenesis followed by selection in tissue culture has led to the production of plants with increased leaf protein content.

The prospect for using gene transfer to improve qualitative traits is quite good. Many of the genes encoding major seed storage proteins from corn, soybean, rice, and sorghum have been isolated and characterized. Legume storage protein genes have been introduced into transgenic tobacco and petunia plants, where they demonstrate normal expression in seeds. Systems of this type will permit the testing of storage proteins in which amino acids have been altered to improve nutritional balance. Excellent progress is also being made in the identification of other plant genes involved in the biosynthesis of starch, fatty acids, and pigments.

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These findings should eventually enable investigators to modify these important qualitative traits.

Plant Breeding. A considerable amount of circumstantial evidence indicates that mutations in mitochondrial DNA are responsible for cytoplasmic male sterility—a phenotype found in a wide range of plant species (e.g., maize, sorghum, sugar beets, and sunflower) characterized by the failure of the mature plant to release functional pollen. This trait, which prevents self-fertilization of the seed parent plant, is used commercially in hybrid seed production. Fusion between somatic cells from sterile male and fertile male species is being used to transfer the male sterile phenotype into a cell with a different nuclear genotype exhibiting desired traits.

Male sterility is often accompanied by sensitivity of the male sterile plant to fungal toxins. Elucidation of the molecular basis of cytoplasmic male sterility and its link to susceptibility to fungal pathogens will have a considerable economic payoff by facilitating the production of plants that are not susceptible to the fungal pathogens.

Reducing Costs

In many crops, control of traits such as herbicide tolerance, ease of harvest, storage properties, shelf life, and baking times are essential for maintaining acceptable profitability. The use of MAb-based or DNA probe-based diagnostic kits to identify diseased seed propagules or fruit will help to eliminate the need for replanting and reduce losses during storage. Such kits will also reduce labor and chemical costs by facilitating the identification of viral, fungal, and insect pathogens, thereby enabling investigators to select the most effective and specific treatments. These kits are expected to be available in the near future.

The development of effective and crop-safe herbicides has been a major contribution to the reduction in farm labor requirements and operating costs. For many crops, however, effective and crop-safe weed controls are not yet available. If genetic engineering can be used to transfer herbicide resistance to crop plants, it could be an alternative and complementary strategy to the usual synthesis, screening, and testing of new herbicides. This approach offers the advantage of allowing proven safe and effective chemicals to be applied to new crops and could result in low cost, effective weed control.

The recent identification of cellular targets for herbicides such as glyphosate, sulfonyl ureas, and triazines indicates that this may indeed

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be possible. Glyphosate inhibits an enzyme, 5-*enol*-pyruvylshikimate-3-phosphate (EPSP) synthase, involved in the synthesis of aromatic amino acids in plants. The expression of either chimeric bacterial or plant EPSP synthase genes in transgenic tobacco and tomato plants increases herbicide resistance. Efforts are currently under way to introduce the chimeric EPSP synthase genes into crop plants where lack of weed control presents a significant problem.

Plant Products

There is a new emphasis on crop improvement as a result of recent technical advances in plant tissue culture and genetic engineering together with heightened commercial interest in the application of biotechnology to plant agriculture. Many cell structures, processes, and pathways are unique to plants and cannot be elucidated by research on animal models. To maximize the applications of biotechnology, therefore, the knowledge base in plant cell biology must be increased. Requiring special attention are needs for the following:

- defined, reproducible conditions for the culture and regeneration of important legume and cereal crop species,
- efficient transformation vectors for cereal plants and for genetic modification of extranuclear (i.e., organelle) genomes, and
- appropriate cellular systems for studying interaction among pathogens, phytohormones, and other agents.

SUMMARY

In summary, the results of basic research in cell biology are being increasingly applied in medicine and in plant and animal agriculture. Such knowledge and techniques provide much of the basis for commercial biotechnology. In medicine, these applications include many new diagnostic techniques and new pharmaceutical health care products that will have a major impact on the prevention, detection, and treatment of human disease. The discovery of, and the ability to produce in large scale, potential new drugs to dissolve blood clots, reduce hypertension, and promote healing of burns and fractures are exciting examples of basic research in cell biology that is being successfully applied in medicine.

There is equally great potential for the application of basic knowledge of cellular processes in animal and plant agriculture. Recent advances provide new approaches for disease treatment, growth promotion, and

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genetic improvement of livestock. Potential crop improvements include increased yield, improved nutritional quality, and reduced labor and production costs. Direct benefits of increased agricultural efficiency include greater profitability for producers, improved food quality and lower cost for consumers, and a reduction in the use of marginal crop and pasture land and associated erosion and pollution.

The elapsed time between the achievement of basic research milestones and their application in medicine or agriculture has shrunk with the advent of large-scale commercial investments in genetic engineering and biotechnology. If there is continued strong support for applications, the rate-limiting step in the development of applications may become the rate of progress in basic research.

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CELL BIOLOGY RESEARCH SUPPORT

The establishment of the American Society of Cell Biology 25 years ago marked the emergence of cell biology as a distinct discipline in the United States. Reflecting the growth and maturation of the field, this organization has grown steadily over the intervening quarter century to its current membership of more than 5,800.

There are now several journals devoted exclusively to research in cell biology and widely used textbooks devoted to the subject. The international nature of the science is evidenced by the significant contributions to the literature made by laboratories in at least 10 foreign countries and by international congresses, which are held every 4 years.

The United States is almost alone in recognizing cell biology as a distinct discipline. In most other countries, cell biology is hidden within animal sciences, agricultural sciences, and biomedical sciences, and it is difficult to identify since its support is included in budgets for education, agriculture, medicine, and other administrative categories. Consequently, it was impossible for the panel to ascertain the amounts of support for cell biology research in other countries.

U.S. GOVERNMENT SUPPORT FOR RESEARCH IN CELL BIOLOGY

In the United States, government support of cell biology research is provided by the National Science Foundation (NSF), the National Institutes of Health (NIH), and, to a lesser degree, by the Department of Agriculture (USDA), the Department of Energy (DoE), and the Office of Naval Research (ONR).

Current support for cell biology research by the named agencies is summarized in Table 1. It is estimated that in FY 1985 the NIH allocated $750 million to research in molecular and cell biology (including $325 million for cell biology) (C. A. Miller, NIH, personal communication, 1986) and the NSF allocated $112 million to research in molecular and cell biology (B. Umminger, NSF, personal communication, 1986). Because these agencies do not categorize their expenditures in the same

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way, the figures are only roughly comparable. Nevertheless, together they represent the bulk of U.S. government support for cell biology research.

The panel was not able to obtain specific figures for support of cell biology research by USDA. The agency's Current Research Information System (CRIS), a databank with information on agency-supported extramural and intramural research activities, was searched by keyword for support of cell biology and related topics in FY 1984. The search yielded 1,755 projects totaling $17.3 million and 472 new projects for which no figures were given. Item-by-item analysis would be required to determine the relevance of individual projects to cell biology (R. Sparks, USDA, personal communication, 1985).

Table 1. Summary of Federal Support for Research in Molecular and Cell Biology, FY 1985

Agency	$ (in millions)
National Institutes of Health	750.0
National Science Foundation	111.8[a]
Department of Energy	21.2
Department of Agriculture	17.3[b]
Office of Naval Research	5.5
TOTAL	905.8

[a] This excludes neurobiology.
[b] FY 1984.

In FY 1985, DoE spent approximately $12.4 million on biological energy research (DoE, 1985). Much of this money supported research on photosynthesis and other aspects of biological energy transduction at the cellular level. For the same fiscal year, DoE's Office of Health and Energy Research, whose mission is to investigate the health and environmental effects of various energy processes, listed support for 11 molecular biology projects totaling $2.5 million and 25 cell biology projects totaling $6.3 million (DoE, 1986), bringing total DoE support for cell biology to approximately $21.2 million (Table 1). ONR support for cell biology in FY 1985 was $5.5 million (R. Newburgh, ONR, personal communication, 1986).

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Even though cell biology enjoys its own budgetary identity at NSF, NIH, and ONR, it is not always possible to identify the total amounts spent by these agencies in support of such research. Because the boundaries of cell biology are not easily defined, much research that may be classified as, for example, genetics, neurophysiology, or agriculture may in fact be molecular or cell biology.

Total support for research in molecular and cell biology for the named agencies in FY 1985 came to approximately $906 million. This figure probably underestimates somewhat the total federal support for research in cell biology for the following reasons:

- It does not include support for cell biology not so classified by the named agencies.
- It does not include several agencies, e.g., the Department of Defense (exclusive of ONR), the National Aeronautics and Space Administration, and the Environmental Protection Agency, that may support some relevant research.

Although departments of cell biology are found at some academic institutions, the discipline is often included under biology, zoology, botany, pathology, anatomy, immunology, biochemistry, and other disciplines. Thus, no attempt was made to determine direct institutional or other nonfederal support for research in cell biology.

Trends in U.S. Research Support

Table 2 summarizes NIH support for research in 1975, 1980, and 1985. The proportionate amount spent by NIH on basic research increased from 44% of the total budget in 1975 to 64% in 1985, whereas the amount spent on applied research declined over the decade from 41% to 29%. The NIH classification system makes it difficult to identify all the cell biology research being supported. However, as noted above, it has been estimated that $750 million was allocated to molecular and cell biology in 1985 at NIH (C. A. Miller, NIH, personal communication, 1986).

Table 3 shows the distribution of a major segment of NIH research support among cell biology and related fields in FY 1975, 1980, and 1985. Distribution by study section in FY 1985 is presented in Table 4.

NSF support for research in cell biology and related areas for FY 1975, 1980, and 1985 is summarized in Table 5. The table reflects the split of the Division of Physiology, Cellular and Molecular Biology

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Table 2. Approximate Amounts Awarded for Research by NIH[a]

	Fiscal Year 1975		Fiscal Year 1980		Fiscal Year 1985	
Category	$ (in millions)	%	$ (in millions)	%	$ (in millions)	%
Total research	1,655	100	2,920	100	4,500	100
Research by type:						
Basic	730	44.1	1,505	51.5	2,857	63.5
Applied	685	41.3	1,055	36.1	1,283	28.5
Development	240	14.6	360	12.4	360	8.0
Converted to constant 1975 dollars:[b]						
Total research	1,655		1,982		2,287	
Basic	730		1,022		1,452	
Applied	685		716		652	
Development	240		244		183	

[a] Data from C. A. Miller, NIH, 1986.

[b] Based on the Biomedical Research and Development Price Index (Department of Commerce, unpublished).

Table 3. Distribution of NIH Research Support by Field of Research as Coded by Traditional Disciplines[a]

	$ (in millions)		
Field of Research	Fiscal Year 1975	Fiscal Year 1980	Fiscal Year 1985
Biochemistry	130	293	440
Genetics	37	76	159
Cell biology	17	55	123[b]
Pathology	27	48	63
TOTAL	211	472	785

[a] Derived from data provided by C. A. Miller, NIH, 1986.

[b] The total amount, corrected for cell biology included in biochemistry, genetics, and other fields, is estimated at approximately $325 million (C. A. Miller, NIH, 1986).

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Table 4. Distribution of NIH Research
Support by Study Section, FY
1985

Study Section	$ (in millions)
Neurology	74
Neurological sciences	34
Cell biology[a]	83
Genetics[b]	183
Immunology	148
Biochemistry[c]	340
Pathology	90
Visual sciences	92
Oral biology	32

[a] Includes molecular cytology.

[b] Includes molecular biology.

[c] Includes biophysical, bioorganic, and
metallorganic chemistry.

into the Division of Molecular Biosciences and the Division of Cellular
Biosciences.

Included in Table 5 is support provided by those components of
the Division of Behavioral and Neural Sciences relevant to cell biology,
namely, Neurobiology (later split into Molecular and Cellular Neuro-
biology, Integrative Neural Systems, and Developmental Neuroscience)
and Sensory Physiology and Perception.

At NSF, the budget for research in all areas of biology more than
doubled over the last 10 years; however, by converting the 1985 figures
to constant 1975 dollars, it can be seen that the actual purchasing power
of this budget increased only 30% (Table 6).

In comparing NIH and NSF support, it is important to note that
whereas the NIH figures represent direct costs only, the NSF figures
include both direct and indirect costs. Thus, in terms of actual support
for research, the NSF figures should be reduced by at least one-third.

Because of cell biology's unclear boundaries and the resulting prob-
lems of definition and categorization, it is difficult to identify with
precision the amounts spent on research in cell biology. To obtain an

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Table 5. Distribution of NSF Support for Research in Cell Biology and Related Fields[a]

NSF Unit	$ (in millions)		
	FY 1975	FY 1980	FY 1985
Division of Physiology, Cellular and Molecular Biology (PCM), later split into Division of Molecular Biosciences (DMB) and Division of Cellular Biosciences (DCB):			
Biological Instrumentation (DMB)	0	3.5	7.7
Biochemistry (DMB)	7.4	9.4	14.0
Biophysics (DMB)	7.3	10.1	13.9
Metabolic Biology (DMB)	6.2	9.9	14.7
Alternate Biological Resources (DMB)	0	1.6	2.5
Genetics, later split into	6.3	9.8	
Procaryotic Genetics (DMB)			8.1
Eucaryotic Genetics (DCB)			8.5
Cell Biology (DCB)	0	6.6	11.0
Cellular Physiology (DCB)	0	4.0	8.0
Developmental Biology (DCB)	5.8	8.6	13.7
Regulatory Biology (DCB)	6.5	8.6	9.6
Human Cell Biology	2.3	0	0
TOTAL, PCM	41.8	72.1	
TOTAL, DMB			60.9
TOTAL, DCB			50.8
Division of Behavioral and Neural Sciences:			
Neurobiology, later split into	3.9	8.8	
Molecular and Cellular Neurobiology			5.2
Integrative Neural Systems			4.9
Developmental Neuroscience			5.3
Sensory Physiology and Perception	2.7	6.9	7.2
TOTAL, DBNS	6.6	15.7	22.6
TOTAL, NSF	48.4	87.8	134.3

[a] Data from B. Umminger, NSF, 1986.

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impressionistic picture, funding for two programs that clearly support cell biology at NSF—Cell Biology and Cellular Physiology—were examined. Since neither of these programs existed in 1975, one can only make the comparisons between 1980 and 1985. During this period, funding in the Cell Biology program increased by 67% and in Cellular Physiology, by 100%. In terms of 1975 dollars, the increases were 27% and 52%, respectively.

Table 6. Approximate Amounts Awarded by NSF for Research in All Areas of Biology[a]

| Category | $ (in millions) | | |
	Fiscal Year 1975	Fiscal Year 1980	Fiscal Year 1985
Total biology	77.2	132.0	197.2
Converted to constant 1975 dollars[b]	77.2	89.7	100.0

[a] Data from B. Umminger, NSF, 1986.

[b] Based on the Biomedical Research and Development Index (Department of Commerce, unpublished).

ONR support of basic research in cell biology in FY 1975, FY 1980, and FY 1985 is shown in Table 7. In constant dollars, ONR support for cell biology increased 87% over the decade.

Table 7. ONR Support for Research in Cell Biology in FY 1975, 1980, and 1985[a]

| Category | $ (in millions) | | |
	Fiscal Year 1975	Fiscal Year 1980	Fiscal Year 1985
Cell biology	1.5	3.5	3.5
Converted to constant 1975 dollars[b]	1.5	2.4	2.8

[a] Data from R. Newburgh, ONR, 1986.

[b] Based on the Biomedical Research and Development Index (Department of Commerce, unpublished).

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These limited data indicate that support for research in cell biology has experienced significant growth over the decade 1975-1985. Much more information would be required, however, to permit a determination of the adequacy of this growth.

Facilities, Instruments, and Services

Continued progress in cell biology research depends on many factors, including research support for trained investigators as well as recruitment of students into the field and support for their training. The panel considered the need for research facilities, including instruments and services, as well as the training of new investigators in molecular and cell biology.

Because of the development of sophisticated and expensive instrumentation, much of which requires operators with special training, some research in cell biology can no longer be conducted in fully self-sufficient laboratories. As a consequence, investigators increasingly seek access to, or services from, special purpose facilities outside their own laboratories or departments. Below we consider several strategies for the provision of these facilities or services, ranging from national facilities to local core facilities and to individual instruments that may be a part of an investigator's own laboratory. We also briefly consider electronic networking in support of collaborative interdisciplinary research.

Core Facilities. In its present state of development, molecular and cell biology does not need large research centers comparable in size and nature and in the cost of instrumentation to the national laboratories now required for many areas of research in physics. There is, however, a critical need for medium-size core facilities for certain research activities, for example, for generating high-quality, state-of-the-art data on glycan structure, amino acid sequences in proteins and peptides, and nucleotide sequences in nucleic acids, and for synthesizing large numbers of high quality oligopeptides and oligonucleotides with well-defined sequences. At least some of these core facilities should include laboratories for the production of monoclonal antibodies by hybridoma procedures.

Core facilities should provide instruments and services that require large capital investments and operating costs and the services of staff with adequate training. Facilities of this kind can rarely if ever be maintained by individual investigators because of prohibitive costs and technological demands. Core facilities can serve the needs of several

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research projects or programs by providing such services in a cost-effective manner. Over the next decades, we can expect an increasing demand for a variety of core facility services from investigators working in the rapidly developing areas of molecular and cell biology mentioned in this report.

A core facility should serve a few individual, well-established research projects or programs and should receive support not only for equipment but also for technically competent personnel, e.g., holders of a Ph.D. degree in bioorganic chemistry or biochemistry and specially trained technicians. The salaries of the professional staff should be sufficiently competitive to attract qualified personnel. Moreover, universities should find a formula that minimizes or eliminates current differences between regular faculty and investigators interested primarily in technology (applications as well as development of new procedures and new instrumentation). Core facilities run by highly competent personnel are needed if academic laboratories in the United States are to compete successfully with foreign academic laboratories and research institutes as well as with U.S. industrial laboratories.

Academic core facilities are also needed to fulfill other important functions generally neglected by industrial research laboratories. The training of students and postdoctoral fellows and the development of new instrumentation and technologies are particularly important among these functions.

National Centers for Specialized Technologies

Transgenic Mice. The panel agreed that research in cell biology would benefit from the establishment of centers with highly specific functions. As an example, it proposes the organization of a national center for undertaking the tedious and expensive procedure of producing transgenic mice. A resource of this kind could produce transgenic mice to order for molecular and cell biology laboratories scattered around the country. An investigator would simply send a probe to the laboratory, where it would be inserted into mouse embryos. The transgenic animals would then be shipped to the initiating laboratory. It is conceivable that every gene or mutated gene would eventually be studied by this system and could be recovered readily for molecular evaluation. Much more research on transgenic animals would probably be conducted if this service were available.

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The center could also function as a national storage facility for transgenic embryos in liquid nitrogen. This would be the only economical way in which large numbers of these animals could be retained for a long time.

Additional National or Regional Facilities. Molecular and cell biologists would benefit from other national or regional facilities, for example:

- genetic libraries for common laboratory animals and for plants of general interest;
- complementary DNA libraries for cell types of widespread interest obtained from such animals and plants;
- repositories of hybridomas and monoclonal antibodies for cell proteins of general interest; and
- data banks for protein and DNA sequences.

These facilities or services might be organized, funded, and administered as national centers, regional centers, or core facilities.

Optical Instrumentation

A new generation of light microscopes is revolutionizing structural studies of dynamic processes in cells (Allen, 1985). By using video images from high-quality light microscopes on line with electronic processors, one can increase contrast and decrease noise sufficiently to detect very small objects in live cells, e.g., vesicles only 40 nm in diameter and microtubules only 25 nm in diameter. This improved imaging has made it possible to detect and characterize vesicle movements along microtubules and exocytosis of synaptic vesicles. The cost of such videomicroscopy units places them beyond the budgets of most investigators. Since they have great potential for accelerating progress on many research frontiers, these units should be available in every major research center.

The resolution now attainable in advanced electron microscopes is better than 2 Å. Atoms can be resolved individually at the edge of gold crystals. But specimen preparation procedures for biological specimens are still lagging far behind. Resolution in scanning electron microscopy is now better than 25 Å because of improvements in instrumentation and specimen preparation, especially by the use of extremely thin (approximately 20 Å) continuous metal films for specimen coating.

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The most interesting and promising new development is the scanning electron tunneling microscope, which can operate at atomic resolution. If it proves as efficient as claimed, it may provide images of macro-molecules and bridge the gap still existing in structural studies between electron microscopy and x-ray diffraction.

Structural Biology and Computer-Assisted Modeling

In molecular and cell biology and related fields such as biochem-istry, biophysics, immunology, and neurobiology, the three-dimensional (tertiary) structure of molecules commands more and more attention. Tertiary structure largely determines the function of macromolecules in all intracellular and intercellular processes. Protein traffic control, receptor-ligand interactions, signal transduction, and regulation of cell-cell interactions (including immunological interactions) are only a few examples of such processes.

At present, only a few laboratories are equipped for studies in biologi-cal structure by x-ray diffraction, nuclear magnetic resonance spectrom-etry, computer graphics, and computer-assisted mathematical modeling. Such laboratories require access to large computers, which could be lo-cated in local or national facilities. The number of laboratories for structural studies should be increased as should the number of investi-gators.

Multidisciplinary Research

Research in many areas of cell biology is likely to become increas-ingly multidisciplinary, crossing the boundaries of cell biology, genetics, whole animal biology, and even animal husbandry. To perform such research, interdisciplinary and interinstitutional consortia of scientists with expertise in particular disciplines could be established. Collabo-rative research between investigators at different institutions or even in different locations within the same institution would be facilitated by the availability of computer terminals with access to electronic mail service, data banks, and other computer-assisted strategies for data storage, retrieval, exchange, and analysis.

Although individual research grant and program projects are clearly time-tested and efficient, the panel believes that multidisciplinary centers, exploiting a multiplicity of different approaches, may represent the best strategy for rapid progress in special areas such as regulation of the cell cycle, signal transduction, and signal-induced intracellular reactions.

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Plant Cell Biology

The recommendations made in the preceding section concerning the needs and support for current and future research in cell biology apply equally as well for research on procaryotic and eucaryotic cells of either animal or plant origin. However, plant cell biology faces special problems more basic than modernization of research facilities. By general consensus, the field is inadequately and in part inefficiently supported, notwithstanding its importance for biological sciences in general and for the well-being of the population and the economy of the United States. It is definitely much less well supported than research on animal cell biology. Given the inadequacy of support, it should not be surprising that many areas of research in the United States lag behind research carried out abroad, for example, in the Federal Republic of Germany, the United Kingdom, and Australia.

In the United States, the major contributor to research in plant biology is USDA. Much of this agency's support is committed to applied work, which is essential for the health of the U.S. agricultural enterprise. Relatively little is devoted to basic research in plant cell biology, and very little of that is available to institutions outside the land-grant system. There is a need for some system to coordinate appropriate support for research in basic and applied plant cell biology from various sources. Only within the last few years has USDA's Office of Competitive Research Grants (CRGO) come into existence. The CRGO provides funding for researchers in specific basic science fields underlying agriculture both in and out of the land-grant system. Other sources of support for basic research in plant biology include NIH, NSF, DoE, and ONR.

Administrators of the research funds in USDA, NSF, and other agencies must always judge whether to fund many individual investigators with small grants or a few people with large grants. CRGO policy has been the former. It might be desirable to review the relative allocation of small and large grants to ensure that important studies requiring the use of expensive new technologies can be funded when appropriate.

By its inherent promise, plant cell biology can attract many young, well-trained molecular and cell biologists, but few finally choose to make their careers in this field. The large majority is discouraged by the limited number of positions available and by the lack of adequate support.

In the United States, the study of photosynthesis in its broadest

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sense, i.e., including the organization of the photosynthetic apparatus and the developmental genetics of chloroplasts, is pursued by a modest number of scientists, considering the complexity and the importance of the subject. Funds for photosynthesis research have been provided primarily by NSF and DoE. In FY 1985, DoE support for such research totaled approximately $3.75 million (DoE, 1985). Smaller amounts have been supplied by the USDA's CRGO and by the NIH. The USDA has also supported a small amount of intramural basic research on photosynthesis.

Analyses of the physical aspects of photosynthesis, including light harvesting and excitation energy transfer, increasingly demand expensive and sophisticated equipment, but operating costs are relatively low. Analysis of photosynthetic membranes and of their component polypeptides has been relatively inexpensive. In contrast, operating costs for research in molecular biology are high, and funds have not been made available to meet these requirements. Thus, not only is insufficient money available for plant cell research but the individual awards are generally inadequate to permit the use of newly developed tools.

As noted elsewhere in this report, a German group was the first to obtain an x-ray diffraction pattern revealing the structure of a membrane protein complex (Deisenhofer *et al.*, 1985), and a Swedish group was the first to obtain useful x-ray patterns leading to a better understanding of the structure of ribulose bisphosphate carboxylase (Schneider *et al.*, 1986). Two Japanese groups are believed to have nearly completed the DNA sequencing of two entire chloroplast DNA molecules. While major progress on the molecular biology, biochemistry, and biophysics of photosynthesis is reported from Japan and Western Europe, progress in this research area lags behind in the United States. The support of research on photosynthesis in the United States is but one example of the limited support for research in plant cell biology. NSF has been very supportive of plant research; however, considering its many other commitments and restricted budget, it is not clear that NSF alone can support the kind of effort that the opportunities warrant.

Plastids. A substantial amount of basic information about the molecular and cell biology of plastids has been amassed, but expansion from this base is likely to be slow. Few people are studying these aspects of plastids, and financial support for such work is meager in relation to its significance for biology and the genetic engineering of crops.

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Plant Cells as Research Models. Although documentation is lacking, anecdotal information received by the panel suggests that the United States lags behind other countries not only in important aspects of research on photosynthesis, but also in other aspects of plant cell biology. For example, the John Innes Institute in England is a leading center for research on the cell surface recognition factors involved in pistil-pollen incompatibility reactions (Roberts *et al.*, 1985).

Since plant function presents an exciting challenge and we are completely dependent upon plants for our existence, it is not clear why there is little funding for plant cell biology in the United States in comparison to the support available for animal cell biology. Perhaps it is because the average U.S. citizen spends a relatively small proportion of total income on food, because of low cost agriculture compared to other countries and the large amount of arable land in proportion to the size of the U.S. population. Moreover, famine and associated diseases are not a threat in the United States. These facts have spawned the attitude that greater funding for plant research is not necessary when U.S. farmers and the taxpayers are already plagued with overproduction. Another reason, however, may lie in the NIH requirement that research proposals include a statement on the relevance of the research to human health, which may be difficult for a researcher using plant models.

In *Models for Biomedical Research*, a National Research Council committee concluded that knowledge of human biology in health and disease is only part of a large, multidimensional matrix of biological knowledge that, because of shared evolution, encompasses all of organismal biology (NRC, 1985). Thus, appropriate models in biomedical research can be found among a wide variety of organisms, including plants. That study group recommended that research support be awarded "without taxonomic or phylogenetic bias." Rigorous application of this recommendation might reduce, if not remove, the disadvantage perceived by many plant biologists in their quest for NIH research support. It should be recognized, however, that NIH supports much of the research done on yeasts and *Neurospora*.

TRAINING

Biological sciences in general and biomedical sciences in particular have enjoyed more support for training at the pre- and postdoctoral levels than most other fields in the natural sciences. This support has been rewarding; it has been one of the important ingredients leading

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to the spectacular progress made in recent times. Funds for training have come from NIH, from the Alcohol, Drug Abuse, and Mental Health Administration, and, in smaller amounts, from NSF. At the predoctoral level, most NIH support was allocated to interdepartmental training programs, which usually include biochemistry, genetics, and molecular and cell biology—a judicious choice meant to facilitate good overall training at the basic science level. The National Institute of General Medical Sciences (NIGMS) has provided funds for approximately 60% of all predoctoral trainees and for about 10% of those at the postdoctoral level, the rest of the support being divided among the other 11 units of NIH (C. Miller, NIH, personal communication, 1986).

Earlier in this report, the point was made that increased support for basic research through research grants, program grants, and appropriate facilities is needed if the U.S. research enterprise is to maintain momentum, retain its international prominence, and take full advantage of the unusual opportunities lying ahead. In contrast, training support is quite different: current and projected levels of funding fall short of covering even present needs.

The number of NIH-supported trainees has decreased continously since 1968, and sharp reductions are expected for the 1986 and 1987 budget years. Table 8 lists the numbers of trainees and the training funds for 1975, 1980, and 1985, as well as projections for 1986 and 1987. Table 9 gives the total number of full-time training positions supported by NIH for 1980 and 1984 and provides a breakdown by the three NIH units most heavily involved in training (NIGMS, the National Cancer Institute [NCI], and the National Heart, Lung, and Blood Institute [NHLBI]). Table 10 provides NIGMS data on predoctoral and postdoctoral training. Molecular and cell biology is the beneficiary of about one-third of the predoctoral training positions. The corresponding fraction for postdoctoral fellowships is more difficult to assess.

The decline in support is clearly evident, and the trend is alarming, especially when the following points are considered:

- NSF is offering only a small number (approximately 25) of postdoctoral fellowships in plant biology and has reduced the number of predoctoral fellowships to 560. .

- The majority of NIH training grants are now funded below the levels recommended by NIH advisory councils.

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Table 8. NIH-Supported Graduate Students and Postdoctoral Fellows

Year	$ (in millions)	% of NIH Budget	FTTP[a]
1975	154.9	10.5	12,272
1980	176.4	7.3	10,664
1985	217.5	5.0	10,624
1986	209.4	—[b]	9,948
1987[c]	198.2	—	9,258

[a] Full-time training positions.

[b] Data not available.

[c] President's proposed budget.

Table 9. Training Support (Predoctoral and Postdoctoral) for NIH as a Whole and for the Three Largest Sources of Support within NIH

	Number of Full-Time Training Positions			
Year	Total	NIGMS	NCI	NHLBI
1980	10,664	3,765	1,531	1,549
1984	10,514	3,581	1,465	1,688

Table 10. NIGMS Training Programs

		Number of Full-Time Training Positions	
Year	$ (in millions)	Predoctoral	Postdoctoral
1972	50.5	4,682	1,393
1980	47.6	2,955	607
1985	57.7	2,355	563
1986	55.2	2,268	369
1987[a]	52.7	2,100	200

[a] The President's budget provides funds only for noncompeting training grants and fellowships.

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- NIGMS will not be able to fund a number of approved training grants in the 1986 fiscal year.
- The President's budget for 1987 allocates funds primarily for continuing NIH training grants; support is not available for either competitive renewals or new grants or for postdoctoral fellowships in the budgets of most NIH institutes.

Despite impressive advances and bright opportunities, we are rapidly approaching a crisis in training. If reductions in training funds were defensible in the late 1960s because of a transient overproduction of young investigators, reductions at present appear shortsighted and inconsistent with our stated goals. We are not maintaining a proper balance between research and training. Research careers in basic biological sciences are becoming less and less attractive, primarily because of the instability introduced by these trends, and the resulting shortage of research personnel will severely affect future research, since it takes about 10 years to train a new investigator adequately.

Training is at present the darkest sector on the horizon of U.S. research in the basic biological sciences, including molecular and cell biology.

RESEARCH SUPPORT FOR CELL BIOLOGY IN OTHER COUNTRIES

The panel sought data on support for cell biology research and applications in other countries. To analyze trends, an attempt was made to obtain data for 1975, 1980, and 1985. But since the academic and governmental institutions of other countries generally do not recognize cell biology as a separate discipline, the effort was thwarted. The panel therefore considered basic biomedical research support as a whole and assumed that cell biology represents a fairly constant segment of that research. It was also assumed that agricultural research and university budgets for education included support for cell biology.

There is a dearth of recent comparative funding data for biomedical research. The most recent multicountry survey was conducted in 1983 by the Organization of Economic Cooperation and Development (OECD) with funding by NIH (Shepard and Durch, 1985). In this study, expenditures for biomedical research were analyzed for 19 industrialized countries. Among the countries examined, the United States spends by far the largest absolute amount for biomedical research and

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development—approximately $6.5 billion in 1980, accounting for 45% of the funds spent by all 19 countries. On a per capita basis, however, U.S. spending is exceeded by that of Sweden. Support from private (non-governmental) sources is considerably more active in other countries: the United States ranks sixth.

INTERNATIONAL TRENDS IN RESEARCH

Irvine *et al.* (1985) concluded that in eight research areas, including categories entitled "biology" and "biomedical research," there was a decline in the world share of publications and citations for the United Kingdom and the United States, whereas increases were found for France, the Federal Republic of Germany, and Japan. The same study showed a 5% increase in the U.S. share of "biology" publications emanating from major industrial countries from 1973 to 1982, in contrast to a 24% increase for Japan. For the same period, there was a 5% increase for the United States and a 64% increase for Japan in contributions to the world's publications in "biomedical research." In citations, the United States fared even worse, with declines of 5% in "biology" and 4% in "biomedical research" for the same period, whereas the Japanese share of citations grew by 32% for "biology" and 83% for "biomedical" research. The Federal Republic of Germany experienced increases of 13% and 48%, respectively, during the same period.

Although the limited data did not permit analysis of current funding patterns or recent trends, some important observations can nevertheless be made. The panel noted, for example, that although the United States enjoys leadership in many aspects of cell biology, there are instances in which research leadership either has already been lost, or may soon be, if present trends continue.

TRACKING U.S. GOVERNMENT SUPPORT FOR RESEARCH IN CELL BIOLOGY

The panel encountered numerous obstacles in its efforts to determine the amount and distribution of government support of research in cell biology. The obstacles can be summarized as essentially twofold:

- Cell biology has hazy boundaries and may often be classified in other disciplines.
- Federal agencies lack common definitions for cell biology; some do not use the designation.

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Despite these obstacles, it seems that the necessary data are available and accessible and that if sufficient resources were committed it would be possible to discern the present pattern of distribution of support for cell biology as well as trends in both the levels of support and their distribution among agencies. The results of such a study would be of great help in ensuring that future allocations of resources are adequate and appropriate for various aspects of animal and plant cell biology. By including a select group of foreign countries in such a study, it would be possible to monitor trends in the levels of support for cell biology in the United States and in other countries.

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APPENDIX A

DIAGRAM OF A REPRESENTATIVE CELL

To illustrate some of the many parts of the cell discussed in this report, the panel selected a diagram of an animal cell, which is depicted along with a detailed caption on the following two pages.

Opposite:

"An animal cell, as interpreted from electron micrographs. Like all cells, this one is bounded by an outer cell membrane, which acts as a selectively permeable barrier to the surrounding environment. All materials that enter or leave the cell, including food, wastes, and chemical messages, must pass through this barrier.

"Within the membrane is found the cytoplasm, which contains the enzymes and other solutes of the cell. The cytoplasm is traversed and subdivided by an elaborate system of membranes, the endoplasmic reticulum, a portion of which is shown here. In some areas, the endoplasmic reticulum is covered with ribosomes, the special structures on which amino acids are assembled into proteins. Ribosomes are also found elsewhere in the cytoplasm.

"Golgi bodies are packaging centers for molecules synthesized within the cell. The mitochondria are the sites of the chemical reactions that provide energy for cellular activities.

"The largest body in the cell is the nucleus. It is surrounded by a double membrane, the nuclear envelope, which is continuous with the endoplasmic reticulum. Within the nuclear envelope is a nucleolus, the site where the ribosomes are formed, and the chromatin, which is the material of the chromosomes in an extended form.

"Not shown in this diagram is the cytoskeleton, an elaborate, highly structured network of protein filaments. These filaments pervade the cytoplasm, anchoring the organelles, maintaining the cell's shape and directing the intracellular molecular traffic."

(Caption and figure from *Biology* by Helena Curtis, Worth Publishers, Inc., New York, with permission from the publisher.)

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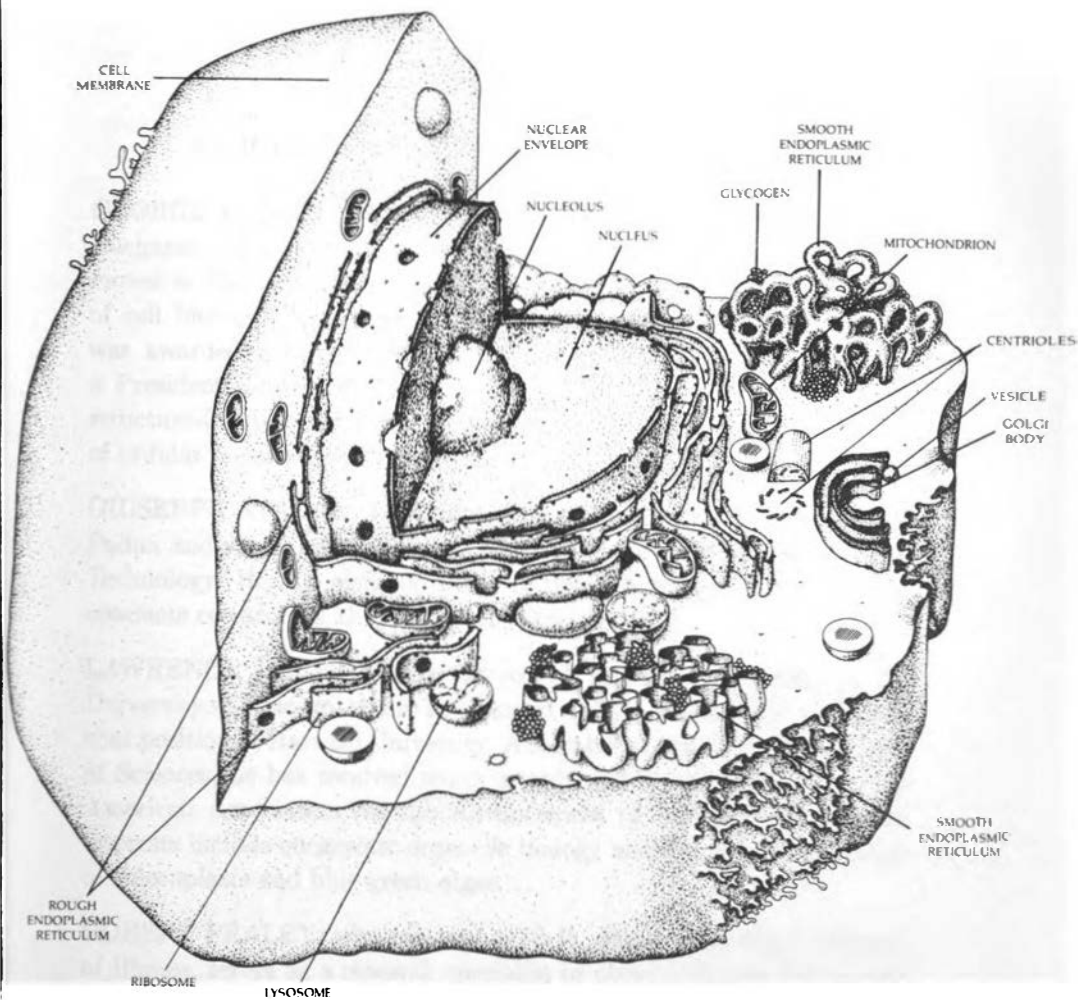
APPENDIX B

CURRICULA VITAE OF PANEL MEMBERS

GEORGE PALADE received an M.D. degree from the University of Bucharest. After serving on the faculty of the Rockefeller University, he moved to the Yale University School of Medicine, where he is professor of cell biology. A member of the National Academy of Sciences, he was awarded a Nobel Prize in Physiology or Medicine in 1974 and a Presidential Science Medal in 1986. His research interests include structure-function correlations at the subcellular level and the biology of cellular membranes.

GIUSEPPE ATTARDI holds an M.D. degree from the University of Padua and serves as professor of biology at the California Institute of Technology. He is a member of the National Academy of Sciences and conducts research on the biology of mitochondria.

LAWRENCE BOGORAD has served as professor of biology at the University of Chicago, where he received a Ph.D. degree, and now holds that position at Harvard University. A member of the National Academy of Sciences, he has received many awards and is now president of the American Association for the Advancement of Science. His research interests include eucaryotic organelle biology and the molecular biology of chloroplasts and blue-green algae.

ROBERT FRALEY, who received a Ph.D. degree from the University of Illinois, serves as a research specialist in plant molecular biology for the Monsanto Company. His principal research interest is the genetic engineering of plants, including the introduction of foreign genes into plant cells.

NORTON GILULA was awarded a Ph.D. degree from the University of California, Berkeley. Formerly professor of cell biology at the Baylor College of Medicine and a member of the faculty of the Rockefeller University, he recently joined the Research Institute of Scripps Clinic at

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La Jolla, California. He is editor-in-chief of the *Journal of Cell Biology* and conducts research on cell-to-cell communication.

THOMAS POLLARD holds an M.D. degree from the Harvard University School of Medicine. He is a professor and director of the Department of Cell Biology and Anatomy at The Johns Hopkins University Medical School. An associate editor of the *Journal of Cell Biology*, his research is focused on the molecular basis of cell motility.

FRANCIS RUDDLE earned a Ph.D. degree at the University of California, Berkeley. He is currently professor of biology and human genetics at Yale University. A member of the National Academy of Sciences, his research is focused on somatic cell genetics and differentiation.